Discordant COVID-19 PCR test results and the implications in long-term care

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SARS-CoV-2, the causative agent of Coronavirus Disease 2019 (COVID-19) has infected over 247 million people worldwide, and is responsible for over 5 million deaths (as of November 2, 2021) [1]. The brunt of the disease has been felt more among the elderly, especially those living in long-term care homes. As public health authorities battle with outbreak management, a clear definition of what constitutes an outbreak is essential. Often, outbreak declarations are triggered by two or more laboratory-confirmed COVID-19 cases with an epidemiological link within a 14-day period where both cases could have reasonably acquired their infection in the same setting [2, 3]. Although this definition seems scientifically appealing, outbreak management and the care of residents may be affected if laboratory test results are not indeed confirmed, or if results appear to be discordant. An example of a discordant result is when a specimen from an individual tests positive, and a subsequent specimen or repeat tests from the same person within the same timeframe using the same or a different assay gives negative results [4, 5].

To understand the concept of discordant results, that is false-positive or false-negative Polymerase Chain Reaction (PCR) tests, it is important to understand the principle behind PCR. Basically, the COVID-19 PCR test is meant to detect the genetic material (ribonucleic acid or RNA) of SARS-CoV-2 virus in a specimen [6]. The test starts with RNA extraction from a respiratory specimen followed by reverse transcription to complementary deoxyribonucleic acid (cDNA), which is then amplified using oligonucleotide primers and fluorescently labelled probe(s) specific to region(s) of the SARS-CoV-2 genome [7, 8]. If SARS-CoV-2 RNA is present in the sample, these oligonucleotide primers attach themselves to target sections of the cDNA. Through a thermocycling reaction, identical copies of the target sections of cDNA are created. It should be noted that PCR assays have cut-off points (the number of cycles it runs), and different laboratories may set different cut-off values. Typically, a standard real time PCR set-up usually goes through about 40 cycles.

As new copies of the viral DNA sections are built, the fluorescent probes attach to the DNA strands and then release a fluorescent signal, which is measured in real time. 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 specimen, the fewer cycles are required for the signal to reach the detection threshold (low Ct value, e.g., Ct<30). The less RNA present in the specimen, the more cycles are required. So, a low Ct value corresponds to a high viral load, while a high Ct value corresponds to a low viral load. For example, the cut-off point for a positive result for public health Ontario laboratories is 38 cycles. This means that if the virus is detected at or before 38 cycles are completed, then the test is considered positive. The cut-off point for a negative result is 40 cycles. If the virus is detected between 38 and 40 cycles, then it is considered as indeterminate or inconclusive [9]. Also, because the test does not detect live virus (only viral nucleic acid), the test could detect RNA, not just from an individual who has an active infection, but also in persons who may be shedding the viral particles from a recent infection and may no longer be infectious [8].

With the understanding of the principle behind PCR testing, it is important to mention that false-positive PCR results could occur due to human or analytical errors. From a human error perspective, samples can get mixed up, software glitches can produce erroneous interpretations of test results, and mistakes can be made when entering or communicating results [10]. From an analytical standpoint, cross-contamination of samples during collection, pipetting, or processing may generate false-positive results [11]. The propensity of false-positive results has also been linked to increased frequency of asymptomatic testing in settings of low SARS-CoV-2 incidence, or low pre-test probability [12].

On the other hand, false-negative results can occur for numerous reasons, including inappropriate specimen type, suboptimal specimen collection, testing too early in the disease process (low viral load), or low analytic sensitivity [13, 14]. Other factors such as the quality of the RNA extracted from the swabs, degradation of purified RNA, the presence of RT-PCR inhibitors, or genomic mutations may cause false-negative results [15]. As discussed above, considering the fact that PCR diagnostic

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performance, including analytical sensitivity and specificity may vary, it is essential that laboratory results are verified before confirmed outbreak declarations are made.

In this Editorial, I would like to focus on false-positive results as every false-positive test has direct negative consequences on outbreak management and the care of residents in long-term care facilities. Staff with false-positive test results and their close contacts are excluded from work, and this can lead to staffing shortages. False-positive results may also lead to unnecessary testing of residents and placement on additional precautions (droplet/contact precautions) for up to 14 days due to the perceived exposure. Unnecessary isolation can worsen loneliness, psychological distress and overall mental health of residents [16]. Misdiagnosis can also result in stigmatization and the fear of infecting others, as well as unnecessary restriction of visitation to the home, and leave of absence of residents.

Besides causing an increase in operational cost to implement outbreak control measures, false-positive results may also lead to overestimating COVID-19 true incidence and the overall burden of the disease. Recently in Saskatchewan, Canada, 255 COVID-19 test results were deemed to be invalid after a testing error was identified at a provincial laboratory. After retesting, 206 results were found to be false positive [17]. Prior to this verification, outbreaks or suspected outbreaks were already declared in several long-term care homes across the region. Also, my team conducted a survey in Ontario, Canada from August 2020 to March 2021, and found that out of 64 suspect or confirmed COVID-19 outbreaks in some long-term care homes, 23 (36%) were deemed to be pseudo-outbreaks (no clinical or epidemiological correlation) with discordant results that were subsequently determined to be false positive (negative). In most of the cases, outbreaks were declared and then called off when further testing of specimens gave negative results (false positive). In other situations, local health units treated the events as true outbreaks even though the results of repeat testing were negative or discordant. These data and those from other sources suggest how errors in laboratory tests may result in outbreak declaration?. Besides the psychological distress of residents due to prolonged confinement, each of these outbreaks require considerable human resource capacity mobilization, outbreak management initiatives, and significant personal protective equipment supply and use.

From an epidemiological standpoint, one of the key steps in outbreak response is verifying the diagnosis, or establishing the existence of an outbreak [18]. Verifying the diagnosis is important to:

(a) ensure that the causative agent has been properly identified, since control measures are often disease-specific;
(b) rule out the possibility of laboratory errors or pseudo-outbreaks; and,
(c) to interpret laboratory findings in line with the clinical and epidemiologic findings [18].

Currently, most surveillance systems exclude persons who have been recently infected with COVID-19 (i.e., within 90 days) from routine surveillance testing. Therefore, persons who were deemed as positive when probably they were not (false positive) are excluded from the surveillance testing and this could create an opportunity of risks as these persons could indeed become infected and spread the virus as they are not included in routine asymptomatic surveillance testing [19].

Together, prior to outbreak declaration, diagnostic verification has often not been fully investigated and facilities have been plunged into outbreak status without a thorough investigation or due diligence on the part of some health units. The need to apply the precautionary principle during uncertainty is understood, but this should not obviate the requirement to definitively establish the existence of an outbreak using epidemiologic, clinical and scientific principles. In fact, declaring a COVID-19 outbreak should not be made solely on the basis of a single positive PCR result, even involving more than two cases, but should include an assessment of signs or symptoms, epidemiologic links and then confirmed by additional PCR tests or other types of tests. Public health authorities must strengthen their diagnostic algorithms in order to guide outbreak declarations and downstream public health interventions.

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