Centers for Disease Control and Prevention

COVID-19 for Laboratories

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What are you looking for?

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General Guidance and Regulatory Requirements

My facility would like to begin SARS-CoV-2 testing. Do we need a Clinical Laboratory Improvement Amendments (CLIA) certificate? Can my facility be granted a waiver from the CLIA certification requirements so that I can begin testing immediately?

Before conducting SARS-CoV-2 viral testing, a laboratory or other facility that performs testing must be CLIA-certified and meet applicable regulatory requirements. The Centers for Medicare and Medicaid Services (CMS) does not have the authority to grant waivers of exceptions that are not established in a statute or regulation. For additional information, please refer to the FAQs on the CMS website: CMS Coronavirus Information .

What is the CLIA test complexity categorization of SARS-CoV-2 tests that do not have an Emergency Use Authorization (EUA)?

Tests for SARS-CoV-2 that are offered prior to or without an EUA have not been reviewed by the Food and Drug Administration (FDA), are not FDA-authorized, and have not received a CLIA categorization . Thus, those tests are considered high complexity by default until they receive an EUA or other FDA review that indicates they may be performed as moderate complexity or waived tests. For more information, visit FDA COVID-19 Resources , and navigate to the section titled “General FAQs.”

How do I apply for a CLIA certificate so that my testing facility can perform SARS-CoV-2 testing?

The federal CLIA program contracts with states to carry out certain oversight and recording functions of the CLIA program. The state in which the laboratory is located processes applications for CLIA certificates. After the laboratory has identified a qualified and certified laboratory director and has provided all required information on the CMS-116 application, a CLIA number will be assigned and the laboratory can begin testing if applicable CLIA requirements have been met. For additional information, please refer to the FAQs on the CMS website: CMS Coronavirus Information .

Can a laboratory without a CLIA certificate conduct surveillance testing?

Yes. If a laboratory conducts surveillance testing on a specimen without a unique identifier and the results of that testing are not returned to the individual, or to the individual's healthcare provider, employer, etc., that laboratory does not need a CLIA certificate. Surveillance testing results may be returned in aggregate to the institution that requested the study. In such cases, surveillance testing may indicate the need to conduct additional and perhaps more targeted diagnostic testing or screening at the individual level in a CLIA-certified laboratory to improve population or setting-specific health. If at any time a facility conducting surveillance testing intends to report a patient-specific testing result, it must first obtain a CLIA certificate and meet all CLIA requirements to perform that testing.

How does my laboratory assess the validity of a specimen that has been obtained through self-collection?

Self-collection of specimens, both unsupervised and supervised by a medical professional, is currently available for specific tests authorized by the FDA. Additional authorized diagnostic tests for the detection of SARS-CoV-2 will likely have this capability as well.

There have been reports of fraudulent specimens being submitted to laboratories for testing, often as a result of unsupervised collection and travel- or work-related requirements. Laboratories should make every effort to confirm the specimen has been obtained correctly and from the individual that is being tested. Generally, CLIA requires laboratories to ensure positive specimen identification and optimum integrity of a patient's specimen using at least two separate (distinct) or unique identifiers, such as patient's name or another unique identifier. Other information that must be provided to the laboratory when requesting a test includes the sex and age or date of birth of the patient; the test(s) to be performed; the specimen source; the date and, if appropriate, the time of specimen collection.

Test Developers

Can test developers reference the EUA for CDC's diagnostic multiplex assay for flu and SARS-CoV-2 when validating or seeking authorization for a test based on the CDC design?

- Yes. CDC has extended right of reference for manufacturers and clinical laboratories to cite the EUA for CDC's Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay (FDA submission number EUA201781). This means clinical laboratories and commercial manufacturers may avoid repeating studies CDC has already conducted in support of its EUA. CDC has published the primers and probes sequences, so other laboratories and companies may manufacture their own reagents. The sequences are identical to those used for the CDC test and may be used by commercial manufacturers and clinical laboratories in the design of their own independent assays. These sequences are labeled *research use only* because the primers and probes manufactured from these sequences cannot be used under CDC's EUA. Only primer and probe sets distributed through the International Reagent Resource may be used with the assay under CDC's EUA.

Where do test developers get the genomic RNA needed to validate test performance for FDA?

Currently, genomic RNA material can be used for validation purposes in biosafety level 2 laboratories (BSL-2). Genomic RNA material is available through BEI Resources. Registration with BEI Resources is required to request SARS-CoV-2 materials. BEI Resources is prioritizing and fast-tracking all SARS-CoV-2 registrations with a 12- to 72-hour turnaround time. Please contact BEI Resources at contact@beiresources.org or 1-800 359-7370 for questions.

Developers are required to sign a material transfer agreement prior to the release of materials.

All BEI Resources reagents are provided worldwide. There is no cost for the reagents themselves. However, shipping and handling charges may apply.

Commercial sources also may have this material.

For Public Health Laboratories: If a kit to detect SARS-CoV-2 is needed, contact the International Reagent Resource .

What is the National Institutes of Health's (NIH) BEI Resources Repository?

BEI Resources Repository was established by the National Institute of Allergy and Infectious Diseases at the National Institutes of Health to provide reagents, tools, and information for studying Category A, B, and C priority pathogens, emerging infectious disease agents, non-pathogenic microbes, and other microbiological materials of relevance to the research community including diagnostic developers. Centralizing these functions within BEI Resources facilitates access to these materials by the scientific community and ensures quality control of the reagents.

Laboratory Biosafety

What safety issues are there with PrimeStore® Molecular Transport Medium (MTM) when used with SARS-CoV-2 testing platforms?

PrimeStore® MTM transport media contains guanidine thiocyanate, which produces a dangerous chemical reaction that releases cyanide gas when exposed to bleach (sodium hypochlorite). The PrimeStore® MTM transport media being provided by state health departments is currently labeled at the bulk box level, but individual vials lack labels to warn users of the reactive ingredient.

Do NOT use PrimeStore® MTM with any Reverse Transcription Polymerase Chain Reaction (RT-PCR) platforms that include a disinfecting step that uses bleach (e.g., Panther® Hologic, Panther Fusion® Systems).

In addition to its reactivity, PrimeStore® MTM may be harmful if inhaled, if it contacts the skin, or if swallowed. Wear appropriate personal protective equipment (PPE) as required by your laboratory protocols, including laboratory coat, safety glasses, and gloves. Dispose of product content and container in accordance with all local, regional, national, and international regulations. Untreated waste should not be disposed into the sewer unless fully compliant with all applicable requirements. See the Material Safety Data Sheet for disposal information.

For more information, see the Longhorn PrimeStore® MTM Fact Sheet .

What safety issues can occur when using a mixture of A549 and Mv 1 Lu cell lines (also referred to as A549/Mv 1 Lu mix or R-Mix™) for culturing respiratory viruses?

It has been shown that Mv 1 Lu cells can support low level replication of SARS-CoV, which could result in the inadvertent growth of SARS-CoV-2. Therefore, CDC recommends that laboratories **discontinue the use** of the A549/Mv 1 Lu mix (R-Mix™) or any other mixture containing Mv 1 Lu cell lines.

Based on the literature (Severe Acute Respiratory Syndrome Coronavirus 2 from Patient with Coronavirus Disease, United States), A549 and MDCK cells lines (which make up R-Mix Too™) do not support SARS-CoV-2 replication. As a result, R-Mix Too™ may be considered for use as an alternative for R-Mix™.

For additional information, see

- SARS-associated Coronavirus Replication in Cell Lines

SARS-associated Coronavirus Replication in Cell Lines

- Severe Acute Respiratory Syndrome Coronavirus 2 from Patient with Coronavirus Disease, United States

Interpreting Results of Diagnostic Tests

What influences the likelihood of false-positive or false-negative diagnostic test results?

The likelihood of obtaining a false-positive or false-negative diagnostic test result is influenced by factors related to the testing scenario and the test being used (e.g., sensitivity and specificity of the diagnostic test). Diagnostic tests perform optimally for detecting an infection when the **pretest probability** is high. Pretest probability is the likelihood that the person being tested actually has the infection. This likelihood is based on both the proportion of people in the test population or group who have the infection at a given time (prevalence) and the clinical presentation (including symptoms and known exposure) of the person being tested. In other words, the pretest probability increases with increasing prevalence in the population and clinical indications of illness in the person being tested. In contrast, tests typically perform best for excluding an infection when the pretest probability is low**. Test sensitivity** is the ability of a test to correctly identify people with infection, whereas **test specificity** is the ability to correctly rule out infection.

What factors have the greatest impact on false-positive rates?

Positive predictive value is the probability that a person who has a positive test result most likely has the infection. Pretest probability and test specificity have the greatest impact on false-positive rates. As the pretest probability and the specificity of the test increases, the false-positive rate decreases and the positive predictive value increases.

What factors have the greatest impact on false-negative rates?

Negative predictive value is the probability that a person who has a negative test result most likely does not have the infection. Pretest probability and test sensitivity have the greatest impact on false-negative rates. As the pretest probability decreases, the false-negative rate decreases and the negative predictive value increases. As the sensitivity of the test increases, the false-negative rate decreases and the negative predictive value increases.

What is the relationship between pretest probability and positive and negative predictive values?

Relationship between pretest probability and positive and negative predictive values

Pretest Probability*	Negative Predictive Value**	Positive Predictive Value**	Impact on Test Results
Low	High	Low	Increased likelihood of False Positives Increased likelihood of True Negatives
High	Low	High	Increased likelihood of True Positives Increased likelihood of False Negatives

*Sensitivity and specificity of tests are not affected by the pretest probability

**Predictive values are affected by the pretest probability

Can a diagnostic RT-PCR test show how infectious someone is?

No. RT-PCR tests are used to identify and diagnose an active infection and cannot be used to show how infectious an individual person is. Get more information about when you can be around others if you had COVID-19.

What is a cycle threshold (Ct) value from a RT-PCR test?

To improve the test's ability to detect virus, a RT-PCR test creates many copies of the same genetic material from the virus in a process called amplification. The Ct value indicates the number of amplification cycles needed for detection of the nucleic acid target during the amplification process. There is an association between the Ct value and the amount of viral genetic material that was present in the specimen.

Can a Ct value determine how much viral genetic material is present in an individual patient specimen?

RT-PCR tests can be either ***qualitative*** or ***quantitative***, and this affects how a Ct value is interpreted. As of August 5, 2021, all diagnostic RT-PCR tests that had received a US Food and Drug Administration (FDA) Emergency Use Authorization (EUA) for SARS-CoV-2 testing were ***qualitative*** tests. In a ***qualitative*** RT-PCR test, known amounts of virus are used during the development of the test to determine what Ct values are associated with positive and negative test results. A Ct value is generated when testing a patient specimen and is used to interpret a test as positive or negative but **cannot** be used to determine how much virus is present in an individual patient specimen.

In a ***quantitative*** RT-PCR test, a range of known numbers of genome copies, called reference samples, are tested alongside each RT-PCR reaction. By comparing the Ct value of a patient specimen to the Ct values from the reference samples, the test can calculate the copy number of target nucleic acid.

The correlation between Ct value and viral load may be useful for comparisons of certain populations (e.g., when comparing symptomatic and asymptomatic people) and can be applied to aggregate data to infer the difference in the relative amount of viral load between the two populations.

Can a Ct value predict how infectious an individual with COVID-19 is?

No. Ct values should not be used to determine an individual's viral load, how infectious an individual person may be, or when an individual person can be released from isolation or quarantine.

Although there is an association between Ct value and the amount of genetic material in a patient sample, attempting to correlate Ct values and the amount of virus in the original specimen is imperfect. Ct values can be affected by many factors other than viral load, including but not limited to improper collection or storage of the specimen, how the specimen was processed, or the sensitivity level of the test performed. Thus, a high Ct value can easily result from factors not related to the amount of virus in the specimen. Ct values should not be used to infer a relationship with the viral load from a person's specimen, nor should they be used to determine the level of infection risk posed by a particular individual.

If a Ct value can be affected by factors like specimen collection, how do I know if my RT-PCR test result is accurate?

In addition to detecting SARS-CoV-2 genetic material, each RT-PCR diagnostic test also includes a test to detect human genetic material. Detecting the human genetic material in the specimen confirms the quality of the specimen and the

processing steps of the test. If the human genetic material is detected, then we can be reasonably sure the specimen was collected and processed correctly, and the test result is accurate.

Can Ct values from different RT-PCR tests be compared?

For a given RT-PCR diagnostic test, a patient specimen must be processed using a specific series of steps to produce a valid test result. However, the steps used to process the genetic material, the specific genetic target being measured, and the amount of specimen used varies among RT-PCR tests. Because the nucleic acid target (the pathogen of interest), platform and format differ, Ct values from different RT-PCR tests cannot be compared.

Can Ct values be used at a population level for public health surveillance purposes?

Yes. Although specific Ct values should not be included in a person's health record or used to influence a person's individual care, median Ct values from a population or group may be valuable for public health to evaluate viral load and transmissibility for a particular SARS-CoV-2 variant, or to compare the viral load between two groups (e.g., between vaccinated and unvaccinated individuals).

Anatomic Pathology

Are pathologists able to sign out cases remotely during the COVID-19 public health emergency?

CMS has indicated that it will allow laboratories to use temporary testing sites for remote review and reporting of laboratory data, slides, and images if specific criteria are met. Please refer to this CMS Memorandum for additional information.

What practices should be followed to prevent SARS-CoV-2 exposure when processing specimens and performing test procedures in anatomic pathology?

Manual processing of fresh unfixed specimens, including frozen sections, should be conducted in a manner that provides a barrier between the specimen and personnel during specimen manipulation. In addition, protect the mucous membranes of the eyes, nose, and mouth during procedures that are likely to generate **splashes, sprays, droplets, and aerosols.** Examples of these barriers include:

- Performing tissue dissection in a certified Class II A1 or A2 biological safety cabinet (BSC) if available
- Working behind a splash shield
- Using combinations of PPE, such as:
  - Surgical mask with attached eye shield
  - Surgical mask and goggles
  - Mask and a face shield that fully cover the front and sides of the face
  - Double gloves or mesh cut-resistant gloves
  - Surgical scrubs, shoe covers, full gown, plastic apron, and hair covering
  - N95 respirators or powered air-purifying respirators (PAPRs) (the use of respiratory protection requires appropriate training and evaluation)

What precautions should clinical and non-clinical support staff take when handling specimen containers that may be contaminated with blood and body fluids?

All laboratories should perform a site- and activity-specific risk assessment and follow Standard Precautions when handling specimen containers and paper requisitions that could have been contaminated by tissue and fluid specimens. This risk assessment may suggest use of some of these mitigation strategies:

- Use face shields and/or work behind a splash guard whenever possible.
- Store human specimens in closed containers that can be decontaminated before moving them to a secure area.

Place specimen containers in closed and clearly labeled plastic bins until pick-up and dispose according to your institutional waste management policies.

What are the biosafety recommendations for performing frozen sectioning on confirmed and suspected COVID-19 patient specimens?

Avoid frozen sectioning of specimens from people with confirmed COVID-19 whenever possible. Talk with the relevant clinical and surgical teams about the clinical necessity and benefit of frozen sectioning and consider appropriate alternatives for suspected and confirmed COVID-19 cases. When frozen sectioning is unavoidable, the following are recommended, if possible:

- Receive specimens in an area apart from administrative staff.
- Consider using a cryostat that has a downdraft and other safety features.
- Use cryostats in a closed room that has inward directional (negative) airflow vented directly to the outside or recirculated through a HEPA filter to avoid contaminating the rest of the surgical pathology suite.
- Provide grossing rooms with inward directional airflow.
- Reduce the number of operators to a minimum.
- Wear appropriate PPE, including but not limited to:
  - Fluid-resistant disposable double gloves and gown
  - Fluid-resistant disposable apron
  - Eye protection (face shield or goggles)
  - N95 respirator or fluid-resistant surgical mask
- Do not use freezing sprays; they are not recommended by the manufacturers of cryostat instrumentation.
- Wear cut-resistant, stainless steel mesh gloves during disassembly, cleaning, and disinfection of microtome knives.
- Collect accumulated instrument shavings and discard them as biohazardous waste.
- Follow local standard decontamination procedures of the cryostat and other surfaces. Ultraviolet lights are not a substitute for terminal cleaning of the instrument.

For additional information, refer to the following:

- Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19)
- Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories (CDC Morbidity and Mortality Weekly Report)

What chemical treatments inactivate SARS-CoV-2 in tissues during histopathology processing?

Human tissues submitted for permanent pathologic examination typically undergo several processing steps with

chemicals that have been shown to inactivate coronaviruses:

- Studies with SARS-CoV-1 and MERS-CoV have shown that virus inactivation for these coronaviruses occurs in a time-dependent fashion with both formalin fixation and temperatures of 56°C or above.
- Alcohol at 70% concentration or higher has been shown to inactivate the virus and tissue processing typically includes a series of alcohol dehydration steps that use 70% to 100% alcohol prior to paraffin embedding.
- In addition, the final step of applying a glass or plastic coverslip to the slide provides an additional barrier between the personnel and the tissue.

For additional information, refer to the following:

- Inactivation of the coronavirus that induces severe acute respiratory syndrome, SARS-CoV
- Inactivation and safety testing of Middle East Respiratory Syndrome Coronavirus
- CAP Practical Guide to Specimen Handling in Surgical Pathology
- Coronavirus disinfection in histopathology
- NSH-COVID-19: Novel Coronavirus Resources for Histology Labs

Does a grossing station that draws air and fumes toward the rear of the unit offer the same protection as a biosafety cabinet?

No. Grossing stations pull formalin fumes away from the person who is doing the dissecting. In general, grossing stations are not as effective as biosafety cabinets at protecting the user from exposure to biological agents.

For additional resources related to biological safety cabinets, refer to:

- Fundamentals of Working Safely in a Biological Safety Cabinet (Provides Free Training CEU)
- Biosafety in Microbiological and Biomedical Laboratories (BMBL) (6th edition) Appendix A, Section III_Biological Safety Cabinets (page 370).

Ordering Supplies (For Public Health Laboratories)

What Is CDC's International Reagent Resource (IRR)?

The International Reagent Resource (IRR) provides registered users with reagents, tools, and information for studying and detecting influenza virus and other pathogens, including SARS-CoV-2. IRR is primarily a resource used for procuring pathogen test components and assembling, qualifying, and distributing these kits to public health laboratories for use in public health activities. This resource supports detection and characterization of pathogens, which will aid in informing interventions. By centralizing these functions within IRR, access to and use of these materials in the scientific and public health community is supported and quality control of the reagents is assured.

IRR has supported the COVID-19 emergency response since February 2020, with a catalog expansion from April to December 2020, to provide more products needed for viral testing, including numerous commercially produced EUA assays. IRR is managed under a CDC contract by American Type Culture Collection (ATCC).

What supplies are being distributed by IRR for testing for SARS-CoV-2?

IRR provides CDC-manufactured kits and controls associated with its EUA applications to registered public health laboratories that perform SARS-CoV-2 testing.

Commercial reagents may be added or removed from the IRR catalog as needed to ensure equitable nationwide testing. For a complete list of IRR's currently available items, visit IRR's FAQ page.

Can I register my lab or hospital with IRR?

CDC limits IRR registration and SARS-CoV-2 diagnostic reagent distribution to US public health laboratories validated to perform SARS-CoV-2 viral testing. During the SARS-CoV-2 pandemic, CDC will defer the decision to authorize new laboratories to the corresponding state public health laboratory. Qualified laboratories must have the appropriate certifications (CLIA) to serve as a diagnostic laboratory as well as appropriate equipment, training, and demonstration of testing proficiency under their state laboratory's stewardship.

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