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IFCC interim guidelines on serological testing of antibodies against SARS-CoV-2

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Abstract: Serological testing for the detection of antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is emerging as an important component of the clinical management of patients with coronavirus disease 2019 (COVID-19) as well as the epidemiological assessment of SARS-CoV-2 exposure worldwide. In addition to molecular testing for the detection of SARS-CoV-2 infection, clinical laboratories have also needed to increase testing capacity to include serological evaluation of patients with suspected or known COVID-19. While regulatory approved serological immunoassays are now widely available from diagnostic manufacturers globally, there is significant debate regarding the clinical utility of these tests, as well as their clinical and analytical performance requirements prior to application. This document by the International Federation for Clinical Chemistry and Laboratory Medicine (IFCC) Taskforce on COVID-19 provides interim guidance on: (A) clinical indications and target populations, (B) assay selection, (C) assay evaluation, and (D) test interpretation and limitations for serological testing of antibodies against SARS-CoV-2 infection. These evidence-based recommendations will provide practical guidance to clinical laboratories in the selection, verification, and implementation of serological assays and are of the utmost importance as we expand our pandemic response from initial case tracing and containment to mitigation strategies to minimize resurgence and further morbidity and mortality.

Keywords: antibody; COVID-19; immunity; laboratory medicine; SARS-CoV-2; serology.

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There is an emerging demand for highly sensitive and specific serological assays for detecting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies. While serological immunoassays are now widely available from many diagnostic manufacturers globally, there is significant debate regarding their clinical utility, as well as the appropriate clinical and analytical performance characteristics for routine applications in this pandemic.

This document by the IFCC Taskforce on COVID-19 provides interim guidance on: (A) clinical indications and

target populations, (B) assay selection, (C) assay evaluation, and (D) test interpretation and limitations for serological testing of antibodies against SARS-CoV-2 infection. It is aimed to assist laboratories in selecting, validating, and implementing regulatory approved serological assays.

Taskforce recommendations – serology

A Clinical indications and target population

[A1] Key clinical indications for serological testing of antibodies against SARS-CoV-2

Serological tests can be broadly defined as blood-based assays used to detect humoral immunity (i.e., antibody production) in response to viral infection. Their value thus lies in identification of viral exposure and past infection. In COVID-19, there is significant interest in the potential role of serological testing for detection of antibodies against SARS-CoV-2 in various clinical and epidemiological contexts. At present, the clinical indications for serological testing in healthcare settings remain limited, due to uncertainties regarding timing, kinetics, and duration of SARS-CoV-2 antibody response in different populations (e.g., asymptomatic, symptomatic, and hospitalized), as well as the clinical and analytical performance of currently available assays [1–6].

Recommendation [A1]: Key clinical indications for serological testing of antibodies against SARS-CoV-2.

The following indications should be regarded as supported by current evidence and of clinical value:

- To serve as adjunct to molecular testing in patients presenting with suggestive clinical features (>14 days post symptom onset), but molecular testing for SARS-CoV-2 is negative, undetermined, or unavailable.
- To serve as adjunct to molecular testing where persistently positive molecular tests occur in the absence of infectious virus, such as late after resolved infection.
- To assist in the diagnostic work-up of multi-system inflammatory syndrome in children (MIS-C).

The following indications should be regarded as potentially valuable in the future, but are not possible using currently approved assays or have minimal associated evidence:

- To identify prior infection in non-hospitalized individuals (asymptomatic and symptomatic) and ascertain community exposure via seroprevalence surveys.
- To quantitatively evaluate the degree of antibody response in COVID-19 patients.
- To assist in identification of potential convalescent plasma donors.
- To assist in identification of immunity and evaluation of antibody response to future vaccines.
- To assist in monitoring the progression of herd immunity.

The following indication should be regarded as not supported due to strong evidence against application:

- To diagnose SARS-CoV-2 infection in the acute phase of illness (0–<14 days).

[A2] Populations that should be <u>prioritized</u> for serological testing of antibodies against SARS-CoV-2

Serological testing is anticipated to be of value in several populations depending on test case use, including asymptomatic, symptomatic, and hospitalized patients. Given the estimated prevalence of COVID-19 globally, prioritizing patients with clinical symptoms or those at higher risk can improve clinical performance and/or diagnostic accuracy of serological testing, and reduce the risk of misdiagnosis.

Recommendation [A2]: Populations that should be <u>prioritized</u> for serological testing.

- Patients presenting with possible COVID-19 symptoms but who were negative by molecular testing (e.g., delayed clinical onset).

B Assay selection

[B1] Importance of immunoglobulin isotype and antigenic target in assay selection

Most available SARS-CoV-2 serological assays detect immunoglobulin M (IgM), immunoglobulin G (IgG), immunoglobulin A (IgA), or total antibodies. The specific dynamics of IgM, IgG, and IgA response and their relation to each other are not well elucidated, but could potentially impact assay performance [7–10]. Current evidence supports that seroconversion occurs within approximately two weeks post symptom onset [11]. Some literature suggests that detection of IgM and IgA antibodies in comparison to IgG may indicate more recent infection, while others report concomitant expression of immunoglobulin isotypes similar to what has been observed in SARS-CoV [12–14]. Varying sensitivities and specificities have been reported for assays detecting IgM, IgG, or total antibodies, with some

studies showing that total antibodies immunoassays may be more sensitive [10, 15]. In comparison to IgM, IgG is anticipated to play a greater role in COVID-19 serological monitoring due to its classically longer lasting response [16].

An additional consideration in assay selection is antigenic target. Available assays currently target either the spike glycoprotein (S) or the nucleocapsid protein (N) of SARS-CoV-2. The S protein consists of two subunits, the N-terminal S1 unit, which contains the receptor binding domain (RBD), and the C-terminal S2 subunit [17]. Some commercially available assays solely target the RBD region of S1. Current evidence is insufficient to prove that assays which employ specific antigens to capture antibodies (e.g., the S1 subunit, specifically the RBD region, vs. nucleocapsid) show a greater correlation to antibody neutralization activity than others [7, 18–20]. Proper neutralization assays should be considered the only technique that can determine the neutralization capacity of patient sera. Most commercially available serology assays do not make an explicit claim against detection of neutralizing antibodies. For these methods, a positive result does not indicate immunity against reinfection. In assay selection, laboratories should consider whether an orthogonal testing strategy will be employed and if so, the assays selected should target different antigenic targets. This would ideally include testing the same patient specimen on an alternate platform to confirm positive results.

Recommendation [B1]: Importance of immunoglobulin isotype and antigenic target in assay selection.

- There is insufficient evidence to support any one specific immunoglobulin isotype as better than others in assay selection.
- No commercially available serological test has proven capability to detect neutralization antibodies, regardless of antigenic target, and positive results should not be used to indicate immunity.
- Neutralization assays should be used to determine the neutralization capacity of patient sera.

[B2] Importance of assay principle in assay selection (Lab-based vs. point-of-care testing [POCT])

Testing principles for SARS-CoV-2 serological assays range from rapid diagnostic tests used at the point of care (lateral flow assays (LFA)), to enzyme-linked immunosorbent assays (ELISA) or chemiluminescent immunoassays (CLIA) run on fully automated clinical laboratory instruments. Varying clinical specificities and sensitivities have been reported across LFA-, CLIA-, and ELISA-based methods, due to variable test validation protocols and often poorly designed diagnostic accuracy studies, and variation in manufacturers' products studied [21–25]. Comprehensive meta-analyses have reported some differences in test technology with CLIA methods appearing more sensitive compared to ELISA or LFA for assays targeting IgG as well as IgM/IgG [2]. No clear differences in sensitivity were reported for IgM assays, and there is little evidence to suggest differences in specificity between technology types [2, 3]. Low sensitivity has been reported for currently available point-of-care serological tests [3].

Recommendation [B2]: Importance of assay principle in assay selection (Lab-based vs. POCT).

- Currently available point-of-care assays for serological detection of antibodies against SARS-CoV-2 compare poorly in sensitivity to lab-based assays and should not be used without extensive clinical and analytical validation. When used, negative results with a high suspicion of infection should be followed up with a lab-based assay.

C Verification of regulatory-approved assays

The following recommendations are meant to provide general guidance to clinical laboratories on test evaluation prior to clinical testing. This guidance is focused on the verification of **regulatory approved test** performance and is not meant for the validation of laboratory developed tests or for validation of new tests by manufacturers. Most current serological assays are qualitative and thus this guidance is designed towards these tests. This guidance will be updated as regulatory-approved quantitative tests become available. Individual laboratories should consider local resource availability as well as regulatory and accreditation requirements, which may differ from those stated below, and modify their evaluation plans accordingly.

(1) Evaluation of analytical performance in the context the assay will be used.
(2) Evaluation of clinical performance in the context the assay will be used.

[C1] Specifications for analytical performance verification of serological tests against SARS-CoV-2

It is desirable to verify the performance of the testing system on all sample matrices that will be encountered during routine testing. It is anticipated that some laboratories will

not have direct access to the samples required for evaluation. This lack of access to samples may be overcome by close collaboration with peers, or with a reference laboratory. All samples used in the evaluation should be stored in conditions that ensure high stability and should be thoroughly homogenized prior to testing. An example analytical assay evaluation protocol is provided in Table 1 for qualitative serological tests.

One major concern regarding the implementation of serological testing into clinical practice is the potential for cross-reactivity, especially given that over 90% of adults are estimated to have antibodies against other commonly circulating coronaviruses [11]. Many manufacturers and most available literature report minimal assay cross-reactivity, although some false positives against endemic coronaviruses and other species have been reported [21, 25–31].

Recommendation [C1]: Specifications for analytical performance verification of serological tests against SARS-CoV-2.

- Laboratories should verify the analytical performance of regulatory approved serological tests, including the parameters described in Table 1, before routine use.
- Laboratories should participate in a Quality Assurance Program for SARS-CoV-2 serology, when possible.

Participation in a recognized SARS-CoV-2 serology Quality Assurance Program (QAP) is also essential, as more options become available [32].

[C2] Specifications for clinical performance verification of serological tests against SARS-CoV-2

Clinical laboratories should agree with clinical users or policy makers on clinical performance requirements, based on intended assay use, prior to proceeding with assay evaluation. This clinical performance requirement could vary based on epidemiological characteristics and patient population in which the assay will be applied. Importantly, the clinical samples used for method verification should be the same as those expected during clinical testing. For example, if the test is intended to be used to confirm past SARS-CoV-2 infection in a population where the prevalence is low, or if the test is to be used in seroprevalence studies, it is important to have a test with high specificity (e.g., ≥99%) to minimize false positive results [33]. Diagnostic accuracy studies and clinical performance specifications should be driven by the actual purpose of test use. Of note, clinical sensitivity determined in validation studies can be considerably lower than manufacturer's claim, although clinical specificity tend to be more consistent [21, 25–31].

Key components when selecting samples for evaluating clinical performance include: patient setting (e.g., inpatient vs. outpatient), severity of cases (e.g., asymptomatic, moderately symptomatic, and severe), and timing of assessment (e.g., 1–2 months or, 3+ months after exposure to the virus). Importantly, if these components are not considered in assay evaluation, findings will not be transferrable to intended clinical practice. Most available literature has evaluated serological tests in patients with severe COVID-19 disease. This fact has likely inflated the diagnostic accuracy of these tests, and findings cannot be simply applied to less severe populations. It is also paramount to ensure that an appropriate reference standard is used in serological tests evaluations. If the serology test is to be used for assessing whether *known* COVID-19 positive cases have developed an antibody response, samples that are obtained from patients confirmed by RT-PCR, in accordance with the WHO and China CDC case definitions, should be used. If the serological test is used for assessing individuals with *suspected* COVID-19 who do not have an RT-PCR result, evaluation design and selection of the reference standard should take extra precaution in confirming or ruling out COVID-19 through additional measures (e.g., clinical follow-up, CT scans, and/or repeat RT-PCR) [2]. In addition to sensitivity analyses, historic samples from pre-COVID-19 patients and/or samples from contemporary PCR-negative patients should be analyzed to evaluate specificity.

Table 2 provides an example test evaluation protocol for evaluation of serological tests in hospitalized patients. This protocol should be modified if test performance is evaluated in other populations. We encourage clinical laboratories to use the Standards for Reporting Diagnostic Accuracy (STARD) guidelines to assist in reporting clinical performance studies [34, 35].

Recommendation [C2]: Specifications for clinical performance verification of serological tests against SARS-CoV-2.

- Laboratories should set clinical performance specifications together with clinicians and policy makers that reflect the intended use of the test in the intended population and clinical setting.
- Clinical performance studies should verify if the test is fit for purpose in the local setting.
- Laboratories should follow the STARD guidelines when designing and reporting clinical performance studies.

Table 1: Analytical verification recommended for clinical laboratories when verifying a **regulatory-approved serological assay** (modified from [41]).

Consideration	Element	Specifications
Imprecision	Design	Prepare positive and negative quality control samples (if they return a quantitative index result), preferably at concentrations where the imprecision claims were made by the manufacturer, and run 5 times daily for five days.
	Evaluation	Calculate mean, standard deviation (SD) and coefficient of variation (CV) for repeatability and within-laboratory imprecision of the index values (e.g., COI/S/Co ratio) and compare against corresponding manufacturer claims. It may be necessary to employ analysis-of-variance for the calculation of each imprecision component.
	Acceptability	The imprecision should fall within manufacturer's claimed limits, where available.
Carry-over	Design	It is highly recommended to assess for carryover contamination by measuring a negative sample in duplicate, followed by a high sample in duplicate, and followed by the negative sample in duplicate again. The high sample should be within 10% of the upper measurement range (e.g., COI/S/Co ratio), if available. Carry-over may not be required when the analytical platform uses disposable sample tips.
	Evaluation	Calculate percentage of carry-over (average of duplicate negative sample after high sample/average of duplicate negative sample before high sample $\times$ 100%)
	Acceptability	The carry-over should be within manufacturer's claim, or <2 times the analytical CV of the COI/S/Co ratio (if available) of the negative sample.
Cross-reactivity	Design	Not required, but recommended if resources allow. If not assessed, positive results should be provided with a comment indicating possible cross-reactivity as per manufacturer information. In consultation with available resources, prepare samples with known cross-reactivity with endemic coronaviruses or other circulating respiratory pathogens[a] ideally collected before COVID-19 appeared in late 2019. It is important to note that cross-reactivity is more likely to be demonstrated when the sample size per species subtype is greater than 10.
	Evaluation	Calculate number of false positives for each species and the overall analytical specificity together with data from "Interference" as described below. Where possible, calculate the 95% confidence intervals (95% CI).
	Acceptability	The overall analytical specificity (and 95% CI, if calculated) meets the manufacturers' claim or the laboratory's *a priori* clinical requirement. When cross-reactivity is observed, this should be reported and considered in test interpretation.
Interference	Design	Not required, but recommended if resources allow. In consultation with available resources, prepare samples with common interfering substances (e.g., icterus, hemolysis, lipemia) from patients with and without COVID-19. It is possible to use a spiking experiment to achieve the desired samples.
	Evaluation	Calculate number of discordant results for each interference and overall analytical specificity together with data from "cross-reactivity" described above. Where possible, calculate the 95% CI.
	Acceptability	The overall analytical specificity (and 95% CI, if calculated) meets the manufacturers' claim or the laboratory's *a priori* clinical requirement.

[a]Verification results for many SARS-CoV-2 serology tests are available from Public Health England, including a list of key species to consider for cross-reactivity (accessible at: https://www.gov.uk/government/publications/covid-19-laboratory-evaluations-of-serological-assays).

D Test interpretation and limitations

[D1] Considerations for test interpretation

It is essential that SARS-CoV-2 antibody test results are interpreted in the context of the time since symptom onset. Importantly, a negative serological result does not confirm definite lack of exposure, as neither a positive result confirms exposure. In order to avoid false negative and false positive results, it is critical that both sensitivity and specificity of SARS-CoV-2 antibody assays are as high as possible in the clinical population for which the assay will be applied [36]. Diagnostic accuracy of any test highly depends on disease prevalence in the studied population [37]. Thus, regional epidemiology must be considered when deciding on sufficient diagnostic sensitivity and specificity. Potential strategies to improve positive predictive value (PPV) estimates include: restricting testing to high-risk populations and performing orthogonal testing where initial positives are tested by a second method, ideally with a different antigenic target. It is also likely that patient age, immune status, exposure history (i.e., mild vs. severe), and potentially ethnicity will impact the immune response to

SARS-CoV-2 infection [38]. As more clinical data become available, it will be important to consider these covariates in result interpretation. Finally, it should be noted and communicated to clinical colleagues that a positive SARS-CoV-2 antibody test result does not mean that the patient is no longer infectious or immune to subsequent infections. Both SARS-CoV-2 viral and serological positivity have been demonstrated, suggesting that an antibody response can be detected when active viral shedding is still occurring [39].

Recommendation [D1]: Considerations for SARS-CoV-2 serology test interpretation.

- **Positive test result:**
  - Antibodies against SARS-CoV-2 were detected in the sample indicating recent or prior COVID-2 infection. The results should be interpreted in the clinical context and considering assay specificity, sensitivity, and population prevalence.
- **Negative test result:**
  - Antibodies against SARS-CoV-2 were not detected in the sample, but lack of SARS-CoV-2 exposure cannot be ruled out. Follow-up testing might be indicated. The results should be interpreted in the clinical context and considering assay specificity, sensitivity, and population prevalence.
- **Indeterminate test result:**
  - Test result cannot be interpreted; follow-up re-testing should be completed to yield a determinate result.

[D2] Current limitations of serological tests

Our current understanding of SARS-CoV-2 immune response and prevalence as well as assay performance characteristics in large cohorts across several populations is limited. It is hence important to exercise caution when implementing serological testing into clinical care, to avoid misinformation and misdiagnosis. Importantly, while serological testing is anticipated to play an important role in the identification of past infection, very few studies have examined assay performance in asymptomatic patients. Further, data are accumulating that the antibody response in some patients wanes over a period of weeks to months, rather than months to years. Thus, combined with the lack of detectable antibody on some assays before 10–14 days post-infection, antibody testing may be falsely negative early and late after infection. Additionally, as previously mentioned, increasing data suggest that the antibody response in severe patients is much stronger than that of mildly symptomatic or asymptomatic patients, and thus assay performance may vary with clinical severity. It is important that clinical laboratories continue to highlight these limitations when reporting serological results. Consideration shall also be made on providing quantitative rather than qualitative test results, due to the association between disease severity and antibodies titer, as well as for the need of monitoring humoral immunity over time [40].

Table 2: Clinical performance verification steps recommended for clinical laboratories verifying a regulatory approved commercial serological assay in **hospitalized patients** (modified from [41]).

Consideration	Element	Description
Clinical performance	Design	A minimum of 10–20 positive samples and 10–20 negative samples is recommended, per matrix. Where resources permit, 40 or more samples for each positive/negative status is preferred. Positive samples should be residual samples from patients with positive molecular test results. Days since symptom onset should be recorded and used in interpretation (ideally, all positive samples should be >14 days post symptom onset). Negative samples should ideally be samples from the pre-COVID-19 era. A patient with negative SARS-CoV-2 RT-PCR does not comprehensively rule out COVID-19 infection, since it may be a false negative test.
	Evaluation	Calculate the clinical sensitivity, clinical specificity, negative predictive value, and positive predictive value of the test, along with the respective confidence intervals.
	Acceptability	The overall clinical sensitivity and specificity (and 95% CI, if calculated) meets the manufacturers' claim or the laboratory's *a priori* clinical requirement. If an initial evaluation failed to meet manufacturer's claim, consider including more samples for reassessment.

This recommended protocol is only to verify manufacturer's claims, which are largely based on hospitalized patients. In order to determine if a serology test is fit for purpose, one must collect samples from a population representative of local prevalence and desired clinical scenario (e.g., asymptomatic, delayed clinical presentation).

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