Clinical presentation in persons infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) ranges from asymptomatic to the life-threatening respiratory distress that can occur with coronavirus disease 2019 (COVID-19). 1 Diagnosis of acute or new cases of SARS-CoV-2 infection at present relies upon molecular-based detection of viral RNA in upper or lower respiratory tract specimens, typically within 2–7 days after exposure. 2,3 In this period, active viral shedding occurs, and individuals who are infected can transmit the virus to others. Although viral RNA may still be detected in respiratory and stool specimens of some people for many weeks after they have recovered, this does not appear to pose a transmission risk. 4,5 Serological testing involves detection of antibodies specific to SARS-CoV-2 infection in blood, serum or plasma. The role of serology is limited in the diagnosis of acute COVID-19 because it usually takes a minimum of 7–14 days or more after symptom onset to develop a reliable and measurable SARS-CoV-2 antibody response. 6,7 However, interest has arisen in the potential application of serological testing for purposes as wide-ranging as authorization of international travel, stratification of reinfection risk in workplaces and the reduction of public anxiety to facilitate resumption of economic activity. 8,9 We review what is currently known regarding SARS-CoV-2 serological testing—a body of basic and clinical science that is still evolving (Box 1); consider its implications for clinical care, the development of appropriate services and test interpretation; and advise on appropriate use of serological testing for clinical and public health purposes.

What are the antibody responses to SARS-CoV-2?

The SARS-CoV-2 genome encodes 4 major structural proteins: surface or spike glycoprotein (S), envelope, membrane and nucleocapsid (N). 10 Currently available serological tests detect antibodies to various epitopes on the S or N structural proteins.

The surface spikes are the “corona” observed on electron micrographs of coronaviruses; they play a critical role in viral pathogenesis, and thus studies have focused on antibodies to specific parts of the S glycoprotein. Each spike protein consists of 2 subunits: the S1 subunit binds to angiotensin-converting-enzyme-2 receptors on cells in multiple organs via its receptor-binding domain; and the S2 subunit mediates fusion between the virus and the cell membrane of the host. The S protein receptor-binding domain is an important vaccine and therapeutic target because a subset of antibodies targeting the receptor-binding domain appear to block viral binding and neutralize viral infectivity in vitro. 11,12

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SARS-CoV-2 (COVID-19) serology: implications for clinical practice, laboratory medicine and public health

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Cite as: CMAJ 2020 August 24;192:E973-9. doi: 10.1503/cmaj.201588; early-released August 4, 2020

A complete list of the members of the 5 authorship groups appears at the end of the article.
Figure 1 shows a schematic of the pattern of antibody response to SARS-CoV-2 infection. Although immunoglobulin M (IgM) and immunoglobulin A (IgA) antibodies are widely regarded to appear early during most acute viral infections, it is uncertain whether this occurs with SARS-CoV-2 infection. With COVID-19, similar to SARS and Middle East respiratory syndrome (MERS), both IgM and IgG antibodies appear at detectable levels concomitantly around 2–3 weeks after symptom onset or exposure. However, in some mild and asymptomatic cases, antibodies may not be detected at all, at least within the time scale as reported in some recent studies (< 46 d).

Commercially available assays target 1 or more of the 3 antibody isotypes (i.e., IgA, IgM or IgG) or total immunoglobulin. The 2 main types of commercial assays are described in Box 2. An updated list of approved clinical diagnostic tests for SARS-CoV-2 antibodies is available through the Health Canada website.

Although several laboratory-based immunoassays have been approved, there is insufficient evidence to support use of point-of-care testing devices for SARS-CoV-2 serology (see Box 2 for a description of these kits) and, at the time of writing, no SARS-CoV-2 serological point-of-care tests have received Health Canada approval for use. Effective use and interpretation of point-of-care tests will require consistent correlation of their results with approved laboratory-based tests, as well as secure supplies of kits that have consistent quality-assured performance.

What considerations affect the interpretation of SARS-CoV-2 serological tests?

Sensitivity, specificity and disease prevalence

The discriminative potential of a test is assessed by its clinical sensitivity and specificity. The sensitivity is a measure of the test’s ability to detect antibodies in matrices such as blood, serum or plasma of patients with SARS-CoV-2 infection (i.e., a true positive result). Specificity, on the other hand, is a measure of the test’s ability to correctly identify the absence of antibodies...
in an individual who has not been infected (i.e., a true negative result). At present, Health Canada recommends a target specificity of 98% or higher, and the minimum required for consideration of approval is 95%. Sensitivity and specificity are inherent elements of test performance, but the predictive value of any test depends on the prevalence of the infection within a given population. For example, if the disease prevalence in the population is only 1%, even a highly specific diagnostic test (99% specificity or only 1 false-positive result out of 100 patient results) would be predicted to lead to roughly 1 false-positive result for every true positive result.

In Canada, the baseline prevalence of SARS-CoV-2 infection as of July 9, 2020, was estimated at 0.3%, based on 106805 confirmed cases of COVID-19. However, the number of Canadians infected is likely many times higher than indicated by the number of confirmed cases for several reasons. Current nucleic acid testing has moderate sensitivity, and tests performed in patients with infection may have returned a negative result in some cases. Furthermore, testing indications and coverage have varied over time and by region, and some people with SARS-CoV-2 infection do not show symptoms; therefore, many people who were infected may not have requested or qualified for testing. The prevalence of asymptomatic infection measured in other jurisdictions ranges widely depending on the target population tested, geographic location and age of the patients.

We posit that the prevalence in most Canadian locations is likely low enough that very small reductions in test specificity will drive up the proportion of false positives that are reported. This can be corrected post hoc in population-level estimates of the prevalence of SARS-CoV-2 infections but creates problems in applying serology results at the individual level — a challenge compounded by biological uncertainties.

**Correlation of SARS-CoV-2 antibodies with virus neutralization**

Commercially available serological assays detect and semiquantitatively determine the amount of antibody binding to various SARS-CoV-2 antigens. (Quantitation may be valuable when a rising level suggests recent infection and associated positive seroconversion.) Depending on the antigen targeted, the bound antibodies detected may correlate with detection of neutralizing antibodies that, as noted above, are antibodies that block viral binding and neutralize viral infection in vitro (hence the term, neutralizing antibodies). Neutralizing antibodies may provide a better indication of immunity, but there is ongoing debate as to whether neutralizing antibodies are the primary mechanism of immune protection against SARS-CoV-2 infection. Instead, a cell-mediated immune response, known to be a key element in viral control for SARS-CoV-1 and MERS-CoV, may be more relevant. Therefore, further studies are required to evaluate the correlation of commercial assays for SARS-CoV-2 antibodies with neutralization capacity, the potential for antibody-dependent cell-mediated immune responses and seroprotection.

**Duration of antibody response**

In mild and asymptomatic cases, antibody responses may not consistently develop or reach levels sufficient to be detectable by antibody tests. Research continues on the extent and duration of antibody responses in the context of infections ranging from asymptomatic to severe, and across different populations, ages, genetic backgrounds and comorbidities. Antibody levels to
Serological test positivity and infectiousness

A positive antibody result cannot be equated to a noninfectious state. Particularly for non-neutralizing antibodies, the presence of antibodies does not preclude active viral shedding through respiratory secretions. Thus, factors such as symptom onset, symptom resolution and days since onset or resolution should guide advice on infectivity.

What are the implications for practitioners and policy-makers?

Consider test performance

Laboratories should strive to implement SARS-CoV-2 serologic tests that have manufacturer-claimed sensitivity of 95% or more and specificity of 99.5% or more based on limited current evidence. In such cases, the time since exposure (if known) or since symptom onset should be considered as seropositivity occurs only 7–14 or more days after symptom onset.

Serological testing may assist in the assessment of patients who present with atypical clinical manifestations such as inflammatory syndromes (e.g., multisystem inflammatory disorder in children and adolescents, COVID toes or unexplained thrombosis). Box 3 discusses antibody testing in the context of multisystem inflammatory syndrome in children and adolescents, based on limited current evidence.

How to report the results of serological tests

A recent United Kingdom report showed variability in the clinical interpretation of SARS-CoV-2 serology results especially with respect to inferring immunity and the infectious status of individuals. Consistent messaging and avoiding misinterpretation of serology test results depends on harmonized reporting across laboratories combined with proactive communication by laboratory staff, medical microbiologists and infectious disease practitioners. Box 4 provides suggestions for some interpretive wording for interim use by clinical and public health laboratories for reporting SARS-CoV-2 serology.
Box 4: Suggestions for the reporting of test results by clinical laboratories (include as appended comments)

Reporting positive/reactive results

- Presence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibody indicates current or previous infection. False-positive results may occur because of cross-reacting antibody or other causes. Currently, it is unknown whether antibodies indicate protective immunity and for how long.
- Presence of SARS-CoV-2 antibody should not be used to infer the infectious status of an individual or immunity.

Reporting negative/nonreactive results

- Nonreactive results do not rule out acute or previous SARS-CoV-2 infection.
- Absence of SARS-CoV-2 antibody should not be used to infer the infectious status of an individual or immunity.

Reporting inconclusive/indeterminate results

- An inconclusive result neither rules in nor rules out previous SARS-CoV-2 infection.

Potential uses of SARS-CoV-2 serology from a public health and research perspective

At present, based on the evidence we have considered, serological test results should not be used to guide patient-level decision-making on measures for infection control, including the use of personal protective equipment, timing of return to work or local physical-distancing policies.

However, seroprevalence studies of SARS-CoV-2 may be used to estimate rates of exposure and the geographic transmission of the virus within communities and populations, as well as within facilities, workplaces and households over time. This information may be used by epidemic modellers to help guide public health policies, by vaccine program planners to help set priorities and by front-line public health practitioners to determine which communities or congregate settings show minimal past exposure to SARS-CoV-2 and, therefore, may be at higher risk of rapid spread. At the interface of clinical and public health applications, while the diagnostic role of antibody testing is strictly adjunctive, seroprevalence studies may be useful in contact tracing when RNA tests are indeterminate.

Longitudinal seroprevalence studies may provide information on the nature and durability of antibody responses in patients with confirmed infection. The aim of such studies may be to determine if previous COVID-19 infection and seropositivity is associated with protection from subsequent reinfection; specialized serology tests, such as neutralization assays, will be particularly useful in this context. Likewise, serological screening of donated blood may reveal which blood samples contain adequate levels of neutralizing antibody to allow for their use in randomized controlled trials that investigate the effectiveness of pooled convalescent plasma treatment for patients with severe COVID-19.

Studies of vaccine effectiveness for SARS-CoV-2 may use serological testing results as a marker of immunity in cohort studies that explore correlates of protection and reinfection risk.

Conclusion

Given measurement and interpretive uncertainties of the tests, the clinical indications for SARS-CoV-2 serological testing are limited, with only a few exceptions. The tests will be useful in diverse research contexts and for policy-making in public health but should not be rolled out for general clinical use based on current evidence. Careful interpretation and reporting of test results is important. The current state of knowledge does not permit definitive inferences about immunity and likelihood of reinfection based on the results of serological testing, and testing cannot, therefore, be used to inform individual-level decisions on changing occupational exposure, the use of personal protective equipment, recommendations on physical distancing by members of the public or advice on international travel.

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Competing interests: Paul Van Caeseele is the director of the Cadham Provincial Public Health Laboratory (Manitoba Health). Mel Krajden has received grants paid to his institution from Roche, Hologic and Siemens. No other competing interests were declared. For competing interests of members of the 5 authorship groups, see Appendix 1, available at https://www.cmaj.ca/lookup/doi/10.1503/cmaj.201588/tab-related-content.

This article has been peer reviewed.

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Contributors: All of the authors contributed to the conception and design of the review; revised the manuscript, which was first drafted by Dana Bailey and Mel Krajden, for important intellectual content; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. Paul Van Caeseele serves as co-chair of the Canadian Public Health Laboratory Network. Dana Bailey serves as the designated corresponding author for the Canadian Society of Clinical Chemists COVID-19 Significant Interest Working Group. Sarah Forgie serves as president of the Association of Medical Microbiology and Infectious Disease Canada. Tanis Dingle serves as president of the Canadian Association for Clinical Microbiology and Infectious Diseases. Mel Krajden leads the Testing Working Group of the COVID-19 Immunity Task Force.

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