CURRENT STATE OF DIAGNOSTIC AND SEROLOGICAL TESTING FOR COVID-19 DISEASE

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In late December 2019, an outbreak of pneumonia cases of unclear etiology was reported in Wuhan City, Hubei Province, China.

Subsequently, it was identified as a novel Coronavirus. The illness is now known as Coronavirus Disease 2019 (COVID-19). Based on genetic homology, the novel coronavirus was officially named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2).

Coronaviruses are enveloped viruses containing a single strand of positive-sense RNA. Virions are mostly spherical, with pronounced spiked glycoprotein (S) embedded in the envelope. Additional structural proteins include envelope (E), matrix (M), and nucleocapsid (N)[1,2].

Coronaviruses belong to the family Coronaviridae which includes four genera, Alphacoronavirus, Betacoronavirus, Deltacoronavirus and Gammecoronavirus.

By March 11, 2020, WHO officially declared a global pandemic caused by SARS-CoV-2.

Molecular Testing for Confirmation of COVID-19 Diagnosis

Accurate molecular diagnostic tests based on Nucleic Acid Amplification Technologies (NAAT) such as real-time reverse-transcription PCR (rRT-PCR) are considered to be the gold standard for diagnosis of an active SARS-CoV-2 infection. Direct detection of viral nucleic acid in respiratory tract specimens is needed to provide accurate results to patient, healthcare institutions, and public health agencies for informed decision making[1,2,3].

Molecular Testing for COVID-19 at LifeLabs

At LifeLabs ON, we started offering RT-PCR testing for Coronavirus COVID-19 using Seegene Allplex kit in March 2020. Nasopharyngeal swabs are the specimens validated for testing in our laboratory.

We detect the presence of 3 target genes; RdRP, E and N genes by RT-PCR.

There are many RT-PCR methods that are being used by different laboratories in Ontario.

Interpretation of Molecular Testing

Specimens with a single target gene detected will be reported as 'COVID-19 virus detected', which is sufficient for laboratory confirmation of COVID-19 infection.

A "positive" PCR result reflects only the detection of viral RNA and does not necessarily indicate presence of a viable virus.

Test positivity starts to decline by week 3 post-onset of symptoms, and subsequently becomes undetectable[4].

False-negative results mainly occur due to inappropriate timing of nasopharyngeal swabs collection in relation to illness onset, and deficiency in sampling technique[4].

Specificity of most of the RT-PCR tests depends on the combination of viral gene targets used, but is generally close to 100% because the primer design is specific to the genome sequence of SARS-CoV-2. Occasional false-positive results may occur due to technical errors and reagent contamination.

Clinical correlation is required when testing asymptomatic patients with no risk factors, since this may increase possibility of false positive results based on the pre-test probability[1]. For public safety, a positive test should be treated as positive.

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Serological Testing (Antibody Testing) for SARS-CoV-2

Two main types of serological (antibody) testing available are rapid-response point of care (POC) tests, based on lateral-flow antibody binding, and laboratory-based tests. Different antibody tests target different viral antigens, where the most common ones include antibodies to viral nucleocapsid (N), spike (S), and receptor binding domain (RBD) epitopes[4,5].

Some serological tests measure total antibody response to viral antigens which include IgM, IgG, and IgA antibodies, and others are isotype-specific such as IgG-specific, IgM-specific or IgA-specific. Even though the kinetics of the humoral immune response is not completely understood, a growing number of studies indicate that IgM and IgG antibodies to SARS-CoV-2 appear within two weeks of symptoms onset in most patients[4].

Varied Quality of Available Serological Tests

Many serological tests currently on the market lack extensive analytical and clinical validation necessary to understand their clinical accuracy, including important aspects such as test sensitivity, specificity, positive and negative predictive values.

Cross-reactivity with related coronaviruses and other pathogens of high prevalence is of particular concern and needs to be determined as well.

In the absence of more information, both false negative and false positive results are possible which makes interpretation of test results difficult, especially in context of a general population where disease prevalence is thought to be low < 2%. With that kind of prevalence, a test with < 99% specificity can have positive predictive value no better than flipping a coin[6].

At the time of writing of this article (May 14, 2020), Health Canada has approved two serological test for COVID-19. Others are under review. The FDA has issued Emergency Use Authorization for 13 serological tests.

Clinical Utility and Test Interpretation of Serological Tests

Serological tests are not recommended for the acute diagnosis of COVID-19, primarily because there is a lag between exposure and the development of humoral immune response to the virus.

There are some indications that serological testing may be used in detection of PCR-negative cases, such as in patients who present later in disease, past the window of viral detection by PCR.

A positive serological test may indicate recent or previous exposure to the virus, but it is not yet known if detection of antibodies to SARS-CoV-2 in immunocompetent children and adults is an indicator of protective immunity.

A better understanding of the humoral response to SARS-CoV-2 is required before serological tests can be considered a test for immunity. Information regarding neutralizing capability of the antibodies, as well as titer and length of protection, needs to be elucidated before these tests can be reliably used in the context of "immunity passports" or return to work testing for health care workers.

On a population level, serological testing can be used for epidemiological surveillance purposes to determine and monitor prevalence of COVID-19 in the population. There is no data on what proportion of infected population may have mild symptoms that go unreported or even be asymptomatic. Surveillance studies may be used to inform public health policies related to disease management strategies in the short and long term: (https://www.covid19immunitytaskforce.ca/).

At the time of writing of this article, serological testing for COVID-19 is not offered by Ontario clinical laboratories.

Many clinical laboratories in the US are offering serological testing, and we plan to do so as soon as we have sufficient information to support the release of a test.

We are working closely with provincial public health agencies, and we are carefully reviewing Health Canada, WHO, and CDC guidance for validation and clinical utility of serological testing, so that once available, we can provide the best quality of testing to Canadians.

POINTS TO REMEMBER:

- PCR-based testing is the most reliable test for the acute diagnosis of COVID-19.
- False-negative PCR results may occur due to inappropriate timing of the sample collection or deficiency in sampling technique.
- Serological testing is not indicated for the acute diagnosis of COVID-19.
- Serological testing may be used as an adjunct to PCR when the patient has a negative PCR result, but displays symptoms consistent with COVID-19, presenting later in their disease.
- Currently, it is unknown whether the antibodies detected by serological testing indicate protective immunity to COVID-19.

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ADDITIONAL RESOURCES:

7. CDC – Serology Testing: https://www.cdc.gov/coronavirus/2019-ncov/lab/serology-testing.html
8. WHO – Serology Testing: https://www.who.int/news-room/commentaries/detail/advice-on-the-use-of-point-of-care-immunodiagnostic-tests-for-covid-19
9. FDA https://www.fda.gov/media/137470/download
10. Health Canada requirements for serological antibody tests submitted under the COVID-19 Interim Order: guidance can be found in: https://www.canada.ca/en/health-canada/services/drugs-health-products/medical-devices/application-information/guidance-documents/covid19-requirements-serological-antibody-tests.html#a64
11. Lab Tests Online COVID-19 Page: https://labtestsonline.org/tests/coronavirus-covid-19-testing

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