



# Considerations for Increasing SARS-CoV-2 Molecular Testing Capacity via Group Testing (Pooling)

## Background

Group testing is a standard approach to screening blood products for infectious diseases and has also been used to detect respiratory viruses from nasopharyngeal (NP) and throat (OP) swab specimens. There is a great deal of interest in using this approach to test for SARS-CoV-2 as a means of decreasing test reagent use and increasing the overall testing capacity for a given population.

The general principle of pooling provides that when specimens are pooled together and tested, the test will result as negative when everyone in the pool is healthy and positive when at least one person is infected. For positive results, each specimen in the pool would have to be retested individually. When disease prevalence in a population is 5% to 8% or less, most test results will be negative, and a greater number of samples can be pooled than when disease prevalence is high (see table). Pooling also affects sensitivity due to the dilution effect of adding fluid from multiple samples together and this is therefore dependent on the size of the pool (see table).

Positivity Rate	Optimal Pool Size*	Reduction in Testing	Fold Increase in Testing Efficiency	Fold Loss in Sensitivity (~ΔCt)
0.1%	32	94%	15	32 (5)
1%	11	80%	5	11 (3.5)
5%	5	57%	~2	5 (2.5)
10%	4	41%	~1.5	4 (2)
29%	3	10%	~1.1	3 (1.5)

## Potential Opportunities and Barriers

### Regulatory

Currently no SARS-CoV-2 diagnostic test has received an emergency use authorization (EUA) from the US Food and Drug Administration (FDA) for use with sample pooling. However, the FDA recently provided guidance for laboratories that intend to incorporate the pooling of specimens into their SARS-CoV-2 diagnostic testing algorithms. Laboratories must validate their molecular assays for use with pooled specimens and submit an EUA request to the FDA. Submission of an EUA request to FDA requires regulatory expertise that is not available to all clinical laboratories.

Laboratories reporting patient-specific results must have a CLIA certificate from the Centers for Medicare and Medicaid Services (CMS).

### Method Performance

Successful application of the pooling approach requires knowledge of the limit of detection (LOD), sensitivity, and specificity of the assay being used, as well as the prevalence of disease in the population. Pooling of specimens dilutes each individual specimen, which could lead to false negative results. Therefore, upper respiratory specimens, associated

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with higher levels of sensitivity, should be utilized for pooling efforts, but those specimens with low pre-pooling sensitivity, such as saliva, should not be used for pooling.

To avoid false negatives, testing of pooled specimens requires a molecular assay that has a low limit of detection and high sensitivity and specificity. The validation of an assay for pooling should follow current FDA guidance. At least 25% of previously tested specimens used in a validation should have a cycle threshold (Ct) shift within 2 to 3 Ct and no more than within 2 to 4 Ct. Additionally, the assay's limit of detection using pooled specimens must be evaluated and sufficient to accurately identify specimens with viral genetic material close to the limit of detection pre-pooling.

Determination of appropriate pool size is based on disease prevalence in the population to be tested. Most publications seem to agree that a pool size of 4 to 5 specimens provides a reasonable balance between conservation of resources and the performance of the test. There are online resources that laboratories can utilize to aid in the determination of appropriate pool size.

As respiratory pathogen season begins, and laboratories pursue methods to simultaneously test for multiple pathogens (e.g. influenza and SARS-CoV-2) the ability to perform group testing using these new multiplex methods will need to be re-evaluated.

### ***Information Technology***

Most laboratory information management systems are configured to track individual specimens. Laboratories will need to establish mechanisms to track individual specimens within pools, permit selection of pooled specimens for retesting if the pool is positive, and report individual test results once confirmed. Absent an electronic process to track specimens and results, manual processes that are more labor-intensive and error-prone may be necessary.

### ***Logistics***

Large scale use of pooling may require laboratory automation to maximize staff efficiency and minimize opportunity errors.

Billing processes for pooled specimens need to be resolved as no reimbursement mechanism currently exists.

Turn around time for positive specimens may double due to the requirement to confirm these results. Coordination of individual retests and initial group testing will be necessary to minimize potential reporting delays.

### ***Research***

CMS issued guidance indicating that facilities performing SARS-CoV-2 surveillance testing using a pooled sampling procedure to report non patient-specific SARS-CoV-2 cohort results will not require CLIA certification. Thus, research laboratories can perform pooled testing but the results cannot be reported to individual identified participants as this testing is not considered by CMS to be diagnostic of SARS-CoV-2 infection. Importantly, any positive pools tested in a research setting will need to be reflexed to a CLIA-certified laboratory for confirmation and reporting. This connection should be established before pooled testing is implemented in a research environment.

### ***Results Reporting***

Establishing a mechanism to report pooled results, negative or positive, to the public health system is essential before starting pooled testing. Reporting both negative and positive results is required per the North Carolina State Health Director Temporary Order.

### ***Scenarios Where Pooling May Be Considered***

Due to the potential for reduced sensitivity with group testing for SARS-CoV-2, pooling has the greatest utility in asymptomatic patient populations with low pre-test probability that the patient has COVID-19. Use of this method should be reserved for instances where the incidence rate is 5% to 8% or less as this will maximize staff efficiency and

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conservation of test reagents. Positive results in this scenario must be confirmed and downstream costs (financial and opportunity) of that confirmatory testing should be considered when making implementation decisions. Below are some example scenarios in which group testing for SARS-CoV-2 may reasonably be used:

- Pre-admission patients
- Patients screened pre-procedure
- Students returning to campus
- Asymptomatic front-line and essential workers
- Population-based surveillance studies

### ***Scenarios Where Pooling Should Not Be Considered***

Due to the lower sensitivity of group testing and need to individually repeat test on pools that initially test positive, pooling should not be used under the following circumstances:

- Emergency or outbreak situations
- High-risk congregate settings with active confirmed cases
- Symptomatic populations
- Hospitalized patients
- Populations with an incidence greater than 5% to 8%

### ***Resources:***

Abdalhamid B, *et al.* Assessment of specimen pooling to conserve SARS-CoV-2 testing resources. *American Journal of Clinical Pathology*, Volume 153, Issue 6, June 2020, Pages 715–718, <https://doi.org/10.1093/ajcp/aqaa064>

Callahan C, *et al.* Nasal-Swab Testing Misses Patients with Low SARS-CoV-2 Viral Loads. medRxiv preprint doi: <https://doi.org/10.1101/2020.06.12.20128736>

Chen JY and Chen ASC. Optimal pool size for COVID-19 group testing. medRxiv preprint: <https://doi.org/10.1101/2020.04.26.20076265>

Eliaz Y, *et al.* POOLKEH finds the optimal pooling strategy for population-wide COVID-19 testing. medRxiv preprint: <https://doi.org/10.1101/2020.04.25.20079343>

Griesemer, S, *et al.* Assessment of sample pooling for clinical SARS-CoV-2 testing. bioRxiv preprint doi: <https://doi.org/10.1101/2020.05.26.118133>

Pikovski A and Bentele K. Remarks on pooling Coronavirus tests. medRxiv preprint: <https://doi.org/10.1101/2020.06.08.20125781>

Pilcher C, *et al.* Group Testing for SARS-Cov-2 to Enable Rapid Scale-Up of Testing and Real-Time Surveillance of Incidence. *The Journal of Infectious Diseases*, jiaa378, <https://doi.org/10.1093/infdis/jiaa378>

Yelin I, *et al.* Evaluation of COVID-19 RT-qPCR test in multi-sample pools. *Clinical Infectious Diseases*, ciaa531, <https://doi.org/10.1093/cid/ciaa531>

Web-based applications for determination of appropriate pool size: <https://www.chrisbilder.com/shiny> and <https://poolkeh.herokuapp.com>