

# Verification procedure for commercial serologic tests with Emergency Use Authorization for detection of antibodies to SARS-CoV-2

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*on behalf of the American Society for Microbiology Clinical and Public Health Microbiology Committee, Subcommittee on Laboratory Practices*

## I. Introduction

The current COVID-19 pandemic has led to the development of several serologic tests to detect antibodies to SARS-CoV-2. As of June 19, 2020, there are [21 serologic tests](#) that have received Emergency Use Authorization (EUA) by the U.S. Food and Drug Administration (FDA). Serologic tests should not be used for diagnosis of acute COVID-19. However, they may be useful in select clinical situations to facilitate diagnosis of COVID-19, such as for patients who present with COVID-19-like symptoms late in clinical disease course, are negative for SARS-CoV-2 by a molecular assay and for whom a lower respiratory sample cannot be collected. In addition, serologic tests advance our understanding of the epidemiology and seroprevalence rates of SARS-CoV-2 at the local, state and national levels. They also play an important role in screening for anti-SARS-CoV-2 antibodies in potential convalescent plasma donors for use in national convalescent plasma therapy clinical trials.

The commercially available serologic assays with EUA detect IgM, IgG or total antibodies against different regions of recombinant SARS-CoV-2 spike proteins (subunit 1 [S1], subunit 2 [S2] or the receptor binding domain [RBD]) or nucleocapsid proteins. These assays are categorized as either lateral flow assays (LFAs), enzyme linked immunosorbent assays (ELISAs), or chemiluminescent immunoassays (CLIAs). They differ in the specimen types accepted, the required specimen volume, cost per test, and throughput, among other factors. The manufacturer's instruction for use (IFU) for serologic tests listed on the [FDA EUA webpage](#) and performance characteristics listed on the [FDA EUA authorized serology test performance webpage](#) provide select information regarding these variable factors and should be reviewed before implementing these tests.

All current EUA SARS-CoV-2 serologic test are qualitative, and are resulted as either "positive", "negative", "indeterminate", or similar such results. These tests should be verified for accuracy and precision. All commercial serology EUA tests should be verified in a similar manner to FDA-approved/cleared diagnostic assays before reporting test results.



## II. Materials Required

- Appropriate biosafety and protective equipment ([CDC biosafety guidelines](#))
- Positive patient samples tested on a compatible comparator assay (matched antibody class and preferably, targeted antigenic region)
- Negative patient specimens collected in the U.S. before December 2019 or negative by a compatible comparator assay
- Contrived samples (as needed) – antibody class and antigenic target compatible reference material, diluted in negative patient matrix or commercial matrix
- Whole blood, plasma, or serum negative matrix, as appropriate
- Test tubes/pipette tips
- Calibrators (provided by test manufacturer)
- SARS-CoV-2 serologic test reagents with FDA EUA
- Positive and negative controls provided by manufacturer or purchased separately
- Reference kit, as necessary

## III. Instruments Required

- Based on [FDA EUA](#) authorized available tests.

## IV. Verification Procedure

The verification procedure described here applies to LFA, ELISA and CLIA serologic tests. It is important to note that for EUA serologic tests the FDA requires manufacturers to perform validation studies for analytic sensitivity and specificity (reactivity/cross reactivity), class specificity (IgG/IgM), and clinical studies. Laboratories should review the summaries of the manufacturer's validation studies included in the IFUs to become familiar with the test performance and the parameters validated by the manufacturer.

### Step 1. Calibration

For tests in which the results are interpreted according to a cut-off, for example determination of relative light units (RLUs), calibration should be performed using the manufacturer-provided calibrators, following the manufacturer's instructions. Note that verification of the manufacturer established cut-off for qualitative results by ELISAs and CLIAs can be established during assay calibration and/or use of calibration materials or QC material near the cutoff value.

### Step 2. Test at least one positive and one negative quality control (QC) sample

Positive and negative QC testing should be performed before beginning verification sample testing to ensure the test cartridges and/or kit reagents and instrumentation are working appropriately. QC should be performed according to the manufacturer's instructions. Refer to the

Section V, QC Testing, for further information. If QC is successful, the verification study can proceed. If QC is not successful, contact the manufacturer.

As of June 16, 2020, an Individualized Quality Control Plan ([IQCP](#)) may be used to decrease the frequency of external controls for EUA assays which would otherwise be IQCP eligible.

### Step 3. Verification procedure

These recommendations are designed to meet the performance specifications required for accuracy and precision. **Table 1** provides sample selection recommendations.

#### ***Positive sample selection:***

- a. Patient samples - Undiluted residual patient specimens that tested positive on a compatible comparator assay are preferred. Dilution of highly positive specimens into patient matrix can supplement. When possible, samples with varied amounts of antibody should be tested.
- b. Contrived samples - If reference material is used, we recommend using a third-party reagent. The control material can be diluted to prepare several verification samples at lower index values or signal to cut-off (S/Co) ratios. If the reference material is a different matrix than that being verified, a minimum dilution of 1:10 in test matrix is recommended to minimize matrix effects from the reference material. Using negative matrix from different patients is preferred.

#### ***Negative sample selection:***

SARS-CoV-2 antibody negative residual patient specimens can be used to evaluate assay specificity and matrix effects. The matrix should be matched as closely as possible. The following are acceptable negative sample sources.

- a. Pre-SARS-CoV-2 patient samples - Samples collected in the U.S. prior to December 2019, particularly from a population with a high prevalence of vaccination against influenza and other viruses.
- b. Samples negative by a comparator assay- Matrix-matched residual patient specimens negative for SARS-CoV-2 antibodies by a verified EUA serology test can be used, ideally targeting the same antibody class(es) and antigenic targets.
- c. Commercial matrix- Commercial negative controls or negative matrix can be used sparingly to supplement patient specimen testing.

#### ***Whole blood sample selection by fingerstick:***

Comparator testing from the same sample is not possible for this specimen type. Venous blood and a fingerstick specimen can be collected side-by-side from individual patients. When fingerstick whole blood is added directly to the test cartridge, specimens should be tested in real-time. The venous blood (prepared as whole blood, plasma, or serum) is subsequently tested on a verified EUA assay and used as the comparator result.

## Accuracy

Accuracy verification for serologic assays detecting anti-SARS-CoV-2 antibodies must include both known positive and known negative samples. The detection of all applicable analytes (e.g. IgG, IgM) should be evaluated, as well as matrix effects. **Table 1** provides an overview of the recommended sample number and characteristics for assays detecting a single analyte, multiple analytes reported as a single result, or multiple individually detected and reported analytes.

**Single analyte assays:** Single analyte assays include serologic assays that detect one antibody class against SARS-CoV-2 and report a single qualitative value. A minimum of 10 positive samples and 10 negative samples is recommended, per matrix. When verifying multiple matrices (i.e. sample types or collection devices) test 10 positive and 10 negative samples for each matrix. If samples or test reagents are limited and multiple matrices are verified, a laboratory may consider using as few as ten total samples for each matrix, including five positive and five negative samples.

**Multiple analytes detected, but not differentiated:** A minimum of 10 positive samples and 10 negative samples is recommended, for each matrix. Positive specimens enabling verification of the assay's ability to detect the different antibody classes is preferred, including for example known IgG and/or IgM positive samples to confirm that the total antibody assay undergoing verification produces a positive result for these specimens.

**Multiple analytes detected and differentiated:** For these assays, the accurate detection of both anti-SARS-CoV-2 IgG and anti-SARS-CoV-2 IgM must be verified. A minimum of 10 positive and 10 negative samples should be evaluated for each analyte (IgM and IgG). Optimally, a combination of IgM+/IgG-, IgM-/IgG+, and IgM+/IgG+ samples are included among the positive specimens to assess performance of each analyte and verify the absence of between-class cross-reactivity. For negative samples, at least five anti-SARS-CoV-2 IgM-/IgG- samples should be included. Additionally, IgM-/IgG+ samples could be included in the negative accuracy analysis of IgM. IgM+/IgG- samples could be included in the negative accuracy analysis of IgG.

## Precision

Precision is verified by repeat measurements of samples within-run (repeatability, intra-precision) and inter-run over a period of time (reproducibility, inter-precision). If possible, multiple positive samples with varied relative levels of antibody (high, medium, and/or low) can be repeatedly evaluated for precision. Pooled positive and negative samples can be used if not enough volume from individual samples is available to complete the precision studies.

Data for inter-run and intra-run precision should be generated over 5 runs:

- Day 1. Test positive and negative patient samples in duplicate or triplicate by one designated laboratory operator;
- Day 2 - 5. Test the same positive and negative samples in duplicate over four additional days, including one or more different operators

For LFAs, within-run precision does not apply as each device is used independently. For inter-run precision studies, testing of a minimum of 5 samples in duplicate for five days is recommended. Because the reading of results, particularly for devices requiring a visual reading, is operator dependent, more than two operators should participate in the precision study to ensure that reading variability is considered for the test precision assessment.

For tests using fingerstick samples, where testing is performed immediately after collection and added directly to the test cartridge, multiple tests (3-5) could be run on day 1 by different operators in samples collected from a known positive and a negative patient.

### **Reportable Range**

As of June 15, 2020, verification of reportable range is not applicable for the available, qualitative serologic tests.

### **Carryover Studies**

For ELISA and CLIA serologic tests, it is highly recommended to assess for carryover contamination by alternating testing of a negative and a positive sample. This can be done during the process of testing for accuracy and precision.

### **Data Analysis**

Data should be analyzed and performance assessed by calculating positive and negative agreement between the verification result and the comparator result. If exclusively pre-December 2019 samples are used for negative sample comparison, specificity can be calculated. At least 95% agreement is generally considered acceptable test performance. In addition,  $\geq 95\%$  agreement across all matrices is acceptable when assessing equivalency across different matrices.

If the test under evaluation can differentiate between IgG and IgM, positive and negative agreement for IgG and IgM should be calculated separately.

Discrepant results between the comparator method and the test being verified should be fully investigated. Samples should be re-tested and if significant discrepancy continues, the manufacturer should be contacted and/or the new assay should not be implemented for patient testing.

## **V. Quality Control Testing**

Daily QC is recommended when verification testing is performed. After verification, external low positive and negative QC must be run every day of patient testing or no less than the manufacturer's instructions. In general, external QC should be run every shift, every 24 hours or every batch, as appropriate. Acceptability of QC criteria should be established for quality assurance. For commercial assays, calibrator material is provided by the manufacturer and



should be used to calibrate the assay per the manufacturer's instructions, every 6 months, or after major instrument maintenance.

Commercial QC products provided by the test manufacturer are preferred over residual patient samples or third-party vendors. If patient samples or third-party material must be used, contrived controls should be reviewed to ensure they are matrix-appropriate for the test being used (eg, plasma matrix), contain the appropriate antibody that is targeted by the assay and do not approach the assay qualitative cut-off threshold.

## VI. Biosafety Requirements

Prior to onboarding SARS-CoV-2 serology testing, the laboratory should perform a site-specific risk assessment to identify and mitigate risk. According to the World Health Organization ([WHO](#)) and [CDC](#) biosafety guidelines, the risk assessment should review the procedures that will be performed, identify the hazards in the processes, assess all equipment and facilities, review staff competencies, and determine the mitigation required to safely perform testing.

Since LFAs, ELISAs or CLIAs use purified proteins and not live virus, these assays can be performed in a BSL-2 laboratory using standard precautions. Likewise, blood specimens do not contain high levels of infectious SARS-CoV-2 virions; therefore, standard precautions are sufficient for handling such specimens.

Laboratories that perform [neutralizing antibody assays](#), which involve incubating serum or plasma with live virus and subsequently infecting cells, must consider use of increased safety precautions if live SARS-CoV-2, rather than recombinant virus, is used. Virus neutralization tests (VNTs), such as the plaque reduction neutralization test (PRNT) and microneutralization assays, may be performed using either a strain of SARS-CoV-2 from a clinical isolate or pseudotyped vesicular stomatitis virus or other viral vector expressing the SARS-CoV-2 spike protein. VNTs using live SARS-CoV-2 virus require BSL-3 conditions, whereas use of chimeric viruses expressing only the SARS-CoV-2 spike protein can typically be performed at BSL-2.

## VII. Limitations

- **Assay/Technical Limitations**  
Small reductions in test specificity, when combined with a low prevalence of disease, lead to difficulty in ascertaining the likelihood that a positive result represents a true positive result. Assay specificity can also be impacted by the portion of the virus that is targeted by the assay (e.g. nucleocapsid vs. spike protein). In addition, the presence of antibodies to SARS-CoV-2 is not yet known to correlate with the presence of protective immunity against re-infection. As a result of this current knowledge gap, the [CDC's interim guidelines](#) specifically state that antibody results for SARS-CoV-2 should not be



used to guide return-to-work policies, decisions regarding congregate settings or use of personal protective equipment for healthcare workers.

- **Clinical Limitations**  
Serologic tests should not be used alone to diagnose acute SARS-CoV-2 infection, but in combination with other testing modalities (e.g. RT-PCR and/or antigen testing), and clinical assessment may contribute to diagnosis.
- **Staff Training**  
Workers interpreting tests that require manual interpretation (such as LFAs) should be provided with clear, unambiguous interpretive guidance and training. For assays which involve manual steps (e.g. plate washing), care should always be taken to adhere to appropriate practices. Competency must be ascertained and maintained according to regulatory requirements similar to all laboratory testing.

## VIII. Supplementary Information

- All U.S. laboratories using these products should have a process in place for reporting all test results to healthcare providers and relevant public health authorities, according to updated guidance under the Coronavirus Aid, Relief, and Economic Security (CARES) Act found here:
  - <https://www.hhs.gov/sites/default/files/covid-19-laboratory-data-reporting-guidance.pdf>
- FDA requires that laboratories performing EUA-approved SARS-CoV-2 serology testing share authorized Fact Sheets with test results reporting. Fact Sheets specific to each EUA assay are available for test recipients (i.e., persons whose samples were tested for SARS-CoV-2 antibodies) and healthcare providers who order the testing. These fact sheets can be found here:
  - <https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization#covidinvitrodev>
- The FDA offers a calculator to aid in the estimation of positive and negative predictive values of a test (or of two independent tests performed on the same individual) based on the estimated prevalence of SARS-CoV-2 in the local population. For this calculation, the tests' analytical sensitivity and specificity must be known. The calculator can be found here:
  - <https://www.fda.gov/medical-devices/emergency-situations-medical-devices/eua-authorized-serology-test-performance>



- Results should be reported following manufacturer's guidelines and will differ based upon the type of assay (i.e., reportable results from LFAs may differ from those from ELISA or CLIAs).
  - Possible reportable results include: negative, non-reactive, not detected; positive, reactive, detected; or equivocal, indeterminate, borderline, or invalid, depending upon the test system.
  - Manufacturer guidelines can be found here:  
<https://www.fda.gov/medical-devices/emergency-situations-medical-devices/eua-authorized-serology-test-performance>
  
- The laboratory should provide clear test interpretive guidance, which may include report comments. Examples of value-added comments are provided in **Table 2** and can be customized for each laboratory or institution.
  
- Results from antibody testing should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to inform infection status.

**Table 1: Accuracy Study Sample Selection Recommendations**

Verification Assay		Single Matrix	Multiple Matrices
Common to all assays	Options for positive sample selection	<ul style="list-style-type: none"> <li>- Positive by a comparator assay targeting one or multiple analytes, differentiated and reported individually</li> <li>- Contrived specimens spiked with target analyte reference material(s)</li> <li>- Contrived specimens made from dilution of positive patient samples</li> <li>- When possible, include samples spanning the detection range of the verification assay (high, medium, low amounts of analyte). Example: <math>\geq 3</math>-4 high positive, <math>\geq 3</math>-4 moderately positive, <math>\geq 2</math>-4 low to moderately-low positive</li> </ul>	
	Options for negative sample selection	<ul style="list-style-type: none"> <li>- Samples collected prior to December 2019</li> <li>- Negative by a comparator assay for only the target analyte(s)</li> <li>- Negative by a comparator assay targeting multiple analytes, including all target analyte(s). Differentiated or undifferentiated comparator assays are acceptable.</li> </ul>	
Single analyte (example: IgG only)	Recommended samples	$\geq 10$ positive samples $\geq 10$ negative samples	$\geq 10$ positive samples, each matrix $\geq 10$ negative samples, each matrix If limited, a minimum of 5 positive and 5 negative samples for each matrix may be tested
	Additional positive sample selection options	<ul style="list-style-type: none"> <li>- Positive by a comparator assay targeting the same analyte (e.g. IgG)</li> </ul>	
Multiple analytes, undifferentiated (examples: total antibody or IgG/IgM)	Recommended samples	$\geq 10$ positive samples $\geq 10$ negative samples	$\geq 10$ positive samples, each matrix $\geq 10$ negative samples, each matrix If limited, a minimum of 5 positive and 5 negative samples for each matrix may be tested
	Additional positive sample selection options	<ul style="list-style-type: none"> <li>- Positive by a comparator assay targeting the same analytes, undifferentiated</li> </ul>	
Multiple analytes, differentiated (example: IgG and IgM)	Recommended samples	$\geq 10$ positive samples, each analyte $\geq 10$ negative samples, each analyte	$\geq 10$ positive samples, each analyte/matrix combination $\geq 10$ negative samples, each analyte/matrix combination If limited, a minimum of 5 positive and 5 negative samples for each analyte/matrix may be tested
	Additional positive sample selection options	Positive by a single-target comparator assay. Include as a positive verification study sample for the known analyte and excluded from analysis for the unknown analyte(s).	
	Additional negative sample selection options	IgM-/IgG+ samples can be included in negative IgM sample count; IgM+/IgG- samples can be included in negative IgG sample count	



**Table 2: Examples of Interpretive “Value-added” Comments for SARS-CoV-2 Serologic Assays**

Result	Comment
Serologic SARS-CoV-2 Testing Comments for IgG or Total Antibody Tests	
Negative, not detected, or non-reactive	No antibodies to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) detected. Negative results do not preclude acute SARS-CoV-2 infection. Negative results may occur in serum collected too soon following infection (ie, if the quantity of antibodies for the SARS-CoV-2 virus present in the specimen is below the detection limit of the assay), if the virus has undergone amino acid mutation(s) in the epitope recognized by the antibody used in the test, or in immunosuppressed patients. Follow-up testing with a molecular SARS-CoV-2 test is recommended in symptomatic persons. This test should not be used to exclude acute/recent coronavirus disease 2019 (COVID-19).
Indeterminate or equivocal or borderline	Repeat testing in 7 to 10 days may be considered to determine definitive serologic status.
Positive, detected, or reactive*	SARS-CoV-2 antibodies detected. Results suggest recent or prior infection with SARS-CoV-2. Correlation with epidemiologic risk factors and other clinical and laboratory findings is recommended. Serologic results should not be used to diagnose acute SARS-CoV-2 infection. False-positive results may occur due to cross-reactivity from pre-existing antibodies or other possible causes. It is currently unknown how long antibodies to SARS-CoV-2 remain present in the body after infection and if they confer immunity to infection.
Additional Comments If Specific Antibody Classes IgG and/or IgM Reported	
IgM positive/ IgG negative	Presence of IgM in the absence of IgG is consistent with an acute or recent SARS-CoV-2 infection, or may be a false positive IgM. (In addition, consider applying general comments above, based on testing scenario.)
IgG positive/ IgM negative	Presence of IgG in the absence of IgM is consistent with recent or prior SARS-CoV-2 infection. The presence of IgG



	antibodies often indicates a past infection but does not exclude recently infected persons who are still contagious. (In addition, consider applying general comments above, based on testing scenario.)
IgG positive/ IgM positive	Presence of IgG and IgM may indicate acute, recent, or prior SARS-CoV-2 virus infection.
General Comment	
Universal EUA comment	This test has received FDA Emergency Use Authorization and has been verified by XXX laboratory. This test is only authorized for the duration of the declaration and the circumstances that exist to justify the authorization of the emergency use of in vitro SARS-CoV-2 serologic assays as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, under section 564 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. § 360bbb-3), unless the authorization is terminated or revoked sooner. This testing was performed at the XXX laboratory located at [Anywhere, USA, 12345] (CLIA Certificate #XXX, Accreditation #XXX, other as appropriate).

\*Positive results by some assays may be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E, or from cross-reactivity from pre-existing antibodies or other causes. Refer to IFUs.

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