File No. CI 20-01-29284

THE QUEEN'S BENCH
Winnipeg Centre

B E T W E E N:

**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.

Reply Affidavit of JARED MANLEY PETER BULLARD
Affirmed, April 29, 2021

Manitoba Justice, Legal Services Branch
Constitutional Law Section

Per: Michael Conner
Heather Leonoff
Denis Guénette
Sean Boyd

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Respondents.

AFFIDAVIT

1. I have previously presented an affidavit in this proceeding. I have reviewed the affidavits filed subsequent to my initial affidavit, and present this affidavit in reply.

2. I have personal knowledge of the facts and matters stated in this affidavit – except where they are based upon information and belief, in which case I believe them to be true.

Applicants' Request for Undertakings

3. By letter dated March 19, 2021, legal counsel for the Applicants wrote to legal counsel for the Respondents to request a breakdown of Ct values on all positive tests performed by the lab.

4. Counsel for the Respondents initially refused to provide the documents on the basis that it would require the lab to undertake further analysis. By letter dated April 1, 2021, the Applicants renewed their request for production of information that would show the Ct thresholds for all positive PCR tests. In particular, the Applicants' request was stated as follows:

> 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, 41, 42, 43, 44, 45 (not simply the percentage as a range from 36-40)

5. While Cadham Provincial Laboratory ("Cadham") did not have the breakdown as requested, we confirmed that we could produce a spreadsheet from data in our computer system showing the Ct value of each of the 15,464 positive PCR tests Cadham had performed from March 11, 2020 to March 31, 2021. I understand from the Respondents' counsel that they provided this information to the Applicants' counsel by way of letter dated April 6, 2021.

6. The spreadsheet provided the date the lab received each test sample, the type of PCR test used and the Ct value. However, some of the entries provided a number that differs from the typical range for Ct discussed in my report included in my first affidavit. These are results from a different type of nucleic acid amplification test (PCR_PANTH_COV19) called transcription

mediated amplification that uses a value known as relative light units (RLU) rather than CT. Despite the shortcut name "PCR_PANTH_COV19", it is not a PCR-based assay.

7. I understand that in a letter dated April 13, 2021, Applicants' counsel subsequently raised some questions about the information provided in the spreadsheet as well as statements I made in my original affidavit about statistics regarding the percentage of Ct values within certain ranges. I respond to those questions below.

Breakdown of tests by Ct value

8. In my report, that is attached as Exhibit C to my first affidavit, I indicated the following at lines 193 to 197:

> In December 2020, 5825 Manitoba SARS-CoV-2 positive results were analyzed and categorized by Ct value. Almost 60% had a Ct value less than 25 (~35% <20 and ~23% 20-25) and of the remaining samples, 18% had Ct 25-30, 18% had Ct 30-36 and 7% had Ct 36-40. The large majority (~68%) of these patients would be considered as higher level risk of infectiousness, just based on Ct value alone.

9. First, I would like to correct a typographical error in this passage. My reference to "~68%" in the last sentence of this passage above should have been "~76%".

10. Counsel for the Applicants suggests that there is a discrepancy because my spreadsheet shows there were only 3,200 positive tests in December with a Ct value, which contradicts the reference to 5,825 tests in lines 193-197 of my report. However, as stated in my report, the analysis of test results was conducted in December, 2020. It was not conducted only on

tests that were done in December. I have asked the scientist who prepared this information and they do not remember precisely what query or criteria was used to run the analysis (it was done through a computer/spreadsheet query). In any event, I asked them to perform the same breakdown with respect to all of the PCR tests performed from 2020 in the spreadsheet (excluding those tests that do not use a Ct value). The result was quite similar to the result in the December analysis of the 5,825 tests:

CT	# of tests	% from all 2020 tests	% from the 5825 tests
<19.99	3039	33%	~35%
20-24.99	2135	23%	~23%
25-29.99	1725	19%	18%
30-35.99	1713	19%	18%
>36	582	6%	7%
total	9194		

Number of Positive PCR tests in Manitoba

11. I understand Applicants' counsel suggests there is a discrepancy between the total number of reported COVID-19 cases in Manitoba and the number of tests Cadham has performed. Applicants' counsel points to Dr. Loeppky's affidavit (Exhibit B, p. 7) which identified 26,785 lab confirmed COVID-19 cases, whereas she said the spreadsheet I provided only showed 15,466 positive test results. There is no discrepancy. Cadham is one of a few laboratories that conducts PCR testing for COVID-19 in Manitoba. Shared Health Inc. conducts PCR testing, primarily for samples taken in hospital settings, and a private laboratory service provider, Dynacare,

conducts many of the community tests for Winnipeg based public testing centres. I provided information that is in the possession of Cadham about the tests it had performed. The balance of the positive tests were performed by these other agencies.

World Health Organization Recommendations for PCR testing Guidelines

12. In Dr. Warren's discussion of my report on page 3 of Exhibit B of his affidavit (sworn March 30, 2021), he asserts that "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'." Dr. Warren takes this quote from a January 13, 2021 notice to laboratory professionals published by the WHO entitled "Information Notice for IVD Users 2020/05". A copy of the January 13, 2021 Notice is attached as **Exhibit A**.

13. Dr. Bhattacharya, in his discussion of my report and the Ct values of PCR tests generally on page 14 of Exhibit "A" to his second affidavit (sworn March 31, 2021), also refers to the January 13, 2021 WHO "Information Notice for IVD Users 2020/05" (**Exhibit A**).

14. I can confirm that I am aware of the WHO guidance around the use of PCR tests and agree that an in-vitro diagnostic medical device (IVD) user must be aware of the limitations of the PCR tests in diagnosing and treating a particular patient. Cadham performs the lab test, but interpretation in the context of clinical and epidemiological information must also be made.

15. WHO guidance on the use of PCR tests includes both the document that Dr. Warren and Dr. Bhattacharya reference, which is a reminder to laboratory professionals to ensure they follow the instructions for the PCR test they are administering, as well as a more comprehensive guidance document entitled "Interim Guidance on Diagnostic Testing for SARS-CoV-2" issued by the WHO on September 11, 2020. A copy of the Interim Guidance document is attached as **Exhibit B.**

16. A number of elements of the September 11, 2020 Interim Guidance (**Exhibit B**) are salient in responding to Dr. Warren's comments, and also the comments of Dr. Kettner in his report at p. 8 of Exhibit B to his affidavit (sworn April 1, 2021). In particularly Dr. Kettner suggests that viral culture is the "gold standard" for determining infectivity. While the ability to culture virus does confirm infectiousness in cells and thus likely in humans, the converse is not true, as discussed in my report. However, the Interim Guidance document provides some helpful context for understanding the role of the PCR test and viral culture in the pandemic response. The WHO advises that viral isolation (culturing virus) is not recommended in the diagnostic process due to the biosafety level required to safety do the tests (BSL-3 or what we call containment laboratory 3/CL3 in Canada). That is, very few labs in Canada are capable of safely culturing the virus. The WHO also suggests that rapid testing is required for planning and design of public health and outbreak control, with approximately 24 hours being the maximum recommended in most settings. Cell culture cannot achieve this.

17. The January 13, 2021 WHO Notice also observes "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".

18. It is certainly true that disease prevalence has an effect on false positives. For example, in the middle of the summer 2020, when there were few confirmed cases of COVID-19 in Manitoba, the likelihood of a false positive may have been expected to be marginally higher. This would not be the case in the fall and winter of 2020 when community transmission of the virus was widespread. Cadham also follows the WHO's recommendation to perform repeat confirmatory testing on tests with a Ct value of 36.5 or greater.

19. The WHO Notice also goes on to recommend providing the Ct value of a test to the requesting health care provider.

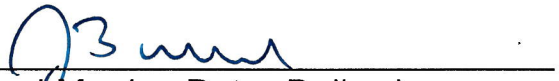
20. Shortly after this recommendation was issued by the WHO, the same subject was considered by the Canadian Public Health Laboratory Network ("CPHLN"). CPHLN is a national association of public health laboratory professionals which acts as a unified voice for federal and provincial member laboratories. CPHLN is composed of provincial public health laboratories and the National Microbiology Laboratory. It provides guidance to Canadian public health laboratories and facilitates collaboration.

21. The CPHLN prepared a FAQ which addresses a number of questions about PCR tests, Ct values and their use. Despite the recommendation in the WHO's January 13, 2021 Notice, the CPHLN reaffirmed the practice of not routinely reporting Ct values with PCR results, and concludes with similar recommendations as the United States Centers for Disease Control and Prevention, the (American) Association of Public Health Laboratories, and the Ontario Ministry of Health. As indicated in the FAQ document this is due to the concern that the Ct value should not be used alone to make clinical or

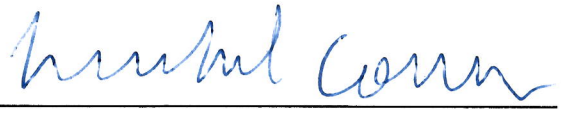
public health judgements. Not all PCR or alternate nucleic acid amplification assays produce Ct values or an equivalent proxy measure of viral load. Further, while Ct values can provide an indication that someone is more or less likely to be infectious, a Ct value cannot be used to determine infectiousness definitively. However, Ct values may be provided to physicians upon request and the lab will consult to assist interpreting the test results. I can confirm that Cadham also does on occasion provide Ct values on request to Medical Officers of Health and institutional infection control practitioners, but only along with a full consultation on the individual's clinical and contact details. It should be noted that consultation with public health professionals is also required as part of good disease control practice. A copy of the FAQ is attached as **Exhibit C.**

22. I make this affidavit *bona fide*.

AFFIRMED BEFORE ME at)
the City of Winnipeg, in)
the Province of Manitoba,)
this 29th day of April, 2021)



Jared Manley Peter Bullard



A Barrister at Law in and for
The Province of Manitoba

This is Exhibit "A" referred to in the Affidavit of Jared Manley Peter Bullard affirmed before me this 29th day of April, 2021.

A Barrister-at-law entitled to practice in and for the Province of Manitoba.

WHO Information Notice for IVD Users 2020/05

Nucleic acid testing (NAT) technologies that use polymerase chain reaction (PCR) for detection of SARS-CoV-2

20 January 2021 | Medical product alert | Geneva | Reading time:

العربية | 中文 | Français | Русский | Español | Português

Product type: Nucleic acid testing (NAT) technologies that use polymerase chain reaction (PCR) for detection of SARS-CoV-2

Date: 13 January 2021

WHO-identifier: 2020/5, version 2

Target audience: laboratory professionals and users of IVDs.

Purpose of this notice: clarify information previously provided by WHO. This notice supersedes WHO Information Notice for In Vitro Diagnostic Medical Device (IVD) Users 2020/05 version 1, issued 14 December 2020.

Description of the problem: WHO requests users to follow the instructions for use (IFU) when interpreting results for specimens tested using PCR methodology.

Users of IVDs must read and follow the IFU carefully to determine if manual adjustment of the PCR positivity threshold is recommended by the manufacturer.

WHO guidance Diagnostic testing for SARS-CoV-2 states that careful interpretation of weak positive results is needed (*1*). The cycle threshold (Ct) needed to detect virus is inversely proportional to the patient's viral load. Where test results do not correspond with the clinical presentation, a new specimen should be taken and retested using the same or different NAT technology.

WHO reminds IVD users that disease prevalence alters the predictive value of test results; as disease prevalence decreases, the risk of false positive increases (*2*). 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.

Most PCR assays are indicated as an aid for diagnosis, therefore, 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.

Actions to be taken by IVD users:

1. Please read carefully the IFU in its entirety.
2. Contact your local representative if there is any aspect of the IFU that is unclear to you.
3. Check the IFU for each incoming consignment to detect any changes to the IFU.
4. Provide the Ct value in the report to the requesting health care provider.

Contact person for further information:

Anita SANDS, Regulation and Prequalification, World Health Organization, e-mail: rapidalert@who.int

References:

1. Diagnostic testing for SARS-CoV-2. Geneva: World Health Organization; 2020, WHO reference number WHO/2019-nCoV/laboratory/2020.6.

2. Altman DG, Bland JM. Diagnostic tests 2: Predictive values. BMJ. 1994 Jul 9;309(6947):102. doi: 10.1136/bmj.309.6947.102.

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This is Exhibit "B" referred to in the Affidavit of Jared Manley Peter Bullard affirmed before me this 29th day of April, 2021.

A Barrister-at-law entitled to practice in and for the Province of Manitoba.

Diagnostic testing for SARS-CoV-2

Interim guidance

11 September 2020

World Health Organization

Introduction

This document provides interim guidance to laboratories and other stakeholders involved in diagnostics for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It covers the main considerations for specimen collection, nucleic acid amplification testing (NAAT), antigen (Ag), antibody (Ab) detection and quality assurance. This document will be updated as new information becomes available. Feedback can be sent to WHElab@who.int.

Changes from the previous version

The title of this interim guidance has changed from "Laboratory testing for COVID-19 in suspected human cases" to "Diagnostic testing for SARS-CoV-2". Additional relevant background information and a clinical diagnostic algorithm has been added to the document. Furthermore, the guidance has been updated with new findings from the literature and best practices.

Relevant WHO documents

WHO has developed interim guidance and technical briefs to assist policy-makers and laboratories on testing for SARS-CoV-2. These documents cover laboratory testing strategy [1], laboratory assessment tool [2], laboratory biosafety [3], advice on the use of point-of-care immunodiagnostic tests [4], antigen detection in diagnosis of SARS-CoV-2 infection using rapid immunoassays [5], guidance for the investigations of clusters [6], public health surveillance [7] and operational considerations for surveillance using GISRS [8]. In addition, early investigation protocols [9] can be used by countries to implement epidemiological studies and enhance understanding of transmission patterns, disease severity and prevalence, clinical features and risk factors of SARS-CoV-2 infection.

Background on SARS-CoV-2

WHO was first alerted to a cluster of pneumonia of unknown etiology in Wuhan, People's Republic of China on 31 December 2019. The virus was initially tentatively named 2019 novel coronavirus (2019-nCoV).

Subsequently the International Committee of Taxonomy of Viruses (ICTV) named the virus SARS-CoV-2 [10]. COVID-19 is the name of the illness caused by SARS-CoV-2.

SARS-CoV-2 is classified within the genus *Betacoronavirus* (subgenus *Sarbecovirus*) of the family *Coronaviridae* [11]. It is an enveloped, positive sense, single-stranded ribonucleic acid (RNA) virus with a 30-kb genome [10]. The virus has an RNA proofreading mechanism keeping the mutation rate relatively low. The genome encodes for non-structural proteins (some of these are essential in forming the replicase transcriptase complex), four structural proteins (spike (S), envelope (E), membrane (M), nucleocapsid (N)) and putative accessory proteins [12-14]. The virus binds to an angiotensin-converting enzyme 2 (ACE2) receptor for cell entry [15-17].

SARS-CoV-2 is the seventh coronavirus identified that is known to infect humans (HCoV). Four of these viruses, HCoV-229E, HCoV-NL63, HCoV-HKU1 and HCoV-OC43, are endemic, seasonal and tend to cause mild respiratory disease. The other two viruses are the more virulent zoonotic Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus type 1 (SARS-CoV-1). SARS-CoV-2 is most genetically similar to SARS-CoV-1, and both of these viruses belong to the subgenus *Sarbecovirus* within the genus *Betacoronavirus* [11]. However, SARS-CoV-1 is currently not known to circulate in the human population.

The clinical presentation of SARS-CoV-2 infection can range from asymptomatic infection to severe disease [18-27]. Mortality rates differ per country [28]. Early laboratory diagnosis of a SARS-CoV-2 infection can aid clinical management and outbreak control. Diagnostic testing can involve detecting the virus itself (viral RNA or antigen) or detecting the human immune response to infection (antibodies or other biomarkers).

While our understanding of SARS-CoV-2 has rapidly expanded, there are still many outstanding questions that need to be addressed. WHO encourages research and the sharing of results that may contribute toward an improved characterization of SARS-CoV-2 [29, 30].

Background information on SARS-CoV-2 RNA detection

Standard confirmation of acute SARS-CoV-2 infections is based on the detection of unique viral sequences by nucleic acid amplification tests (NAATs), such as real-time reverse-transcription polymerase chain reaction (rRT-PCR). The assays' targets include regions on the E, RdRP, N and S genes.

Once an individual has been infected by the virus, the mean time it takes to develop symptoms (incubation period) is 5-6 days, with a range of between 1 and 14 days following exposure [31-35]. The virus may be detectable in the upper respiratory tract (URT) 1-3 days before the onset of symptoms. The concentration of SARS-CoV-2 in the URT is highest around the time of symptom onset, after which it gradually declines [36-42]. Some studies report higher viral loads in the severely ill compared with patients with mild disease, while other studies do not report such differences [36, 43-49]. The presence of viral RNA in the lower respiratory tract (LRT), and for a subset of individuals in the faeces, increases during the second week of illness [38]. In some patients the viral RNA may only be detectable for several days, while in other patients it can be detected for several weeks, possibly months [44, 50-60]. Prolonged presence of viral RNA does not necessarily signify prolonged infectiousness. Several studies describe the correlation between reduced infectiousness and i) increased number of days that have elapsed since symptom onset and resolution, ii) decrease in viral load in respiratory secretions [37, 61-64] and iii) an increase in neutralizing antibodies [37, 61]. More information can be found on this in Criteria to release COVID-19 patients from isolation [65].

Respiratory secretions may be quite variable in composition, and the adequacy of sampling efforts may also vary, which can occasionally result in false-negative PCR results [40, 42, 58, 66-74]. In patients for whom SARS-CoV-2 infection is strongly suspected and URT swabs are negative, viral RNA may be detected in LRT secretions, such as sputum or bronchoalveolar lavage [70, 71, 75, 76]. Faeces or rectal swabs have been shown to be positive for SARS-CoV-2 RNA in a subset of patients, with some studies suggesting that this positivity is prolonged compared to that of respiratory tract specimens [46, 56, 59, 75, 77]. In some patients, SARS-CoV-2 RNA detection in blood samples has been reported and some studies suggest that detection in the blood is associated with disease severity, however, more studies on this potential association are required [75, 78-81]. In oral fluid specimens (e.g. induced saliva) [28, 49, 82-88], reported detection rates compared with URT specimens from the same patient vary widely, and limited data are available on adequacy of SARS-CoV-2 detection in gargling/mouth washes [85]. The striking differences in sensitivity of oral fluids evaluations are potentially due to large differences in collection, transport and storage techniques, as well as the evaluation of different testing populations. Occasionally, SARS-CoV-2 can be detected in ocular fluids in patients with and without signs of conjunctivitis [89-93]. Some studies have not detected SARS-CoV-2 in urine [58, 75, 94], while others were able to detect viral RNA in urine in a limited number of patients [57, 95]. One study reported several patients with positive semen samples [96]. In addition, positive RNA detection for brain tissue [97] and cerebrospinal fluid [98] have been described in case reports. Thus, SARS-CoV-2 can be detected in a wide range of other body fluids and compartments, but it is most frequently detected in respiratory material and, therefore, respiratory samples remain the sample type of choice for diagnostics.

Laboratory testing guiding principles

The decision to test should be based on both clinical and epidemiological factors. See the interim guidance clinical management of COVID-19 [99], investigations of clusters [6] and public health surveillance [7].

Rapid collection of appropriate specimens from and accurate laboratory diagnosis of patients in whom SARS-CoV-2 infection is strongly suspected are the two priorities to support clinical management of patients and infection control measures. Given the complexity of adequate sampling, laboratory analysis, and interpretation of results, collection and laboratory diagnosis should be performed by trained and competent operators.

Individuals infected with SARS-CoV-2 may never develop symptoms (asymptomatic cases), they may have very mild disease (pauci-symptomatic), or they may develop moderate to severe COVID-19 disease [18-26]. The most robust evidence for viral infection comes from the detection of fragments of the virus, such as proteins or nucleic acids, through virological testing. Infected individuals may test positive for viral nucleic acids or viral proteins without symptoms (asymptomatic), or before symptom onset (pre-symptomatic), and throughout a disease episode (symptomatic). For those who develop COVID-19 illness, symptoms can be wide-ranging at initial presentation of disease. Individuals may present with very mild symptoms, with apparent pneumonia, febrile illnesses/sepsis, and less commonly with gastro-enteritis or neurological symptoms [99]. If required for case management, patients should also be tested for other pathogens, as recommended in local clinical management guidelines, but this should never delay testing for SARS-CoV-2 [99, 100]. Co-infections of SARS-CoV-2 with other pathogens have been reported, thus a positive test for another pathogen does not rule out COVID-19 and vice versa [27, 101-109]. Cases of false positive dengue antibody test results using a dengue rapid diagnostic test (RDT) in COVID-19 patients have been reported [110, 111]. There is also a risk of false positive or false negative SARS-CoV-2 results, if testing is not performed with adequate assays or not done under adequate conditions.

Specimen collection, shipment and storage

Safety procedures during specimen collection

Ensure that health workers collecting clinical specimens from suspect cases adhere rigorously to infection prevention and control guidelines (IPC) and wear appropriate personal protective equipment (PPE), see also COVID-19 WHO interim guidance on infection prevention and control during health care [7].

Ensure that adequate standard operating procedures (SOP) are in place and that staff are appropriately trained in specimen collection, packaging, shipment and storage. It should be assumed that all specimens collected for investigations may be infected with SARS-CoV-2 and other pathogens. See also WHO interim guidance on laboratory biosafety for SARS-CoV-2 [3]. Local guidelines, including on informed consent, should be followed for specimen collection, testing, storage and research.

Specimens to be collected

The optimal specimen depends on clinical presentation and time since symptom onset. At minimum, respiratory specimens should be collected.

Respiratory specimens

- **Upper respiratory specimens** are adequate for testing early-stage infections, especially in asymptomatic or mild cases. Testing combined nasopharyngeal and oropharyngeal swabs from one individual has been shown to increase sensitivity for detection of respiratory viruses and improve the reliability of the result [60, 86, 112-114]. Two individual swabs can be combined in one collection tube or a combined nasopharyngeal and oropharyngeal swab can be taken [115]. A few studies have found that individual nasopharyngeal swabs yield a more reliable result than oropharyngeal swabs [40, 75, 76, 114].
- **Lower respiratory specimens** are advised if collected later in the course of the COVID-19 disease or in patients with a negative URT sampling and there is a strong clinical suspicion of COVID-19 [70, 71, 75, 76, 86]. LRT specimens can consist of sputum, if spontaneously produced (induced sputum is not recommended as this poses an increased risk of aerosol transmission [99]) and/or endotracheal aspirate or bronchoalveolar lavage in patients with more severe respiratory disease. Caution should be exercised due to the high risk of aerosolization; therefore strict adherence to IPC procedures during sample collection is required. The indication for an invasive procedure should be evaluated by a physician.

Before implementing other respiratory or oral fluid sampling methods, the sampling method should first pass validation in the laboratory for the intended patient groups.

Simplified and optimized specimen collection

There is a high demand for simplified and optimized specimen collection for SARS-CoV-2 detection. Studies on combined oropharyngeal and nares/nasal swab [116, 117], others on midturbinate [118-120] or lower nasal or nares swabs [120, 121] or tongue swab [120] either by a trained sampler or by self-sampling have been conducted. While some of these studies show that these approaches perform reasonably well, these studies focus mostly on specific patient groups and their sample sizes are limited. Before broad implementation of these alternatives can be recommended, further assessment and validation is needed to determine the indications for which these collection methods serve as appropriate alternatives.

There are specific cases where collecting nasopharyngeal and oropharyngeal swabs can be problematic, such as mass screening in schools or nursing homes, especially when elderly people with dementia or young children are involved. In these scenarios, oral fluids could potentially be a suitable specimen, as the collection methods are less invasive and there is a lower risk of exposure to others upon collection, as compared with the collection of URT specimens.

Oral fluid collection methods vary widely: from posterior oropharyngeal fluids/saliva collected by spitting or drooling, or collection of oral fluid with pipet or special sponges. Gargling with saline solutions is another alternative that has been studied. Sensitivity of these specimens has a wide performance range compared with naso- and/or oropharyngeal sampling [28, 49, 82, 83, 85-88, 122-125]. Due to the large variety of collection methods and processing steps, laboratories must collect their own performance data linked to the local method of collection and in the relevant population for testing. At this time, WHO does not recommend the use of saliva as the sole specimen type for routine clinical diagnostics. If nonstandard collection methods are intended to be used to diagnose other respiratory pathogens, the detection of these pathogens needs to be part of the validation procedure.

Faecal specimens

From the second week after symptom onset and onwards, NAAT can be considered for faecal specimens in cases where URT and LRT are negative and the clinical suspicion of a COVID-19 infection remains [126]. When testing faeces, ensure that the intended extraction method and NAAT has been validated for this type of sample.

Post-mortem specimens

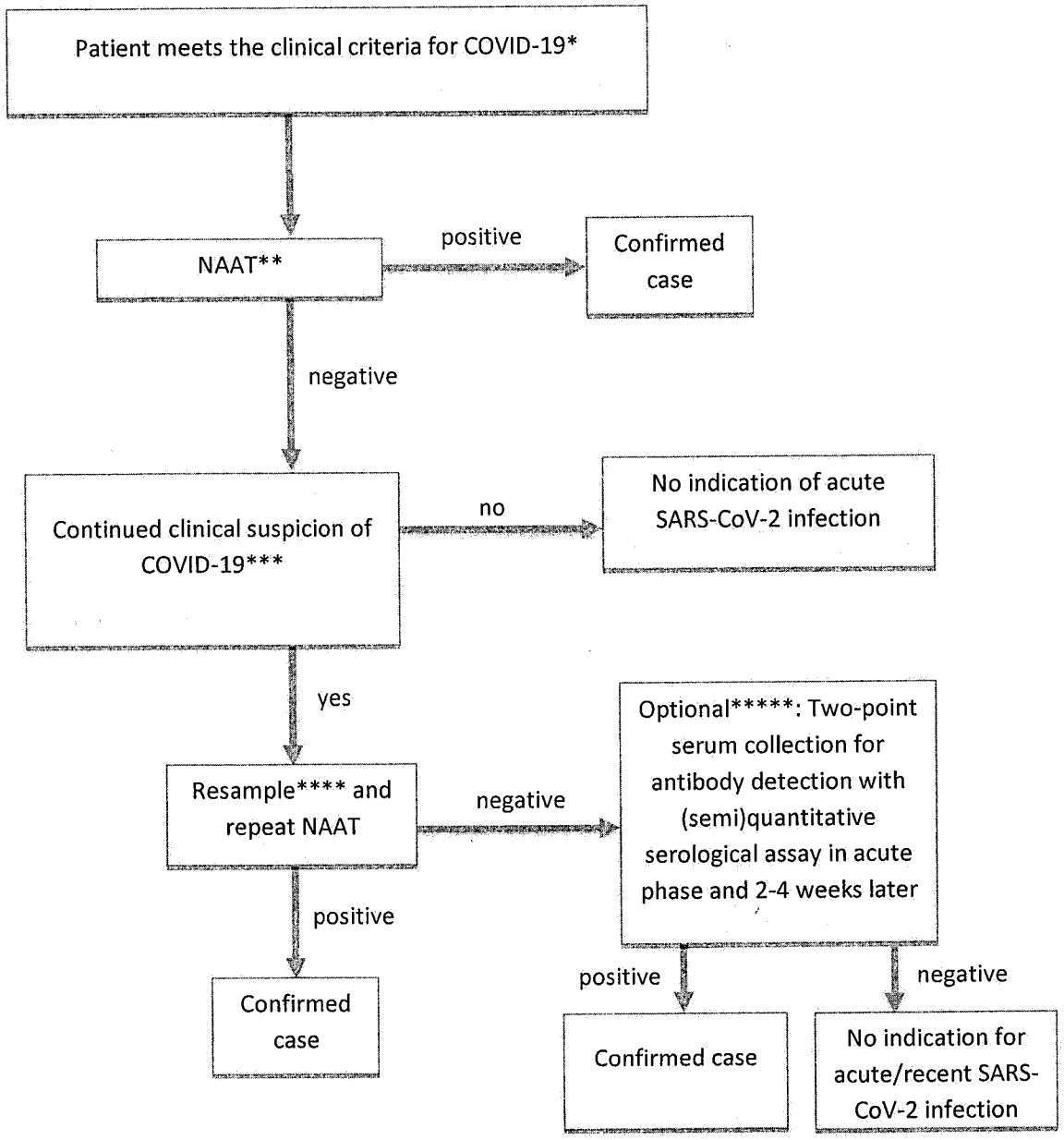
If the person is deceased, consider taking a post-mortem swab, needle biopsy or tissue specimens from the autopsy, including lung tissue for further pathological and microbiological testing [127-133].

Serum specimens

If negative NAAT results are obtained from a patient in whom SARS-CoV-2 infection is strongly suspected, a paired serum specimen could be collected. One specimen taken in the acute phase and one in the convalescent phase 2-4 weeks later can be used to look for seroconversion or a rise in antibody titres. These two samples can be used retrospectively to determine whether the individual has had COVID-19, especially when the infection could not be detected using NAAT.

See Figure 1 for the diagnostic algorithm for cases requiring clinical care and are suspected to have COVID-19.

Figure 1: Diagnostic flow diagram for the detection of acute SARS-CoV-2 infection in individuals with clinical suspicion for COVID-19



* Clinical management of COVID-19 (Interim Guidance), World Health Organization [99].

** If antigen detection would be incorporated into the testing algorithm, how this needs to be done depends on the sensitivity and specificity of the antigen test and on the prevalence of SARS-CoV-2 infection in the intended testing population. For more information see section below on "Rapid diagnostic tests based on antigen detection" and the specific guidance Interim guidance on antigen-detection in diagnosis of SARS-CoV-2 infection using rapid immunoassays [5].

*** Continued clinical suspicion can, for example, be the absence of another obvious etiology, the presence of an epidemiological link, or suggestive clinical finding (e.g. typical radiological signs).

**** The selection of specimen type will depend on the clinical presentation, see section "Specimens to be collected". Increasing the number of samples tested will also increase the sensitivity of testing for COVID-19. More than two samples might be needed on some occasions to detect SARS-CoV-2 [73].

***** For interpretation of serology, see section "Implementation and interpretation of antibody testing in the clinical laboratory". Serology cannot be used as a standalone diagnostic for acute SARS-CoV-2 infections and clinical management.

Packaging and shipment of clinical specimens

Specimens for virus detection should reach the laboratory as soon as possible after collection. Correct handling of specimens during transportation and in the laboratory is essential. For guidance on this see Annex 1.

Transportation of specimens within national borders should comply with the applicable national regulations. International transport of specimens that may contain SARS-CoV-2 should follow the United Nations Model Regulations, Biological Substance, Category B (UN 3373), and any other applicable regulations depending on the mode of transport.

More information may be found in the WHO Guidance on regulations for the transport of infectious substances 2019-2020 [134] and specific SARS-CoV-2 laboratory biosafety guidance [3] and shipment instructions [135].

Maintain open and efficient lines of communication with the laboratory and provide all requested information. Specimens should be correctly labelled and accompanied by a diagnostic request form (see Annex 2 for a request form template, including minimal required clinical information). Alerting the laboratory before sending specimens and providing the essential background information with the diagnostic request allows for the proper and timely processing of specimens and reporting of results.

Biosafety practices in the laboratory

Laboratories undertaking testing for SARS-CoV-2 should adhere strictly to the appropriate biosafety practices. Testing clinical specimens that may contain SARS-CoV-2 should be performed in appropriately equipped laboratories by staff trained in the relevant technical and safety procedures. National guidelines on laboratory biosafety should be followed in all circumstances. Specimen handling for molecular testing using standard rRT-PCR requires biosafety level (BSL) 2 or equivalent facilities with the use of a biosafety cabinet (BSC) or a primary containment device that is recommended for sample manipulation before inactivation.

Attempts to isolate the virus in cell culture require BSL-3 facilities at a minimum. When performing viral culture on potentially SARS-CoV-2 positive clinical specimens for other purposes, a risk assessment needs to be conducted followed by required safety measures and procedures [136].

Specific considerations of biosafety requirements may allow certain point-of-care (POC) or near-patient assays to be performed outside a biosafety cabinet, once local regulations have been reviewed, after performing risk assessment and having put in place adequate risk-mitigation measures. For further details on laboratory biosafety, see specific laboratory biosafety interim guidance [3]. For general laboratory biosafety guidelines, see the WHO Laboratory biosafety manual, 3rd edition [136].

Testing for SARS-CoV-2

Nucleic acid amplification test (NAAT)

Wherever possible, suspected active SARS-CoV-2 infections should be tested with NAAT, such as rRT-PCR. NAAT assays should target the SARS-CoV-2 genome. Since there is currently no known circulation of SARS-CoV-1 globally, a sarbecovirus-specific sequence is also a reasonable target. For commercial assays, interpretation of results should be done according to the instructions for use. Optimal diagnostics consist of a NAAT assay with at least two independent targets on the SARS-CoV-2 genome, however, in areas with widespread transmission of SARS-CoV-2, a simple algorithm might be adopted with one single discriminatory target. When using a one-target assay, it is recommended to have a strategy in place to monitor for mutations that might affect performance. For more details, see section below on "Background information on monitoring for mutations in primer and probe regions".

Background information on monitoring for mutations in primer and probe regions

As SARS-CoV-2 continues to acquire genetic changes over time, mismatches between primers and/or probes, and corresponding binding sites within SARS-CoV-2 genomes may reduce NAAT sensitivity. Where feasible, monitor for primer and probe mismatches due to SARS-CoV-2 mutations, and assess their impact. By routinely testing all specimens with two different primer/probe sets that target different genomic regions it is possible to reduce the risk of false-negative results. Several tools monitoring for relevant mutations are available, including searches done by GISAID (the Global Initiative on Sharing All Influenza Data) and other tools including PrimerCheck (Erasmus Medical Centre), PrimerScan (European Centre for Disease prevention and Control) and CoV-GLUE (COVID-19 UK Genomics Consortium and MRC-University of Glasgow Centre for Virus Research). Primercheck and COV-GLUE allows researchers to use their own sequence data confidentially as input. Not all mutations in primer/probe regions lead to significant changes in performance. *In silico* predictions of binding efficiency are insufficient to quantify the effect of a mismatch on the sensitivity of a NAAT, so it is essential to do an experimental comparison of the assay's sensitivity for both variant and reference virus isolates. For commercial assays, it is vital to keep track of possible incidents of suboptimal performance. Inform the manufacturer of the assay and WHO of any concerns you may experience with a specific assay.

Many in-house and commercial rRT-PCR assays have become available and several have been independently validated [137-143]. Some considerations for selecting the right NAAT for the laboratory are listed in Annex 3. A few of the NAAT systems have the capacity for fully automated testing that integrates sample processing as well as the capacity for RNA extraction, amplification and

reporting. Such systems provide access to testing in locations with limited laboratory capacity and rapid turnaround time when used for near-patient testing. Validation data of some of these assays are now available [144]. When implementing these assays in specific settings, staff performing the test should be appropriately trained, performance should be assessed in those specific settings and a system to monitor quality should be put in place. Additional potentially valuable amplification/detection methods, such as CRISPR (targeting clustered regularly interspaced short palindromic repeats), isothermal nucleic acid amplification technologies (e.g. reverse transcription loop-mediated isothermal amplification (RT-LAMP), and molecular microarray assays are under development or in the process of being commercialized [145-147]. Validation of the analytic and clinical performance of these assays, demonstration of their potential operational utility, rapid sharing of data, as well as emergency regulatory review of manufacturable, well-performing tests are encouraged to increase access to SARS-CoV-2 testing.

Careful interpretation of weak positive NAAT results is needed, as some of the assays have shown to produce false signals at high Ct values. When test results turn out to be invalid or questionable, the patient should be resampled and retested. If additional samples from the patient are not available, RNA should be re-extracted from the original samples and retested by highly experienced staff. Results can be confirmed by an alternative NAAT test or via virus sequencing if the viral load is sufficiently high. Laboratories are urged to seek reference laboratory confirmation of any unexpected results.

One or more negative results do not necessarily rule out the SARS-CoV-2 infection [40, 42, 58, 66-74]. A number of factors could lead to a negative result in an infected individual, including:

- poor quality of the specimen, because it contains too little patient material;
- the specimen was collected late in the course of the disease, or the specimen was taken from a body compartment that did not contain the virus at that given time;
- the specimen was not handled and/or shipped appropriately;
- technical reasons inherent in the test, e.g. PCR inhibition or virus mutation.

For clinical case management a proposed testing algorithm is depicted in Figure 1.

Alternatives to RNA extraction

Most conventional molecular diagnostic workflows require RNA extraction before an rRT-PCR test is conducted. However, there are global shortages of commercial extraction kits due to the COVID-19 pandemic. Direct rRT-PCR from nasopharyngeal swabs may provide an emergency or temporary alternative to RNA extraction, but limitations to the input volume, as well as an increased risk of RNA degradation and PCR inhibition can lead to a loss of sensitivity of the assay [148, 149]. Heat treatment prior to sample processing can affect the RNA quality [149, 150]. Other factors that can affect RNA quality and which should be evaluated before implementation are the addition of detergents, transport media, the volume of the specimen used, and the polymerase enzyme used [148, 151-154]. The biosafety implications of alternative extraction workflows should also be considered. Laboratories considering alternative methods that bypass the need for RNA extraction should validate their protocols thoroughly and conduct a risk assessment that weighs the benefits and risks, before integrating such protocols into a diagnostic workflow.

Pooling of specimens for NAAT

Pooling of samples from multiple individuals can increase the diagnostic capacity for detecting SARS-CoV-2 when the rate of testing does not meet the demand in some settings [155-159]. There are several strategies for pooling specimens. If the pooled result is negative, all individual specimens in the pool are regarded as negative. If the pool tests positive the follow-up steps depend on the strategy, but in general each specimen needs to undergo individual testing (pool deconvolution) to identify the positive specimen(s). Another approach is matrix pooling. This means that pools are made per row and per column, and tested by PCR, the position in the matrix identifies the positive specimen without additional testing if prevalence is sufficiently low. Depending how robust the matrix testing method is in the specific context, it might still be advisable to retest the identified positive samples for confirmation. Pooling of specimens could be considered in population groups with a low/very low expected prevalence of SARS-CoV-2 infection, but not for cases or cohorts that more likely to be infected with SARS-CoV-2. Routine use of the pooling of specimens from multiple individuals in clinical care and for contact tracing purposes is not recommended. Studies have been conducted to determine the optimal sample pooling number and design pooling strategies in different outbreak settings [156, 160-162].

Before any sample pooling protocols can be implemented, they must be validated in the appropriate populations and settings. An inappropriate testing strategy may lead to missed cases or other laboratory errors that may, in turn, negatively affect patient management and public health control measures. In addition, the risk of cross-contamination and the potential increase in workload complexity and volume must be considered. To perform reliable pooling, adequate automation is key (e.g. robotic systems, software supporting the algorithms to identify positive samples, laboratory information systems and middle-ware that can work with sample pooling).

Based on currently available data, intra-individual pooling (multiple specimens from one individual that are pooled and tested as a single sample) from upper respiratory tract samples can be used. Intra-individual pooling of sputum and faeces with URT samples is not recommended because the former may contain compounds that inhibit rRT-PCR.

Rapid diagnostic tests based on antigen detection

Rapid diagnostic tests that detect the presence of SARS-CoV-2 viral proteins (antigens) in respiratory tract specimens are being developed and commercialized. Most of these are lateral flow immunoassays (LFI), which are typically completed within 30 minutes. In contrast to NAATs, there is no amplification of the target that is detected, making antigen tests less sensitive. Additionally, false-positive (indicating that a person is infected when they are not) results may occur if the antibodies on the test strip also recognize antigens of viruses other than SARS-CoV-2, such other human coronaviruses.

The sensitivity of different RDTs compared to rRT-PCR in specimens from URT (nasopharyngeal swabs) appears to be highly variable [144, 163-165], but specificity is consistently reported to be high. Currently, data on antigen performance in the clinical setting is still limited: paired NAAT and antigen validations in clinical studies are encouraged to identify which of the antigen detection tests that are either under development or have already been commercialized demonstrate acceptable performance in representative field studies. When performance is acceptable, antigen RDTs could be implemented in a diagnostic algorithm to reduce the number of molecular tests that need to be performed and to support rapid identification and management of COVID-19 cases. How antigen detection would be incorporated into the testing algorithm depends on the sensitivity and specificity of the antigen test and on the prevalence of SARS-CoV-2 infection in the intended testing population. Higher viral loads are associated with improved antigen test performance; therefore test performance is expected to be best around symptom onset and in the initial phase of a SARS-CoV-2 infection. For specific guidance on antigen detection tests see the WHO Interim guidance on antigen-detection in diagnosis of SARS-CoV-2 infection using rapid immunoassays [5].

Antibody testing

Serological assays that detect antibodies produced by the human body in response to infection with the SARS-CoV-2 can be useful in various settings.

For example, serosurveillance studies can be used to support the investigation of an ongoing outbreak and to support the retrospective assessment of the attack rate or the size of an outbreak [9]. As SARS-CoV-2 is a novel pathogen, our understanding of the antibody responses it engenders is still emerging and therefore antibody detection tests should be used with caution, and not used to determine acute infections.

Non-quantitative assays (e.g. lateral flow assays) cannot detect an increase in antibody titres, in contrast to (semi)quantitative or quantitative assays. Lateral flow antibody detection assays (or other non-quantitative assays) are currently not recommended for acute diagnosis and clinical management and their role in epidemiologic surveys is being studied. For more information on the utility of rapid immunodiagnostic tests, we refer to the WHO scientific brief with advice on the specific SARS-CoV-2 point-of-care immunodiagnostic tests [4].

Serology should not be used as a standalone diagnostic to identify acute cases in clinical care or for contact tracing purposes. Interpretations should be made by an expert and are dependent on several factors including the timing of the disease, clinical morbidity, the epidemiology and prevalence within the setting, the type of test used, the validation method, and the reliability of the results.

Seroconversion (development of measurable antibody response after infection) has been observed to be more robust and faster in patients with severe disease compared to those with milder disease or asymptomatic infections. Antibodies have been detected as early as in the end of the first week of illness in a fraction of patients, but can also take weeks to develop in patients with subclinical/mild infection [37, 166-173]. A reliable diagnosis of COVID-19 infection based on patients' antibody response will often only be possible in the recovery phase, when opportunities for clinical intervention or interruption of disease transmission have passed. Therefore, serology is not a suitable replacement for virological assays to inform contact tracing or clinical management. The duration of the persistence of antibodies generated in response to SARS-CoV-2 is still under study [49, 174]. Furthermore, the presence of antibodies that bind to SARS-CoV-2 does not guarantee that they are neutralizing antibodies, or that they offer protective immunity.

Available serological tests for detecting antibodies

Commercial and non-commercial tests measuring binding antibodies (Total immunoglobulins (Ig), IgG, IgM, and/or IgA in different combinations) utilizing various techniques including LFI, enzyme-linked immunosorbent assay (ELISA) and chemiluminescence immunoassay (CLIA) have become available. A number of validations and systematic reviews on these assays have been published [170, 171, 173, 175-177]. The performance of serologic assays varies widely in different testing groups (such as in patients with mild versus moderate-to-severe disease as well as in young versus old), timing of testing and the target viral protein. Understanding these performance variations will require further study. Antibody detection tests for coronavirus may also cross-react with other pathogens, including other human coronaviruses, [167, 178-180] or with pre-existing conditions (e.g. pregnancy, autoimmune diseases) and thus yield false-positive results.

Virus neutralization assays are considered to be the gold standard test for detecting the presence of functional antibodies. These tests require highly skilled staff and BSL-3 culture facilities and, therefore, are unsuitable for use in routine diagnostic testing.

Implementation and interpretation of antibody testing in the clinical laboratory

When implementing serological assays in the clinical laboratory, an in-house validation or verification of the specific assays is advisable. Even if commercial tests have been authorized for use in emergencies, an in-house verification (or if required by local authorities a validation) is still required. Protocols and examples with suggestions as to how to do this are now available [170, 171, 181].

Each serological test is different. With regard to commercial tests, follow the manufacturer's instructions for use. Studies show that several commercial assays measuring total Ig or IgG have performed well. Most of these studies show no advantage of IgM over IgG, as IgM does not appear much earlier than IgG [173]. The additional role of IgA testing in routine diagnostics has not been established. For confirmation of a recent infection, acute and convalescent sera must be tested using a validated (semi)quantitative or quantitative assay. The first sample should be collected during the acute phase of illness, and the second sample at least 14 days after the initial sera was collected. Maximum antibody levels are expected to occur in the third/fourth week after symptom onset. Seroconversion or a rise in antibody titres in paired sera will help to confirm whether the infection is recent and/or acute. If the initial sample tests positive, this result could be due to a past infection that is not related to the current illness.

The first known case of reinfection with SARS-CoV-2 has been documented [182]. Only limited information is available on the interpretation of SARS-CoV-2 antibody tests after a previous infection with SARS-CoV-2 and on the dynamics of SARS-CoV-2 serology if a subsequent infection with another coronavirus occurs. In these two sets of circumstances interpretation of serology may be extremely challenging.

Viral isolation

Virus isolation is not recommended as a routine diagnostic procedure. All procedures involving viral isolation in cell culture require trained staff and BSL-3 facilities. A thorough risk assessment should be carried out when culturing specimens from potential SARS-CoV-2 patients for other respiratory viruses because SARS-CoV-2 has been shown to grow on a variety of cell lines [183].

Genomic sequencing for SARS-CoV-2

Genomic sequencing for SARS-CoV-2 can be used to investigate the dynamics of the outbreak, including changes in the size of an epidemic over time, its spatiotemporal spread, and testing hypotheses about transmission routes. In addition, genomic sequences can be used to decide which diagnostic assays, drugs and vaccines may be suitable candidates for further exploration. Analysis of SARS-CoV-2 virus genomes can, therefore, complement, augment and support strategies to reduce the disease burden of COVID-19. However, the potentially high cost and volume of the work required for genomic sequencing means that laboratories should have clarity about the expected returns from such investment and what is required to maximize the utility of such genomic sequence data. WHO guidance on SARS-CoV-2 genomic sequencing is currently being developed.

Quality assurance

Before introducing a new testing method, a new assay, new batches of materials, or a new PCR technician into the laboratory, a validation or verification should be carried out, to ensure that the laboratory testing system is performing adequately.

For manual PCR systems, each NAAT sample should include internal controls and ideally a specimen collection control (human gene target). Additionally, external controls are recommended for each test run. Laboratories that order their own primers and probes should carry out entry testing or validation looking at functionality and potential contaminants [184].

Laboratories are encouraged to define their assays' detection limits, and senior staff should recognize how disease prevalence alters the predictive value of their test results. Once the number of cases goes down, the positive predictive value will decrease, therefore the interpretation of tests should continue to be part of a stringent quality assurance scheme, with interpretation based on: timing of sampling, sample type, test specifics, clinical data and epidemiological data.

Laboratories should put measures in place to reduce the potential for false positive rRT-PCR results and have a strategy for the management of equivocal results. See Annex 4 for a checklist.

In general, laboratories should have a quality assurance system in place and are encouraged to participate in external quality assessment (EQA) schemes or perform result comparison between laboratories of a subset of samples.

WHO has previously advised national laboratories to ensure quality performance by confirmation of testing results for the first 5 positive specimens and the first 10 negative specimens (collected from patients that fit the case definition) by referring them to one of the WHO reference laboratories that provide confirmatory testing for SARS-CoV-2. WHO provided support to national laboratories to facilitate specimen shipment to one of the dedicated reference laboratories. For more information, consult the WHO website for the list of reference laboratories [185] and shipment instructions [135]. Strengthened national reference laboratories and growing access to EQA schemes for SARS-CoV-2 reduce the need to use this mechanism. If testing for SARS-CoV-2 is not yet available in a country, efforts should be made to establish national capacity.

Reporting of cases and test results

Rapid communication of test results is important for planning and design of public health and outbreak control interventions. Laboratories should follow national reporting requirements. In general, all test results, positive or negative, should be immediately reported to the national authorities. States Parties to the International Health Regulations (IHR) are reminded of their obligation to share with WHO relevant public health information for events for which they must notify WHO, using the decision instrument in Annex 2 of the IHR (2005) [186].

Regular interaction between public health experts, clinicians and local laboratory experts to discuss strategies, potential problems and solutions, should be considered to be an essential part of an adequate COVID-19 response. This response includes the development of guidance and (clinical-, epidemiological-, and trial) study protocols.

A rapid turnaround time of test results can, in turn, have a positive impact on the outbreak [187, 188]. More studies are needed to fine tune the maximum acceptable time from symptom onset to sample result to have impact on clinical management and outbreak control; currently a maximum of 24 hours is considered reasonable in most settings. As laboratories often have control only over the time between sample arrival and the test result, it is critical to ensure that samples arrive in the laboratory without delay.

Methods

This document was developed in consultation with experts from the SARS-CoV-2 laboratory expert network. Experts in the network completed a confidentiality agreement and declaration of interest. The declaration of interest forms were reviewed, and no conflicts regarding the support of this guidance document were identified. Relevant WHO guidance has been used in this document [136, 185, 189-194]. This is the sixth edition (version 2020.6) and was originally adapted from *Laboratory testing for Middle East Respiratory Syndrome Coronavirus* [189].

A broad spectrum of clinical laboratory experts from different regions were engaged in the development of this document. The internal experts involved in the development include WHO regional laboratory focal points, epidemiologists and clinical experts. This version of the guidance incorporates the novel understanding and characteristics of the virus and addresses questions and issues received from WHO's country and regional offices and other channels.

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WHO continues to monitor the situation closely for any changes that may affect this interim guidance. Should any factors change, WHO will issue a further update. Otherwise, this interim guidance will expire 1 year after the date of publication.

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WHO reference number: WHO/2019-nCoV-19/laboratory/2020.6

Annex 1: Specimen collection and storage

Specimen type	Collection materials	Recommended temperature for storage and/or shipment to the laboratory and until testing (from date of specimen collection) #
Nasopharyngeal and oropharyngeal swab	Dacron or polyester flocked swabs with VTM *	2-8 °C if ≤12 days* –70 °C (dry ice) if > 12 days
Bronchoalveolar lavage	Sterile container with viral transport medium **	2-8 °C if ≤ 2 days –70 °C (dry ice) if > 2 days
(Endo)tracheal aspirate, nasopharyngeal or nasal wash/aspirate	Sterile container with viral transport medium**	2-8 °C if ≤2 days –70 °C (dry ice) if > 2 days
Sputum	Sterile container	2-8 °C if ≤ 2 days –70 °C (dry ice) if > 2 days
Tissue from biopsy or autopsy including from lung	Sterile container with saline or VTM	2-8 °C if ≤ 24 hours –70 °C (dry ice) if > 24 hours
Serum	Serum separator tubes (adults: collect 3-5 ml whole blood)	2-8 °C if ≤ 5 days –70 °C (dry ice) if > 5 days
Whole blood	Collection tube	2-8 °C if ≤5 days –70 °C (dry ice) if > 5 days
Stool	Stool container	2-8 °C if ≤5 days –70 °C (dry ice) if > 5 days

Avoid repeated freezing and thawing of specimens. If no access to –70 °C consider storing at -20 °C.

* For transport of specimens for viral detection, use preferentially viral transport medium (VTM) containing antifungal and antibiotic supplements. If VTM is not available, other solutions may be used after validation. Such solution may include phosphate buffered saline (PBS), 0.9% sterile saline, minimum essential medium (with storage at +4C up to 7 to 14 days) [195-197]. In case other viruses such as influenza should also be tested, do not store samples for more than 5 days at 4-8 degrees but –70 °C or dry ice [194],.

** If VTM is not available, sterile saline may be used [198]. Duration of specimen storage at 2-8 °C may be different from what is indicated above.

Apart from specific collection materials indicated in the table, ensure that other materials and equipment are available: e.g. transport containers and specimen collection bags and packaging, coolers, and cold packs or dry ice, sterile blood-drawing equipment (e.g. needles, syringes and tubes), labels and permanent markers, PPE, materials for decontamination of surfaces, etc.

Annex 2: laboratory request form

SARS-CoV-2 LABORATORY TEST REQUEST FORM[1]

Submitter information

NAME OF SUBMITTING HOSPITAL, LABORATORY, or OTHER FACILITY*
Physician
Address
Phone number
Case definition[2]: ☐ Suspected case ☐ Probable case ☐ Other:

Patient info

First name **Last name**
Patient ID number **Date of Birth** **Age:**
Address **Sex** ☐ Male ☐ Female ☐ Unknown

Phone number

Specimen information

Type ☐ Nasopharyngeal and oropharyngeal swab ☐ Bronchoalveolar lavage ☐ Endotracheal aspirate ☐ Nasopharyngeal aspirate ☐ Nasal wash ☐ Sputum ☐ Lung tissue ☐ Serum ☐ Whole blood ☐ Stool ☐ Other: ....

All specimens collected should be regarded as potentially infectious **and you <u>must contact</u> the reference laboratory <u>before</u> sending specimens to them.**
All specimens must be sent in accordance with category B transport requirements.

Please tick the box if your clinical specimen is post mortem ☐
Date of collection **Time of collection**
Priority status

Clinical details

Date of symptom onset:
Has the patient had a recent history of travelling to an affected area? ☐ Yes ☐ No **Country** **Return date**

Has the patient had contact with a confirmed case? ☐ Yes ☐ No ☐ Unknown ☐ Other exposure:
Additional Comments (e.g. antimicrobial treatment, immunosuppressants)

[1] Form in accordance with ISO 15189:2012 requirements
[2] Public health surveillance for COVID-19: interim guidance

Annex 3: Considerations when selecting the optimal NAAT for the use setting

Aspect	Considerations
Manufacturing quality	CE-IVD, WHO EUL, PQ, EU-FDA or other approval. Independent validation data. Manufacture under ISO.
Targets	Number of targets, specificity for SARS-CoV-2 or other sarbecoviruses.
Controls	For manual NAAT testing, a positive template control (PTC) and at least one negative template control (NTC) should be included. Use of an extraction control and an internal human housekeeping gene specimen adequacy control is also recommended.
Instrumentation	Is the assay compatible with available systems in the laboratory or country? Ease of use and operational utility. Opportunity to multiplex with other respiratory pathogens. Costing of platform and maintenance. Ease of access to maintenance provider/troubleshooting.
Workflow	Can the kit be implemented in the existing workflow of the laboratory, while assuring minimal disruption on other diagnostics?
Ease of use	Complexity of assay. Number of steps. Required training and staff.
Storage and shipment requirement	Many kits require cold chain conditions during shipment and storage, in some circumstances this might pose a challenge. Some kits contain lyophilized enzymes that do not require the kit to be shipped and sometimes stored cold. Shelf life: To be prepared for periods of intense testing stocks might be needed, a longer shelf life is needed to ensure adequate use of resources.
Training needs and access	Instructions for use (IFU) available, training available by company or others, troubleshooting options provided and accessible help line in local language.
Need for ancillary reagents	Complete kit for sampling/extraction/amplification or the PCR kit requires additional reagents or tools. Compatibility with laboratories' extraction method. Compatibility with procurable polymerases if needed. Special equipment needed (e.g. calibration panel before running the test, extraction platforms, heat block, vortex, magnetic stand or centrifuge).
Continuity of supply	Long term supply agreement. Secured routes of delivery if lockdowns occur. Assay and ancillary reagents costs.

Annex 4: Suggestions for checklist to reduce possible cases of false positive rRT-PCR results and handling of equivocal results

Laboratories should have a standard operating procedure in place to reduce the possible false positive rRT-PCR results and how to handle equivocal results. This checklist provides the laboratories with suggestions and considerations. The checklist is formulated for manual rRT-PCRs but many aspects can also be used for other NAATs.

CLERICAL

- ❑ Eliminate or reduce transcription
- ❑ If transcribe, method of checking
- ❑ Sorting, aliquoting and labelling
- ❑ Double identifiers
- ❑ Entering results

CROSS CONTAMINATION

- ❑ Preparation area
- ❑ Manipulation of tubes
- ❑ Aerosol generation
- ❑ Nucleic acid concentration and extraction setup
- ❑ PCR format and steps
- ❑ Check other positives in same run
- ❑ Environmental
- ❑ Contaminated reagents
- ❑ Disposal

EQUIPMENT and TEST KITS

- ❑ Calibration method
- ❑ Equipment validated for test kit
- ❑ Assess new equipment for contamination risk

PRACTICE

- ❑ For mass screening, separate high-prevalence from low-prevalence groups.
- ❑ Visual inspection of run
- ❑ Analytical – examination of raw data
- ❑ Extend run when necessary for late Ct

EQUIVOCAL RESULTS

- ❑ Follow manufacturer's instructions
- ❑ Laboratory policy for equivocal results
- ❑ Any additional laboratory criteria for equivocal category
- ❑ Communication of interpretation to users
- ❑ Criteria for repeat testing, if any
- ❑ Use of alternative test or PCR target
- ❑ Communication with clinical and public health staff

This is Exhibit "C" referred to in the Affidavit of Jared Manley Peter Bullard affirmed before me this 29th day of April, 2021.

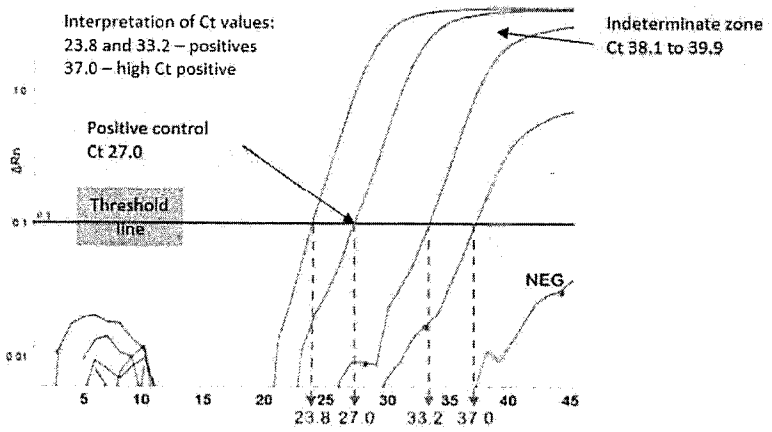
A Barrister-at-law entitled to practice in and for the Province of Manitoba.

PCR and Ct values in COVID-19 testing – Frequently Asked Questions and Important Considerations

Canadian Public Health Laboratory Network (CPHLN)
Respiratory Virus Infections Working Group

What is a cycle threshold (Ct) value? Most tests that detect the RNA or genetic finger print of the virus that causes COVID-19 (e.g. PCR) do so through a process where specific bits of the genetic finger print are amplified using a temperature cycling reaction that repeats up to 45 times (called amplification cycles). The amount of genetic material doubles after each cycle (Figure 1). The number of amplification cycles required to create enough copies of the viral RNA to be detected is called the cycle threshold or Ct value. The more RNA that is present in the patient sample, the fewer cycles are required for the signal to reach the detection threshold (low Ct value). The fewer RNA present in the clinical sample, the more cycles are required. So a low Ct value corresponds to a high viral load, while a high Ct value correspondsto a low viral load.

Figure 1: An Example of a Real-Time PCR Amplification Curve



Note: This amplification curve is presented on a logarithmic scale. Curves can also be viewed on a linear scale, which will look different but does not change the Ct interpretation. Not all commercial real-time PCR assays provide Ct values or amplification curves for viewing by the user. In addition, some molecular assays are based on other technologies (e.g. flow cytometry), and hence, do not provide Ct values.

Source: Public Health Ontario: An Overview of Cycle Threshold Values and their Role in SARS-CoV-2 Real-Time PCR TestInterpretation

How are Ct values used? The fewer amplification cycles it takes to pass this threshold (a low Ct value) the more virus is likely to present in the initial sample. The more cycles required to amplify the viral genes above the threshold (a high Ct value) suggests a lower amount of virus present in the initial sample.There can be up to 45 total number of cycles for many molecular tests. The Ct value is the cut-off that calls a test positive, which is defined by the manufacturer of the test or the laboratory during the validation process to make sure that the PCR test is correctly detecting the presence of the virus and not false signals.

Does a certain Ct value predict who is infectious? This is a complex issue. Higher viral loads among patients admitted to hospital have been linked to a higher 30 day mortality. However, it is not possible to directly translate a Ct value into degree of infectiousness. There is good evidence that when more than 35cycles are required to detect virus, the virus concentration is so low that it is unlikely to grow the virus inthe laboratory. However, the cells used in the laboratory to grow the virus are different from the cells in the back of the throat and nose (nasopharynx) or the lungs in people. So just because one can't grow the virus in a laboratory that does not mean that it won't transmit.

Many believe that with low viral RNA copy numbers (high Ct value) the virus is not likely to be transmitted. A recent study which followed symptomatic patients not requiring hospitalization showed that those with higher viral loads (lower Cts) infected a higher proportion of their immediate contacts. But we do not know how much virus is actually required to cause an infection in someone and there are other important factors that may influence infectiousness, including the health of the person exposed and the type of exposure that has happened.

Important factors to consider in interpreting Ct values:

1) *Ct values will depend on the stage of infection* – Between exposure to the virus and symptom onset (i.e.incubation or pre-symptomatic period), the amount of virus in a person's sample can be initially too low to be detectable (negative). A person with an initially negative result may progress to give a test with a high Ct value i.e. >30 (low viral load), then to a lower Ct value (increased viral load) dramatically within a couple of days. Laboratories across the country have seen many cases where the person is tested early during their course of infection and the initial sample had a very high Ct value ~35 (low virus RNA concentration) and the following day the Ct was ~14 (high virus RNA concentration).

2) *Ct values are affected by the type of the sample taken from the person* - Nasopharyngeal swabs (those that go deep into the nose to swab the back of the upper throat) are the most sensitive specimen type for people who do not need admission to hospital; throat/nasal swabs, and gargles/saliva may not have as much virus in them (so they would give a positive test with a higher Ct value). In people where COVID-19 has infected their lungs, these samples from the nose/throat can be negative and a deeper sample like sputum is needed to detect the virus. In addition, the type of swabs used for collecting samples may also influence the Ct value.

3) *Ct values are affected by the quality of the sample taken from the person* - The quality of the sample collected is very important. If you don't get the best possible sample, less virus will be in it and this can lead to a sample with an artificially high Ct value in a person who could have lots of virus in their system.

4) *Ct values cannot be compared between different PCR tests* - There is no standard yet to be able to compare one test to another so the Ct range can greatly differ by the type of test used, that may use different signal detection methods. In fact, even when testing identical samples using different PCR tests,the results can differ by up to 8 Ct values (e.g. from 22 to 30). This has been observed in the laboratories from different jurisdictions (e.g. ON, BC and SK).

5) *The genetic fingerprint of the virus can be picked up long after the virus is no longer infectious* – PCR can be positive for over 100 days or more after infection, usually with tests that have high Ct values but in most cases are unlikely to transmit to others beyond 10 days post symptom onset. This finding has been considered in the Infection Prevention and Control (IPAC) and public health practice that recommends patient isolation based on symptom onset, disease severity and the presence of any underlying, immunocompromising conditions instead of on PCR results alone both in some healthcare facilities and more so in the community setting.

6) *The impact of new variants on Ct values is not clear* – Our current tests can detect the new COVID-19 variants of concern (VOCs) - B.1.1.7 (first detected in the United Kingdom), B.1.351 (first detected in South Africa) and P.1 (first detected in Brazil). It has been documented that B.1.1.7 and B.1.351 variants are more infectious, and patients with B.1.1.7 infections have lower Ct values (higher viral loads) compared with those infected with the originally circulating (non-variant) SARS-CoV-2 virus. B.1.351 and P.1 are undergoing further study. We are closely following the VOC-positive samples in Canada to better understand the impact of these variants on our laboratory tests.

Key Points and Recommendations:

1. Ct values can sometimes be used by practitioners, in combination with clinical and epidemiologic information, to make judgment-based decisions. Ct values should not be used alone to make concrete clinical or public health decisions.
2. Not all nucleic acid amplification assays produce Ct values or an equivalent proxy measure of viral 'RNA load'.
3. High Ct values are not yet proven to be able to declare someone non-infectious, only that they are less likely to be infectious.
4. As a result, it is not recommended that Ct values be routinely be clinically reported with SARS-CoV-2 RT-PCR results.

5. If a laboratory chooses to routinely report Ct values, it is recommended that clear language regarding uncertainty in interpretation and which authorities may need to be consulted for decision making be included in the report.

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