

Wadsworth Center Validation Procedure for SARS-CoV-2 LDTs

The U.S. Food and Drug Administration (FDA) has given the Wadsworth Center authority to approve laboratory developed tests (LDTs) for SARS-CoV-2 for specific qualified laboratories during this public health emergency. This includes New York State (NYS) permitted clinical laboratories that have the category of Virology, including molecular methods, on their permits, or laboratories that have obtained temporary approval in accordance with Executive Order 202.10. Executive Order 202.10 allows laboratories holding a Clinical Laboratory Improvement Acts (CLIA) certificate and meeting the CLIA quality standards described in 42 CFR Subparts H, J, K and M, to perform testing for the detection of SARS-CoV-2 in specimens collected from individuals suspected of suffering from a COVID-19 infection. Laboratories should have experience in the development and validation of laboratory developed tests.

A streamlined validation procedure has been established based on previous recommendations from the FDA for assay validation under an Emergency Use Authorization (EUA) that your laboratory can use to perform validation studies. The information below describes minimum validation requirements for performing testing on single specimens and pooled specimens.

Validation Procedure: Testing of Single Specimens

The information below needs to be submitted for review. Note: The validation must be completed and produce satisfactory results, prior to initiating testing of clinical samples.

Overview:

Provide a full description of the testing procedure you are using, including extraction, amplification and detection.

Specimen matrix:

For respiratory specimens, limit of detection (LoD) and clinical evaluation studies (described below) need only be performed on one respiratory specimen type, e.g. nasopharyngeal swab(s) in Viral Transport Medium. Validation of larger respiratory panels or additional respiratory sample types is at the discretion of the lab directors at each site. We would, however, caution against the testing of sputum samples without previously validating the performance of the test in that matrix, as performance differences have been observed. When validating non-respiratory specimens (e.g., saliva, stool, CSF, etc.), limit of detection (LoD) and clinical evaluation studies need to be performed on each specimen type.

Limit of Detection (LoD)

A preliminary range-finding experiment should be performed initially by testing, in triplicate, a 10-fold serial dilution of quantitated whole viral genomic RNA or virus, spiked into pooled specimen matrix. Suitable material for spiking is available from a number of sources including BEI, LGC SeraCare and Zepmetrix. The LoD observed in this experiment should be confirmed by testing 20 replicates spiked at that concentration into individual specimens, tested from extraction. Each extraction method for which authorization is being requested must be separately validated. A $\geq 95\%$ detection (at least 19 of the 20 replicates testing positive) must be demonstrated at this concentration.

Analytical Specificity

For assays using primers and probes that are used in both the New York and CDC SARS-CoV-2 real-time RT-PCR assays, extensive specificity studies have already been performed

at those two facilities and need not be repeated. For other assay designs, the potential cross-reactivity should be tested against a range of respiratory pathogens, as well as other agents likely to be found in the same specimen type(s) that will be tested in the assay. At a minimum, this should include representative strains of circulating influenza A and B viruses, and a number of agents from each of the following: parainfluenza viruses, respiratory adenoviruses, rhinoviruses, respiratory enteroviruses, human metapneumovirus, RSV, and at least one representative of each of the six other human coronaviruses (OC43, 229E, NL63, HK-U1, MERS and SARS). Also, human herpes viruses 1 – 6, and a selection of common respiratory bacterial pathogens. Where possible, agents should be tested empirically at concentrations representative of those likely to be present in biological specimens and in general, not less than 10⁶gc/ml. For testing in a non-respiratory specimen, additional specificity testing against relevant non-respiratory pathogens is also required.

For agents where empirical testing is not possible, *in silico* analysis should be performed. A result summary of the *in silico* blast analysis should be provided, showing any significant potential for amplification of non-target sequences, with individual blast results for each primer and probe and percent sequence homology for each organism relative to the target analyte primers/probes in the assay. Analysis should include sequence data from multiple representative strains for each organism. The omission of agents from empirical testing should be supported by a justification.

Reproducibility Studies

Inter-assay, intra-assay, inter-user and inter-instrument reproducibility studies are not required.

Interfering Substances

Data on testing for interference by endogenous substances is not required if the extraction procedure is a well-established method (e.g. Boom).

Comparative LoD

A comparative LoD study is not required. However, a comment on comparative LoD should be provided in your submission by comparing the LoD in the submitted test with those already granted EUA by the FDA.

Specimen and Reagent Stability

Quality control of all reagents should be closely monitored as issues have been reported in the past. Briefly describe the method used for determining ongoing verification of the performance of any reagents for which the manufacturer does not designate an expiration date, and for validation of the testing of freeze/thawed reagents and specimens (if applicable).

Clinical Evaluation

Perform testing on 30 positive and 30 negative clinical specimens; 20 of the positive specimens should be in the weak-positive range (CT >30). If positive clinical specimens are not available, contrived positive specimens can be used, with 20 of the 30 positive samples spiked at concentrations to generate CT values in the weak positive range.

Modification to approved tests

Bridging studies comparing the performance of a previously approved FDA EUA or NYS method on a new extraction or amplification/detection platform should be performed with a LOD range finding 10-fold dilution series on pooled matrix, followed by 20 replicate confirmation at the

estimated LOD on individual specimens. Comparable performance with no significant loss of sensitivity must be demonstrated. No additional clinical sample testing is required.

Where the modified equipment uses a very similar chemistry and platform (e.g. easyMAG and eMAG, 7500 FAST and 7500 FAST Dx) the data need not be submitted for review but kept on file at the laboratory.

Where the equipment or platform is dissimilar (e.g. easyMAG and Qiagen HT extractor), the data should be submitted for review.

Modifications to specimen type should be validated as for a full LDT described above and the data submitted to NYS, unless the specimen type is extremely similar (e.g. nasal swab and mid-nasal turbinate, tracheal aspirate and bronchial aspirate) in which case specimens are considered equivalent and do not require bridging studies.

Validation Requirements for pooled samples when using an FDA EUA or NYS authorized SARS-CoV-2 test on pooled specimens

A laboratory performing testing on pooled samples with a test that has FDA Emergency Use Authorization (EUA) or NYS approval will need to compare the performance of the assay on single specimens to that in pooled specimens. The goal is to determine if there is a reduction in assay analytical sensitivity (as observed by the shift in Ct score for RT-PCR assays) with respect to the number of samples to be pooled, to ensure that the pooling strategy will maintain appropriate sensitivity. The information below needs to be submitted for review. Note: The validation must be completed and produce satisfactory results, prior to initiating testing of clinical samples.

Considerations when performing SARS-CoV-2 Surveillance (non-diagnostic) Testing on Pooled Samples

- There are multiple ways to pool specimens for testing. For example:
 - Multiple swabs are placed into a single tube of transport medium at the time of collection and an aliquot of transport media from the pooled specimens is tested. This requires performing a repeat specimen collection on each patient, to facilitate retesting, should the pool test as positive.
 - Individual swabs are placed into individual tubes of transport media, an aliquot of transport media is taken from each individual sample, pooled into another tube and then an aliquot of the pooled specimen is added into the first stage of the sample processing tube (lysis buffer). The individual residual specimens remain separate and available for retesting if needed.
 - Individual swabs are placed into individual tubes of transport media and an aliquot of transport media is taken from each individual sample and added into the first stage of the sample processing tube (lysis buffer). The individual residual specimens remain separate and available for retesting if needed.
- It is recommended that the published literature on this topic be reviewed before making a final decision on which sample pooling method to use.
- The pooling strategy is only useful when disease prevalence is low, otherwise pools are too frequently positive and too frequently have to be deconvoluted for retesting of individual specimens.
- Some methods have an inherent risk of reduced sensitivity and pooling large numbers of specimens will always reduce the detection sensitivity.

- Even when there is no calculated theoretical loss of sensitivity, it is imperative that laboratories perform empirical testing to determine the impact on the limit of detection and ensure that it has not been significantly adversely impacted by the process.
- The impact on the entire laboratory process needs to be carefully assessed prior to implementing a pooling strategy. Other aspects of the testing process should be considered, such as specimen accessioning, the protocols that need to be established to identify patient specimens that need retesting if a pooled specimen is positive, and reporting of results, which are not streamlined and may be complicated by pooling procedures.

Overview

A full description of how samples will be pooled and how identification of samples will be tracked throughout the testing process such that results can be deconvoluted for reporting. If individual samples will be used for pooling, a full description of how samples will be stored in the event that they need to be retested.

A full description of the testing procedure you are using, including extraction, amplification and detection.

Results from the testing of positive specimen pools.

- A minimum of 20 positive specimens need to be used to prepare 20 separate positive pools. Each pool should consist of a specimen that was positive by the assay when tested individually, pooled with pretested, randomly selected negative specimens.
- Prior to selection of the samples, analyze existing laboratory data and evaluate the Ct scores of positive samples.
- Select positive samples for the validation that have Ct scores representative of the range of Ct values in the samples tested at the submitting laboratory.
- Compare the detection of the positive samples when tested individually and when tested in pools, including the Ct values observed in both formats.
- Provide tables of Ct values obtained for each positive sample when run individually and when pooled and the Ct change for each sample. Also provide the average Ct change observed for the entire study and the standard deviation.
- Include data on Ct values for internal control assays, demonstrating that there has not been an adverse effect on the inhibition rate.
 - Note – in general, an average Ct change of more than 1.5 is not acceptable.

Results from the testing of negative specimen pools.

A minimum of 20 negative pools need to be tested to confirm that negative samples remain negative when pooled.

Validation Requirements for pooled samples when using a test that does NOT have FDA EUA or NYS authorization

A laboratory performing testing on pooled samples with a test that does NOT have FDA EUA or NYS approval will need to:

- Perform the validation procedure for the testing of single specimens described above AND
- Perform validation of pooled samples as described above with the exception that a minimum of 30 positive specimens need to be used to prepare 30 separate positive pools.