Pooled Testing Frequently Asked Questions

What is pooled testing?

Pooling samples involves mixing several samples together in a "batch" or pooled sample, then testing the pooled sample. This approach increases the number of individuals that can be tested while reducing resources required for testing. For example, four samples may be tested together, using only the resources needed for a single test. However, according to the FDA, because samples are diluted, there is a greater likelihood of false negative results, particularly if the test and the pooling method are not properly validated. Pooling samples works well when there is a **low prevalence (less than 5-6%)** of cases, because more negative results are expected than positive results.

When is pooled testing used?

Pooled testing can be used for two different purposes.

1. Surveillance testing is used to monitor community or population-level occurrence and is not patient-specific (nondiagnostic). Results of surveillance tests are not reported to individual patients by the laboratory or anyone else and the results are not available for clinical or public health purposes.
2. Pooled testing can also be used for individual patient-specific diagnosis or screening. For this type of pooled testing, a positive result from pooled testing must be followed by individual testing of each member of the pool to identify which people are infected and allow for appropriate clinical and public health measures to occur. Results of diagnostic or screening tests are reported to individual patients.

The requirements for laboratories conducting pooled testing are different depending on whether the laboratory is doing surveillance (nondiagnostic) or screening/diagnostic testing.

Does a Massachusetts laboratory performing SARS-CoV-2 (nondiagnostic) surveillance testing on pooled specimens need a DPH clinical laboratory license?

No. In accordance with CMS guidance, a laboratory performing SARS-CoV-2 (nondiagnostic) surveillance testing using pooled specimens is not required to hold CLIA certification. Accordingly, Massachusetts does not require laboratories performing (nondiagnostic) surveillance testing to hold a state clinical laboratory license.

If pooled testing is being used for diagnostic or screening purposes and patient specific test results will be reported by the laboratory appropriate CLIA certification and state laboratory licensure are required. In addition, laboratories performing **SARS-CoV-2 diagnostic or** screening testing (as opposed to surveillance testing) are required to use a test approved by CDC and FDA and need to have the appropriate CLIA certification.

Does a laboratory performing SARS-CoV-2 (nondiagnostic) surveillance testing on pooled specimens need to use a test that has received FDA EUA authorization?

No. In accordance with CMS guidance, a laboratory performing SARS-CoV-2 (nondiagnostic) surveillance testing on pooled specimens collected in Massachusetts will not be required to use an FDA EUA laboratory test. However, the Centers for Disease Control and Prevention and the Massachusetts Department of Public Health recommend that laboratories conducting surveillance testing with pooling should use an assay and test system that has received an EUA from FDA.

Laboratories performing **SARS-CoV-2** diagnostic or screening testing (as opposed to surveillance testing) are required to use a test approved by CDC and FDA and need to have the appropriate CLIA certification.

Can unlicensed staff collect specimens?

Yes. Department of Public Health guidance requires all personnel performing specimen collection, including nasopharyngeal (NP) or anterior nares (AN) specimen collection to be trained and demonstrate competency to be able to safely collect specimens. While specific licensure is not required, all unlicensed healthcare personnel should be appropriately supervised.

Do (nondiagnostic) surveillance pooled specimens require an order from a licensed healthcare provider?

No, only patient specific diagnostic tests require an order under M.G.L. c. 111D, section 8 (7).

Do the results of (nondiagnostic) surveillance pooled specimens need to be reported to the Department of Public Health?

No, only results of patient specific diagnostic or screening tests must be reported to DPH under 105 CMR 180.044 and 105 CMR 300.170.

CLIA certified laboratories conducting diagnostic or screening tests are required to report negative results for each individual in a pool that tests negative and to perform and report diagnostic testing of individual specimens from each pool that tests positive.

Considerations when using a SARS-CoV-2 (nondiagnostic) Surveillance Testing Method on Pooled Samples

- The pooling strategy is only useful when disease prevalence is low **(less than 5-6%)** of cases, otherwise pools are too frequently positive and retesting of individual specimens must occur frequently.
- The Centers for Disease Control and Prevention and the Massachusetts Department of Public Health recommend that all laboratories conducting testing with pooling should use an assay and test system that has received an EUA from FDA.

- Some laboratory tests have a low sensitivity and pooling large numbers of specimens will always further reduce the detection sensitivity. Ask the laboratory for data on their validation of the pooled test and consider using laboratories that have smaller pool sizes.
- Testing turnaround time may be impacted due to the need to retest individual samples from positive pooled samples.
- Populations that may benefit from pooled testing when disease prevalence is low include:
    - Health care workers
    - First responders
    - Essential workers
    - Correctional facilities: prisoners and staff
    - Congregate care facilities: residents and staff
    - Education sector
    - Businesses for return-to-work

Information and resources for laboratories considering performing pooled testing

Pooling approaches can be divided into at least two categories: pooling aliquots of transport media from individual patients, or addition of individual swabs into a single volume of transport media. To support strong public health measures with good laboratory testing practices, all pooling strategies should be evaluated and have met an acceptable minimum performance standard of ≥ 85% percent positive agreement (PPA) when compared to the same test performed on individual samples before being implemented.

Because pooling will result in decreased test sensitivity by the very nature of dilution of the primary patient sample and its utility decreases above a certain positivity threshold, all pooling procedures must have an ongoing mechanism to monitor the positivity rate and the performance of the pooled test. Per FDA guidance, n-sample pooling (where n is the number of transport media samples in a pool) is interpreted for negative and positive pooled results as follows: a negative pooled result implies that all samples in the pool are negative and a positive pooled result implies that at least one of the samples in the pool is positive. As the positivity rate in the population increases, the n-sample pooling size must decrease to maintain adequate test sensitivity. Laboratories performing (nondiagnostic) surveillance testing should follow FDA's guidance of maximum pool sizes based on positivity rates (n=5 for 5-6%; n=4 for 7-12%; n=3 for 13-25%) unless they have validated a different pool size and have specific, and ongoing, supporting documentation.

The Food and Drug Administration (FDA) provides a Molecular Diagnostic Template for Laboratories, including guidance for validation of SARS-CoV-2 molecular tests for specimen pooling.

The FDA COVID EUA website posts updates to EUA protocols Some test kit manufacturers are applying for on-label claims for pooled testing.

The U.S. Centers for Medicare &Medicaid Services (CMS) has also released regulatory guidance pertaining to sample pooling.