File No: AI22-30-09740

QB/PC File No: CI-20-01-29284

IN THE COURT OF APPEAL

BETWEEN:

GATEWAY BIBLE BAPTIST CHURCH, PEMBINA VALLEY BAPTIST CHURCH, REDEEMING GRACE BIBLE CHURCH, THOMAS REMPEL, GRACE COVENANT CHURCH, SLAVIC BAPTIST CHURCH, CHRISTIAN CHURCH OF MORDEN, BIBLE BAPTIST CHURCH, TOBIAS TISSEN and ROSS MACKAY

(Applicants) Appellants

-and-

HER MAJESTY THE QUEEN IN RIGHT OF THE PROVINCE OF MANITOBA, and DR. BRENT ROUSSIN in his capacity as CHIEF PUBLIC HEALTH OFFICER OF MANITOBA, and DR. JAZZ ATWAL in his capacity as ACTING DEPUTY CHIEF OFFICER OF HEALTH MANITOBA

(Respondents) Respondents

APPELLANTS' APPEAL BOOK

VOLUME 3 (Pages AB522 to AB796)

May 20, 2022

Supreme Advocacy LLP

Eugene Meehan, Q.C.
Thomas Slade

[redacted]

Pejovic Law

Allison Kindle Pejovic

[redacted]

Counsel for the (Applicants), Appellants

AND TO: **Manitoba Justice, Legal Services Branch**
Constitutional Law Section
[redacted]

Michael Conner
Denis Guénette
[redacted]

Counsel for the Respondents

AND TO: **The Association for Reformed Political Action (ARPA) Canada**
[redacted]

André Schutten
[redacted]

Tabitha Ewert
[redacted]

Counsel for the Intervener
before the Court of Queen's Bench

INDEX

Tabs

VOLUME 1

VOLUME 2

VOLUME 3

VOLUME 4

VOLUME 5

VOLUME 6

3. Affidavits of the Respondent

VOLUME 7

VOLUME 8

VOLUME 9

VOLUME 11

File No. CI 20-01-29284

THE QUEEN'S BENCH
Winnipeg Centre

APPLICATION UNDER: *The Constitutional Questions Act*, C.C.S.M., c. 180

AND UNDER: The Court of Queen's Bench Rules, M.R. 553/88

IN THE MATTER OF: *The Public Health Act*, C.C.S.M. c. P210

BETWEEN:

GATEWAY BIBLE BAPTIST CHURCH, PEMBINA VALLEY BAPTIST CHURCH, REDEEMING GRACE BIBLE CHURCH, THOMAS REMPEL, GRACE COVENANT CHURCH, SLAVIC BAPTIST CHURCH, CHRISTIAN CHURCH OF MORDEN, BIBLE BAPTIST CHURCH, TOBIAS TISSEN, ROSS MACKAY

Applicants,

– and –

HER MAJESTY THE QUEEN IN RIGHT OF THE PROVINCE OF MANITOBA, DR. BRENT ROUSSIN in his capacity as CHIEF PUBLIC HEALTH OFFICER OF MANITOBA, and DR. JAZZ ATWAL in his capacity as ACTING DEPUTY CHIEF OFFICER OF HEALTH MANITOBA

Respondents.

AFFIDAVIT OF THOMAS WARREN
SWORN MARCH 30, 2021

JUSTICE CENTRE FOR CONSTITUTIONAL FREEDOMS
D. Jared Brown / Allison Kindle Pejovic / Jay Cameron
[redacted]

THE QUEEN'S BENCH
Winnipeg Centre

APPLICATION UNDER: *The Constitutional Questions Act*, C.C.S.M., c. 180

AND UNDER: The Court of Queen's Bench Rules, M.R. 553/88

IN THE MATTER OF: *The Public Health Act*, C.C.S.M. c. P210

BETWEEN:

GATEWAY BIBLE BAPTIST CHURCH, PEMBINA VALLEY BAPTIST CHURCH, REDEEMING GRACE BIBLE CHURCH, THOMAS REMPEL, GRACE COVENANT CHURCH, SLAVIC BAPTIST CHURCH, CHRISTIAN CHURCH OF MORDEN, BIBLE BAPTIST CHURCH, TOBIAS TISSEN, ROSS MACKAY

Applicants,

– and –

HER MAJESTY THE QUEEN IN RIGHT OF THE PROVINCE OF MANITOBA, DR. BRENT ROUSSIN in his capacity as CHIEF PUBLIC HEALTH OFFICER OF MANITOBA, and DR. JAZZ ATWAL in his capacity as ACTING DEPUTY CHIEF OFFICER OF HEALTH MANITOBA

Respondents.

AFFIDAVIT OF THOMAS WARREN

I, THOMAS WARREN of the City of Oakville, in the Province of Ontario,

MAKE OATH AND SAY AS FOLLOWS:

1. I have personal knowledge of the facts and matters hereinafter deposed to by me, except where same are stated to be based upon information and belief, and those I do verily believe to be true.

2. I am an Infectious Diseases specialist and Medical Microbiologist currently practicing in the locations of Oakville, Milton and Georgetown in Ontario.

3. I obtained my Doctor of Medicine (MD) from the University of Western Ontario in 2005, after which I completed a three-year residency in Internal Medicine through the University of Ottawa. Following my Internal Medicine residency, I completed a Fellowship in Infectious Diseases and a second residency in Medical Microbiology, both at the University of Toronto. During my residencies and fellowship I regularly taught medical students and junior residents.

4. I have practiced in these specialty areas for ten (10) years. As part of my clinical practice, I teach through my appointment as an Assistant Clinical Professor (Adjunct) at McMaster University in Hamilton, ON. This includes supervising Infectious Diseases Clinical Rotations for physician assistant students, medical students, and Infectious Diseases fellows.

5. I am currently enrolled in a Master's of Science (Epidemiology) at the London School of Hygiene and Tropical Medicine, University of London, with an expected completion date of 2022. Areas of study include the framework for understanding the epidemiology of infectious diseases and the mathematical theory underlying epidemiological studies.

6. In my medical microbiology residency I was trained to develop, use and interpret reverse transcription polymerase chain reaction (RT-PCR) testing. I have practiced as a microbiologist for ten years in a microbiology laboratory that uses a variety of PCR tests. As an infectious diseases consultant I interpret PCR test results in the context of clinical care.

7. A copy of my curriculum vitae is attached hereto and marked as **Exhibit "A"**.

8. The Applicants' counsel contacted me about providing expert testimony in response to the Affidavit of Dr. Brent Roussin, and the Affidavits and expert reports of Dr. Jared Bullard and Dr. Jason Kindrachuk. Specifically, I have been asked to respond to their evidence in respect of important issues surrounding the virus SARS-CoV-2 and Covid-19 disease, specifically: their description, PCR testing, and asymptomatic transmission.

9. A copy of my expert report is attached hereto and marked as **Exhibit "B"**.

10. I acknowledge that in preparing this report and providing expert evidence, the Applicants' counsel explained that my role is to assist the court to determine the matters in issue. I further acknowledge that it is my duty to provide evidence that is fair, objective and non-partisan and to opine only on matters that are within my area of expertise. This duty prevails over any obligation that I may owe to any party on whose behalf I am engaged.

11. I make this affidavit *bona fide*.

SWORN before me in the City of Winnipeg, in the Province of Manitoba, through use of video conferencing as permitted by order under *The Emergency Measures Act*, this 30th day of March, 2021.

L Lah

Commissioner of Oaths in and for the Province of Manitoba
My Commission Expires: July 8/21

T

THOMAS WARREN

THIS IS EXHIBIT "A" TO THE
AFFIDAVIT OF THOMAS WARREN
SWORN BEOFRE ME IN THE CITY
OF WINNIPEG THIS 30th DAY
OF MARCH, 2021

L Loeb

A COMMISIONER OF OATHS IN AND
FOR THE PROVINCE OF MANITOBA
MY COMMISSION EXPIRES: July 8/21

AB528

Thomas A. Warren, MD

[redacted]

Employment

2011 -	**Infectious Diseases consultant & Medical Microbiologist** Halton Healthcare, Oakville ON
2010-2011	**Internal Medicine specialist – locum coverage** St. Michael's Hospital, Toronto ON Hamilton Health Sciences, Hamilton ON Lakeridge Health, Oshawa ON
2010-2011	**University of Toronto** Department of Laboratory Medicine & Pathobiology, Toronto ON *Resident, Medical Microbiology*
2008-2010	**University of Toronto** Department of Medicine, Division of Infectious Diseases, Toronto ON *Fellow, Infectious Diseases*
2005-2008	**University of Ottawa** Department of Medicine, Ottawa ON *Resident, Internal Medicine*
1997-2003	**University of Western Ontario** Department of Medicine, London ON *Computer Programmer & Web Developer*

Education

2018 -	**London School of Hygiene and Tropical Medicine, University of London** *Master's of Science (Epidemiology)* Expected Completion 2022
2010-2011	**Royal College of Physicians & Surgeons of Canada** *Residency in Medical Microbiology*

2008-2010	**Royal College of Physicians & Surgeons of Canada** *Fellowship in Infectious Diseases*
2005-2008	**Royal College of Physicians & Surgeons of Canada** *Residency in Internal Medicine*
2001-2005	**University of Western Ontario** Schulich School of Medicine & Dentistry *Doctor of Medicine*
1997-2001	**University of Western Ontario** *Bachelor of Science - Honors Microbiology & Immunology* *(Scholar's Electives Program)* Graduated With Distinction

Continuing Medical Education

2018	**IDEAS Foundations of Quality Improvement Program** May 30 McMaster University Hamilton, ON
2018	**Clinical Teaching Fundamentals** January – March McMaster University Hamilton, ON

Peer-Reviewed Publications

2015	**Warren T**, Lau R, Ralevski F, Rau N, Boggild AK. Fever in a visitor to Canada: a case of mistaken identity. *J Clin Microbiol*. 53:1783-1785.
2012	**Warren TA**, Yau Y, Ratjen F, Tullis E, Waters V. Serum galactomannan in cystic fibrosis patients colonized with Aspergillus species. *Medical Mycology*. 2012; 50: 658-660.
2010	**Warren TA**, McTaggart L, Richardson SE, Zhang SX. Candida bracarensis Bloodstream Infection in an Immunocompromised Patient. *Journal of Clinical Microbiology*. 2010; 48: 4677–4679.

Abstracts & Conference Presentations

2011 **Warren TA**, Yau Y, Waters V.
Serum galactomannan in cystic fibrosis patients colonized with Aspergillus species.
Poster session presented at: Association of Medical Microbiology and Infectious Disease (AMMI) Canada 2011 Annual Conference
2011 April 7-9; Montreal, QC.

2010 **Warren TA**, Yau Y, Waters V.
Serum galactomannan in cystic fibrosis patients colonized with Aspergillus species.
Poster session presented at: North American Cystic Fibrosis Conference
2010 October 21-23; Baltimore, MD.

2010 **Warren TA**, Govindapillai S, Tullis E, Devlin HR, Ferris W, Matukas LM.
Evaluation of Etest Combination Testing of Antibiotics Against Isolates from Patients with Cystic Fibrosis.
Poster session presented at: 50th Interscience Conference on Antimicrobial Agents and Chemotherapy
2010 September 12-15; Boston, MA.

2010 **Warren TA**, Rotstein C, Cole EH, Singer LG, Keshavjee S4, Husain S.
Posaconazole therapy in solid organ transplant recipients refractory to or intolerant of standard therapy.
Poster session presented at: Canadian Society for Transplantation Annual Conference
2010 August 12-15; Vancouver, BC.

2010 **Warren TA**, McTaggart L, Zhang S. Candida bracarensis
Blood Stream Infection in an Immunocompromised Patient: Case Report.
Poster session presented at: Focus on Fungal Infections
2010 March 3-5; New Orleans, LA.

2007 **Warren TA**, McCarthy AE.
A Ten-Year Retrospective Study of Vaccination Rates, Prophylactic Antibiotic Use, Serious Infection and Overwhelming Postsplenectomy Sepsis Rates in Splenectomized Patients.
Poster session presented at: Annual Meeting of the Infectious Diseases Society of America
2007 October 4-7; San Diego, CA.

Awards

2011 **Best Student Poster Award – 2011 Annual Conference**
Association of Medical Microbiology and Infectious Disease (AMMI) Canada
Montreal, QC

2010 **ASM ICAAC Infectious Diseases Fellows Grant**
2010 Interscience Conference on Antimicrobial Agents and Chemotherapy
Boston, MA

2008 **Internal Medicine CanMeds Award for Communication**
University of Ottawa, Department of Medicine
Ottawa, ON

2006 **Resident Research Day Award of Excellence – PGY1**
University of Ottawa, Department of Medicine
Ottawa, ON

2001 **Laurene Paterson scholarship**
University of Western Ontario
London, ON

1997-2001 **Dean's Honor List**
University of Western Ontario, Faculty of Science
London, ON

1997 **Western Scholarship of Excellence**
University of Western Ontario
London, ON

Appointments

2013 - **McMaster University**
Assistant Clinical Professor (Adjunct)
Department of Medicine, Faculty of Health Sciences
Hamilton, ON

Teaching

2012-2021 **Infectious Diseases – Clinical Rotations**
Supervised physician assistant students, medical students, residents and infectious diseases fellows from the University of Toronto and McMaster University
Oakville, ON

2009	**Pathobiology of Disease** *Taught microbiology to second year medical students* University of Toronto Toronto, ON
2008	**Pathobiology of Disease** *Taught microbiology to second year medical students* University of Toronto Toronto, ON
2008	**Physical Skills Development Course** *Taught physical exam skills to first year medical students* University of Ottawa Ottawa, ON

Memberships

Association of Medical Microbiology and Infectious Diseases Canada

Canadian Medical Association

Canadian Medical Protective Association

College of Physicians and Surgeons of Ontario

Ontario Medical Association

Royal College of Physicians and Surgeons of Canada

THIS IS EXHIBIT "B" TO THE
AFFIDAVIT OF THOMAS WARREN
SWORN BEOFRE ME IN THE CITY
OF WINNIPEG THIS 30th DAY
OF MARCH, 2021

L Loeb

A COMMISIONER OF OATHS IN AND
FOR THE PROVINCE OF MANITOBA
MY COMMISSION EXPIRES: July 8/21

THOMAS WARREN M.D. – RESPONDING EXPERT REPORT ON SARS-Co-V-2 and COVID-19, PCR TESTS, AND ASYMPTOMATIC TRANSMISSION

March 29, 2021

SARS-CoV-2 and COVID-19 – Response to Dr. Roussin's Description

Dr. Roussin explains and describes SARS-CoV-2 and COVID-19 in his affidavit (paragraphs 20-26). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel coronavirus. There are six other coronaviruses that are known to infect humans. Four coronaviruses, HCoV-NL63, HCoV-HKU1, HCoV-229E, and HCoV-OC43 circulate worldwide and together are the second most common cause of the common cold[1,2]. Severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) infected 8096 people in 2003 resulting in 774 deaths[3]. After 2003 there has not been any further human to human transmission. Middle East respiratory syndrome coronavirus (MERS-CoV) was first identified in humans in 2012[4]. MERS-CoV continues to cause sporadic infection and outbreaks in the Arabian peninsula, as well as occasional other cases and outbreaks in other parts of the world linked to travelers to the Arabian peninsula[5].

Bats were the source of SARS-CoV-1[6] and are known to be a natural reservoir for related coronaviruses[7,8]. SARS-CoV-2 was likely circulating in bats for decades[9]. In late 2019, SARS-CoV-2 was first detected in humans and is established as the cause of the disease now designated coronavirus disease 2019 (COVID-19). Approximately 10-20% of persons with SARS-CoV-2 infection are asymptomatic[10,11]. In those who are symptomatic, there is a wide range of illness from those with mild symptoms such as runny nose to those with severe disease affecting particularly the respiratory tract with high mortality[12]. Most people with SARS-CoV-2 infection are asymptomatic or have mild-moderate symptoms not requiring hospitalization. In one study of a relatively healthy population, those with COVID-19 requiring hospital care was < 2%, and the mortality rate was < 0.1%[11].

What is PCR? – Response to Dr. Bullard

Dr. Bullard's report describes PCR technology and the utility of PCR in the identification of SARS-CoV-2 and diagnosis of COVID-19. Polymerase chain reaction (PCR) is a technology to amplify DNA fragments[13,14]. It is widely used in molecular biology, biotechnology, and medicine. Real time reverse transcription-polymerase chain reaction (real time RT-PCR) is a modification of PCR with an additional step of reverse transcription (RT) of RNA to DNA to enable amplification of an RNA target rather than a DNA target.

PCR is a relatively quick and inexpensive process that is highly sensitive. The process detects genetic material (DNA or RNA), even in minute quantities, and then copies it in a series of steps (cycles) that is usually done by a machine in a fully automated process. Each cycle doubles the amount of target DNA, and the newly created DNA is labeled with a fluorescent dye for detection. If a certain level of fluorescence is surpassed and detected by the machine, the test is considered positive. The cycle of the test that passes this threshold is called the cycle threshold (Ct).

PCR is commonly used in microbiology laboratories for the diagnosis of infectious diseases. While PCR can be the best diagnostic tool to diagnose many infections, it does have important limitations that also need to be considered. It is the limitations of PCR that are inadequately addressed in Dr. Bullard's report.

Limitations of PCR: Response to Dr. Bullard

The World Health Organization (WHO) recognizes the limitations of PCR and advises that "health care providers must consider any result in combination with timing of sampling, specimen type, assay specifics, clinical observations, patient history, confirmed status of any contacts, and epidemiological information"[15]. The WHO guidance goes on to say that "disease prevalence alters the predictive value of test results; as disease prevalence decreases, the risk of false positive increases. This means that the probability that a person who has a positive result (SARS-CoV-2 detected) is truly infected with SARS-CoV-2 decreases as prevalence decreases, irrespective of the claimed specificity."

In his affidavit, Dr. Bullard states, "Regardless of the particular Ct value, a positive RT-PCR result represents a true positive case of the SARS-CoV-2 virus." (paragraph 10). It is true that a positive RT-PCR result represents the identification of SARS-CoV-2 virus fragments, but a positive RT-PCR result does not necessarily indicate the entire virus is present, replication competent virus is present, or the patient has COVID-19. If the entire virus is not present in the person, or the virus is not replication competent, then the person is not infectious.

A recent study from Singapore[16] showed that the higher the Ct value, the larger degree of viral fragmentation and the less likely that an entire viral genome is actually present. In other words, the higher the Ct, the more likely it is that only bits of virus are being

detected, and less likely that entire virus is present in the patient. Only complete virus particles can be replication competent and therefore infectious and transmissible.

Dr. Bullard asserts that "while less likely, some individuals might still be infectious even at a higher Ct value" (paragraph 12), and "higher Ct values are associated with a lower likelihood of growing SARS-CoV-2 in cell culture, but this cannot rule that the person was or was not infectious at the time of sample collection."(lines 164-166) Many high quality studies published in leading peer-reviewed journals[17-23], including Dr. Bullard's own study published in *Clinical Infectious Diseases*[24], have convincingly shown that the higher the Ct, the less likely replication-competent virus (infectious virus) can be detected through cell culture. An editorial in *Clinical Infectious Diseases* regarding Dr. Bullard's study concluded "that PCR positivity is likely not a reliable surrogate marker for determining the infectious status of COVID-19 patients". A systematic review on the topic, also published in *Clinical Infectious Diseases*, concluded that test results "with high cycle threshold are unlikely to have infectious potential."[25]

That a positive test does not necessarily indicate infectiousness is indicated by recommendations for stopping isolation in persons previously positive for SARS-CoV-2. The WHO[26-27], CDC[28], and Canadian jurisdictions[29] recommend discontinuing isolation of persons with COVID-19 ten days after symptom onset, and in persons who have tested positive for SARS-CoV-2 without symptoms ten days after their first positive RT-PCR result, even though it is well established that many persons in these groups will continue to have positive RT-PCR results after those time frames. Those guidelines recommend against RT-PCR testing in these groups because it is known that positive tests in these groups does not indicate infectiousness. This is a concrete application of the evidence that late in the course of SARS-CoV-2 infection, which corresponds to increasing Ct, there is no risk of transmission. In another example, Ontario uses point-of-care tests, that are less sensitive than RT-PCR, to rule out SARS-CoV-2 infection[30] in persons who are symptomatic without known contact with a positive case. In this case, even though a more sensitive test such as RT-PCR might detect more positives, a less sensitive test[31] is sufficient to rule out significant infection.

Dr. Bullard states that "It is challenging to know where in their disease trajectory an individual with a high Ct is, thus it remains essential to identify them as a case, at a minimum to identify and investigate contacts in order to minimize secondary SARS-CoV-2 spread." (lines 209-212) What the evidence in fact shows, as outlined below, is that the higher the Ct value, the more likely it is that a person is in the later stages of the

infection, and therefore less infectious. The nearer the Ct value approaches 40, the closer the likelihood that the patient is infectious approaches zero.

A report from the Emerging Sciences Group of the Public Health Agency of Canada[32] concludes that in symptomatic persons there is "a peak in viral load ranging from just before to during the first week after onset of illness" and in asymptomatic persons "viable virus and viral RNA was highest during the first week of infection and declined in subsequent weeks." In persons that are asymptomatic or mildly symptomatic, late in the course of illness the Ct value is higher[21] and viable virus cannot be detected though cell culture. The likelihood of a positive cell culture correlates with disease severity[33], and therefore risk of infectiousness correlates with disease severity.

Dr. Bullard further asserts that "a positive test at a higher Ct value (indicating a lower viral load) may result because the individual is only at the early stages of the COVID-19 disease." (paragraph 12); however, a January 2021 peer-reviewed study[34] published in the *Journal of Clinical Medicine* clearly showed that the Ct value early in the course of illness is significantly lower than the Ct value late in the course of illness. The Ct value of pre-symptomatic persons has been shown to be low, and not be significantly different from symptomatic persons; in one peer-reviewed study published in the *New England Journal of Medicine*, the Ct value was 23.1 for pre-symptomatic persons compared to 24.8 in persons with typical symptoms[10]. There is a clear association between Ct value and stage of infection; the higher the cycle threshold[33], the more likely the patient is in the later stages of the infection, and the less likely the patient is infectious or at risk of transmitting the virus to another person.

SARS-CoV-2 infection versus COVID-19 disease – Response to Dr. Bullard

It is important to recognize the difference between SARS-CoV-2 infection and COVID-19 disease. This is an important distinction that is made with many other infections. As noted in a *British Medical Journal* editorial: "Unusually in disease management, a positive test result is the sole criterion for a Covid-19 case. Normally, a test is a support for clinical diagnosis, not a substitute."[35] In other words, for COVID-19, a positive test is sufficient to make the diagnosis, which is not done in other infections that are similarly mild and short-lived.

Dr. Bullard states that RT-PCR has "a specificity of greater than 99.9% (less than 1 in 1000 will have a false positive result)" (lines 135-136) and uses that to conclude that "If

the individual tests positive, they have the SARS-CoV-2 pathogen detectable and have been diagnosed with COVID-19." (lines 217-218) A positive test means there is a 99.9% likelihood that the person has or recently had SARS-CoV-2 (virus) in their body; however, it does not mean that the person is infectious or that they have COVID-19 disease (i.e. symptoms). If we wanted to define specificity as diagnosing infectiousness or disease (i.e. symptoms or pathology), then the specificity of RT-PCR would be dramatically lower. This distinction can be better understood if we look at how PCR is used in other infections.

To cite just a few examples, positive PCR tests for Group A Streptococcus, Salmonella, E. coli O157, Campylobacter, C. difficile, Epstein-Barr Virus (EBV), Cytomegalovirus (CMV) do not necessarily indicate disease. In the absence of symptoms or other tests indicating pathological effects, persons with positive PCR tests for those infections are not considered to have disease and they are usually not treated. The distinction between infection and disease is important. Over 50% of adult Canadians will be infected with EBV or CMV for most of their life; once acquired, those infections persist lifelong and PCR tests can detect those viruses, to varying degrees, throughout their lifetime. It would be inaccurate to say that over 50% of Canadians have disease associated with those viruses even though they can be detected by PCR.

A positive PCR result also does not necessarily indicate infectiousness. At any time, about 10% of school-aged children will have throat swabs positive for Group A Streptococcus (GAS)[36,37]. GAS can cause pharyngitis (strep throat), scarlet fever, rheumatic fever, and necrotizing fasciitis (flesh eating disease), but a positive test in an asymptomatic person is not considered significant in most cases.

Even a positive RT-PCR result in a person living with HIV does not necessarily mean that the person is considered infectious. According to Canadian law[38], "the combined effect of condom use and low viral load precludes a realistic possibility of transmission of HIV". Similarly, the Ontario Court of Appeal stated that "viral loads below a defined level, standing on their own, are sufficient to negate the realistic possibility of HIV transmission"[39].

The presence of SARS-CoV-2 virus as detected by PCR is necessary but not sufficient to indicate either infectiousness or COVID-19 *disease* properly defined. If a true positive is defined as the presence of complete virus, or replication competent virus (i.e. infectious virus) then the specificity of PCR is much lower and the number of false positives associated with PCR would be considered much higher.

Asymptomatic transmission: Response to Dr. Roussin and Dr. Kindrachuk

The affidavit from Dr. Roussin and the affidavit and expert report from Dr. Kindrachuk do not adequately synthesize or contextualize the risk of transmission in asymptomatic persons compared to pre-symptomatic or symptomatic persons.

A *British Medical Journal* editorial concisely summarizes the risk of asymptomatic transmission: "The transmission rates to contacts within a specific group (secondary attack rate) may be 3-25 times lower for people who are asymptomatic than for those with symptoms."[35] This is consistent with the conclusions from several peer-reviewed systematic reviews and meta-analyses[40-43].

To further exemplify the risk of asymptomatic transmission, it is useful to look specifically at a few large or comprehensive studies. A very large study, published in a leading peer-reviewed journal (*Nature Communications*) in Wuhan China of 9,899,828 city residents found 300 asymptomatic cases but there were no positive tests amongst 1,174 close contacts of asymptomatic cases[44]. Similarly, in a very thorough study of 100 cases from Taiwan, published in the peer-reviewed *Journal of the American Medical Association Internal Medicine*, found that "none of the 9 asymptomatic case patients transmitted a secondary case."[45]

Household transmission is one of the most important modes of transmission. In a meta-analysis of household transmission, published in the peer-reviewed *Journal of the American Medical Association Network Open*, which included 54 studies and 77 758 participants[46], transmission from asymptomatic cases was 0.7% compared to 18% transmission from symptomatic cases. In other words, symptomatic transmission was roughly 25 times higher than asymptomatic transmission.

In conclusion, asymptomatic transmission does occur but the rates of transmission from asymptomatic persons is substantially less than from symptomatic persons and does not warrant being considered a significant contributor to the overall transmission burden. There is no justification to limit the activities of asymptomatic persons since the risk of transmission is negligible compared to symptomatic persons.

REFERENCES CITED

1. Killerby ME, Biggs HM, Haynes A, Dahl RM, Mustaquim D, Gerber SI, Watson JT. Human coronavirus circulation in the United States 2014-2017. *J Clin Virol*. 2018 Apr;101:52-56.

2. Su S, Wong G, Shi W, Liu J, Lai ACK, Zhou J, Liu W, Bi Y, Gao GF. Epidemiology, Genetic Recombination, and Pathogenesis of Coronaviruses. *Trends Microbiol*. 2016 Jun;24(6):490-502.

3. https://www.who.int/publications/m/item/summary-of-probable-sars-cases-with-onset-of-illness-from-1-november-2002-to-31-july-2003

4. Dawson P, Malik MR, Parvez F, Morse SS. What Have We Learned About Middle East Respiratory Syndrome Coronavirus Emergence in Humans? A Systematic Literature Review. *Vector Borne Zoonotic Dis*. 2019 Mar;19(3):174-192.

5. https://www.who.int/health-topics/middle-east-respiratory-syndrome-coronavirus-mers#tab=tab_1

6. de Wit E, van Doremalen N, Falzarano D, Munster VJ. SARS and MERS: recent insights into emerging coronaviruses. *Nat Rev Microbiol*. 2016 Aug;14(8):523-34.

7. Wang LF, Shi Z, Zhang S, Field H, Daszak P, Eaton BT. Review of bats and SARS. *Emerg Infect Dis*. 2006 Dec;12(12):1834-40.

8. Li W, Shi Z, Yu M, Ren W, Smith C, Epstein JH, Wang H, Crameri G, Hu Z, Zhang H, Zhang J, McEachern J, Field H, Daszak P, Eaton BT, Zhang S, Wang LF. Bats are natural reservoirs of SARS-like coronaviruses. *Science*. 2005 Oct 28;310(5748):676-9.

9. Boni MF, Lemey P, Jiang X, Lam TT, Perry BW, Castoe TA, Rambaut A, Robertson DL. Evolutionary origins of the SARS-CoV-2 sarbecovirus lineage responsible for the COVID-19 pandemic. *Nat Microbiol*. 2020 Nov;5(11):1408-1417.

10. Arons MM, Hatfield KM, Reddy SC, Kimball A, James A, Jacobs JR, Taylor J, Spicer K, Bardossy AC, Oakley LP, Tanwar S, Dyal JW, Harney J, Chisty Z, Bell JM, Methner

M, Paul P, Carlson CM, McLaughlin HP, Thornburg N, Tong S, Tamin A, Tao Y, Uehara A, Harcourt J, Clark S, Brostrom-Smith C, Page LC, Kay M, Lewis J, Montgomery P, Stone ND, Clark TA, Honein MA, Duchin JS, Jernigan JA; Public Health–Seattle and King County and CDC COVID-19 Investigation Team. Presymptomatic SARS-CoV-2 Infections and Transmission in a Skilled Nursing Facility. *N Engl J Med*. 2020 May 28;382(22):2081-2090.

11. Kasper MR, Geibe JR, Sears CL, Riegodedios AJ, Luse T, Von Thun AM, McGinnis MB, Olson N, Houskamp D, Fenequito R, Burgess TH, Armstrong AW, DeLong G, Hawkins RJ, Gillingham BL. An Outbreak of Covid-19 on an Aircraft Carrier. *N Engl J Med*. 2020 Dec 17;383(25):2417-2426.

12. Berlin DA, Gulick RM, Martinez FJ. Severe Covid-19. *N Engl J Med*. 2020 Dec 17;383(25):2451-2460.

13. Templeton NS. The polymerase chain reaction. History, methods, and applications. *Diagn Mol Pathol*. 1992 Mar;1(1):58-72.

14. Garcia JG, Ma SF. Polymerase chain reaction: a landmark in the history of gene technology. *Crit Care Med*. 2005 Dec;33(12 Suppl):S429-32.

15. World Health Organization. WHO Information Notice for IVD Users 2020/05. 20 January 2021.

16. Choudhury Y, Cher CY, Wan ZY, Xie C, Lim JS, Virk RK, Tan MH, Teo AKJ, Hsu LY. A Viral Fragmentation Signature for SARS-CoV-2 in Clinical Samples Correlating with Contagiousness. medRxiv. doi: https://doi.org/10.1101/2021.01.11.21249265

17. Walsh KA, Spillane S, Comber L, Cardwell K, Harrington P, Connell J, Teljeur C, Broderick N, de Gascun CF, Smith SM, Ryan M, O'Neill M. The duration of infectiousness of individuals infected with SARS-CoV-2. *J Infect*. 2020 Dec;81(6):847-856.

18. Jaafar R, Aherfi S, Wurtz N, Grimaldier C, Hoang VT, Colson P, Raoult D, La Scola B. Correlation between 3790 qPCR positives samples and positive cell cultures including 1941 SARS-CoV-2 isolates. *Clin Infect Dis*. 2020 Sep 28:ciaa1491.

19. Singanayagam A, Patel M, Charlett A, Lopez Bernal J, Saliba V, Ellis J, Ladhani S, Zambon M, Gopal R. Duration of infectiousness and correlation with RT-PCR cycle threshold values in cases of COVID-19, England, January to May 2020. *Euro Surveill.* 2020 Aug;25(32):2001483.

20. La Scola B, Le Bideau M, Andreani J, Hoang VT, Grimaldier C, Colson P, Gautret P, Raoult D. Viral RNA load as determined by cell culture as a management tool for discharge of SARS-CoV-2 patients from infectious disease wards. *Eur J Clin Microbiol Infect Dis.* 2020 Jun;39(6):1059-1061.

21. Sohn Y, Jeong SJ, Chung WS, Hyun JH, Baek YJ, Cho Y, Kim JH, Ahn JY, Choi JY, Yeom JS. Assessing Viral Shedding and Infectivity of Asymptomatic or Mildly Symptomatic Patients with COVID-19 in a Later Phase. *J Clin Med.* 2020 Sep 10;9(9):2924.

22. van Kampen JJA, van de Vijver DAMC, Fraaij PLA, Haagmans BL, Lamers MM, Okba N, van den Akker JPC, Endeman H, Gommers DAMPJ, Cornelissen JJ, Hoek RAS, van der Eerden MM, Hesselink DA, Metselaar HJ, Verbon A, de Steenwinkel JEM, Aron GI, van Gorp ECM, van Boheemen S, Voermans JC, Boucher CAB, Molenkamp R, Koopmans MPG, Geurtsvankessel C, van der Eijk AA. Duration and key determinants of infectious virus shedding in hospitalized patients with coronavirus disease-2019 (COVID-19). *Nat Commun.* 2021 Jan 11;12(1):267.

23. Young BE, Ong SWX, Ng LFP, Anderson DE, Chia WN, Chia PY, Ang LW, Mak TM, Kalimuddin S, Chai LYA, Pada S, Tan SY, Sun L, Parthasarathy P, Fong SW, Chan YH, Tan CW, Lee B, Rötzschke O, Ding Y, Tambyah P, Low JGH, Cui L, Barkham T, Lin RTP, Leo YS, Renia L, Wang LF, Lye DC; Singapore 2019 Novel Coronavirus Outbreak Research team. Viral dynamics and immune correlates of COVID-19 disease severity. *Clin Infect Dis.* 2020 Aug 28:ciaa1280.

24. Bullard J, Dust K, Funk D, Strong JE, Alexander D, Garnett L, Boodman C, Bello A, Hedley A, Schiffman Z, Doan K, Bastien N, Li Y, Van Caeseele PG, Poliquin G. Predicting Infectious Severe Acute Respiratory Syndrome Coronavirus 2 From Diagnostic Samples. *Clin Infect Dis.* 2020 Dec 17;71(10):2663-2666.

25. Jefferson T, Spencer EA, Brassey J, Heneghan C. Viral cultures for COVID-19 infectious potential assessment - a systematic review. *Clin Infect Dis.* 2020 Dec 3:ciaa1764.

26. World Health Organization. Criteria for releasing COVID-19 patients from isolation. Scientific brief. 17 June 2020.

27. World Health Organization. COVID-19 Clinical management. Living guidance. 25 January 2021. WHO/2019-nCoV/clinical/2021.1

28. Centers for Disease Control and Prevention. Interim Guidance on Duration of Isolation and Precautions for Adults with COVID-19. Feb. 13, 2021.

29. Province of Ontario. Ministry of Health. COVID-19 Quick Reference Public Health Guidance on Testing and Clearance. Version 13.0 March 5, 2021.

30. Province of Ontario. Appendix 9: Management of Individuals with Point-of-Care Testing Results. Version 3.0 March 5, 2021.

31. Canadian Public Health Laboratory Network and the Canadian Society of Clinical Chemists. Interim guidance on the use of the Abbott ID NOW™ instrument and COVID-19 assay. *Can Commun Dis Rep* 2020;46(11/12):422–6. https://doi.org/10.14745/ccdr.v46i1112a09.

32. Emerging Sciences Group of the Public Health Agency of Canada. COVID BRIEF. How long are people with COVID-19 infectious? CCDR. November 5, 2020. Vol. 46 No. 11/1. Page 436.

33. Basile K, McPhie K, Carter I, Alderson S, Rahman H, Donovan L, Kumar S, Tran T, Ko D, Sivaruban T, Ngo C, Toi C, O'Sullivan MV, Sintchenko V, Chen SC, Maddocks S, Dwyer DE, Kok J. Cell-based culture of SARS-CoV-2 informs infectivity and safe de-isolation assessments during COVID-19. *Clin Infect Dis*. 2020 Oct 24:ciaa1579.

34. Manzulli V, Scioscia G, Giganti G, Capobianchi MR, Lacedonia D, Pace L, Cipolletta D, Tondo P, De Nittis R, Rondinone V, Serrecchia L, Parisi A, Galante D, Lo Caputo S, Santantonio TA, Moschetta D, Dattoli V, Fasanella A, Foschino Barbaro MP. Real Time PCR and Culture-Based Virus Isolation Test in Clinically Recovered Patients: Is the Subject Still Infectious for SARS-CoV2? *J Clin Med*. 2021 Jan 15;10(2):309.

35. Pollack AM, Lancaster J. Asymptomatic transmission of covid-19. *BMJ*. 2020;371:m4851.

36. Martin JM, Green M, Barbadora KA, Wald ER. Group A streptococci among school-aged children: clinical characteristics and the carrier state. *Pediatrics*. 2004 Nov;114(5):1212-9.

37. Shaikh N, Leonard E, Martin JM. Prevalence of streptococcal pharyngitis and streptococcal carriage in children: a meta-analysis. *Pediatrics*. 2010 Sep;126(3):e557-64.

38. *R v Maibor* 2012 SCC 47 at para 103.

39. *R v N.G.*, 2020 ONCA 494 at para 80.

40. Buitrago-Garcia D, Egli-Gany D, Counotte MJ, Hossmann S, Imeri H, Ipekci AM, Salanti G, Low N. Occurrence and transmission potential of asymptomatic and presymptomatic SARS-CoV-2 infections: A living systematic review and meta-analysis. PLoS Med. 2020 Sep 22;17(9):e1003346.

41. Byambasuren O, Cardona M, Bell K, Clark J, McLaws M-L, Glasziou P. Estimating the extent of asymptomatic COVID-19 and its potential for community transmission: Systematic review and meta-analysis. *JAMMI*. 5.4, 2020. 223-234.

42. Koh WC, Naing L, Chaw L, Rosledzana MA, Alikhan MF, Jamaludin SA, Amin F, Omar A, Shazli A, Griffith M, Pastore R, Wong J. What do we know about SARS-CoV-2 transmission? A systematic review and meta-analysis of the secondary attack rate and associated risk factors. PLoS One. 2020 Oct 8;15(10):e0240205.

43. Qiu X, Nergiz AI, Maraolo AE, Bogoch II, Low N, Cevik M. Defining the role of asymptomatic and pre-symptomatic SARS-CoV-2 transmission - a living systematic review. *Clin Microbiol Infect*. 2021 Jan 20:S1198-743X(21)00038-0.

44. Cao S, Gan Y, Wang C, Bachmann M, Wei S, Gong J, Huang Y, Wang T, Li L, Lu K, Jiang H, Gong Y, Xu H, Shen X, Tian Q, Lv C, Song F, Yin X, Lu Z. Post-lockdown SARS-CoV-2 nucleic acid screening in nearly ten million residents of Wuhan, China. *Nat Commun*. 2020 Nov 20;11(1):5917.

45. Cheng HY, Jian SW, Liu DP, Ng TC, Huang WT, Lin HH; Taiwan COVID-19 Outbreak Investigation Team. Contact Tracing Assessment of COVID-19 Transmission

Dynamics in Taiwan and Risk at Different Exposure Periods Before and After Symptom Onset. *JAMA Intern Med*. 2020 Sep 1;180(9):1156-1163.

46. Madewell ZJ, Yang Y, Longini IM Jr, Halloran ME, Dean NE. Household Transmission of SARS-CoV-2: A Systematic Review and Meta-analysis. *JAMA Netw Open*. 2020 Dec 1;3(12):e2031756.

File No. CI 20-01-29284

THE QUEEN'S BENCH
Winnipeg Centre

APPLICATION UNDER: *The Constitutional Questions Act*, C.C.S.M., c. 180

AND UNDER: The Court of Queen's Bench Rules, M.R. 553/88

IN THE MATTER OF: *The Public Health Act*, C.C.S.M. c. P210

BETWEEN:

GATEWAY BIBLE BAPTIST CHURCH, PEMBINA VALLEY BAPTIST CHURCH, REDEEMING GRACE BIBLE CHURCH, THOMAS REMPEL, GRACE COVENANT CHURCH, SLAVIC BAPTIST CHURCH, CHRISTIAN CHURCH OF MORDEN, BIBLE BAPTIST CHURCH, TOBIAS TISSEN, ROSS MACKAY

Applicants,

– and –

HER MAJESTY THE QUEEN IN RIGHT OF THE PROVINCE OF MANITOBA, DR. BRENT ROUSSIN in his capacity as CHIEF PUBLIC HEALTH OFFICER OF MANITOBA, and DR. JAZZ ATWAL in his capacity as ACTING DEPUTY CHIEF OFFICER OF HEALTH OF MANITOBA

Respondents.

AFFIDAVIT OF JAY BHATTACHARYA
SWORN MARCH 31, 2021

JUSTICE CENTRE FOR CONSTITUTIONAL FREEDOMS
D. Jared Brown / Allison Kindle Pejovic / Jay Cameron
[redacted]

THE QUEEN'S BENCH
Winnipeg Centre

APPLICATION UNDER: *The Constitutional Questions Act*, C.C.S.M., c. 180

AND UNDER: The Court of Queen's Bench Rules, M.R. 553/88

IN THE MATTER OF: *The Public Health Act*, C.C.S.M. c. P210

BETWEEN:

GATEWAY BIBLE BAPTIST CHURCH, PEMBINA VALLEY BAPTIST CHURCH, REDEEMING GRACE BIBLE CHURCH, THOMAS REMPEL, GRACE COVENANT CHURCH, SLAVIC BAPTIST CHURCH, CHRISTIAN CHURCH OF MORDEN, BIBLE BAPTIST CHURCH, TOBIAS TISSEN, ROSS MACKAY

Applicants,

– and –

HER MAJESTY THE QUEEN IN RIGHT OF THE PROVINCE OF MANITOBA, DR. BRENT ROUSSIN in his capacity as CHIEF PUBLIC HEALTH OFFICER OF MANITOBA, and DR. JAZZ ATWAL in his capacity as ACTING DEPUTY CHIEF OFFICER OF HEALTH OF MANITOBA

Respondents.

AFFIDAVIT OF JAY BHATTACHARYA

I, JAY BHATTACHARYA of the City of Los Altos, in the County of Santa Clara, in the State of California,

MAKE OATH AND SAY AS FOLLOWS:

1. I have personal knowledge of the facts and matters hereinafter deposed to by me, except where same are stated to be based upon information and belief, and those I do verily believe to be true.

2. I have reviewed the affidavits filed by the Respondents in this matter, and I make this affidavit and a responding expert report in order to address the evidence provided by the Respondents in support of their Covid-19 Public Health Orders.

3. While the Respondents' affiants and experts address many topics related to my January 5, 2021 expert report, I note at the outset one overarching issue that their affiants did not address. In particular, nowhere do their affiants provide a formal analysis of the marginal benefits and vast harms of the various lockdown policies – church closures, restrictions on outdoor gatherings, restrictions on private in-home gatherings, restrictions on social interactions, etc. – that the Respondents have imposed. The Respondents' affiants provide their views and analyses on the benefits of these policies in terms of reduced COVID-19 disease spread, but do not provide any formal analyses of the harms of these policies, many of which I documented with reference to the scientific literature in my January 5, 2021 expert report. This insufficient consideration of a policy's harms violates a basic principle of public health, which I outlined in my expert report and which the Respondents did not contest.

4. In my responding expert report, I organize my responses to the Respondents' affiants on 9 topics: (1) the lack of a causal link between lockdown policies and subsequent growth in COVID-19 cases; (2) the evidence on emergence of mutated variants of the SARS-CoV-2 virus; (3) the relative inefficiency of asymptomatic viral carriers to infect others; (4)

errors in the polymerase chain reaction (PCR) test use to identity viral presence; (5) the futility of contact tracing programs to eliminate COVID-19 disease spread; (6) the Respondents' affiants' mischaracterization of the Great Barrington Declaration; (7) the possibility of effective focused protection of the vulnerable; (8) a problematic analysis by Dr. Jason Kindrachuk on the possibility of herd immunity as the long run outcome; and (9) a failure by the Respondents to consider the collateral harms of lockdown policies.

5. As of March 29, 2021, the online signature count on the Great Barrington Declaration has increased to include 13,796 medical & public health scientists, 41,890 medical practitioners and 764,085 concerned citizens around the world.

6. I acknowledge that in preparing my January 5, 2021 and March 31, 2021 expert reports and providing expert evidence, the Applicants' counsel explained that my role is to assist the court in determining the matters in issue. I further acknowledge that it is my duty to provide evidence that is fair, objective and non-partisan and to opine only on matters that are within my area of expertise. This duty prevails over any obligation that I may owe to any party on whose behalf I am engaged.

7. I make this affidavit *bona fide*.

SWORN before me in the City of Winnipeg, in the Province of Manitoba, through the use of video conferencing as permitted by order under *The Emergency Measures Act*, this 31st day of March, 2021.

JAY BHATTACHARYA

A Commissioner of Oaths in and
For the Province of Manitoba
My Commission Expires: July 8/21

THIS IS EXHIBIT "A" TO THE
AFFIDAVIT OF JAY BHATTACHARYA
SWORN BEFORE ME IN THE CITY
OF WINNIPEG THIS 31st DAY
OF MARCH, 2021

A COMMISIONER OF OATHS IN AND
FOR THE PROVINCE OF MANITOBA
MY COMMISSION EXPIRES: July 9/21

AB553

JAY BHATTACHARYA M.D., Ph.D. – RESPONDING EXPERT REPORT ON THE COVID-19 EPIDEMIC RESPONSE IN MANITOBA

March 31, 2021

There is no established causal link between lockdown policies and COVID-19 case growth and mortality rate

The Respondents' arguments are all premised on the assertion that lockdown policies, such as prohibitions on gatherings, in-person worship and non-essential business closures, etc. work to reduce the risk of COVID-19 infection in the community. Much of the evidence they refer to in their affidavits/reports (especially, Kindrachuk) is based on modeling studies, which as noted in the original January 5, 2021 expert report, have a poor track record.

Now, however, there has emerged a growing peer-reviewed empirical literature that demonstrates the futility of lockdowns to control COVID case growth over a long period of time. This may be illustrated by describing one peer-reviewed study recently published in the *European Journal of Clinical Investigation*, of which I am a co-author. This study compares the effectiveness of mandatory lockdown orders (stay-at-home orders and forced business closures) versus less restrictive policies adopted by ten European and Asian countries on case growth in Spring 2020.[1] This study re-analyzes and revises the results from an earlier study by using countries that did not introduce mandatory stay-at-home orders and business closures over this period (like Sweden and South Korea) as a comparison with countries that did.[2] The main conclusion arising from this analysis is that "While small benefits cannot be excluded, [my co-authors and I] do not find significant benefits on case growth of more restrictive NPIs. Similar reductions in case growth may be achievable with less restrictive interventions."

Other peer-reviews papers, using different methodologies, and different comparison countries and regions, confirm this finding.[3,4,5] Perhaps the best peer-reviewed study evaluating the efficacy of lockdowns was published this past month in the prestigious journal, *Scientific Reports*. The analysis considers the effects of non-pharmaceutical interventions such as those imposed in Canada on COVID-19 related mortality in 87 regions around the world. The primary finding is that in the vast majority of cases there is no detectable effect of lockdowns on COVID

---

[1] Bendavid E, Oh C, Bhattacharya J, Ioannidis J (2020) "Assessing Mandatory Stay-at-Home and Business Closure Effects on the Spread of COVID-19" *European Journal of Clinical Investigation*. 5 January 2020. doi:10.1111/eci.13484

[2] Hsiang S, Allen D, Annan-Phan S, et al. The effect of large-scale anti-contagion policies on the COVID-19 pandemic. *Nature*. 2020;584(7820):262-267. doi:10.1038/s41586-020-2404-8

[3] Savaris, R. F., Pumi, G., Dalzochio, J., & Kunst, R. (2021). Stay-at-home policy is a case of exception fallacy: an internet-based ecological study. Scientific Reports, 11(1), 5313. https://doi.org/10.1038/s41598-021-84092-1

[4] Berry, C. R., Fowler, A., Glazer, T., Handel-Meyer, S., & MacMillen, A. (2021). Evaluating the effects of shelter-in-place policies during the COVID-19 pandemic. Proceedings of the National Academy of Sciences of the United States of America, 118(15). https://doi.org/10.1073/pnas.2019706118

[5] Karáth, K. (2020). Covid-19: How does Belarus have one of the lowest death rates in Europe? The BMJ, 370. https://doi.org/10.1136/bmj.m3543

mortality. The authors conclude that "With our results, we were not able to explain if COVID-19 mortality is reduced by staying at home in ~ 98% of the comparisons after epidemiological weeks 9 to 34." Earlier work[6], which used modeling methodologies that held as a fixed assumption that lockdowns reduce disease spread to conclude that lockdowns were effective, have been criticized on methodological grounds.[7] The observational data analyses cited here do not suffer from this methodological flaw.

Another response to the Respondents' conclusions that lockdowns (Public Health Orders) are needed in order to reduce transmission, reduce cases and prevent death is to examine a case study contrasting COVID results in California (which has implemented extended lockdowns, including mandatory stay-at-home orders, curfews, school, church, and business closures, among other strategies at various points during the epidemic), and Florida which is demographically similar to California, but has not implemented harsh lockdown since May 2020 (and entirely lifted lockdowns in September 2020).

Through March 28th, 2021, 8.9% of all Californians have been identified as COVID cases – 3.6 million cases.[8] Since most infections are not recognized as cases, a much larger fraction of the population has been infected with COVID.[9] Through March 31st, nearly 58,000 people have died in California with COVID.[10] In sharp contrast with California, Florida partially lifted its lockdown in May 2020[11] and then further relaxed restrictions in September 2020.[12] Most Florida schools and universities have been open for in-person instruction since the fall, normal human

---

[6] Flaxman S, Mishra S, Gandy A, Unwin HJT, Mellan TA, Coupland H, Whittaker C, Zhu H, Berah T, Eaton JW, Monod M; Imperial College COVID-19 Response Team, Ghani AC, Donnelly CA, Riley S, Vollmer MAC, Ferguson NM, Okell LC, Bhatt S. Estimating the effects of non-pharmaceutical interventions on COVID-19 in Europe. *Nature*. 2020 Aug;584(7820):257-261. doi: 10.1038/s41586-020-2405-7. Epub 2020 Jun 8. PMID: 32512579.

[7] Kuhbandner, C., & Homburg, S. (2020). Commentary: Estimating the effects of non-pharmaceutical interventions on COVID-19 in Europe. *Frontiers in Medicine*, 7, 257–261. https://doi.org/10.3389/fmed.2020.580361

[8] Financial Times COVID Tracker (2021) "Cumulative Confirmed Cases of COVID-19 in Florida and California" ? https://ig.ft.com/coronavirus-chart/?areas=can&areas=swe&areasRegional=usfl&areasRegional=usca&cumulative=0&logScale=0&per100K=1&startDate=2020-03-01&values=cases Accessed March 31, 2021.

[9] Sood N, Simon P, Ebner P, Eichner D, Reynolds J, Bendavid E, Bhattacharya J. Seroprevalence of SARS-CoV-2-Specific Antibodies Among Adults in Los Angeles County, California, on April 10-11, 2020. JAMA. 2020 Jun 16;323(23):2425-2427. doi: 10.1001/jama.2020.8279. PMID: 32421144; PMCID: PMC7235907.

[10] Financial Times COVID Tracker (2021) "Cumulative Deaths Attributed to COVID-19 in Florida and California" https://ig.ft.com/coronavirus-chart/?areas=can&areasRegional=usfl&areasRegional=usca&cumulative=0&logScale=0&per100K=1&startDate=2020-03-01&values=deaths Accessed March 31, 2021.

[11] Emily Crane (2020) "Florida is Back in Business!" *Daily Mail*. May 4, 2020. https://www.dailymail.co.uk/news/article-8285211/Florida-reopens-economy-states-continue-lift-COVID-19-lockdowns.html

[12] Greg Allen (2020) "Florida's Governor Lifts All COVID-19 Restricitons on Businesses Statewide" National Public Radio KQED. September 25, 2020. https://www.npr.org/sections/coronavirus-live-updates/2020/09/25/916969969/floridas-governor-lifts-all-covid-19-restrictions-on-businesses-statewide

activities – sports, church-going, visits to the park – occur with regularity, and businesses have been open for in-person activities.[13] Local ordinances can recommend masks and social distancing and impose indoor capacity limitations but cannot mandate closures, as is the case in California. Disneyworld in Orlando, Florida has been open since July.[14] At the same time, Florida increased testing and protection of its nursing homes to reduce the risk of COVID among its most vulnerable residents.

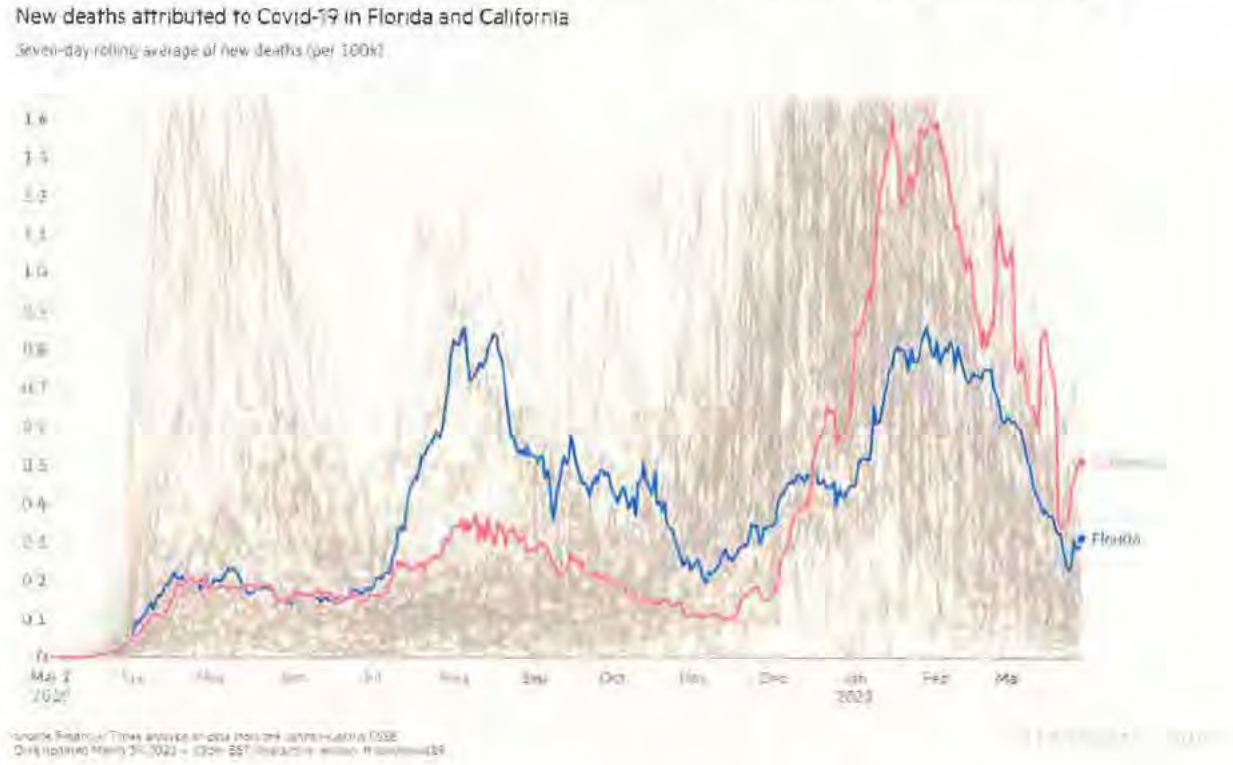
Despite the dramatically different policies, the infection control results to date in Florida look remarkably similar to California's, and in some ways better. Through March 28th, 2021, 9.5% of Floridians have been identified as COVID cases.[15] Once we account for the fact that Florida has the fifth oldest population in the country and California the seventh youngest,[16] the death rates with COVID through January 20th are, by my calculations, almost identical in the two states. In fact, the COVID mortality rate for *both* the under-65 population and the over-65 population are lower to date in Florida than in California.

Figure 1, immediately below, illustrates the numbers cited above; it compares the trend in COVID-19 deaths in California and Florida through the entire epidemic. Despite one of the sharpest lockdowns in the United States (including closed schools, shuttered businesses and churches, periodic shelter-in-place orders and curfews, and mask mandates), California has had higher COVID-19 mortality since December 2020. At best, one can say that the lockdowns delayed spread of the disease in California by a few months, at enormous harm to the population.

---

[13] USA Today (2020/1) "COVID-19 Restrictions. Map of COVID-19 Case Trends, Restrictions, and Mobility" https://www.usatoday.com/storytelling/coronavirus-reopening-america-map/ Accessed February 18, 2021.
[14] Janine Puhak and Michael Bartiromo (2020) "Disney World Targets July 11 as Reopening Date for Theme Park" Fox News. https://www.foxnews.com/travel/disney-world-present-reopening-plans-theme-park.
[15] Financial Times COVID Tracker (2021) "Cumulative Confirmed Cases of COVID-19 in Florida and California" https://ig.ft.com/coronavirus-chart/?areas=can&areasRegional=usfl&areasRegional=usca&cumulative=0&logScale=0&per100K=1&startDate=2020-03-01&values=deaths Accessed March 31, 2021.
[16] World Population Review (2021) Median Age by State 2021. https://worldpopulationreview.com/state-rankings/median-age-by-state. Accessed March 31, 2021.

Figure 1: COVID-19 Deaths in California vs. Florida. March 2020 – March 2021.



The Respondents in their affidavits/reports seem to think of lockdowns as the only possible way to protect the population from exposure to COVID risk. In reality, the California lockdowns and elsewhere have served to protect only a portion of the population – the rich. Data from L.A. County, where a large fraction of COVID cases in California has occurred, put this fact in stark relief.[17] Through January 23rd, in the wealthiest parts of L.A. county (with less than 10% poverty), the age-adjusted death rate with COVID-19 was 76 people per 100,000 population. As we look in poorer and poorer areas, the death rate mounts; areas with more than 30% poverty have faced a death rate of 263 people per 100,000 population – more than three times as many deaths. Hispanics in L.A. have borne the worst of the pandemic, with a death rate of 219 per 100,000 people. By contrast, black, Asian, and white residents have experienced 131, 96, and 78 deaths per 100k residents, respectively. The California and Canadian[18] lockdowns are a form of trickle-down epidemiology. In Florida, by contrast, there is little difference between races in

[17] LA County Public Health (2021) "Age-Adjusted Death Rates due to COVID-19 per 100K." http://publichealth.lacounty.gov/media/Coronavirus/data/index.htm#graph-deathrate. Accessed January 23, 2021.

[18] Kulldorff M and Gupta S (2020) Canada's COVID-19 Strategy is an Assault on the Working Class. Toronto Sun, Nov. 28, 2020. https://torontosun.com/opinion/columnists/opinion-canadas-covid-19-strategy-is-an-assault-on-the-working-class

COVID-related death rates throughout the epidemic. with the Hispanic population dying at lower rates than the white population.[19]

The Emergence of Variant Strains of the SARS-CoV-2 Virus Does Not Justify Continuing Lockdowns

Kindrachuk and Roussin both express concern about Covid variants in their affidavits/reports and suggest a continuance of measures which would reduce community transmission. Their assertion echoes predictions made by the Canadian public health forecast dated February 19th 2021. The Canadian Public Health Agency's forecast examined the impact of new mutated variants of the SARS-CoV-2 virus on future case growth. In particular, the Agency's model predicted that the spread of particular variants common in the U.S. and in the U.K. throughout Canada would lead to a sharp increase in the number of COVID-19 cases throughout Canada in the coming months. In February, The Public Health Agency of Canada predicted an imminent catastrophic new wave of cases of greater magnitude than the cases in the first and second wave combined, based on the assumptions that a mutated variant of the SARS-CoV-2 virus will spread throughout Canada in the next weeks.[20] Figure 2, immediately below, shows this official Canadian forecast. In the figure, there are three lines, one corresponding to the lifting of all restrictions, one corresponding to the maintenance of the lockdowns as they were in mid-February, and a third corresponding to a tightening of restrictions. The first two predicted a sharp growth in Canadian cases, while the third predicted a decline in cases. In point of fact, several Canadian provinces started lifting restrictions in February 2021.[21, 22]

---

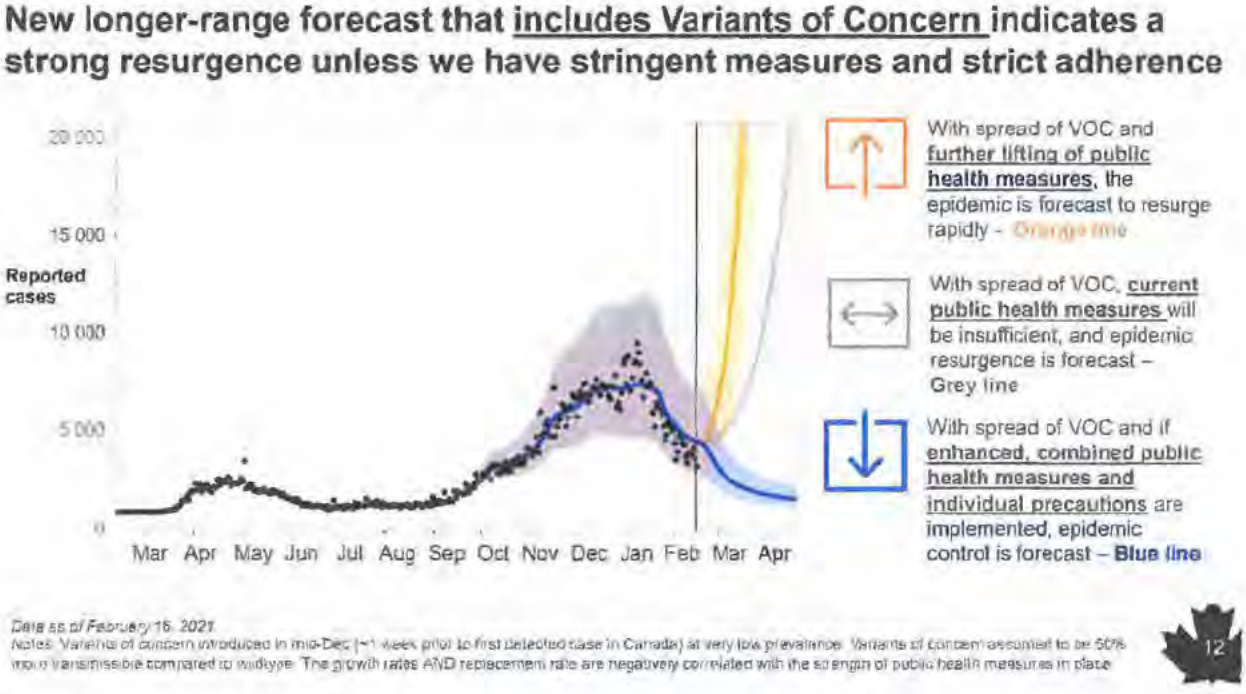
[19] COVID Tracking Project (2021) "The Data: Florida" https://covidtracking.com/data/state/florida. Accessed January 23, 2021.

[20] Public Health Agency of Canada (2021) *Update on COVID-19 in Canada: Epidemiology and Modelling*. https://www.canada.ca/content/dam/phac-aspc/documents/services/diseases-maladies/coronavirus-disease-covid-19/epidemiological-economic-research-data/update-covid-19-canada-epidemiology-modelling-20210219-en.pdf

[21] Serebrin J (2021) COVID-19 Restrictions Relaxed in Several Provinces, but Variant Concerns Persist. Canada's National Observer. February 9, 2021. https://www.nationalobserver.com/2021/02/08/news/covid-19-restrictions-relaxed-provinces-variant

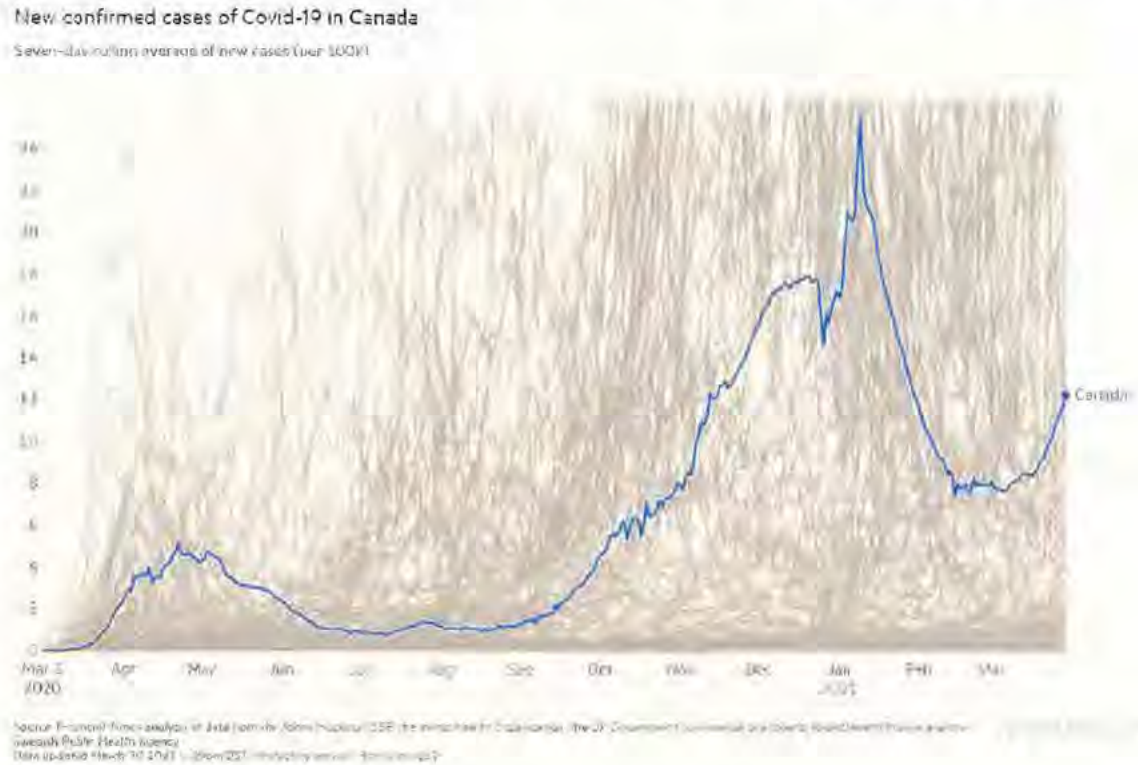
[22] The Canadian Press (2021) Quebec to Ease COVID-19 Restrictions Outside Montreal Area March 8. March 4, 2020. https://www.msn.com/en-ca/news/canada/quebec-to-ease-covid-19-restrictions-outside-montreal-area-march-8/vi-BB1edsPl?parent-subcat=cookingschool+%22+target

Figure 2: February 29th 2021 Canadian Forecast of COVID-19 Case Growth Assuming Spread of Variants



The experience in Canada through March has contradicted those predictions. Figure 3, immediately below, plots the number of cases in Canada from March 2020 through the end of March 2021. Rather than the sharp increase in cases predicted by Canadian public health unless sharp new restrictions were implemented, Canada experienced a mild increase in cases through March 2021. Please notice that the increase in cases through March that Canada actually experienced remained below the peak of cases during the previous wave, contrary to the February forecasting model by Canadian public health.

Figure 3: COVID-19 Confirmed Cases in Canada– March 2020 to March 2021



That the actual case estimates have diverged from the modeling predictions should not be surprising, as epidemic forecasting has proved unreliable (typically in the direction of overestimating disease spread) throughout the epidemic.

The empirical literature belies these predictions. First, the mutant variants do not escape the immunity provided by previous infection with the wild-type virus, or by the vaccines.[23,24,25] Although it is possible for a reinfection to occur, people who have been previously infected by the wild-type (non-variant) virus are unlikely to have a severe outcome (hospitalization or death) after exposure to a variant virus. This means that the presence of a variant circulating in the

---

[23] Alison Tarke, A., Sidney, J., Methot, N., Zhang, Y., Dan, J. M., Goodwin, B., Rubiro, P., Sutherland, A., da Silva Antunes, R., Frazier, A., Rawlings, S. A., Smith, D. M., Peters, B., Scheuermann, R. H., Weiskopf, D., Crotty, S., Grifoni, A., & Sette, A. (2021). Negligible impact of SARS-CoV-2 variants on CD4 + and CD8 + T cell reactivity in COVID-19 exposed donors and vaccinees. BioRxiv, 2021.02.27.433180. https://doi.org/10.1101/2021.02.27.433180

[24] Wu, K., Werner, A. P., Moliva, J. I., Koch, M., Choi, A., Stewart-Jones, G. B. E., Bennett, H., Boyoglu-Barnum, S., Shi, W., Graham, B. S., Carfi, A., Corbett, K. S., Seder, R. A., & Edwards, D. K. (2021). mRNA-1273 vaccine induces neutralizing antibodies against spike mutants from global SARS-CoV-2 variants. BioRxiv : The Preprint Server for Biology, 2021.01.25.427948. https://doi.org/10.1101/2021.01.25.427948

[25] Redd, A. D., Nardin, A., Kared, H., Bloch, E. M., Pekosz, A., Laeyendecker, O., Abel, B., Fehlings, M., Quinn, T. C., & Tobian, A. A. (2021). CD8+ T cell responses in COVID-19 convalescent individuals target conserved epitopes from multiple prominent SARS-CoV-2 circulating variants. MedRxiv : The Preprint Server for Health Sciences, 2021.02.11.21251585. https://doi.org/10.1101/2021.02.11.21251585

population poses little additional risk of hospital overcrowding or excess mortality due to viral infection.

As a general matter, such predictions are based on compartment models that are known to rely on faulty assumptions and have proven to be unreliable guides to the effects of COVID-19 containment policies on the track of the epidemic. One comprehensive peer-reviewed assessment of these models cautions against their use:[26]

> "Epidemic forecasting has a dubious track-record, and its failures became more prominent with COVID-19. Poor data input, wrong modeling assumptions, high sensitivity of estimates, lack of incorporation of epidemiological features, poor past evidence on effects of available interventions, lack of transparency, errors, lack of determinacy, looking at only one or a few dimensions of the problem at hand, lack of expertise in crucial disciplines, groupthink and bandwagon effects and selective reporting are some of the causes of these failures. ... When major decisions (e.g. draconian lockdowns) are based on forecasts, the harms (in terms of health, economy, and society at large) and the asymmetry of risks need to be approached in a holistic fashion, considering the totality of the evidence."

Second, theoretical work suggests that lockdowns place selective pressure that promote the development and establishment of more deadly variants. This, in part explains why the most concerning variants have emerged in places like the UK, South Africa, and California, where severe lockdowns have been imposed for extended periods of time.[27] While this hypothesis awaits a definitive empirical test, it is consistent with the *prima facie* evidence on mutant variants' development. None of these facts is accounted for in the Canadian Public Health Agency's or Institut National de Sante Publique Quebec's forecasts.[28]

Third, the variants have been widely spreading in many countries these past months, even as cases have been dropping. This is true, for instance, in Florida, where the UK variant B.1.1.7 is widespread[29], but cases have dropped sharply over the same period that variant has been spreading. That variants with a small infectivity advantage – but no more lethality -- make up a larger fraction of a smaller number of cases is an interesting scientific observation but not important for public health policy.

---

[26] Ioannidis JPA, Cripps S, Tanner MA. Forecasting for COVID-19 has failed. Int J Forecast. 2020 Aug 25. doi: 10.1016/j.ijforecast.2020.08.004. Epub ahead of print. PMID: 32863495; PMCID: PMC7447267.

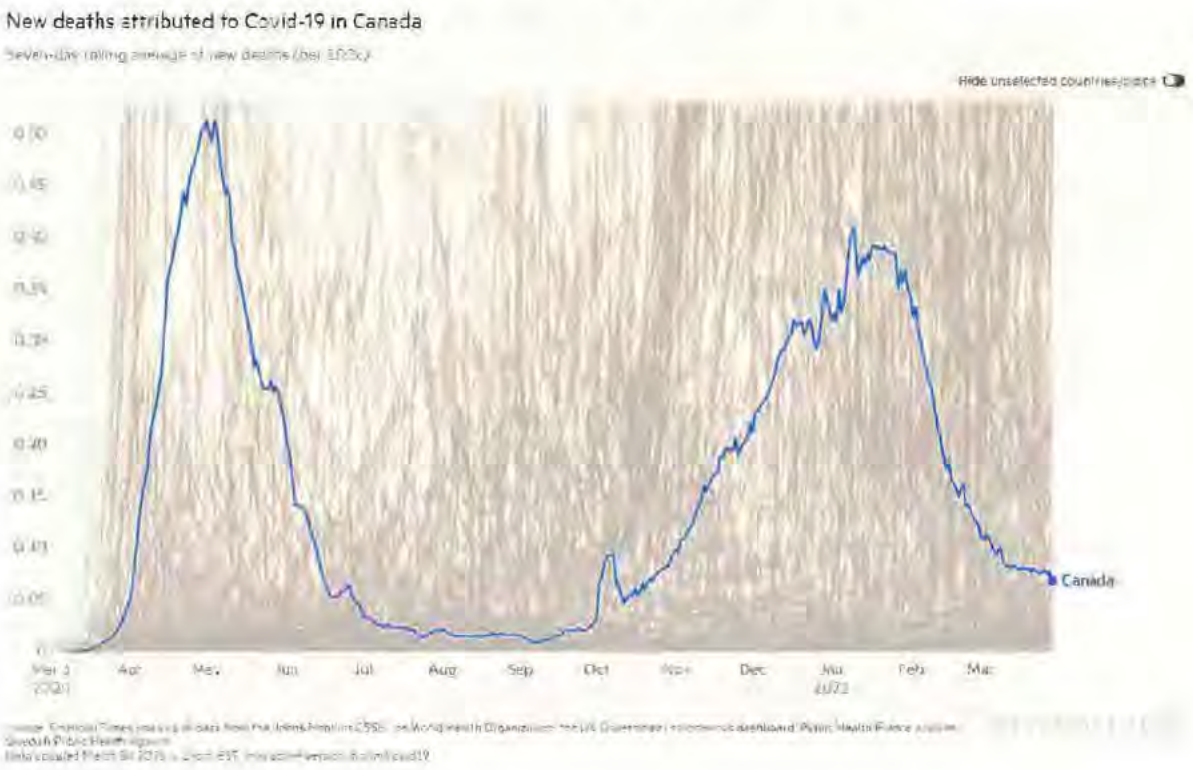
[27] Moran J. (2021) Mutant variations and the danger of lockdowns. The Critic Magazine. March 2, 2021. https://thecritic.co.uk/mutant-variations-and-the-danger-of-lockdowns/

[28] Brisson M. et al. (2021) *Modélisation de l'impact potentiel d'un variant COVID-19 plus transmissible au Québec*. Institut National de Sante Publique Québec. https://www.inspq.qc.ca/sites/default/files/covid/projections/inspq-projections-4mars2021.pdf

[29] US Centers for Disease Control (2021) US COVID-19 Cases Caused by Variants. https://www.cdc.gov/coronavirus/2019-ncov/transmission/variant-cases.html

Fourth, the dissemination of vaccines that protect against hospitalizations and deaths upon COVID-19 infection throughout the older population in Canada have decoupled the growth in COVID-19 cases from COVID-19 mortality. Vaccinated people can still perhaps be infected, but rarely have severe symptoms in response to infection. Figure 4 plots the number of COVID-19 deaths in Canada over this same time period as cases were plotted in Figure 3. Strikingly, the number of COVID-19 deaths have declined in Canada in February and March 2021 despite the mildly increasing number of cases. Throughout last year, a rise in cases has inevitably been accompanied by an increase in deaths with a two to three week lag. However, during this most recent wave, there has been no rise in deaths to accompany the rise in cases because of the deployment of the vaccine in the vulnerable older population in Canada. This is true despite the spread of new variant forms of the virus throughout Canada in February and March of 2020.[30] Because of the success of the Canadian vaccination effort among the vulnerable elderly, COVID-19 cases and COVID-19 deaths are now effectively decoupled.

Figure 4: COVID-19 Deaths in Canada – March 2020 to March 2021.



[30] Bensadoun E (2021) Coronavirus variants are spreading throughout Canada. Is it still safe to reopen? Global News. Feb. 9, 2021. https://globalnews.ca/news/7627391/coronavirus-variants-canada-provinces-reopen/

Fifth, and perhaps most importantly, even if it is accepted the increased transmissibility of the new variants, the harsh lockdowns that Manitoba has implemented over the past year as its primary infection control policy are unlikely to work to limit the number of COVID-19 infections. Despite the harsh lockdowns and the circulation of the somewhat less infectious wild type virus, over the previous year, nearly a million Canadians have been infected, and tens of thousands of Canadians have died with COVID-19. If the lockdowns did not work to protect Canadians from the less infectious wild type virus (and they did not – see the discussion in the next section) – then there is little reason to expect that they would work to suppress a more infectious variant.

Scientific Evidence Indicates Asymptomatic and Pre-symptomatic Individuals Spread the Disease Inefficiently

In my original expert report, I provided evidence from a large meta-analysis of within household spread of the virus, from an infected person to someone else living in the same home, where none of the safeguards that restaurants recommended by the CDC are typically applied. This study represents the most comprehensive survey of the vast empirical literature on asymptomatic spread; because it focuses on a single setting (household transmission), it is not subject to the same problems that other studies on this topic might have. The primary result is that symptomatic patients passed on the disease to household members in 18% of instances, while asymptomatic and pre-symptomatic patients passed on the disease to household members in 0.7% of instances.[31] Kindrachuk does not address this evidence on the relatively low risk of asymptomatic disease spread drawn from real-world transmission data and focuses instead on modeling studies that require a substantial number of unverifiable assumptions. In particular, these models often make the *assumption* that lockdowns actually work in reducing interactions between individuals in ways that reduce disease transmission risk. It is inappropriate to then conclude from such modeling studies that lockdowns work in a way predicted by the model. The Respondents' affidavits provide no evidence that they have conducted any validation exercises which would suggest that the models on which they rely to infer the efficacy of church closures have actually match real-world evidence I provided from the scientific literature of low levels of asymptomatic spread. Many of the studies cited by Kindrachuk were taken into consideration within the large meta-analysis, which ultimately found, after analyzing 54 studies (including Kindrachuk's cited studies and others) a very low chance of asymptomatic and pre-symptomatic disease spread.

One clear implication of the small likelihood of asymptomatic and pre-symptomatic disease spread and a higher likelihood of symptomatic spread is that the Respondents have available a

[31] (Madewell ZJ, Yang Y, Longini IM, Halloran ME, Dean NE. Household Transmission of SARS-CoV-2: A Systematic Review and Meta-analysis. *JAMA Netw Open.* 2020;3(12):e2031756. doi:10.1001/jamanetworkopen.2020.31756)

simple policy that would have similar infection control properties but with substantially lower harms. In response to Roussin's and Kindrachuk's concerns about the spread of Covid in church settings, the Respondents could require churches to screen for COVID-like symptoms at the door and ask congregants who have such symptoms to not worship indoors. They could also provide public health advice (through advertisements and other means) to tell the public to stay at home if they experience such symptoms. There is no rational basis for the Respondents' current policy given the availability of this less harmful and equally effective alternate policy. Since asymptomatic and pre-symptomatic disease spread from an infected individual to an uninfected individual is unlikely to occur inside of a household, it is even less likely to occur within a church, especially a church that asks its members to socially distance and wear masks.

In response to Roussin's and Kindrachuk's concerns about the spread of Covid indoors, the insights from this literature could also be used to replace the Respondents' draconian policy restricting in-home private gatherings – with a less draconian policy. In particular, the public health authorities could inform citizens of the higher risk of disease transmission posed by symptomatic individuals and advise people with symptoms consistent with COVID-19 infection to stay at home and avoid private gatherings with people outside of their households. If people without symptoms are gathering, even if they come from different households, the likelihood of disease spread occurring is an order of magnitude lower than if symptomatic people gather alongside uninfected people.

A special consideration for church services involves the risk posed by singing in terms of disease spread. The evidence cited by the Respondents regarding the risk of "super spreader" events in churches comes from locations (e.g. South Korea, early in the epidemic) where no precautions were taken for social distancing or mask wearing in service. However, there is evidence that churches that ask congregants to wear masks, and asks congregants with symptoms to stay at home, can safely worship indoors, and permit singing, without an undue risk of causing a super spread event.

The Errors in PCR Testing Render them Unfit for Public Health Decision Making

This section is in response to Bullard's and Roussin's affidavits. In the January 5, 2021 report, it was explained that the to scientific evidence[32] that the test on which Canada bases its count of COVID infections – the RT-PCR test for the presence of the SARS-CoV-2 virus – will often generate a positive result even when an individual is not infectious (that is, does not pose a danger of infecting other people). The difficulty is that the RT-PCR test permits too many doubling cycles of viral particles before declaring a negative test. The functional false positive rate increases with the number of cycles (known as a Ct value) required to produce a positive

[32] (T. Jefferson, et al., *Viral Cultures for COVID-19 Infectivity Assessment – A Systematic Review (Update 3)* (Sept. 3, 2020), medRxiv, https://www.medrxiv.org/content/10.1101/2020.08.04.20167932v3.full.pdf.)

result. As was stated in the January 5, 2021 report, according to a careful study published in *Eurosurveillance* (a top journal in the field of epidemiology), if 27 cycles are needed for a positive test, the false positive rate is 34%; if 32 cycles are needed for a positive test, the false positive rate is 72%, and if 37 cycles are need for a positive test, the false positive rate is 92%.[33] If more than 40 cycles are needed for a positive test, the functional false positive rate is nearly 100%. Many laboratories in Canada run the RT-PCR test up to 45 cycles, so false positive results are not just a theoretical possibility.

Bullard states in his report that the term "functional false positive" does not exist in the literature. This is false. I introduced the term in the published scientific literature[34] to make a key distinction between a test that is a gold standard for viral presence (the PCR test) and a gold standard for infectivity (a viral culture test). A true positive PCR test, which indicates viral presence, may be a functional false positive result with regard to viral infectivity if the number of duplication cycles needed to find a positive result is sufficiently high. This key distinction has been made by other scientists as well in the published literature[35], and the concept of a "functional false positive" provides an easy way to refer to the phenomenon.

This error in the test is a major problem with Manitoba's epidemic policy making because it relies on the accuracy of the RT-PCR tests to determine whether an individual is infected with the virus. The PCR test's inaccuracies imply Manitoba's epidemic planning does not reflect the risk of community spread of the virus because a high case count or positivity rate may be due instead to functional false positive outcomes. Given this scientific evidence, it is certain that Canadian provinces are imposing sharp lockdowns – along with their attendant costs– even when the risk of community spread of COVID-19 does not warrant it.

Surprisingly, none of the Respondents' affiants dispute any of these points. Instead, they assert that the RT-PCR test is a "gold standard" test for checking for the presence of SARS-CoV-2 virus – a fact not in dispute. The important question is not whether RT-PCR is a "gold standard" test for viral presence, but rather whether it is a gold standard test for determining whether a patient is infectious, which it is not. Rather, the gold standard test for infectivity involves checking whether a sample taken from the nasopharynx of a patient can infect, in vitro, a cell culture. Infectious samples are known as "culture positive", while non-infectious samples are known as "culture negative". From an epidemiological point of view, infectivity measurement is

---

[33] Singanayagam A, Patel M, Charlett A, Lopez Bernal J, Saliba V, Ellis J, et al. Duration of infectiousness and correlation with RT-PCR cycle threshold values in cases of COVID-19, England, January to May 2020. Eurosurveillance. 2020;25(32):2001483. 2020

[34] Bhattacharya J and Packalen M (2020) On the Futility of Contact Tracing. Inference 5(3) : 1/5-5/5 September 28, 2020. https://inference-review.com/assets/pdf/articles/on-the-futility-of-contact-tracing.pdf

[35] Jefferson T, Heneghan C, Spencer E, Brassey J (2020) Are You Infectious If You Have a A Positive PCR Test Result for COVID-19? The Centre for Evidence-Based Medicine. Oxford University. August 5, 2020. https://www.cebm.net/covid-19/infectious-positive-pcr-test-result-covid-19/

more important than a measurement of whether the virus is present, since it is possible for a patient to have non-viable viral fragments present, a positive PCR test, and yet not be infectious.

The relevant question then, is whether the RT-PCR test is sufficiently accurate to use as a tool to decide whether to sharply curtail the normal activities of more than a million people living in Manitoba, imposing untold harm on them related to the lockdown. None of the Respondents' affiants provide any argument or analysis in support of an affirmative answer to that question. Instead, they provide details about standardization procedures that the province uses to correct for other problems in the province's case estimates that the original January 5, 2021 did not discuss. None of these standardization procedures fix the problem of functional false positives.

Bullard provides data from December 2020 that out of 5825 positive PCR results in Manitoba, 18% had a Ct of 25-30, 18% had a Ct of 30-36, and 7% had a Ct of 36-40. Bullard argues that it is good public health practice to ignore the errors of the PCR test because it is in the interest of Canadian public health to identify every single person virally infected person and quarantine them, whether or not they pose any risk whatsoever in spreading the virus. The assertion that we need to capture every case, regardless of the risk that the person poses to infection spread, ignores a basic public health principle, which is that both the costs and benefits of public health actions should be considered. Quarantining people who are positive but not infectious imposes costs on the quarantined, with no benefit whatsoever for the population. According to Bullard's own report, 25% of the 5825 people that Manitoba considered a case in December of 2020 had Ct values that strongly suggest they were not infectious. Nevertheless, an estimated 1,456 people were forced to quarantine, with their civil liberties violated, but with no discernable public health benefit in terms of infection control. This is poor public health practice.

It is also problematic that there is no mention in any of the Respondents' affidavits of a communication between the lab and Roussin of the Ct values. Such information is critical to inform good decision making and good public health policy in terms of the risk that a person who tests positive presents to the public, and in balancing the costs and benefits of making public health orders based on that information. Roussin states in his affidavit that some of the factors that determines what special measures are necessary to reduce the threat of Covid include the total number of cases, and the test positivity rate and trend. Without knowing the Ct value of those positive tests, it is impossible to determine whether the proportion of people in the population who are at risk of spreading the disease is increasing or decreasing. Faulty case counts that do not correct for this issue with Ct values do not reflect the risk that the identified cases pose to the population, and thus cannot provide a scientific basis for drastic public health orders (such as lockdown orders) that violate basic civil liberties.

Although the Respondents' affiants do not address the topic, there are simple alternative tracking methods available – using existing technology – that would yield more accurate information about disease risk. In particular, Canadian provinces could premise epidemic policy making on the number of cycles necessary to achieve a positive RT-PCR test result (a number already

produced by the PCR test, but not used by Canadian policy makers in decision making about lockdowns). The Respondents' affiants dismiss this possibility by arguing that the RT-PCR is a qualitative test rather than a quantitative test. This is not responsive, since the number of cycles to achieve a positive result is a readily available (though unreported) output of the RT-PCR test currently in use in clinical laboratories throughout Canada. It is suggested that a patient should only be counted as a case if the RT-PCR test result indicates that the patient is very likely infectious, and not counted otherwise. There is support for this approach from even pro-lockdown scientists, like Harvard University epidemiology professor Marc Lipsitch, who recently wrote:[36]

> "Our findings suggest that instead of discarding individual Ct values from positive specimens, incorporation of viral loads into public health data streams offers a new approach for real-time resource allocation and assessment of outbreak mitigation strategies, even where repeat incidence data is not available. Ct values or similar viral load data should be regularly reported to public health officials by testing centers and incorporated into monitoring programs."

Since the January 5, 2021 expert report, the World Health Organization on January 13th, 2021, issued a technical report that supports the points made in that report.[37] The report emphasizes two things. First, it points out that a positive COVID test does not necessarily mean that someone has any capacity of infecting someone else with the virus. Therefore, it instructs laboratories to report the replication number, as I suggested. And second, the WHO warns against relying on a single test for patients without considering clinical COVID-19 symptoms, as Manitoba does. There is no mention in the Respondents' affidavits that a positive case (patient) must be assessed clinically after diagnosis with Covid based on that positive test. Manitoba decision making about the lockdowns is thus not aligned with WHO guidelines for using the PCR test data.

On the Futility of Contact Tracing to Control Disease Spread

Most of the Respondents' affiants explain that the Respondents rely on contact tracing programs as a means to control the spread of COVID-19 disease. Contact tracing programs require people who have been identified as COVID-19 cases to divulge to public health officials all the people with whom they have been in contact with during their illness, as well as all the locations they may have visited. Health officials have asked Canadians to install a phone application that aids in contact tracing by providing officials information about the locations where a person has

---

[36] Hay JA, Kennedy-Shaffer L, Kanjilal S, Lipsitch M, Mina M. (2020) Estimating Epidemiologic Dynamics from Single Cross-Sectional Viral Load Distributions. medRxiv preprint. https://www.medrxiv.org/content/10.1101/2020.10.08.20204222v1

[37] WHO (2021) "WHO Information Notice for IVD Users 2020/05" January 13, 2021. https://www.who.int/news/item/20-01-2021-who-information-notice-for-ivd-users-2020-05

frequented. In Manitoba, public health officials have recently implemented an "aggressive" stance toward contact tracing, including asking restaurants to report on the names of all the customers who have patronized a location.[38]

Despite extensive expenditures devoted to these efforts, there has been no demonstration that contact tracing programs in Canada have contributed to limiting disease spread. Manitoba has not provided any data to illustrate the effectiveness of contact tracing. Rather, news reports suggest that contact tracing programs have been overwhelmed throughout Canada whenever COVID-19 case frequency starts to rise.[39, 40,41,42] Canadian researchers who have examined the topic concluded that Canadians were wary of the COVID Alert app because they do not believe that their privacy will be protected.[43] The failure of contact tracing programs to control disease spread is not unique to Canada; a recent government report in the UK concluded that there was no clear evidence that it had accomplished much, despite an expenditure of 37 billion pounds over a two year span.[44]

The futility of contact tracing to control COVID-19 disease spread is entirely predictable. While contact tracing is a useful public health technique for diseases where the location of disease spread is readily identifiable (e.g. sexually transmitted diseases), it is less efficacious for diseases like COVID-19, where the moment of disease transmission is harder to identify. This is especially true since a large fraction of COVID-19 cases involve no symptoms at all. Though asymptomatic disease spread is much less efficient than symptomatic disease spread, it does occur (0.7% of the time in intimate household settings), and it renders contact tracing efforts less likely to succeed. Errors in the PCR testing, which render it unable to distinguish a COVID-19 patient who is highly infectious from a patient who has recovered from the disease, still has non-infectious viral fragments detectable, and is no longer a threat to spread the disease, also make contact tracing efforts less likely to succeed. When contact tracers are overwhelmed, delays in identifying, contacting, and testing contacts makes it more likely that contacts will be found long

---

[38] Erik Pindera (2021) Passive-'aggressive' contact tracing raises questions. Winnipeg Free Press March 17, 2021. https://www.winnipegfreepress.com/special/coronavirus/passive-aggressive-contact-tracing-raises-questions-574012022.html#long-story

[39] CBC News (2020) Ottawa Public Health to focus contact tracing on high-risk spreaders. Oct. 6, 2020. https://ca.news.yahoo.com/ottawa-public-health-focus-contact-152847281.html

[40] CBC News (2020) Overwhelmed by increase in cases, Montreal public health narrows contact-tracing efforts Social Sharing. Sept. 24, 2020. https://www.cbc.ca/news/canada/montreal/montreal-public-health-officials-reduce-contact-tracing-amid-shortage-1.5737248

[41] Joel Dryden (2020) Alberta's contact tracers are now overwhelmed at a critical time, infectious disease expert says. CBC News. November 6, 2020. https://www.msn.com/en-ca/news/canada/alberta-s-contact-tracers-are-now-overwhelmed-at-a-critical-time-infectious-disease-expert-says/ar-BB1aK0AU

[42] Gary Mason (2021) Canada's overwhelmed contact-tracing efforts have been a gross failure. The Globe and Mail. Jan. 7, 2021. https://www.theglobeandmail.com/opinion/article-canadas-overwhelmed-contact-tracing-efforts-have-been-a-gross-failure/

[43] Lynn Desjardins (2021) Why People Don't Use COVID Contact Tracing Apps. Radio Canada International. March 22, 2021. https://www.rcinet.ca/en/2021/03/22/why-people-dont-use-covid-contract-tracing-apps/

[44] Lizzy Buchan (2021) 'No clear evidence' Test and Trace is effective despite 'unimaginable' £37billion cost. UK Mirror. March 10, 2021. https://www.mirror.co.uk/news/politics/no-clear-evidence-test-trace-23649758

after they pose any risk of disease spread. Finally, from a privacy point of view, the reluctance of Canadians (and others) to cooperate with contact tracers is entirely understandable – there is little to no private benefit derived by the infected patient from reporting on their friends, family, churches, or favorite restaurants, and there is some possible social harm from the unwanted attention and privacy violations inherent in contact tracing. I discuss many of these issues in a paper entitled "On the Futility of Contact Tracing", that I published in September of last year.[45]

Criticism of the Great Barrington Declaration

Roussin criticizes the Great Barrington Declaration (hereafter, GBD)[46] in his affidavit. The logic of the GBD is that the return to normal life will improve health and other outcomes for the non-vulnerable by reducing lockdown harms, while focused protection policies will protect the vulnerable. The aim of focused protection is to minimize overall mortality from *both* COVID-19 *and* other diseases by balancing the need to protect high-risk individuals from COVID-19 while reducing the harm that lockdowns have had on other aspects of medical care and public health. The GBD represents a return to standard public health practices, which acknowledge that human health requires more than just infection control and is instead concerned with the health and well-being of populations in a much broader way.[47]

The Possibility of Effective Focused Protection of the Vulnerable

Roussin writes skeptically about the possibility of protecting vulnerable people (the elderly primarily, but also others with certain chronic conditions for whom COVID-19 infection poses a high mortality risk) from infection without lockdowns. He argues that the only way to protect the vulnerable is to reduce community disease spread. In particular, he argues that focused protection of the vulnerable – as described in the Great Barrington Declaration – is impossible without lockdown. He mischaracterizes focused protections as requiring a complete segregation of vulnerable and non-vulnerable populations, when what is necessary are policies that reduce the probability that infected people will have extended contact with vulnerable people in a context where the spread of the disease is likely. The former is impossible, while the latter is certainly possible, especially since the leading meta-analysis study discussed above shows that asymptomatic spread is exceedingly rare. There are several other major problems with Roussin's argument.

---

[45] Bhattacharya J, Packalen M. On the Futility of Contact Tracing. *Inference* 5(3) September (2020) https://inference-review.com/article/on-the-futility-of-contact-tracing

[46] Kulldorff M, Gupta S, and Bhattacharya J (2020) Great Barrington Declaration, Oct. 4, 2020. https://gbdeclaration.org/

[47] Public Health Leadership Society (2002) Principles of the Ethical Practice of Public Health. American Public Health Association. https://www.apha.org/-/media/files/pdf/membergroups/ethics/ethics_brochure.ashx

As we have seen, there is good theoretical and empirical evidence that lockdowns do not and cannot control community spread of the disease over an extended period of time. Even if lockdowns slow the spread, vulnerable people will ultimately be infected. The best example of success of the Focused Protection approach is in Florida, which, as discussed above, has reduced its death and case count without lockdown, and has fared slightly better than California (with some of the harshest lockdown restrictions in the US) in terms of its overall Covid death rate accounting for age.[48]

Focused protection is possible as long as public health experts deeply understand the particular living circumstances of the vulnerable and are creative in designing effective interventions based on that understanding. Empirical evidence from around the world shows that focused protection of nursing homes is possible. During the first wave of the epidemic, there was an unfortunately high rate of exposure of nursing home residents to COVID-19 infections – a failure of focused protection. In the US, nearly half of all COVID-19 deaths occurred in nursing home settings, fueled by policies – famously adopted by New York state – that sent elderly COVID-19 infected patients back to nursing homes that could not effectively quarantine them.[49] The same was true in Quebec and elsewhere in Canada. The proportion of COVID-19 deaths in nursing homes dropped sharply during the second wave of COVID-19 infections over the summer as these facilities adopted better policies to protect their elderly residents.[50]

A strategy of focused protection involves a suite of policies that protect people who are particularly vulnerable (e.g. the elderly) from COVID-19 infection. Those strategies have been discussed thoroughly in the January 5, 2021 report.

Finally, and most importantly, the new and effective vaccines make it relatively simple to implement a policy of focused protection. By prioritizing the older, most vulnerable, population for vaccination, it is possible to provide near perfect focused protection, even without adopting any of the policy suggestions outlined above. Certainly, no lockdown is necessary for reducing hospitalization and deaths from COVID, as long as the older population is prioritized for vaccination.

---

[48] "Vindication for Ron DeSantis" *Wall Street Journal*, Allysia Finley, March 5, 2021, online: https://www.wsj.com/articles/vindication-for-ron-desantis-11614986751

[49] Perrett C (2020) Gov. Cuomo's controversial order requiring nursing homes to admit COVID-19 patients was reportedly removed from New York's health website. *Business Insider*. May 27, 2020. https://www.businessinsider.com/new-york-deleted-cuomos-order-nursing-homes-order-2020-5. Accessed Dec. 7, 2020.

[50] Ioannidis JPA, Axfors C, Contopoulos-Ionnidis DG (2020) Second versus first wave of COVID-19 deaths: shifts in age distribution and in nursing home fatalities. medRxiv. https://www.medrxiv.org/content/10.1101/2020.11.28.20240366v1.full-text (accessed Dec. 7, 2020)

Problematic Analysis of the Possibility of Herd Immunity

Kindrachuk also provides a misleading analysis of the role that herd immunity plays in the control of the epidemic. Herd immunity – also known as endemic equilibrium – occurs when enough people have immunity so that most infected people cannot find new uninfected people to infect, leading to the end of the epidemic.[51] This means that the epidemic will end before everyone is infected, although it will continue in endemic form with low rates of infections. Sooner or later, herd immunity will be reached either through natural infection or through a combination of vaccinations and natural infection. Since worldwide zero COVID is impossible, herd immunity is the endpoint of this epidemic regardless of whether we choose lockdowns or focused protection to address it.

Kindrachuk cites the experience of Manaus, Brazil to assert that herd immunity cannot be achieved. The basic fact cited is that Manaus has experienced two very large epidemic waves, and that high levels of population immunity achieved during the first wave did not protect the population from a large second epidemic wave. The major problem with this reasoning is that it is based on a single, flawed, seroprevalence study conducted in Manaus in the middle of 2020. The 76% estimate was not based on a random survey, but on blood donors, who are a very select group of people in the developing world. Moreover, the seroprevalence among the blood donors was 52%, which was adjusted upwards based on questionable mathematical modelling of the waning of anti-bodies. Hence, we do not really know the level of immunity in Manaus from earlier this year.

Apart from this factual problem, there are several other explanations for the Manaus, Brazil experience that Kindrachuk does not consider, and would need to be ruled out from a scientific point of view before accepting the proposition that herd immunity failed in Brazil. First, residential segregation in Manaus (along socio-economic lines) could lead to a separation in the peaks of epidemics occurring in different communities. An unfortunate feature of the reporting of figures during this pandemic has been the misleading aggregation of data from different geographical locations. For instance, the impression of a bigger 'second wave' occurring within the same jurisdiction, may be due to a bigger area being affected during the second wave compared to the first. But even within the same location, residential and socio-economic segregation can create the conditions for a second wave to occur more or less independently of the first.

Second, the herd immunity threshold is not a single constant that is known in the literature, but instead is likely to vary substantially from place to place and by season of the year since interaction patterns between people – and disease contagion risk – vary along these dimensions.

---

[51] Fine P, Eames K, Heymann DL. (2011) "Herd Immunity": A Rough Guide. *Clinical Infectious Disease* 52(7):911-6. doi: 10.1093/cid/cir007

The herd immunity thresholds differ sharply by location and time, depending upon factors such as population density, living arrangements, social interactions, climate, season and hygiene. It is not a universal constant determined by biological characteristics of the virus alone. One cannot learn much about herd immunity thresholds in Manitoba from the experience of Manaus, Brazil.

Third, based on a location (Manaus, Brazil) with a largely uncontrolled epidemic, it is impossible to conclude that lockdowns are a good strategy to control the epidemic. It is scientifically unconvincing to attempt to make inferences about the efficacy of lockdowns from one single location where lockdowns were not implemented. A similar serosurvey conducted in the Dharavi slums in Mumbai, India,– the focus of an intense lockdown through May and only limited reopening in June, 2020 – found a seroprevalence of 57% in early July, 2020.[52] One of the researchers who conducted the study conveyed the hypothesis to me that the lockdown may have intensified the spread of the disease in the densely packed region by forcing residents to spend long days in packed rooms with poor ventilation. Similarly, nearly 40% of the population of Lima, Peru has SARS-CoV-2 specific antibodies, despite one of the longest lasting and harshest lockdown policies in the world.[53]

Fourth, the experience of Manaus, Brazil does not rule out the possibility of replacing Manitoba lockdowns with a policy of focused protection with good results. Manaus, Brazil did not adopt a focused protection strategy. As expected with a largely uncontrolled epidemic, the seroprevalence was roughly equal across the age-distribution in Manaus, which makes it similar to lockdown countries like Spain.[54] As a contrast, in Sweden seroprevalence (which adopted something more akin to a focused protection strategy) was more than twice as high among ages 20-64 compared to those over 65, belying the assertion that focused protection is impossible.

No Consideration of Harms of Lockdown Restrictions

Although a fundamental principle of public health requires that officials conduct a careful consideration of *both the costs and benefits* before imposing any policy, the Respondents' affidavits do not show any evidence that Manitoba has conducted a rigorous evaluation of the lockdown policies it has adopted. It is clear from the Respondents' affidavits that the Province has worked to quantify the purported public health benefit from its lockdown policy (though this analysis has its problems that are addressed in the original expert report and here). However, it

---

[52] Biswas S (2020) India coronavirus: 'More than half of Mumbai slum-dwellers had Covid-19. BBC News. July 29, 2020, https://www.bbc.com/news/world-asia-india-53576653

[53] Andina: Agencia Peruana de Noticas (2020) Peru: Nearly 4 million people may already have had COVID-19 in Lima Metropolitan Area. Dec. 29, 2020. https://andina.pe/Ingles/noticia-peru-nearly-4-million-people-may-already-have-had-covid19-in-lima-metropolitan-area-827959.aspx

[54] Baral S, Chandler R, Prieto RG, Gupta S, Mishra S, Kulldorff M. Leveraging epidemiological principles to evaluate Sweden's COVID-19 response. Ann Epidemiol. 2021 Feb;54:21-26. doi: 10.1016/j.annepidem.2020.11.005. Epub 2020 Nov 23. PMID: 33242596; PMCID: PMC7682427

is striking that there is no discussion whatsoever of the collateral harms from these forced closures.

For instance, the forced closure of churches has had and is likely to have a substantial impact on the financial viability of churches, including on the ability of churches to employ its staff. The forced limitation of church activities is also likely to have ripple impacts on the businesses from which churches purchase goods and services. Many churches are also active in communities in the provision of social services to indigent populations and in the organization of charitable giving to providers of such services. The forced closure or limitation of church activities is likely to impact the ability of churches and other religious organization to provide such services. The Respondents have conducted no analysis of the direct or indirect economic impacts of their closure orders, and yet have continued to impose them on religious organizations.

Instead, the Respondents have offered the testimony of an expert (Komlodi) who discusses at length the various financial programs that the Province has put in place to offset the financial harms from the lockdowns to businesses. None of this testimony addresses the distributional effects of the harms (poor are hardest hit), nor does it establish whether the financial programs sufficiently offset the lockdown induced financial harm to the businesses affected. Most importantly, however, these programs cannot possibly offset the harms done by the lockdown policies to church members, whose fundamental right to worship freely have been violated. No pecuniary renumeration would be sufficient to offset this harm, which can only be addressed by once more permitting the free exercise of religion in Canada.

Loeppky's affidavit reports significant increases in alcohol abuse, hospitalizations for suicide attempts, and intentional injuries in mid-2020. In response, Komlodi discusses how the Province has offered two free sessions of online counselling to people plus a "help line" in order to cope with mental health issues arising over the past year. What Komlodi and the government have not established – because it would not be accurate – is that these counseling sessions are sufficient to undo the psychological and other harms caused by the lockdowns.

In my original expert report, I discussed some of the scientific evidence for the psychological benefits of church attendance. It is also clear from the Respondents' affidavits that they make no attempt to quantify or consider in any way the positive public health benefits forgone by shutting down churches and banning worship, both for congregants and the positive ripple effects in the community. Policies enacted without a careful consideration of *both* its costs and benefits cannot possibly be construed to have a rational basis.

File No. CI 20-01-29284

THE QUEEN'S BENCH
Winnipeg Centre

APPLICATION UNDER: *The Constitutional Questions Act*, C.C.S.M., c. 180

AND UNDER: The Court of Queen's Bench Rules, M.R. 553/88

IN THE MATTER OF: *The Public Health Act*, C.C.S.M. c. P210

BETWEEN:

GATEWAY BIBLE BAPTIST CHURCH, PEMBINA VALLEY BAPTIST CHURCH, REDEEMING GRACE BIBLE CHURCH, THOMAS REMPEL, GRACE COVENANT CHURCH, SLAVIC BAPTIST CHURCH, CHRISTIAN CHURCH OF MORDEN, BIBLE BAPTIST CHURCH, TOBIAS TISSEN, ROSS MACKAY

Applicants,

– and –

HER MAJESTY THE QUEEN IN RIGHT OF THE PROVINCE OF MANITOBA and DR. BRENT ROUSSIN in his capacity as CHIEF PUBLIC HEALTH OFFICER OF MANITOBA, and DR. JAZZ ATWAL in his capacity as ACTING DEPUTY CHIEF OFFICER OF HEALTH OF MANITOBA,

Respondents.

AFFIDAVIT OF JOEL KETTNER
SWORN APRIL 1, 2021

D. Jared Brown / Allison Kindle Pejovic / Jay Cameron

[redacted]

THE QUEEN'S BENCH
Winnipeg Centre

APPLICATION UNDER: *The Constitutional Questions Act*, C.C.S.M., c. 180

AND UNDER: The Court of Queen's Bench Rules, M.R. 553/88

IN THE MATTER OF: *The Public Health Act*, C.C.S.M. c. P210

BETWEEN:

GATEWAY BIBLE BAPTIST CHURCH, PEMBINA VALLEY BAPTIST CHURCH, REDEEMING GRACE BIBLE CHURCH, THOMAS REMPEL, GRACE COVENANT CHURCH, SLAVIC BAPTIST CHURCH, CHRISTIAN CHURCH OF MORDEN, BIBLE BAPTIST CHURCH, TOBIAS TISSEN, ROSS MACKAY

Applicants,

– and –

HER MAJESTY THE QUEEN IN RIGHT OF THE PROVINCE OF MANITOBA and DR. BRENT ROUSSIN in his capacity as CHIEF PUBLIC HEALTH OFFICER OF MANITOBA, and DR. JAZZ ATWAL in his capacity as ACTING DEPUTY CHIEF OFFICER OF HEALTH OF MANITOBA,

Respondents.

AFFIDAVIT OF JOEL KETTNER

I, JOEL KETTNER of the City of Winnipeg, in the Province of Manitoba,

MAKE OATH AND SAY AS FOLLOWS:

1. I have personal knowledge of the facts and matters hereinafter deposed to by me, except where same are stated to be based upon information and belief, and those I do verily believe to be true.

2. I am an associate professor in the Department of Community Health Sciences at the College of Medicine, University of Manitoba.

3. I hold Canadian Royal College fellowship certifications in Public Health and Preventive Medicine as well as General Surgery. I have a Master of Science in Epidemiology from the London School of Hygiene and Tropical Medicine, University of London, United Kingdom.

4. My previous employments include: Chief Medical Officer of Health and Chief Public Health Officer for the Province of Manitoba (1999-2012), regional medical officer of health in urban, rural and northern parts of Manitoba (1990-1999), and clinical work in general practice, emergency urgent care medicine.

5. As part of my 12-year tenure as Manitoba's chief medical officer of health, I led the Province's public health responses to several outbreaks including the SARS Coronavirus-1 and the H1N1 pandemic influenza.

6. Following the SARS outbreak, I was part of the Canadian delegation to the World Health Organization special meeting in Geneva to develop the fourth edition of the International Health Regulation which introduced the concept, definition, and expectations of countries during a Public Health Emergency of International Concern (PHEIC).

7. I played a leading role at the World Health Organization Pan American Health Organization special H1N1 meeting in Washington DC. In

addition to a plenary presentation describing Manitoba's experience with the first wave, I led a working group to develop guidance for the prevention and treatment of H1N1 in low resourced parts of the world. At that same meeting, I collaborated as an author of the first comprehensive review article of H1N1 influenza published in the New England Journal of Medicine.

8. Following my tenure as chief medical officer of health, I undertook a number of relevant roles and responsibilities including scientific director of the Public Health Agency of Canada's National Collaborating Centre for Infectious Diseases, medical director of the International Centre for Infectious Diseases in Winnipeg, board director of the Canadian Public Health Association, and president of the Public Health Physicians of Canada.

9. Specific relevant roles at the University of Manitoba have included director of the Medical College undergraduate program in Community Health Sciences, and Director of the Masters in Public Health program. I continue to teach public health and epidemiology at the undergraduate, graduate, and post-graduate levels. During this COVID-19 pandemic, I have been active organizing learning events and providing expert opinion and dialogue in a variety of academic and public media platforms.

10. A copy of my curriculum vitae is attached hereto and marked as **Exhibit "A"**.

11. During my tenure as Chief Public Health Officer of Manitoba, the *Public Health Act* was amended to provide the Chief Public Health Officer with the power to take special measures (Section 67(3) in response to a public health emergency). Section 67(2) lists restrictive measures that can

only be taken with the approval of the minister. I was well aware of section 3 of the *Act* and the requirement that someone in my position would have to ensure that any measures taken in response to a public health emergency that infringed rights and freedoms had to be shown to be reasonably necessary. I also was aware that section 3 of the *Act* was consistent with section 1 of the *Charter of Rights and Freedoms*, and that it was up to the Minister of Health and the Chief Public Health Officer to justify to the public the need for such measures.

12. I also, as a physician, knew that I had to abide by the code of ethics of the Manitoba College of Physicians and Surgeons, including taking "all reasonable steps to prevent or minimize harm to the patient; disclose to the patient if there is a risk of harm or if harm has occurred". (Note that in public health medicine "the patient" refers to the population at large.) Other relevant commitments include "recognize the balance of potential benefits and harms associated with any medical act; act to bring about a positive balance of benefits over harms", "always respect the autonomy of the patient", and "promote the well-being of communities and populations by striving to improve health outcomes and access to care, reduce health inequities and disparities in care, and promote social accountability."

13. Technically, I saw a pandemic every year during my tenure as CMHO. It was called Influenza. Some years were worse than others. During my tenure, I can confirm that hospitals and ICUs were often stressed in a similar way to what has been described during the waves of this past year. Surgeries and other medical procedures were often delayed for several weeks. Most nursing homes had "outbreaks" of influenza during one or more flu seasons. How much of the respiratory illness during "flu

season" was attributed to influenza was not known because of the limited number of tests that were done.

14. During the H1N1 influenza pandemic 11 deaths were officially associated with that virus, although it was suspected that in many more deaths that influenza was a contributing cause. Although some infectious diseases specialists recommended the closure of schools, it was judged by me and others that the benefits of that would not outweigh the harms. This was despite the fact that number of reported deaths from influenza in children have exceeded in every previous year the rare reports COVID-19-associated deaths in children. With an understanding that it is very impractical to suppress the spread of a contagious respiratory virus, we focused on general measures such as handwashing, cough etiquette, staying home when sick, limiting exposure to those at highest risk for severe illness (influenza has similar risk factors to COVID-19), obtaining timely care when symptoms are more severe - especially if at high risk, and priorisation of vaccine administration to highest risk groups including Indigenous populations, elderly, and people with significant health conditions. I would describe our usual approach to influenza as focused protection.

15. The Applicants' counsel contacted me about providing expert testimony in response to the evidence provided by Respondents to justify the need for various Covid-19 Public Health Orders. I agreed to provide an expert report with my professional opinion on these matters.

16. A copy of my responding expert report as described above is attached hereto and marked as **Exhibit "B"**.

17. I acknowledge that in preparing this report and providing expert evidence, the Applicants' counsel explained that my role is to assist the court to determine the matters in issue. I further acknowledge that it is my duty to provide evidence that is fair, objective and non-partisan and to opine only on matters that are within my area of expertise. This duty prevails over any obligation that I may owe to any party on whose behalf I am engaged.

18. I make this affidavit *bona fide*.

SWORN before me in the City of Winnipeg, in the Province of Manitoba, on April 1, 2021.

A Commissioner of Oaths in and for the Province of Manitoba
My Commission Expires: July 8/21

JOEL KETTNER

THIS IS **EXHIBIT "A"** TO THE AFFIDAVIT OF JOEL KETTNER affirmed before me at the City of Winnipeg, in the Province of Manitoba, the 1st day of April, 2021.

L Loch

A Commissioner for Oaths in and for the Province of Manitoba. My commission expires July 8, 2021.

CURRICULUM VITAE

Joel David Kettner
MSc MD FRCSC FRCPC

March 31, 2021

TABLE OF CONTENTS

PERSONAL AND PROFESSIONAL INFORMATION, CONTACT INFORMATION

Home Address:	[redacted]
Home Telephone Numbers:	[redacted]
Work Phone Number:	[redacted]
Mobile Phone	[redacted]
Work Emails:	[redacted]
UM Address:	University of Manitoba Dept. of Community Health Sciences College of Medicine, Faculty of Health Sciences University of Manitoba [redacted]

Personal History

Date of Birth:	[redacted]
Place of Birth:	Minneapolis, Minnesota, U.S.A. (Canadian citizen born abroad)
Citizenship:	Canadian
Marital Status:	Married, six children, seven grandchildren

Places of Residence

1951 – 1955	Minneapolis, Minnesota, U.S.A.
1955 – 1967	Winnipeg, Manitoba, Canada
1967 – 1968	London, England, United Kingdom
1968 – 1985	Winnipeg, Manitoba, Canada
1985 – 1988	London, England, United Kingdom
1988 – present	Winnipeg, Manitoba, Canada

PRESENT EMPLOYMENT

University of Manitoba	Associate Professor, Departments of Community Health Sciences and Surgery (since 1990) Associate Director, Public Health clerkship rotation Postgraduate Medical Education CanMEDS roles advisor, and co-chair, Postgraduate Medical Education Truth and Reconciliation Action Plan Working Group
University of Winnipeg	Adjunct professor, Dept of Indigenous Studies
Self-Employment	Independent consultant Consultant to several organizations with respect to COVID-19. Lead and administrator, WhatsApp chat group for COVID-19 Open Minded Critical Thinkers (physicians from across Canada) Consultant, Advisory Circle, Health Transformation Project, Southern Chief's Organization, Manitoba. Vaccinator, First Nations Communities COVID-19 vaccine project.

EDUCATION and TRAINING

Pre-University

1968 – 1969	St. John's High School, Winnipeg, Canada
1967 – 1968	Woodhouse Grammar School, London, England
1964 – 1967	St. John's High School, Winnipeg, Canada

University – Undergraduate

1972 – 1976	Faculty of Medicine, University of Manitoba, Dean A. Naimark Winnipeg, Canada
1969 – 1971	"Pre-med" Arts & Science" University of Manitoba, Winnipeg, Canada

University – Graduate and Post – Graduate

2000	Medical Assistance in Dying Addictions medicine, opiate agonist therapy
1989 – 1990 (6 months)	Family Medicine Weekly clinics, Family Medicine Centre, University of Manitoba Winnipeg, Canada
1988 – 1990	Community Medicine (now Public Health and Preventive Medicine) Residency, Dept. of Community Health Sciences, Faculty of Medicine University of Manitoba Winnipeg, Canada
1987 – 1988	Clinical Research Fellow, Imperial Cancer Research Fund Colorectoral Cancer Unit, St. Mark's Hospital, London, England
1986 – 1987	Clinical Research Fellow, Hepato-biliary Surgical Unit, Dept. of Surgery, University of London Royal Postgraduate Medical School and Hammersmith Hospital, London, England

1985 – 1986	Master of Science, Epidemiology, Faculty of Medicine, University of London, England, London School of Hygiene and Tropical Medicine
1985	Post – fellowship, Gastrointestinal Endoscopy, Gastrointestinal Surgery and Gastroenterology (Health Sciences Centre and St. Boniface General Hospital, Winnipeg Canada
1979 – 1984	General Surgery Residency, Dept. Faculty of Medicine, University of Manitoba (Health Sciences Centre and St. Boniface General Hospital), Winnipeg, Canada
1977	Extended Internship, Intensive Care (voluntary), Health Sciences Centre and St. Boniface General Hospital, Winnipeg, Canada
1976 – 1977	Rotating Internship, University of Manitoba, Faculty of Medicine (Manitoba Affiliated Teaching Hospitals – Health Sciences Centre and St. Boniface General Hospital, Winnipeg, Canada)

UNIVERSITY DEGREES AND CERTIFICATES

1991	Specialist Certification, Community Medicine (now Public Health and Preventive Medicine), Royal College of Physicians of Canada (FRCPC)
1985	Master of Science in Epidemiology, London School of Hygiene and Tropical Medicine, Faculty of Medicine, University of London, England, (MSc) (MSc Thesis – Epidemiology for Surgeons)
1984	Specialist Certification, General Surgery, Royal College Surgeons of Canada (FRCSC)
1976	Doctor of Medicine (MD), University of Manitoba, Winnipeg, Canada
1976	Licentiate, Medical Council of Canada (LMCC)

FELLOWSHIPS, ACADEMIC PRIZES, DISTINCTIONS AND AWARDS

1991-2020	Nominated for best teacher of the year by undergraduate medical students in most years; most recently for small group teaching, inspiration, innovation, and mentorship by first and second year medical students.
2016	Long Service Award in Recognition and Appreciation of Twenty-five Years of Loyal Service, University of Manitoba.
2012-2014	McArthur Foundation Fellowship (two years), Masters Development Practice program, University of Winnipeg
2012	Nominated for Manitoba Civil Service Excellence Team Award – CPPHO Report on the Health of Manitobans report-team (leader).
2010	Winner of Manitoba Civil Service Excellence Team Award - Manitoba Health Pandemic H1N1 Influenza Incident Command Team (Medical lead)
1987 – 1988	University of Manitoba Faculty Fund Fellowship for studies in the clinical epidemiology of colorectal cancer.
1987 – 1988	Visiting Clinical Research Fellowship, Imperial Cancer Research Fund, UK, to study clinical epidemiology and Screening of colorectal cancer at the ICRF Colorectal Cancer Unit, St. Mark's Hospital, London, England
1985 – 1987	J.H.F. Knight Fellowship (University of London, England) to study epidemiology and screening for colorectal cancer
1985 – 1987	R.S. McLaughlin Foundation Fellowship (University of Manitoba) to study epidemiology and surgery at the University of London, England
1983	Davis and Geck Award for Best Surgical Resident of the Year
1982	Second Prize for paper presented at the American College of Surgeons (Manitoba Chapter), Manitoba
1969 – 1971	Dean's Honour List, both years of Pre-Medicine, Faculty of Science, University of Manitoba

MEDICAL WORK EXPERIENCE

Current	See "Present Employment"
2017	Consultant to Manitoba Keewatinowi Okimakanak, Inc. re northern health clinical transformation
2012-2017	Medical director, International Centre for Infectious Diseases
2012-2015	Director, Master of Public Health program, University of Manitoba
2012-2015	Scientific director, National Collaborating Centre for Infectious Diseases, International Centre for Infectious Diseases.
2012-2014	University of Winnipeg Visiting Professor and Senior Fellow Masters in Development Practice Program Faculty of Graduate Studies
2008-2012	Chief Provincial Public Health Officer of Manitoba
1999 – 2008	Chief Medical Officer of Health Province of Manitoba
1999	Medical Officer of Health Winnipeg Community Health Authority
1995 – 1999	Medical Officer of Health Winnipeg Region, Manitoba
1995 - 1999	Part-time general medical practice and travel clinics, Winnipeg City Clinic, 385 River Avenue, Winnipeg
1995 – 2010	Casual employment as emergency room physician, urgent care physician, and surgical assistant, Seven Oaks General Hospital Concordia General Hospital, Misericordia General Hospital, Grace Hospital, Victoria Hospital
1991 – 1995	Medical Officer of Health Thompson, Norman and Interlake Regions, Manitoba Health
1990	Attending surgeon, Surgical Intensive Care Unit, Health Sciences Centre

1986 – 1988	Locum tenens as senior registrar in Surgery, Hammersmith and St. Mark's Hospitals, London, England
1984 – 1985	Surgical Assistant, Cardiac, Surgery Unit, Health Sciences entre, Winnipeg, Canada
1977 – 1979	Full-time emergency room physician, St. Boniface General Hospital, Winnipeg, Canada

SELECTED CONTINUING PROFESSIONAL DEVELOPMENT

2020	Weekly Dept of Community Health Sciences Colloquia, on-line sessions, webinars, and conferences on topics including medical education and COVID-19.
2019	Many family medicine sessions and teaching development sessions at the University Office of Continuing Professional Development and the Office of Educational and Faculty Development. Annual Scientific Assembly, Manitoba College of Family Physicians, Canadian Conference of Medical Education, Niagara Falls. Canadian Public Health Association annual conference, Ottawa Public Health Physicians of Canada annual Continuing Professional Development Symposium, Ottawa.
2018	Canadian Conference Medical Education, Halifax. Canadian Public Health Association annual meeting, Montreal. Public Health Physicians of Canada annual meeting, Montreal. Weekly Colloquia, Department of Community Health Sciences. CPD sessions, Office of Educational and Faculty Development. Preparation for CAPE (clinical assessment and professional enhancement for re-entry to clinical practice.
2017	Canadian Conference Medical Education, Winnipeg. Canadian Public Health Association annual meeting. Public Health Physicians of Canada annual meeting. Weekly Colloquia, Department of Community Health Sciences.
2015-2016	Canadian Conference Medical Education, Montreal. Canadian Public Health Association Annual Meeting, Toronto. Choosing Wisely symposium, Public Health Physicians of Canada, Toronto. Association of Medical Microbiology and Infectious Diseases Annual Meeting, Vancouver. Annual BIO Conference, San Francisco. Weekly Colloquia, Department of Community Health Sciences and Weekly Medical Microbiology Case Presentations. Peer Mentoring session for instructors of Indigenous health course.
2014	Faculty Development Workshop - Community Health Sciences June 12, 2014
2012	Medical Education Workshops, University of Manitoba Learning Styles in the Classroom Feb 16/12 Teaching Clinical Reasoning April 10/12 Teaching Critical Thinking May 22/12

2012 (most recent)	Basic Life Support Joe Doupe Centre Faculty of Medicine University of Manitoba
2012 (most recent)	Advanced Trauma Life Support University of Manitoba, Winnipeg, Canada
2012 (most recent)	Advanced Cardiac Life Support Canadian Heart and Stroke Foundation, Winnipeg, Canada
2007	Queen's University Executive Leadership Course
1994-1995	Observation and supervised experience in Emergency Medicine, Seven Oaks Hospital, Winnipeg Canada (organized by Dr. Kopelow, Department of Continuing Medical Education)
1993	Clinician's Assessment and Enhancement Program, Department of Continuing Medical Education, Faculty of Medicine, University of Manitoba, Winnipeg, Canada

PROFESSIONAL MEMBERSHIPS, ORGANIZATIONS AND LICENSES

2020	Lead, WhatsApp Chat Group, Open-Minded Critical Thinkers, COVID-19
2013 – 2016	President, Public Health Physicians of Canada.
2012 – present	Member, Board of Directors, Canadian Association of Medical Education Foundation, currently liaison member to the Canadian Medical Education Journal.
2012 – 2015	Executive member, Clinical Teachers Association of Manitoba
2012 – 2014	Member, Board of Directors, Canadian Public Health Association of Canada
1999 – present	Member, Public Health Physicians of Canada, previously National Specialty Society of Community Medicine
1993 - present	Member, College of Family Physicians of Canada
2000 – present	Member, Canadian Association of Medical Education
1991 – present	Fellow of the Royal College of Physicians of Canada (Community Medicine – now Public Health and Preventive Medicine)
1990 – 2012	Assistant Professor, Depts. of Community Medicines, Surgery and Family Medicine, Faculty of Medicine, University of Manitoba
2012 - present	Associate Professor, Depts. of Community Medicines, Surgery and Family Medicine, College of Medicine, Faculty of Health Sciences, University of Manitoba
1990 – present	Member of the Canadian Association of Teachers of Community Health
1988 – present	Member of the Canadian Public Health Association and the Manitoba Public Health Association
1984 – present	Fellow of the Royal College of Surgeons of Canada (General Surgery)
1976 – present	Licentiate of the College of Physicians and Surgeons of Manitoba, Current license, General Practice, with Specialty privileges in General Surgery and Community Medicine

1976 – present	Licentiate of the Medical Council of Canada
1976 – present	Member of the Canadian Medical Association (Manitoba Division)
1976 – present	Member of the Canadian Medical Protective Association

UNIVERSITY AND OTHER ACADEMIC ACTIVITIES

2020	Faculty appointee, Undergraduate Medical Education Financial Award Committee
2018 - 2020	Member, Postgraduate Medical Education Assessments Committee, Professional Curriculum Committee, Education Advisory Committee, Accreditation Working Group, and Competency-based Medical Education Committee.
2019 – present	Chair, Post-graduate Medical Education Truth and Reconciliation Action Plan Working Group
2017 - present	Post-graduate medical education CanMEDs subject advisor
2015 - present	Associate director, Public Health part of Family Medicine/Public Health Clerkship.
2013- 2017	Member, Healthy Campus Advisory Committee, University of Winnipeg
1991- present	Member (and previous chair), Dept of Community Health Sciences Undergraduate Committee
2012-2015	Director, Master of Public Health program, University of Manitoba
2012-2014	Visiting professor and senior fellow, University of Winnipeg, Masters in Development Practice program, Faculty of Graduate Studies
2012	Promoted to associate professor, University of Manitoba
2012-2015	Elected to University of Manitoba Senate by the Faculty Council of Medicine
2011-2012	Co-chair Curriculum Renewal Task Group on Health systems, Public Health, and Environmental and Occupational Health and member of the Curriculum Renewal Steering Committee, Faculty of Medicine, University of Manitoba

2007-2012	Founding member of the first national Public Health Educators Network, and participant author of its first national on-line learning resource for medical students (The Primer);
1995, 2006, 2010	Member, Search Committees for Head of the Department Community Health Sciences, Department of Community Health Sciences, Faculty of Medicine, University of Manitoba
1992-1994	MSc thesis advisor for Anita Kozyrskyj: Validation of an Electronic Prescription Database in Manitoba: An Opportunity to Evaluate Pharmacist Participation in Drug Utilization Review.
1994 – 1996	Member, Med I and II Curriculum Reform Committee –Core Concepts Block, Faculty of Medicine, University of Manitoba
1994 - 1995	Member, Search Committee for new tenure-track position, Department of Community Health Sciences, Faculty of Medicine, University of Manitoba
1991 – 2011	Member, Executive Committee, Department Community Health Sciences, Faculty of Medicine, University of Manitoba
1991 – 2015	Member, Committee of Evaluation, Faculty of Medicine, University of Manitoba
1991 – 2015	Member, Clerkship Curriculum Committee, Faculty of Medicine, University of Manitoba
1991 – 2011	Director, Undergraduate Program, Department of Community Health Sciences, Faculty of Medicine, University of Manitoba (special teaching responsibilities include Course Director, Line and major clerkship-Family Medicine Community Medicine, graduate course teaching, thesis supervision and teaching and supervision of community medicine residents).

Undergraduate courses taught at University of Manitoba

2015 - present	Small group teaching in the population and public health pre-clerkship and clerkship programs and the Indigenous health longitudinal course, totaling now more than 100 hours per year.
1991- 2014	Average of more than 50 hours per year in undergraduate teaching, including 2-5 lectures and 2-3 tutorials in Population Health and Medicine, including Introduction to Health and Medicine (first lecture to first year medical students), Natural History of Disease and Levels of Prevention, Measurements of Health and Disease, Determinants of Health, Social Responsibility of Physicians; Public Health part of the Family Medicine/Public Health clerkship rotation (8 rotations per year), including orientation, community health status assessment, a "hot" current topic, followed after the rotation by a debrief; Annual summary presentation of Population and public health (invited consistently by 4th year students) as part of the LMCC QE Part I exam review.

Graduate and Postgraduate courses taught at University of Manitoba

2004 – present	Graduate teaching (MPH, MSc and PhD level): Problem Solving in Public Health (formerly Current Topics in Community Medicine 93.7510)
2016 - present	Invited speaker on Population Health and Health Care Organization to surgical residents as part of their Principles of Surgery training program.
2019	Invited speaker, Clinical Investigators Program: Health advocacy and health advocacy research.
1991- 2015	Annual guest teaching of "Principles of Prevention" in Epidemiology I and "Risk Communication" in Epidemiology II
1995-2008	Designer, supervisor, and lecturer in a recurring series of learning sessions in Epidemiology, Statistics, and Critical Appraisal in the PGME Core curriculum for all residents at the Faculty of Medicine;

Graduate Student Supervision

2015-2016	Supervised practicum of MPH student at International Centre for Infectious Diseases and National Collaborating Centre for Infectious Diseases
1994 - 2015	Supervisor for PGME students in Public Health and Preventive Medicine (average one - two per year for one to four month rotations)
2012-2015	Advisor to 13 MPH students, including field placement supervision.
1992-1994	MSc thesis advisor for Anita Kozyrskyj: Validation of an Electronic Prescription Database in Manitoba: An Opportunity to Evaluate Pharmacist Participation in Drug Utilization Review.

Current Research Activities

2013 – present	Health mentor, Grand Challenges Phase 1 Grant (total $100,000) "Improving Maternal and Child Health at the Root through Village Level Biotechnologies" with International Institute of Sustainable Development (co-PI) and CTx Green (P.I.)

SELECTED SERVICES, PROVINCIAL COMMITTEES AND OTHER RELEVANT ACTIVITIES

2012 – present	Member, Manitoba Provincial Vaccine Advisory Committee
2015-2016	Member, planning committee, *Conference to develop a federal framework on Lyme disease, Ottawa, May 15-17, 2016*
1994 – 2018	Examiner, Medical Council of Canada Part II Qualifying Exam
2014 - 2016	Member, Winnipeg Harvest Health and Hunger Committee
2015 - 2016	Member, Advisory committee to the Public Interest Law Committee research study on guaranteed annual income.
2003 – 2015	Statistics Canada Canadian Health Measures Survey Expert Advisory Committee
2013-2015	Member, Public Health Infrastructure Task Group to develop a blueprint for a federated surveillance system in Canada
2006 – 2012	Member of the Advisory Committee, National Collaborating Centre for Infectious Disease
2003 – 2007	Healthy Living Issue Group of the Population Health Promotion Expert Group, Canadian Public Health Network responsible for leading the writing of the Pan-Canadian Healthy Living Strategy,
2006 – 2011	Federal Provincial Territorial Roles & Responsibilities in Pandemic Preparedness and Response Task Group, Public Health Network Council
2006	Member of the selection committee for scientific director, National Collaborating Centre for Infectious Disease
2006 – 2008	Medical Advisory Committee, Health Science Centre, Winnipeg, Manitoba, representing Department of Community Health Sciences
2002 – 2009	Emergency Preparedness Expert Group, Canadian Public Health Network
2002 – 2006	Manitoba member, Federal Provincial Territorial Deputy Ministers of Health Advisory Committee Population Health and Health Security

2002 – 2003	Co-chair, Health Disparities Task Group, Federal Provincial Territorial Deputy Ministers of Health Advisory Committee Population Health and Health Security
2000-2001	Chair, Province of Manitoba Drinking Water Advisory Committee and sole accountable author of Report on Bacterial Safety of Drinking Water In Manitoba
1999 – 2002	Chair, Federal Provincial Territorial Deputy Ministers of Health Advisory Committee on Population Health
1999 – 2012	Council of Chief Medical Officers of Health of Canada (CCMOH)
1995 – 1999	Co-chair, Project Team, Community Health Status Assessments, Epidemiology Unit, Manitoba Health
1995	Participant, Federal-Provincial Working Group/Workshop for present the Prevention of Neural Tube Defects, Manitoba Health and Health Canada, Ottawa
1995	Member, Provincial Committee on Hepatitis A, B and C amongst Winnipeg street-evolved youth
1995 – 1999	Member, core committee to review the Public Health Act of Manitoba
1995	Member, Advisory Committee to the Baby Alert Program
1994 – 1995	Member, Steering Committee for Psychiatric Day Hospital and Community Services in Mental Health for Winnipeg, Manitoba Health
1994 – 1999	Member of the Manitoba Health Communicable Disease Control Surveillance Review Committee and Chairman, Subcommittee on Analysis and Dissemination of Results.
1994 – 1999	Member of the Winnipeg Air Quality Index Committee
1993 – 1995	Member, Provincial Cancer Control Committee and Chair of Subcommittee on Secondary Prevention of Cancer, Manitoba Health
1993-1994	Member, Working Group for Psychogeriatric Services in Winnipeg, Manitoba Health
1993-1994	Member, Committee to Define Core Services for Rural Health Associations, Manitoba Health
1993-1994	Member, Provincial Surgery Committee, Manitoba Health

1993	Participant, national workshop and consensus conference on the training of community medicine specialists, Toronto
1991 – 1995	Member, National Population Health Survey Provincial Advisory Committee, Manitoba Health
1989	Member, Provincial Task Force on Breast Cancer Screening in Manitoba, Manitoba Health
1986-1988	Member, Public Health Alliance of Britain
1985-1988	Member, International Physicians for the Prevention of Nuclear War
1977-1985	President, Progressive Medical Association, Winnipeg
1974-1976	Founding member of "The Community Medicine Group" medical students concerned about social and public health issues
1974-1976	Founding co-editor (with Dr. Brian Postl) of "The Meditoban", medical school student newspaper
1974-1976	Founding board member, NorWest Health Co-op, Winnipeg

PUBLISHED BOOKS

Northover, John M.A., Kettner, Joel D. and Mr. Barry Paraskeva PhD, FRCS. Your Guide to Bowel Cancer (Royal Society of Medicine). A Hodder Arnold Publication, 2007

Northover, John M.A. and Kettner, Joel D. Bowel Cancer: The Facts. New York, Oxford University Press, 1992

SIGNIFICANT REPORTS

Chief Provincial Public Health Officers' "Report on the Health Status of Manitobans 2010: Priorities for Prevention – Everyone, Every Place, Every Day" (published November, 2011)

PUBLISHED PAPERS

- SM Moghadas, M Haworth-Brockman, H Isfeld-Kiely, J Kettner. Improving public health policy through infection transmission modelling: Guidelines for creating a Community of Practice. Can J Infect Dis Med Microbiol 2015;26(X):1-5.
- Mahmud S, Hammond G, Elliott L, Hilderman T, Kurbis C, Caetano P, Van Caeseele P, Kettner J, Dawood M. Effectiveness of the pandemic H1N1 influenza vaccines against laboratory-confirmed H1N1 infections: population-based case-control study. **Vaccine**. 2011 Oct 19;29(45):7975-81. Epub 2011 Aug 30.
- Writing Committee of the WHO Consultation on Clinical Aspects of Pandemic (H1N1) 2009 Influenza, Bautista E, Chotpitayasunondh T, Gao Z, Harper SA, Shaw M, Uyeki TM, Zaki SR, Hayden FG, Hui DS, Kettner JD, Kumar A, Lim M, Shindo N, Penn C, Nicholson KG. Clinical aspects of pandemic 2009 influenza A (H1N1) virus infection. Review. **N Engl J Med.** 2010 May 6;362(18):1708-19.
- Zarychanski R, Stuart TL, Kumar A, Doucette S, Elliott L, Kettner J, Plummer F. Correlates of severe disease in patients with 2009 pandemic influenza (H1N1) virus infection. **CMAJ.** 2010 Feb 23; 182(3): 257-64. Epub 2010 Jan 21, 2010
- Verne J, Kettner J, Mant D *et al.* Self-administered faecal occult blood tests do not increase compliance with screening for colorectal cancer: results of a randomized controlled trial. **Eur J Cancer Prev** 1993; Jul: 301-305
- Yassi A, Kettner J, Hammond, G *et al.* Effectiveness and costs-benefit of an Influenza Vaccine Program for Healthcare Workers. **Can J In Dis** 1991: 101-108;
- Kettner, JD, Whatrup C, Verne JE *et al.* Is there a preference for different ways of performing faecal occult blood tests? **Int J. Colorectal Dis** 1990; May:82-86;

PUBLISHED ABSTRACTS

Kettner JD, Whatrup C, Miller K. A comparative study of three patient approach methods for faecal occult b1000 testing in a North London general practice. Coloproctology. 1988;10:129

Kettner JD, Whatrup C, Young K. A within-person comparison of efficacy and individual preference for two methods of faecal occult blood detection. Coloproctology 1988;10:123

Kettner JD, Whatrup C, Miller K *et al.* Evaluation of new faecal occult blood test-a comparison of individual preference and efficacy using Early Detector and Haemoccult. Theoretical Surgery 1987;2:82

Kettner JD, Whatrup C, Miller K *et al.* A randomized trail of invitation methods for occult blood screening. Theoretical Surgery 1987;2:81-82

Kettner J, Paetkau D, Slykerman L *et al.* Effect of treatment on cardiac performance when right ventricular afterload is gradually increased in dogs. Critical Care Medicine 1983; 11;3:217

Paetkau D, Kettner J, Girling L, Slykerman L, Prewitt RM. What is the appropriate therapy to maintain cardiac output as pulmonary vascular resistance increases? Anaesthesiology, 57;3:A-56, September, 1982

PUBLISHED LETTERS

Kettner, J. Quebec's Public Health Cuts Canadian Journal of Public Health 2015:106:3 March/April.

Scholefield JH, Kettner, JD, Northover JMA. Papillomavirus infection and progress to abnormal cervical smears. Lancet, 1988:i:1405;

Scholefield JH, Kettner, JD, Northover JMA. Problems with anal cancer demographics. Diseases of the Colon and Rectum; 1988:31:10:831;

Kettner JD, Mant D, Northover JMA. Ethics of preventive medicine. Lancet; 1988;ii:44-45;

Kettner Joel and Northover, JM. Screening for colorectal cancer, Lancet 1986;i:562-563;

Kettner Joel and Northover, JM. Occult-blood screening, Lancet 1986;ii:110;

PRESENTATIONS, WEBINARS AND OTHER SCHOLARLY AND EDUCATIONAL ACTIVITIES

2016 Planning consultant and facilitator, NCCID-York University Workshop on Mathematical Modelling in Public Health Infectious Diseases, York University, Toronto, October 3-4, 2016

2016 Guest (as Infectious Diseases Public Health specialist) on This Hour Has 22 Minutes, CBC Television.

2016 Member of scientific planning committee, Lyme Disease symposium, May 15-17, 2016, Ottawa.

Public Health 2016 (annual conference of the Canadian Public Health Association)

- Member, Conference Scientific Planning Committee
- Welcoming remarks on behalf of the Public Health Physicians of Canada at the opening ceremony
- Organized and participated in a panel discussion on "Public Health Inspectors, Public Health Nurses, and Public Health Physicians As Leaders: A Candid Conversation about Collaboration and Change "

Moderator, and member of the scientific planning committee, International Centre for Infectious Diseases National Grand Rounds:

- February 18, 2016: *Zika virus - What to Know, What to Do*, University of Manitoba, in collaboration with the Dept of Community Health Sciences Bold Ideas Colloquium Series.

Moderator, and member of the scientific planning committee, International Centre for Infectious Diseases International Webinars:

- December 1, 2016: *Difficult-to-treat Gram Negative Pathogens*
- November 8, 2016: *The Burden and Preventability of Non-respiratory Complications of Influenza in Older Adults*
- October 27, 2016: *Antibacterial Resistance in Gram-Negatives: Prevalence, risk factors, and impact of inappropriate therapy*
- October 13, 2016: *Pneumococcal Immunization for Older Adults.*
- August 17, 2016: *Pneumococcal conjugate vaccines for infants: What have we learned since their introduction?*
- June 22, 2016: *HPV Immunization Programs: What is the advantage of including males?*
- February 25, 2016: *Vaccine Hesitancy: What is it, Why is it, What to do about it?*
- January 13, 2016: *Mind your T's and Q's - What do we know about today's influenza vaccine options?* (moderator) and speaker: *Today's Menu of Vaccine Choices – the Basics and the New Ingredients*

2017 Radio interview re: legal age of marijuana purchase and use in Manitoba.

2015-2016	Radio, Television, and Media interviews on subjects including Ebola, ZikaVirus, Malathion, Influenza.
2015-2016	Designer, moderator, and speaker of ICID National Grand Rounds (Influenza vaccine for under 2 year olds, Influenza vaccine choices for seniors, Zika virus) and webinars (e.g. HPV vaccine, new vaccine options including quadrivalent, pneumococcal disease)
2015-2016	Co-chair (International Centre for Infectious Diseases/National Foundation for Infectious Diseases) of scientific planning committee and chair of international advisory committee for an accredited on-line learning module produced by MDBriefcase on *Seasonal Influenza in Older Adults: Immunization Challenges and Options for Vaccination Strategies*

2015:

Moderator, and member of the scientific planning committee, International Centre for Infectious Diseases National Grand Rounds:

- December 17, 2015: *Influenza Vaccines for Adults Over 65: Evaluating the Evidence*, University of Manitoba Medical College
- October 27, 2015: *Flu Vaccines for Little Kids - What's New, What's True*, University of Toronto

Moderator, and member of the scientific planning committee, International Centre for Infectious Diseases International Webinars:

- May 6, 2015: *Males and HPV: Burden of Disease and Prevention through Immunization*

November 25, 2015: Invited speaker, Public Health Physicians of Canada Residents' national educational webinar series: *Life After Residency.*

Lyme Disease Best Brains Exchange in Ottawa, June, 2015.

Chaired panel discussion at annual meeting of CHVI RD Alliance Coordinating Office at Canadian Association of HIV Research annual meeting, Toronto, 2015.

DCHS Colloquium presentation on the NCCID program: with Ms. Margaret Haworth Brockman: Ebola Virus Disease and other Challenges and Opportunities for the NCCID

Activities at Public Health 2015 (annual conference of the Canadian Public Health Association)

- Welcoming remarks on behalf of the Public Health Physicians of Canada at the opening ceremony
- Organized and chaired a panel discussion on "The ebola outbreak: What have we learned that we didn't know before?"

- Facilitated a workshop on Burden of Illness in Infectious Diseases

Association of Medical Microbiology and Infectious Diseases annual conference, Charlottetown, May, 2015.

- Poster presentation: AMR, Public Health, and Knowledge Translation: A Forward Approach

2014	Reviewer of research proposals for CIHR SPOR projects, Institutes of Population and Public Health and Health Services Delivery.
2013-2014	Member, scientific planning committee, Consensus Conference on Antimicrobial Resistant Organisms, University of and Institute of Health Economics, June 18-20, 2014
2014	Invited speaker, Consensus Conference on Antimicrobial Resistant Organisms, University of Alberta Institute of Health Economics, June 18-20, 2014: "What is surveillance? What is screening? How are they related?"
2014	Series of four public lectures at the University of Winnipeg on Public Health in the 21st Century: - *Public Health Unpacked: What is it? Who needs it?* - *Priorities for Prevention in Manitoba: our Provincial Profile* - *Public Health ahead: What are the Possibilities? How can we prevent the threats that we do not see or know?* - *Power, Process, and Public Policy: The Peculiar Ethics and Politics of Public Health and its relationship to Sustainable Development.*
2013-2014	National webinars for Public Health and Preventive Medicine residents and public health physicians hosted by the National Collaborating Centres for Public Health. Topic: - "Treatment as Prevention" with Drs. A. Ronald and J. Montaner - " Knowledge Translation for Emerging Diseases"
2013	Options (VIII) for the Control of Influenza, September 5-9, Capetown, South Africa - Paper: Rapid Knowledge Translation during the 2009 influenza pandemic - Poster: A project to translate and exchange knowledge towards more effective, efficient and equitable public health and primary care strategies for influenza and influenza-like illness (ILI) in Canada. JD Kettner, E Cheuk
2013	Innovation in Medicine and Health Care, University of Piraeus, Piraeus, Greece - Paper: Knowledge Translation for Emerging Infectious Diseases: Challenges and Opportunities

2013	University of Winnipeg Summer Institute Course: Hosted a morning session and presented a lecture on "Principles of prevention of infectious and chronic diseases"
2014	Series of four public lectures on public health, University of Winnipeg.
2012	Surgery Grand Rounds: "A Surgeon's Career in Public Health – the Long and the Short of It"
2003-2011	Annual lecture (most years) at "Bug Day" including SARS, "Little Bugs in the Big Picture", H1N1, and tuberculosis.
2010	National Collaborating Centre for Public Health, Making Connections, Opening Ceremony and plenary, keynote speaker, and co-presenter with Dr. Pat Martens on partnerships between government and university in public health policy setting, Summer Institute of the National Collaborating Centres of Canada
2010	The Manitoba College of Family Physicians, 52 Annual Scientific Assembly, key note speaker: H1N1 De-Brief
2010	Doctors Manitoba, Western Conference of Provincial/Territorial Medical Association, *"How to Survive a Pandemic –What have we learned?"*
2010	International College of Dentists Annual meeting, Winnipeg. *Public Health and the H1N1 Pandemic Influenza*
2009	Continuing Medical Education, Mini Medical School, University of Manitoba 2009;
2009	Presented on H1N1 for disadvantaged populations and led a practice guidelines consensus session at the Pan-American Health Organization of the World Health Organization consultation conference in October, 2009 in Washington, D.C.,
2008	Mini-university lecture on what on public health and evidence for the news
2007-2013	Annual lecture on *Issues and Trends in Public Health* at Red River Community College Issues and Trends in Health course taught by Jim Hayes as part of the Health management course for employees in regional health authorities
2007	Plenary speaker and panel discussant: Ethical issues in the practice of public health. The First Canadian Roundtable on Public Health: Exploring the Foundations, Montreal, Quebec.

CONTRACTED AND OTHER REPORTS

Manitoba Health Provincial Health Indicators, member of Working Group. 1999. https://www.gov.mb.ca/health/documents/ind-all.pdf

Kettner, Joel D. Community Health Status Assessment, Waterhen First nation: 1993 (for Waterhen First Nation, Manitoba)

Kettner, Joel D. and Postl, B Community Health Status Assessment: a tool to understand and improve the health of Aboriginal communities: 1991 (Northern Health Research Unit for Medical Services Branch, Health Canada)

Kettner, Joel D. Community Health Status Assessment, Cross Lake, Manitoba; 1989 (for Medical Services Branch, Health Canada)

INVITED REVIEWS

2017- 2021: Canadian Journal of Public Health

2018-2021: Canadian Journal of Medical Education

SELECTED MEDIA, COVID 19

Winnipeg Free Press panel, Dec 10, 2020
https://www.youtube.com/watch?v=9l52CWsUGTE

Toronto Caribbean interview, November 26, 2020
https://www.youtube.com/watch?v=cpjk53umB_0&feature=emb_title

CBC West of Centre panel discussion
Circuit Breakers and Personal Freedom, November 12, 2020.
https://www.cbc.ca/listen/cbc-podcasts/407-west-of-centre/episode/15808413-circuit-breakers-and-personal-freedom

Open letter to first ministers, July 29, 2020
https://healthydebate.ca/opinions/an-open-letter-to-pm-covid19

Opinion piece CBC Manitoba, July 25, 2020
A new normal, or new abnormal? Change in direction needed on COVID-19 response
https://www.cbc.ca/news/canada/manitoba/joel-kettner-opinion-covid-19-response-1.5654062

Letter to the editor, Winnipeg Free Press, June, 27, 2020
https://www.winnipegfreepress.com/search/?keywords=clergy+kettner&searchSubmitted=y&sortBy=-startDate

Cross-country Check-up, March 15, 2020.
https://www.cbc.ca/listen/live-radio/1-13-cross-country-checkup/clip/15765826-march-15-2020-is-enough-done-slow-covid-19

THIS IS **EXHIBIT "B"** TO THE AFFIDAVIT OF JOEL KETTNER affirmed before me at the City of Winnipeg, in the Province of Manitoba, the 1st day of April, 2021.

A Commissioner for Oaths in and for the Province of Manitoba. My commission expires July 8, 2021.

AB612

RESPONDING EXPERT REPORT ON THE COVID-19 PANDEMIC RESPONSE IN MANITOBA

JOEL KETTNER M.D.

April 1, 2021

Introduction

This report is in response to the affidavits of the Respondents. From a public health perspective, and as the former Chief Public Health Officer of Manitoba, it is my view that the Respondents' affidavits do not include sufficient justification or evidence to show that the restrictions on places of worship and public gatherings have been no greater than is reasonably necessary to respond to the COVID-19 threat to public health.

To meet the requirements of good public health practice, the Manitoba Public Health Act, and the Canadian Charter of Rights and Freedoms, public health officials are required to show that the severity of the threat has justified the restrictive interventions, that the effectiveness and benefits of the interventions have sufficiently outweighed the harms, and that there were no alternative strategies that would have been less restrictive, equally or more effective, and less harmful.

These requirements are not only about rights and freedoms, important as those are from an ethical and legal perspective. These requirements are about good public health practice to maximize benefit of interventions while minimizing harms, especially for those that are disadvantaged. It is about evidence-based and rational decision-making for optimal outcomes.

Principles of a public health approach to a communicable disease outbreak

A viral respiratory illness outbreak with pandemic transmission is a complex biological and social phenomenon. Decision-making by public health officials and governments – especially in an emergent and evolving situation – is challenging.

The importance of a plan and strategy

To meet this challenge, public health practitioners are expected to develop and implement a comprehensive, effective, efficient, and equitable strategy that is based on evidence, reason, and fairness.

Neither a previous nor current Manitoba plan or strategy for COVID-19 or any other respiratory virus public health emergency has been included in any of the affidavits or exhibits of the Respondents. Nor has an overall plan or strategy been made available to the public.

The strategy should be developed on the foundation of an appropriate and current response plan. The plan should include a framework for action to assess the severity of the threat, to set clear short-term and long-term goals and objectives, to survey the most relevant data, and to

guide rational, ethical, fair, and evidence-based decision-making. It should be able to monitor in real time outcomes from the disease as well as the beneficial and harmful effects of interventions. The prepared plans and current strategies should be shared transparently with the public for the purpose of achieving informed engagement and consultation, establishing and/or maintaining trust and understanding, and optimizing the effectiveness.

These expectations are reflected in the recently released Auditor-General's report.

> Section 8.1 of the Auditor-General's 2021 report on Pandemic Preparedness, Surveillance, and Border Control Measures states:
>
> "This audit is important because a well-planned and informed public health response is crucial to limiting the spread and public health impact of an infectious disease during a pandemic. In particular, timely and comprehensive surveillance information is needed to direct public health efforts."[1]

Both the preparedness plan and the strategies should be explainable and comprehendible to government officials that adopt it, health professionals, the media, and all members and organizations of the public.

A plan and strategy are important because that is the document that sets out in an accountable and transparent way the broad goals and specific measurable objectives of the response. Although goals are referred to in various parts of the Respondent's affidavits, no specific measurable objectives were found.

Without stated goals and specific measurable objectives, interventions cannot be rationally selected and surveillance and evaluation cannot be operationally relevant. The response plan would be expected to contain the methods of surveillance, definitions and protocols for assessing the frequency and severity of cases, processes for assessing and prioritizing types of exposures and settings for transmission of infection, and ways of systematically monitoring and evaluating the effectiveness, benefits, and harms of interventions – not only for the disease of interest but for all other causes of morbidity and mortality, including the social, economic, and other determinants of health.

Without a plan with measurable objectives, the large amounts of data and information contained in the Respondents' affidavits have not shown in a quantified and comprehendible way that the severity of the threat of COVID-19 has justified the restrictive interventions, nor have they shown that the effectiveness and benefits of the interventions have sufficiently outweighed the harms.

---

[1] https://www.oag-bvg.gc.ca/internet/English/parl_oag_202103_03_e_43785.html#hd3c

Roussin's Affidavit at paragraphs 54, 56 and exhibits 9 and 10 accurately sets out the principles that underlie the ethics of public health, the principles to guide in the justification of public health intervention, and the five main building blocks of public health practice. Of particular note is the document referred to in exhibit 10 of Roussin's Affidavit – Public Health: A Conceptual Framework. It states "Prior to taking action on a specific issue, *a risk assessment is necessary to estimate the nature and likelihood of negative health outcomes in individuals*." (emphasis added) With regard to effectiveness, it is stated on page 12 that "Outcome evaluations measure progress in the program's targeted public health challenge, and may include short-, intermediate-, and long-term results, that are also based on quantitative and qualitative data."

Further, in the Objectives of Training in the Specialty of Public Health and Preventive Medicine – exhibit 9 of Roussin's Affidavit - there are objectives of training pertaining to risk assessment. Of note are:
5.1.1.1. Characterize the hazard identified, *both quantitively and qualitatively*.
5.1.2. Integrate hazard identification, characterization, and *assessment into an estimate of the adverse events likely to occur in a population*, based on a hazard found in that population. (emphasis added)

What must be concluded and emphasized from these excerpts from paragraphs 54 and 56 and exhibits 9 and 10 of Roussin's Affidavit, is that none of the five principles that guide sound decision-making and none of the four principles to guide in the justification of public health intervention can be applied or met without quantitative estimates of risk and quantitative estimates of intervention effectiveness. For risk assessment, this includes estimations of likelihoods (probabilities), and degrees of risk (i.e. severity). For effectiveness of interventions, this includes measurements and estimations of quantitative outcomes that are relevant to the objectives and targets of the program. Valid and comparable quantitative estimates of risk and valid quantitative estimates of effectiveness are fundamental to the process of demonstrating that public health interventions are proportional to the threat and reasonably necessary. These include basic epidemiology descriptors and indicators such as probabilities, rates, ratios, and proportions. Accurate estimates of these quantitative measures are necessary but not sufficient for reasonable decision-making. The numbers must be analysed to assess their validity, relevance, meaning, and use.

Some examples of the quantitative estimates which would be expected to be found in the Respondents' affidavits but were not, are listed here:

- **Probabilities of event occurrence.** For example, statements that "COVID-19 has been proven to be highly communicable and contagious among people" and "... certain settings...have led to a higher risk of transmission"[2] are not meaningful or useful unless accompanied by quantitative estimates of absolute risk (probability) of transmission.

[2] Roussin's affidavit, Paragraph 27

- **Incidence rates of cases with appropriate numerators, denominators, and time periods, stratified by relevant characteristics.** For example, rather than only the crude number of cases in each region or a cluster, it is necessary to describe the proportion of the population affected, whether that be demographic, geographical, or setting type. Without information that includes relevant denominators, the numbers may seem disproportionately large either in absolute or relative terms – and cannot be compared in a meaningful way.

- **Population-based rates of hospitalizations and deaths, stratified by relevant characteristics.** For example, rather than a list of deaths by age and sex[3], it is necessary - as has been done in other provinces – to calculate actual rates of mortality and fatality, using denominators of population size and case counts, respectively, stratified by age, and health conditions, and other relevant factors. Without information that includes relevant denominators, the numbers may seem disproportionately large either in absolute or relative terms – and cannot be compared in a meaningful way. Stratification – e.g. age-specific rates provide objective information about the size of risk differences between people of different ages.

- **Ratios of probabilities of events associated with the disease or outcomes of interventions.** These are necessary for the purposes of valid comparisons. For example, listing the number of cases associated with each of "ten clusters associated with attendance at faith-based events"[4] does not provide sufficient data for a risk assessment. At the very least there should be an estimation of a denominator such as the number of Manitobans that attend a place of worship during the relevant time period. More relevant, an assessment of the effectiveness of closing places of worship should be based on the estimated ratio of the probability of getting infected per week with one or two hours of church attendance in comparison with the probability of getting infected per week without church attendance.

- **Proportions are a way to compare events or characteristics and to put them into better perspective.** For example, rather than merely counting the number of cases associated with attendance at faith-based events[5], additional information such as the total number of cases would help to put the numbers in perspective. It is not clear over what time period these data have been collected, but 172 "primary" cases and 30 "secondary" cases were identified in 10 "clusters" between August 2020 and February 2021. Assuming that the church exposures were their sources of transmission, and using all cases in Manitoba until January 14, 2021[6] as the denominator, the data provided represents an estimate of 202/26954 = 0.7% or, equivalently, one church-associated case per 133 of all Manitoba cases. Based on the data provided, one per 2500 Manitobans

[3] Loeppky affidavit, exhibit B, page 16-33
[4] Loeppky's affidavit, Paragraph 14
[5] Loeppky's affidavit,
[6] Loeppky's affidavit, exhibit B page 1.

that attend a worship setting at least once per month, have been identified as a case during the past year.

To achieve the general public health goals of optimizing health of the population, public health decisions must consider many dimensions. The decisions about public health interventions must consider short-term and long-term benefits and harms for society as a whole. These considerations must include all matters pertaining to health. Even when one specific disease becomes the focus of attention, they must consider the morbidity and mortality from all diseases and injury, especially when interventions for one disease may increase the rates or severity of other conditions. These considerations must also include causes and risk factors of all diseases and injuries; these factors are often referred to as social, educational, and economic determinants of health.

Assessment of the Public Health Response.

In order to respond to the Respondents' explanation of and justification for the impugned Public Health Orders, the following questions will be answered with specific reference to the Respondents' affidavits:

1. Has the PCR test and have the PCR test results – and other information - been used in a reasonable and reliable way to accurately estimate the frequency of infections, fatality-rate of infections, hospitalizations, deaths, and years of life lost attributable to COVID-19 and to justify the reasonable necessity of restrictions of rights and freedoms, including quarantine and isolation?

2. Have the public health orders which have restricted rights and freedoms of people at very low risk for severe illness or death been shown to be reasonably necessary, fair, and sufficiently effective to protect those at higher risk and to maintain hospital capacity for all Manitobans?

3. Has it been shown reasonably by the Respondents by use of valid models and other methods that alternative less restrictive strategies could not have achieved better health outcomes with less harm?

4. Have the Respondents demonstrated how they have ensured that the restrictions of their policies have been no greater than reasonably necessary by anticipating, considering, estimating, and surveilling the observed health and social harms of the restrictions, including their impact on all determinants of health?

5. Have the Respondents reasonably explained their estimation of the absolute (actual) and relative risk of transmission of COVID-19 causally associated with attending church services?

Approach to address these questions

Section three of the Manitoba Public Health Act – an act that was revised during my tenure as chief medical officer of health - states

> "If the exercise of a power under this Act restricts rights or freedoms, the restriction must be no greater than is reasonably necessary, in the circumstances, to respond to a health hazard, a communicable disease, a public health emergency or any other threat to public health."

This wording is consistent with Section One of the Canadian Charter of Rights of Freedoms which states:

> The Canadian Charter of Rights and Freedoms guarantees the rights and freedoms set out in it subject only to such reasonable limits prescribed by law as can be demonstrably justified in a free and democratic society.

At the time when the new Manitoba Public Health Act was proclaimed in 2009 the onus then fell on the Minister of Health and the Chief Provincial Public Health Officer to justify the need for such measures. The Act has not changed; it is still the onus and responsibility of the government and the chief provincial health officer to explain the need for the restrictions that have been implemented in the past year.

The Respondents' affidavits lack the necessary evidence to demonstrate they met the standard required from a public health perspective in section three of the Act, that the restrictions have been reasonably necessary to respond to the circulation of the respiratory virus SARS-CoV-2 . The basis for this opinion is that the goals and objectives of the strategy have not been adequately described, that the size of the actual threat from COVID-19 has not been reasonably estimated, that the effectiveness of the restrictions have not been reasonably demonstrated, that the harms resulting from these interventions have not been adequately described, and that alternative, less restrictive, and less harmful interventions have not been transparently considered.

Public health interventions – especially restrictive ones of the kind we have seen during the past year – can only be justified if the size of threat is big enough, the interventions are effective enough, and the harms from the interventions are small enough. Without reasonably accurate estimates of these three factors, and without an adequate explanation of why there were no less restrictive and harmful interventions that could have been used to achieve the goals and objectives, the Respondents have not met the public health standard set out in section three of the Act.

1. Response to Dr. Roussin and Dr. Bullard - Has the PCR test and have the PCR test results – and other methods - been used in a reasonable and reliable way to estimate accurately frequency of infections, fatality-rate of infections, hospitalizations, deaths, and years of life lost attributable to COVID-19 and to justify the reasonable necessity of restrictions of rights and freedoms, including quarantine and isolation?

It is appropriate that this this should be the first question to address. As stated, some of the fundamental requirements of communicable disease public health response are the case definitions, surveillance, and analysis of data needed to understand the frequency, severity, transmission, and impact of the disease as well as to monitor the trends and impacts of interventions.

For most outbreaks, laboratory testing has played an important role for some of these. In this outbreak, laboratory testing – primarily using the PCR test – has been the basis for all of them.

For this reason, it is important to explore fully not only the accuracy and validity of the test, but how the test has been interpreted and used.

PCR tests: What they tell us; what they do not tell us

This is a very important issue, because descriptions of COVID-19 disease frequency, severity and death have been based mostly on results of the PCR test.

The PCR test is considered quite accurate with respect to identifying the presence of a long enough chain of amino acids to confirm whether the type of virus being tested for is in the sample.

Paragraph of 10 Bullard's Affidavit states that "the RT-PCR test is the most accurate test available for identifying cases of SARS-COV-2". It is important to distinguish the meaning and definition of the terms SARS-COV-2 and COVID-19. SARS-COV-2 is the name given to the coronavirus, first identified in Wuhan, China. COVID-19 is the name given to the disease that is caused by a SARS-COV-2 infection. Infection in this context means that the virus has actually invaded cells and multiplied within the person, whether symptoms have resulted or not. It is important to clarify that the mere finding of genetic material of SARS-COV-2 in a sample from the back of the nose of a person does not necessarily mean that the person is infected, had, has or will have symptoms, or should be classified as a "case" of COVID-19. That would depend on case definition, not the mere identification of genetic material of a virus.

PCR tests: Cycle thresholds (Ct)

The cycle threshold describes the number of cycles of amplification needed to strengthen a weak signal to enable the identification of the amino acid sequence of the virus being tested for. It is an indicator of how much genetic material is present in the sample. The higher the Ct to obtain a positive signal, the lower the volume of genetic material in the sample. The Ct number would be expected to be documented by the Lab for every positive test result.

The interpretation of this number is important and it is agreed that the higher the Ct, the less likely it is that the person is infectious at the time that the sample was obtained. It is also agreed that the longer the period of time since the beginning of symptoms, the less likely it is that a positive PCR test will correctly identify the presence of viable, replicable, infectious virus. It is also agreed that a PCR test can be positive several weeks or months after a symptomatic or asymptomatic infection has resolved. These observations have been verified by many microbiologists and laboratories including Drs. Bullard and Van Caeseele at the Manitoba Cadham Provincial Laboratory (CPL), whose published study (the "Bullard et al Study") is referred to on line 142 of the report of Drs. Bullard and Van Caeseele (the "Bullard/Van Caeseele Report") (Exhibit C to Bullard's Affidavit).

Lines 85-86 of of the Bullard/Van Caeseele Report dated February 24, 2021 states that the current test at CPL uses a maximum of 40 cycles of amplification (Ct). This means that if it takes 40 or less cycles before a positive signal is produced, the test result is considered positive.

The Bullard et al Study cited in the Bullard/Van Caeseele Report was published in *Clinical Infectious Diseases* on 22 May 2020[7]. It compared PCR test results with the results of virus cultures, a reasonable gold standard that they used to determine the presence of viable, replicable and potentially infectious virus in the samples submitted to the lab.

The fourth line of the Results section of the abstract of the Bullard et al Study, states that "there was no growth in samples with a Ct > 24 or an STT (symptom onset to test) of greater than eight days".

With respect to Ct levels, the Bullard et al Study stated that "there was no growth in samples with a Ct greater than 25."

In the Discussion part of the Bullard et al Study, the authors discuss the implication of their observations for public health policy issues, including unnecessary isolation. They concluded that "These data, if confirmed, may help guide isolation, contact tracing, and testing guidelines."

One way to confirm these data, would be to compare them with epidemiological evidence. Despite this suggestion in the Bullard et al Study of 10 months ago, no epidemiological evidence

[7] https://academic.oup.com/cid/article/71/10/2663/5842165

has been cited in any of the Respondents' affidavits. Analysis of even a sample of the data from contact tracing of 34,000 cases could have provided reasonable estimates of the rates of transmission to contacts stratified by Ct levels and stratified by the numbers of days between onset of symptoms and the taking of the sample.

If this information is known, why hasn't it been previously made available and why hasn't it been included in any of the affidavits? If it is not known, why were these questions not prioritized to ensure that unnecessary isolations and quarantines were avoided? Either way, this data is important to establish whether the impositions caused by isolation and quarantine have been reasonably necessary.

In other words, imposing quarantine on people whose contact with the index (primary) case occurred eight or more days after the onset of symptoms in the index case and their Ct was > 25 would be an unreasonable interference with their autonomy. This is because of the negligible probably that the index case was infected at the time of contact. Current knowledge that the probability of infectiousness is negligible more than eight days after the onset of symptoms means that it is not reasonable to isolate a case for more than eight days after the onset of symptoms, especially if their symptoms have resolved or are resolving. The latter circumstances – presence of symptoms after eight days - could be reviewed on a case by case basis.

PCR tests: How they have been used for surveillance and case definition?

A confirmed case is defined by the Respondents as "a person with a laboratory confirmation of infection with the virus that causes COVID-19 performed at a community, hospital or reference laboratory (NML or a provincial public health laboratory) running a validated assay. This consists of detection of at least one specific gene target by a NAAT assay (e.g. real-time PCR or nucleic acid sequencing)".[8]

Paragraph 10 of the Bullard/Van Caeseele Report states that "Regardless of the particular Ct value, a positive RT-PCR result represents a true positive case of the SARS-Cov-2 virus." This statement cannot be interpreted without defining "a true positive case". Tests do not normally "represent" or equal a case. The use of test results is one criterion of case definitions. Other criteria include clinical (e.g. onset and type of symptoms, clinical signs, diagnostic tests) and epidemiological (e.g. contact history, type and settings of exposure, occupations, travel). These are the criteria used to define the case categories – typically four types, namely possible, suspect, probable, and confirmed. Manitoba Health's case definitions are posted on their website.

Paragraph 12 of the Bullard/Van Caeseele Report states "From a public health perspective, it is important to identify and report all positive cases of SARS-CoV-2, regardless of the Ct value." It should be clarified that it is not normally the role of a lab to "report cases" to Public Health. The

[8] https://www.gov.mb.ca/health/publichealth/surveillance/covid-19/resources/Notes.html

lab's role is to report test results, which in this case should include the Ct level with each positive result. The product insert for the cobas SARS-COV-2 in vitro test, used by the Cadham Provincial Lab as a PCR test, states "Results should only be interpreted in conjunction with information available from clinical evaluation of the patient and patient history."[9] The product insert for Aptima SARS-CoV-2, also used by that lab, states "A positive result indicates the detection of nucleic acid from the relevant virus. Nucleic acid may persist even after the virus is no longer viable."[10] Whether it is the practice of the lab to report Ct levels for each test has not been stated. (The importance of this will be discussed further in the next question.)

PCR tests: How they have been used for quarantine and isolation decisions?

Based on the data from the Bullard et al Study and others, there would be little reason to isolate persons who have had a positive test with a Ct > 25 or when their test was done more than eight days after the onset of symptoms. This is because of the negligible probability of being infectious to others. Manitoba's policy has been mandatory isolation for 14 days after a positive PCR, regardless of the Ct level or the duration of time since symptoms.

Based on data provided in the Bullard/Van Caeseele Report beginning at line 193, more than 40% of 5852 positive PCR tests had a Ct value greater than 25; 25% had a Ct value greater than 30. There is no reference to a report containing these data or other relevant information. For example, there are no data about the time since onset of symptoms or what has been learned through contact tracing about transmission of infection from these cases.

Nonetheless, using the findings from the published Bullard et al Study, the best estimate of the proportion of PCR-positive persons that were not infectious at the time of testing is 40%.

There is no detailed data of the number of cases and contacts that have been required to self-isolate or self-quarantine, respectively. Based on government policy 34,000 Manitobans have had to self-isolate for 14 days and several more than that have had to self-quarantine. What proportion of those have been unnecessary because of the very low probability that the cases have been infectious? At 40% of PCR positive tests with a Ct > 25, a reasonable estimate would be 34,000 X .4 = 14,000 cases and 40% of all contacts. From an operational public health perspective, a more appropriate measure of specificity, using these data with a false-positive rate of 40%, should be 60% - not 99.9%.

The Bullard/Van Caeseele Report argues at lines 157 – 170 that despite the fact that "higher Ct values are associated with a lower likelihood of growing SARS-CoV-2 in cell culture, this cannot rule (sic) that the person was or was not infectious at the time of sample collection." This statement can be made about any test. Public health policy should not be made on the basis of possibilities; it should be based on probabilities. If it is not important to determine how well a PCR test result correlates with infectiousness, why did Drs. Bullard and Van Caeseele conduct

[9] Qualitative assay for use on the cobas® 6800/8800 Systems product insert **09179909001-02EN Doc Rev 2.0** p.18
[10] Aptima SARS-CoV-2 Panther System AW-21677-001 REV. 0002

their study at all and why did they conclude that their findings "may help guide isolation, contact tracing, and testing guidelines"?

At line 70, the Bullard/Van Caeseele Report states that no single PCR Ct value "in isolation can be used to determine infectiousness of a case and must be interpreted in the overall clinical context". If that is true, what is the protocol to make that interpretation? How have the results of that interpretation been used to make decisions about isolation, contact tracing, and quarantine? Where is the report to describe the outcomes from those interpretations? This information would be expected to be included in the Respondents' affidavits to meet the public health standard required by section 3 of the Act to justify the public health interventions. Without this information, one cannot determine if the isolations and quarantines imposed have reasonably necessary.

PCR tests: How they have been used for counting cases, hospital diagnoses, death certification and estimation of premature death and potential years of life lost?

Cases

According to information provided in the Respondents' affidavits, Manitoba Health's case definitions include two categories- probable or confirmed[11].

A probable case must have:

- clinical symptoms compatible with an acute respiratory illness,
- meet exposure criteria,
- and have an inconclusive or no test result.

One type of "inconclusive" result is called "indeterminate" and "is defined as a late amplification signal in a real-time PCR reaction at a predetermined high cycle threshold value". It is further stated that in these situations, "when clinically relevant" – other test methods that are "equally or more sensitive" should be used or another sample should be obtained.

A definition of that "high cycle value" referred to in the case definition has not been found.

A confirmed case has only one criterion – a positive test result at a lab running a validated assay. There are no clinical, epidemiological or exposure criteria.

The Respondents have not provided a breakdown between probable and confirmed cases. What proportion of all cases, all hospitalized cases, all ICU cases, and all deaths are probable cases - i.e. those cases without a positive PCR test. In those probable cases, a judgment must be exercised with respect to clinical, epidemiological, and laboratory criteria.

[11] https://www.gov.mb.ca/health/publichealth/surveillance/covid-19/resources/Notes.html

By disaggregating (splitting) data, more understanding can be gained than when it is aggregated (lumped). How many of the nursing home deaths have been confirmed cases (with a positive PCR) and how many have been probable cases (e.g. untested, but in a pch with an "outbreak")? These disaggregated results can enable analysis about under or over-diagnosing COVID-19 and under- or over-attributing deaths to COVID-19.

Similarly, disaggregating PCR test results by level of Ct can help us learn from and manage cases and contacts with different likelihoods of infectiousness.

However, if Ct information is not linked to positive test results or to other clinical information such as case or contact status, outbreaks, hospitalization, or death, little can be monitored or learned about the importance of Ct levels.

For example, observation and analysis of the frequency of infections amongst contacts could be stratified by the Ct level of the index case test. This would provide Manitoba-specific information about transmission which could be used to adjust policies regarding isolation and quarantine that would enable them to be least restrictive and most effective at reducing spread. In addition to the increased autonomy, less work would be missed, including health care workers.

Similarly, more information about the Ct level and onset of symptoms would better delineate the probability of active infection in a hospitalized patient or PCH resident. This could guide policies about isolation and quarantine in those settings.

Apparently the Respondents do not have possession of Ct values or access to death certificates or medical files. It also seems that real-time tracking and monitoring of the outcomes associated with the contact tracing program has not occurred or the results are not available for sharing.

Apparently, "specific information about hospitalization, ICU, and deaths of individuals who isolated after contact tracing is not readily available." These data are needed to analyze and better understand what settings and types of exposures were associated with severe cases. This information could be used for more evidence and risk-based strategies for more focused protection. It can also be used to evaluate the effectiveness of strategies to interrupt transmission. This was the reply to a request for the following data:
a) the proportion of traced contacts that became symptomatic during their quarantine period,
b) the proportion of traced contacts that tested positive for COVID-19 during their quarantine period,
c) the proportion of symptomatic contacts that were hospitalized, needed ICU, or died,
d) the estimated number and rate of prevented hospitalizations, ICU admissions or deaths attributable to contact tracing, quarantine and isolation.

Hospital diagnoses

Accurate classification of hospitalized patients is important. Two goals of the Respondents' strategy as Roussin states are to reduce severe disease and to maintain hospital capacity.

Therefore, it is critical to know which hospitalized patients have been admitted because of infection with SARS-CoV-2.

Page 8 of Exhibit E of Loeppky's Affidavit shows a table that includes hospitalizations. How is it determined that the reason for admission is a COVID infection?

The following excerpt is from the Provincial Respiratory Surveillance Report COVID-19 Technical Notes[12]

> "COVID-19 Associated Severe Outcome
> Hospitalizations and ICU submissions in COVID-19 cases are extracted from the provincial data system, Admission, Discharge and Transmission. ***Due to a need for timely reporting, hospitalizations and ICU submissions do not need to be directly attributed to COVID-19. Instead an association to a positive COVID-19 laboratory result is sufficient.*** Duplicate submissions for the same patient within the same illness episode are excluded. In this report, only Manitoba residents are included. ICU admissions are also included in hospitalizations."

These instructions indicate that the daily counts of hospitalized patients with a diagnosis of COVID-19 include all patients that have had a positive PCR test regardless of their clinical diagnosis and regardless of whether their admission is "directly attributable to COVID-19". What proportion of hospitalized patients had a mild or asymptomatic infection with SARS-CoV-2 within the previous weeks or months? A positive PCR test at that time or at the time of admission to hospital would be sufficient to count their admission as a COVID-19 case. Furthermore, a patient that has symptoms compatible with COVID-19 (e.g. symptoms of a common cold) and has been exposed to a setting with a cluster or outbreak meets the criteria of a probable case. An outbreak in a nursing home or other high risk setting is defined as one or more confirmed cases.[13] Without validation by clinical investigation, it has not been determined what proportion of hospitalizations should be attributed to COVID-19 and to what extent the count is an overestimation.

Cause of death

The same problem exists with respect to accuracy of ascertaining the cause of death. Manitoba's guidance for surveillance of deaths is consistent with recommendations of the World Health Organization and the U.S. Centers for Disease Control which significantly changed the rules and protocols for determining cause of death for confirmed or probable cases of COVID-19.[14]

Before these changes, standard protocols for determining cause of death – i.e. the underlying cause of death – have been based on the WHO International Classification of Diseases guidelines.

---

[12] https://www.gov.mb.ca/health/publichealth/surveillance/covid-19/resources/Notes.html
[13] https://manitoba.ca/asset_library/en/coronavirus/interim_guidance.pdf
[14] https://www.who.int/classifications/icd/Guidelines_Cause_of_Death_COVID-19.pdf

The modifications of the protocol, now direct medical death certifiers to name COVID-19 as the underlying cause of death unless there is an obvious cause for which COVID-19 could not have been even a contributing cause. Compare this with the following excerpt from the WHO ICD-10 2016 guidance for assigning any type of pneumonia as the immediate cause of death while listing as the underlying cause conditions that impair the immune system, wasting diseases, paralysis, dementia, and many other chronic conditions that are common in elderly people, especially those that are most vulnerable in personal care homes. In other words, before the revised death certificate protocols, death with pneumonia – of any known or unknown virus or bacterium - in a 90 year old person with severe dementia and wasting would be certified as dementia/wasting as the underlying cause of death and pneumonia (e.g. COVID-19) as the immediate cause of death. It would be have been coded as dementia – not COVID – as the cause of death.

Before March 20, 2020, these are WHO guidelines for classifying pneumonia as the immediate cause of death, not the underlying cause of death.

> Pneumonia. Consider Dependence syndrome due to use of alcohol (F10.2) as the obvious cause of Lobar pneumonia, unspecified (J18.1). Consider conditions that impair the immune system, wasting diseases (such as malignant neoplasms and malnutrition), diseases causing paralysis (such as cerebral haemorrhage and thrombosis), serious respiratory conditions, communicable diseases, conditions that affect the process of swallowing, other diseases that limit the ability to care for oneself, including dementia and degenerative diseases of the nervous system, poisoning and serious injuries (grade 1–4 according to the injury priority list in Annex 7.7) as obvious causes of any pneumonia (J12–J18, J69.0 and J69.8).[15]

The Respondents' affidavits reveal that Manitoba defines a COVID death for surveillance as a death resulting from a clinically compatible illness, unless there is a clear alternative cause of death that cannot be related to COVID disease (e.g. trauma). There should be no period of complete recovery* from COVID-19 between illness and death.

*Recovery in this context means no residual effects or complications from COVID-19, and does not refer to the status of "recovered", which refers to clients who are off isolation or precautions and are no longer considered infectious.

In April, 2020, both the World Health Organization[16] and the Centers for Disease Control and Prevention announced the very same changes in the protocols for filling out a death certificate and determining the underlying cause of death. What follows is the explanation of why and how the protocol for determining the cause of death has changed. Italics are mine for emphasis.

---

[15] https://icd.who.int/browse10/Content/statichtml/ICD10Volume2_en_2016.pdf
[16] https://www.who.int/classifications/icd/Guidelines_Cause_of_Death_COVID-19.pdf

"ICD-10 Cause of Death coding of COVID-19 Certifiers use a range of terms to describe COVID-19 as a cause of death, a sample can be found in the annex of this document.

Although both categories, U07.1 (COVID-19, virus identified) and U07.2 (COVID-19, virus not identified) are suitable for cause of death coding, it is recognized that in many countries detail as to the laboratory confirmation of COVID-19 will NOT be reported on the death certificate. *In the absence of this detail, it is recommended, for mortality purposes only, to code COVID-19 provisionally to U07.1* unless it is stated as "probable" or "suspected".

The international rules and guidelines for selecting the underlying cause of death for statistical tabulation apply when COVID-19 is reported on a death certificate *but, given the intense public health requirements for data*, COVID-19 is not considered as due to, or as an obvious consequence of, anything else in analogy to the coding rules applied for INFLUENZA.

Further to this, there is no provision in the classification to link COVID-19 to other causes or modify its coding in any way. With reference to section 4.2.3 of volume 2 of ICD-10, the *purpose of mortality classification (coding) is to produce the most useful cause of death statistics possible*. Thus, whether a sequence is listed as 'rejected' or 'accepted' may reflect interests of importance for public health *rather than what is acceptable from a purely medical point of view. Therefore, always apply these instructions, whether they can be considered medically correct or not.* Individual countries should not correct what is COVID-19 - GUIDELINES FOR DEATH CERTIFICATION AND CODING assumed to be an error, since changes at the national level will lead to data that are less comparable to data from other countries, and thus less useful for analysis. *A manual plausibility check is recommended for certificates where COVID-19 is reported, in particular for certificates where COVID-19 was reported but not selected as the underlying cause of death for statistical tabulation.*"

A striking example of a new protocol, unprecedented in Manitoba (even during pandemic H1N1 or other influenza outbreaks) is the guidance for obtaining a nasopharyngeal sample from every deceased person – including those that only have "very mild" symptoms compatible with COVID-19. Depending on the circumstances of the setting, such a case might be classified as a probable case if the PCR test is negative or, regardless of the circumstances of the setting, a confirmed case if the PCR test result is positive. Either way, the death would be classified as a COVID-19 death – even if the symptoms of COVID are "very mild". The protocol follows.

Testing Individuals After Death In the interest of identifying all deaths related to COVID-19 and to better understand the burden of disease in Manitoba, collection of a post-mortem nasopharyngeal (NP) swab for COVID-19 testing should be considered if the following are true: Part A: Prior testing 1) The deceased did not have a NP swab positive for COVID-19 prior to death OR 2) The deceased did not have two or more NP swabs

> negative for COVID-19 in the past week AND Part B: Symptoms or cause of death 1) Death was preceded by influenza-like illness (ILI), upper or lower respiratory tract infection, or any symptoms compatible with COVID-19, even if very mild OR 2) Cause of death is unclear If a previous swab was positive, no further testing is required.[17]

The Respondents' affidavits do not comment on this change of rules or protocols to identify underlying causes of death – or the implications for accurate estimating of the attribution of deaths to COVID-19. For example, there is no information on the proportion of deaths attributed to COVID-19 in personal care homes in which pneumonia has been the immediate cause of death but has not been identified in the death certificate as the underlying cause of death. What proportion of "COVID-19 deaths" in PCH's have occurred in residents that have been classified as probable cases without a positive lab test for SARS-CoV-2? (See above for "hospital diagnosis".)

On page 16 of Loeppky's affidavit, *Table 2. Deaths due to COVID-19 in Manitoba* is a misnomer because there is no description of how it was established that COVID-19 was the underlying cause of death. As shown above, the definition of a "death due to COVID-19" is "A death resulting from a clinically compatible illness, unless there is a clear alternative cause of death that cannot be related to COVID disease (e.g. trauma). How was it established that there was a "clinically compatible illness"?

Manitoba's guidelines state:

> "To understand risk factors for severe outcomes, for all deaths related to COVID-19 ensure complete documentation of underlying illnesses (in risk factors), and, if known, the cause of death and any contributing factors. For deaths under the age of 65 years, when underlying illness is unknown, regional Medical Officers of Health should follow-up with the attending physician to obtain further information."

The Respondents' affidavits do not provide information to describe how many deaths under the age of 65 were followed up with the attending physician and/or the findings and conclusions from such follow-up. If all deaths are counted as equal with respect to estimating burden of illness, why is this policy limited to deaths under the age of 65? Without this kind of information, it is not possible to have an accurate estimate of the severity of any cause of respiratory infection or to compare them with other pneumonia-associated deaths such as influenza.

For example, has a 90 year old frail person with dementia, a weak cough reflex, who frequently aspirates, dies from pneumonia, and has had a positive PCR test (of any Ct level and of any duration since the onset of symptoms), been listed as a "death due to COVID-19"? In fact, even without a positive PCR test, the probable case definition includes anyone with a "clinically compatible illness" and has been exposed to a PCH or hospital setting where there has been one of more confirmed cases.

---

[17] https://manitoba.ca/asset_library/en/coronavirus/interim_guidance.pdf

Every death causes great sadness and grieving for loved ones, but if the person had a very short life expectancy from advanced cancer, a "do not resuscitate" order, and a health care directive for "comfort care only", would her death reasonably be attributed to COVID-19 and compared equally to a death in a younger person without other health conditions – whether associated with COVID-19 or not?

There is no data in any of the Respondents' affidavits comparing the putative 755 COVID-19 deaths over 9 and 1/2 months with other causes of death. Even assuming that COVID-19 was the underlying cause of death in all 755 deaths, this would equate to 950/11,000 = 9% of all deaths. This is significantly less than deaths from heart disease, cancer, and other diseases that are attributable to diabetes, smoking, and alcohol – diseases and conditions that are not described as a cause of death in any of the Respondents' affidavits and have been given little attention in government or public health communications in the past year.

Perhaps more significantly, the median age of deaths listed in Table 2 on page 16 of Loeppky's Affidavit is 85 for females and 80 for males. 60% of these deaths occurred in people over the age of 80, 80% occurred over the age of 70, and 90% occurred over the age of 60. There is no data in the Respondents' affidavits showing the average life expectancy or annual death rates of residents in personal care homes stratified by age and health status. There is no data in the Respondents' affidavits showing what proportion of personal care home residents – prior to COVID-19 – usually die from pneumonia and how often that has been certified as the underlying cause of death. This is relevant because of the importance of measuring and analyzing the impact of COVID-19 – or any disease - age of death and on life expectancy. What has not been shown by the Respondents is an estimation of the potential years of life lost that can be attributed to COVID-19. Without that information, the number of deaths is not a meaningful measure of the burden of the illness or the magnitude of the public health threat. Pneumonia is a common immediate cause of death for people in an advanced stage of illness or frailty, but it is rarely listed as the cause of death – i.e. underlying cause of death – when it is the last stage of a series of conditions. The decisions to list COVID-19 as the cause of death regardless of other underlying factors can only have the effect of overestimating the burden and lethality of this disease – especially in comparison to previous causes of pneumonia as the immediate cause of death.

Estimation of premature death and potential years of life lost

Paragraph 53 of Roussin's Affidavit states "Public health intervention seeks to reduce mortality and morbidity, and places emphasis on disease prevention and health promotion for a community".

From a population health perspective, modern assessments of the impact of deaths - from any or all causes - focus on quality-adjusted or disability-adjusted potential years of life lost rather than crude counts of death that do not differentiate deaths by age, quality of life or life expectancy. The Manitoba Health Provincial Health Indicators include potential years of life lost

and premature deaths as indicators of importance to priorize causes of death[18]. Statistics Canada provides reports on these measures.[19]

Priorizing health problems with consideration for their impact on life expectancy and quality of life are not new ideas, but it has taken time to integrate them into routine and transparent measures. The World Health Organization initiated the Global Burden of Disease Project in 1990.[20] It emphasizes the importance of assessing and comparing disease burden by loss of quality-adjusted life years, stating "the framework for integrating, validating, analysing and disseminating such information is needed to *assess the comparative importance of diseases, injuries and risk factors in causing premature death, loss of health and disability* in different populations. Countries can combine this type of evidence along with information about policies and their costs to decide how to set their health agenda" (emphasis added).

To measure more meaningfully the impact of COVID-I9 on the lives of Manitobans, and the need for restrictive health orders, would require a more accurate assessment of the causes of death, assessments of life expectancy, and assessments of quality of life. There is no indication in the affidavits of the Respondents that any of these measurements or analyses have been undertaken.

In addition to obtaining a reasonable estimate of the impact of COVID-19 on life expectancy and quality-adjusted life years, it would be important to compare this with other causes of death – underlying and immediate. It would also be useful to guide policy with respect to public health interventions that restrict gatherings of family members. Given the shorter life expectancy of Manitobans that are at highest risk, the enforcement of social isolation for the duration of their remaining life is not warranted by the evidence that has been provided.

[18] https://www.gov.mb.ca/health/documents/ind-all.pdf
[19] https://open.canada.ca/data/en/dataset/a04b5362-7534-4fb7-846c-262275eea1c3
[20] https://www.who.int/healthinfo/global_burden_disease/about/en/

2. .Have the public health orders which have restricted rights and freedoms of people at very low risk for severe illness or death been shown to be reasonably necessary with regard to the principles of public health and the standards under the Act, fair, and sufficiently effective to protect those at higher risk and to maintain hospital capacity for all Manitobans?

As stated on page 60 in Roussin's affidavit the "focus" of the approach is "to minimize severe illness and death from COVID-19 by maintaining the spread of virus to manageable levels so it does not overwhelm our health care resources."

A primary "focus" or end is to avoid overwhelming health care resources. The means to that end is to minimize severe illness by maintaining the spread of the virus to manageable levels. There are no measurable objectives referred to or reported. Specific targets have not been specified beyond which the health care system would be considered to be overwhelmed. It has been reported often that intensive care units are full - or nearly full – operating "overcapacity" – an oxymoron in need of explanation. Intensive care units are always "full" or "nearly full". During "flu" seasons, most ICU patients have been diagnosed with influenza and elective surgery and other procedures have been delayed for weeks. As has been demonstrated many times in the past, the capacity of Manitoba's health system to provide acute care may be stretched, but has not been exceeded – depending of course on definitions of these terms and objective standards for measurement. The Respondents have failed to provide information as to Manitoba's ICU capacity, surge capacity, and the degree to which the ICU was "overwhelmed" due to Covid-19. Without this information, decision-makers and the public may be mislead about the severity and danger imposed by claims of the exceedance of or "on the brink of" exceedance of capacity. Noe doubt, like in past "flu seasons" hospitals and ICU's are "stressed". The issue is that when big decisions like "lockdowns" are made on the basis of concerns – often legitimate concerns – of hospital capacity there should be more measured, objective and transparent information and analysis to explain and justify those decisions.

From the community perspective, "manageable levels of spread" have not been defined. A reasonable and achievable target for minimizing severe illness and death has not been explained. No goals or objectives for other causes of severe illness and death – i.e. all causes of morbidity and mortality have been part of the "focus". No specific and distinct goals and objectives have been stated for people and communities at highest risk compared to those at lowest risk. No goals or objectives have been stated for other health consequences of the public health orders, including mental health, addictions, social isolation, loss of employment and income, and other social, educational, and economic determinants of health.

Without defining and setting goals, objectives, limits, and triggers, - especially for the most important indicators – meeting the required public health standard under the Act of justification for restrictive directives is more difficult to demonstrate.

The first priority is to identify and estimate quantitatively who is at highest risk and who is at lowest risk for severe illness and the need for hospitalization. This enables strategies to be more targeted towards protection and early care for those at highest risk.

The measures of most importance are rates of hospitalization, intensive care hospitalization, and death. As discussed earlier with respect to cause of death, it is important to minimize misclassification of attribution to COVID-19. Without accurate ascertainment, other estimates of risk and rates may be biased.

The list of deaths on pages 14-33 of Exhibit B of Loeppky's affidavit include the date of death, sex, age and health region. They are not summarized or analysed by any of the fields of data in the table. Using the data provided, the following calculations have been made.

Pivot tables derived from the Affidavit of Carla Loeppky pages 17-33. The imported raw data was double checked and corrected against the pdf. Pivot table calculations were also double-checked. No third-party has verified my dataset or calculations.

Deaths by Year	Count
Unknown	3
2020	679
2021	73
Grand Total	755

Deaths by Gender	Count
Female	396
Male	359
Grand Total	755

Row Labels	Min of Age	Max of Age
Female	28	105
Male	8	105

Average Age at Time of Death	Average of Age
Female	82
Male	77
Both*	80

*Properly calculated, not by merely adding totals and averaging.

Median Age Among Deaths	Median Age
Female	85
Male	80
Both*	83

*Also properly calculated.

Age at death by Decile	Count	%	Cumulative %
0-9	1	0.001	1.00
20-29	2	0.003	1.00
30-39	11	0.015	1.00
40-49	22	0.029	0.98
50-59	40	0.053	0.95
60-69	76	0.101	0.90
70-79	153	0.203	0.80
80-89	252	0.334	0.60
90-99	182	0.241	0.26
100+	16	0.021	0.02
Grand Total	755	1.000	

This data shows that that the median age of death is 83, meaning that one-half of all deaths have occurred at a higher age than 83.

This data shows that 80% of deaths have occurred in people over the age of 70 and 90% of deaths have occurred over the age of 60.

This data shows that 10% of deaths have occurred under the age of 60 and 2% of deaths have occurred under the age of 40.

Using the denominator of cases or of estimated infections, rates can be used to estimate the probability of severe illness amongst cases, such as the case-fatality ratio. Using the denominator of the population, these rates represent population-based rates, such as the mortality rate.

Even more so than influenza and other infectious diseases, severe illness from COVID-19 is highly associated with age, certain chronic health conditions, and socio-economic characteristics such as Indigenous identity, as Roussin points out in his affidavit. Combinations of these increase that risk further. For these reasons, it is important to fully understand the actual and relative risks of those at highest and lowest risk. This requires that these rates be stratified by the most relevant factors.

Unlike some provinces, Manitoba's data provided in the Respondents' affidavits has not been summarized or analyzed to readily provide a quantitative assessment of actual and relative risks for severe illness, stratified by factors of most importance. Without this information and analyses, there is less ability to show that the Respondents meet the public health standard that restrictions of those at lowest risk for severe illness have been reasonably necessary, fair, and sufficiently effective to protect those at higher risk and to maintain hospital capacity.

Has it been shown reasonably by the Respondents with respect to the relevant public health standards that by use of valid models and other methods that alternative less restrictive strategies could not have achieved better health outcomes with less harm?

Section three of the Manitoba Public Health Act and section one of the Canadian Charter of Rights and Freedoms make it necessary for alternative strategies to be considered by government and why it must be shown transparently that there were no less restrictive strategies that could have achieved an equal or better balance of benefits and harms. Section three of the Manitoba Public Health Act states that *"the restriction must be no greater than is reasonably necessary". Without considering and comparing alternative strategies which cause less restrictions, the test of "reasonably necessary" cannot be explained or defended.*

Mathematical models: Uses and limitations

Mathematical models may be used to forecast or predict outcomes. They can be used to better understand and to demonstrate which factors are associated with those outcomes. Like all models, which are a conceptual simulation and simplification of more complex realities, they can be helpful or unhelpful, depending on how realistically they are constructed, how valid are the inputs, and how meaningful are the outputs. More important than the quality of the models – which are less than perfect, at best – is the quality of their interpretation and application to decision-making.

As stated on page 25 of exhibit F of Loeppky's Affidavit, "as with models trying to predict the future, its results must be interpreted with caution". The expectation would be therefore, that the caution used in the interpretation or application of any model's predictions would be specifically described and clearly explained before drawing conclusions about how their results should be used. Such descriptions and explanations have not been evident in the affidavits submitted by the Respondents. Either such cautions have not been used or the cautions have not been disclosed. This raises concerns about how these models have been interpreted and how they have been used to make decisions and for communication to the public to justify those decisions.

Structure, processes, inputs, and outputs

The "Made-in-Manitoba" Agent-based Modelling Simulations are described on pages 23-33 of exhibit F of Loeppky's affidavit. There is insufficient information to describe the actual design of the model, how the inputs are estimated or used, and how to interpret the outputs.

The only output variable shown by the model projections results on pages 30-33 of exhibit F to Loeppky's Affidavit are the numbers of cases. More relevant outputs, such as the proportion of those cases expected to need hospital care or expected to die are not described. That would be dependent on the age, health status, and other risk factors of the population of cases. Also, there do not appear to be any harmful outcomes projected by this model (e.g unemployment, social isolation)

The information the Respondents have provided about the "165 parameters" referred to on page 25 of exhibit F to Loeppky's Affidavit is insufficient to assess the validity and usefulness of the model. Without more detailed definitions of each parameter and what estimates have been used as inputs, one cannot assess the appropriateness of the model or how its results should be used.

For example, with respect to transmission dynamics, there are three parameters for a "transmission probability reduction factor for asymptomatic infections" – lower limit, mode, upper limit. As noted elsewhere in this report, the estimated prevalence of asymptomatic or pre-symptomatic infections and the estimated probability of transmission from asymptomatic or pre-symptomatic infections under different circumstances are very important considerations in risk assessment spread of the virus.

Without information about the probability values that have been used in the model simulations, it is not reasonable to expect an evaluation of the model nor to gain the confidence of others in its forecasts.

There is a parameter "probability of a random contact happening outside social compartments three times per week". Another is "daily probability of participating in a larger event". Another is "number of random interactions in a large event". Another is "number of random interactions on a day." No information has been made available by the Respondents about how these parameters were "made in Manitoba", how they were measured, and how they have been validated.

One use of a model is to perform sensitivity analyses on the input parameters to determine which variables have the biggest effect on the outcomes of main interest. Sensitivity analyses were not mentioned in the Respondents' Reports. Either sensitivity analyses were not done or their results have not been shared. Without sensitivity analyses, a model cannot be evaluated with respect to the most important and least important input parameters. Without that evaluation, the usefulness of the model is limited and should be used with even more caution than usual.

Other strategies to consider

Despite what one might conclude from mainstream media and government communications, there has been since the beginning of this pandemic the expected scientific and other debates amongst "experts" and non-experts alike.

An open communication to all first ministers in July, 2020 *"Dealing with COVID-19: A Balanced Approach"* [21] was signed by 18 Canadian physicians currently or previously in leadership positions in public health and health care, including two Manitoban physicians at the University of Manitoba. The letter contained 12 recommendations to support the overall goal: *Minimize the*

[21] https://healthydebate.ca/opinions/an-open-letter-to-pm-covid19/

impact of COVID-19 using methods that are practical, effective and compatible with our values and sense of social justice. We need to focus on preventing deaths and serious illness by protecting the vulnerable while allowing society to function.

This approach is similar to that known as the Great Barrington Declaration, initiated by three prominent international experts in medicine, epidemiology, and infectious diseases. It was released on October 5, 2020 and has been signed by 14,000 medical and public health scientists, 42,000 medical practitioners, and 760,000 concerned citizens. The essence of the declaration – "focused protection" - is recommended as *"The most compassionate approach that balances the risks and benefits of reaching herd immunity, is to allow those who are at minimal risk of death to live their lives normally to build up immunity to the virus through natural infection, while better protecting those who are at highest risk. We call this Focused Protection".*

Paragraphs 161 – 179 of Roussin's affidavit constitute the arguments in support of Manitoba's policies and directives, including the restrictions on faith-based and outdoor gatherings. Because these arguments are central to the requirement that the government justifies that its public health directives are proportionate and reasonably necessary, it is important to respond to each of them.

There have been and continue to be variations in the public health orders with respects to geographic regions, settings, and activities. What is not mentioned in paragraph 162 of Roussin's Affidavit is the essence of the concept of focused protection, namely a focus on people that are at highest risk of severe illness and need for hospitalization.

The statements at paragraphs 163 and 164 of Roussin's Affidavit are true that severe illness and death has not only occurred in residents of personal care homes and that there is a risk that younger persons working or visiting long term care facilities will transmit the disease. Whether the risk will be greater if young people "were allowed to circulate freely in the community" is a matter of opinion. It would be important to provide evidence and an estimate of the risk before rejecting this concept out of hand. None was provided. It would be necessary to show that the probability (point prevalence) of asymptomatic infection or the probability of concealing a symptomatic illness would be more significant factors than compliance with non-pharmaceutical interventions (distancing, masks, hygiene) when interacting with people at highest risk.

In reference to the experience with children and schools, paragraph 165 of Roussin's Affidavit provides good examples of focused protection. It is true that SARS-CoV-2 is communicable. It is likely that "outbreaks" have occurred in all settings, especially when they are defined as one or two cases. Whether PCH deaths would be less if there was less ability of visitors and PCH staff to "circulate freely in the community" would need to be explained and quantified.

The numbers of deaths cannot be changed. It is a 1:1 ratio with births. When death will occur, from what causes, and how many quality-adjusted life years can be saved are epidemiological questions. The Respondents' Affidavits have not provided data on the residents of personal care homes that have died and those that have not. What was the age and sex distribution of the PCH

deaths? What was their health status, quality of life, and actuarial life expectancy? How many had a health care directive for "do not resuscitate" and "comfort care" only? How many of them and their families would have accepted the risk of "catching pneumonia" to have visits and care from loved ones? These are epidemiology questions also.

These questions are objective and epidemiological. They are not intended to imply values or expectations or preferences. They are intended to provide a fuller understanding of the end-of-life circumstances, priorities, and risks for 10,000 Manitobans who live the last part of their lives in personal care homes.

With reference to paragraph 166 and 167 of Roussin's Affidavit, it is true that most people over the age of 60 do not live in long-term care facilities or other congregate settings. Most of them would not be considered vulnerable. They are, in general, healthier and less likely to be frail or have severe dementia. They are less likely to be at an advanced stage of cancer, chronic heart or lung disease – and are more likely to have an intact immune system. They are more likely to be able to sit up and walk without assistance, have a good cough reflex, and strong respiratory muscles. Perhaps most importantly, they are more likely to have a high tolerance for oxygen desaturation from pneumonia - one of the most life-threatening conditions faced by people at higher risk, whether it is initiated by a common cold or other respiratory tract. With more public education about who is at higher risk for severe illness, how they can be protected without socially isolating ("compartmentalizing") them, and when to seek timely medical care for them – as was the approach taken in Manitoba during the pandemic H1N1 influenza – what evidence has been presented that less restrictions on the majority of the population at lowest risk would result in higher rates of hospitalization and death?

For clarity, it is better to separate hospitalizations and deaths. This is because most people under 60 survive their hospitalization. 5% of all deaths in Manitoba have occurred in people under the age of 60. However, with respect to hospital capacity, it is a valid point. It would have been useful to include in the Respondents' Affidavits more data on the characterization of hospitalized patients, duration of stay, and outcomes. Similarly, it would have been useful for there to be more data on the occurrence of deaths with respect to location of deaths, i.e. hospitals, ICU, long term care facilities, palliative care units or other chronic disease settings, or home. This would help us to understand the pressure on hospital capacity associated with deaths and what stage of life those persons were at when they died. The points raised in paragraph 167 emphasize the importance of ascertaining the immediate and underlying causes of deaths for people that have died and had a positive PCR test. The changes in the medical certification of death protocols proposed by the World Health Organization and U.S. Centers for Disease Control appear to have been adopted by Manitoba Health[22]. Adopting this protocol, a reversal of decades of policy, will make it difficult to identify and monitor the importance of underlying causes of death and the sequence of clinic-pathological states towards the immediate cause. As is known to any physician, the immediate cause of death is often the "last straw". For example, a

[22] https://www.gov.mb.ca/health/publichealth/surveillance/covid-19/resources/Notes.html

person in an advanced stage of pancreatic cancer with a prognosis of "months" rather than "years" does not die of cancer per se. The immediate cause of death is something that interferes with adequate blood circulation, oxygenation, or biochemical imbalance. Either the heart stops first or the breathing stops first. Pneumonia is one of the most common immediate causes of death, but unless it is a primary pneumonia and is not part of the causal pathway to death from an underlying disease such as cancer, heart disease, dementia, neuromuscular disease, liver or kidney failure, then it would not be listed as the cause of death (underlying cause of death).

If we ever test for influenza the way we have been testing for SARS-CoV-2, the public health response could be even more challenging, especially because of influenza's higher rates of severe illness and death for healthy people under the age of 60, especially children.

At paragraph 168 of Roussin's Affidavit we find what could be considered an example of risk-based strategy with some features of focused protection. When a whole population – because of racialized or geographic status - is at increased risk in addition to individual age and other risk factors, it is appropriate, in collaboration with the community and with Indigenous health leaders, to implement targeted strategies for all Indigenous people and in specific Indigenous communities.

Contrary to paragraph 169 of Roussin's Affidavit, the primary principle of focused protection is to maximize protection of those at highest risk while enabling those at lowest risk to maintain and support social, educational, economic, and recreational living for all that are safe to participate. The anticipated benefit of natural immunity is secondary. Functionally, it is evident that the innate immunity against SARS-CoV-2 has been effective in limiting the impact of the infection for the vast majority of the population. For healthy people under the age of 60, the infection-fatality ratio has been estimated to be in the range of one per 10,000 infections. The issue of re-infection is a matter of probability, not possibility. One anecdotal report of a variant "re-infection" one year after the onset of a pandemic is not a very strong signal for a common event. Regardless of the merits of an anecdotal case report, experience with previous viral infections would suggest that re-infection, not surprisingly, happens. What is more challenging is to estimate its frequency and its epidemiological significance. The very same question and challenge applies to the duration of immunity from a vaccine.

With respect to paragraph 170 of Roussin's Affidavit and the Manaus, Brazil situation attested to in the affidavit of Jason Kindrachuk ("Kindrachuk's Affidavit"), drawing conclusions from afar about events in other places should be done with caution. Accurate and representative estimates of the prevalence of infected people are difficult to achieve under the best of conditions. Explaining patterns of rising and falling rates of infection without consideration of many biological and social factors should be done with caution.

Herd immunity is not an absolute categorical state; it is a continuum. This is especially true with in places where populations are not isolated or closed off from others. As the immune proportion of the population increases, the effective reproduction number – the average number of people infected by one case – decreases.

The issue is not whether there is "clear evidence" that "lasting herd immunity can be achieved by allowing less vulnerable people to circulate freely". What is clear evidence? A randomized controlled trial of comparative strategies? Valid evidence of that type has not been available to justify the lockdown or other restrictive strategies that have been in use. What is needed to consider alternative strategies is a fair and objective comparison of estimated benefits and harms based on existing science and current analysis of relevant data collected by comprehensive and detailed surveillance.

Regarding Roussin's Affidavit's reference to variants, there are a variety of factors which may be associated with the number and frequency of variants, one of which is our ability to test for them. A statement of fact that "permitting the virus to replicate more widely results in more variants" should not be made without clarification, explanation and evidence. Perhaps it was intended to be stated as a hypothesis.

While paragraph 171 of Roussin's Affidavit attempts to make a case that natural herd immunity would come at a much greater cost of deaths and severe outcomes, this requires at the least, a statement of assumptions and estimates. A decision analysis or other type of model could provide more clarity. No further analysis or information was provided, therefore this is not an evidence-based conclusion.

As set out at paragraph 173 of Roussin's Affidavit, on October 14, 2020, another declaration – known as the John Snow Memorandum – was released. It has been signed by 6,900 scientists, researchers, and health care professionals. It advocates for more general restrictions until a vaccine strategy has been implemented. It states: "The evidence is very clear: controlling community spread of COVID-19 is the best way to protect our societies and economies until safe and effective vaccines and therapeutics arrive within the coming months. We cannot afford distractions that undermine an effective response; it is essential that we act urgently based on the evidence."

This report has opined that there is no clear evidence for more general restrictions in Manitoba. Reasonable arguments for more effective, less harmful, and less restrictive alternatives are not "distractions". On the contrary, the arguments presented to the First Ministers for a more risk-based, less restrictive, and balanced approach and the arguments presented in the Great Barrington Declaration for strategies for focused protection are those that must be refuted by the Manitoba government in order to justify its actions and to be compliant with the standards inherent in the Public Health Act and the Charter of Rights and Freedoms.

To varying degrees, within Canada, and in other countries, less and more restrictive measures have been used without clear evidence of significant long-term differences in morbidity and mortality associated with COVID-19. NEED EXAMPLES HERE. Less restrictive and less harmful strategies should be considered and compared in a transparent way.

4. Have the Respondents demonstrated how they met the public health standard and ensured that the restrictions of their policies have been no greater than reasonably necessary by anticipating, considering, estimating, and surveilling the observed health and social harms of the restrictions, including their impact on all determinants of health?

Public health work is not about only one disease or one goal, even in a crisis or emergency situation. In clinical medicine, it is important that the treatment should not be worse than the disease. In public health, it is important that a prevention strategy is not worse than the disease.

It is reasonable to have goals to prevent severe illness and death from COVID-19. It is reasonable to have goals to maintain hospital and other health care capacity for all who need it. But from a public health perspective, there should be other goals. There should be goals to minimize the harmful impacts of public health interventions, to minimize societal disruption, and to minimize negative impacts on the determinants of health. Short term and long term impacts on health should be considered.

Paragraph 87 of Roussin's affidavit states:

> Public health officials also consider potential collateral effects of restrictions such as unintended adverse economic or mental health impacts. By their nature, pandemics are very hard on a population. In addition to the direct health impacts of the disease, pandemics may cause fear and anxiety among the public. Public Health Orders that restrict gatherings or temporarily close places can also adversely affect peoples' economic status and their mental health. This is why we seek to impose the least restrictive measures necessary. It is a difficult balance, which must be re-evaluated in a dynamic way as the pandemic progresses. Governments also attempt to alleviate these hardships by providing mental health supports and economic relief.

This paragraph acknowledges some of the significant adverse impacts restrictive public health interventions and the importance of a balanced approach. It is not evident how this balance is being achieved? What specifically is being monitored and how has it been re-evaluated, other than a rise or fall in case counts, deaths, and hospitalization rates from one disease only? What are the goals and measurable objectives? What are the important indicators? What are the ethical values? What are the priorities? How are inequities measured and prevented? What are the decision-making processes? What are the mechanisms for consultation and engagement? The Respondents have not provided a transparent strategy and response plan. Without it, this information is scattered and incomplete. Without this information, the Respondents have not demonstrated how they have met the relevant public health standards and ensured that the restrictions of their policies have been no greater than reasonably necessary.

It has been long recognized that, in addition to health care, there are many other determinants of health – of individuals and communities. From Health Canada's official set of twelve, seven determinants of particular relevance are listed here. How have they been affected by COVID-19 and by the government and public health response?

Paragraph 12 of Loeppky's Affidavit states that the Epidemiology and Surveillance Unit is monitoring the impacts of the COVID-19 pandemic on various Manitoba health indicators. Exhibit D to Loeppky's Affidavit is the report "Impacts of COVID-19 PUBLIC HEALTH MEASURES ON VARIOUS HEALTH INDICATORS IN MANITOBA, November 1, 2020."

The Report contains 52 figures of various data. Many of these are important indicators to monitor and much of the data can be useful. The data is described, but there are few commentaries about the validity, limitations, or implications of the findings. It is not stated why these particular descriptors were chosen and what phenomena they are meant to indicate.

The conclusion of the Report contains some descriptions that may be misleading. For example, it is stated that "during COVID-19 period, the monthly number of MMRV childhood immunization doses administered in Manitoba increased from April 2020 to August 2020 by 103%". However, the rate in April 2020 had already dropped significantly and the rate in August 2020 was less than that in August 2019. Although difficult to quantify from the graph, it is evident that less children have been vaccinated from March to August in 2020 than were vaccinated during the same period in 2019. There are significant concerns about the impact of the pandemic response on preventive care for children and others. It is important to monitor, quantify and explain these and other indicators in a more robust and clear manner.

The patterns of increased utilization of health services for mental health and behavioural disorders referenced in Loeppky's affidavit including substance use, overdoses, and injuries, are of particular concern because of the increasing awareness of the impacts of restrictions of the determinants of health, especially social supports and coping skills, healthy behaviours, childhood experiences, education and literacy, employment and working conditions, and income and social status.

5.Have the Respondents reasonably explained their estimation of the absolute (actual) and relative risk of transmission of COVID-19 causally associated with attending church services?

Paragraph 26 of Roussin's affidavit states the following with respect to transmission of SARS-CoV-2:

"COVID-19 has been proven to be highly communicable and contagious among people."
"asymptomatic and especially pre-symptomatic transmission of SARS-CoV-2 does occur."
"Children can transmit the virus."

"There is evidence that certain activities like singing and talking loudly can pose a greater risk of transmission."

Paragraph 27 of Roussin's affidavit states "It has become understood that certain settings, including indoor crowded spaces with poor ventilation, have led to a higher risk of transmission.

Paragraph 31 of Roussin's affidavit states that "for a certain segment of the population who become infected, COVID-19 engages very serious symptoms that can only be treated through hospitalization", "some individuals require admission to an Intensive Care Unit and ventilation", and "COVID-19 can be fatal for the most severely affected segment of the population."

Whereas the general truth of each of these statements are uncontestable, none of them meet the expectations for sound decision-making as described in paragraph 54 of Roussin's affidavit. This is because there are no quantitative estimates. What is the quantitative meaning of "highly communicable"?

Paragraphs 155-160 of Roussin's affidavit describes reasons that "places of worship and faith-based gathering have their own potential for virus transmission and outbreaks" and the decision in November 2020 that places of worship had to temporarily closed. No data is provided to estimate the asserted increased risk other than reference to a total of 19 clusters or outbreaks in Canada and the United States – 10 of which are discussed below. Although there are limitations of cluster analyses with respect to ascertainment of the numbers of infections and ascertainment of sources of exposures, there is no doubt that there have been and will be transmission of the SARS-CoV-2 and many other coronavirus respiratory viruses, influenza, adenovirus, and rhinoviruses. The issue of importance from a public health perspective is quantitative risk – frequency and severity. Without objective and reasonably accurate estimates of the risks of attending church, how can the Respondents claim that they've met the relevant public health standards that their restrictive orders are proportionate to the risk and reasonably necessary?

Paragraph 56 of Roussin's Affidavit states the Canadian Public Health Association's five main building blocks of public health practice: evidence, risk assessment, policy, intervention, and evaluation. The document referred to – exhibit 10 – Public Health: A Conceptual Framework states "Prior to taking action on a specific issue, ***a risk assessment is necessary to estimate the nature and likelihood*** of negative health outcomes in individuals." With regard to effectiveness, it is stated on page 12 that "Outcome evaluations measure progress in the program's targeted public health challenge, and may include short-, intermediate-, and long-term results, that are also based on quantitative and qualitative data."

- Ratios of probabilities of events associated with the disease or outcomes of interventions. These are necessary for the purposes of valid comparisons. For example, listing the number of cases associated with each of "ten clusters associated with

attendance at faith-based events"[23] does not provide sufficient data for a risk assessment. At the very least there should be an estimation of a denominator such as the number of Manitobans that attend a place of worship during the relevant time period. More relevant, an assessment of the effectiveness of closing places of worship should be based on the estimated ratio of the probability of getting infected per week when that week includes one or two hours of church attendance in comparison with the probability of getting infected per week when that week does not include any church attendance.

- Proportions are a way to compare events or characteristics and to put them into better perspective. For example, rather than merely counting the number of cases associated with attendance at faith-based events[24], additional information such as the comparison with the total number of cases would help to put the numbers in perspective. It is not clear over what time period, these data have been collected, but 172 "primary" cases and 30 "secondary" cases were identified in 10 "clusters" between August 2020 and February 2021. Even assuming that the church exposure was actually the one responsible for their infections and that this included all worship, and using all cases in Manitoba until January 14, 2021[25] as the denominator, the data provided represents an estimate of 202/26954 = 0.7% or one per 133 of all Manitoba cases over a one year period. These data provided by the Respondents show that the risk from attendance at settings of worship are significantly less than other settings which have remained – to some degree and intermittently – open.

Estimation of actual risk of transmission in places of worship and outdoor gatherings

Estimates of the actual and relative risk of transmission of COVID-19 in places of worship or public gatherings can be modeled theoretically or analysed from observational data.

Theoretical modelling would include a number of parameters such as prevalence of asymptomatic or pre-symptomatic infected people, compliance with non-attendance by people with COVID-like symptoms, type of activities with respect to close contact exposures, separation of households, distancing, masks, duration of exposure, etc.

Such modelling or estimation of the actual risks has not been included in the affidavits of the Respondents either for attendance at places of worship or outdoor gatherings.

One would expect that in addition to the reports of clusters, data from contact tracing would provide more information about the observed rate of transmission associated with attendance at a place of worship or an outdoor gathering. No summaries or analyses of the results from the

[23] Loeppky's affidavit, Paragraph 14
[24] Loeppky's affidavit,
[25] Loeppky's affidavit, exhibit B page 1.

contact tracing of more than 30,000 reported cases have been included in the affidavits or posted on Manitoba's COVID-19 websites. Without that information, the opportunity for population-based risk-assessment has been missed, relying instead on more anecdotal and speculative information.

Estimation of actual risk of transmission in places of worship

According to a report by the Pew Research Centre in 2013 and Statistics Canada in 2003, 1/3 of people in Manitoba over the age 15 attend a religious service at least once per month.[26,27] The number of Manitobans over the age of 15 is 1.1 million[28]. Based on these numbers a reasonable estimate of Manitobans in a religious service at least once per month is 370,000. Using the Respondents' health reports, there have been 10 clusters with 202 cases. Using as the denominator 370,000 multiplied by 10 months of COVID-19 reporting, there have been 3,700,000 person-exposure. Using an average of one hour per service, this equates to 3,700,000 person-hours of exposure. The rate of cases per hour of exposure can be estimated, therefore, at 202 divided by 3,700,000 = one case per 18,000 hours of exposure. This level of risk would not normally be considered as a reasonable basis to justify prohibition of attending religious services.

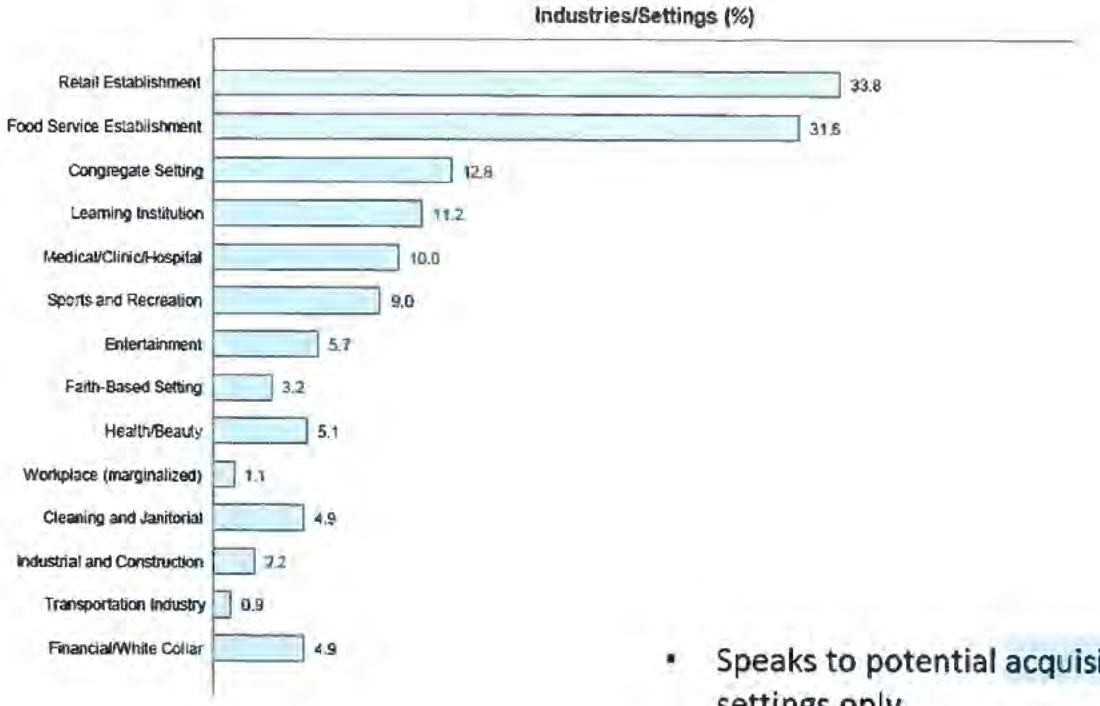
Regarding the number of cases, this could be an underestimate because of incomplete testing or because of incomplete surveillance ascertainment. If it is believed that this number of cases is an underestimate of the actual case rate or infection rate, an alternative estimate should have been provided. Contact tracing of 30,000 should have generated more accurate and complete statistics.

Without a transparent protocol for determining the most probable source and setting of transmission, one cannot assess the likelihood that these cases actually acquired their virus at a religious service. Even at one hour per week, this exposure period compares to 16 hours per day multiplied by 7 days per week of awake time = 112 hours. In other words, it is reasonable to estimate that for people that attend services four times the average Manitoban, less than one percent of their awake time is spent in a place of worship. Without a clear and reasonable protocol to determine the likeliest source of exposure, the probability that transmission happen elsewhere – such as a retail outlet, educational setting, or restaurant, is, by exposure proportion, more likely.

Of 633 cases reported in September, 2020, 3.2% were "potentially" acquired at a faith-based setting, ranking 11th out of 14 industries and settings exposures. The 10 highest-ranking settings accounted for 92% of cases, two-thirds of which were at retail and food service establishments. (Page17, Exhibit E, Loeppky's affidavit)

---

[26] https://www.pewforum.org/2013/06/27/canadas-changing-religious-landscape/
[27] https://www.statcan.gc.ca/sites/default/files/6493-eng.pdf
[28] https://www.manitoba.ca/health/population/pr2019.pdf



It is correct and important for this graph to be qualified by the caveat of "potential acquisition". In contact tracing, one cannot assume which setting or exposure was the source of the infection of the "secondary case". That is because of the many number of possible exposures during the time period of relevance. Also people may not remember all of the facts or may not want to disclose them. Many assumptions and estimates are made before drawing the conclusion about the setting of exposure.

Summary and Conclusions.

1. Has the PCR test and have the PCR test results – and other methods - been used in a reasonable and reliable way to estimate accurately frequency of infections, fatality-rate of infections, hospitalizations, deaths, and years of life lost attributable to COVID-19 and to justify the reasonable necessity of restrictions of rights and freedoms, including quarantine and isolation?

For the following reasons, the answer to this question is "no".

- A positive PCR test result is the only criterion necessary to define a "confirmed case" despite instructions in product inserts that the tests should be interpreted in the context of the individual's clinical information and despite Dr. Bullard's own admission that "no single PCR Ct value in isolation can be used to determine infectiousness of a case and

must be interpreted in the overall clinical context" – a process for which no description has been given and from which no data has been provided.

- A positive PCR test result is the only criterion necessary to classify a hospitalized patient as a COVID patient (with the exception of injury).
- A positive PCR test result is the only criterion necessary to classify an intensive care patient as a COVID patient (with the exception of injury).
- A positive PCR test result is the only criterion necessary to classify a death as a COVID death (with the exception of injury).
- No estimates of potential years of life lost from COVID have been described.
- No clinic-pathological analyses of patients or deaths associated with a positive PCR have been described.
- A positive PCR test – in the absence of clinical and epidemiological information - does not indicate if or when a person was infected or is infectious.
- A positive PCR test result of any Ct level is used and interpreted and used in the same way, regardless of the estimated probability that the host of the sample was infectious at the time of the swab.
- The lab does not provide public health with the Ct values of positive cases.
- Based on data provided for December 2020, 40% of positive PCR tests have been associated with virus fragments that are not infectious (or have a far lower probability of being infectious) Isolating cases more than eight days after the onset of symptoms of with a Ct>25 is unnecessary, unfair, and is harmful. Quarantining contacts that were exposed to a case eight or more days after the onset of symptoms or after a positive test with Ct > 25 is unnecessary, unfair and harmful. Aside from inconvenience, mental stress and social isolation, isolation and quarantine interferes with care-giving, income, education, other necessary and healthy activities, and employment including health care services.

2. Have the public health orders which have restricted rights and freedoms of people at very low risk for severe illness or death been shown to be reasonably necessary, fair, and sufficiently effective with reference to the public health standards to protect those at higher risk and to maintain hospital capacity for all Manitobans?

   For the following reasons, my answer to this question is "no".

   - After one year, 30,000 cases, 1000 deaths, the Respondents have not used or had access to data to answer the following questions with sufficient detail and quantity:
     - What are the most important factors for highest risk for severe illness, hospitalization, and death;
     - In what settings and by what types of exposures has transmission occurred to those at highest risk;
     - To what degree has the presence of the virus SARS-CoV-2 contributed to the morbidity and mortality of Manitobans, quality-adjusted years of life lost,

hospitalizations, and deaths where sole criterion of a positive PCR test has been used to make the diagnosis of COVID-19 and surveillance have attributed hospitalizations and deaths to COVID-19 on the basis of a PCR test only.

3. Has it been shown reasonably by the Respondents by use of valid models and other methods that alternative less restrictive strategies could not have achieved better health outcomes with less harm?

   For the following reasons, my answer to this question is "no".

   - Without clear goals and measurable objectives of a response plan, there is no objective basis for determining which health outcomes and to what degree they could have been met with less restrictive measures.
   - None of the Respondents' models nor the international review included measures or estimates of harm caused by the interventions.
   - None of the Respondents' models defined clearly their outputs, inputs, the estimates and ranges used for their input parameters, the design and formulae of the model, or showed through sensitivity analysis which of the parameters were most important in correlating with the outcomes.
   - It is stated, reasonably, that "as with any models trying to predict the future, its results must be interpreted with caution". How the models have been used to shape policy has not been included in the Reports. Which cautions have been the most important have not been described.

4. Have the Respondents demonstrated how they have ensured that the restrictions of their policies have been no greater than reasonably necessary in accordance with the public health standards by anticipating, considering, estimating, and surveilling the observed health and social harms of the restrictions, including their impact on all determinants of health?

   For the following reasons, my answer to this question is "no".

   - There has not been a systematic monitoring or communicating of harmful consequences from the pandemic response, especially on individuals and communities most disadvantaged
   - There has not been a transparent and clear comparison of anticipated benefits and harms from strategies with different levels of restrictions

5. Have the Respondents reasonably explained their estimation of the absolute (actual) and relative risk of transmission of COVID-19 causally associated with attending church services?

   For the following reasons, the answer to this question is "no".

- No data from contact tracing of 30,000 Manitoba cases was provided in any of the affidavits of the Respondents. The frequencies of cases, exposures, transmission, and outcomes – and other relevant information – could have provided a rational basis for decisions to restrict or prohibit gatherings.
- Raw data of 10 faith-based clusters of 202 cases was described but no analysis or risk assessment was provided in any of the Respondents' affidavits.
- Anecdotal reports of "clusters" from other provinces and the USA are referred to but no representative analysis or overall quantitative risk is provided.
- Calculations using data from the affidavits of the Respondents, show that less than one percent of all reported COVID cases have been associated with attendance at a faith-based gathering.
- In one assessment of cases "potentially" acquired at settings during September, 2020, 3% of cases were acquired at faith-based settings
- Using data provided in the affidavits of the Respondents, a reasonable overall estimate of the risk of transmission during attendance at a church service in Manitoba was calculated to be one case per 18,000 hours of attendance.

Conclusion

To meet the requirements and standards of good public health practice, the Respondents are required to show that the severity of the threat has justified the restrictive interventions, that the effectiveness and benefits of the interventions have sufficiently outweighed the harms, and that there were no alternative strategies that would have been less restrictive, equally or more effective, and less harmful.

This report has provided evidence and arguments that the affidavits of the Respondents show that the severity of the threat has not been scientifically estimated, that the effectiveness of the restrictions have not been scientifically evaluated, that the harms caused by the restrictions have not been systematically assessed, and that alternative less restrictive interventions have not been described or compared with the restrictive interventions.

Accurate estimates have not been described of the illness severity, premature deaths, years of life lost, and pressure on the health system that can be attributed to COVID-19. Its impact appears to be overestimated.

Similarly, accurate estimates have not been described of the harms associated with the restrictive policies. Their harmful impacts appear to have been underestimated.

Finally, accurate estimates of the effectiveness of the restrictive policies have not been described sufficiently to justify their continuation without more rigorous evaluation. Their effectiveness appears to have been overestimated.

File No. CI 20-01-29284

THE QUEEN'S BENCH
Winnipeg Centre

APPLICATION UNDER: *The Constitutional Questions Act*, C.C.S.M., c. 180

AND UNDER: The Court of Queen's Bench Rules, M.R. 553/88

IN THE MATTER OF: *The Public Health Act*, C.C.S.M. c. P210

BETWEEN:

GATEWAY BIBLE BAPTIST CHURCH, PEMBINA VALLEY BAPTIST CHURCH, REDEEMING GRACE BIBLE CHURCH, THOMAS REMPEL, GRACE COVENANT CHURCH, SLAVIC BAPTIST CHURCH, CHRISTIAN CHURCH OF MORDEN, BIBLE BAPTIST CHURCH, TOBIAS TISSEN, ROSS MACKAY

Applicants,

– and –

HER MAJESTY THE QUEEN IN RIGHT OF THE PROVINCE OF MANITOBA, DR. BRENT ROUSSIN in his capacity as CHIEF PUBLIC HEALTH OFFICER OF MANITOBA, and DR. JAZZ ATWAL in his capacity as ACTING DEPUTY CHIEF OFFICER OF HEALTH OF MANITOBA

Respondents.

AFFIDAVIT OF ROSS MACKAY
AFFIRMED APRIL 1, 2021

JUSTICE CENTRE FOR CONSTITUTIONAL FREEDOMS
D. Jared Brown / Allison Kindle Pejovic / Jay Cameron
[redacted]

THE QUEEN'S BENCH
Winnipeg Centre

APPLICATION UNDER: *The Constitutional Questions Act*, C.C.S.M., c. 180

AND UNDER: The Court of Queen's Bench Rules, M.R. 553/88

IN THE MATTER OF: *The Public Health Act*, C.C.S.M. c. P210

BETWEEN:

GATEWAY BIBLE BAPTIST CHURCH, PEMBINA VALLEY BAPTIST CHURCH, REDEEMING GRACE BIBLE CHURCH, THOMAS REMPEL, GRACE COVENANT CHURCH, SLAVIC BAPTIST CHURCH, CHRISTIAN CHURCH OF MORDEN, BIBLE BAPTIST CHURCH, TOBIAS TISSEN, ROSS MACKAY

Applicants,

– and –

HER MAJESTY THE QUEEN IN RIGHT OF THE PROVINCE OF MANITOBA, DR. BRENT ROUSSIN in his capacity as CHIEF PUBLIC HEALTH OFFICER OF MANITOBA, and DR. JAZZ ATWAL in his capacity as ACTING DEPUTY CHIEF OFFICER OF HEALTH OF MANITOBA

Respondents.

AFFIDAVIT OF ROSS MACKAY

I, Ross MacKay of the City of Winnipeg, in the Province of Manitoba,

MAKE OATH AND SAY AS FOLLOWS:

1. I have personal knowledge of the facts and matters hereinafter deposed to by me, except where same are stated to be based upon information and belief, and those I do verily believe to be true.

2. I write this affidavit in response to the affidavits filed by the Respondents.

3. I was surprised when I read about the provincial mental health programs cited in Mr. Komlodi's affidavit, such as online counselling, a help line and online recreational activities. I had never heard of any of them. The Respondents never reached out to me to offer counselling or any other support for my inability to have my daughter fly home to see me for Christmas, to have unrestricted visits with friends and family, or to peacefully protest these restrictions with more than a couple of other people who have similar concerns about the Public Health Orders.

4. I believe that in order for me to get through these stressful times, family support is critical. I will respect people who want to live in their basements, but I would rather have liberty and freedom. My own review of the data on Covid-19 and its primary threat to the elderly and immunocompromised is a factor that I, as a rational and thinking person, has a right to weigh when I determine how I should behave. I do not believe that two hours of free online counselling will help me to overcome the stress and anxiety that these restrictions are causing me. I believe that being able to enjoy my life again, without restrictions on gatherings and expression, will be far more effective in ending my despair than two hours of online counselling, calling a help line, or doing online recreation.

5. All of this impersonal contact with people over devices only adds to my frustration and distress. I went to the hospital over the Christmas holidays because I thought I was having a heart attack. I feel immense stress and anxiety over these Public Health Orders, and there is nothing I want more than for things to go back to normal.

6. I make this affidavit *bona fide*.

SWORN before me in the City of Winnipeg, in the Province of Manitoba, this 1st day of April, 2021

Cheryl Schoch

A Commissioner of Oaths in and for the Province of Manitoba

My Commission expires: July 28/21

Ross MacKay

ROSS MACKAY

File No. CI 20-01-29284

THE QUEEN'S BENCH
Winnipeg Centre

APPLICATION UNDER: *The Constitutional Questions Act*, C.C.S.M., c. 180

AND UNDER: The Court of Queen's Bench Rules, M.R. 553/88

IN THE MATTER OF: *The Public Health Act*, C.C.S.M. c. P210

BETWEEN:

GATEWAY BIBLE BAPTIST CHURCH, PEMBINA VALLEY BAPTIST CHURCH, REDEEMING GRACE BIBLE CHURCH, THOMAS REMPEL, GRACE COVENANT CHURCH, SLAVIC BAPTIST CHURCH, CHRISTIAN CHURCH OF MORDEN, BIBLE BAPTIST CHURCH, TOBIAS TISSEN, ROSS MACKAY

Applicants,

– and –

HER MAJESTY THE QUEEN IN RIGHT OF THE PROVINCE OF MANITOBA, DR. BRENT ROUSSIN in his capacity as CHIEF PUBLIC HEALTH OFFICER OF MANITOBA, and DR. JAZZ ATWAL in his capacity as ACTING DEPUTY CHIEF OFFICER OF HEALTH MANITOBA

Respondents.

AFFIDAVIT OF DAVID HERSEY
SWORN APRIL 20, 2021

JUSTICE CENTRE FOR CONSTITUTIONAL FREEDOMS
D. Jared Brown / Allison Kindle Pejovic / Jay Cameron
[redacted]

THE QUEEN'S BENCH
Winnipeg Centre

APPLICATION UNDER: *The Constitutional Questions Act*, C.C.S.M., c. 180

AND UNDER: The Court of Queen's Bench Rules, M.R. 553/88

IN THE MATTER OF: *The Public Health Act*, C.C.S.M. c. P210

BETWEEN:

GATEWAY BIBLE BAPTIST CHURCH, PEMBINA VALLEY BAPTIST CHURCH, REDEEMING GRACE BIBLE CHURCH, THOMAS REMPEL, GRACE COVENANT CHURCH, SLAVIC BAPTIST CHURCH, CHRISTIAN CHURCH OF MORDEN, BIBLE BAPTIST CHURCH, TOBIAS TISSEN, ROSS MACKAY

Applicants,

– and –

HER MAJESTY THE QUEEN IN RIGHT OF THE PROVINCE OF MANITOBA, DR. BRENT ROUSSIN in his capacity as CHIEF PUBLIC HEALTH OFFICER OF MANITOBA, and DR. JAZZ ATWAL in his capacity as ACTING DEPUTY CHIEF OFFICER OF HEALTH MANITOBA

Respondents.

AFFIDAVIT OF DAVID HERSEY

I, DAVID HERSEY, of the City of Calgary, in the Province of Alberta,

MAKE OATH AND SAY AS FOLLOWS:

1. I have personal knowledge of the facts and matters hereinafter deposed to by me, except where same are stated to be based upon information and belief, and those I do verily believe to be true.

2. I am a Senior Paralegal at the Justice Centre for Constitutional Freedoms in Calgary, Alberta. I have worked at the Justice Centre since September 2018.

3. I have reviewed the correspondence between the Applicants' and the Respondents' in anticipation of the hearing of this Application.

4. On March 19, 2021, the Applicants sent a letter to the Respondents. A copy of that letter is attached hereto and marked as **Exhibit "A"** to this my affidavit.

5. On March 30, 2021, the Respondents sent a letter to the Applicants by email which contained attachments. Copies of that email, letter, and the attachments to that email are attached hereto and marked as **Exhibit "B"** to this my affidavit.

6. On April 1, 2021, the Applicants sent a letter by email to the Respondents. Copies of that email and letter are attached hereto and marked as **Exhibit "C"** to this my affidavit.

7. On April 6, 2021, the Respondents sent a letter by email to the Applicants. A spreadsheet was attached to that email. Copies of that email, letter, and the spreadsheet are attached hereto and marked as **Exhibit "D"** to this my affidavit.

8. On April 8, 2021, the Respondents emailed the Applicants. A spreadsheet was attached to that email. Copies of that email and the spreadsheet are attached hereto and marked as **Exhibit "E"** to this my affidavit.

9. On April 13, 2021, the Applicants sent a letter to the Respondents. A copy of that letter is attached hereto and marked as **Exhibit "F"** to this my affidavit.

10. On April 14, 2021, the Respondents sent an email to the Applicants which attached one document. Copies of that email and the attached document are attached hereto and marked as **Exhibit "G"** to this my affidavit.

11. I make this affidavit *bona fide*.

SWORN before me in the City of Calgary, in the Province of Alberta, on April 20, 2021)

A Barrister entitled-to-practice in the Province of Alberta)

John V. Carpay

DAVID HERSEY

EXHIBIT "A"

AB658

THIS IS EXH ..... "
referred to in the Affidavit of
David Hersey
Sworn before me this 20
day of April A.D. 20 21
A Commissioner in and for the Province of Alberta
John Carpay
Barrister and Solicitor

Justice Centre
for Constitutional Freedoms

March 19, 2021

Via-email

Department of Justice
Constitutional Law Branch
1205-405 Broadway
Winnipeg, MB R3C 3L6

Attention: Heather Leonoff/Michael Conner/Denis Guenette/Sean Boyd

Dear Madam/Sir:

RE: Gateway Bible Baptist Church et al. v. Manitoba and Dr. Roussin – File No. CI 20-01-29284

Upon review of your filed affidavits in this matter, the Applicants request the following information in advance of the hearing which is relevant to both the Respondents' affidavit evidence and the issues in the proceeding:

Specifically, we request that you provide us with the following (subject to further agreement on medium of production and delivery timeline):

1. Affidavit of Carla Loeppky, Exhibit B, page 17
   For all Manitoba Covid deaths listed:
   a. anonymized lab reports with CT threshold used in achieving the Covid-positive test result,
   b. Document(s) identifying whether the deceased was a symptomatic or asymptomatic case,
   c. anonymized death certificates with primary and secondary causes of death

2. Affidavit of Jared Bullard
   Package inserts/manufacturer's instructions from all Covid-19 diagnostic test kits (PCR or otherwise) that Manitoba uses to diagnose Covid-19

[redacted]
CRA reg[redacted]

3. Affidavit of Jared Bullard, lines 193-199

    a. Document(s) with CT thresholds by percentages of all positive cases between March 2020-March 2021, and specifically, what percentage of cases per month resulted from a positive PCR test with a CT of 36, 37, 38, 39, 40, 40+ (not simply the percentage as a range from 36-40)
    b. for every positive case, the anonymized lab report confirming the CT value used

4. Affidavit of Brent Roussin, para. 70
   Documentation on the contact tracing program:
    a. the proportion of traced contacts that became symptomatic during their quarantine period,
    b. the proportion of traced contacts that tested positive for Covid-19 during their quarantine period,
    c. the proportion of symptomatic contacts that were hospitalized, needed ICU, or died,
    d. estimated number and rate of prevented hospitalizations, ICU admissions, or deaths attributable to contact tracing, quarantine and isolation

5. Affidavit of Carla Loeppky, Exhibit B, pp. 16-33
   Document(s) or policies used to determine whether a death is "related to Covid-19" or is a death "due to Covid-19", and any document(s) outlining any changes in the usual method of death certification (prior to 2020) with respect to Covid-19's designation in Part 1 or Part 2 of the death certificate.

6. Affidavit of Carla Loeppky, Exhibit D, Affidavit of Lanette Siragusa, para. 10
   Document(s) providing the number of total deaths in Manitoba in 2020 due to the following conditions as the primary cause of death:
    a. Malignant neoplasms
    b. Diseases of heart
    c. Cerebrovascular disease
    d. Chronic lower respiratory disease (excluding Covid-19)
    e. Accidents (unintentional injuries)

[redacted]

7. Affidavit of Carla Loeppky, Exhibit F, p. 25
   Document(s) which sets out the 165 parameters in respect of the modelling

8. Affidavit of Carla Loeppky, para. 14
   Document(s) or policies defining a "cluster"

9. Affidavit of Brent Roussin, para. 177
   Document(s) that show:
   a. the total number of ICU beds available in Manitoba for the years 2015-2020
   b. the surge capacity (how many extra beds could be made available for ICU patients) in Manitoba for the years 2015-2020
   c. by month, the highest number of ICU patients in Manitoba for the years 2015-2020 and up to March 2021
   d. how many days per month in the years 2015-2020 and up until March 2021 did ICU patients exceed the number of available ICU beds before and after (if applicable) surge capacity was reached?

10. Documents showing that out of all of the PCR positive cases of Covid-19 in Manitoba, how many of those people were also tested for Influenza within the same time frame as they were tested for Covid-19

11. Please provide Manitoba's Pandemic Response Plan (or similar such emergency plan) for the past 5 years.

We look forward to receiving the foregoing. Please feel free to contact the undersigned should you wish to discuss the method and timeline for delivery. We are content to rely on electronic delivery (i.e. by USB) should that prove more convenient.

Best regards,

Allison Kindle Pejovic
Barrister and Solicitor
Justice Centre for Constitutional Freedoms

cc: Jay Cameron, Litigation Manager, Justice Centre for Constitutional Freedoms,
[redacted]

Phone: [redacted]

Jared Brown, Lead Counsel, Brown Litigation, [redacted]

Heather Leonoff, Legal Services Branch, Constitutional Law Section, Manitoba Justice, [redacted]

Denis Guenette, Legal Services Branch, Civil Legal Services, Manitoba Justice, [redacted]

Michael Conner, Legal Services Branch, Constitutional Law Section, Manitoba Justice, [redacted]

Sean Boyd, Legal Services Branch, Civil Legal Services, Manitoba Justice, [redacted]

[redacted]

EXHIBIT "**B**"

AB663

THIS IS EXHIBIT " B "
referred to in the Affidavit of
David Hersey
Sworn before me this 20
day of April A.D. 20 21
A Commissioner in and for the Province of Alberta
John Carpay
Barrister and Solicitor

From: "Conner, Michael (JUS)" <[redacted]>
Date: Tuesday, March 30, 2021 at 8:59 AM
To: Allison Pejovic[redacted]a>
Cc: Jared Brown <[redacted], Jay Cameron [redacted], "Leonoff, Heather (JUS)" [redacted]
Subject: RE: Gateway [redacted]s

Good morning,

Please see the enclosed letter with attachments in reference to your request for undertakings from our affiants.

Regards,

Michael Conner

Michael Conner
General Counsel
Constitutional Law Section
Legal Services Branch, Manitoba Justice
[redacted]

From: Allison Pejovic <apejovic@jccf.ca>
Sent: March 19, 2021 12:49 PM
To: Leonoff, Heather (JUS) <[redacted]>; Boyd, Sean (JUS) [redacted]>; Conner, Michael (JUS) <[redacted]; Guenette, Denis (JUS) [redacted]
Cc: Jared Brown [redacted]>; Jay Cameron [redacted]
Subject: [Caution: Suspicious Email] Gateway Bible Baptist Church v. MB - Letter Requesting Additional Materials

Hello everyone,

Please find enclosed correspondence from the Applicants in respect of the upcoming hearing.

Please be advised that a third counsel has joined the Applicants' team. Jared Brown is Lead Counsel at Brown Litigation in Toronto and he is assisting us on this file. Please copy him on all future correspondence.

Best regards,

Allison Kindle Pejovic, B.A., LL.B., LL.M.
Barrister and Solicitor
Justice Centre for Constitutional Freedoms
[redacted]

www.jccf.ca

"Defending the constitutional freedoms of Canadians"

Manitoba

Justice
Constitutional Law Section, Legal Services Branch
Crown Law Division
Room 1230 Woodsworth Building
405 Broadway
Winnipeg MB R3C 3L6

In reply, please refer to:

Michael Conner
General Counsel

[redacted]

March 30, 2021

Justice Centre for Constitutional Freedoms
#253, 7260 Elbow Drive SW
Calgary, AB T2V 1K2

Attention: Allison Kindle Pejovic

Dear Ms Pejovic:

Re: ***Gateway Bible Baptist Church et al. v. Manitoba et al.*** **– File No. CI 20-01-29284**

This is in response to your letter of March 19, 2021, in which you seek undertakings from the Respondents' affiants for the purpose of cross examination.

As you are aware, there is no entitlement to examinations for discovery in an Application. While undertakings can be requested under Rule 39.03.1, the rules contemplate this would occur during the course of cross examination and not as a means of advance discovery. An undertaking can be refused if it does not relate to an important issue, it would be overly onerous or the information would not significantly assist the court in determining the application.

In our view, many of the items requested are of marginal relevance or significance to the ultimate issue to be decided. Nonetheless, in the interest of being cooperative, we will provide the documents requested if they are readily available. Below, we respond to each item.

Item 1: Affidavit of Carla Loeppky

We decline to give this undertaking.

a) The affiant does not have possession of CT values. Further, the lab has no knowledge of whether a particular individual subsequently died of COVID-19 and therefore does not have a record of the CT values used for persons who later died of COVID-19. It would be unduly onerous to try to reconstruct this information.

b) The affiant does not have access to updated medical files of patients indicating whether the deceased was a symptomatic or asymptomatic case of COVID-19 at the time of death.

c) The affiant does not have possession or control over death certificates. Death certificates are prepared by attending physicians and provided to Vital Statistics in accordance with *The Vital Statistics Act*.

Item 2: Affidavit of Jared Bullard

We have attached manufacturers' inserts for PCR tests used by Cadham Provincial Laboratory.

Item 3: Affidavit of Jared Bullard

We decline to provide this undertaking as requested. The lab reports state whether the case is positive for COVID-19 but do not include CT values. The lab would have to undertake further analysis to provide the information requested.

Item 4: Affidavit of Brent Roussin

We decline this undertaking. Available information about the total number of COVID-19 hospitalizations, ICU admissions and deaths has been provided in the affidavit of Carla Loeppky. Specific information about hospitalization, ICU and deaths of individuals who isolated after contact tracing is not readily available.

Item 5: Affidavit of Carla Loeppky

The information requested is not relevant. The Chief Public Health Officers relies on COVID-19 deaths as reported to Epidemiology and Surveillance by hospitals or health officials in the community. Nonetheless, we can provide the following information.

Public Health has published a document for epidemiology and surveillance purposes entitled "COVID-19 Technical Notes", which is part of its Provincial Respiratory Surveillance Report. For reporting COVID-19 deaths, the document follows the "Word Health Organization Guidelines for Certification and Classification (Coding) of COVID-19 as a Cause of Death". These guidelines are consistent with the Public Health Agency of Canada guidelines entitled "National Case Definition".

The Technical Notes can be found here:
https://www.gov.mb.ca/health/publichealth/surveillance/covid-19/resources/Notes.html

The WHO Guidelines can be found here:
https://www.who.int/classifications/icd/Guidelines_Cause_of_Death_COVID-19.pdf

The Public Health Agency of Canada guidelines can be found here:
https://www.canada.ca/en/public-health/services/diseases/2019-novel-coronavirus-infection/health-professionals/national-case-definition.html

The affiant has no role in determining the cause of death. The Government of Manitoba has not issued any changes to how death certificates are prepared with respect to COVID-19. Death

Certificates are prepared in accordance with *The Vital Statistics Act*, which also follows WHO guidelines.

Item 6: Affidavits of Carla Loeppky and Lanette Siragusa

The number of deaths in Manitoba resulting from other non-communicable diseases is irrelevant to this Application. Nonetheless, the 2020 data is published by Statistics Canada.

Table 1: Number of total deaths in Manitoba from December 29, 2019 to November 14, 2020 by primary cause of death

Primary cause of death	Number
Malignant neoplasms	2020
Diseases of heart	1425
Cerebrovascular diseases	385
Chronic lower respiratory diseases	330
Accidents (unintentional injuries)	325

Data source: Statistics Canada. Table 13-10-0810-01 Selected grouped causes of death, by week

Item 7: Affidavit of Carla Loeppky

The list of modelling parameters is attached.

Item 8: Affidavit of Carla Loeppky

The definition of "cluster" is found at Appendix B of the Interim Guidance Public Health Measures. This document is cited at footnote 158 of Dr. Bhattacharya's report. An updated version of this document can be found at:
https://manitoba.ca/asset_library/en/coronavirus/interim_guidance.pdf

Item 9: Affidavit of Brent Roussin

We decline this undertaking. The information requested is not in the possession or control of the affiant. In any event, the historical ICU and surge capacity is not relevant to the Application.

Item 10: How many COVID-19 PCR positive cases were also tested for influenza

The Public Health Agency of Canada's FluWatch Report is attached. At page 2, the report indicates there were 38,500 influenza tests done in Manitoba from August 23, 2020 to March 20, 2021. Only 1 case tested positive for influenza. Since September 1, 2020, all would also have received a test for SARS-CoV-2.

Item 11: Manitoba's Pandemic Response Plan for the past 5 years

A copy of the Manitoba Emergency Plan can be found at:
https://www.gov.mb.ca/emo/pdfs/MEP.pdf

Sincerely,

"Original signed by"

Michael Conner,
General Counsel

c. Jay Cameron and Jared Brown, counsel for the Applicants
Heather Leonoff, Q.C., Denis Guénette and Sean Boyd, counsel for the Respondents

AB670

Rx Only

cobas® SARS-CoV-2

Qualitative assay for use on the cobas® 6800/8800 Systems

For in vitro diagnostic use

cobas® SARS-CoV-2	P/N: 09175431190
cobas® SARS-CoV-2 Control Kit	P/N: 09175440190
cobas® 6800/8800 Buffer Negative Control Kit	P/N: 07002238190

Table of Contents

Intended use

cobas® SARS-CoV-2 for use on the **cobas**® 6800/8800 Systems is a real-time RT-PCR test intended for the qualitative detection of nucleic acids from SARS-CoV-2 in clinician-instructed self-collected nasal swab specimens (collected on site), and clinician-collected nasal, nasopharyngeal and oropharyngeal swab samples from patients with signs and symptoms suggestive of COVID-19 (e.g., fever and/or symptoms of acute respiratory illness).

Results are for the detection of SARS-CoV-2 RNA that are detectable in nasal, nasopharyngeal and oropharyngeal swab samples during infection. Positive results are indicative of SARS-CoV-2 RNA detection, but may not represent the presence of transmissible virus.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

cobas® SARS-CoV-2 is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

Summary and explanation of the test

Explanation of the test

cobas® SARS-CoV-2 is a qualitative test for use on the **cobas**® 6800 System and **cobas**® 8800 System for the detection of the 2019 novel coronavirus (SARS-CoV-2) RNA in nasal, nasopharyngeal and oropharyngeal swab samples collected in Copan Universal Transport Medium System (UTM-RT), BD™ Universal Viral Transport System (UVT), **cobas**® PCR Media, or 0.9% physiological saline. The RNA Internal Control, used to monitor the entire sample preparation and PCR amplification process, is introduced into each specimen during sample processing. In addition, the test utilizes external controls (low titer positive control and a negative control).

Principles of the procedure

cobas® SARS-CoV-2 is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The **cobas**® 6800/8800 Systems consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the **cobas**® 6800/8800 software, which assigns test results for all tests. Results can be reviewed directly on the system screen, and printed as a report.

Nucleic acid from patient samples and added internal control RNA (RNA IC) molecules are simultaneously extracted. Nucleic acid is released by addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured protein, cellular debris and potential PCR inhibitors, are removed with subsequent wash steps and purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature. External controls (positive and negative) are processed in the same way with each **cobas**® SARS-CoV-2 run.

Selective amplification of target nucleic acid from the sample is achieved by the use of target-specific forward and reverse primers for ORF1a/b non-structural region that is unique to SARS-CoV-2. Additionally, a conserved region in the structural protein envelope E-gene were chosen for pan-Sarbecovirus detection. The pan-Sarbecovirus detection sets will also detect SARS-CoV-2 virus.

Selective amplification of RNA Internal Control is achieved by the use of non-competitive sequence specific forward and reverse primers which have no homology with the coronavirus genome. A thermostable DNA polymerase enzyme is used for amplification.

The **cobas**® SARS-CoV-2 master mix contains detection probes which are specific for the coronavirus type SARS-CoV-2, members of the Sarbecovirus subgenus, and the RNA Internal Control nucleic acid. The coronavirus and RNA Internal Control detection probes are each labeled with unique fluorescent dyes that act as a reporter. Each probe also has a second dye which acts as a quencher. When not bound to the target sequence, the fluorescent signals of the intact probes are suppressed by the quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by the 5' to 3' exonuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye increases concomitantly. Each reporter dye is measured at defined wavelengths, which enables simultaneous detection and discrimination of the amplified coronavirus target and the RNA Internal Control. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythimidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon). Any contaminating amplicons from previous PCR runs are destroyed by the AmpErase enzyme [uracil-N-glycosylase], which is included in the PCR mix, when heated in the first thermal cycling step. However, newly formed amplicons are not destroyed since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

Reagents and materials

The materials provided for **cobas**® SARS-CoV-2 can be found in Table 1. Materials required, but not provided can be found in Table 2, Table 3, Table 4, Table 7, and Table 8.

Refer to the **Reagents and materials** section and **Precautions and handling requirements** section for the hazard information for the product.

cobas® SARS-CoV-2 reagents and controls

All unopened reagents and controls shall be stored as recommended in Table 1 to Table 4.

Table 1 cobas® SARS-CoV-2

cobas® SARS-CoV-2 Store at 2-8°C 192 test cassette (P/N 09175431190)		
Kit components	**Reagent ingredients**	**Quantity per kit 192 tests**
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% proteinase EUH210: Safety data sheet available on request. EUH208: Contains Subtilisin. May produce an allergic reaction.	22.3 mL
RNA Internal Control (RNA IC)	Tris buffer, <0.05% EDTA, <0.001% non-Sarbecovirus related armored RNA construct containing primer and probe specific primer sequence regions (non-infectious RNA in MS2 bacteriophage), <0.1% sodium azide	21.2 mL
Elution Buffer (EB)	Tris buffer, 0.2% methyl-4 hydroxybenzoate	21.2 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, potassium hydroxide, <0.1% sodium azide	7.5 mL
SARS-CoV-2 Master Mix Reagent 2 (SARS-CoV-2 MMX-R2)	Tricine buffer, potassium acetate, < 18% dimethyl sulfoxide, glycerol, < 0.1% Tween 20, EDTA, < 0.12% dATP, dCTP, dGTP, dUTPs, < 0.01% upstream and downstream SARS-CoV-2 and Sarbecovirus primers, < 0.01% Internal Control forward and reverse primers, < 0.01% fluorescent-labeled oligonucleotide probes specific for SARS-CoV-2, Sarbecovirus, and the RNA Internal Control, < 0.01% oligonucleotide aptamer, < 0.1% Z05D DNA polymerase, < 0.10% AmpErase (uracil-N-glycosylase) enzyme (microbial), < 0.1% sodium azide	9.7 mL

Table 2 cobas® SARS-CoV-2 Control Kit

cobas® SARS-CoV-2 Control Kit Store at 2–8°C (P/N 09175440190)		
Kit components	**Reagent ingredients**	**Quantity per kit**
SARS-CoV-2 Positive Control (SARS-CoV-2 (+)C)	Tris buffer, < 0.05% Sodium azide, < 0.005% EDTA, < 0.003% Poly rA, < 0.01% Non-infectious plasmid DNA (microbial) containing SARS-CoV-2 sequence, < 0.01% Non-infectious plasmid DNA (microbial) containing pan-Sarbecovirus 1 sequence, < 0.01% Non-infectious plasmid DNA (microbial) containing pan-Sarbecovirus sequence	16 mL (16 x 1 mL)

Table 3 cobas® Buffer Negative Control Kit

cobas® Buffer Negative Control Kit Store at 2-8°C (P/N 07002238190)		
Kit components	**Reagent ingredients**	**Quantity per kit**
cobas® Buffer Negative Control (BUF (-) C)	Tris buffer, < 0.1% sodium azide, EDTA, < 0.002% Poly rA RNA (synthetic)	16 mL (16 x 1 mL)

cobas omni reagents for sample preparation

Table 4 **cobas omni** reagents for sample preparation*

Reagents	Reagent ingredients	Quantity per kit	Safety symbol and warning**
cobas omni MGP Reagent (MGP) Store at 2–8°C (P/N 06997546190)	Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	480 tests	Not applicable
cobas omni Specimen Diluent (SPEC DIL) Store at 2–8°C (P/N 06997511190)	Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	4 x 875 mL	Not applicable
cobas omni Lysis Reagent (LYS) Store at 2–8°C (P/N 06997538190)	43% (w/w) guanidine thiocyanate***, 5% (w/v) polydocanol***, 2% (w/v) dithiothreitol***, dihydro sodium citrate	4 x 875 mL	 **DANGER** H302 + H332: Harmful if swallowed or if inhaled. H314: Causes severe burns and eye damage. H412: Harmful to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. P261: Avoid breathing dust/fume/gas/mist/vapours/ spray. P273: Avoid release to the environment. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection. P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor. 593-84-0 Guanidinium thiocyanate 9002-92-0 Polidocanol 3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol
cobas omni Wash Reagent (WASH) Store at 15–30°C (P/N 06997503190)	Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate	4.2 L	Not applicable

* These reagents are not included in the **cobas**® SARS-CoV-2 test kit. See listing of additional materials required (Table 7).

** Product safety labeling primarily follows EU GHS guidance

***Hazardous substance

Reagent storage and handling requirements

Reagents shall be stored and will be handled as specified in Table 5 and Table 6.

When reagents are not loaded on the **cobas**® 6800/8800 Systems, store them at the corresponding temperature specified in Table 5.

Table 5 Reagent storage (when reagent is not on the system)

Reagent	Storage temperature
cobas® SARS-CoV-2 -192	2–8°C
cobas® SARS-CoV-2 Control Kit	2–8°C
cobas® Buffer Negative Control Kit	2–8°C
cobas omni Lysis Reagent	2–8°C
cobas omni MGP Reagent	2–8°C
cobas omni Specimen Diluent	2–8°C
cobas omni Wash Reagent	15–30°C

Reagents loaded onto the **cobas**® 6800/8800 Systems are stored at appropriate temperatures and their expiration is monitored by the system. The **cobas**® 6800/8800 Systems allow reagents to be used only if all of the conditions shown in Table 6 are met. The system automatically prevents use of expired reagents. Table 6 allows the user to understand the reagent handling conditions enforced by the **cobas**® 6800/8800 Systems.

Table 6 Reagent expiry conditions enforced by the **cobas**® 6800/8800 Systems

Reagent	Kit expiration date	Open-kit stability	Number of runs for which this kit can be used	On-board stability (cumulative time on board outside refrigerator)
cobas® SARS-CoV-2 – 192	Date not passed[1]	90 days from first usage[1,2]	Max 40 runs[1]	Max 40 hours[1]
cobas® SARS-CoV-2 Control Kit	Date not passed[1]	Not applicable[3]	Not applicable	Max 8 hours[1]
cobas® Buffer Negative Control Kit	Date not passed	Not applicable[3]	Not applicable	Max 10 hours
cobas omni Lysis Reagent	Date not passed	30 days from loading[2]	Not applicable	Not applicable
cobas omni MGP Reagent	Date not passed	30 days from loading[2]	Not applicable	Not applicable
cobas omni Specimen Diluent	Date not passed	30 days from loading[2]	Not applicable	Not applicable
cobas omni Wash Reagent	Date not passed	30 days from loading[2]	Not applicable	Not applicable

[1] The performance has not been established for suggested use cycles and time, but is based on similar reagents used on the same system.

[2] Time is measured from the first time that reagent is loaded onto the **cobas**® 6800/8800 Systems.

[3] Single use reagents

Additional materials required

Table 7 Materials and consumables for use on **cobas**® 6800/8800 Systems

Material	P/N
cobas omni Processing Plate	05534917001
cobas omni Amplification Plate	05534941001
cobas omni Pipette Tips	05534925001
cobas omni Liquid Waste Container	07094388001
cobas omni Lysis Reagent	06997538190
cobas omni MGP Reagent	06997546190
cobas omni Specimen Diluent	06997511190
cobas omni Wash Reagent	06997503190
Solid Waste Bag	07435967001
Solid Waste Bag and Solid Waste Container or Solid Waste Bag With Insert and Kit Drawer	07435967001 and 07094361001 or 08030073001 and 08387281001
Solid Waste Container	07094361001
cobas omni Secondary Tubes 13x75 (optional)	06438776001

Instrumentation and software required

The **cobas**® 6800/8800 software and **cobas**® SARS-CoV-2 analysis package must be installed on the instrument(s). The Instrument Gateway (IG) server will be provided with the system.

Table 8 Instrumentation

Equipment	P/N
cobas® 6800 System (Moveable Platform)	05524245001 and 06379672001
cobas® 6800 System (Fixed Platform)	05524245001 and 06379664001
cobas® 8800 System	05412722001
Sample Supply Module	06301037001
Instrument Gateway	06349595001

For additional information, please refer to the **cobas**® 6800/8800 Systems – User Assistance and/or User Guide.

Note: Contact your local Roche representative for a detailed order list for sample racks, racks for clotted tips and rack trays accepted on the instruments.

Precautions and handling requirements

Warnings and precautions

As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.

- For *in vitro* diagnostic use.
- Positive results are indicative of presence of SARS-CoV-2 RNA.
- All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.[1,2] Only personnel proficient in handling infectious materials and the use of **cobas**® SARS-CoV-2 and **cobas**® 6800/8800 Systems should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10) or follow appropriate site procedures.
- The use of sterile disposable pipettes and nuclease-free pipette tips is recommended. Use only supplied or specified required consumables to ensure optimal test performance.
- Safety Data Sheets (SDS) are available on request from your local Roche representative.
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.
- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.

Reagent handling

- Handle all reagents, controls, and samples according to good laboratory practice in order to prevent carryover of samples or controls.
- Before use, visually inspect each reagent cassette, diluent, lysis reagent, and wash reagent to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.
- **cobas omni** Lysis Reagent contains guanidine thiocyanate, a potentially hazardous chemical. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur.
- **cobas**® SARS-CoV-2 test kit, **cobas**® SARS-CoV-2 Control kit, **cobas**® Buffer Negative Control kit, **cobas omni** MGP Reagent, and **cobas omni** Specimen Diluent contain sodium azide as a preservative. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry.
- Do not allow **cobas omni** Lysis Reagent, which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.
- Dispose of all materials that have come in contact with samples and reagents in accordance with country, state, and local regulations.

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink, or smoke in designated work areas.
- Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents. Gloves must be changed between handling samples and **cobas**® SARS-CoV-2 kits, **cobas**® SARS-CoV-2 Control kit, **cobas**® Buffer Negative Control kit and **cobas omni** reagents to prevent contamination. Avoid contaminating gloves when handling samples and controls.
- Wash hands thoroughly after handling samples and kit reagents, and after removing the gloves.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.
- If spills occur on the **cobas**® 6800/8800 instrument, follow the instructions in the **cobas**® 6800/8800 Systems – User Assistance and/or User Guide to properly clean and decontaminate the surface of instrument(s).

Sample collection, transport, and storage

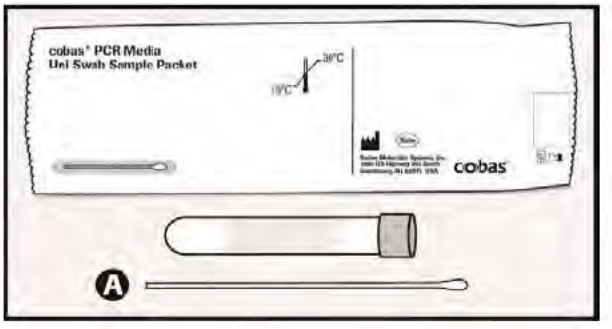
Note: Handle all samples and controls as if they are capable of transmitting infectious agents.

Sample collection

- Collect nasal, nasopharyngeal and oropharyngeal specimens according to standard collection technique using flocked or polyester-tipped swabs and immediately place in 3 mL of Copan Universal Transport Medium (UTM-RT) or BD™ Universal Viral Transport (UVT).
- Collect nasal specimens according to standard collection technique using flocked or polyester-tipped swabs and immediately place into **cobas**® PCR Media tube from **cobas**® PCR Media Kit (P/N 06466281190).
- Collect nasal specimens using the **cobas**® PCR Media Uni Swab Sample Kit (P/N 07958030190) or **cobas**® PCR Media Dual Swab Sample Kit (P/N 07958021190) according to instructions below.

Nasal (anterior nares) swab specimen collection – clinician or self-collected on site

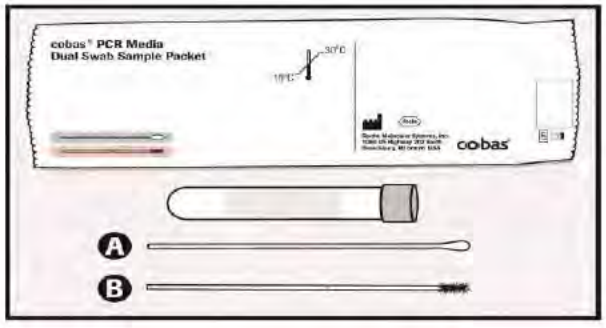
WARNING: DO NOT PRE-WET SWAB IN cobas® PCR MEDIA BEFORE COLLECTION!



The **cobas**® PCR Media Uni Swab Sample kit contains:

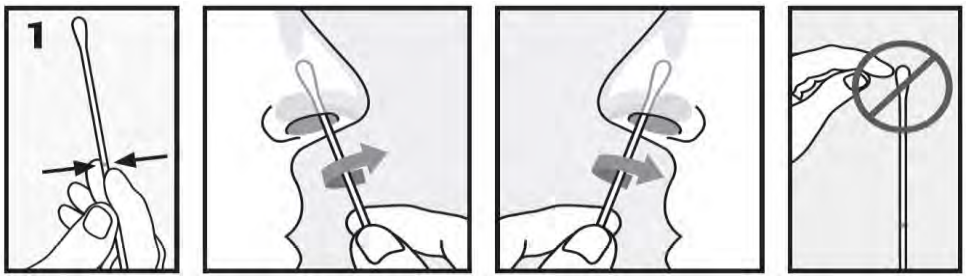
cobas® PCR Media tube
Woven Swab: A

OR



The **cobas**® PCR Media Dual Swab Sample kit contains:

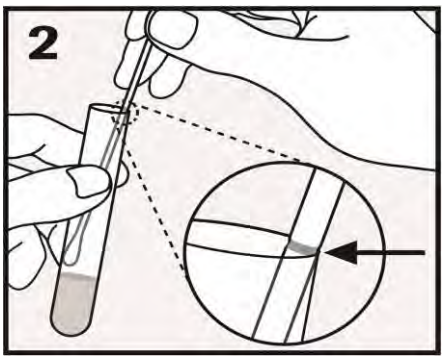
cobas® PCR Media Tube
Woven Swab: A
Flocked Swab: B



DO NOT PRE-WET SWAB IN cobas® PCR MEDIA BEFORE COLLECTION!

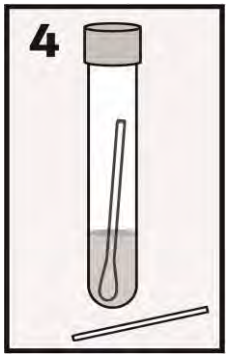
1. **COLLECT:** Hold the woven swab (Swab A) or the flocked swab (Swab B) with the scoreline above your hand. Insert the swab 1-2 cm into one of the anterior nares. Rotate the swab against the nasal mucosa for about 3 seconds and withdraw. Repeat with the other anterior nare using the same swab.

Do not let the swab touch any surface before placing it into the collection tube.



2. **ALIGN:** Remove the cap from the **cobas**® PCR Media Tube and lower the swab specimen into the tube until the visible scoreline on the swab is aligned with the tube rim.

3. **BREAK:** Carefully leverage the swab against the tube rim to break the swab shaft at the scoreline.



4. **CLOSE: Tightly** re-cap the **cobas**® PCR Media Tube. The specimen is now ready for transport. Discard the top portion of the swab.

- Collect nasal specimens according to standard collection technique using flocked or polyester-tipped swabs and immediately place in 3 mL of 0.9% physiological saline.

Transport and storage

Transportation of collected specimens must comply with all applicable regulations for the transport of etiologic agents.

- Transport and store samples collected in **cobas**® PCR Media or 0.9% physiological saline as follows:
  - After collection, specimens in **cobas**® PCR Media or 0.9% physiological saline should be stored at 2-8°C and processed within 48 hours.
- Sample stability when using **cobas**® SARS-CoV-2 has not been established for suggested temperatures and time, but is based on viability data from testing similar viruses in the UTM-RT or UVT Systems as stated in Copan UTM-RT System Instructions For Use and shown below:
  - After collection, the specimen should be stored at 2-25°C and processed within 48 hours.
  - If delivery and processing exceed 48 hours, specimens should be transported in dry ice and once in laboratory frozen at -70°C or colder.

Instructions for use

Procedural notes

- Do not use **cobas**® SARS-CoV-2 reagents, **cobas**® SARS-CoV-2 Control Kit, **cobas**® Buffer Negative Control Kit, or **cobas omni** reagents after their expiry dates.
- Do not reuse consumables. They are for one-time use only.
- Refer to the **cobas**® 6800/8800 Systems – User Assistance and/or User Guide for proper maintenance of instruments.

Running cobas® SARS-CoV-2

cobas® SARS-CoV-2 can be run with a minimum required sample volume of 0.6 mL.

Always use caution when transferring specimens from a primary collection tube to a secondary tube.

Use pipettes with aerosol-barrier or positive-displacement tips to handle specimens.

Always use a new pipette tip for each specimen.

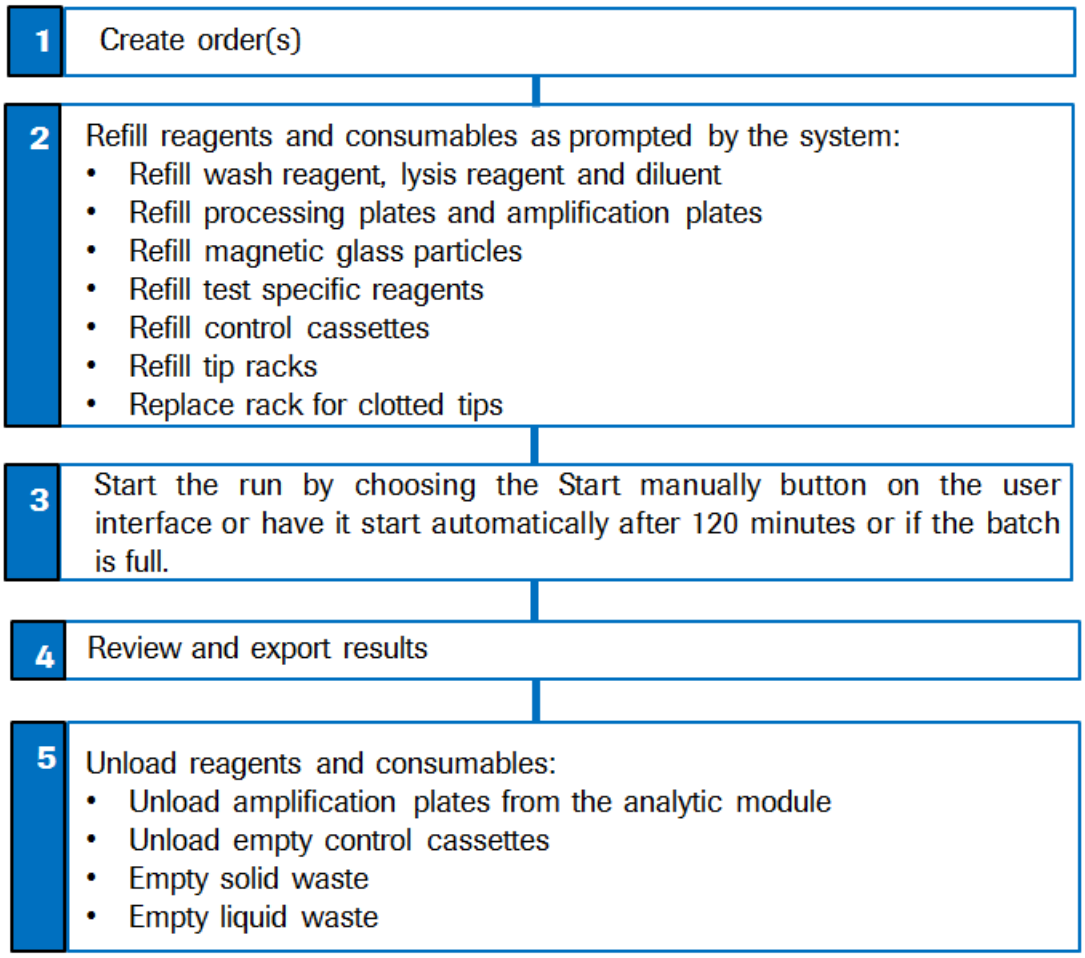
Ensure samples are equilibrated to room temperature prior to transfer into a cobas omni Secondary Tube.

Follow the steps below to transfer patient sample from a primary collection tube into a **cobas omni** Secondary Tube:

- Unscrew the primary sample tube cap.
- Lift the cap and any attached swab to allow a pipette to be inserted into the sample tube.
- Transfer 0.6 mL into the prepared barcoded secondary tube.
- Transfer secondary tube to a rack. Close the primary sample tube cap.

The test procedure is described in detail in the **cobas**® 6800/8800 Systems – User Assistance and/or User Guide. Figure 1 below summarizes the procedure.

Figure 1 **cobas**® SARS-CoV-2 procedure



Results

The **cobas**® 6800/8800 Systems automatically detect the SARS-CoV-2 , for each individually processed sample and control, displaying individual target results for samples as well as test validity and overall results for controls.

Quality control and validity of results

- One **cobas**® Buffer Negative Control [(-) Ctrl] and one [SARS-CoV-2 (+)C] are processed with each batch.
- In the **cobas**® 6800/8800 software and/or report, check for flags and their associated results to ensure the batch validity.
- All flags are described in the **cobas**® 6800/8800 Systems User Guide.
- The batch is valid if no flags appear for any controls. If the batch is invalid, repeat testing of the entire batch.

Validation of results is performed automatically by the **cobas**® 6800/8800 software based on negative and positive control performance.

Interpretation of results

cobas® SARS-CoV-2 for System Software v1.2

Display examples for **cobas®** SARS-CoV-2 for System Software v1.2 or higher are shown in Figure 2.

Figure 2 Example of **cobas®** SARS-CoV-2 results display for System Software v1.2

Test	Sample ID	Valid*	Flags	Sample type	Overall result*	Target 1	Target 2
SARS-CoV-2 400 µL	Swab_01	Yes		Swab	**Negative**	Negative	Negative
SARS-CoV-2 400 µL	Swab _C1	No	Y40T	Swab	**Invalid**	Invalid	Invalid
SARS-CoV-2 400 µL	Swab _B1	Yes		Swab	**Reactive**	Negative	Positive
SARS-CoV-2 400 µL	Swab _B2	Yes		Swab	**Positive**	Positive	Positive
SARS-CoV-2 400 µL	Swab _D1	Yes		Swab	**Negative**	Negative	Negative
SARS-CoV-2 400 µL	Swab _A6	Yes		Swab	**Reactive**	Positive	Negative
SARS-CoV-2 400 µL	Swab _E1	No	C01H2	Swab	**Invalid**	Positive	Invalid
SARS-CoV-2 400 µL	Swab _A2	No	C01H1	Swab	**Invalid**	Invalid	Positive
SARS-CoV-2	C161420284090428828404	Yes		(-) Ctrl	**Valid**	Valid	Valid
SARS-CoV-2	C16142028409300958026 4	Yes		SARS-CoV-2 (+) C	**Valid**	Valid	Valid

* The "Valid" and "Overall Result" columns are not applicable to sample results for the **cobas®** SARS-CoV-2. Values reported in these columns are not applicable and do not impact the validity of results reported within individual Target Result columns. Refer to Table 9, **cobas®** SARS-CoV-2 results interpretation, for specific instructions on test results interpretation.

cobas® SARS-CoV-2 for System Software v1.3 or higher

Display examples for **cobas®** SARS-CoV-2 for System Software v1.3 or higher are shown in Figure 3.

Figure 3 Example of **cobas®** SARS-CoV-2 results display for System Software v1.3 or higher

Test	Sample ID	Valid*	Flags	Sample type	Overall result*	Target 1	Target 2
SARS-CoV-2 400 µL	Swab_01	NA		Swab	**NA**	Negative	Negative
SARS-CoV-2 400 µL	Swab _C1	NA	Y40T	Swab	**NA**	Invalid	Invalid
SARS-CoV-2 400 µL	Swab _B1	NA		Swab	**NA**	Negative	Positive
SARS-CoV-2 400 µL	Swab _B2	NA		Swab	**NA**	Positive	Positive
SARS-CoV-2 400 µL	Swab _D1	NA		Swab	**NA**	Negative	Negative
SARS-CoV-2 400 µL	Swab _A6	NA		Swab	**NA**	Positive	Negative
SARS-CoV-2 400 µL	Swab _E1	NA	C01H2	Swab	**NA**	Positive	Invalid
SARS-CoV-2 400 µL	Swab _A2	NA	C01H1	Swab	**NA**	Invalid	Positive
SARS-CoV-2	C161420284090428828404	Yes		(-) Ctrl	**Valid**	Valid	Valid
SARS-CoV-2	C16142028409300958026 4	Yes		SARS-CoV-2 (+) C	**Valid**	Valid	Valid

* The "Valid" and "Overall Result" columns are not applicable to sample results for the **cobas®** SARS-CoV-2. Values reported in these columns are not applicable and do not impact the validity of results reported within individual Target Result columns. Refer to Table 9, **cobas®** SARS-CoV-2 results interpretation, for specific instructions on test results interpretation.

Interpretation of results

The following result interpretation applies to both **cobas**® 6800/8800 software version 1.2 and **cobas**® 6800/8800 software version 1.3 and higher.

For a valid batch, check each individual sample for flags in the **cobas**® 6800/8800 software and/or report. The result interpretation should be as follows:

- A valid batch may include both valid and invalid sample results.
- **The "Valid" and "Overall Result" columns are not applicable to sample results for the cobas® SARS-CoV-2. Values reported in these columns are not applicable and do not impact the validity of results reported within individual Target Result columns.**
- Invalid results for one or more target combinations are possible and are reported out specifically for each channel.
- Results of this test should only be interpreted in conjunction with information available from clinical evaluation of the patient and patient history.

Results and their corresponding interpretation for detecting SARS-CoV-2 are shown below (Table 9).

Table 9 **cobas®** SARS-CoV-2 results interpretation

Target 1	Target 2	Interpretation
Positive	**Positive**	All Target Results were valid. Result for SARS-CoV-2 RNA is Detected.
Positive	**Negative**	All Target Results were valid. Result for SARS-CoV-2 RNA is Detected. A positive Target 1 result and a negative Target 2 result is suggestive of 1) a sample at concentrations near or below the limit of detection of the test, 2) a mutation in the Target 2, target region, or 3) other factors.
Negative	**Positive**	All Target Results were valid. Result for SARS-CoV-2 RNA is Presumptive Positive. A negative Target 1 result and a positive Target 2 result is suggestive of 1) a sample at concentrations near or below the limit of detection of the test, 2) a mutation in the Target 1 target region in the oligo binding sites, or 3) infection with some other Sarbecovirus (e.g., SARS-CoV or some other Sarbecovirus previously unknown to infect humans), or 4) other factors. Sample should be retested. For samples with a repeated Presumptive Positive result, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management.
Negative	**Negative**	All Target Results were valid. Result for SARS-CoV-2 RNA is Not Detected.
Positive	**Invalid**	Not all Target Results were valid. Result for SARS-CoV-2 RNA is Detected.
Invalid	**Positive**	Not all Target Results were valid. Result for SARS-CoV-2 RNA is Presumptive Positive. Sample should be retested. For samples with a repeated Presumptive Positive result, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management.
Negative	**Invalid**	Not all Target Results were valid. Sample should be retested. If the result is still invalid, a new specimen should be obtained.
Invalid	**Negative**	Not all Target Results were valid. Sample should be retested. If the result is still invalid, a new specimen should be obtained.
Invalid	**Invalid**	All Target Results were invalid. Sample should be retested. If the result is still invalid, a new specimen should be obtained.

Procedural limitations

- **cobas**® SARS-CoV-2 has been evaluated only for use in combination with the **cobas**® SARS-CoV-2 Control Kit, **cobas**® Buffer Negative Control Kit, **cobas omni** MGP Reagent, **cobas omni** Lysis Reagent, **cobas omni** Specimen Diluent, and **cobas omni** Wash Reagent for use on the **cobas**® 6800/8800 Systems.
- Reliable results depend on proper sample collection, storage and handling procedures.
- This test is intended to be used for the detection of SARS-CoV-2 RNA in nasal, nasopharyngeal and oropharyngeal swab samples collected in a Copan UTM-RT System (UTM-RT) or BD™ Universal Viral Transport System (UVT), and nasal swab samples collected in **cobas**® PCR Media and 0.9% physiological saline. Testing of other sample types with **cobas**® SARS-CoV-2 may result in inaccurate results.
- Detection of SARS-CoV-2 RNA may be affected by sample collection methods, patient factors (e.g., presence of symptoms), and/or stage of infection.
- As with any molecular test, mutations within the target regions of **cobas**® SARS-CoV-2 could affect primer and/or probe binding resulting in failure to detect the presence of virus.
- Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. One hundred percent agreement between the results should not be expected due to aforementioned differences between technologies. Users should follow their own specific policies/procedures.
- False negative or invalid results may occur due to interference. The Internal Control is included in **cobas**® SARS-CoV-2 to help identify the specimens containing substances that may interfere with nucleic acid isolation and PCR amplification.
- The addition of AmpErase enzyme into the **cobas**® SARS-CoV-2 Master Mix reagent enables selective amplification of target RNA; however, good laboratory practices and careful adherence to the procedures specified in this Instructions For Use document are necessary to avoid contamination of reagents.

Non-clinical performance evaluation

Key performance characteristics

Analytical sensitivity

Limit of detection (LoD) studies determine the lowest detectable concentration of SARS-CoV-2 at which greater or equal to 95% of all (true positive) replicates test positive.

To determine the LoD, a cultured virus of an isolate from a US patient (USA-WA1/2020, catalog number NR-52281, lot number 70033175, 2.8E+05 $TCID_{50}$/mL[§]) was serially diluted in simulated clinical matrix. A total of 7 concentration levels, with 3-fold serial dilutions between the levels, were tested with a total of 21 replicates per concentration, with an additional 10 replicates of a blank sample (i.e, simulated clinical matrix).

As shown in Table 10, the concentration level with observed hit rates greater than or equal to 95% were 0.009 and 0.003 $TCID_{50}$/mL for SARS-CoV-2 (Target 1) and pan-Sarbecovirus (Target 2), respectively. As shown in Table 11, the Probit predicted 95% hit rates were 0.007 and 0.004 $TCID_{50}$/mL for SARS-CoV-2 (Target 1) and pan-Sarbecovirus (Target 2), respectively.

Table 10 LoD determination using USA-WA1/2020 strain

Strain	Concentration [$TCID_{50}$/mL]	Total valid results	Hit rate [%]^		Mean Ct*	
			Target 1	Target 2	Target 1	Target 2
USA-WA1/2020[§] (stock concentration 2.8E+05 $TCID_{50}$/mL)	0.084	21	100	100	31.0	33.0
	0.028	21	100	100	31.8	34.1
	0.009	21	100	100	32.7	35.2
	0.003	21	38.1	100	33.5	36.4
	0.001	21	0	52.4	n/a	37.9
	0.0003	21	0	14.3	n/a	37.2
	0.0001	21	0	9.5	n/a	38.5
	0 (blank)	10	0	0	n/a	n/a

[§] Reagent was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH: SARS-Related Coronavirus 2, Isolate USA-WA1/2020, NR-52281

^ All replicates where Target 1 was positive were also positive for Target 2.

* Calculations only include positive results.

Table 11 Probit predicted 95% hit rates using USA-WA1/2020 strain

Strain	Probit Predicted 95% Hit Rate [$TCID_{50}$/mL]	
	Target 1	Target 2
USA-WA1/2020 (stock concentration 2.8E+05 $TCID_{50}$/mL)	0.007 (95% CI: 0.005 – 0.036)	0.004 (95% CI: 0.002 – 0.009)

Cross-reactivity

In silico analysis

The *in silico* analysis for possible cross-reactions with all the organisms listed in Table 12 was conducted by mapping primers in **cobas**® SARS-CoV-2 individually to the sequences downloaded from NCBI and GISAID databases. If any two of the primers were mapped to a sequence on opposite strands with short distance apart, potential amplifications were flagged. No potential unintended cross reactivity is expected based on this *in silico* analysis.

Table 12 In silico analysis for SARS-CoV-2

Strain	*In Silico* Analysis for % Identity to Target 1 (nCoV)	*In Silico* Analysis for % Identity to Target 2 (Pan-Sarbecovirus 1)
CoV 229E	74.47	No alignment was found*
CoV OC43	72.26	No alignment was found*
CoV HKU1	76.52	No alignment was found*
CoV NL63	71.32	No alignment was found*
SARS-CoV	95.04	100
MERS	No alignment was found*	No alignment was found*
AdV	No alignment was found*	No alignment was found*
HMPV	No alignment was found*	No alignment was found*
HPIV1	No alignment was found*	No alignment was found*
HPIV2	No alignment was found*	No alignment was found*
HPIV3	No alignment was found*	No alignment was found*
HPIV4	No alignment was found*	No alignment was found*
Flu A	No alignment was found*	No alignment was found*
Flu B	No alignment was found*	No alignment was found*
EV	No alignment was found*	No alignment was found*
RSV	No alignment was found*	No alignment was found*
RV	No alignment was found*	No alignment was found*
Chlamydia pneumoniae	No alignment was found*	No alignment was found*
Haemophilus influenzae	No alignment was found*	No alignment was found*

Strain	***In Silico* Analysis for % Identity to Target 1 (nCoV)**	***In Silico* Analysis for % Identity to Target 2 (Pan-Sarbecovirus 1)**
Legionella pneumophila	No alignment was found*	No alignment was found*
MTB Mycobacterium bovis subsp. Bovis	No alignment was found*	No alignment was found*
Streptococcus pneumoniae	No alignment was found*	No alignment was found*
Streptococcus pyrogenes	No alignment was found*	No alignment was found*
Bordetella pertussis	No alignment was found*	No alignment was found*
Mycoplasma pneumoniae	No alignment was found*	No alignment was found*
Pneumocystis jirovecii	No alignment was found*	No alignment was found*
Influenza C	No alignment was found*	No alignment was found*
Parechovirus	No alignment was found*	No alignment was found*
Candida albicans	No alignment was found*	No alignment was found*
Corynebacterium diphtheriae	No alignment was found*	No alignment was found*
Legionella non-pneumophila	No alignment was found*	No alignment was found*
Bacillus anthracis (Anthrax)	No alignment was found*	No alignment was found*
Moraxella catarrhalis	No alignment was found*	No alignment was found*
Neisseria elongate and meningitides	No alignment was found*	No alignment was found*
Pseudomonas aeruginosa	No alignment was found*	No alignment was found*
Staphylococcus epidermidis	No alignment was found*	No alignment was found*
Staphylococcus salivarius	No alignment was found*	No alignment was found*
Leptospira	No alignment was found*	No alignment was found*
Chlamydia psittaci	No alignment was found*	No alignment was found*
Coxiella burnetii (Q-Fever)	No alignment was found*	No alignment was found*
Staphylococcus aureus	No alignment was found*	No alignment was found*

Note: * The amplicon sequences were blasted against all the exclusive sequences with very low stringency cutoff (50% and 100bp). No alignment were found passing the cutoff and no concerns for cross-reactivity were observed.

Cross reactivity testing

Cross-reactivity of **cobas**® SARS-CoV-2 was evaluated by testing whole organisms. As listed in Table 13, a panel of multiple unique sub-species of microorganisms were tested. High titer stocks of the potentially cross-reacting microorganisms were spiked into negative simulated clinical matrix to a concentration level of 1.0E+05 units/mL for viruses and 1.0E+06 units/mL for other microorganisms, unless otherwise noted.

None of the organisms tested interfered with **cobas**® SARS-CoV-2 performance by generating false positive results.

Table 13 Cross-reactivity test results

Microorganism	Concentration	Target 1 Result	Target 2 Result
Human coronavirus 229E	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Human coronavirus OC43	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Human coronavirus HKU1	1.0E+05 cp/mL	Negative	Negative
Human coronavirus NL63	1.0E+05 $TCID_{50}$/mL	Negative	Negative
MERS coronavirus	1.0E+05 genomic equivalent/mL	Negative	Negative
SARS coronavirus	1.0E+05 PFU/mL	Negative	Positive
Adenovirus B (Type 34)	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Human Metapneumovirus (hMPV)	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Parainfluenza virus Type 1	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Parainfluenza virus Type 2	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Parainfluenza virus Type 3	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Parainfluenza virus Type 4	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Influenza A (H1N1)	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Influenza B	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Enterovirus E (Type 1)	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Respiratory syncytial virus	1.0E+05 PFU/mL	Negative	Negative
Rhinovirus	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Chlamydia pneumonia	1.0E+06 $TCID_{50}$/mL	Negative	Negative
Haemophilus influenzae	1.0E+06 CFU/mL	Negative	Negative
Legionella pneumophila	1.0E+06 CFU/mL	Negative	Negative
Mycobacterium tuberculosis	1.0E+06 cells/mL	Negative	Negative
Streptococcus pneumonia	1.0E+06 CFU/mL	Negative	Negative
Streptococcus pyrogenes	1.0E+06 CFU/mL	Negative	Negative
Bordetella pertussis	1.0E+06 CFU/mL	Negative	Negative
Mycoplasma pneumoniae	1.0E+06 CFU/mL	Negative	Negative
Pooled human nasal wash	5 - 50%	Negative	Negative

Sample type equivalency

Equivalence between nasopharyngeal swab (NPS) and oropharyngeal swab (OPS) sample types was evaluated using cultured virus (USA-WA1/2020 strain) spiked into paired negative samples (individual samples, not pooled) to prepare contrived low positive (approximately 1.5x Target 1 LoD) and moderate positive (approximately 4x Target 1 LoD) samples for each sample type. A total of 21 low positive paired samples, 11 moderate positive paired samples, and 11 negative paired samples were tested.

As shown in Table 14, all low positive and moderate positive paired samples were positive in both sample matrices. All negative paired samples were negative in both sample types. The observed Ct values for contrived positive samples were comparable in both sample types.

Table 14 Result comparison of nasopharyngeal to oropharyngeal sample types

Sample Type	Sample Concentration	N	Target 1		Target 2	
			% Positive	Mean Ct (95% CI)	% Positive	Mean Ct (95% CI)
NPS	~1.5x LoD (Target 1)	21	100	31.9 (31.7 – 32.0)	100	33.6 (33.5 – 33.7)
OPS			100	32.2 (31.8 – 32.6)	100	33.7 (33.4 – 34.1)
NPS	~4x LoD (Target 1)	11	100	30.9 (30.3 – 31.5)	100	32.2 (31.6 – 32.9)
OPS			100	31.5 (31.2 – 31.9)	100	32.7 (32.4 – 33.0)
NPS	Negative	11	0	n/a	0	n/a
OPS			0	n/a	0	n/a

Matrix equivalency – UTM-RT and cobas® PCR Media

Equivalence between samples collected in UTM-RT and **cobas**® PCR Media (CPM) was evaluated using cultured virus (USA-WA1/2020 strain) spiked into paired negative nasopharyngeal samples from patients with signs and symptoms of an upper respiratory infection (individual samples, not pooled) to prepare contrived low positive (approximately 1.5x LoD) and moderate positive (approximately 4x LoD) samples for each collection media. A total of 21 low positive paired samples, 11 moderate positive paired samples, and 11 negative paired samples were tested.

As shown in Table 15, all low positive and moderate positive paired samples were positive in both sample matrices. All negative paired samples were negative in both sample matrices. The observed Ct values for contrived positive samples were comparable in both sample matrices.

Table 15 Result comparison of UTM-RT to **cobas**® PCR Media

Collection Media	Sample Concentration	N	Target 1		Target 2	
			% Positive	Mean Ct (95% CI)	% Positive	Mean Ct (95% CI)
UTM	~1.5x LoD	21	100	31.8 (31.6 - 32.0)	100	34.0 (33.8 - 34.2)
CPM			100	32.2 (31.9 - 32.4)	100	34.7 (34.4 - 33.8)
UTM	~4x LoD	11	100	30.7 (30.1 - 31.2)	100	32.4 (31.8 - 33.1)
CPM			100	31.6 (31.0 - 32.1)	100	33.7 (33.0 - 34.5)
UTM	Negative	11	0	n/a	0	n/a
CPM			0	n/a	0	n/a

Matrix equivalency –UTM-RT and 0.9% physiological saline

Equivalence between samples collected in UTM-RT and 0.9% physiological saline was evaluated using cultured virus (USA-WA1/2020 strain) spiked into paired negative samples (individual samples, not pooled) to prepare contrived low positive (approximately 1.5x LoD) and moderate positive (approximately 4x LoD) samples for each collection media. Three samples were collected from each of 45 healthy donors using swabs from **cobas**® PCR Media Dual Swab Sample Kit; two nasal sample (NS) collected using dual flocked/woven polyester swabs stored in UTM and one nasal sample (other nostril) collected using a woven polyester swab stored in 0/9% physiological saline. A total of 17 low positive paired samples, 11 moderate positive paired samples, and 45 negative paired samples were tested.

As shown in Table 16, all low positive and moderate positive paired samples were positive in both sample matrices. All negative paired samples were negative in both sample matrices. The observed Ct values for contrived positive samples were comparable in both sample matrices.

Table 16 Result comparison of UTM-RT to 0.9% physiological saline

Collection Device	Sample Concentration	N	Target 1		Target 2	
			% Positive	Mean Ct (95% CI)	% Positive	Mean Ct (95% CI)
Flocked Swab in UTM-RT	~1.5x LoD	17	100	32.2 (32.0 - 32.4)	100	33.6 (33.6 - 33.7)
Woven Swab in UTM-RT		16	100	31.6 (31.1 - 32.1)	100	33.2 (32.7 - 33.8)
Woven Swab in Saline		17	100	31.7 (31.4 - 32.0)	100	33.5 (33.2 - 33.8)
Flocked Swab in UTM-RT	~4x LoD	11	100	31.2 (31.1 - 31.4)	100	32.6 (32.4 - 32.7)
Woven Swab in UTM-RT			100	30.9 (30.4 - 31.4)	100	32.4 (31.9 - 33.0)
Woven Swab in Saline			100	31.0 (30.8 - 31.3)	100	32.6 (32.5 - 32.7)
Flocked Swab in UTM-RT	Negative	45	0	n/a	0	n/a
Woven Swab in UTM-RT			0	n/a	0	n/a
Woven Swab in Saline			0	n/a	0	n/a

Clinical evaluation

The performance of **cobas**® SARS-CoV-2 with prospectively collected nasopharyngeal swab clinical samples was evaluated using 100 individual negative clinical samples and 50 contrived positive clinical samples collected from patients with signs and symptoms of an upper respiratory infection.

Clinical samples were collected by qualified personnel according to the package insert of the collection device. Samples were handled as described in the package insert of the collection device and stored frozen until use. Samples were tested to be negative by a commercially available nucleic acid test for the qualitative detection of microorganisms associated with common upper respiratory tract infections.

Low positive and moderate positive contrived positive clinical samples were prepared by spiking cultured virus (USA-WA1/2020 strain) into individual negative clinical samples to approximately ~1.5x LoD (Target 1) (25 samples) and ~4x LoD (Target 1) (25 samples), respectively.

As shown in Table 17 all low positive and moderate positive samples were positive and all negative samples were negative in the background of individual clinical sample matrix.

Table 17 Clinical evaluation with nasopharyngeal swab samples

Sample Concentration	N	Target 1		Target 2	
		% positive (two-sided 95% CI)	Mean Ct	% positive (two-sided 95% CI)	Mean Ct
~1.5x LoD	25	100 (86.7 – 100)	31.6	100 (86.7 – 100)	33.2
~4x LoD	25	100 (86.7 – 100)	31.1	100 (86.7 – 100)	32.4
Negative	100	0 (n/a)	n/a	0 (n/a)	n/a

Performance against the expected results are:

Positive Percent Agreement 50/50 = 100% (95% CI: 92.9% - 100%)
Negative Percent Agreement 100/100 = 100% (95% CI: 96.3% - 100%)

Additional information

Key test features

Sample type	Nasopharyngeal and oropharyngeal swab samples collected in the Copan UTM-RT System or the BD™ UVT System Nasal swab samples collected in the Copan UTM-RT System, the BD™ UVT System, the **cobas**® PCR Media, and 0.9% physiological saline
Minimum amount of sample required	0.6 mL*
Sample processing volume	0.4 mL
Test duration	Results are available within less than 3.5 hours after loading the sample on the system.

*Dead volume of 0.2 mL is identified for the **cobas omni** Secondary tubes. Other tubes compatible with **cobas**® 6800/8800 Systems (consult User Assistance Guide) may have different dead volume and require more or less minimum volume.

Symbols

The following symbols are used in labeling for Roche PCR diagnostic products.

Table 18 Symbols used in labeling for Roche PCR diagnostics products

SW	Ancillary Software	LLR	Lower Limit of Assigned Range	CONTROL –	Negative Control
EC REP	Authorized representative in the European community	ULR	Upper Limit of Assigned Range	CONTROL +	Positive Control
BARCODE	Barcode Data Sheet		Store in the dark	CONTROL	Control
LOT	Batch code	Σ	Contains sufficient for <*n*> tests	Assigned Range [copies/mL]	Assigned Range (copies/mL)
	Biological risks		Temperature limit	Assigned Range [IU/mL]	Assigned Range (IU/mL)
REF	Catalogue number	TDF	Test Definition File	Procedure Standard	Standard Procedure
	Consult instructions for use		Manufacturer	Procedure UltraSensitive	Ultrasensitive Procedure
Cont.	Contents of kit		Use-by date	QS copies/PCR	QS copies per PCR reaction, use the QS copies per PCR reaction in calculation of the results.
D	Distributed by	GTIN	Global Trade Item Number	QS IU/PCR	QS IU per PCR reaction, use the QS International Units (IU) per PCR reaction in calculation of the results.
	For IVD performance evaluation only	SN	Serial number	CE	This product fulfills the requirements of the European Directive 98/79 EC for *in vitro* diagnostic medical devices.
Rx Only	US Only: Federal law restricts this device to sale by or on the order of a physician.		Date of manufacture		
IVD	*In Vitro* diagnostic medical device		Do not reuse		

US Customer Technical Support 1-800-526-1247

Manufacturer and distributors

Table 19 Manufacturer and distributors

Roche Molecular Systems, Inc.
1080 US Highway 202 South
Branchburg, NJ 08876 USA
www.roche.com

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany

Roche Diagnostics
9115 Hague Road
Indianapolis, IN 46250-0457 USA
(For Technical Assistance call the
Roche Response Center
toll-free: 1-800-526-1247)

Trademarks and patents

See http://www.roche-diagnostics.us/patents

Copyright

Roche Diagnostics GmbH
Sandhofer Str. 116
68305 Mannheim
Germany

References

1. Center for Disease Control and Prevention. Biosafety in Microbiological and Biomedical Laboratories, 5th ed. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health HHS Publication No. (CDC) 21-1112, revised December 2009.
2. Clinical and Laboratory Standards Institute (CLSI). Protection of laboratory workers from occupationally acquired infections. Approved Guideline-Fourth Edition. CLSI Document M29-A4:Wayne, PA;CLSI, 2014.

Document revision

Document Revision Information	
Doc Rev. 1.0 03/2020	First Publishing.
Doc Rev. 2.0 04/2020	Corrected typographical errors, organism names, and table references. Added nasal swabs (self-collected on site or by the physician), collected in UTM-RT, VTM, **cobas**® PCR Media and 0.9% physiological saline. Addition of the analytical performance data related to the added specimen and media types. Replaced “container” with “collection tube” to improve clarity. Please contact your local Roche Representative if you have any questions.

Aptima™ SARS-CoV-2 Assay (Panther™ System)

For *in vitro* diagnostic use.

For U.S. Export only.

CONTENTS

General Information

Intended Use

The Aptima™ SARS-CoV-2 assay is a nucleic acid amplification *in vitro* diagnostic test intended for the qualitative detection of RNA from SARS-CoV-2 isolated and purified from nasopharyngeal (NP), nasal, mid-turbinate and oropharyngeal (OP) swab specimens, nasopharyngeal wash/ aspirate or nasal aspirates obtained from individuals meeting COVID-19 clinical and/or epidemiological criteria.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA, clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The Aptima SARS-CoV-2 assay on the Panther™ and Panther Fusion™ system is intended for use by clinical laboratory personnel specifically instructed and trained in the operation of the Panther and Panther Fusion systems and in vitro diagnostic procedures.

Summary and Explanation of the Test

Coronaviruses are a large family of viruses which may cause illness in animals or humans. In humans, several coronaviruses are known to cause respiratory infections ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS). The most recently discovered coronavirus, SARS-CoV-2, causes the associated coronavirus disease COVID-19. This new virus and disease were unknown before the outbreak began in Wuhan, China, in December 2019.[1]

The most common symptoms of COVID-19 are fever, tiredness, and dry cough. Some patients may have aches and pains, nasal congestion, runny nose, sore throat, new loss of taste or smell, or diarrhea. These symptoms are usually mild and begin gradually. Some people become infected but don't develop any symptoms and don't feel unwell. The disease can spread through respiratory droplets produced when an infected person coughs or sneezes. These droplets can land in the mouths or noses of people who are nearby or possibly be inhaled into the lungs.[2] These droplets also can land on objects and surfaces around the person. Other people may acquire SARS-CoV-2 by touching these objects or surfaces, then touching their eyes, nose, or mouth.

The virus that causes COVID-19 is infecting people and spreading easily from person to person.[3] On March 11, 2020, the COVID-19 outbreak was characterized as a pandemic by the World Health Organization (WHO).[4,5]

Principles of the Procedure

The Aptima SARS-CoV-2 assay combines the technologies of target capture, Transcription Mediated Amplification (TMA), and Dual Kinetic Assay (DKA).

Specimens are collected and transferred into their respective specimen transport tubes. The transport solutions in these tubes release the RNA target and protect them from degradation during storage. When the Aptima SARS-CoV-2 assay is performed in the laboratory, the target RNA molecules are isolated from specimens by use of capture oligomers via target capture that utilizes magnetic microparticles. The capture oligomers contain sequences complementary to specific regions of the target molecules as well as a string of deoxyadenosine residues. A separate capture oligomer is used for each target. During the hybridization step, the sequence specific regions of the capture oligomers bind to specific regions of the target molecules. The capture oligomer:target complex is then captured out of solution by decreasing the temperature of the reaction to room temperature. This temperature reduction allows hybridization to occur between the deoxyadenosine region on the capture oligomer and the poly-deoxythymidine molecules that are covalently attached to the magnetic particles. The microparticles, including the captured target molecules bound to them, are pulled to the side of the reaction vessel using magnets and the supernatant is aspirated. The particles are washed to remove residual specimen matrix that may contain amplification reaction inhibitors. After the target capture steps are completed, the specimens are ready for amplification.

Target amplification assays are based on the ability of complementary oligonucleotide primers to specifically anneal and allow enzymatic amplification of the target nucleic acid strands. The Aptima SARS-CoV-2 assay replicates specific regions of the RNA from SARS-CoV-2 virus. Detection of the RNA amplification product sequences (amplicon) is achieved using nucleic acid hybridization. Single-stranded chemiluminescent nucleic acid probes, which are unique and complementary to a region of each target amplicon and Internal Control (IC) amplicon, are labeled with different acridinium ester (AE) molecules. The AE labeled probes combine with amplicon to form stable hybrids. The Selection Reagent differentiates hybridized from unhybridized probe, eliminating the generation of signal from unhybridized probe. During the detection step, light emitted from the labeled hybrids is measured as photon signals in a luminometer, and are reported as Relative Light Units (RLU). In DKA, differences in the kinetic profiles of the labeled probes allow for the differentiation of signal; kinetic profiles are derived from measurements of photon output during the detection read time. The chemiluminescent detection reaction for the IC signal has very rapid kinetics and has the “flasher” kinetic type. The chemiluminescent detection reaction for the SARS-CoV-2 signal is relatively slower and has the “glower” kinetic type. Assay results are determined by a cut-off based on the total RLU and the kinetic curve type.

The Aptima SARS-CoV-2 assay amplifies and detects two conserved regions of the ORF1ab gene in the same reaction, using the same “glower” kinetic type. The two regions are not differentiated and amplification of either or both regions leads to RLU signal. The assay results are determined by a cut-off based on the total RLU and the kinetic curve type.

Warnings and Precautions

A. For *in vitro* diagnostic use. Carefully read this entire package insert and the *Panther/Panther Fusion System Operator’s Manual*.

B. Only personnel adequately trained on the use of this assay and in handling potentially infectious materials should perform these procedures. If a spill occurs, immediately disinfect using appropriate site procedures.

C. Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019-nCoV. https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html.

D. Specimens may be infectious. Use Universal Precautions when performing this assay. Proper handling and disposal methods should be established by the laboratory director. Only personnel adequately trained in handling infectious materials should be permitted to perform this diagnostic procedure.[6]

E. If infection with SARS-CoV-2 is suspected based on current clinical screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions.

F. Use only supplied or specified disposable laboratory ware.

G. Use appropriate personal protective equipment when collecting and handling specimens from individuals suspected of being infected with SARS-CoV-2 as outlined in CDC Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019 Novel Coronavirus (2019-nCoV).

H. Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling specimens and reagents. Wash hands thoroughly after handling specimens and reagents.

I. Dispose of all material that has come into contact with specimens and reagents in accordance with applicable national, international, and regional regulations.

J. Expiration dates listed on the Panther Fusion Specimen Lysis Tubes, Hologic Specimen Lysis Tubes, the Aptima Multitest Collection Kit, the Aptima Swab Unisex Specimen Collection Kit and the Aptima Specimen Transfer Kit pertain to the transfer of sample into the tube and not to testing of the sample. Specimens collected/transferred any time prior to these expiration dates are valid for testing provided they are transported and stored in accordance with the appropriate package insert, even if these expiration dates have passed.

K. Maintain proper storage conditions during specimen shipping to ensure the integrity of the specimen. Specimen stability under shipping conditions other than those recommended has not been evaluated.

L. Avoid cross-contamination during the specimen handling steps. Specimens can contain extremely high levels of virus or other organisms. Ensure that specimen containers do not come in contact with one another, and discard used materials without passing them over any open containers. Change gloves if they come in contact with specimens.

M. Do not use the reagents and controls after the expiration date.

N. Store assay components at the recommended storage condition. See *Reagent Storage and Handling Requirements (*page 5), and *Panther System Test Procedure* (page 12) for more information.

O. Do not combine any assay reagents or fluids. Do not top off reagents or fluids; the Panther system verifies reagent levels.

P. Avoid microbial and ribonuclease contamination of reagents.

Q. Do not use material that may contain Guanidinium thiocyanate or any guanidine-containing materials on the instrument. Highly reactive and/or toxic compounds may form if combined with sodium hypochlorite.

R. A reagent in this kit is labeled with risk and safety symbols.

Note: Hazard Communication reflects the EU Safety Data Sheets (SDS) classification. For hazard communication information specific to your region, refer to the region specific SDS on the Safety Data Sheet Library at www.hologicsds.com.

Selection Reagent
BORIC ACID 1-5%
WARNING
H315 - Causes skin irritation

Reagent Storage and Handling Requirements

A. The following reagents are stable when stored at 2°C to 8°C (refrigerated):

Aptima SARS-CoV-2 Amplification Reagent

Aptima SARS-CoV-2 Enzyme Reagent

Aptima SARS-CoV-2 Probe Reagent

Aptima SARS-CoV-2 Internal Control

Aptima SARS-CoV-2 Positive Control

Aptima SARS-CoV-2 Negative Control

B. The following reagents are stable when stored at 2°C to 30°C:

Aptima SARS-CoV-2 Amplification Reconstitution Solution

Aptima SARS-CoV-2 Enzyme Reconstitution Solution

Aptima SARS-CoV-2 Probe Reconstitution Solution

Aptima SARS-CoV-2 Selection Reagent

C. The following reagents are stable when stored at 15°C to 30°C (room temperature):

Aptima SARS-CoV-2 Target Capture Reagent

Aptima Wash Solution

Aptima Buffer for Deactivation Fluid

Aptima Oil Reagent

D. Working Target Capture Reagent (wTCR) is stable for 30 days when stored at 15°C to 30°C. Do not refrigerate.

E. After reconstitution, the Enzyme Reagent, Amplification Reagent, and Probe Reagent are stable for 30 days when stored at 2°C to 8°C.

F. Discard any unused reconstituted reagents and wTCR after 30 days or after the Master Lot expiration date, whichever comes first.

G. Controls are stable until the date indicated on the vials.

H. Reagents stored on-board the Panther System have 72 hours of on-board stability.

I. The Probe Reagent and Reconstituted Probe Reagent are photosensitive. Store the reagents protected from light. The specified reconstituted stability is based on 12 hours exposure of the Reconstituted Probe Reagent to two 60W fluorescent bulbs, at a distance of 17 inches (43 cm), and temperature less than 30°C. Light exposure of the Reconstituted Probe Reagent should be limited accordingly.

J. Upon warming to room temperature, some control tubes may appear cloudy or contain precipitates. Cloudiness or precipitation associated with controls does not affect control performance. The controls may be used whether they are clear or cloudy/precipitated. If clear controls are desired, solubilization may be expedited by incubating them at the upper end of the room temperature range (15°C to 30°C).

K. Do not freeze the reagents.

Specimen Collection and Storage

Specimens - Clinical material collected from patient placed in an appropriate transport system. For the Aptima SARS-CoV-2 assay, this includes NP, nasal, midturbinate and OP swab specimens, or nasopharyngeal wash/aspirate and nasal aspirate specimen collection in viral transport medium (VTM/UTM), saline, Liquid Amies, or specimen transport medium (STM).

Samples - Represents a more generic term to describe any material for testing on the Panther System including specimens, specimens transferred into a Panther Fusion Specimen Lysis Tube and controls.

Note: *Handle all specimens as if they contain potentially infectious agents. Use Universal Precautions.*

Note: *Take care to avoid cross-contamination during specimen handling steps. For example, discard used material without passing over open tubes.*

Swab Specimen Collection

Collect NP swab, nasal swab, and OP swab specimens according to standard technique using a polyester-, rayon-, or nylon-tipped swab. Immediately place the swab specimen into 3mL of VTM or UTM. Swab specimens may alternatively be added to saline, Liquid Amies or STM. The Aptima Multitest Swab Specimen Collection Kit may be used for the collection of OP and nasal swab samples.

After collection, specimens collected in VTM/UTM can be stored at 2°C to 8°C up to 96 hours before transferring to the Specimen Lysis Tube or transfer tube as described in the specimen processing section below. Remaining specimen volumes can be stored at ≤-70°C.

After collection, specimens in the Aptima Multitest Tube may be stored at 2°C to 30°C up to 6 days.

Note: *It is recommended that specimens transferred to the Aptima Multitest Tube are stored capped and upright in a rack.*

The following types of VTM/UTM can be used.

- Remel MicroTest M4, M4RT, M5 or M6 formulations
- Copan Universal Transport Medium
- BD Universal Viral Transport Medium

Note: *Do not use medium that may contain Guanidium thiocyanate or any guanidine-containing material.*

Nasopharyngeal Wash/aspirate and Nasal Aspirate Specimen Collection

Collect nasopharyngeal wash/aspirate and nasal aspirate specimens according to standard techniques.

Specimen Processing using the Panther Fusion Specimen Lysis Tube

A. Prior to testing on the Panther system, transfer 500 µL of the collected specimen* to a Panther Fusion Specimen Lysis Tube.

***Note:** When testing frozen specimen, allow specimen to reach room temperature prior to processing.*

***Note:** When using the Aptima SARS-CoV-2 uncapped tube assay software, prepare the Panther Fusion Specimen Lysis Tube as described below in Specimen* Processing using the Hologic Specimen Lysis Tube with Solid Cap.

Note:

Specimen Processing using the Hologic Specimen Lysis Tube with Solid Cap

A. Uncap the Hologic Specimen Lysis Tube and retain the cap.

B. Prior to testing on the Panther system, transfer 500 uL of the specimen to the Hologic Specimen Lysis Tube

C. It is recommended to recap the tube and gently invert three times to ensure viral inactivation and a homogeneous mixture.

D. To avoid contact with the top of the tube, loosen the cap and place the sample tube into the sample rack.

E. Remove and discard the cap. Inspect the sample tube. If bubbles are present, carefully remove from the sample tube (for example, use the tip of a sterile swab or similar method).

F. Place the rack retainer on the sample rack and load the rack into the instrument.

Note: *Specimen processing using the Hologic Specimen Lysis Tube is for use with the Aptima SARS-CoV-2 uncapped tube assay software.*

Specimen Processing using a Custom Specimen Lysis Tube

A. Using a sterile or non-sterile generic tube made of siliconized glass, polypropylene plastic or similar material that is 12 mm to 13 mm in outer diameter and 75 mm to 100 mm in height, aliquot 0.78 mL ± 0.07 mL of bulk STM into the tube using a pipet or repeat pipettor.

   Note: *If tubes are prepared prior to use, recap the tube and store at* 15°C to 30°C until use in specimen processing.

B. Uncap the custom Specimen Lysis Tube containing STM and retain the cap.

C. Prior to testing on the Panther system, transfer 500 µL of the specimen to the custom Specimen Lysis Tube containing STM.

D. It is recommended to recap the sample tube and gently invert three times to ensure viral inactivation and a homogeneous mixture.

E. To avoid contact with the top of the tube, loosen the cap and place the sample tube into the sample rack.

F. Remove and discard the cap. Inspect the sample tube. If bubbles are present, carefully remove from the tube (for example, use the tip of a sterile swab or similar method).

G. Place the rack retainer on the sample rack and load the rack into the instrument.

Note: *Specimen processing using the custom Specimen Lysis Tube is for use with the Aptima SARS-CoV-2 uncapped tube assay software.*

Specimen Processing using the Aptima Specimen Transfer Tube

A. Prior to testing on the Panther system, transfer 1 mL of the collected specimen* to an Aptima Specimen Transfer Tube**.

   Note: *When testing frozen specimen, allow specimen to reach room temperature prior to processing.*

   ****Note:*** *Alternatively, an unused Aptima Multitest Tube or Aptima Unisex Tube can be used.*

B. Recap the Aptima Specimen Transfer Tube tightly.

C. Gently invert the tube 2 to 3 times to ensure complete mixture of the specimen.

   Note: *The Aptima Specimen Transfer Tube cannot be tested on a system using the Aptima SARS-CoV-2 uncapped tube assay software.*

Specimen Processing for Specimen Collected with the Aptima Multitest Collection Kit

A. After placing the collected specimen* into the Aptima Multitest Tube using the Aptima Multitest Collection Kit, no further processing is required.

   Note: *When testing frozen specimen, allow specimen to reach room temperature prior to processing.*

   Note: *On a system using the Aptima SARS-CoV-2 uncapped tube assay software, transfer the collected specimen from the Aptima Multitest Tube to a Hologic Specimen Lysis Tube or custom Specimen Lysis Tube as described in the specimen processing sections above.*

Sample Storage

A. Samples on board the Panther system may be archived for additional testing at a later time.

B. Storing samples before or after testing

1. Samples in the Aptima Multitest Tube, Aptima Specimen Tube, or Specimen Lysis Tube should be stored upright in the rack under the following condition:
   - 2°C to 30°C up to 6 days
2. The samples should be covered with a new, clean plastic film or foil barrier.
3. If assayed samples need to be frozen or shipped, remove the penetrable cap and place a new non-penetrable cap on the specimen tubes. If samples need to be shipped for testing at another facility, recommended temperatures must be maintained. Prior to uncapping, specimen transport tubes must be centrifuged for 5 minutes at 420 Relative Centrifugal Force (RCF) to bring all of the liquid down to the bottom of the tube. Avoid splashing and cross-contamination.

   Note: *The Fisherbrand™ VersaClosure™ tube closure should not be used to cover tubes for freezing or shipping.*

Specimen Transport

Maintain specimen storage conditions as described in the *Specimen Collection and Storage section on* page 6.

Note: *Specimens must be shipped in accordance with applicable national, international, and regional transportation regulations.*

Panther System

Reagents for the Aptima SARS-CoV-2 assay are listed below for the Panther System. Reagent Identification Symbols are also listed next to the reagent name.

Reagents and Materials Provided

Aptima SARS-CoV-2 Assay Kit PRD-06419

250 tests (2 boxes)

Aptima SARS-CoV-2 Refrigerated Box (Box 1 of 2)
(store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity 250 test kit
A	**Aptima SARS-CoV-2 Amplification Reagent** *Non-infectious nucleic acids dried in buffered solution containing < 5% bulking agent.*	1 vial
E	**Aptima SARS-CoV-2 Enzyme Reagent** *Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing < 10% bulking reagent.*	1 vial
P	**Aptima SARS-CoV-2 Probe Reagent** *Non-infectious chemiluminescent DNA probes dried in succinate buffered solution containing < 5% detergent.*	1 vial
IC	**Aptima SARS-CoV-2 Internal Control**	1 vial

Aptima SARS-CoV-2 Room Temperature Box (Box 2 of 2)
(store at 15°C to 30°C upon receipt)

Symbol	Component	Quantity 250 test kit
AR	**Aptima SARS-CoV-2 Amplification Reconstitution Solution** *Aqueous solution containing preservatives.*	1 x 27.7 mL
ER	**Aptima SARS-CoV-2 Enzyme Reconstitution Solution** *HEPES buffered solution containing a surfactant and glycerol.*	1 x 11.1 mL
PR	**Aptima SARS-CoV-2 Probe Reconstitution Solution** *Succinate buffered solution containing < 5% detergent.*	1 x 35.4 mL
S	**Aptima SARS-CoV-2 Selection Reagent** *600 mM borate buffered solution containing surfactant.*	1 x 108 mL
TCR	**Aptima SARS-CoV-2 Target Capture Reagent** *Buffered salt solution containing solid phase and capture oligomers.*	1 x 54 mL
	Reconstitution Collars	3
	Master Lot Barcode Sheet	1 sheet

Materials Required and Available Separately

Note: *Materials available from Hologic have catalog numbers listed, unless otherwise specified.*

	Cat. No.
Panther System	303095
Aptima Assay Fluids Kit *(Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent)*	303014 (1000 tests)
Aptima Auto Detect Kit	303013 (1000 tests)
Multi-tube units (MTUs)	104772-02
Panther Waste Bag Kit	902731
Panther Waste Bin Cover	504405
Or Panther Run Kit *contains MTUs, waste bags, waste bin covers, assay fluids, and auto detects*	303096 (5000 tests)
Tips, 1000 µL conductive, liquid sensing	10612513 (Tecan)
Aptima SARS-CoV-2 Controls Kit *PC - Aptima SARS-CoV-2 Positive Control. Non-infectious nucleic acid in a buffered solution containing < 5% detergent. Quantity 5 x 1.7 mL* *NC - Aptima SARS-CoV-2 Negative Control. A buffered solution containing <5% detergent. Quantity 5 x 1.7 mL*	PRD-06420
Aptima Multitest Swab Specimen Collection Kit	PRD-03546
Aptima Specimen Transfer Kit	301154C
Aptima Specimen Transfer Kit - printable	PRD-05110
Aptima Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens	301041
Panther Fusion Specimen Lysis Tubes, 100 per bag *tube contains 0.71 mL of STM with a penetrable cap*	PRD-04339
Hologic Specimen Lysis Tubes, 100 each *tube contains 0.71 mL of STM with a solid cap*	PRD-06554
Hologic Specimen Lysis Tubes, 1200 each *tube contains 0.71 mL of STM with a solid cap*	PRD-06660
Specimen Transport Medium, 1 bottle, 80 mL	PRD-04423
Specimen Transport Medium, 1 bottle, 120 mL	PRD-06657
Bleach, 5% to 7% (0.7M to 1.0M) sodium hypochlorite solution	—
Disposable gloves	—
Hologic Solid Replacement Caps, 100 per bag	PRD-06720
Fisherbrand VersaClosure Tube Closures*, 1000 per pack **a single-use tube cover for the Hologic Specimen Lysis Tube (PRD-06554 only) after testing*	02-707

	Cat. No.
Replacement Caps for the 250-test kits	—
Amplification and Probe reagent reconstitution solutions	*CL0041 (100 caps)*
Enzyme Reagent reconstitution solution	*501616 (100 caps)*
TCR and Selection reagent	*CL0040 (100 caps)*

Optional Materials

	Cat. No.
Hologic Bleach Enhancer for Cleaning *for routine cleaning of surfaces and equipment*	302101
Tube rocker	—

Panther System Test Procedure

Note: *Refer to the Panther/Panther System Operator's Manual for additional procedural information.*

A. Work Area Preparation

Clean work surfaces where reagents and samples will be prepared. Wipe down work surfaces with 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution. Allow the sodium hypochlorite solution to contact surfaces for at least 1 minute and then follow with a water rinse. Do not allow the sodium hypochlorite solution to dry. Cover the bench surface on which the reagents and samples will be prepared with clean, plastic-backed absorbent laboratory bench covers.

B. Reagent Reconstitution/Preparation of a New Kit

Note: *Reagent reconstitution should be performed prior to beginning any work on the Panther System.*

1. To reconstitute Amplification, Enzyme, and Probe Reagents, combine the bottles of lyophilized reagent with the reconstitution solution. If refrigerated, allow the reconstitution solutions to reach room temperature before use.
   a. Pair each reconstitution solution with its lyophilized reagent. Ensure that the reconstitution solution and reagent have matching label colors before attaching the reconstitution collar.
   b. Check the lot numbers on the Master Lot Barcode Sheet to ensure that the appropriate reagents are paired.
   c. Open the lyophilized reagent vial and firmly insert the notched end of the reconstitution collar into the vial opening (Figure 1, Step 1).
   d. Open the matching reconstitution solution, and set the cap on a clean, covered work surface.
   e. While holding the reconstitution solution bottle on the bench, firmly insert the other end of the reconstitution collar into the bottle opening (Figure 1, Step 2).

f. Slowly invert the assembled bottles. Allow the solution to drain from the bottle into the glass vial (Figure 1, Step 3).

g. Thoroughly mix the solution in the glass vial by swirling (Figure 1, Step 4).

h. Wait for the lyophilized reagent to go into solution, then invert the assembled bottles again, tilting at a 45° angle to minimize foaming (Figure 1, Step 5). Allow all of the liquid to drain back into the plastic bottle.

i. Remove the reconstitution collar and glass vial (Figure 1, Step 6).

j. Recap the plastic bottle. Record operator initials and reconstitution date on the label (Figure 1, Step 7).

k. Discard the reconstitution collar and glass vial (Figure 1, Step 8).

Option: Additional mixing of the Amplification, Enzyme, and Probe Reagents using a tube rocker is allowed. The reagents may be mixed by placing the recapped plastic bottle on a tube rocker set to 20 RPM (or equivalent) for a minimum of 5 minutes.

Warning: *Avoid creating foam when reconstituting reagents. Foam compromises the level-sensing in the Panther System.*

Warning: *Adequate mixing of the reagents is necessary to achieve expected assay results.*

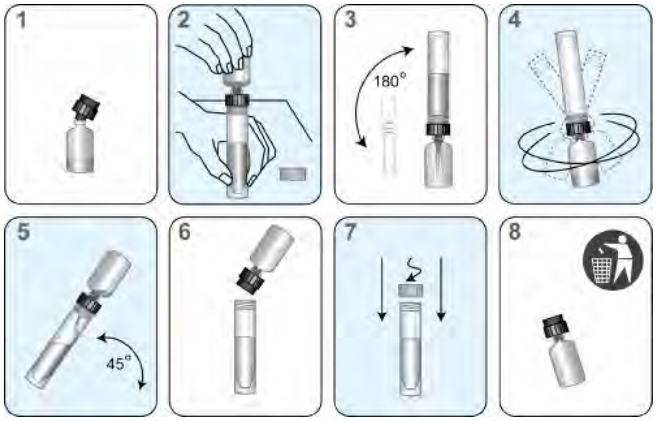


Figure 1. Panther System Reconstitution Process

2. Prepare Working Target Capture Reagent (wTCR)

a. Pair the appropriate bottles of TCR and IC.

b. Check the reagent lot numbers on the Master Lot Barcode Sheet to make sure that the appropriate reagents in the kit are paired.

c. Open the bottle of TCR, and set the cap on a clean, covered work surface.

d. Open the IC bottle and pour the entire contents into the bottle of TCR. Expect a small amount of liquid to remain in the IC bottle.

e. Cap the bottle of TCR and gently swirl the solution to mix the contents. Avoid creating foam during this step.

f. Record operator initials and the current date on the label.

g. Discard the IC bottle and cap.

3. Prepare Selection Reagent
   a. Check the lot number on the reagent bottle to make sure it matches the lot number on the Master Lot Barcode Sheet.
   b. Record operator initials and the current date on the label.

Note: *Thoroughly mix by gently inverting all reagents prior to loading on the system. Avoid creating foam during inversion of reagents.*

C. Reagent Preparation for Previously Reconstituted Reagents

1. Previously reconstituted Amplification, Enzyme, and Probe Reagents must reach room temperature (15°C to 30°C) prior to the start of the assay.

   Option: The reagents may be brought to room temperature by placing the reconstituted Amplification, Enzyme, and Probe Reagents on a tube rocker set to 20 RPM (or equivalent) for a minimum of 25 minutes.

2. If reconstituted Probe Reagent contains precipitate that does not return to solution at room temperature, heat the capped bottle at a temperature that does not exceed 62°C for 1 to 2 minutes. After this heat step, the Probe Reagent may be used even if residual precipitate remains. Mix Probe Reagent by inversion, being careful not to induce foam, prior to loading onto the system.
3. Thoroughly mix each reagent by gently inverting prior to loading on the system. Avoid creating foam during inversion of reagents. This step is not required if reagents are loaded onto the system directly after mixing on the tube rocker.
4. Do not top off reagent bottles. The Panther System will recognize and reject bottles that have been topped off.
5. *Adequate mixing of the reagents is necessary to achieve expected assay results.*

D. Specimen Handling using Panther Fusion Specimen Lysis Tube or Aptima Specimen Transfer Tube

Note: *Prepare specimens per the Specimen Processing instructions in the Specimen Collection and Storage section before loading specimens onto the Panther system.*

1. Inspect sample tubes before loading into the rack. If a sample tube contains bubbles or has a lower volume than is typically observed, gently tap the bottom of the tube to bring contents to the bottom.

Note: *For samples transferred to the Panther Fusion Specimen Lysis Tube or the Aptima Specimen Transfer Tube, to avoid a processing error, ensure adequate specimen volume is added to the tube. When adequate collected specimen is added to the tube, there is sufficient volume to perform 3 nucleic acid extractions.*

E. Specimen Handling using Hologic Specimen Lysis Tube or custom Specimen Lysis Tube

1. Prepare specimens per the specimen processing instructions in the *Specimen Collection and Storage* section.

Note: *For samples transferred to the Hologic Specimen Lysis Tube or a custom Specimen Lysis Tube, to avoid a processing error, ensure adequate specimen volume is added to the tube. When adequate collected specimen is added to the tube, there is sufficient volume to perform 2 nucleic acid extractions.*

Note: *When using the Aptima SARS-CoV-2 uncapped tube assay software, remove the cap from the Positive and Negative control before loading onto the Panther system.*

F. System Preparation

1. Set up the system according to the instructions in the *Panther/Panther Fusion System Operator's Manual* and *Procedural Notes*. Make sure that the appropriately sized reagent racks and TCR adapters are used.
2. Load samples.

Procedural Notes

A. Controls

1. To work properly with the Aptima Assay software for the Panther system, one pair of controls is required. The Aptima SARS-CoV-2 positive and negative controls can be loaded in any rack position or in any Sample Bay Lane on the Panther system. Patient specimen pipetting will begin when one of the following two conditions has been met:
   a. A pair of controls is currently being processed by the system.
   b. Valid results for the controls are registered on the system.
2. Once the control tubes have been pipetted and are processing for a specific reagent kit, patient specimens can be run with the associated kit up to 24 hours unless:
   a. Controls results are invalid.
   b. The associated assay reagent kit is removed from the system.
   c. The associated assay reagent kit has exceeded stability limits.
3. Each Aptima control tube can be tested once. Attempts to pipette more than once from the tube can lead to processing errors.
4. Patient specimen pipetting begins when one of the following two conditions is met:
   a. Valid results for the controls are registered on the system.
   b. A pair of controls is currently in process on the system.

B. Temperature

Room temperature is defined as 15°C to 30°C.

C. Glove Powder

As in any reagent system, excess powder on some gloves may cause contamination of opened tubes. Powderless gloves are recommended.

D. Lab Contamination Monitoring Protocol for the Panther System

There are many laboratory-specific factors that may contribute to contamination, including testing volume, workflow, disease prevalence and various other laboratory activities. These factors should be taken into consideration when contamination monitoring frequency is being established. Intervals for contamination monitoring should be established based on each laboratory's practices and procedures.

To monitor for laboratory contamination, the following procedure may be performed using the Aptima Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens:

1. Label swab transport tubes with numbers corresponding to the areas to be tested.
2. Remove the specimen collection swab (blue shaft swab with green printing) from its packaging, wet the swab in the specimen transport medium (STM), and swab the designated area using a circular motion.
3. Immediately insert the swab into transport tube.
4. Carefully break the swab shaft at the score line; use care to avoid splashing of the contents.
5. Recap the swab transport tube tightly.
6. Repeat Steps 2 to 5 for each area to be swabbed.

E. If the results are positive, see *Interpretation of Results*. For additional Panther system-specific contamination monitoring information, contact Hologic Technical Support.

Quality Control

A run or specimen result may be invalidated by the Panther system if problems occur while performing the assay. Specimens with invalid results must be retested.

Negative and Positive Controls

To generate valid results, a set of assay controls must be tested. One replicate of the negative assay control and positive assay control must be tested each time a new kit is loaded on the Panther system or when the current set of valid controls have expired.

The Panther system is configured to require assay controls run at an administrator-specified interval of up to 24 hours. Software on the Panther system alerts the operator when assay controls are required and does not start new tests until the assay controls are loaded and have started processing.

During processing, criteria for acceptance of the assay controls are automatically verified by the Panther system. To generate valid results, the assay controls must pass a series of validity checks performed by the Panther system.

If the assay controls pass all validity checks, they are considered valid for the administrator-specified time interval. When the time interval has passed, the assay controls are expired by the Panther system which requires a new set of assay controls be tested prior to starting any new samples.

If any one of the assay controls fails the validity checks, the Panther system automatically invalidates the affected samples and requires a new set of assay controls be tested prior to starting any new samples.

Internal Control

An internal control is added to each sample with the wTCR. During processing, the internal control acceptance criteria are automatically verified by the Panther system software. Detection of the internal control is not required for samples that are positive for SARS-CoV-2. The internal control must be detected in all samples that are negative for SARS-CoV-2 targets; samples that fail to meet that criteria will be reported as Invalid. Each sample with an Invalid result must be retested.

The Panther system is designed to accurately verify processes when procedures are performed following the instructions provided in this package insert and the *Panther/Panther Fusion System Operator's Manual.*

Interpretation of Results

The Panther system automatically determines the test results for samples and controls. A test result may be negative, positive, or invalid.

Table1 shows the possible results reported in a valid run with result interpretations.

Table 1: Result Interpretation

SARS-CoV-2 Result	IC Result	Interpretation
Neg	Valid	SARS-CoV-2 not detected.
POS	Valid	SARS-CoV-2 detected.
Invalid	Invalid	Invalid. There was an error in the generation of the result; retest sample.

Note: Detection of internal control is not required for samples that are positive for SARS-CoV-2.

Limitations

A. Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.

B. Reliable results are dependent on adequate specimen collection, transport, storage, and processing.

C. Avoid contamination by adhering to good laboratory practices and to the procedures specified in this package insert.

D. A positive result indicates the detection of nucleic acid from the relevant virus. Nucleic acid may persist even after the virus is no longer viable.

E. Nasopharyngeal wash/aspirate or nasal aspirates and self-collected or healthcare provider collected nasal and midturbinate nasal swabs are additional acceptable upper respiratory specimens that can be tested with the Aptima SARS-CoV-2 assay; however, performance with these specimen types have not been determined.

Panther SARS-CoV-2 Assay Performance

Analytical Sensitivity

The analytical sensitivity (limit of detection or LoD) of the Aptima SARS-CoV-2 assay was determined by testing serial dilutions of pooled negative clinical nasopharyngeal swab specimens spiked with inactivated cultured SARS-CoV-2 virus (USA-WA1/2020; BEI Resources; NR-52281). Ten replicates of each serial dilution were evaluated using each of two assay reagent lots across two Panther systems. The LoD was determined to be 0.01 $TCID_{50}$/mL and verified by testing an additional 20 replicates with one assay reagent lot. The LoD was also confirmed using saline, Liquid Amies and specimen transport medium (STM) swab collection media.

The analytical sensitivity of the Aptima SARS-CoV-2 assay was additionally evaluated using reference material from three commercial vendors. Serial dilutions of the reference material were made in STM and 20 or more replicates at each level were tested using each of two assay reagent lots across two Panther systems. The reference materials and the lowest dilution levels resulting in ≥ 95% detection are listed in Table 2.

Table 2: Analytical Sensitivity Evaluation of Commercial Reference Material

Vendor	Name	Reference #	Lot #	Analytical Sensitivity
ZeptoMetrix	SARS-CoV-2 External Run control	NATSARS(COV2)-ERC	324332	83 Copies/mL
SeraCare	AccuPlex SARS-Cov-2 Reference Material	0505-0126	10483977	83 Copies/mL
Exact Diagnostic	SARS-CoV-2 Standard	COV019	20033001	83 Copies/mL

Analytical Sensitivity with the Aptima Specimen Transfer Tube Workflow

The determined 0.01 $TCID_{50}$/mL analytical sensitivity (limit of detection) of the Aptima SARS-CoV-2 assay was confirmed using the Aptima Specimen Transfer tube specimen preparation workflow. Confirmation was performed using inactivated cultured SARS-CoV-2 virus (USA-QA1/2020; BEI Resources; NR-52281) in negative clinical nasopharyngeal (NP) swab, saline, Liquid Amies and specimen transport medium (STM) swab collection media by testing 20 replicates with one reagent lot (Table 3).

Table 3: LoD Confirmation with the Aptima Specimen Transfer Workflow

Target	Matrix	N Valid	N Positive	% Positive	Avg kRLU	StdDev kRLU	%CV
Inactivated SARS-CoV-2 virus	NP Swab	20	20	100%	1063	61	5.8%
	STM	20	20	100%	1064	116	10.9%
	Saline	20	20	100%	1102	60	5.4%
	Liquid Amies	20	20	100%	1101	51	4.7%

Carryover Contamination

The carryover contamination rate of the Aptima SARS-CoV-2 assay for samples tested with the capped tube and uncapped tube workflows was determined. The evaluation consisted of testing high titer SARS-CoV-2 target panels ~5 logs above the assay LoD in a checkerboard pattern with negative panels in four runs on three Panther systems. The capped tube workflow had an observed carryover rate of 0%, whereas the uncapped tube workflow carryover rate was 0.67% with 5 of 744 negative samples evaluated giving a false positive result.

Inclusivity

The inclusivity of the Aptima SARS-CoV-2 assay was evaluated using *in silico* analysis of the assay target capture oligos, amplification primers, and detection probes in relation to 9,896 SARS-CoV-2 sequences available in the NCBI and GISAID gene databases. Any sequence with missing or ambiguous sequence information was removed from the analysis, resulting in 9,879 sequences evaluated for the first target region of the assay and 9,880 for the second target region. The *in silico* analysis showed 100% homology to the assay oligos of both target systems for 9,749 (98.5%) of the evaluated sequences and 100% homology to the assay oligos of at least one target system for all 9,896 sequences. There were no evaluated sequences with identified mismatches predicted to impact binding or performance of both target systems.

Analytical Specificity and Microbial Interference

The analytical specificity of the Aptima SARS-CoV-2 assay was evaluated by testing 30 microorganisms representing common respiratory pathogens or closely related species (Table 4). Bacteria were tested at 10^6 CFU/mL and viruses were tested at 10^5 $TCID_{50}$/mL, except where noted. Microorganisms were tested with and without the presence of SARS-CoV-2 inactivated virus at 3x LoD. Analytical specificity of the Aptima SARS-CoV-2 assay was 100% with no evidence of microbial interference.

In addition to microorganism testing, *in silico* analysis was performed to assess the specificity of the assay in relation to the microorganisms listed in Table 4. The *in silico* analysis showed no probable cross reactivity to any of the 112 GenBank sequences evaluated.

Table 4: Aptima SARS-CoV-2 Analytical Specificity and Microbial Interference Microorganisms

Microorganism	Concentration	Microorganism	Concentration
Human coronavirus 229E	1E+5 $TCID_{50}$/mL	Parainfluenza virus 1	1E+5 $TCID_{50}$/mL
Human coronavirus OC43	1E+5 $TCID_{50}$/mL	Parainfluenza virus 2	1E+5 $TCID_{50}$/mL
Human coronavirus HKU1[1]	1E+6 copies/mL	Parainfluenza virus 3	1E+5 $TCID_{50}$/mL
Human coronavirus NL63	1E+4 $TCID_{50}$/mL	Parainfluenza virus 4	1E+3 $TCID_{50}$/mL
SARS-coronavirus[1]	1E+6 copies/mL	Influenza A	1E+5 $TCID_{50}$/mL
MERS-coronavirus	1E+4 $TCID_{50}$/mL	Influenza B	2E+3 $TCID_{50}$/mL
Adenovirus (e.g. C1 Ad. 71)	1E+5 $TCID_{50}$/mL	Enterovirus (e.g. EV68)	1E+5 $TCID_{50}$/mL
Human Metapneumovirus (hMPV)	1E+6 $TCID_{50}$/mL	Rhinovirus	1E+4 $TCID_{50}$/mL
Respiratory syncytial virus	1E+5 $TCID_{50}$/mL	*Legionella pneumophila*	1E+6 CFU/mL
Chlamydia pneumoniae	1E+6 IFU/mL	*Mycobacterium tuberculosis*	1E+6 $TCID_{50}$/mL
Haemophilus influenzae	1E+6 CFU/mL	*Streptococcus pneumoniae*	1E+6 CFU/mL
Bordetella pertussis	1E+6 CFU/mL	*Streptococcus pyogenes*	1E+6 CFU/mL
Pneumocystis jirovecii (PJP)	1E+6 nuc/mL	*Streptococcus salivarius*	1E+6 CFU/mL
Candida albicans	1E+6 CFU/mL	*Mycoplasma pneumoniae*	1E+6 CFU/mL
Staphylococcus epidermidis	1E+6 CFU/mL	*Pseudomonas aeruginosa*	1E+6 CFU/mL
Pooled human nasal wash[2] - *to represent diverse microbial flora in human respiratory tract*	N/A		

[1] Cultured virus and whole genome purified nucleic acid for Human coronavirus HKU1 and SARS-coronavirus are not readily available. HKU1 and SARS-coronavirus IVTs corresponding to the ORF1ab gene regions targeted by the assay were used to evaluate cross-reactivity and microbial interference.

[2] In place of evaluating pooled human nasal wash, testing of 30 individual negative clinical NP swab specimens was performed to represent diverse microbial flora in the human respiratory tract.

Clinical Performance

The clinical performance of the Aptima SARS-CoV-2 assay was evaluated in comparison to the Panther Fusion SARS-CoV-2 assay (Hologic, Inc.) using a panel of remnant clinical specimens. For the study, remnant clinical nasopharyngeal specimens were collected from US patients with signs and symptoms of respiratory infection.

The Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) was calculated in relation to the Panther Fusion assay as the reference result, as shown in Table 5. The Aptima SARS-CoV-2 assay showed positive and negative agreements of 100% and 98.2%, respectively.

Nasopharyngeal wash/aspirate, nasal aspirates, nasal swabs and midturbinate nasal swabs are acceptable specimens to test for viral respiratory infections. However, performance with these specimen types has not been specifically evaluated with the Aptima SARS-CoV-2 assay.

Table 5: Aptima SARS-CoV-2 Clinical Agreement

		Panther Fusion SARS-CoV-2 Assay	
		Positive	Negative
Aptima SARS-CoV-2 Assay	Positive	50	1
	Negative	0	54

Positive Percent Agreement: (95% CI): 100% (92.9% – 100%)
Negative Percent Agreement: (95% CI): 98.2% (90.4% – 99.7%)
Overall Agreement: (95% CI): 99.0% (94.8% – 99.8%)

Clinical Performance with Contrived Panel

The clinical performance of the Aptima SARS-CoV-2 assay using the Aptima Specimen Transfer tube specimen preparation workflow was evaluated in comparison to a panel of contrived specimens. For the study, a panel of 115 remnant clinical nasopharyngeal specimens was tested using both the Panther Fusion Specimen Lysis Tube (Specimen Lysis Tube) and Aptima Specimen Transfer tube workflows. All specimens were collected from US patients with signs and symptoms of respiratory infection. The panel consisted of 65 SARS-CoV-2 positive and 50 SARS-CoV-2 negative specimens. Of the 65 positive specimens, 40 were at concentrations 0.5-2x LoD and 25 were at concentrations 3-5x LoD using inactivated cultured SARS-CoV-2 virus (USA-QA1/2020; BEI Resources; NR-52281) as the target.

The Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) for both specimen preparation workflows were calculated in relation to the expected result of the contrived specimen panel, as shown in Table 6 for the Aptima Specimen Transfer Tube and Table 7 for the Specimen Lysis Tube. Detection characteristics for the contrived specimens were calculated by target concentration, as shown in Table 8. Both specimen preparation workflows showed 100% agreement for the evaluated panels.

Table 6: Performance of the Aptima Specimen Transfer Tube Workflow Relative to Expected Results

		Expected Result		
		Positive	Negative	Total
Aptima Specimen Transfer Result	Positive	65	0	65
	Negative	0	50	50
	Total	65	50	115

Overall Agreement: 100% (96.8% – 100%)
Positive Agreement: 100% (94.4% – 100%)
Negative Agreement: 100% (92.9% – 100%)

Table 7: Performance of the Specimen Lysis Tube Workflow Relative to Expected Results

		Expected Result		
		Positive	Negative	Total
Specimen Lysis Tube Result	Positive	65	0	65
	Negative	0	50	50
	Total	65	50	115

Overall Agreement: 100% (96.8% – 100%)
Positive Agreement: 100% (94.4% – 100%)
Negative Agreement: 100% (92.9% – 100%)

Table 8: Detection Characteristics for Contrived Nasopharyngeal Swab Specimens

	Aptima Specimen Transfer Sample Workflow						Specimen Lysis Tube Sample Workflow					
Target Conc.	**n Valid**	**n Positive**	**% Positive**	**Average kRLU**	**St Dev kRLU**	**%CV**	**n Valid**	**n Positive**	**% Positive**	**Average kRLU**	**St Dev kRLU**	**%CV**
Neg	50	0	0	299	9.7	3.2	50	0	0	300	9.3	3.1
0.5x LoD	10	10	100	1050	208.5	19.9	10	10	100	1153	113.0	9.8
1.0x LoD	10	10	100	1176	102.1	8.7	10	10	100	1205	24.3	2.0
1.5x LoD	10	10	100	1222	31.6	2.6	10	10	100	1223	21.9	1.8
2.0x LoD	10	10	100	1225	22.6	1.8	10	10	100	1237	26.0	2.1
3.0x LoD	10	10	100	1228	13.6	1.1	10	10	100	1215	25.5	2.1
4.0x LoD	5	5	100	1238	16.7	1.4	5	5	100	1212	12.5	1.0
5.0x LoD	10	10	100	1237	18.2	1.5	10	10	100	1246	28.3	2.3

Clinical Performance with Naturally Infected Positive Specimens

The clinical performance of the Aptima SARS-CoV-2 assay using the Aptima Specimen Transfer tube specimen preparation workflow was evaluated in comparison to the Specimen Lysis Tube workflow tested with both the Aptima and Panther Fusion SARS-CoV-2 assays. For the study, three dilutions of 15 unique SARS-CoV-2 positive nasopharyngeal swab specimens were prepared and processed using both workflows. SARS-CoV-2 samples were previously determined to be positive using a non-Hologic molecular assay.

The positive percent agreement between the Aptima SARS-CoV-2 Assay using the Aptima Specimen Transfer Tube and the Specimen Lysis Tube workflows were 97.5% (87.1% – 99.6%) and 100% (91.0% – 100%), respectively, when compared to the Panther Fusion SARS-CoV-2 assay using the Specimen Lysis Tube workflow as reference. The positive percent agreement of the Aptima Specimen Transfer tube workflow was 95.0% (83.5% – 98.6%) when compared to the Specimen Lysis Tube workflow as reference.

Bibliography

1. **World Health Organization.** Q&A on coronaviruses (COVID-19). March 9, 2020. World Health Organization Web site https://www.who.int/news-room/q-a-detail/q-a-coronaviruses. Accessed March 10, 2020.
2. **Centers for Disease Control and Prevention.** https://www.cdc.gov/coronavirus/2019-ncov/prevent-getting-sick/how-covid-spreads.html. Accessed June 17, 2020.
3. **Centers for Disease Control and Prevention.** Coronavirus Disease 2019-(COVID-19) in the U.S. Updated March 10, 2020. Centers for Disease Control and Prevention Web site https://www.cdc.gov/coronavirus/2019-ncov/cases-in-us.html. Accessed March 10, 2020.
4. **Centers for Disease Control and Prevention.** Coronavirus Disease 2019 Information for Travel. Page last reviewed March 8, 2020. Centers for Disease Control and Prevention Web site https://www.cdc.gov/coronavirus/2019-ncov/travelers/index.html. Accessed March 10, 2020.
5. **Centers for Disease Control and Prevention.** Coronavirus Disease 2019-(COVID-19) Situation Summary. Updated March 9, 2020. Centers for Disease Control and Prevention Web site https://www.cdc.gov/coronavirus/2019-ncov/summary.html. Accessed March 10, 2020.
6. **Clinical & Laboratory Standards Institute.** Document M29 Protection of Laboratory Workers from Occupationally Acquired Infections. CLSI Web site https://clsi.org/standards/products/microbiology/documents/m29/. Accessed September 2017.

IVD

Hologic, Inc.
10210 Genetic Center Drive
San Diego, CA 92121 USA

Customer Support: +1 800 442 9892
customersupport@hologic.com

Technical Support: +1 888 484 4747
molecularsupport@hologic.com

For more contact information visit www.hologic.com.

AW-21677-001 Rev. 002
2020-10

AB726

GeneXpert
Powered By CEPHEID INNOVATION

Xpert® Xpress SARS-CoV-2

Instructions for Use
For Use Under an Emergency Use Authorization (EUA) Only

℞ only

REF XPRSARS-COV2-10

For Use with GeneXpert Dx or GeneXpert Infinity Systems

Trademark, Patents and Copyright Statements

Cepheid
904 Caribbean Drive
Sunnyvale, CA 94089 USA
Phone: +1 408 541 4191
Fax: +1 408 541 4192

Xpert® Xpress SARS-CoV-2

For use under the Emergency Use Authorization (EUA) only.

1 Proprietary Name

Xpert® Xpress SARS-CoV-2

2 Common or Usual Name

Xpert Xpress SARS-CoV-2

3 Intended Use

The Xpert Xpress SARS-CoV-2 test is a rapid, real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in upper respiratory specimens (such as nasopharyngeal, oropharyngeal, nasal, or mid-turbinate swab and/or nasal wash/ aspirate) collected from individuals suspected of COVID-19 by their healthcare provider.

Testing of nasopharyngeal, oropharyngeal, nasal, or mid-turbinate swab and nasal wash/aspirate specimens using the Xpert Xpress SARS-CoV-2 test run on the GeneXpert Dx and GeneXpert Infinity systems is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high and moderate complexity tests.

Testing of nasopharyngeal, nasal, or mid-turbinate swab specimens using the Xpert Xpress SARS-CoV-2 test run on the GeneXpert Xpress System (Tablet and Hub Configurations) is authorized to be distributed and used in patient care settings outside of the clinical laboratory environment.

Results are for the detection of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of active infection with SARS-CoV-2; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

Testing with the Xpert Xpress SARS-CoV-2 test is intended for use by trained operators who are proficient in performing tests using either GeneXpert Dx, GeneXpert Infinity and/or GeneXpert Xpress systems. The Xpert Xpress SARS-CoV-2 test is only for use under the Food and Drug Administration's Emergency Use Authorization.

4 Summary and Explanation

An outbreak of respiratory illness of unknown etiology in Wuhan City, Hubei Province, China was initially reported to the World Health Organization (WHO) on December 31, 2019.[1] Chinese authorities identified a novel coronavirus (2019-nCoV), which has resulted in thousands of confirmed human infections in multiple provinces throughout China and exported cases in several Southeast Asian countries and more recently the United States. Cases of severe illness and some deaths have been reported. The International Committee for Taxonomy of Viruses (ICTV) renamed the virus SARS-CoV-2.[2]

The Xpert Xpress SARS-CoV-2 test is a molecular *in vitro* diagnostic test that aids in the detection and diagnosis SARS-CoV-2 and is based on widely used nucleic acid amplification technology. The Xpert Xpress SARS-CoV-2 test contains primers and probes and internal controls used in RT-PCR for the *in vitro* qualitative detection of SARS-CoV-2 RNA in upper respiratory specimens.

The term "qualified laboratories" refers to laboratories in which all users, analysts, and any person reporting results from use of this device are proficient in performing real-time RT-PCR assays.

5 Principle of the Procedure

The Xpert Xpress SARS-CoV-2 test is an automated *in vitro* diagnostic test for qualitative detection of nucleic acid from SARS-CoV-2. The Xpert Xpress SARS-CoV-2 test is performed on GeneXpert Instrument Systems.

The GeneXpert Instrument Systems automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequences in simple or complex samples using real-time PCR assays. The systems consist of an instrument, computer, and preloaded software for running tests and viewing the results. The systems require the use of single-use disposable cartridges that hold the RT-PCR reagents and host the RT-PCR process. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the systems, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*.

The Xpert Xpress SARS-CoV-2 test includes reagents for the detection of RNA from SARS-CoV-2 in nasopharyngeal, oropharyngeal, nasal, or mid-turbinate swab and/or nasal wash/aspirate specimens. A Sample Processing Control (SPC) and a Probe Check Control (PCC) are also included in the cartridge utilized by the GeneXpert instrument. The SPC is present to control for adequate processing of the sample and to monitor for the presence of potential inhibitor(s) in the RT-PCR reaction. The SPC also ensures that the RT-PCR reaction conditions (temperature and time) are appropriate for the amplification reaction and that the RT-PCR reagents are functional. The PCC verifies reagent rehydration, PCR tube filling, and confirms that all reaction components are present in the cartridge including monitoring for probe integrity and dye stability.

The nasopharyngeal, oropharyngeal, nasal, or mid-turbinate swab specimen and/or nasal wash/aspirate specimen is collected and placed into a viral transport tube containing 3 mL transport medium or 3 mL of saline. The specimen is briefly mixed by rapidly inverting the collection tube 5 times. Using the supplied transfer pipette, the sample is transferred to the sample chamber of the Xpert Xpress SARS-CoV-2 cartridge. The GeneXpert cartridge is loaded onto the GeneXpert Instrument System platform, which performs hands-off, automated sample processing, and real-time RT-PCR for detection of viral RNA.

6 Reagents and Instruments

6.1 Materials Provided

The Xpert Xpress SARS-CoV-2 kit contains sufficient reagents to process 10 specimens or quality control samples. The kit contains the following:

Xpert Xpress SARS-CoV-2 Cartridges with Integrated Reaction Tubes	**10**
• Bead 1, Bead 2, and Bead 3 (freeze-dried)	1 of each per cartridge
• Lysis Reagent	1.5 mL per cartridge
• Binding Reagent	1.5 mL per cartridge
• Elution Reagent	3.0 mL per cartridge
Disposable Transfer Pipettes	**12 per kit**
CD	**1 per kit**
• Assay Definition File (ADF)	
• Instructions to import ADF into GeneXpert software	
Flyer	**1 per kit**
• Directions to locate the Product Insert on www.cepheid.com	

Note Safety Data Sheets (SDS) are available at www.cepheidinternational.com under the **SUPPORT** tab.

Note The bovine serum albumin (BSA) in the beads within this product was produced and manufactured exclusively from bovine plasma sourced in the United States. No ruminant protein or other animal protein was fed to the animals; the animals passed ante- and postmortem testing. During processing, there was no mixing of the material with other animal materials.

7 Storage and Handling

- Store the Xpert Xpress SARS-CoV-2 cartridges at 2-28°C.
- Do not open a cartridge lid until you are ready to perform testing.
- Do not use a cartridge that is wet or has leaked.

8 Materials Required but Not Provided

- GeneXpert Dx or GeneXpert Infinity systems (catalog number varies by configuration): GeneXpert instrument, computer, barcode scanner, operator manual.

  For GeneXpert Dx System: GeneXpert Dx software version 4.7b or higher

  For GeneXpert Infinity-80 and Infinity-48s systems: Xpertise software version 6.4b or higher

9 Materials Available but Not Provided

SeraCare AccuPlex™ Reference Material Kit, catalog number 0505-0126 (Order Code CEPHEID)

10 Warnings and Precautions

10.1 General

- For *in vitro* diagnostic use.
- For emergency use only.
- Positive results are indicative of presence of SARS-CoV-2-RNA.
- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
- Performance characteristics of this test have been established with the specimen types listed in the Intended Use Section only. The performance of this assay with other specimen types or samples has not been evaluated.
-  Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological specimens should be handled using standard precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention[3] and the Clinical and Laboratory Standards Institute.[4]
- Follow safety procedures set by your institution for working with chemicals and handling biological specimens.
- Consult your institution's environmental waste personnel on proper disposal of used cartridges, which may contain amplified material. This material may exhibit characteristics of federal EPA Resource Conservation and Recovery Act (RCRA) hazardous waste requiring specific disposal requirements. Check state and local regulations as they may differ from federal disposal regulations. Institutions should check the hazardous waste disposal requirements within their respective countries.

10.2 Specimens

- Maintain proper storage conditions during specimen transport to ensure the integrity of the specimen (see Section 12, Specimen Collection, Transport, and Storage). Specimen stability under shipping conditions other than those recommended has not been evaluated.

10.3 Assay/Reagent

- Do not open the Xpert Xpress SARS-CoV-2 cartridge lid except when adding specimen.
- Do not use a cartridge that has been dropped after removing it from the packaging.
- Do not shake the cartridge. Shaking or dropping the cartridge after opening the cartridge lid may yield non-determinate results.
- Do not place the sample ID label on the cartridge lid or on the barcode label on the cartridge.
- Do not use a cartridge with a damaged barcode label.
- Do not use a cartridge that has a damaged reaction tube.
-  Each single-use Xpert Xpress SARS-CoV-2 cartridge is used to process one test. Do not reuse processed cartridges.
-  Each single-use disposable pipette is used to transfer one specimen. Do not reuse disposable pipettes.
- Do not use a cartridge if it appears wet or if the lid seal appears to have been broken.
- Wear clean lab coats and gloves. Change gloves between the handling of each specimen.
- In the event of a spill of specimens or controls, wear gloves and absorb the spill with paper towels. Then, thoroughly clean the contaminated area with a 10% freshly prepared household chlorine bleach. Allow a minimum of two minutes of contact time. Ensure the work area is dry before using 70% denatured ethanol to remove bleach residue. Allow surface to dry completely before proceeding. Or, follow your institution's standard procedures for a contamination or spill event. For equipment, follow the manufacturer's recommendations for decontamination of equipment.

- Biological specimens, transfer devices, and used cartridges should be considered capable of transmitting infectious agents requiring standard precautions. Follow your institution's environmental waste procedures for proper disposal of used cartridges and unused reagents. These materials may exhibit characteristics of chemical hazardous waste requiring specific disposal. If country or regional regulations do not provide clear direction on proper disposal, biological specimens and used cartridges should be disposed per WHO [World Health Organization] medical waste handling and disposal guidelines.

11 Chemical Hazards[5,6]

- Signal Word: WARNING
- **UN GHS Hazard Statements**
  - Harmful if swallowed.
  - May be harmful in contact with skin.
  - Causes eye irritation.
- **UN GHS Precautionary Statements**
  - **Prevention**
    - Wash hands thoroughly after handling.
  - **Response**
    - Call a POISON CENTER or doctor/physician if you feel unwell.
    - If skin irritation occurs: Get medical advice/attention.
    - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
    - If eye irritation persists: Get medical advice/attention.

12 Specimen Collection, Transport, and Storage

Proper specimen collection, storage, and transport are critical to the performance of this test. Inadequate specimen collection, improper specimen handling and/or transport may yield a false result. See Section 12.1 for nasopharyngeal swab collection procedure, Section 12.2 for oropharyngeal swab collection procedure, Section 12.3 for nasal swab collection procedure, Section 12.4 for mid-turbinate swab collection procedure, and Section 12.5 for nasal wash/aspirate procedure. Nasopharyngeal, nasal, and mid-turbinate swabs and nasal wash/aspirate specimens can be stored at room temperature (15-30 °C) for up to 8 hours and refrigerated (2-8 °C) up to seven days until testing is performed on the GeneXpert Instrument Systems. For oropharyngeal swab specimen transport and storage requirements and additional information, refer to the CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons Under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19) using the link provided below.

https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html

12.1 Nasopharyngeal Swab Collection Procedure

Insert the swab into either nostril, passing it into the posterior nasopharynx (see Figure 1). Rotate swab by firmly brushing against the nasopharynx several times. Remove and place the swab into the tube containing 3 mL of viral transport medium or 3 mL of saline. Break swab at the indicated break line and cap the specimen collection tube tightly.

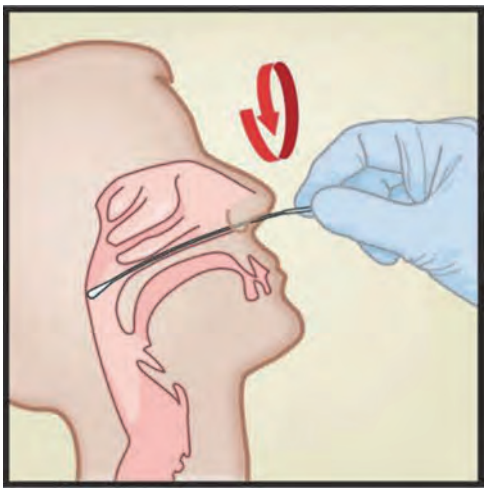


Figure 1. Nasopharyngeal Swab Collection

12.2 Oropharyngeal Swab Collection Procedure

1. Swab the posterior pharynx, tonsils, and other inflamed areas. Avoid touching the tongue, cheeks, and teeth with the swab when collecting specimens.
2. Remove and place the swab into the tube containing 3 mL of viral transport medium or 3 mL of saline. Break swab at the indicated break line and cap the specimen collection tube tightly.

12.3 Nasal Swab Collection Procedure

1. Insert a nasal swab 1 to 1.5 cm into a nostril. Rotate the swab against the inside of the nostril for 3 seconds while applying pressure with a finger to the outside of the nostril (see Figure 2).

Figure 2. Nasal Swab Collection for First Nostril

2. Repeat on the other nostril with the same swab, using external pressure on the outside of the other nostril (see Figure 3). To avoid specimen contamination, do not touch the swab tip to anything other than the inside of the nostril.

Figure 3. Nasal Swab Collection for Second Nostril

3. Remove and place the swab into the tube containing 3 mL of viral transport medium or 3 mL of saline. Break swab at the indicated break line and cap the specimen collection tube tightly.

12.4 Mid-Turbinate Swab Collection Procedure

1. Insert the mid-turbinate swab into either nostril, passing it into the mid-turbinate area (see Figure 4). Rotate swab by firmly brushing against the mid-turbinate area several times.
2. Remove and place the swab into the tube containing 3 mL of viral transport medium or 3 mL of saline. Break swab at the indicated break line and cap the specimen collection tube tightly.

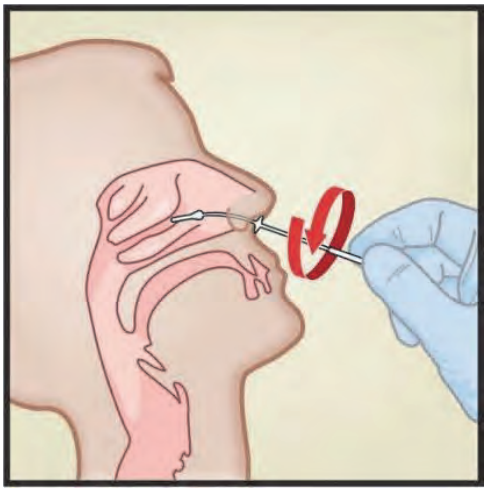


Figure 4. Mid-turbinate Swab Specimen Collection

12.5 Nasal Wash/Aspirate Procedure

Using a clean transfer pipette, transfer 600 µL of the sample into the tube containing 3 mL of viral transport medium or 3 mL of saline and then cap the tube.

13 Procedure

13.1 Preparing the Cartridge

Important **Start the test within 30 minutes of adding the sample to the cartridge.**

1. Remove a cartridge from the package.
2. Check the specimen transport tube is closed.
3. Mix specimen by rapidly inverting the specimen transport tube 5 times. Open cap on the specimen transport tube.

4. Open the cartridge lid.
5. Remove the transfer pipette from the wrapper.
6. Squeeze the top bulb of the transfer pipette completely and then place the pipette tip in the specimen transport tube (see Figure 5).

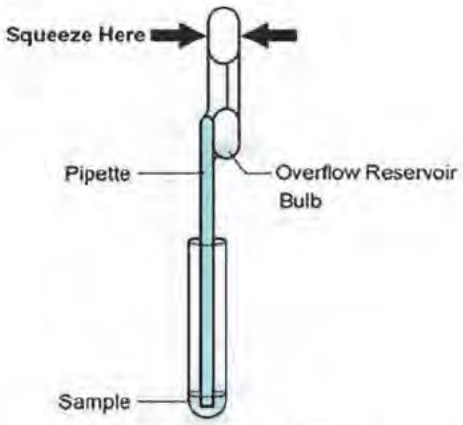


Figure 5. Transfer Pipette

7. Release the top bulb of the pipette to fill the pipette before removing from the tube. After filling pipette, excess sample will be seen in the overflow reservoir bulb of the pipette (see Figure 5). Check that the pipette does not contain bubbles.
8. To transfer the sample to the cartridge, squeeze the top bulb of the transfer pipette completely again to empty the contents of the pipette (300 µL) into the large opening (Sample Chamber) in the cartridge shown in Figure 6. Dispose of the used pipette.

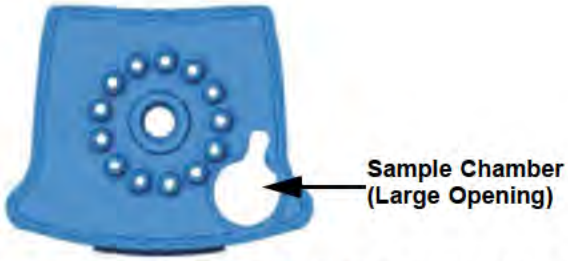


Figure 6. Xpert Xpress SARS-CoV-2 Cartridge (Top View)

Note Take care to dispense the entire volume of liquid into the Sample Chamber. False negative results may occur if insufficient sample is added to the cartridge.

9. Close the cartridge lid.

13.2 External Controls

External controls described in Section 9 are available but not provided and may be used in accordance with local, state, and federal accrediting organizations, as applicable.

To run a control using the Xpert Xpress SARS-CoV-2 test, perform the following steps:

1. Mix control by rapidly inverting the external control tube 5 times. Open cap on external control tube.
2. Open the cartridge lid.
3. Using a clean transfer pipette, transfer one draw of the external control sample (300 µL) into the large opening (Sample Chamber) in the cartridge shown in Figure 6.
4. Close cartridge lid.

13.3 Starting the Test

Note Before you start the test, make sure that the system contains modules with GeneXpert Dx software version 4.7b or higher or Infinity Xpertise software 6.4b or higher, and that the Xpert Xpress SARS-CoV-2 Assay Definition File is imported into the software.

This section lists the default steps to operate the GeneXpert Instrument System. For detailed instructions, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*, depending on the model that is being used.

Note The steps you follow may be different if the system administrator has changed the default workflow of the system.

1. Turn on the GeneXpert Instrument System:
   - **GeneXpert Dx:**
     If using the GeneXpert Dx instrument, first turn on the instrument and then turn on the computer. Log into the Windows operating system. The GeneXpert software may launch automatically or may require double-clicking on the GeneXpert Dx shortcut icon on the Windows® desktop.

     or
   - **GeneXpert Infinity System:**
     If using the GeneXpert Infinity instrument, power up the instrument by turning the power switch clockwise to the **ON** position. On the Windows desktop, double-click the Xpertise Software shortcut icon to launch the software.
2. Log on to the System software. The login screen appears. Type your user name and password.
3. In the GeneXpert System window, click **Create Test** (GeneXpert Dx) or **Orders** followed by **Order Test** (Infinity).
4. Scan or type in the Patient ID (optional). If typing the Patient ID, make sure the Patient ID is typed correctly. The Patient ID is shown on the left side of the View Results window and is associated with the test result.
5. Scan or type in the Sample ID. If typing the Sample ID, make sure the Sample ID is typed correctly. The Sample ID is shown on the left side of the View Results window and is associated with the test result.
6. Scan the barcode on the Xpert Xpress SARS-CoV-2 cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Reagent Lot ID, Cartridge SN, Expiration Date and Selected Assay.

Note If the barcode on the Xpert Xpress SARS-CoV-2 cartridge does not scan, then repeat the test with a new cartridge.

7. Click **Start Test** (GeneXpert Dx) or **Submit** (Infinity) if Auto-Submit is not enabled. In the dialog box that appears, type your password, if required.

   For the GeneXpert Dx Instrument

   A. Locate the module with the blinking green light, open the instrument module door and load the cartridge.

   B. Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off and the door will unlock. Remove the cartridge.

   C. Dispose of used cartridges in the appropriate sample waste containers according to your institution's standard practices.

   or

   For the GeneXpert Infinity System

   A. After clicking **Submit**, you will be asked to place the cartridge on the conveyor belt. After placing the cartridge, click **OK** to continue. The cartridge will be automatically loaded, the test will run and the used cartridge will be placed onto the waste shelf for disposal.

   B. When all samples are loaded, click on the **End Order Test** icon.

Note Do not turn off or unplug the instruments while a test is in progress. Turning off or unplugging the GeneXpert instrument or computer will stop the test.

14 Viewing and Printing Results

For detailed instructions on how to view and print the results, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*.

15 Quality Control

15.1 Internal Controls

CONTROL

Each cartridge includes a Sample Processing Control (SPC) and Probe Check Control (PCC).

Sample Processing Control (SPC) - Ensures that the sample was processed correctly. The SPC verifies that sample processing is adequate. Additionally, this control detects sample-associated inhibition of the real-time PCR assay, ensures that the PCR reaction conditions (temperature and time) are appropriate for the amplification reaction, and that the PCR reagents are functional. The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria.

Probe Check Control (PCC) - Before the start of the PCR reaction, the GeneXpert System measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, probe integrity, and dye stability. The PCC passes if it meets the validated acceptance criteria.

15.2 External Controls

External controls should be used in accordance with local, state, and federal accrediting organizations as applicable.

16 Interpretation of Results

The results are interpreted automatically by the GeneXpert System and are clearly shown in the **View Results** window. The Xpert Xpress SARS-CoV-2 test provides test results based on the detection of two gene targets according to the algorithms shown in Table 1.

Table 1. Xpert Xpress SARS-CoV-2 Possible Results

Result Text	N2	E	SPC
SARS-CoV-2 POSITIVE	+	+/-	+/-
SARS-CoV-2 PRESUMPTIVE POS	-	+	+/-
SARS-CoV-2 NEGATIVE	-	-	+
INVALID	-	-	-

See Table 2 to interpret test result statements for the Xpert Xpress SARS-CoV-2 test.

Table 2. Xpert Xpress SARS-CoV-2 Results and Interpretation

Result	Interpretation
SARS-CoV-2 POSITIVE	The 2019 novel coronavirus (SARS-CoV-2) target nucleic acids are detected. • The SARS-CoV-2 signal for the N2 nucleic acid target or signals for both nucleic acid targets (N2 and E) have a Ct within the valid range and endpoint above the minimum setting • SPC: NA; SPC is ignored because coronavirus target amplification occurred • Probe Check: PASS; all probe check results pass
SARS-CoV-2 PRESUMPTIVE POS	The 2019 novel coronavirus (SARS-CoV-2) nucleic acids may be present. Sample should be retested according to the Retest Procedure in Section 17.2. For samples with a repeated presumptive positive result, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management. • The SARS-CoV-2 signal for only the E nucleic acid target has a Ct within the valid range and endpoint above the minimum setting • SPC: NA; SPC is ignored because a target amplification has occurred. • Probe Check: PASS; all probe check results pass.

Table 2. Xpert Xpress SARS-CoV-2 Results and Interpretation (Continued)

Result	Interpretation
SARS-CoV-2 NEGATIVE	The 2019 novel coronavirus (SARS-CoV-2) target nucleic acids are not detected. • The SARS-CoV-2 signals for two nucleic acid targets (N2 and E) do not have a Ct within the valid range and endpoint above the minimum setting • SPC: PASS; SPC has a Ct within the valid range and endpoint above the minimum setting • Probe Check: PASS; all probe check results pass
INVALID	SPC does not meet acceptance criteria. Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. Repeat test according to the Retest Procedure in Section 17.2. • SPC: FAIL; SPC and SARS-CoV-2 signals do not have a Ct within valid range and endpoint below minimum setting • Probe Check: PASS; all probe check results pass
ERROR	Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. Repeat test according to the Retest Procedure in Section 17.2. • SARS-CoV-2: NO RESULT • SPC: NO RESULT • Probe Check: FAIL[1]; all or one of the probe check results fail [1] If the probe check passes, the error is caused by the maximum pressure limit exceeding the acceptable range, no sample added, or by a system component failure.
NO RESULT	Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. Repeat test according to the Retest Procedure in Section 17.2. A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress. • SARS-CoV-2: NO RESULT • SPC: NO RESULT • Probe Check: NA (not applicable)

The Xpert Xpress SARS-CoV-2 test includes an Early Assay Termination (EAT) function which will provide earlier time to results in high titer specimens. When SARS-CoV-2 titers are high enough to initiate the EAT function, the SPC amplification curve may not be seen and its results may not be reported.

17 Retests

17.1 Reasons to Repeat the Assay

If any of the test results mentioned below occur, repeat the test once according to instructions in Section 17.2, Retest Procedure.

- A **PRESUMPTIVE POS** result indicates the 2019 novel coronavirus (SARS-CoV-2) nucleic acids may be present. Only one of the SARS-CoV-2 nucleic acid target was detected (E gene) while the other SARS-CoV-2 nucleic acid target (N2 gene) was not detected.
- An **INVALID** result indicates that the control SPC failed. The sample was not properly processed, PCR is inhibited, or the sample was not properly collected.
- An **ERROR** result could be due to, but not limited to, Probe Check Control failure, system component failure, no sample added, or the maximum pressure limits were exceeded.
- A **NO RESULT** indicates that insufficient data were collected. For example, cartridge failed integrity test, the operator stopped a test that was in progress, or a power failure occurred.

If an External Control fails to perform as expected, repeat external control test and/or contact Cepheid for assistance.

17.2 Retest Procedure

To retest a non-determinate result (**INVALID**, **NO RESULT**, or **ERROR)** or a **PRESUMPTIVE POS** result, use a new cartridge.

Use the leftover sample from the original specimen transport medium tube or new external control tube.

1. Put on a clean pair of gloves. Obtain a new Xpert Xpress SARS-CoV-2 cartridge and a new transfer pipette.
2. Check the specimen transport tube or external control tube is closed.
3. Mix the sample by rapidly invert the specimen transport medium tube or external control tube 5 times. Open the cap on the specimen transport tube or external control tube.
4. Open the cartridge lid.
5. Using a clean transfer pipette (supplied), transfer sample (one draw) to the sample chamber with the large opening in the cartridge.
6. Close the cartridge lid.

18 Limitations

- Performance of the Xpert Xpress SARS-CoV-2 test has only been established in nasopharyngeal swab and nasal wash/aspirate specimens. Use of the Xpert Xpress SARS-CoV-2 test with other specimen types has not been assessed and performance characteristics are unknown.
- Oropharyngeal, nasal swabs and mid-turbinate swabs are considered acceptable specimen types for use with the Xpert Xpress SARS-CoV-2 test but performance with these specimen types has not been established. Testing of nasal and mid-turbinate nasal swabs (self-collected under supervision of or collected by a healthcare provider) is limited to patients with symptoms of
COVID-19. Please refer to FDA's FAQs on Diagnostic testing for SARS-CoV-2 for additional information.
- A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if inadequate numbers of organisms are present in the specimen.
- As with any molecular test, mutations within the target regions of Xpert Xpress SARS-CoV-2 could affect primer and/or probe binding resulting in failure to detect the presence of virus.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.

19 Conditions of Authorization for Laboratory and Patient Care Settings

The Cepheid Xpert Xpress SARS-CoV-2 Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd

However, to assist clinical laboratories and/or Patient Care Settings using the Xpert Xpress SARS-CoV-2 (referred to in the Letter of Authorization as "Your Product"), the relevant Conditions of Authorization are listed below.

- Authorized laboratories[1] and patient care settings using your product will include with result reports of the Xpert Xpress SARS-CoV-2 test, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories using your product will use your product as outlined in the Xpert Xpress SARS-CoV-2 Instructions for Use - For Use with GeneXpert Dx or GeneXpert Infinity systems. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use the Xpert Xpress SARS-CoV-2 test are not permitted.
- Patient Care Settings using your product will use your product as outlined in the Xpress SARS-CoV-2 Instructions for Use - For Use with GeneXpert Xpress System and associated Quick Reference Instructions for Xpert Xpress SARS-CoV-2 and GeneXpert Xpress System (Hub configuration), and Quick Reference Instructions for Xpert Xpress SARS-CoV-2 and GeneXpert Xpress System (Tablet configuration). Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.
- Authorized laboratories and patient care settings will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories and patient care settings that receive your product will notify the relevant public health authorities of their intent to run your product prior to initiating testing.

- Authorized laboratories and patient care settings using the Xpert Xpress SARS-CoV-2 test will collect information on the performance of the test and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA Reporting@fda.hhs.gov) and Cepheid (+1 888.838.3222 or techsupport@cepheid.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.
- All operators using your product must be appropriately trained in performing and interpreting the results of your product, use appropriate personal protective equipment when handling this kit, and use your product in accordance with the authorized labeling.
- You, authorized distributors, and authorized laboratories and patient care settings using your product will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[1] The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform moderate or high complexity tests" as "authorized laboratories."

20 Performance Characteristics

20.1 Clinical Evaluation - AccuPlex SARS-CoV-2 Reference Material

The performance of the Xpert Xpress SARS-CoV-2 test was evaluated using contrived clinical NP swab specimens in viral transport medium obtained from U.S. patients with signs and symptoms of respiratory infection. The samples were prepared by spiking each individual negative clinical NP swab sample with AccuPlex SARS-CoV-2 (a quantitated reference material - recombinant Sindbis virus particle containing target sequences from the SARS-CoV-2 genome) at 2x LoD, 3x LoD and 5x LoD levels. The NP swab samples were determined to be negative for SARS-CoV-2 prior to spiking. Individual negative NP swab samples were also tested in the study. All positive and negative samples in the study were tested in a randomized and blinded fashion.

Table 3 shows the number of concordant results out of the total number of samples tested for each target concentration of AccuPlex SARS-CoV-2, the mean Ct values for each of the E and N2 nucleic acid targets as well as the percent agreement with the 95% CI where appropriate. The results show 100% agreement with the expected results in the AccuPlex SARS-CoV-2 spiked samples and 100% agreement with the expected results in the negative samples.

Table 3. Xpert Xpress SARS-CoV-2 Test Agreement with the Expected Results by Target Concentration

Target Concentration	Number Concordant/ Number Tested	E Mean Ct	N2 Mean Ct	% Agreement [95% CI]
2x LoD	20/20	34.8	38.0	100% [83.9% - 100%]
3x LoD	5/5	33.7	37.1	100% [NA*]
5x LoD	5/5	33.7	36.8	100% [NA*]
Negative	35/35	NA	NA	100% [90.1% - 100%]

*95% CI not computed for sample concentrations with sample size of 5 or less.

20.2 Clinical Evaluation – Live SARS-CoV-2 Virus

The performance of the Xpert Xpress SARS-CoV-2 test was evaluated using contrived clinical NP swab specimens in viral transport medium obtained from U.S. patients with signs and symptoms of respiratory infection. The samples were prepared by spiking each individual negative clinical NP swab sample with live SARS-CoV-2 virus (USA_WA1/2020) at 2x LoD, 3x LoD and 5x LoD levels. The NP swab samples were determined to be negative for SARS-CoV-2 prior to spiking. Individual negative NP swab samples were also tested in the study. All positive and negative samples in the study were tested in a randomized and blinded fashion.

Table 4 shows the number of concordant results out of the total number of samples tested for each target concentration of live SARS-CoV-2 virus, the mean Ct values for each of the E and N2 nucleic acid targets as well as the percent agreement with the 95% confidence interval (95% CI), where appropriate. The results show 100% agreement with the expected results in the live SARS-CoV-2 virus spiked samples and 100% agreement with the expected results in the negative samples.

Table 4. Xpert Xpress SARS-CoV-2 Test Agreement with the Expected Results by Target Concentration

Target Concentration	Number Concordant/ Number Tested	E Mean Ct	N2 Mean Ct	% Agreement [95% CI]
2x LoD	20/20	35.4	38.4	100% [83.9% - 100%]
3x LoD	5/5	34.2	37.2	100% [NA*]
5x LoD	5/5	33.9	37.0	100% [NA*]
Negative	30/30	NA	NA	100% [88.7% - 100%]

* 95% CI not computed for sample concentrations with sample size of 5 or less.

21 Analytical Performance

21.1 Analytical Sensitivity (Limit of Detection) - AccuPlex SARS-CoV-2 Reference Material

Studies were performed to determine the analytical limit of detection (LoD) of the Xpert Xpress SARS-CoV-2. The LoD of Xpert Xpress SARS-CoV-2 was established using one lot of reagent and limiting dilutions of AccuPlex SARS-CoV-2 prepared in simulated background matrix and NP swab clinical matrix and probit analysis. Verification of the estimated LoD claim was performed on one reagent lot in replicates of 35 prepared in pooled NP swab clinical matrix. The LoD is the lowest concentration (reported as copies/mL) of AccuPlex SARS-Cov-2 recombinant viral sequence that can be reproducibly distinguished from negative samples ≥ 95% of the time with 95% confidence. The claimed LoD for the assay is 250 copies/mL (Table 5).

Table 5. Limit of Detection of the Xpert Xpress SARS-CoV-2

Material	Claimed LoD (copies/mL)	Positives/ Replicates
SARS-CoV-2 Reference Material	250	35/35

21.2 Analytical Sensitivity (Limit of Detection) – Live SARS-CoV-2 Virus

Studies were performed to determine the analytical limit of detection (LoD) of the Xpert Xpress SARS-CoV-2. The LoD of Xpert Xpress SARS-CoV-2 was established using one lot of reagent and limiting dilutions of live SARS-CoV-2 virus (USA_WA1/2020) prepared in viral transport medium and NP swab clinical matrix and probit analysis. Verification of the estimated LoD claim was performed on one reagent lot in replicates of 22 prepared in pooled NP swab clinical matrix. The LoD is the lowest concentration (reported as PFU/mL) of live SARS-CoV-2 virus samples that can be reproducibly distinguished from negative samples ≥ 95% of the time with 95% confidence. The claimed LoD for the assay is 0.0100 PFU/mL (Table 6).

Table 6. Limit of Detection of the Xpert Xpress SARS-CoV-2

Strain	Claimed LoD (PFU/mL)	E Mean Ct	N2 Mean Ct	Positives/ Replicates
SARS-CoV-2 virus (USA_WA1/2020)	0.0100	35.9	38.9	22/22

21.3 Analytical Reactivity (Inclusivity)

The inclusivity of Xpert Xpress SARS-CoV-2 was evaluated using *in silico* analysis of the assay primers and probes in relation to 324 SARS-CoV-2 sequences available in the GISAID gene database for two targets, E and N2.

For the E target, Xpert Xpress SARS-CoV-2 had 100% match to all sequences with the exception of 4 sequences that had a single mismatch. For the N2 target, Xpert Xpress SARS-CoV-2 had 100% match to all sequences with the exception of 2 sequences that had a single mismatch. None of these mismatches found for both targets are predicted to have a negative impact on the performance of the assay, given the location of the mutations in the primer and probe regions respectively for the two variants. These mutations are not predicted to adversely affect the probe and primer binding to the sequences or reduce assay efficiency.

21.4 Analytical Specificity (Exclusivity)

An *in silico* analysis for possible cross-reactions with all the organisms listed in Table 7 was conducted by mapping primers and probes in the Xpert Xpress SARS-CoV-2 test individually to the sequences downloaded from the GISAID database. E primers and probes are not specific for SARS-CoV-2 and will detect Human and Bat SARS-coronavirus. No potential unintended cross reactivity with other organisms listed in Table 7 is expected based on the *in silico* analysis.

Table 7. Xpert Xpress SARS-CoV-2 Analytical Specificity Microorganisms

Microorganisms from the Same Genetic Family	High Priority Organisms
Human coronavirus 229E	Adenovirus (e.g. C1 Ad. 71)
Human coronavirus OC43	Human Metapneumovirus (hMPV)
Human coronavirus HKU1	Parainfluenza virus 1-4
Human coronavirus NL63	Influenza A
SARS-coronavirus	Influenza B
MERS-coronavirus	Influenza C
Bat coronavirus	Enterovirus (e.g. EV68)
	Respiratory syncytial virus
	Rhinovirus
	Chlamydia pneumoniae
	Haemophilus influenzae
	Legionella pneumophila
	Mycobacterium tuberculosis
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Bordetella pertussis
	Mycoplasma pneumoniae
	Pneumocystis jirovecii (PJP)
	Parechovirus
	Candida albicans
	Corynebacterium diphtheriae
	Legionella non-pneumophila
	Bacillus anthracis (Anthrax)
	Moraxella catarrhalis
	Neisseria elongate and meningitidis
	Pseudomonas aeruginosa
	Staphylococcus epidermidis
	Staphylococcus salivarius
	Leptospira
	Chlamydia psittaci
	Coxiella burnetii (Q-Fever)
	Staphylococcus aureus

22 References

1. Centers for Disease Control and Prevention. https://www.cdc.gov/coronavirus/2019-ncov/index.html. Accessed February 9, 2020.
2. bioRxiv. (https://www.biorxiv.org/content/10.1101/2020.02.07.937862v1). Accessed March 3, 2020.
3. Centers for Disease Control and Prevention. *Biosafety in Microbiological and Biomedical laboratories* (refer to latest edition). http://www.cdc.gov/biosafety/publications/
4. Clinical and Laboratory Standards Institute. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline.* Document M29 (refer to latest edition).
5. REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on the classification labeling and packaging of substances and mixtures amending and repealing, List of Precautionary Statements, Directives 67/548/EEC and 1999/45/EC (amending Regulation (EC) No 1907/2007).
6. Occupational Safety and Health Standards, Hazard Communication, Toxic and Hazard Substances (March 26, 2012) (29 C.F.R., pt. 1910, subpt. Z).

23 Cepheid Headquarters Locations

Corporate Headquarters	European Headquarters
Cepheid 904 Caribbean Drive Sunnyvale, CA 94089 USA	Cepheid Europe SAS Vira Solelh 81470 Maurens-Scopont France
Telephone: +1 408 541 4191	Telephone: +33 563 825 300
Fax: +1 408 541 4192	Fax: +33 563 825 301
www.cepheid.com	www.cepheidinternational.com

24 Technical Assistance

Before contacting Cepheid Technical Support, collect the following information:

- Product name
- Lot number
- Serial number of the instrument
- Error messages (if any)
- Software version and, if applicable, Computer Service Tag number

Region	Telephone	Email
US	+1 888.838.3222	techsupport@cepheid.com
France	+33 563 825 319	support@cepheideurope.com
Australia New Zealand	+1800 130 821 +0800 001 028	techsupportANZ@cepheid.com

Contact information for all Cepheid Technical Support offices is available on our website: www.cepheid.com/en/CustomerSupport.

25 Table of Symbols

Symbol	Meaning
REF	Catalog number
IVD	*In vitro* diagnostic medical device
	Do not re-use
LOT	Batch code
	Consult instructions for use
	Caution
	Manufacturer
	Country of manufacture
	Contains sufficient for <n> tests
CONTROL	Control
	Expiration date
	Temperature limitation
	Biological risks
Rx only	For prescription use only

Cepheid
904 Caribbean Drive
Sunnyvale, CA 94089 USA
Phone: +1 408 541 4191
Fax: +1 408 541 4192

IVD

For use under Emergency Use Authorization (EUA) Only

AB744

GeneXpert
Powered By CEPHEID INNOVATION

Xpert® Xpress SARS-CoV-2/Flu/RSV

Instructions for Use
For Use Under an Emergency Use Authorization (EUA) Only

℞only

REF **XPCOV2/FLU/RSV-10**

For Use with GeneXpert Xpress System (point of care system)

Cepheid

For use under an Emergency Use Authorization (EUA) Only

IVD

302-4419, Rev. C January 2021

For *In Vitro* Diagnostic use

Trademark, Patents and Copyright Statements

Cepheid®, the Cepheid logo, GeneXpert® and Xpert® are trademarks of Cepheid.
Windows® is a trademark of Microsoft Corporation.

THE PURCHASE OF THIS PRODUCT CONVEYS TO THE BUYER THE NON-TRANSFERABLE RIGHT TO USE IT IN ACCORDANCE WITH THIS INSTRUCTIONS FOR USE. NO OTHER RIGHTS ARE CONVEYED EXPRESSLY, BY IMPLICATION OR BY ESTOPPEL. FURTHERMORE, NO RIGHTS FOR RESALE ARE CONFERRED WITH THE PURCHASE OF THIS PRODUCT.

Cepheid
904 Caribbean Drive
Sunnyvale, CA 94089 USA
Phone: +1 408 541 4191
Fax: +1 408 541 4192

Xpert Xpress SARS-CoV-2/Flu/RSV

For use under the Emergency Use Authorization (EUA) only.

1 Proprietary Name

Xpert® Xpress SARS-CoV-2/Flu/RSV

2 Common or Usual Name

Xpert Xpress SARS-CoV-2/Flu/RSV

3 Intended Use

The Xpert Xpress SARS-CoV-2/Flu/RSV test is a rapid, multiplexed real-time RT-PCR test intended for the simultaneous qualitative detection and differentiation of SARS-CoV-2, influenza A, influenza B, and respiratory syncytial virus (RSV) viral RNA in either nasopharyngeal swab, nasal swab or nasal wash/ aspirate specimens collected from individuals suspected of respiratory viral infection consistent with COVID-19 by their healthcare provider.[1] Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2, influenza, and RSV can be similar.

Testing of nasopharyngeal swab, nasal swab, or nasal wash/aspirate specimens using the Xpert Xpress SARS-CoV-2/Flu/RSV test run on the GeneXpert Dx and GeneXpert Infinity systems is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, that meet requirements to perform high or moderate complexity tests.

Testing of nasopharyngeal or nasal swab specimens using the Xpert Xpress SARS-CoV-2/Flu/RSV test run on the GeneXpert Xpress System (Tablet and Hub Configurations) is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation.

Results are for the simultaneous detection and differentiation of SARS-CoV-2, influenza A virus, influenza B virus and RSV nucleic acids in clinical specimens and is not intended to detect influenza C virus. SARS-CoV-2, influenza A, influenza B and RSV RNA identified by this test are generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of the identified virus, but do not rule out bacterial infection or co-infection with other pathogens not detected by the test.

Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all SARS-CoV-2 results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2, influenza A virus, influenza B virus and/or RSV infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and/or epidemiological information.

[1] For this EUA, a healthcare provider includes, but is not limited to, physicians, nurses, pharmacists, technologists, laboratory directors, epidemiologists, or any other practitioners or allied health professionals.

Testing with the Xpert Xpress SARS-CoV-2/Flu/RSV test is intended for use by trained operators who are proficient in performing tests using either GeneXpert Dx, GeneXpert Infinity and/or GeneXpert Xpress systems. The Xpert Xpress SARS-CoV-2/Flu/RSV test is only for use under the Food and Drug Administration's Emergency Use Authorization.

4 Summary and Explanation

An outbreak of respiratory illness of unknown etiology in Wuhan City, Hubei Province, China was initially reported to the World Health Organization (WHO) on December 31, 2019.[1] Chinese authorities identified a novel coronavirus (2019-nCoV), which has since spread globally, resulting in a pandemic of coronavirus disease 2019 (COVID-19). COVID-19 is associated with a variety of clinical outcomes, including asymptomatic infection, mild upper respiratory infection, severe lower respiratory disease including pneumonia and respiratory failure, and in some cases, death. The International Committee on Taxonomy of Viruses (ICTV) renamed the virus SARS-CoV-2.[2]

Influenza, or the flu, is a contagious viral infection of the respiratory tract. Transmission of influenza is primarily airborne (i.e., coughing or sneezing) and the peak of transmission usually occurs in the winter months. Symptoms commonly include fever, chills, headache, malaise, cough and sinus congestion. Gastrointestinal symptoms (i.e., nausea, vomiting or diarrhea) may also occur, primarily in children, but are less common. Symptoms generally appear within two days of exposure to an infected person. Pneumonia may develop as a complication due to influenza infection, causing increased morbidity and mortality in pediatric, elderly, and immunocompromised populations.[3, 4]

Influenza viruses are classified into types A, B, and C, the former two of which cause the most human infections. Influenza A (Flu A) is the most common type of influenza virus in humans and is generally responsible for seasonal flu epidemics and potentially pandemics. Flu A viruses can also infect animals such as birds, pigs, and horses. Infections with influenza B (Flu B) virus are generally restricted to humans and less frequently cause epidemics.[5] Flu A viruses are further divided into subtypes on the basis of two surface proteins: hemagglutinin (H) and neuraminidase (N). Seasonal flu is normally caused by influenza A subtypes H1, H2, H3, N1 and N2.

Respiratory Syncytial Virus (RSV), a member of the *Pneumoviridae* family (formerly *Paramyxoviridae*), consisting of two strains (subgroups A and B) is also the cause of a contagious disease that affects primarily infants, and the elderly who are immunocompromised (e.g. patients with chronic lung disease or undergoing treatment for conditions that reduce the strength of their immune system).[6] The virus can remain infectious for hours on countertops and toys and can cause both upper respiratory infections, such as colds, and lower respiratory infections manifesting as bronchiolitis and pneumonia.[6] By the age of two years, most children have already been infected by RSV and because only weak immunity develops, both children and adults can be re-infected.[6] Symptoms appear four to six days after infection and are usually self-limiting, lasting approximately one to two weeks in infants. In adults, infection lasts about 5 days and presents as symptoms consistent with a cold, such as rhinorrhea, fatigue, headache, and fever. The RSV season mirrors influenza somewhat as infections begin to rise during the fall through early spring.[5, 6]

Active surveillance programs in conjunction with infection prevention precautions are important components for preventing transmission of SARS-CoV-2, influenza and RSV.

The use of assays providing rapid results to identify patients infected with these viruses can be an important factor for effective control, proper choice of treatment, and prevention of widespread outbreaks.

The Xpert Xpress SARS-CoV-2/Flu/RSV test is a molecular *in vitro* diagnostic test that aids in the detection and differentiation of RNA from Flu A, Flu B, RSV and SARS-CoV-2 virus and is based on widely used nucleic acid amplification technology. The Xpert Xpress SARS-CoV-2/Flu/RSV test contains primers and probes and internal controls used in RT-PCR for the *in vitro* qualitative detection and differentiation of RNA from Flu A, Flu B, RSV and SARS-CoV-2 virus in upper respiratory specimens.

5 Principle of the Procedure

The Xpert Xpress SARS-CoV-2/Flu/RSV test is an automated *in vitro* diagnostic test for qualitative detection and differentiation of RNA from Flu A, Flu B, RSV and SARS-CoV-2 virus. The Xpert Xpress SARS-CoV-2/Flu/RSV test is performed on GeneXpert Xpress System.

The GeneXpert Xpress System automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequences in simple or complex samples using real-time PCR assays. The systems consist of an instrument, computer, and preloaded software for running tests and viewing the results. The systems require the use of single-use disposable cartridges that hold the RT-PCR reagents and host the RT-PCR process. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the systems, see the GeneXpert Xpress System User's Guide.

The Xpert Xpress SARS-CoV-2/Flu/RSV test includes reagents for the detection of RNA from Flu A, Flu B, RSV and SARS-CoV-2 virus in either nasopharyngeal swab or nasal swab specimens. A Sample Processing Control (SPC) and a Probe Check Control (PCC) are also included in the cartridge utilized by the GeneXpert instrument. The SPC is present to control for adequate processing of the sample and to monitor for the presence of potential inhibitor(s) in the RT-PCR reaction. The SPC also ensures that the RT-PCR reaction conditions (temperature and time) are appropriate for the amplification reaction and that the RT-PCR reagents are functional. The PCC verifies reagent rehydration, PCR tube filling, and confirms that all reaction components are present in the cartridge including monitoring for probe integrity and dye stability.

The nasopharyngeal swab or nasal swab specimen is collected and placed into a transport tube containing 3 mL of viral transport medium or 3mL of saline. The specimen is briefly mixed by rapidly inverting the collection tube 5 times. Using the supplied transfer pipette, the sample is transferred to the sample chamber of the Xpert Xpress SARS-CoV-2/Flu/RSV cartridge. The GeneXpert cartridge is loaded onto the GeneXpert Xpress System platform, which performs hands-off, automated sample processing, and real-time RT-PCR for detection of viral RNA.

6 Reagents and Instruments

6.1 Materials Provided

The Xpert Xpress SARS-CoV-2/Flu/RSV kit contains sufficient reagents to process 10 specimens or quality control samples. The kit contains the following:

Xpert Xpress SARS-CoV-2/Flu/RSV Cartridges with Integrated Reaction Tubes	**10**
• Bead 1, Bead 2, and Bead 3 (freeze-dried)	**1 of each per cartridge**
• Lysis Reagent	**1.0 mL per cartridge**
• Binding Reagent	**1.0 mL per cartridge**
• Elution Reagent	**3.0 mL per cartridge**
• Wash Reagent	**0.4 mL per cartridge**
Disposable Transfer Pipettes	**10-12 per kit**
Flyer	**1 per kit**

- Instructions to locate (and import) the ADF and EUA documentation such as the Product Insert on www.cepheid.com

Quick Reference Instructions (For use with the GeneXpert Xpress Systems – Tablet and Hub Configuration)	**2 per kit**

Note Safety Data Sheets (SDS) are available at www.cepheid.com or www.cepheidinternational.com under the **SUPPORT** tab.

Note The bovine serum albumin (BSA) in the beads within this product was produced and manufactured exclusively from bovine plasma sourced in the United States. No ruminant protein or other animal protein was fed to the animals; the animals passed ante- and post-mortem testing. During processing, there was no mixing of the material with other animal materials.

7 Storage and Handling

- Store the Xpert Xpress SARS-CoV-2/Flu/RSV cartridges at 2-28°C.
- Do not open a cartridge lid until you are ready to perform testing.
- Do not use a cartridge that is wet or has leaked.

8 Materials Required but Not Provided

- GeneXpert Xpress System (Tablet configuration): GeneXpert Xpress II and IV instruments with proprietary GeneXpert Xpress Software Version 5.0 and 5.1, tablet computer device with touchscreen, barcode scanner, external CD drive, wireless printer, Getting Started Guide, and GeneXpert Xpress System User's Guide.
- GeneXpert Xpress System (Hub configuration): GeneXpert Xpress IV instrument, GeneXpert Hub with proprietary GeneXpert Xpress Software Version 6.1 or higher,

GeneXpert Hub with integrated computer, touchscreen monitor and barcode scanner, external CD drive, Getting Started Guide, and GeneXpert Xpress System User's Guide.

9 Materials Available but Not Provided

External controls in the form of inactivated virus(es) are available from ZeptoMetrix (Buffalo, NY).

- External Positive Control: Catalog # NATFRC-6C (NATtrol Flu/RSV/SARS-CoV-2)
- External Negative Control: Catalog #NATCV9-6C (Coxsackievirus A9)

10 Warnings and Precautions

10.1 General

- For *in vitro* diagnostic use.
- For emergency use only.
- Positive results are indicative of presence of Flu A, Flu B, RSV, or SARS-CoV-2 RNA.
- Laboratories within the United States and its territories are required to report all SARS-CoV-2 results to the appropriate public health authorities.
- Performance characteristics of this test have been established with the specimen types listed in the Intended Use Section only. The performance of this assay with other specimen types or samples has not been evaluated.
- Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological specimens should be handled using standard precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention[7] and the Clinical and Laboratory Standards Institute.[8]
- Follow safety procedures set by your institution for working with chemicals and handling biological specimens.
- Consult your institution's environmental waste personnel on proper disposal of used cartridges, which may contain amplified material. This material may exhibit characteristics of federal EPA Resource Conservation and Recovery Act (RCRA) hazardous waste requiring specific disposal requirements. Check state and local regulations as they may differ from federal disposal regulations. Institutions should check the hazardous waste disposal requirements within their respective countries.

10.2 Specimens

- Maintain proper storage conditions during specimen transport to ensure the integrity of the specimen (see Section 12, Specimen Collection, Transport, and Storage). Specimen stability under shipping conditions other than those recommended has not been evaluated.

10.3 Assay/Reagent

- Do not open the Xpert Xpress SARS-CoV-2/Flu/RSV cartridge lid except when adding specimen.
- Do not use a cartridge that has been dropped after removing it from the packaging.
- Do not shake the cartridge. Shaking or dropping the cartridge after opening the cartridge lid may yield non-determinate results.
- Do not place the sample ID label on the cartridge lid or on the barcode label on the cartridge.
- Do not use a cartridge with a damaged barcode label.
- Do not use a cartridge that has a damaged reaction tube.
- Do not use reagents beyond their expiry date.
- Each single-use Xpert Xpress SARS-CoV-2/Flu/RSV cartridge is used to process one test. Do not reuse processed cartridges.
- Each single-use disposable pipette is used to transfer one specimen. Do not reuse disposable pipettes.
- Do not use a cartridge if it appears wet or if the lid seal appears to have been broken.
- Wear clean lab coats and gloves. Change gloves between the handling of each specimen.
- In the event of a spill of specimens or controls, wear gloves and absorb the spill with paper towels. Then, thoroughly clean the contaminated area with a 10% freshly prepared household chlorine bleach. Allow a minimum of two minutes of contact time. Ensure the work area is dry before using 70% denatured ethanol to remove bleach residue. Allow surface to dry completely before proceeding. Or, follow your institution's standard procedures for a contamination or spill event. For equipment, follow the manufacturer's recommendations for decontamination of equipment.
- Biological specimens, transfer devices, and used cartridges should be considered capable of transmitting infectious agents requiring standard precautions. Follow your institution's environmental waste procedures for proper disposal of used cartridges and unused reagents. These materials may exhibit characteristics of chemical hazardous waste requiring specific disposal. If country or regional regulations do not provide clear direction on proper disposal, biological specimens and used cartridges should be disposed per WHO [World Health Organization] medical waste handling and disposal guidelines.

11 Chemical Hazards[9,10]

- **Signal Word: Warning**
- **UN GHS Hazard Statements**
  - Harmful if swallowed
  - May be harmful in contact with skin
  - Causes eye irritation

- **UN GHS Precautionary Statements**
  - **Prevention**
    - Wash hands thoroughly after handling.
  - **Response**
    - Call a POISON CENTER or doctor/physician if you feel unwell.
    - If skin irritation occurs: Get medical advice/attention.
    - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
    - If eye irritation persists: Get medical advice/attention.

12 Specimen Collection, Transport, and Storage

Proper specimen collection, storage, and transport are critical to the performance of this test. Inadequate specimen collection, improper specimen handling and/or transport may yield a false result. See Section 12.1 for nasopharyngeal swab collection procedure and Section 12.2 for nasal swab collection procedure.

Nasopharyngeal and nasal swab specimens can be stored at room temperature (15–30 °C) for up to 24 hours in viral transport medium or 48 hours in saline until testing is performed on the GeneXpert Instrument Systems. Alternatively, nasopharyngeal and nasal swab specimens can be stored refrigerated (2–8 °C) up to seven days in viral transport medium or saline until testing is performed on the GeneXpert Xpress System.

Refer to the CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons Under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19)

https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html.

12.1 Nasopharyngeal Swab Collection Procedure

Insert the swab into either nostril, passing it into the posterior nasopharynx (see Figure 1). Rotate swab by firmly brushing against the nasopharynx several times. Remove and place the swab into the tube containing 3 mL of viral transport medium or 3mL of saline. Break swab at the indicated break line and cap the specimen collection tube tightly.

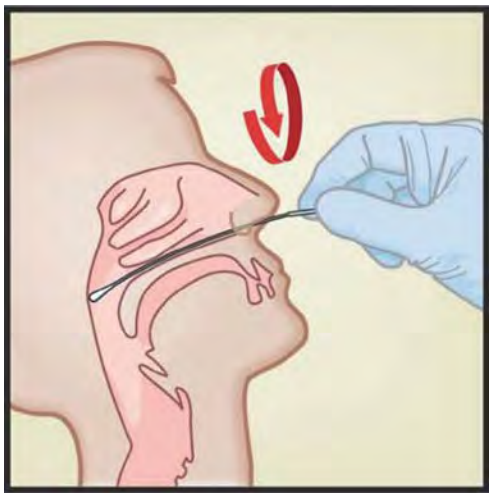


Figure 1. Nasopharyngeal Swab Collection

12.2 Nasal Swab Collection Procedure

1. Insert a nasal swab 1 to 1.5 cm into a nostril. Rotate the swab against the inside of the nostril for 3 seconds while applying pressure with a finger to the outside of the nostril (see Figure 2).

Figure 2. Nasal Swab Collection for First Nostril

2. Repeat on the other nostril with the same swab, using external pressure on the outside of the other nostril (see Figure 3). To avoid specimen contamination, do not touch the swab tip to anything other than the inside of the nostril.

Figure 3. Nasal Swab Collection for Second Nostril

3. Remove and place the swab into the tube containing 3 mL of viral transport medium or 3mL of saline. Break swab at the indicated break line and cap the specimen collection tube tightly.

13 Starting the System

The recommended environmental operating conditions for Xpert Xpress SARS-CoV-2/Flu/RSV test are 15-30°C (59-86 °F), 20-80% relative humidity, noncondensing.

1. Put on a clean pair of gloves.
2. Determine which system configuration you have (Figure 4).

Tablet Configuration **Hub Configuration**

Figure 4. Tablet and Hub System Configurations

- For the *Tablet* configuration, see Section 13.1, Starting the Tablet Configuration.
- For the *Hub* configuration, see Section 13.2, Starting the Hub Configuration.

13.1 Starting the Tablet Configuration

1. Turn on the GeneXpert Xpress instrument (GeneXpert Xpress II or GeneXpert Xpress IV).
2. Turn on the tablet computer:
   - *Windows 7*: The Windows® 7 account screen appears. Touch the **Cepheid-Admin** icon to continue.
   - *Windows 10*: The Windows Lock screen appears. Swipe up to continue.

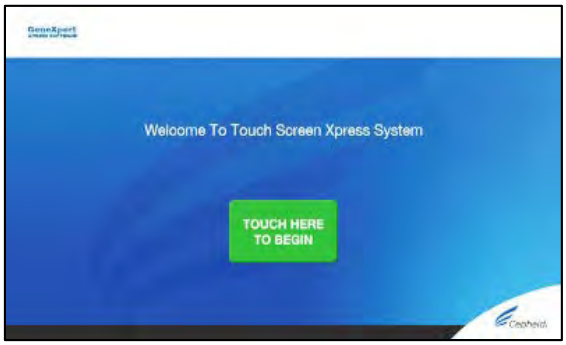
   The Windows Password screen appears.
3. Touch **Password** to display the keyboard, then type your password.
4. Touch the arrow button at the right of the password entry area. The GeneXpert Xpress Software starts.

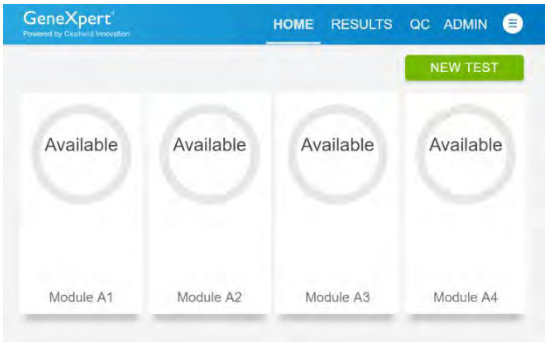
13.2 Starting the Hub Configuration

1. Turn on the GeneXpert Xpress IV instrument (in two or four modules configuration).
2. Turn on the Hub computer. The Windows Lock screen appears.
3. Swipe up to continue. The Windows Password screen appears.
4. Touch **Password** to display the keyboard, then type your Windows password.
5. Touch the arrow button at the right of the password entry area. The GeneXpert Xpress Software starts and a login screen appears.
6. If enabled, you may log in by scanning a barcode on your institutional ID, using the barcode scanner (located behind the right side of the touchscreen). Then proceed to Step 9. Otherwise, follow the steps below to login manually.
7. Enter your User Name and Password (the virtual keyboard appears once you touch the entry fields).
8. Touch the **X** in the upper right of the virtual keyboard. The keyboard disappears and the **LOGIN** button appears at the bottom of the screen. Touch the **LOGIN** button to continue.
9. The Database Maintenance Reminder screen and the Archive Tests Reminder dialog boxes may appear, depending on your system configuration. For more information, see the *GeneXpert Xpress System User's Guide*.

13.3 Determining Your Software Version

When your Xpress opening screen appears, you can determine your software version and the procedure to follow, based on one of the following two screens (see Figure 5).





Software Version 5.0 or Software Version 5.1 **Software Version 6.1 or Higher**

Figure 5. Xpress Opening Screens and Software Versions

- For Software Version 5.0 or Software Version 5.1, see Section 14.
- For Software Version 6.1 or higher, see Section 16.

14 GeneXpert Xpress Software Version 5.0 or Software Version 5.1

1. On the Welcome screen, touch the **TOUCH HERE TO BEGIN** button (see Figure 6).

Figure 6. Welcome Screen

2. The **VIEW PREVIOUS TESTS** button appears. The **RUN NEW TEST** button will appear on the Home screen within 3 minutes.

Note If the Home screen does not display **RUN NEW TEST**, the instrument was not powered up or is no longer powered on. Exit the software using the **EXIT** button. The GeneXpert Xpress instrument must first be turned on then turn on the computer. Click on software icon to launch software and enter password.

14.1 Starting a Test

Note Instructions showing how to prepare the sample and the cartridge are shown on-screen in a video and are also described in the Quick Reference Instructions (QRI).

Important Start the test within 30 minutes of adding the sample to the cartridge.

1. Put on a new pair of gloves if performing a new test. Touch the **RUN NEW TEST** button on the Home screen (see Figure 7) to run a patient specimen or an external control.

Figure 7. RUN NEW TEST button on Home Screen (GeneXpert Xpress IV screen shown)

2. Check that the specimen transport medium tube cap is closed.
3. If there is a Patient/Sample ID barcode, touch the **YES** button, then scan the Patient/Sample ID with the scanner. If there is no Patient/Sample ID barcode, touch the **NO** button, then manually enter the Patient/Sample ID and touch the **OK** button. For external control, type **Positive Control** or **Negative Control**.
4. Confirm the Patient/Sample ID. Touch **YES** if the Patient/Sample ID is correct.

14.2 Preparing the Specimen or External Control and Cartridge

It is recommended that external controls be tested at the frequency noted below.

- Each time a new lot of Xpert Xpress SARS-CoV-2/Flu/RSV kits is received.
- Each time a new shipment of Xpert Xpress SARS-CoV-2/Flu/RSV kits is received even if it is the same lot previously received.
- Each time a new operator is performing the test (i.e., operator who has not performed the test recently).
- When problems (storage, operator, instrument, or other) are suspected or identified.
- If otherwise required by your institution's standard Quality Control (QC) procedures.

1. Remove a cartridge and a transfer pipette from the cartridge kit box.
2. Scan the barcode on the cartridge with the scanner.

Note If the barcode on the Xpert Xpress SARS-CoV-2/Flu/RSV cartridge does not scan or scanning the barcode results in an error message stating the cartridge is expired, then repeat the test with a new cartridge.

If you have scanned the cartridge barcode in the Xpress software and the assay definition file is not available, a screen will appear indicating the assay definition file is not loaded on the system. If this screen appears, contact Cepheid Technical Support.

3. Make the appropriate selection from the Select Assay menu, as shown in Figure 8.
   - SARS-CoV-2, Flu A, Flu B and RSV: Select **Xpert Xpress_SARS-CoV-2_Flu_RSV**
   - SARS-CoV-2 and Flu only: Select **Xpert Xpress_SARS-CoV-2_Flu**
   - SARS-CoV-2 only: Select **Xpert Xpress_SARS-CoV-2**

Only the test result for the assay selected at this step will be collected once the test is started. SARS-CoV-2, Flu A, Flu B, and RSV results will only be collected if the Xpert Xpress_SARS-CoV-2_Flu_RSV assay is selected.

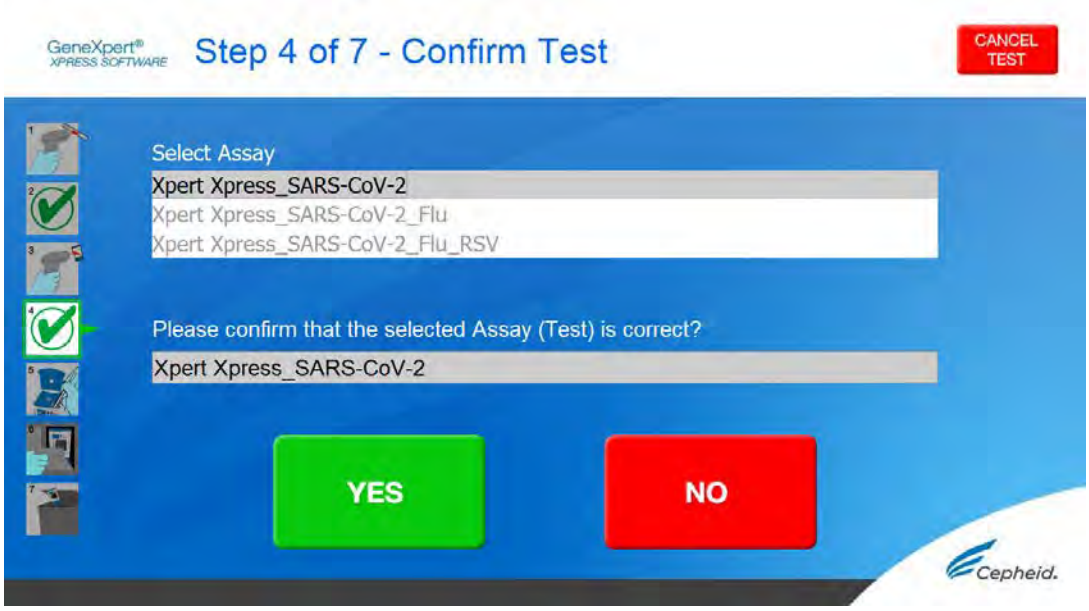


Figure 8. Confirm Test Screen – Select Assay

4. Confirm the selected test from the Select Assay menu (shown in Figure 9 below) and touch **YES** if the displayed information is correct. Enter your user name and password if prompted.

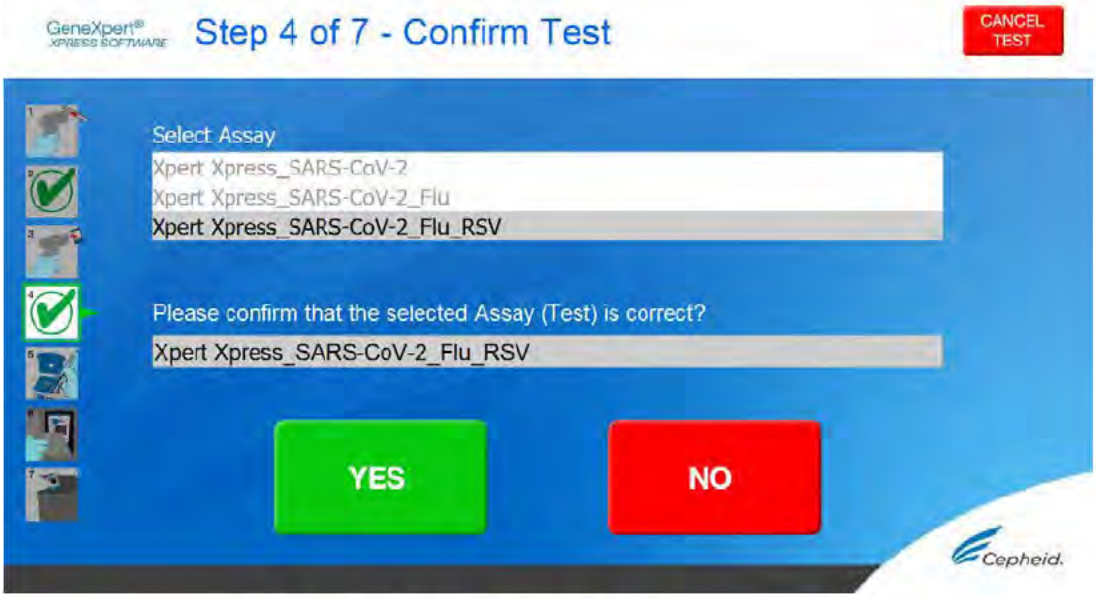


Figure 9. Confirm Test Screen

In the following steps, keep the cartridges upright when handling or scanning. Do not rotate or tip the cartridge, because damage to the contents or injury to personnel may occur.

5. Watch the video before continuing. The video will repeat. Touch the **SKIP VIDEO AND CONTINUE** button to exit video. The **Load Cartridge** screen appears.
6. Mix sample by rapidly inverting the specimen transport tube or external control tube 5 times. Open cap on the specimen transport tube or external control tube.
7. Open the cartridge lid by lifting the front of the cartridge lid.
8. Remove the transfer pipette from the wrapper

Note Do not place unwrapped pipette on the workbench.

9. Squeeze the top bulb of the transfer pipette **completely until the top bulb is fully flat**. While continuing to hold the bulb fully flat, place the pipette tip in the specimen transport tube. (see Figure 10).

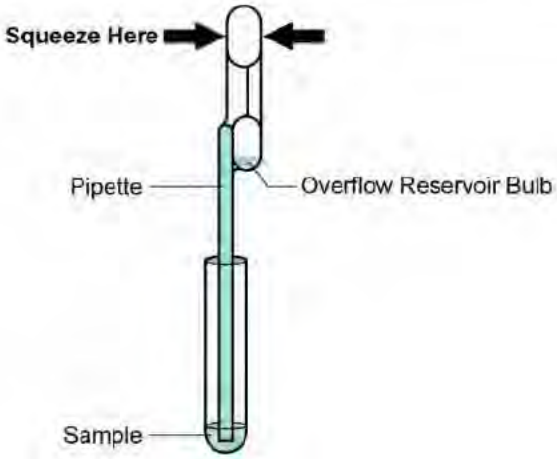


Figure 10. Transfer Pipette

10. Keeping the pipette below the surface of the liquid, release the top bulb of the pipette slowly to fill the pipette with sample before removing from the tube. It is okay if liquid goes into the overflow reservoir (see Figure 10). Check that the pipette does not contain bubbles.

11. To transfer the sample to the cartridge, squeeze the top bulb of the pipette completely again until it is fully flat to empty the contents of the pipette (300 µL) into the large opening (Sample Chamber) in the cartridge shown in Figure 11. Some liquid may remain in the overflow reservoir. Dispose of the used pipette.

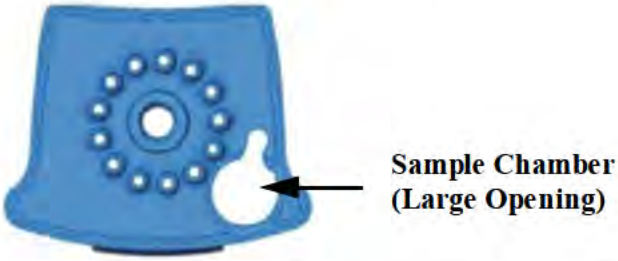


Figure 11. Xpert Xpress SARS-CoV-2/Flu/RSV Cartridge (Top View)

Note Take care to dispense the entire volume of liquid into the Sample Chamber. False negative results may occur if insufficient sample is added to the cartridge.

12. Close the cartridge lid.

14.3 Loading the Cartridge

1. Pull open the module door with the flashing green light.
2. Load the cartridge with the barcode facing the operator onto the cartridge bay platform. Do not try to insert the cartridge past the cartridge bay platform.
3. Close the door until it clicks. The green light will stop flashing and the test starts.

The **Test in Progress** screen appears. When the test is completed (green light goes out), the door will automatically unlock and the **Remove Cartridge** screen appears.

4. Follow the on-screen instructions to remove the cartridge and to reset the module for a new test.
5. Touch **CONTINUE** to view the result of the test.
6. To print results, touch the **PRINT RESULT** button.
7. Remove cartridge. Dispose of the used cartridge and gloves according to your institution's standard practices.
8. To log out, touch the **SIGN OUT** button.

Note Do not turn off or unplug the instruments while a test is in progress. Turning off or unplugging the GeneXpert Xpress instrument or computer will stop the test.

Note If the barcode on the Xpert Xpress SARS-CoV-2/Flu/RSV cartridge does not scan or scanning the barcode results in an error message stating that the cartridge is expired, then repeat the test with a new cartridge.

Note If you have scanned the cartridge barcode in the Xpress software and the assay definition file is not available, a screen will appear indicating that the assay definition file is not loaded or that the product code was not found on the system. If this screen appears, contact Cepheid Technical Support.

14.4 Start A New Test While a Test is Running

1. Put on a clean pair of gloves if performing a new test.
2. Touch the **HOME** button to go to the Home Screen.
3. Touch the **SIGN OUT** button to log out the previous user, if applicable.
4. Start a new test following the steps in Section 14.1, Starting a Test.

15 View Status of Tests in Progress, Completed Tests, and View Results of Past Tests

15.1 Tests in Progress

1. Touch the **HOME** button to view the status of tests in progress.
2. To view a test in progress, touch the **Test in progress touch for status** button. The time remaining to complete the testing will appear on the progress bar at the bottom of the **Test in Progress** screen.

15.2 Completed Tests

1. When a test is completed, touch the **Test complete, touch to continue** button. The **Remove Cartridge** screen appears.
2. Follow the on-screen instructions to remove the cartridge. Touch the **CONTINUE** button to view the result of the test. To print results, touch the **PRINT RESULT** button.

15.3 Results of Past Tests

1. Touch the **VIEW PREVIOUS TESTS** button on the Home screen shown in Figure 12.

Figure 12. VIEW PREVIOUS TESTS button on Home Screen

2. Select the test by either touching the test name or using the arrows to select the test.
3. Touch the **SELECT** button shown in Figure 13 to view results.
4. To print results, touch the **PRINT RESULT** button.

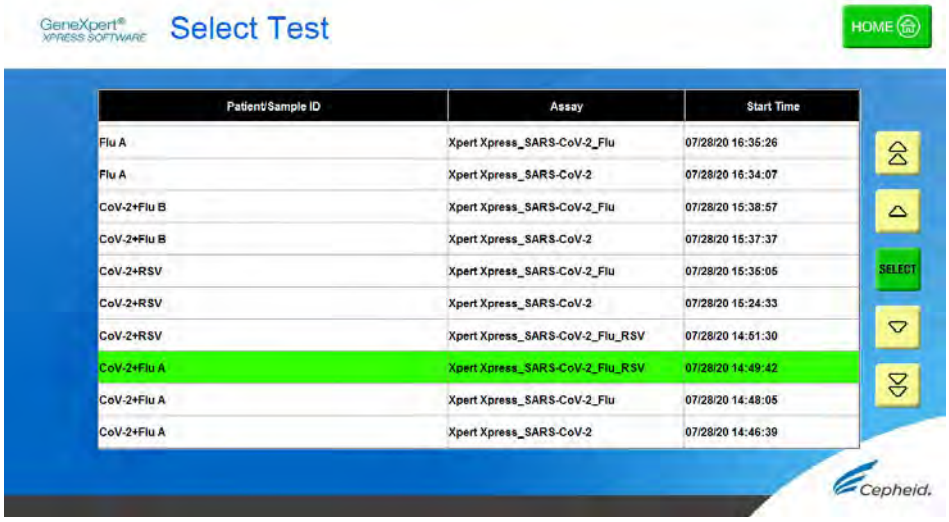


Figure 13. SELECT button

16 GeneXpert Xpress Software Version 6.1 or Higher

16.1 Starting a Test

Note Instructions showing how to prepare the sample and the cartridge are shown on-screen in videos and in the following procedure.

Important Start the test within 30 minutes of adding the sample to the cartridge.

1. Put on a new pair of gloves if performing a new test.
2. Touch the **NEW TEST** button on the Home screen (see Figure 14).

Figure 14. Home Screen

3. Check that the specimen transport medium tube cap is closed.

   If Patient Information is configured by an administrator, then the Patient Information screen appears (see Figure 15). If Patient Information is not configured, the Sample ID screen appears.
4. Skip to Section 16.2 if the Sample ID screen appears.

Figure 15. Patient Information Screen

5. Scan patient ID barcode or manually enter the Patient ID.
6. Touch **CONTINUE**. The Confirm Patient Information screen appears.
7. Verify the Patient ID and touch **CONFIRM**. The Sample ID screen appears.

16.2 Preparing the Specimen

1. Remove a cartridge and a transfer pipette from the cartridge kit box.

2. Check that the transport medium tube cap is closed. Scan Sample ID barcode or manually enter the Sample ID for patient specimen.
3. Touch **CONTINUE**. The Confirm Sample ID screen appears.
4. Verify the Sample ID and touch **CONFIRM**. The Scan Cartridge Barcode screen appears (see Figure 16).

In the following steps, keep the cartridges upright when handling or scanning. Do not rotate or tip the cartridge, because damage to the contents or injury to personnel may occur.

Note If the barcode on the Xpert Xpress SARS-CoV-2/Flu/RSV cartridge does not scan or scanning the barcode results in an error message stating that the cartridge is expired, then repeat the test with a new cartridge.

If you have scanned the cartridge barcode in the Xpress software and the assay definition file is not available, a screen will appear indicating the assay definition file is not loaded on the system. If this screen appears, contact Cepheid Technical Support.

Figure 16. Scan Cartridge Barcode Screen

5. Select the appropriate cartridge with the sample and scan the cartridge barcode. After scanning, the Select Test screen appears.
6. Select the test to run (see Figure 17):
   - SARS-CoV-2, Flu A, Flu B and RSV: Select **Xpert Xpress_SARS-CoV-2_Flu_RSV**
   - SARS-CoV-2 and Flu only: Select **Xpert Xpress_SARS-CoV-2_Flu**
   - SARS-CoV-2 only: Select **Xpert Xpress_SARS-CoV-2**

   Only the test result for the assay selected at this step will be collected once the test is started. SARS CoV-2, Flu A, Flu B, and RSV results will only be collected if the Xpert Xpress_SARS-CoV-2_Flu_RSV assay is selected.

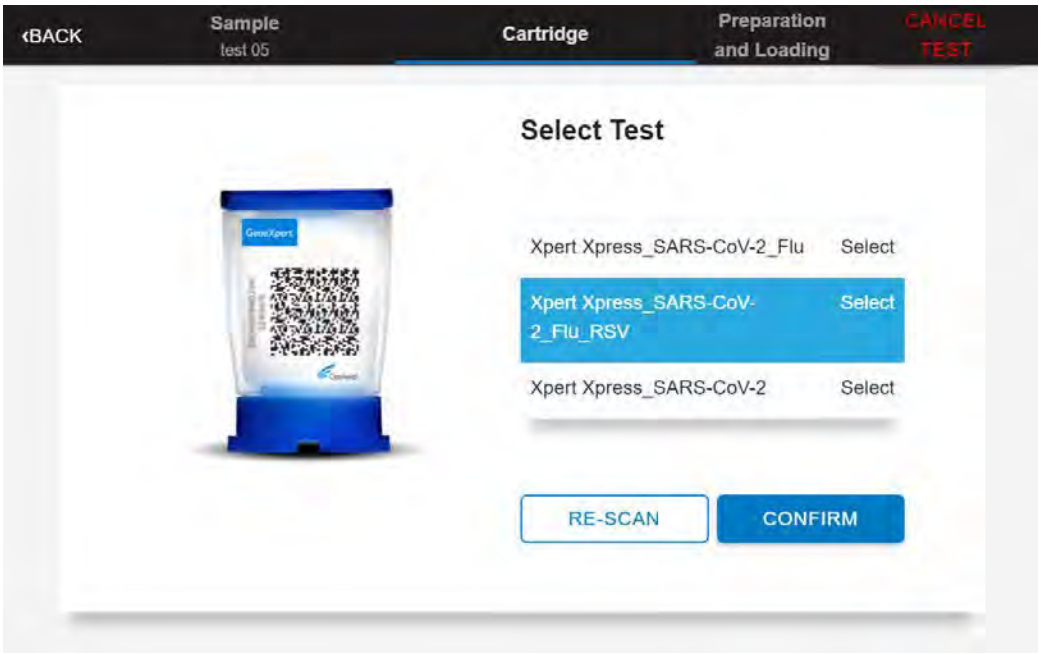


Figure 17. Select Test Screen

7. Verify that the correct cartridge has been scanned and that the assay name matches the name of the assay on the cartridge (see Figure 18).

Figure 18. Confirm Test Information Screen

8. Touch **CONFIRM** if the displayed information is correct.
9. Depending on your configuration, the Enter Credentials to Continue screen may appear (see Figure 19). If enabled, you may log in by scanning your institutional ID. Otherwise, manually enter your User Name and Password and touch **LOGIN** to continue.

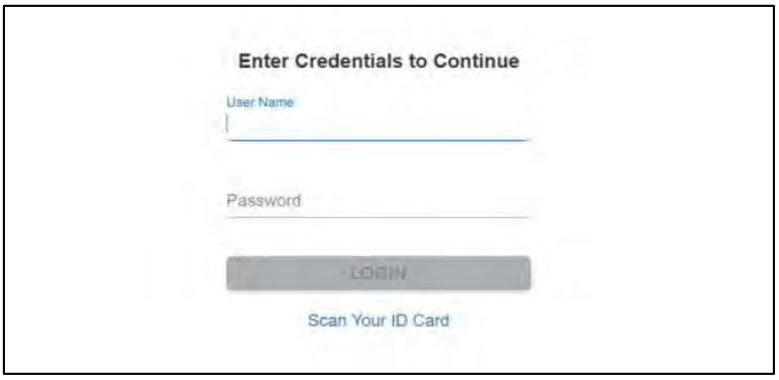


Figure 19. Enter Credentials to Continue Screen

10. The Cartridge Preparation screen appears (see Figure 20).

Figure 20. Cartridge Preparation Screen

11. Watch the video before continuing. The video will repeat. Touch the **SKIP VIDEO AND CONTINUE** button to exit video.
12. Mix specimen by rapidly inverting the specimen transport tube 5 times. Open the lid on the specimen transport tube.
13. Open the cartridge lid by lifting the front of the cartridge lid.
14. Remove the transfer pipette from the wrapper.

Note **Do not place unwrapped pipette on the workbench.**

15. Squeeze the top bulb of the transfer pipette **completely until the top bulb is fully flat**. While continuing to hold the bulb fully flat, place the pipette tip in the specimen transport tube (see Figure 21).

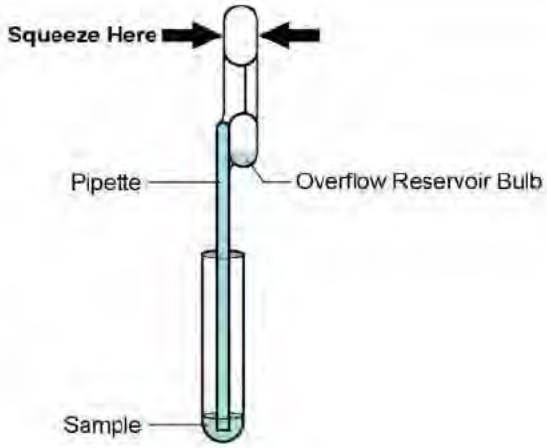


Figure 21. Transfer Pipette

16. Keeping the pipette below the surface of the liquid, release the top bulb of the pipette slowly to fill the pipette with sample before removing from the tube. It is okay if liquid goes into the overflow reservoir (see Figure 21). Check that the pipette does not contain bubbles.
17. To transfer the sample to the cartridge, squeeze the top bulb of the pipette **completely** again until it is fully flat to empty the contents of the pipette (300 µL) into the large opening (Sample Chamber) of the cartridge shown in Figure 22. Some liquid may remain in the overflow reservoir. Dispose of the used pipette.

Figure 22. Xpert Xpress SARS-CoV-2/Flu/RSV Cartridge (Top View)

Note Take care to dispense the entire volume of liquid into the Sample Chamber. False negative results may occur if insufficient sample is added to the cartridge.

18. Close the cartridge lid.
19. Go to Section 16.4, Loading the Cartridge.

16.3 Running External Controls

It is recommended that external controls be tested at the frequency noted below.

- Each time a new lot of Xpert Xpress SARS-CoV-2/Flu/RSV kits is received.
- Each time a new shipment of Xpert Xpress SARS-CoV-2/Flu/RSV kits is received even if it is the same lot previously received.
- Each time a new operator is performing the test (i.e., operator who has not performed the test recently)
- When problems (storage, operator, instrument, or other) are suspected or identified
- If otherwise required by your institution's standard Quality Control (QC) procedures

1. Put on a new pair of gloves if performing a new test. Touch the **QC** button on the Home screen (see Figure 23).

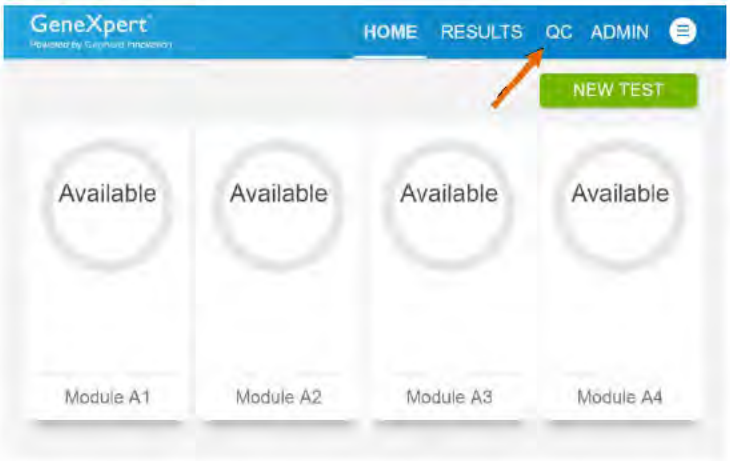


Figure 23. Home Screen

2. The Quality Control screen appears. Touch **RUN QC POSITIVE Test**, **RUN QC NEGATIVE TEST** or **RUN PROFICIENCY TEST** option (Figure 24).

Figure 24. Quality Control Screen

3. The Sample ID appears.
4. Enter the Sample ID, by typing **Positive Control** or **Negative Control** or scan the Sample ID barcode.
5. Touch **CONTINUE**. The Confirm Sample ID screen appears.
6. Verify the Sample ID and touch **CONFIRM**. The Scan Cartridge Barcode screen appears (see Figure 25).

In the following steps, keep the cartridges upright when handling or scanning. Do not rotate or tip the cartridge, because damage to the contents or injury to personnel may occur.

Note If the barcode on the Xpert Xpress SARS-CoV-2/Flu/RSV cartridge does not scan or scanning the barcode results in an error message stating that the cartridge is expired, then repeat the test with a new cartridge.

If you have scanned the cartridge barcode in the Xpress software and the assay definition file is not available, a screen will appear indicating the assay definition file is not loaded on the system. If this screen appears, contact Cepheid Technical Support.

Figure 25. Scan Cartridge Barcode Screen

7. Select the appropriate cartridge with the sample and scan the cartridge barcode. After scanning, the Select Test screen appears.
8. Select **Xpert Xpress_SARS-CoV-2_Flu_RSV** from the Select Assay menu.
9. Confirm the test information is correct then touch **CONFIRM** (see Figure 26).

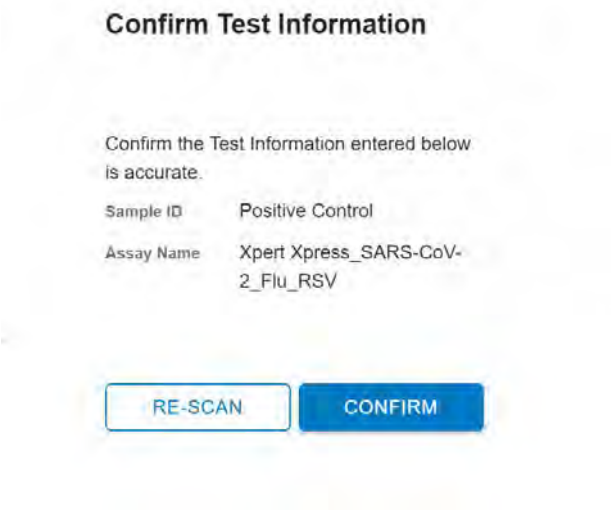


Figure 26. Confirm Test Information

10. Watch the video before continuing. The video will repeat. Touch the **CONTINUE** button to exit video.
11. Mix control by rapidly inverting the external control tube 5 times. Open the lid on the external control tube.
12. Open the cartridge lid by lifting the front of the cartridge lid.
13. Remove the transfer pipette from the wrapper.

Note Do not place unwrapped pipette on the workbench.

14. Squeeze the top bulb of the transfer pipette **completely until the bulb is fully flat.** While continuing to hold the bulb fully flat, place the pipette tip in the specimen transport tube (see Figure 27).

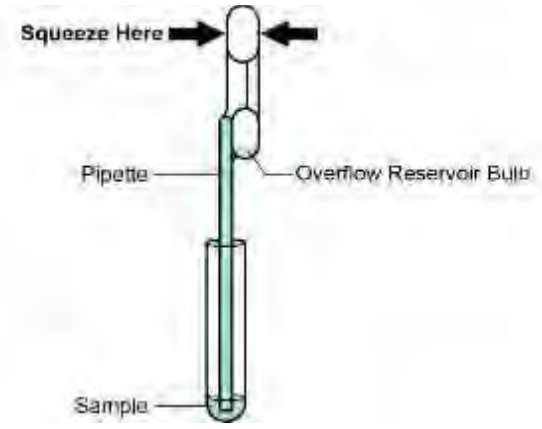


Figure 27. Transfer Pipette

15. Keeping the pipette below the surface of the liquid, release the top bulb of the pipette slowly to fill the pipette before removing from the tube. It is okay if liquid goes into the overflow reservoir (see Figure 27). Check that the pipette does not contain bubbles.
16. To transfer the external control to the cartridge, squeeze the top bulb of the pipette **completely** again until it is fully flat to empty the contents of the pipette into the large opening (Sample Chamber) of the cartridge shown in Figure 28. Dispose of the used pipette.

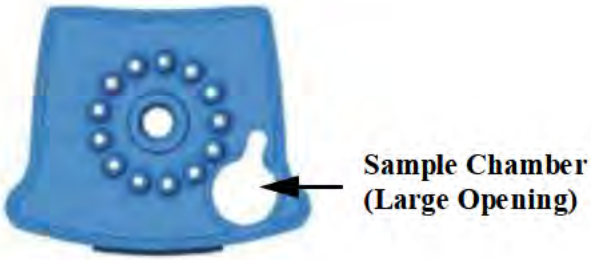


Figure 28. Xpert Xpress SARS-CoV-2/Flu/RSV Cartridge (Top View)

Note Take care to dispense the entire volume of liquid into the Sample Chamber. False negative results may occur if insufficient sample is added to the cartridge.

17. Close the cartridge lid.
18. Go to Section 16.4, Loading the Cartridge.

16.4 Loading the Cartridge

1. Touch the **CONTINUE** button on the Cartridge Preparation screen. The Load Cartridge into Module screen appears (see Figure 29).
2. Open the module door with the flashing green light.

Figure 29. Load Cartridge into Module Screen

3. Load the cartridge with the barcode facing the operator on the cartridge bay platform. Do not try to insert the cartridge past the cartridge bay platform.
4. Close the door until it clicks. The green light will stop blinking and the test starts.
5. When the cartridge is loaded, the Test Loading screen appears, followed by the Test Running screen showing that the test is running. A circular graphic indicator at the right indicates the progress of the test and the time remaining until a test result is available.

Note While a test is running, you can start another test. See Section 16.5, Start a New Test While a Test is Running.

Note Do not turn off or unplug the instrument while a test is in progress. Turning off or unplugging the GeneXpert Xpress instrument or Hub stops the test. If necessary, touch the **STOP TEST** button to cancel a test while it is loading or running.

6. When the test is done, the green light goes out and the door automatically unlocks. The screen text changes to Test Completed. The Test Completed screen provides the results for the test just completed.

Note If an unexpected result occurs (e.g., Negative Quality Control result is positive or Positive Quality Control result is negative), test a new Quality Control sample using a new cartridge. If an unexpected result occurs upon retest, contact Cepheid Technical Support.

7. Open the module door, remove the used cartridge, and properly dispose of the cartridge according to your institution's policy.
8. Touch **HOME** to go back to the Home screen.
9. To log out, touch the **User Menu** icon, then select Logout.

16.5 Start a New Test While a Test is Running

You can start a new test while another test is in progress.

1. Touch the **HOME** button on the Test Running screen.
2. For a new user log in, touch the **User Menu** icon to log in.
3. Repeat the steps in Section 16.1, Starting a Test, Section 16.2, Preparing the Specimen, and Section 16.4, Loading the Cartridge.
4. After a second test has started, touch the **HOME** button. The status of both tests appears. The Home screen displays the module(s) in use with a circular graphic indicator around each test, and Patient Identification below the module graphic (see Figure 30).

Figure 30. Home Screen showing Two Tests Running

5. After a test has completed, the module icon text changes to Complete (see Figure 31). Touch Complete View Result to view test results.

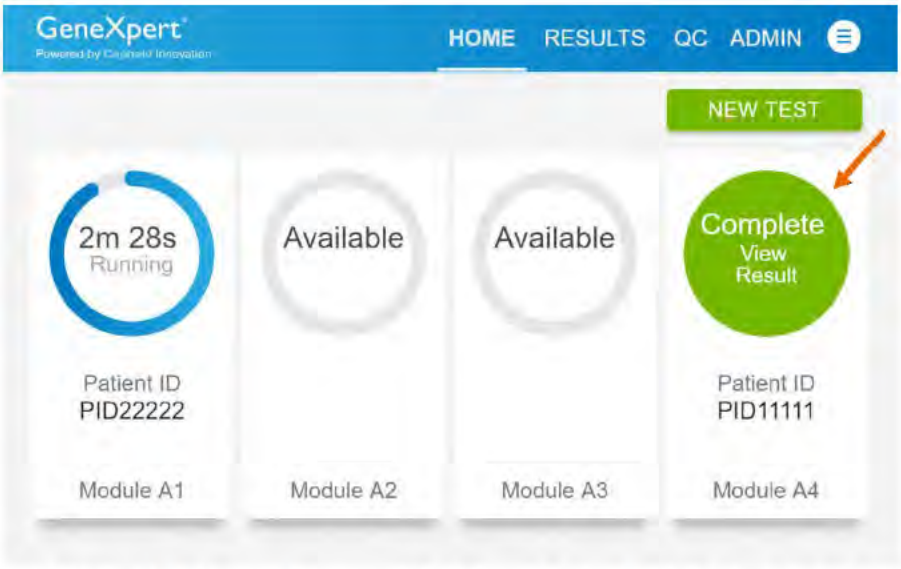


Figure 31. Home Screen with One of Two Tests Completed

16.6 Viewing Test Results

1. Touch the **RESULTS** button located on the panel at the top of the screen (see Figure 31). The Results screen appears (see Figure 32). Test results are, by default, in order of the date and time that the test was run. Navigate through the test result pages by touching the numbered buttons or arrows at the bottom of the screen.

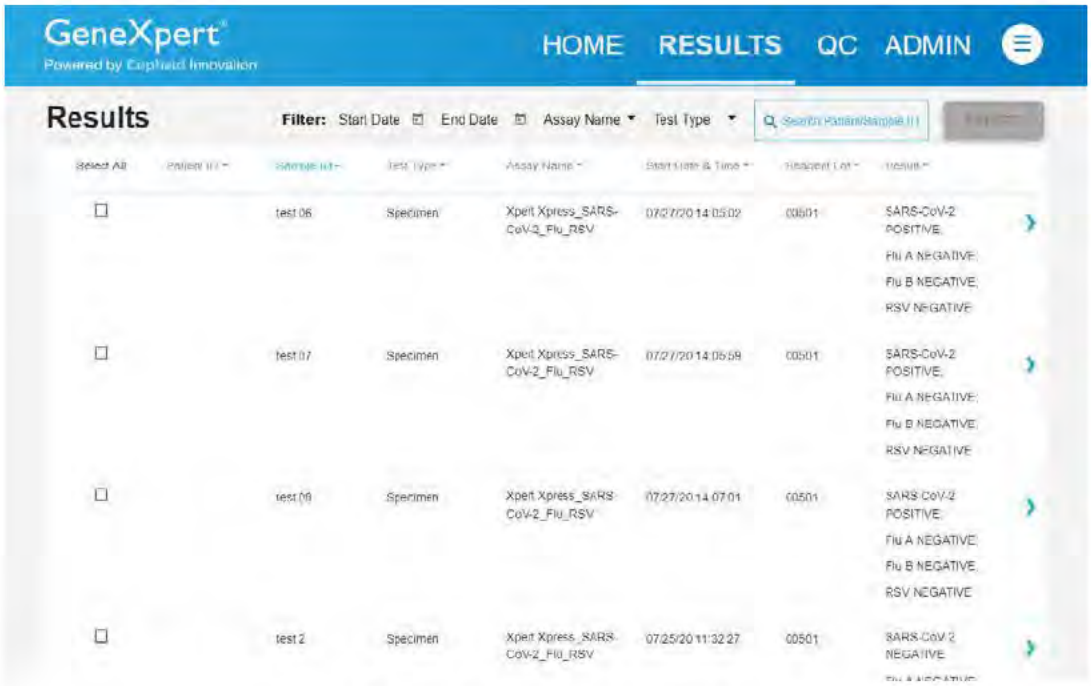


Figure 32. Results Screen

2. Touch the desired result to open the Test Result screen (see Figure 33).
3. To view test report, touch the **REPORT** button then swipe across the screen from left to right to minimize screen and view report.

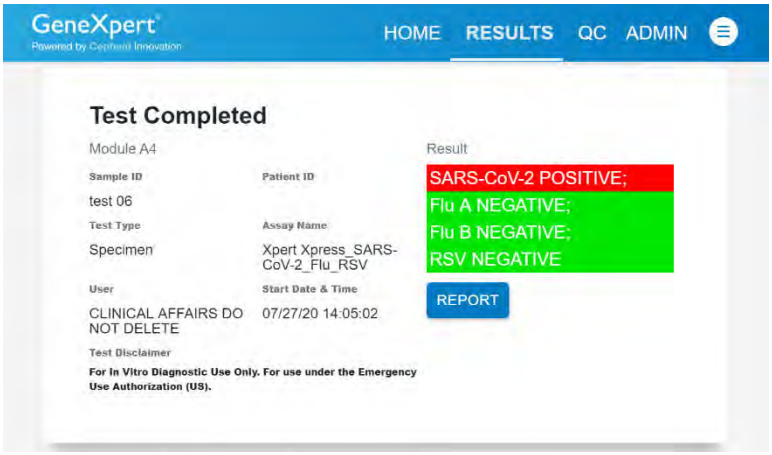


Figure 33. Test Result Screen (Example)

Note If an unexpected result occurs (e.g., Negative Quality Control result is positive or Positive Quality Control result is negative), test a new Quality Control sample using a new cartridge. If an unexpected result occurs upon retest, contact Cepheid Technical Support.

17 Quality Control

17.1 Internal Controls

CONTROL

Each cartridge includes a Sample Processing Control (SPC) and Probe Check Control (PCC).

Sample Processing Control (SPC) – Ensures that the sample was processed correctly. The SPC verifies that sample processing is adequate. Additionally, this control detects sample-associated inhibition of the real-time PCR assay, ensures that the PCR reaction conditions (temperature and time) are appropriate for the amplification reaction, and that the PCR reagents are functional. The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria.

Probe Check Control (PCC) – Before the start of the PCR reaction, the GeneXpert System measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, probe integrity, and dye stability. The PCC passes if it meets the validated acceptance criteria.

17.2 External Controls

External controls should be used in accordance with local, state, and federal accrediting organizations as applicable.

18 Interpretation of Results

The results are interpreted automatically by the GeneXpert Xpress System and are clearly shown in the **View Results** window. The Xpert Xpress SARS-CoV-2/Flu/RSV test provides test results based on the detection of respective gene targets according to the algorithms.

The format of the test results presented will vary depending on the user's choice to run either an Xpert Xpress_SARS-CoV-2_Flu_RSV, Xpert Xpress_SARS-CoV-2_Flu or Xpert Xpress_SARS-CoV-2 test.
Table 1 shows the possible result outcomes when the Xpert Xpress_SARS-CoV-2_Flu_RSV test mode is selected.

Table 1. Xpert Xpress_SARS-CoV-2/Flu/RSV Possible Results and Interpretation

Result	Interpretation
SARS-CoV-2 POSITIVE	The SARS-CoV-2 target RNA is detected. • The SARS-CoV-2 signal has a Ct within the valid range and endpoint above the minimum setting. • SPC: NA (not applicable); SPC is ignored because SARS-CoV-2 target amplification occurred. • Probe Check: PASS; all probe check results pass.
Flu A POSITIVE	• The Flu A signal for either the Flu A1 RNA target or the Flu A2 RNA target or signals for both RNA targets has a Ct within the valid range and endpoint above the threshold setting. • SPC – NA; SPC is ignored because the Flu A target amplification occurred. • Probe Check – PASS; all probe check results pass.
Flu B POSITIVE	• The Flu B signal has a Ct within the valid range and endpoint above the minimum setting. • SPC: NA; SPC is ignored because Flu B target amplification occurred. • Probe Check: PASS; all probe check results pass.
RSV POSITIVE	• The RSV signal has a Ct within the valid range and endpoint above the minimum setting. • SPC: NA; SPC is ignored because RSV target amplification occurred. • Probe Check: PASS; all probe check results pass
SARS-CoV-2 NEGATIVE; Flu A NEGATIVE; Flu B NEGATIVE; RSV NEGATIVE	SARS-CoV-2 target RNA is not detected; Flu A target RNA is not detected; Flu B target RNA is not detected; RSV target RNA is not detected. • SARS-CoV-2, Flu A, Flu B and RSV target RNAs are not detected. • SPC – PASS; SPC has a Ct within the valid range and endpoint above the minimum setting. • Probe Check – PASS; all probe check results pass.
NO RESULT-REPEAT TEST	If result is **NO RESULT - REPEAT TEST**, retest with a new cartridge according to the Retest Procedure in Section 19.2. If retest is **NO RESULT - REPEAT TEST**, obtain a new specimen for testing.

Result	Interpretation
INSTRUMENT ERROR	If result is **INSTRUMENT ERROR**, touch **CLEAR ERROR** and follow the on-screen instructions. When the Home screen appears, repeat the test using a new cartridge according to the Retest Procedure in Section 19.2.
If the SPC is negative and the results for any of the targets are positive, the results for all targets are considered valid.	

If only one viral target is positive but coinfection with multiple targets is suspected, the sample should be re-tested with another FDA cleared, approved, or authorized test, if coinfection would change clinical management.

Table 2 shows the possible result outcomes when the Xpert Xpress_SARS-CoV-2_Flu test mode is selected.

Table 2. Xpert Xpress_SARS-CoV-2_Flu Possible Results and Interpretation

Result	Interpretation
SARS-CoV-2 POSITIVE	The SARS-CoV-2 target RNA is detected. • The SARS-CoV-2 signal has a Ct within the valid range and endpoint above the minimum setting. • SPC: NA (not applicable); SPC is ignored because SARS-CoV-2 target amplification occurred. • Probe Check: PASS; all probe check results pass.
Flu A POSITIVE	• The Flu A signal for either the Flu A1 RNA target or the Flu A2 RNA target or signals for both RNA targets has a Ct within the valid range and endpoint above the threshold setting. • SPC – NA; SPC is ignored because the Flu A target amplification occurred. • Probe Check – PASS; all probe check results pass.
Flu B POSITIVE	• The Flu B signal has a Ct within the valid range and endpoint above the minimum setting. • SPC: NA; SPC is ignored because Flu B target amplification occurred. • Probe Check: PASS; all probe check results pass.
SARS-CoV-2 NEGATIVE; Flu A NEGATIVE; Flu B NEGATIVE	SARS-CoV-2 target RNA is not detected; Flu A target RNA is not detected; Flu B target RNA is not detected. • SARS-CoV-2, Flu A and Flu B target RNAs are not detected. • SPC – PASS; SPC has a Ct within the valid range and endpoint above the minimum setting. • Probe Check – PASS; all probe check results pass.
NO RESULT-REPEAT TEST	If result is **NO RESULT - REPEAT TEST**, retest with a new cartridge according to the Retest Procedure in Section 19.2. If retest is **NO RESULT - REPEAT TEST**, obtain a new specimen for testing.

Result	Interpretation
INSTRUMENT ERROR	If result is **INSTRUMENT ERROR**, touch **CLEAR ERROR** and follow the on-screen instructions. When the Home screen appears, repeat the test using a new cartridge according to the Retest Procedure in Section 19.2.
If the SPC is negative and the results for any of the targets are positive, the results for all targets are considered valid.	

If only one viral target is positive but coinfection with multiple targets is suspected, the sample should be re-tested with another FDA cleared, approved, or authorized test, if coinfection would change clinical management.

Table 3 shows the possible result outcomes when the Xpert Xpress_SARS-CoV-2 test mode is selected.

Table 3. Xpert Xpress_SARS-CoV-2 Possible Results and Interpretation

Result	Interpretation
SARS-CoV-2 POSITIVE	The SARS-CoV-2 target RNA is detected. • The SARS-CoV-2 signal has a Ct within the valid range and endpoint above the minimum setting. • SPC: NA (not applicable); SPC is ignored because SARS-CoV-2 target amplification occurred. • Probe Check: PASS; all probe check results pass.
SARS-CoV-2 NEGATIVE	SARS-CoV-2 target RNA is not detected. • SARS-CoV-2 target RNA is not detected. • SPC – PASS; SPC has a Ct within the valid range and endpoint above the minimum setting. • Probe Check – PASS; all probe check results pass.
NO RESULT-REPEAT TEST	If result is **NO RESULT - REPEAT TEST**, retest with a new cartridge according to the Retest Procedure in Section 19.2. If retest is **NO RESULT - REPEAT TEST**, obtain a new specimen for testing.
INSTRUMENT ERROR	If result is **INSTRUMENT ERROR**, touch **CLEAR ERROR** and follow the on-screen instructions. When the Home screen appears, repeat the test using a new cartridge according to the Retest Procedure in Section 19.2.

The Xpert Xpress SARS-CoV-2/Flu/RSV test can be run to detect SARS-CoV-2, Flu and RSV by selecting Xpert Xpress_SARS-CoV-2_Flu_RSV from the Select Test menu; SARS-CoV-2 and Flu only by selecting Xpert Xpress_SARS-CoV-2_Flu; or SARS-CoV-2 only by selecting Xpert Xpress_SARS-CoV-2. The Xpert Xpress_SARS-CoV-2 test mode

includes an Early Assay Termination (EAT) function which will provide earlier time to results in high titer specimens if the signal from the SARS-CoV-2 target reaches a predetermined threshold before the full 45 PCR cycles have been completed. When SARS-CoV-2 titers are high enough to initiate the EAT function, the SPC amplification curve may not be seen and its results may not be reported.

19 Retests

19.1 Reasons to Repeat the Test

If any of the test results mentioned below occur, repeat the test once according to instructions in Section 19.2, Retest Procedure.

- An **INSTRUMENT ERROR** result could be due to, but not limited to, a system component failure, or the maximum pressure limits were exceeded.
- A **NO RESULT- REPEAT TEST** indicates that insufficient data were collected. For example, cartridge failed integrity test, Probe Check Control failure, no sample added, the operator stopped a test that was in progress, or a power failure occurred.

If an External Control fails to perform as expected, repeat external control test and/or contact Cepheid Technical Support for assistance.

19.2 Retest Procedure

To retest a non-determinate result **(NO RESULT-REPEAT TEST, INSTRUMENT ERROR)**, use a new cartridge.

Use the leftover sample from the original specimen transport tube or new external control tube.

1. Put on a clean pair of gloves. Obtain a new Xpert Xpress SARS-CoV-2/Flu/RSV cartridge and a new transfer pipette.
2. Check the specimen transport tube or external control tube is closed.
3. Mix the sample by rapidly invert the specimen transport medium tube or external control tube 5 times. Open the cap on the specimen transport tube or external control tube.
4. Open the cartridge lid by lifting the front of the cartridge lid.
5. Using a clean transfer pipette (supplied), transfer sample (one draw) to the sample chamber with the large opening in the cartridge.
6. Close the cartridge lid.

20 Limitations

- Performance of the Xpert Xpress SARS-CoV-2/Flu/RSV has only been established in nasopharyngeal swab specimens. Use of the Xpert Xpress SARS-CoV-2/Flu/RSV test with other specimen types has not been assessed and performance characteristics are unknown.
- Nasal swabs (self-collected under supervision of, or collected by, a healthcare provider) are considered acceptable specimen types for use with the Xpert Xpress SARS-CoV-2/Flu/RSV test but performance with these specimen types has not been established.
- As with any molecular test, mutations within the target regions of Xpert Xpress SARS-CoV-2/Flu/RSV could affect primer and/or probe binding resulting in failure to detect the presence of virus.
- As with any molecular test, mutations within the target regions of the Xpert Xpress SARS-CoV-2/Flu/RSV test could affect primer and/or probe binding resulting in failure to detect the presence of virus or the virus being detected less predictably.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- The performance of this test was validated using the procedures provided in this package insert only. Modifications to these procedures may alter the performance of the test.
- Erroneous test results might occur from improper specimen collection; failure to follow the recommended sample collection, handling, and storage procedures; technical error; or sample mix-up. Careful compliance with the instructions in this insert is necessary to avoid erroneous results.
- False negative results may occur if virus is present at levels below the analytical limit of detection.
- Negative results do not preclude SARS-CoV-2, influenza or RSV infection and should not be used as the sole basis for treatment or other patient management decisions.
- Results from the Xpert Xpress SARS-CoV-2/Flu/RSV test should be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient.
- Viral nucleic acid may persist *in vivo*, independent of virus viability. Detection of analyte target(s) does not imply that the corresponding virus(es) are infectious or are the causative agents for clinical symptoms.
- This test has been evaluated for use with human specimen material only.
- This test is a qualitative test and does not provide the quantitative value of detected organism present.
- This test has not been evaluated for patients without signs and symptoms of respiratory tract infection.
- This test has not been evaluated for monitoring treatment of infection.
- This test has not been evaluated for screening of blood or blood products for the

presence of SARS-CoV-2, influenza or RSV.

- The effect of interfering substances has only been evaluated for those listed within the labeling. Interference by substances other than those described can lead to erroneous results.
- Results from analytical studies with contrived co-infected samples showed potential for competitive interference when SARS-CoV-2, influenza or RSV was present at 1X LoD levels.
- Cross-reactivity with respiratory tract organisms other than those described herein can lead to erroneous results.
- Recent patient exposure to FluMist® or other live attenuated influenza vaccines may cause inaccurate positive results.
- As the Xpert Xpress SARS-CoV-2/Flu/RSV test does not differentiate between the N2 and E gene targets, the presence of other coronaviruses in the B lineage, *Betacoronavirus* genus, including SARS-CoV-1 may cause a false positive result. None of these other coronaviruses is known to currently circulate in the human population.
- This test is not intended to differentiate RSV subgroups, influenza A subtypes or influenza B lineages. If differentiation of specific RSV or influenza subtypes and strains is needed, additional testing, in consultation with state or local public health departments, is required.
- Specimen transport media that contain guanidine thiocyanate (GTC) may interfere with the test causing false negative results.
- The performance of this device has not been assessed in a population vaccinated against COVID-19.
- This test has not been FDA cleared or approved.
- This test has been authorized by FDA under an EUA for use by authorized laboratories.
- This test has been authorized only for the simultaneous qualitative detection and differentiation of nucleic acids from SARS-CoV-2, influenza A, influenza B, and respiratory syncytial virus (RSV), and not for any other viruses or pathogens.
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

21 Conditions of Authorization for Laboratory and Patient Care Settings

The Cepheid Xpert Xpress SARS-CoV-2/Flu/RSV Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas.

However, to assist clinical laboratories and/or Patient Care Settings using the Xpert Xpress SARS-CoV-2/Flu/RSV (referred to in the Letter of Authorization as "Your Product"), the relevant Conditions of Authorization are listed below.

- Authorized laboratories[ii] using your product will include with result reports of the Xpert Xpress SARS-CoV-2/Flu/RSV test, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories using your product will use your product as outlined in the Xpert Xpress SARS-CoV-2/Flu/RSV Instructions for Use - For Use with GeneXpert Dx or GeneXpert Infinity systems. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use the Xpert Xpress SARS-CoV-2/Flu/RSV test are not permitted.
- Authorized laboratories operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation using your product will use your product as outlined in the Xpress SARS-CoV-2/Flu/RSV Instructions for Use - For Use with GeneXpert Xpress System and associated Quick Reference Instructions for Xpert Xpress SARS-CoV-2/Flu/RSV and GeneXpert Xpress System (Hub configuration), and Quick Reference Instructions for Xpert Xpress SARS-CoV-2/Flu/RSV and GeneXpert Xpress System (Tablet configuration). Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.
- Authorized laboratories that receive your product will notify the relevant public health authorities of their intent to run your product prior to initiating testing.
- Authorized laboratories using the Xpert Xpress SARS-CoV-2/Flu/RSV test will have a process in place for reporting test results to healthcare providers and relevant public health

[ii] The letter of authorization refers to "authorized laboratories as follows: (1) testing of nasopharyngeal swab, nasal swab, or nasal wash/aspirate specimens using the Xpert SARS-CoV-2/Flu/RSV test run on the GeneXpert Dx and GeneXpert Infinity systems is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, that meet requirements to perform high or moderate complexity tests and (2) testing of nasopharyngeal or nasal swab specimens using the Xpert Xpress SARS-CoV-2/Flu/RSV test run on the GeneXpert Xpress System (Tablet and Hub Configurations) is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation.

authorities, as appropriate.

- Authorized laboratories and will collect information on the performance of the test and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Cepheid (+ 1 888 838 3222 or techsupport@cepheid.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.
- All operators using your product must be appropriately trained in performing and interpreting the results of your product, use appropriate personal protective equipment when handling this kit, and use your product in accordance with the authorized labeling.
- Cepheid, authorized distributors, and authorized laboratories and patient care settings using your product will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

22 Performance Characteristics

22.1 Clinical Evaluation

The performance of the Xpert Xpress SARS-CoV-2/Flu/RSV test was evaluated using archived clinical nasopharyngeal (NP) swab specimens in viral transport medium. Archived specimens were selected consecutively by date and previously known analyte result. A total of 240 NP swab specimens were tested with Xpert Xpress SARS-CoV-2/Flu/RSV side by side with a SARS-CoV-2 EUA RT-PCR test and the FDA-cleared Xpert Xpress Flu/RSV test in a randomized and blinded fashion.

Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were determined by comparing the results of the Xpert Xpress SARS-CoV-2/Flu/RSV test relative to the results of a SARS-CoV-2 EUA RT-PCR test for the SARS-CoV-2 target, and Xpert Xpress Flu/RSV for the Flu A, Flu B, and RSV targets, respectively.

Xpert Xpress SARS-CoV-2/Flu/RSV demonstrated a PPA and NPA of 97.9% and 100.0% for SARS-CoV-2, respectively; 100.0% and 100.0% for Flu A, respectively; 100.0% and 99.0% for Flu B, respectively; 100.0% and 100.0% for RSV, respectively (Table 4).

Table 4. Xpert Xpress SARS-CoV-2/Flu/RSV Performance Results

Target	Number of Specimens	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
SARS-CoV-2	240	46	0	193	1	97.9% (88.9% - 99.6%)	100.0% (98.1% - 100.0%)
Flu A	240	48	0	192	0	100.0% (92.6% - 100.0%)	100.0% (98.0% - 100.0%)
Flu B	240	46	2	192	0	100.0% (92.3% - 100.0%)	99.0% (96.3% - 99.7%)
RSV	240	47	0	193	0	100.0% (92.4% - 100.0%)	100.0% (98.1% - 100.0%)

TP: True Positive; FP: False Positive; TN: True Negative; FN: False Negative; CI: Confidence Interval

22.2 Analytical Sensitivity (Limit of Detection)

The analytical sensitivity of the Xpert Xpress SARS-CoV-2/Flu/RSV test was assessed with one lot of reagent and limiting dilutions of the six respiratory viruses (NATtrol SARS-CoV-2, Flu A H1, Flu A H3, Flu B, RSV A and RSV B) into pooled negative clinical NP swab matrix following the guidance in Clinical and Laboratory Standards Institute (CLSI) document EP17-A2. The estimated LoD values as determined by Probit regression analysis were verified using two lots of Xpert Xpress SARS-CoV-2/Flu/RSV reagents. The verified LoD values for the viruses tested are summarized in Table 5.

Table 5. Xpert Xpress SARS-CoV-2/Flu/RSV Limit of Detection

Virus/Strain	LoD Concentration
SARS-CoV-2 (USA-WA1/2020)	131 copies/mL
Influenza A/ California/7/2009	0.004 $TCID_{50}$/mL
Influenza A/Victoria/361/2011	0.087 $TCID_{50}$/mL
Influenza B/Mass/2/2012	0.04 $TCID_{50}$/mL
RSV A/2/Australia/61	0.43 $TCID_{50}$/mL
RSV B/Wash/18537/62	0.22 $TCID_{50}$/mL

22.3 Analytical Reactivity (Inclusivity)

The inclusivity of Xpert Xpress SARS-CoV-2/Flu/RSV was evaluated using *in silico* analysis of the assay amplicons in relation to 48,461 SARS-CoV-2 sequences available in the GISAID gene database for two targets, E and N2.

For analysis of the E target, 113 sequences were excluded due to ambiguous nucleotides, which reduced the total to 48,348 sequences. Of the 48,348 GISAID sequences, 48,108 (99.5%) were an exact match to the SARS-CoV-2 E target amplicon generated in the Xpert Xpress SARS-CoV-2/Flu/RSV test. Single nucleotide mismatches were observed for 223 sequences and two mismatches were observed for 17 sequences. Of the 17 sequences with two mismatches, two sequences contained 2 mismatches in the forward primer region, three sequences have a 'GA" dinucleotide in the reverse primer, and twelve sequences contained a 'AA' dinucleotide that lies between the oligonucleotides used in the assay. None of these mismatches are expected to affect the performance of the assay.

For analysis of the N2 target, 129 sequences were excluded due to ambiguous nucleotides, which reduced the total used in the evaluation to 48,332 sequences. Of the 48,332 GISAID sequences, 47,962 (99.2%) were an exact match to the SARS-CoV-2 N2 target amplicon generated in the Xpert Xpress SARS-CoV-2/Flu/RSV test. Single nucleotide mismatches were observed for 369 sequences and three (3) mismatches were observed for one sequence. For the one sequence with three variant positions, two of the mismatched nucleotides are in the probe region and could have an impact on probe binding. None of the other mismatches are predicted to have a negative impact on the performance of the assay.

The inclusivity of the Xpert Xpress SARS-CoV-2/Flu/RSV for Flu and RSV viruses are as reported for the analytical reactivity evaluation of the Xpert Xpress Flu/RSV test.

Xpert Xpress Flu/RSV test was evaluated against multiple strains of influenza A H1N1 (seasonal pre-2009), influenza A H1N1 (pandemic 2009), influenza A H3N2 (seasonal), avian influenza A (H5N1, H5N2, H6N2, H7N2, H7N3, H2N2, H7N9, and H9N2), influenza B (representing strains from both Victoria and Yamagata lineages), and respiratory syncytial virus subgroups A and B (RSV A and RSV B) at levels near the analytical LoD. A total of 53 strains comprised of 48 influenza viruses (35 influenza A and 13 influenza B) and 5 RSV strains were tested in this study with the Xpert Xpress Flu/RSV test. Three replicates were tested for each strain. All Flu and RSV strains tested positive in all three replicates, except for one Flu A H1N1 strain (A/New Jersey/8/76), which tested positive in 2 of 3 replicates at 0.1 $TCID_{50}$/mL. Results are shown in Table 6. Predicted cross reactivity from *in silico* analyses showed 100% sequence homology for additional pH1N1 strains.

Table 6. Analytical Reactivity (Inclusivity) of the Xpert Xpress Flu/RSV Test

Virus	Strain	Target Concentration	Result		
			Flu A	Flu B	RSV
No Template Control		N/A	NEG	NEG	NEG
Influenza A H1N1 (pre-2009)	A/swine/Iowa/15/30	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/WS/33	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/PR/8/34	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/Mal/302/54	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/Denver/1/57	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/New Jersey/8/76	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/New Caledonia/20/1999	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/New York/55/2004	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/Solomon Island/3/2006	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/Taiwan/42/06	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/Brisbane/59/2007	0.1 $TCID_{50}$/mL	POS	NEG	NEG
Influenza A H1N1 (pdm2009)	A/swine/NY/02/2009	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/Colorado/14/2012	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/Washington/24/2012	0.1 $TCID_{50}$/mL	POS	NEG	NEG
Influenza A H3N2 (Seasonal)	A/Aichi/2/68	2.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Hong Kong/8/68	2.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Port Chalmers/1/73	2.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Hawaii/15/2001	2.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Wisconsin/67/05	2.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Brisbane/10/2007	2.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Minnesota/11/2010 (H3N2)v	2.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Indiana/08/2011 (H3N2)v	2.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Texas/50/2012	2.0 $TCID_{50}$/mL	POS	NEG	NEG
Avian influenza A	A/duck/Hunan/795/2002 (H5N1)	≤ 1 pg/µL[a]	POS	NEG	NEG
	A/chicken/Hubei/327/2004 (H5N1)	≤ 1 pg/µL[a]	POS	NEG	NEG
	A/Anhui/01/2005 (H5N1)	≤ 1 pg/µL[a]	POS	NEG	NEG
	A/Japanese white eye/Hong Kong/ 1038/2006 (H5N1)	≤ 1 pg/µL[a]	POS	NEG	NEG
	A/mallard/WI/34/75 (H5N2)	≤ 1 pg/µL[a]	POS	NEG	NEG
	A/chicken/CA431/00 (H6N2)	≤ 1 pg/µL[a]	POS	NEG	NEG
	A/duck/LTC-10-82743/1943 (H7N2)	≤ 1 pg/µL[a]	POS	NEG	NEG
	A/chicken/NJ/15086-3/94 (H7N3)	≤ 1 pg/µL[a]	POS	NEG	NEG
	A/Anhui/1/2013 (H7N9)	N/A[b]	POS	NEG	NEG
	A/Shanghai/1/2013 (H7N9)	N/A[b]	POS	NEG	NEG
	A/chicken/Korea/38349-p96323/ 1996 (H9N2)	≤ 1 pg/µL[a]	POS	NEG	NEG
	A/Mallard/NY/6750/78 (H2N2)	≤ 1 pg/µL[a]	POS	NEG	NEG
Influenza B	B/Lee/40	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/Allen/45	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/GL/1739/54	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/Maryland/1/59	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/Panama/45/90[c]	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/Florida/07/2004[d]	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/Florida/02/06[c]	1.0 $TCID_{50}$/mL	NEG	POS	NEG

Virus	Strain	Target Concentration	Result		
			Flu A	Flu B	RSV
	B/Florida/04/06[d]	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/Hong Kong/5/72	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/Wisconsin/01/2011[d]	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/Malaysia/2506/04[c]	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/Taiwan/2/62	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/Brisbane/60/2008[c]	1.0 $TCID_{50}$/mL	NEG	POS	NEG
RSV A	RSV-A/NY (Clinical unknown)	3.0 $TCID_{50}$/mL	NEG	NEG	POS
	RSV-A/WI/629-8-2/2007	3.0 $TCID_{50}$/mL	NEG	NEG	POS
	RSV-A/WI/629-11-1/2008	3.0 $TCID_{50}$/mL	NEG	NEG	POS
RSV B	RSV-B/WV14617/85	7.0 $TCID_{50}$/mL	NEG	NEG	POS
	RSV-B/CH93(18)-18	7.0 $TCID_{50}$/mL	NEG	NEG	POS

a. Purified viral RNA in simulated background matrix was used for avian influenza A viruses due to biosafety regulations.
b. Inactivated avian influenza A (H7N9) viruses without viral titer was diluted 100,000-fold in simulated background matrix and tested due to biosafety regulations.
c. Known Victoria lineage.
d. Known Yamagata lineage.

22.4 Analytical Specificity (Exclusivity)

An *in silico* analysis for possible cross-reactions with all the organisms listed in Table 7 was conducted by mapping primers and probes in the Xpert Xpress SARS-CoV-2/Flu/RSV test individually to the sequences downloaded from the GISAID database. E primers and probes are not specific for SARS-CoV-2 and will detect Human and Bat SARS-coronavirus. No potential unintended cross reactivity with other organisms listed in Table 7 is expected based on the *in silico* analysis.

Table 7. Xpert Xpress SARS-CoV-2/Flu/RSV Analytical Specificity Microorganisms

Microorganisms from the Same Genetic Family	High Priority Organisms
Human coronavirus 229E	Adenovirus (e.g. C1 Ad. 71)
Human coronavirus OC43	Human metapneumovirus (hMPV)
Human coronavirus HKU1	Parainfluenza viruses 1-4
Human coronavirus NL63	Influenza A
SARS-coronavirus	Influenza B
MERS-coronavirus	Influenza C
Bat coronavirus	Enterovirus (e.g. EV68)
	Respiratory syncytial virus
	Rhinovirus
	Chlamydia pneumoniae
	Haemophilus influenzae
	Legionella pneumophila
	Mycobacterium tuberculosis
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Bordetella pertussis
	Mycoplasma pneumoniae
	Pneumocystis jirovecii (PJP)

Microorganisms from the Same Genetic Family	High Priority Organisms
	Parechovirus
	Candida albicans
	Corynebacterium diphtheriae
	Legionella non-*pneumophila*
	Bacillus anthracis (Anthrax)
	Moraxella catarrhalis
	Neisseria elongata and N. meningitidis
	Pseudomonas aeruginosa
	Staphylococcus epidermidis
	Streptococcus salivarius
	Leptospira
	Chlamydia psittaci
	Coxiella burnetii (Q-Fever)
	Staphylococcus aureus

The analytical specificity of the Xpert Xpress SARS-CoV-2/Flu/RSV for Flu A, Flu B and RSV viruses are as reported for the analytical exclusivity evaluation of the Xpert Xpress Flu/RSV test. The analytical specificity of the Xpert Xpress Flu/RSV test was evaluated by testing a panel of 44 cultures consisting of 16 viral, 26 bacterial, and two yeast strains representing common respiratory pathogens or those potentially encountered in the nasopharynx. Three replicates of each bacterial and yeast strain were tested at concentrations of ≥ 1 x 10^6 CFU/mL with the exception of one strain that was tested at 1 x 10^5 CFU/mL (*Chlamydia pneumoniae*). Three replicates of each virus were tested at concentrations of ≥ 1 x 10^5 $TCID_{50}$/mL. The analytical specificity was 100%. Results are shown in Table 8.

Table 8. Analytical Specificity of the Xpert Xpress Flu/RSV Test

Organism	Concentration	Influenza A	Influenza B	RSV
No Template Control	N/A	NEG	NEG	NEG
Adenovirus Type 1	1.12E+06 $TCID_{50}$/mL	NEG	NEG	NEG
Adenovirus Type 7	1.87E+05 $TCID_{50}$/mL	NEG	NEG	NEG
Human coronavirus OC43	2.85E+05 $TCID_{50}$/mL	NEG	NEG	NEG
Human coronavirus 229E	1.00E+05 $TCID_{50}$/mL	NEG	NEG	NEG
Cytomegalovirus	1.00E+05 $TCID_{50}$/mL	NEG	NEG	NEG
Echovirus	3.31E+07 $TCID_{50}$/mL	NEG	NEG	NEG
Enterovirus	3.55E+05 $TCID_{50}$/mL	NEG	NEG	NEG
Epstein Barr Virus	7.16E+07 $TCID_{50}$/mL	NEG	NEG	NEG
Herpes simplexvirus	8.90E+05 $TCID_{50}$/mL	NEG	NEG	NEG
Measles	6.31E+05 $TCID_{50}$/mL	NEG	NEG	NEG
Human metapneumovirus	1.00E+05 $TCID_{50}$/mL	NEG	NEG	NEG
Mumps virus	6.31E+06 $TCID_{50}$/mL	NEG	NEG	NEG

Organism	Concentration	Influenza A	Influenza B	RSV
Human parainfluenza virus Type 1	1.15E+06 $TCID_{50}$/mL	NEG	NEG	NEG
Human parainfluenza virus Type 2	6.31E+05 $TCID_{50}$/mL	NEG	NEG	NEG
Human parainfluenza virus Type 3	3.55E+06 $TCID_{50}$/mL	NEG	NEG	NEG
Rhinovirus Type 1A	1.26E+05 $TCID_{50}$/mL	NEG	NEG	NEG
Acinetobacter baumannii	1.00E+06 CFU/mL	NEG	NEG	NEG
Burkholderia cepacia	3.30E+06 CFU/mL	NEG	NEG	NEG
Candida albicans	3.20E+06 CFU/mL	NEG	NEG	NEG
Candida parapsilosis	3.00E+06 CFU/mL	NEG	NEG	NEG
Bordetella pertussis	3.30E+06 CFU/mL	NEG	NEG	NEG
Chlamydia pneumoniae	1.00E+05 CFU/mL	NEG	NEG	NEG
Citrobacter freundii	3.30E+06 CFU/mL	NEG	NEG	NEG
Corynebacterium sp.	3.30E+06 CFU/mL	NEG	NEG	NEG
Escherichia coli	1.00E+07 CFU/mL	NEG	NEG	NEG
Enterococcus faecalis	1.30E+06 CFU/mL	NEG	NEG	NEG
Hemophilus influenzae	1.00E+06 CFU/mL	NEG	NEG	NEG
Lactobacillus reuteri	1.00E+06 CFU/mL	NEG	NEG	NEG
Legionella spp.	1.00E+06 CFU/mL	NEG	NEG	NEG
Moraxella catarrhalis	1.00E+07 CFU/mL	NEG	NEG	NEG
Mycobacterium tuberculosis (avirulent)	1.00E+06 CFU/mL	NEG	NEG	NEG
Mycoplasma pneumoniae	1.00E+06 CFU/mL	NEG	NEG	NEG
Neisseria meningitidis	2.15E+06 CFU/mL	NEG	NEG	NEG
Neisseria mucosa	1.00E+07 CFU/mL	NEG	NEG	NEG
Propionibacterium acnes	2.40E+07 CFU/mL	NEG	NEG	NEG
Pseudomonas aeruginosa	3.70E+06 CFU/mL	NEG	NEG	NEG
Staphylococcus aureus (protein A producer)	2.20E+06 CFU/mL	NEG	NEG	NEG
Staphylococcus epidermidis	3.40E+06 CFU/mL	NEG	NEG	NEG
Staphylococcus haemolyticus	4.00E+06 CFU/mL	NEG	NEG	NEG
Streptococcus agalactiae	3.50E+06 CFU/mL	NEG	NEG	NEG
Streptococcus pneumoniae	1.00E+06 CFU/mL	NEG	NEG	NEG
Streptococcus pyogenes	1.00E+07 CFU/mL	NEG	NEG	NEG
Streptococcus salivarius	1.00E+07 CFU/mL	NEG	NEG	NEG
Streptococcus sanguinis	3.10E+06 CFU/mL	NEG	NEG	NEG

22.5 Competitive Interference

Competitive interference of the Xpert Xpress SARS-CoV-2/Flu/RSV caused by co-infections were evaluated by testing individual SARS-CoV-2, Flu A, Flu B or RSV strains at 1X LoD in the presence of different target strains at a higher concentration in a simulated background matrix. The concentration at LoD was 131 copies/mL for SARS-CoV-2 and ranged from 0.004 $TCID_{50}$/mL to 0.43 $TCID_{50}$/mL for the Flu and RSV strains; the competitive strains were evaluated at 10^4 titer units (copies/mL, $TCID_{50}$/mL, $CEID_{50}$/mL or PFU/mL). The corresponding concentration of RNA (copies/mL) for the Flu and RSV strains was determined by ddPCR.

Analytical competitive interference was assessed using a strain of SARS-CoV-2 (inactivated USA-WA1/2020), Flu A H3 (H3/Victoria/361/2011), Flu B (B/Mass/02/2012), RSV A (RSV-A/2/Australia/61), and RSV B (RSV-B/Wash/18537/62). Replicates of 20 were tested for each target strain and each competitive strain combination. The normal binomial distribution with 20 replicate samples at LoD is between 17 and 20 positive results based on the binomial distribution with N=20, p=0.95 (X~Bin(20,0.95)). Therefore, sets of 20 with 16 or less positives would be rare and an indication of a competitive inhibitory effect due to high levels of a competing analyte. Below is a summary of the results:

Table 9. Summary of Results for Competitive Interference

		Correct Calls (n/20)					
		Test Strain at LoD and Interferent at:					
Test Strain at LoD	**Interferent Strain**	**10^4* (2.1e7 cp/mL)**	**10^3* (2.1e6 cp/mL)**	**10^2* (2.1e5 cp/mL)**	**10* (2.1e4 cp/mL)**	**1* (2.1e3 cp/mL)**	**0.1* (2.1e2 cp/mL)**
Flu B	Flu A	*6/20*	**20/20**				
RSV A	Flu A	*9/20*	**17/20**				
RSV B	Flu A	*11/20*	**18/20**				
SARS-CoV-2	Flu A	*6/20*	**17/20**	**20/20**			
Test Strain at LoD	**Interferent Strain**	**10^4* (5.2e7 cp/mL)**	**10^3* (5.2e6 cp/mL)**	**10^2* (5.2e5 cp/mL)**	**10* (5.2e4 cp/mL)**	**1* (5.2e3 cp/mL)**	**0.1* (5.2e2 cp/mL)**
Flu A	Flu B	*1/20*	*4/20*	*8/20*	*9/19*	*15/20*	**20/20**
RSV A	Flu B	*0/20*	*0/20*	*3/20*	**18/20**		
RSV B	Flu B	*7/20*	*8/20*	*11/20*	**18/20**		
SARS-CoV-2	Flu B	*3/20*	*4/20*	*11/20*	**17/20**	**20/20**	
Test Strain at LoD	**Interferent Strain**	**10^4* (3.7e7 cp/mL)**	**10^3* (3.7e6 cp/mL)**	**10^2* (3.7e5 cp/mL)**	**10* (3.7e4 cp/mL)**	**1* (3.7e3 cp/mL)**	**0.1* (3.7e2 cp/mL)**
Flu A	RSV A	*15/20*	**12/20**	**20/20**			
Flu B	RSV A	*15/20*	**17/20**				
SARS-CoV-2	RSV A	**17/20**	**19/20**				
Test Strain at LoD	**Interferent Strain**	**10^4* (1.1e7 cp/mL)**	**10^3* (1.1e6 cp/mL)**	**10^2* (1.1e5 cp/mL)**	**10* (1.1e4 cp/mL)**	**1* (1.1e3 cp/mL)**	**0.1* (1.1e2 cp/mL)**
Flu A	RSV B	*9/20*	*7/20*	*6/20*	*14/20*	**20/20**	
Flu B	RSV B	*10/20*	*10/20*	*16/20*	**19/20**		
SARS-CoV-2	RSV B	**17/20**	*16/20*	*15/20*	**20/20**		

		Correct Calls (n/20)					
		Test Strain at LoD and Interferent at:					
Test Strain at LoD	**Interferent Strain**	**10^4***	**10^3***	**10^2***	**10***	**1***	**0.1***
Flu A	SARS-CoV-2	**19/20**					
Flu B	SARS-CoV-2	**18/20**					
RSV A	SARS-CoV-2	**19/20**					
RSV B	SARS-CoV-2	**19/20**					

* Units for the concentration of each organism are as follows: Flu A H3 – $CEID_{50}$/mL; Flu B and RSV B – $TCID_{50}$/mL; RSV A – PFU/mL; SARS-CoV-2 – copies/mL

Italicized font indicates inhibitory effects

Bold font indicates no inhibition (SARS-CoV-2 tested to ≥19/20)

Flu A/Victoria/361/2011 at a concentration of 1 x 10^4 $CEID_{50}$/mL (2.1e7 copies/mL), inhibited Flu B, RSV A, RSV B and SARS-CoV-2 at the LoD.

Flu B/Mass/2/2012 at concentrations shown in Table 9, inhibited SARS-CoV-2, Flu A, RSV A and RSV B at concentrations at the LoD of those targets.

RSV A/2/Australia/61 at a concentration of 1 x 10^4 PFU/mL (3.7e7 copies/mL), inhibited SARS-CoV-2, Flu A and Flu B at the LoD.

RSV-B/Wash/18537/62 at concentrations shown in Table 9, inhibited SARS-CoV-2, Flu A and Flu B at concentrations at the LoD of those targets.

22.6 Potentially Interfering Substances

Potentially interfering substances that could be present in the nasopharynx (or introduced during specimen collection and handling) and interfere with accurate detection of SARS-CoV-2, Flu A, Flu B and RSV were evaluated with select direct testing on the Xpert Xpress SARS-CoV-2/Flu/RSV. Additional substances have also been previously evaluated on the Xpert Xpress Flu/RSV assay.

Potentially interfering substances in the nasal passage and nasopharynx may include, but are not limited to: blood, nasal secretions or mucus, and nasal and throat medications used to relieve congestion, nasal dryness, irritation, or asthma and allergy symptoms, as well as antibiotics and antivirals. Positive and negative samples were prepared in simulated nasal matrix. Negative samples (N = 8) were tested in the presence of each substance to determine the effect on the performance of the sample processing control (SPC). Positive samples (N = 8) were tested per substance with viruses spiked at 3x the analytical LoD determined for each strain. Positive samples tested with the Xpert Xpress SARS-CoV-2/Flu/RSV included one SARS-CoV-2, two influenza A, one influenza B and two RSV (RSV A and RSV B) strains, whereas those tested with the Xpert Xpress Flu/RSV consisted of six influenza (four influenza A and two influenza B) and four RSV (two RSV A and two RSV B). The substances evaluated are listed in Table 10 with active ingredients and final concentrations tested shown. None of the substances caused interference of the assay performance at the concentrations tested in this study. All positive and negative replicates were correctly identified by the Xpert Xpress SARS-CoV-2/Flu/RSV and/or Xpert Xpress Flu/RSV tests.

Table 10. Potentially Interfering Substances in the Xpert Xpress SARS-CoV-2/Flu/RSV Test and/or Xpert Xpress Flu/RSV Test

Substance/Class	Description/Active Ingredient	Concentration Tested
Control	Simulated nasal matrix	100% (v/v)
Beta-adrenergic bronchodilator[a]	Albuterol Sulfate	0.83 mg/mL (equivalent to 1 dose per day)
Blood	Blood (Human)	2% (v/v)
BD Universal Transport System	Transport Media	100% (v/v)
Remel M4®	Transport Media	100% (v/v)
Remel M4RT®	Transport Media	100% (v/v)
Remel M5®	Transport Media	100% (v/v)
Remel M6®	Transport Media	100% (v/v)
Throat lozenges, oral anesthetic and analgesic[a]	Benzocaine, Menthol	1.7 mg/mL
Mucin[a]	Purified Mucin protein (Bovine or porcine submaxillary gland)	0.1% (w/v)[b]
Antibiotic, nasal ointment[a]	Mupirocin	10 mg/mL
Saline Nasal Spray[a]	Sodium Chloride (0.65%)	15% (v/v)
Anefrin Nasal Spray	Oxymetazoline, 0.05%	15% (v/v)
PHNY Nasal Drops	Phenylephrine, 0.5%	15% (v/v)
Tamiflu anti-viral drugs[a]	Zanamivir	7.5 mg/mL
Antibacterial, systemic	Tobramycin	4 µg/mL
Zicam Nasal Gel	Luffa opperculata, Galphimia glauca, Histaminum hydrochloricum Sulfur	15% (w/v)
Nasal corticosteroid	Fluticasone Propionate	5 µg/mL

a. Substances/active ingredients and concentrations specifically evaluated with the Xpert Xpress SARS-CoV-2/Flu/RSV test.

b. No interference to the Xpert Xpress Flu/RSV performance observed at a concentration of 2.5%

22.7 Carry-Over Contamination

Carry-over studies to establish that single-use, self-contained GeneXpert cartridges prevent carry-over contamination have been conducted for previous Xpert tests developed for the GeneXpert systems, including the Xpert Xpress Flu/RSV. The studies demonstrated that a negative sample when preceded by very a high positive sample in the same GeneXpert module resulted in no carry-over.

23 References

1. Centers for Disease Control and Prevention. https://www.cdc.gov/coronavirus/2019-ncov/index.html. Accessed February 9, 2020.
2. bioRxiv. (https://www.biorxiv.org/content/10.1101/2020.02.07.937862v1). Accessed March 3, 2020.
3. Petric M, Comanor L, Petti CA. Role of the laboratory in diagnosis of influenza during seasonal epidemics and potential pandemics. J Infect Dis. 2006;194:S98-110.
4. Schweiger B, Zadow I, Heckler R, et al. Application of a fluorogenic PCR assay for typing and subtyping of influenza viruses in respiratory samples. J Clin Micro. 2000;38:1552-1558.
5. http://www.cdc.gov/flu/about/viruses/types.htm. Accessed on May 19, 2016.
6. http://www.cdc.gov/RSV/index.html. Accessed on March 14, 2013.
7. Centers for Disease Control and Prevention. *Biosafety in Microbiological and Biomedical laboratories* (refer to latest edition). http://www.cdc.gov/biosafety/publications/
8. Clinical and Laboratory Standards Institute. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline*. Document M29 (refer to latest edition).
9. REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on the classification labeling and packaging of substances and mixtures amending and repealing, List of Precautionary Statements, Directives 67/548/EEC and 1999/45/EC (amending Regulation (EC) No 1907/2007).
10. Occupational Safety and Health Standards, Hazard Communication, Toxic and Hazard Substances (March 26, 2012) (29 C.F.R., pt. 1910, subpt. Z).

24 Cepheid Headquarters Locations

Corporate Headquarters	European Headquarters
Cepheid 904 Caribbean Drive Sunnyvale, CA 94089 USA	Cepheid Europe SAS Vira Solelh 81470 Maurens-Scopont France
Telephone: +1 408 541 4191	Telephone: +33 563 825 300
Fax: +1 408 541 4192	Fax: +33 563 825 301
www.cepheid.com	www.cepheidinternational.com

25 Technical Assistance

Before contacting Cepheid Technical Support, collect the following information:

- Product name
- Lot number
- Serial number of the instrument
- Error messages (if any)
- Software version and, if applicable, Computer Service Tag number

Region	Telephone	Email
US	+ 1 888 838 3222	techsupport@cepheid.com
France	+ 33 563 825 319	support@cepheideurope.com
Australia New Zealand	1800 130 821 0800 001 028	techsupportANZ@cepheid.com

Contact information for all Cepheid Technical Support offices is available on our website: www.cepheid.com/en_US/support/contact-us.

26 Table of Symbols

Symbol	Meaning
REF	Catalog number
IVD	*In vitro* diagnostic medical device
	Do not re-use
LOT	Batch code
	Consult instructions for use
	Caution
	Manufacturer
CC	Country of manufacture
Σ	Contains sufficient for <n> tests
CONTROL	Control
	Expiration date
°C	Temperature limitation
	Biological risks
Rx only	For prescription use only

Cepheid
904 Caribbean Drive
Sunnyvale, CA 94089 USA
Phone: +1 408 541 4191
Fax: +1 408 541 4192

IVD

For use under Emergency Use Authorization (EUA) Only