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The COVID-19 Lab Pool Testing: Operational Playbook is to be used for informational purposes and includes regulatory content taken from different sources as listed in the Resource section. Content and materials are to inform labs and manufacturers interested in developing a pool testing strategy. This RADx produced Operational Playbook does not replace official FDA, CMS or CDC guidance or regulations from these agencies and is not endorsed by them.
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Introduction and Background

The COVID-19 pandemic resulted in widespread closures of schools and businesses across the United States. Although these closures were intended to minimize the risk of disease transmission, early studies have shown that these school closures may be having an unintentional adverse social and economic impact on approximately 56.4 million school-aged children.

Given the impact of the closure, the return of children and adults to school and work has taken on extreme urgency and testing remains a critical focus. However, based on production and testing capacity estimates provided by test developers and the anticipated demand by schools, workplaces and other groups setting up testing programs, the need for testing remains greater than available resources can support. Pool testing can improve efficiency, extend supply chain, and reduce cost.

**Pooling**—sometimes referred to as pool testing or pooled testing—involves combining samples from several people and conducting one lab test on the combined pool of samples. Pooling allows a lab to address the need for testing, while conserving reagents and consumables.

This Operational Playbook serves as a practical aid for labs and other parties interested in developing a pooling strategy. It introduces a standard workflow together with elements that should be considered in the development of that strategy. Further, it provides a comprehensive high-level overview of the regulatory guidance from multiple federal agencies in support of pooling. While this practical playbook has been developed for lab companies considering or refining a pooling strategy, it is not a substitute for a comprehensive understanding of the actual guidance documents related to the planning and execution of the pooling strategy. It is also not a substitute for regulatory requirements that may exist at the federal, state, and local levels or for institutional or accreditation requirements which may be more stringent.
Pooling Workflow

For companies submitting a pooling protocol Emergency Use Authorization (EUA) for a SARS-CoV-2 molecular diagnostic test, FDA requires a brief description of the pooling strategy (i.e., number of samples pooled using swabs or media). There are three main phases in a pool testing workflow to consider: Pre-Analytic, Analytic, and Post-Analytic. In short, the three phases involve sample collection, lab pool testing, and results reporting. To ensure consistent high-quality testing, each phase of the pooling workflow requires compliance with standard operating procedures (SOPs) and regulatory guidelines.

The following diagram lists the main steps in each of the three phases of a lab pooling workflow and will be briefly defined in the following pages. Please note that for the purpose of this Operational Playbook, sample collection is included in the pre-analytical phase for compliance with the Clinical Laboratory Improvement Amendments (CLIA) or CLIA Regulations¹, even though FDA views sample collection as occurring before the pre-analytical phase.

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1. https://www.ecfr.gov/cgi-bin/text-idx?SID=6f488b9617820333cbd55cd4c68b80f2&mc=true&node=pt42.5.493&rgn=div5
Collection of a sample as part of a serial testing program may be performed by a healthcare provider (HCP) or be self-collected (based on age, ability, and regulatory guidance) [Home collection template2]. It should also be noted that a human specimen control for home-collected specimens is required unless low invalid rates (from negative human specimen control) can be demonstrated. Adherence to Instructions for Use of the specific test and regulatory guidance is critical to properly conduct sample collection.

Collection Site and Kit

The collection site location and general workflow, where sample collection and the option for on-site sample pooling occurs, needs to be well planned, safe, and accessible. Site management, resources, mapping participant flow, and efficient scheduling all contribute to minimizing backlogs and exposure to risks. Additionally, collection kits designed for use outside of a lab must contain all supplies needed for sample collection along with guidance on use and disposal of kit and sample stability measures. Each testing solution and environment is unique. Therefore, discussion and input from key stakeholders, including community leaders and partners, local health officials, and regulatory/legal experts is highly recommended before initiating a pooling testing program. Here is a list of helpful resources for setting up pool testing programs at schools or other types of organizations:

- Rockefeller Foundation has developed a comprehensive Logistics Plan Playbook3 specifically geared towards point-of-care SARS-CoV-2 testing sites. It covers high-level logistics planning, demand forecasting, detailed processes and activities, and metrics tracking.
- Texas Division of Emergency Management’s K-12 Testing Guide5 is applicable to many testing scenarios. The Best Practices for Schools6 guide covers considerations for physical testing space and testing workflow.
- When to Test7 site and COVID-19 Testing Implementation Guide8.

Sample Types

When testing for COVID-19, the type of specimen collected is based on the test being performed and the manufacturer’s instructions. Some of the specimen types may not be appropriate for all tests. For initial individual diagnostic testing for current COVID-19 infections, CDC recommends collecting and testing an upper respiratory specimen, either a swab sample taken from the nose or throat or a saliva sample. FDA and CDC both offer guidance for healthcare providers or health department staff9 who are collecting specimens from others in a healthcare setting10 or at the point-of-care and for self-collection of specimens11.

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3 https://www.rockefellerfoundation.org/report/covid-19-testing-in-k-12-settings-a-playbook-for-educators-and-leaders/
4 https://covidtesting.com/
5 https://tdem.texas.gov/k-12testing/#1603896349124-052a3611-de4c
6 https://tdem.texas.gov/k-12testing/#1603302672904-21f1f1930-822c
7 https://whentotest.org/
8 https://whentotest.org/implementation-guide/
For pool testing, FDA Amendment Letter, April 20, 2021\(^2\) limits the sample type to an anterior nasal swab specimen and does not include other specimen types, such as saliva. Although saliva as a sample for pooling has not gained widespread acceptance, FDA has recently granted EUAs using pooled saliva\(^3\). Labs considering other types of respiratory specimens (e.g., saliva, sputum, throat/tongue swabs, or nasal aspirates) or non-respiratory specimens (e.g., stool, etc.), should contact FDA at CDRH-EUA-Templates\(^4\) to discuss validation strategies.

### Pooling Methods

Currently, FDA recommends the following two approaches to patient specimen pooling and states that as more data becomes available and new approaches are identified, FDA recommendations may evolve.

1. **Swab pooling:** Adding swabs from multiple patients into a single volume of transport media on-site at the time of collection. Dry sample swabs may also be combined in a single tube and transported dry to the lab for further processing.

2. **Sample/media pooling:** Pooling aliquots of transport media, each containing a single patient sample can take place in the lab during the pre-analytic or analytic phase.

Based on the design of the pooling workflow, sample pooling may take place in the pre-analytical phase on-site at the place of sample collection or sample pooling may be done in the lab during the pre-analytical or analytical phase as described in the following scenarios:

1. Samples are collected and pooled on-site and packaged, and then sent to a lab for testing
   - On-site sample collection and sample pooling may be organized by groups—referred to as pod pooling (e.g., classroom, cohort, group activity) or by random collection at designated areas

2. Individual samples are collected and packaged on-site and then sent to a lab for sample pooling and testing

3. At-home individual samples are collected and sent to lab for sample pooling and testing
   - At-home kits, also called self-collection kits, can be used at home or at any location such as pharmacies, physician offices, retail clinics, schools, workplaces, long term care facilities, drive-through community centers, in mobile vehicles, or in publicly accessible sites managed by local organizations
   - Self-collection kits are available in a pharmacy or retail store either by prescription or over-the-counter without a prescription. Most collections kits will follow a procedure in which samples are collected, a specimen tube is placed in a biohazard bag, into a sample box, then finally into a package according to the manufacturer’s instructions and sent with a pre-paid shipping label to the lab

\(^4\) CDRH-EUA-Templates@fda.hhs.gov
Pool Size

Pool size is the number of samples that are combined in a pool for PCR-based lab tests. Even though pool testing is a viable strategy for SARS-CoV-2, the following factors impact and limit the pool size.

Positivity Rate

Computing the optimal group-size for pooled tests is related to the positivity rate of COVID-19 in the local area, as measured by the lab over a 7-10 day period. When the positivity rate is high, fewer samples or no samples should be pooled. The efficiency of testing at various pool sizes varies according to the community rate of infection. For example, if the positivity rate is high, then the need for deconvolution and repeat testing would be high, thereby decreasing the efficiency of testing large pools. The lab would be more efficient by testing smaller pools in this scenario. Here are references for statistical and probabilistic methods to calculate the best pool size for any prevalence:

- Poisson distribution where each pool is equivalent to a time interval\(^{15}\)
- A robust pooled testing approach to expand COVID-19 screening capacity\(^ {16}\)
- Considerations for Group Testing: A Practical Approach for the Clinical Laboratory\(^ {17}\)
- Optimal pool size based on prevalence for Dorfman Protocol Table\(^ {18}\)
- Boosting test-efficiency by pooled testing for SARS-CoV-2—Formula for optimal pool size\(^ {19}\)

Software/Data Management (reporting and data systems)

Setting up a pool testing program involves manual and/or automated processes that may require IT, data systems, Laboratory Information Management System (LIMS), and other means to provide registration, ID tracking, sample barcoding and scanning, and reporting sample results. As pool testing service providers, labs will manage procurement, oversee contracting and onboarding, and maintain systems used to collect data on testing, inventory, procedures, and a results reporting strategy. Integrating new data systems with a client’s existing technical infrastructure along with cyber security protocols and compliance with applicable law are other considerations that labs need to address.

- How to Report COVID-19 Laboratory Data\(^ {20}\)
- COVID-19 At-Anywhere Diagnostics Design-A-Thon\(^ {21}\)

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16 https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0246285
17 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7731934/
18 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7731934/table/t1-cbr-41-79/
19 https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0240652
Transport and Sample Stability

Sample handling procedures including sample stability, storage, and transport requirements will vary based on the test used. Labs will need to devise a transport plan that outlines proper sample handling, packaging, and shipping procedures, agreed upon turn-around time from sample collection to test processing, and pick-up schedule and method of transport to the lab (i.e., courier, UPS, FedEx overnight/priority). Authorized collection kits should contain supplies, packaging, and instructions located in the test’s IFU and pre-paid shipping labels. The following are transport and sample stability references:

- Interim Guidelines for Collecting and Handling Clinical Specimens for COVID-19 Testing
- Guidance on regulations for the transport of infectious substances

Clinical Performance

A decrease in performance is likely with pooling strategies due to dilution of the primary clinical sample and other factors. Because pooling may impact clinical sensitivity, every effort should be made to minimize this. The additional validation studies required for pooling should be designed to check for minimal decrease in clinical sensitivity: this is further discussed in the following Test Specifications section.

Screening using a highly sensitive test, especially given the asymptomatic testing population, leads to the most accurate results when rapid turnaround times are available. FDA has provided pooling strategies for tests that have already been authorized and demonstrate a positive percent agreement rate of at least 95%. Also, FDA encourages developers who want to offer a less sensitive test for screening to discuss validation approaches with FDA (refer to FDA Amendment Letter, April 20, 2021).

For compliance with CLIA, if FDA has granted an EUA authorization (or clearance) for a specific test, when the manufacturer provides verification procedures and the EUA is unmodified, labs are required to verify performance specifications according to those established by the manufacturer in accordance with 42 CFR §493.1252(a) and per 42 CFR 493.1253(b)(1) of the CLIA Regulations. For non-waived, modified FDA-cleared or approved test systems, the requirements at §493.1253 state that labs must establish certain parameters such as, but not limited to, accuracy and precision according to §493.1253(b)(2)(i)(A), before reporting patient test results. Therefore, each instrument (e.g., thermocycler) must be verified or established as applicable for all tests run on each specific instrument as you would for individual test systems prior to performing patient testing. Helpful links: CMS QSO-18-19-CLIA and Verification of Performance Specifications.

References:

23 https://www.who.int/publications/i/item/9789240019720
25 https://www.ecfr.gov/cgi-bin/text-idx?SID=6f488bb9617820333cb4d2c66b80f2&mc=true&node=pt42.493.1252(a)&rgn=div5
26 https://www.ecfr.gov/cgi-bin/text-idx?SID=1248e43899e5e5f936e55315402bc38b&mc=true&node=pt42.5.493&rgn=div5
Test Specifications

High throughput testing capacity enables labs to address SARS-CoV-2 testing needs during periods when the testing demand is very high. The number of individual tests that can be run with normal operation in a 24-hour period is dependent upon the assay platform. However, that number can be increased 5, 10 and sometimes 20-fold if pooling protocols are implemented. FDA has presented two ways in which sponsors can validate pooling claims:

1. Test a panel of samples that contain virus across the measuring range of an assay, with at least 25% low positives
2. Generate a panel of samples that contain a single sample in the pool that has been diluted to 2-3xLoD.

In the first case, results of single sample testing would be compared to results from the corresponding pool. This method is described in the molecular template and in the April 20th Amendment Letter. The pooling protocol would meet acceptance criteria using this method if:

- ≥ 85% PPA between pooled testing and individual testing
- Invalid rate does not exceed 5%

The second case is used for a previously authorized test with multiple SARS-CoV-2 targets testing anterior nasal swabs in a serial testing program. This method tests 20-30 pooled samples where each positive sample is diluted to a low concentration (2-3xLoD). This method is designed to demonstrate the effect of dilution of a single sample near the LoD on the final result of the pooled test. Acceptance criteria for this study design are:

- ≥ 95% of pooled replicates are detected as positive
- Ct score between pooled and single does not exceed 1.7Ct
- Invalid rate does not exceed 5%

Turn Around Time (TAT)

Labs are expected to have policies that define TAT and the ongoing assessment and monitoring of their own compliance in their quality assessment plan. These processes must be approved by the lab director, and are to align with the labs written policy and Instructions for Use (IFU).

CLIA

Labs that conduct diagnostic or screening testing for COVID-19 must also comply with the CLIA Regulations. For more information on how to obtain a CLIA certificate and meet requirements to perform testing see the CDC’s Interim Guidance and the CMS CLIA Quick Start Guide for CMS CLIA certification.

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29 https://www.ecfr.gov/cgi-bin/text-idx?SID=6f488b9617820333cbd55cd4c68b80f2&mc=true&node=pt42.5.493&rgn=div5
Impact of Variants

Molecular tests are designed to detect the virus by targeting a specific region(s) of the viral genome. False negative results may occur if mutations are in the part of the viral genome assessed by that test and reduce a test’s ability to detect the viral RNA genome. Molecular tests designed to detect multiple SARS-CoV-2 genetic targets are less susceptible to the effects of genetic variation than tests designed to detect a single genetic target. The impact of genetic variants on molecular test performance is influenced by the sequence of the variant, the design of the test, and the prevalence of the variant in the population. If the test fails to detect a virus variant and the variant increases in prevalence in a community, there may be an increase in the percentage of false negative results.

FDA recommends that developers: 1) design their test to minimize the impact of viral mutations on test performance; 2) routinely monitor for viral mutations that may impact test performance; and 3) clearly convey any test limitations in the test’s labeling. The following are CDC references on variants:

- About Variants of the Virus that Causes COVID-19
- Variants and Genomic Surveillance for SARS-CoV-2
- US COVID-19 Cases Caused by Variants

The National Institutes of Health (NIH) Rapid Acceleration of Diagnostics (RADx™) Variant Task Force oversees a program to assess the impact of variants of concern/interest circulating in the United States on both NIH RADx-supported pre- and post-authorized nucleic acid amplification tests (NAAT) and antigen tests. This program is an integrated approach which combines: 1) the *in silico* analysis for both NAAT and antigen tests with publicly available SARS-CoV-2 genomes to determine the extent to which mutations may impact test performance; and 2) performing *in vitro* testing of clinical samples containing the specific mutations that may impact a test’s performance.
Action on Pool Test Results

Screening pertains to routine testing of individuals without symptoms or any reason to suspect exposure. The objective is to reduce transmission by identifying potentially infected individuals faster to protect public health. Test results from pooling are intended to keep schools and other organizations open and are not aimed at individual diagnostics. Authorized labs must consider pool testing reporting protocols and results reporting guidelines for clients, health departments, and government institutions. According to FDA, labs must have a pooling protocol that includes instructions for follow-up for positive and invalid pools, including follow-up instructions to be provided to the organizer of the testing program.

When performed by a lab, for sample/media pooling, presumptive positive or invalid pools must include deconvolution to retest individual samples. Labs will need a process for tracking and maintaining individual samples.

In general, positive and invalid pools must have a process for tracking and reporting presumptive positive pools. Instructions must include a process for retesting individually as well as isolation instructions for everyone in the pooled test. Although, most individual tests will be negative, each person should be instructed to isolate until individual testing results are reported. The “presumed positive” report must include instructions to collect a new specimen to be tested individually and must indicate that such individuals should isolate until a negative test result when re-tested individually and should not be grouped with other individuals who have received a positive or presumptive positive result. If a pool test result is negative, then all specimens are “presumed negative” and normal activity can continue.
Typically, where the pooling is completed will determine whether the lab can deconvolute a positive sample or not. If the lab pools the samples, they can deconvolute a positive. If the samples are pooled on-site then resampling individuals in a positive pool is necessary. The following are actual pool testing workflow scenarios from sample collection to results reporting:

1. Pooled samples sent to lab using on-site sample collection or sample pooling: see Fig. 1. *In this scenario, a presumptive positive pool, will be followed up with re-sampling and reflex testing of individuals in that pool.*

   ![](Fig_1.png)

   **Fig. 1:** Pooling samples on-site, send pooled samples to lab for testing

2. On-site individual sample collection or at-home individual sample collection, samples sent to lab for sample pooling and testing, see Fig. 2. *In this scenario, a presumptive positive pool, will be followed up with in-lab reflex testing of individuals in that pool.*

   ![](Fig_2.png)

   **Fig. 2:** Collect individual samples on-site, send samples to lab for testing
Pool Test Results Reporting

COVID-19 cases are required to be reported by healthcare providers and labs to state, tribal, local, and territorial (STLT) health departments. All case investigation and contact tracing support activities in a given school should be in coordination and agreement with the local Public Health Department.

Authorized labs must keep records of specimen pooling test result data, daily testing totals including number of pooled test results, the number of individuals tested and daily running average of percent positive results. For the first 12 months from the date of their creation, such records must be made available to FDA upon request within 48 business hours. CLIA labs are generally subject to initial and subsequent routine biennial re-certification inspections (every two years), therefore all records for the lab must be available from the time of the previous inspection to the current one. States, territories, and accreditation organizations may have more stringent record-retention requirements. As such, the lab must adhere to the more stringent requirement.

CLIA regulatory requirements vary according to the category of test(s) each lab conducts. If at any time a facility intends to report a patient-specific test result, or the results are acted upon, it must first obtain a CLIA certificate and meet all requirements to perform testing. See the following CDC testing results chart and CDC’s Interim Guidance and CLIA Regulations. In addition, the HHS Secretary’s U.S. Health and Human Services Secretary’s June 4, 2020 Guidance and CDC interim guidance states that SARS-CoV-2 test results need to be reported to the state or local health department in which the patient resides. In order to be in compliance with the CLIA reporting requirements outlined in the CLIA Interim Final Rule, a lab will need to have documentation that it reported SARS-CoV-2 results, or at least attempted to report the results (see QSO-21-10-CLIA).

Repeat or Reflex Testing

If a pool test is presumptive positive, then the pool is deconvoluted, meaning that each individual is retested. Retesting may occur on the same device, referred to as repeat testing, or on a different device, often referred to as reflex testing. Samples are either collected again from every individual (swab pooling) or are re-aliquoted from the original specimen (sample/media pooling) and analyzed through an individual, diagnostic workflow. If retesting is not possible, then the entire pool should isolate, quarantine, contact trace, and follow safety protocols to mitigate risk.

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36 https://www.ecfr.gov/cgi-bin/text-idx?SID=6f488b9617820333cbd55cd4c68b80f2&mc=true&node=pt42.5.493&rgn=div5
Involvement of Federal Agencies in Public Health Emergencies

The Food and Drug Administration (FDA), Center for Medicaid Services (CMS), and the Centers for Disease Control and Prevention (CDC) each play a critical role in responding to public health emergencies. CMS provides guidance to labs during public health emergencies on meeting CLIA requirements to ensure labs continue to produce accurate, reliable, and timely results. FDA, CMS, and CDC are responsible for CLIA, and each agency has a unique role in assuring quality lab testing. For more information about CLIA and the interaction of FDA, CMS and CDC see the following FDA resources: About CLIA and Tri-Agency Task Force for Emergency Diagnostics.

Essential Definitions

It is critical that companies have a clear understanding of the definitions of Diagnostic, Screening, and Surveillance Testing for SARS-CoV-2. The definitions below are taken from the following CDC website pages: Overview of Testing for SARS-CoV-2, and Testing Strategies for SARS-CoV-2. The U.S. Food and Drug Administration’s (FDA) FAQs on Testing for SARS-CoV-2 also addresses diagnostic testing, screening testing and surveillance testing for SARS-CoV-2. See also CMS CLIA’s:

- Frequently Asked Questions About SARS-CoV-2 Surveillance Testing
- Frequently Asked Questions FAQs CLIA Guidance During the COVID-19 Emergency.

Diagnostic Testing

Diagnostic testing for SARS-CoV-2 is intended to identify occurrence at the individual level and is performed when there is a reason to suspect that an individual may be infected, such as having symptoms or suspected recent exposure, or to determine resolution of infection.

Screening Testing

Screening tests for SARS-CoV-2 are intended to identify occurrence at the individual level even if there is no reason to suspect infection e.g., there is no known exposure. Screening tests are intended to identify infected individuals without, or prior to development of, symptoms who may be contagious so that measures can be taken to prevent further transmission.

Surveillance Testing

Surveillance for SARS-CoV-2 includes ongoing systematic activities, including collection, analysis, and interpretation of health-related data that are essential to planning, implementing, and evaluating public health practice. Surveillance testing is generally used to monitor for a community- or population-level occurrence, such as an infectious disease outbreak, or to characterize the occurrence once detected, such as looking at the incidence and prevalence of the occurrence. Surveillance testing is used to gain information at a population level, rather than an individual level.

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Emergency Use Authorization

Diagnostic tests for emerging diseases such as COVID-19, can be quickly made available to meet response needs during a crisis through the EUA process. FDA has provided recommendations and information regarding EUA requests for SARS-CoV-2 diagnostic tests in the Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency (Revised) (“Policy for COVID-19 Tests”) and the EUA templates (discussed below) referenced in that policy. Detailed information for all relevant SARS-CoV-2 EUAs, including authorizations and fact sheets is provided in the In Vitro Diagnostics EUAs.

Diagnostic or Screening Testing Using a Pooling Strategy

Labs certified under CLIA can use a specimen pooling strategy to expand SARS-CoV-2 nucleic acid diagnostic or screening testing capacity when using a test authorized for such use by FDA.

If a pooled test result is negative, then all specimens can be presumed negative with the single test. If the test result is positive or indeterminate, then all the specimens used to create a sample/media pool, if available, need to be retested individually. The advantages of this two-stage specimen pooling strategy include preserving testing reagents and resources, reducing the amount of time required to test large numbers of specimens, and lowering the overall cost of testing. In the case of swab pooling, a presumptive positive pool will be followed up with re-sampling and reflex testing of individuals in that pool.

A pooling strategy depends on the community prevalence of virus. Thus, the pool size will need to be adjusted accordingly. The CDC recommends that labs should determine prevalence based on a rolling average of the positivity rate of their own SARS-CoV-2 testing over the previous 7–10 days. Labs should use a standardized methodology or calculator that factors in the sensitivity of the assay they are using and their costs of testing to determine when the positivity rate is low enough to justify the implementation of a pooling strategy. Labs should also understand and, where appropriate, communicate the limitations associated with pooled testing, which are described in greater detail below.

46 https://www.fda.gov/media/135659/download
Regulatory Requirements for Pooling of Diagnostic or Screening Testing

FDA has also outlined various policies in their guidance regarding the use of SARS-CoV-2 tests prior to authorization. A lab that wishes to use pooling with a SARS-CoV-2 nucleic acid test assay would be expected to evaluate and validate the performance of an assay for a pooling strategy and submit an EUA request to FDA.

However, if the lab modifies that authorized assay by incorporating alternative components, including extraction methods, polymerase chain reaction (PCR) instruments, and software versions, the lab should evaluate and validate the performance of the component changes.

Labs that conduct diagnostic or screening testing for SARS-CoV-2 must also comply with CLIA. *If at any time a facility intends to report patient-specific test results, or take action on those results, it is not considered surveillance and it must first obtain a CLIA certificate and meet all requirements to perform testing.*

In addition, all SARS-CoV-2 tests without at EUA or without full approval, to include those that have been modified and not yet authorized or cleared for use by FDA, are deemed high-complexity. As such, these labs must meet the CLIA requirements for high-complexity testing. For more information, see the CLIA Regulations.

Reporting Pooled Diagnostic or Screening Testing Results to Health Departments

It is a legal requirement of the Coronavirus Aid, Relief, and Economic Security (CARES) Act, that "every lab that performs or analyzes a test that is intended to detect COVID-19 or to diagnose a possible case of COVID-19" to report the results to state, local, tribal, or territory public health departments. In addition, the HHS Secretary's *June 4, 2020 Guidance* and CDC interim guidance states that SARS-CoV-2 test results need to be reported to the state or local health department in which the patient resides. HHS has published guidance on *COVID-19 Pandemic Response, Laboratory Data Reporting: CARES Act Section 18115* that specifies what data must be reported by labs along with SARS-CoV-2 diagnostic or screening test results.

In order to be in compliance with the CLIA reporting requirements outlined in the CLIA Interim Final Rule, a lab will need to have documentation that it reported SARS-CoV-2 results, or at least attempted to report the results. See QSO-20-37-CLIA,NH and QSO-21-10-CLIA for more information.

A CLIA-certified lab that allows for pooling must report diagnostic or screening negative test results to the participants in the pool according to the instructions for use of FDA-authorized SARS-CoV-2 in vitro diagnostic device that the lab used. The test report given to the individuals in the pool must indicate that the testing procedure involved specimen pooling and explain the limitations of that type of testing. The CLIA-certified lab must also report those diagnostic or screening negative test results to the local, state, tribal, or territory health department.

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48 https://www.ecfr.gov/cgi-bin/text-idx?SID=6f488b9617820333cbd55cd4c68b80f2&mc=true&node=pt42.5.493&rgn=div5
The CLIA-certified lab should not report positive or indeterminate results of a pooled test to either the participants in the pool, or the local, state, tribal, or territory health department. All participant specimens that were in a pooled test with a positive or indeterminate result should be retested separately, and the subsequent individual diagnostic or screening results must be reported to the local, state, tribal, or territory health department.

**Limitations of Pooled Diagnostic or Screening Testing**

Based on limited data, using a pooling testing procedure for SARS-CoV-2 has some limitations. In some pooling procedures, the lab cannot ensure the diagnostic integrity of an individual specimen because it is combined with other specimens before testing. Specimen integrity can be affected by the quality of swab specimen collection, which could result in some swabs having limited amounts of viral genetic material for detection. Inadequate individual specimens, including those with limited amounts of viral genetic material, might not be eliminated from the pooled specimen before testing. Even if each individual specimen in a pool is adequate, the specimens in a pooled procedure may be diluted or contain a higher concentration of interferents, which could result in a low concentration of viral genetic material below the limit of detection of a given test or inhibition of detection. These limitations mean that monitoring the positivity rate and properly validating the assay and the instrumentation are important to limit the potential for false-negative results. In general, the larger the pool of specimens, the higher the likelihood of generating false-negative results.
Templates

FDA has developed and published templates for EUA submissions to help facilitate the preparation, submission, and authorization of an EUA. These include Diagnostic Templates (Molecular and Antigen) and Serology/Antibody Templates. The following templates are relevant to pooling in the context of lab tests and are briefly described below:

- **Molecular Diagnostic Template**
  - FDA recommends that all developers of molecular SARS-CoV-2 tests include the Molecular EUA Template Cover Sheet when submitting their EUA.
- **Molecular Diagnostic Home Specimen Collection Template**
- **Supplemental Template for Developers of Molecular and Antigen Diagnostic COVID-19 Tests for Screening with Serial Testing**

The templates reflect FDA's guidance on the data and information that developers should submit to facilitate the EUA process. The templates provide information and recommendations and are updated as appropriate. Please always check that you are operating from the most recent document.

Developers who intend to use alternative approaches should seek FDA's feedback or recommendations to help them through the EUA process. Test developers interested in pursuing an EUA may submit a pre-EUA to begin discussions with FDA or may submit an EUA request to CDRH-EUA-Templates@fda.hhs.gov. For additional information see the FAQs on Testing for SARS-CoV-2.

**Molecular Diagnostic Template**

The Molecular Diagnostic Template includes FDA's current recommendations for labs concerning what data and information they should submit to support an EUA request for a molecular diagnostic for SARS-CoV-2 developed for use in a single CLIA certified high-complexity lab. It also contains critical information for labs planning to adopt a pooling strategy.

**Specimen Pooling**

To establish performance of a test with pooling, FDA recommends conducting a clinical validation study in the intended use population that includes testing each sample individually and using the proposed pooling strategy.

Currently, FDA recommends two approaches to patient specimen pooling: 1) pooling aliquots of transport media which each contain a single patient sample (media pooling) or 2) adding swabs from multiple patients into a single volume of transport media (swab pooling). Please see template for detailed information.

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54 https://www.fda.gov/media/152768/download
56 https://www.fda.gov/media/146695/download
57 CDRH-EUA-Templates@fda.hhs.gov
Molecular Diagnostic Home Specimen Collection Template

This template provides FDA’s current recommendations concerning what data and information should be submitted to FDA in support of a pre-EUA/EUA submission for prescription use only home collection devices used by an individual to collect certain clinical specimen(s) that are then sent to a clinical lab for testing with a molecular diagnostic for SARS-CoV-2 that is authorized for use with the home collection kit. The template is intended to help manufacturers provide appropriate validation data and other information to FDA, but alternative approaches can be used. It does not speak to pooling of samples but is an important reference document for home specimen collection.

Authorization of a home collection kit must be accompanied by authorization of one or more molecular assays that has been validated with specimens collected and transported with the subject home collection kit. There are three options for how to authorize a molecular assay with a home collection kit:

- Within the same EUA at one time (i.e., when the same developer makes the home collection kit and molecular assay and seeks authorization at the same time for the assay and kit).
- In one or more EUAs (i.e., when the developer of the home collection kit is different from the developer(s) of the molecular assay(s)); or
- As an amendment to the EUA of a previously authorized assay to add home collection with the specific home collection kit.

The template contains guidance and/or required text for Intended Use statements, Special Conditions for Use Statement, Device Description (Home Collection Kit Ordering and Processing), Specimen Collection Control and Partnering Labs. Guidance on Product Manufacturing and validation of the device are provided. Recommendations for real time stability and Human Usability studies are also included.

Supplemental Template for Developers of Molecular and Antigen Diagnostic COVID-19 Tests for Screening with Serial Testing

This template is intended to provide supplemental recommendations for developers of molecular and antigen tests seeking claims for screening with serial testing without studying asymptomatic individuals prior to authorization, including for point-of-care (POC) and at-home tests.

Amendment Letter, April 20, 2021

On April 20, 2021, in response to public health needs to expand the nation’s testing capacity, FDA issued a letter authorizing additional indications for EUAs with respect to pooling. Use is limited to lab certified under CLIA and meet requirements to perform high complexity tests. Tests amended by this letter are authorized for use with pooled anterior nasal specimens for screening (i.e., testing individuals without symptoms or other epidemiological reasons to suspect COVID-19) when used as part of a serial testing program. This means that tests with EUAs that are amended by this authorization may be used with pooled anterior nasal respiratory specimens from individuals without known or suspected COVID-19 when such individuals are tested as part of a testing program that includes testing at regular intervals, at least once per week, such as those implemented by schools, workplaces, and community groups.

The Amendment Letter outlines critical guidance for lab planning to obtain a pooling claim as part of a previously authorized test. Requirements for validation of a pooling claim is summarized in the following table Moderate/High Complexity Lab Pooling Requirements for SARS-CoV-2 Molecular Tests. This table contains key points outlined in the April 20, 2021, EUA Amendment Guidance.
# Moderate/High Complexity Lab Pooling Requirements

(Diagnostic and Screening)

EUA Pooling Amendment: [https://www.fda.gov/media/147737/download](https://www.fda.gov/media/147737/download)

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<table>
<thead>
<tr>
<th>Criteria to add pooling claim</th>
<th>Validation Requirements: Appendices</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. RT PCR, AN swabs only, ≥ 95% PPA</td>
<td>Appendix A: swab n=3*</td>
</tr>
<tr>
<td>2. Detects 2 or more targets</td>
<td>Appendix B: swab n=5</td>
</tr>
<tr>
<td>3. Chemical lysis step, extraction</td>
<td>Appendix C: swab n=10</td>
</tr>
<tr>
<td>4. Detects only SARS-CoV-2</td>
<td>Appendix D: media n=3</td>
</tr>
<tr>
<td>5. Does not amend tests not authorized for pooling</td>
<td>Appendix E: media n=5, option 1</td>
</tr>
<tr>
<td>6. Does not amend tests previously not authorized for screening.</td>
<td>Appendix F: media n=10, option 1</td>
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</tbody>
</table>

### Swab Pooling: LoD testing:

- Generate standard curve with positive samples or inactivated virus. **Use final concentration at 3x LoD of authorized assay in negative AN matrix.**
- Test at least 20 + samples without pooling
- Test at least 20 + pools with samples at 3x LoD (one pos. swab and the rest neg. swabs)
  - ≥ 95% of pooled replicates are detected as positive
  - Ct score between pooled and single does not exceed 1.7Ct
  - Invalid rate does not exceed 5%

### Swab Pooling: High Viral Concentrations (e.g., 5 swabs)

- Spike 10^6 cp/ml of virus onto swab
- Add 3 pos swabs and 2 neg swabs to 10 pools
  - All 10 replicates are positive
  - Invalid rate does not exceed 5%

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<table>
<thead>
<tr>
<th>Media Pooling Option 1: LoD testing</th>
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<tbody>
<tr>
<td>- Generate standard curve with positive samples or inactivated virus. <strong>Prepare 5x or 10x LoD samples in negative AN matrix.</strong></td>
</tr>
<tr>
<td>- Test at least 20 + samples without pooling</td>
</tr>
<tr>
<td>- Test at least 20 + pools with a single pos sample and the rest neg.</td>
</tr>
</tbody>
</table>
  - ≥ 95% of pooled replicates are detected as positive
  - Ct score between pooled and single does not exceed 1.7Ct
  - Invalid rate does not exceed 5%

### Media Pooling Option 2 (effect on % agreement): LoD testing

- Test at least 20 + pools with a single pos sample and the rest neg.
  - 20% of samples should be low positive – within 2.32Ct of mean LoD of authorized test
  - May dilute high pos samples
  - ≥ 85% between pooled testing and individual testing
  - Invalid rate does not exceed 5%

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<table>
<thead>
<tr>
<th>Condition of Authorization: Appendix A</th>
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<tbody>
<tr>
<td>- Update IFU</td>
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<tr>
<td>- Update Fact Sheets for Health Care Providers and Patients</td>
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<tr>
<td>- Amended EUA will be posted on FDAs webpage</td>
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<tr>
<td>- Pooling may start when FDA confirms validation</td>
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</tbody>
</table>

**Note:** If test had not been authorized for screening, then must submit data within 6 months to support this claim.

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<table>
<thead>
<tr>
<th>Condition of Authorization: Appendix C</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Notify FDA by sending a message to Addition to Exhibit 1 of the Pooling and Serial Testing Amendment” to <a href="mailto:CDRH-EUA-Templates@fda.hhs.gov">CDRH-EUA-Templates@fda.hhs.gov</a></td>
</tr>
<tr>
<td>- Validation data</td>
</tr>
<tr>
<td>- Revised redline and final labeling</td>
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<tr>
<td>- Testing capacity information</td>
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<td>- Statement certifying that 6 criteria are met</td>
</tr>
</tbody>
</table>

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*Note: No additional validation required for pooling 3 sample swabs or media!*
Resources

Pool Testing

- Interim Guidance for Use of Pooling Procedures in SARS-CoV-2 Diagnostic, Screening, and Surveillance Testing
- Facilitating Diagnostic Test Availability for Asymptomatic Testing and Sample Pooling
- FDA Approach to Add Pooled Serial Screening Claims to Certain Authorized Tests for Use in Serial Testing
- Pooled Sample Testing and Screening Testing for COVID-19
- Diagnostics EUAs
- Overview of Testing for SARS-CoV-2 (COVID-19)
- Getting Started: In-Lab Pooled Testing
- COVID-19 Testing Settings: FAQs on Testing for SARS-CoV-2
- Amendment Letter, April 20, 2021

Pooling Methods

- Considerations for Group Testing: A Practical Approach for the Clinical lab
- Comparing two sample pooling strategies for SARS-CoV-2 RNA detection for efficient screening of COVID-19

Testing Implementation

- WhenToTest.org
- When to Test K-12 Playbook
- COVID-19 Testing Implementation Guide (CTIG)