

MSDH Approval of SARS-CoV-2 NUCLEIC ACID AMPLIFICATION ASSAYS

Please complete all information requested below.

SECTION 1: General Information

Laboratory Name: _____

CLIA Number: _____

Locations that test will be performed:

Contact Person: _____

Phone: _____ Fax: _____ Contact E-mail: _____

Validated Specimen Type(s) _____

Check One	Assay Type	Necessary Forms for Package Submission
	Laboratory developed test that does not utilize Analyte Specific Reagents (ASRs)	See Section A
	Laboratory developed test that utilizes Analyte Specific Reagents	See Section A
	Kit labeled for Investigational Use Only (IUO) or Research Use Only (RUO)	See Section A
	Modification of a FDA-approved assay	See Section A

SECTION A: Required Validation Package

The checklist below provides a list of all validation steps that must be performed to request Emergency Use Authorization for nucleic acid amplification tests for SARS-CoV-2 testing. The information submitted must be organized as numbered or tabbed attachments. Indicate the **page numbers, appendices and/or tabs where** the items and/or attachments can be found. **SUBMISSIONS THAT ARE NOT ORGANIZED AS DESCRIBED MAY BE RETURNED AND THE REVIEW SIGNIFICANTLY DELAYED.**

1: Assay Documentation Requirements

Method Standard Operating Procedure including:

- detailed step-by-step protocol that provides all steps for performing the assay in sequential order
- specimen collection (where applicable), processing and storage requirements
- acceptable specimen types and collection materials (i.e. tube types), specimen transport requirements (i.e. temperature, time to receipt) and specimen rejection criteria
- sources of reagents and equipment including: concentrations and volumes of stock and working reagents. List all RT-PCR instruments, software, manual extraction kits/processes, automated extraction systems.
- a description of all controls and calibrators used in the assay including the function of each control (see additional description and guidance below). Preparation, concentration and storage requirements of controls should be included. Note the frequency of use in the testing protocol as well as acceptable control limits and action(s) to be taken when controls exceed the defined tolerance limit.
- algorithm for defining all possible results and procedures for interpreting or reporting each type of result (ex. positive, negative, quantitative values, indeterminate, invalid and inconclusive)
- examples of all calculations needed to produce interpretable results.
- technical limitations of the assay, potential sources of error, and trouble-shooting protocols, and any other information relevant to performing the assay
- a description of standard molecular workflow and the strategy used for separating pre-and post-amplification areas, as well as the implementation of methods to minimize amplicon contamination (such as the use of unidirectional workflow and aerosol-resistant pipette tips)

Required Controls: The following controls are necessary for nucleic acid amplification tests. The procedure should contain details on how each of the following functions is controlled in the assay, including the composition of the control and the step in which the control is included in the assay.

Additional guidance on acceptable control materials and procedures is provided below:

- **Extraction Control**

The extraction control will monitor the performance of the entire assay, including the lysis/extraction process, to ensure that it is performing as expected.

- **Reagent Contamination Control**

A reagent contamination control (i.e. no-template control) ensures that the amplification reagents are not contaminated. This control should consist of water or buffer in a reaction tube that contains all of the reagents for amplification, but does not contain nucleic acid template.

- **Negative controls**

The negative sample control is used to evaluate the complete assay procedure, including extraction. Negative results for this control confirm that signals are not obtained in the absence of target sequences (e.g., due to non-specific priming or detection)

- **Positive Controls**

The positive control contains target nucleic acids, and is used to test the entire assay process, including sample lysis, nucleic acid extraction, amplification, and detection. It is designed to mimic a patient specimen and is run as a separate assay, concurrently with patient specimens.

- **Internal Control**

The internal control is a non-target nucleic acid sequence that is co-extracted and/or co-amplified with the target nucleic acid. It controls for integrity of the reagents (e.g., polymerase, primers, etc.), equipment function (e.g., thermal cycler), and the presence of inhibitors in the sample. Examples of acceptable internal control materials include:

- Human nucleic acid co-extracted with MTB-complex and primers amplifying human housekeeping genes (e.g., RNaseP, β -actin).
- A non-infectious DNA plasmid containing the non-target nucleic acid that is added to the sample either prior to or after sample lysis and extraction.
- A complete non-MTB complex organism that is added to the sample prior to sample lysis and extraction.
- Packaged RNA for assays that target ribosomal RNA.

- **Extraction Control**

The extraction control is an external control that verifies that lysis of and subsequent nucleic acid isolation has occurred efficiently. This control should contain whole organism (bacterial cells, viruses, parasites, fungi) or, if these are unavailable, nucleic acid. This control should be included at a low but easily detectable concentration and should be run through the entire assay. The positive control for the complete assay, or the internal control, may serve as an extraction control if run through the entire testing process.

2: Test Reports	
	<p>Provide examples of test reports containing all findings (e.g. positive (quantitative or qualitative), negative, indeterminate, inconclusive, etc.) with interpretive text, assay limitations and any disclaimers required by the federal government for tests. This description must indicate the cut-off values for all outputs of the assay and include the following information:</p> <ul style="list-style-type: none"> • In particular, you must provide the cut-off value for defining a negative result of the assay. If the assay has only two output results (e.g., detected and not detected), this cut-off also defines a positive result of the assay. • If the assay has an indeterminate range, you must provide limits for the indeterminate range. • If the assay has an invalid result, you must describe how an invalid result is defined.
3: Validation Requirements	
	<p>Validation Data Summary: Use the provided MSDH SARS-CoV-2 Validation Study template to document how the new assay was validated and to provide the validation results. For each assay, data must be provided for Specificity, Sensitivity, Precision (Inter-assay Reproducibility and Intra-assay Reproducibility) and accuracy. All data must meet the stated acceptance criteria.</p>
	<p>Specificity:</p> <ul style="list-style-type: none"> • Perform an in silico analysis of the assay primer and probes compared to common respiratory flora and other viral pathogens or provide a list of all organisms tested in the specificity study and the verification run, including the source and concentration of each organism or nucleic acid target if whole organism is not available. Provide the results of the specificity study. If there is any cross-reactivity, provide additional information on how the results will be resolved or interpreted. The <i>FDA</i> defines in silico cross-reactivity as greater than 80% homology between one of the primers/probes and any sequence present in the targeted microorganism • Specificity of the assay should be demonstrated using genetically-related organisms, organisms that can produce similar symptoms or illness, and other organisms that can be present in the specimen matrix/matrices to which the assay will be applied. If any cross-reacting organisms are noted, they should be clearly specified in the application, as well as on the patient report of test findings. In addition, assay a minimum of 5 different strains/isolates of the intended target organism/virus if available. If there are additional genotypes/serotypes/subtypes/variants, these should be included as well.
	<p>Sensitivity:</p> <ul style="list-style-type: none"> • Provide a brief description of experiments used to demonstrate the limit of detection (LOD) of the assay. • Provide the results of the LOD experiments for each specimen matrix tested. It is acceptable to spike RNA or inactivated virus into clinical matrix for LOD determination. <p>Laboratories should test a 2-3 fold dilution series of three extraction replicates per concentration, and then confirm the final concentration with 20 replicates. FDA defines LOD as the lowest concentration at which 19/20 replicates are positive. If multiple clinical matrices are intended for clinical testing, laboratories should submit the results from all matrices. If needed, we recommend that you follow the most current version of the CLSI standard, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures (CLSI EP17)</p>
	<p>Inter-assay Reproducibility:</p> <ul style="list-style-type: none"> • Provide a brief experimental description and results demonstrating inter-assay reproducibility.
	<p>Guidance: At least 3 authentic clinical samples or spiked clinical samples should be run on three different days. If spiked, the three samples should include one concentration at or near the limit of detection. Alternatively, a single positive control run repeatedly over many days (3 or more) may be used.</p>
	<p>Intra-assay Reproducibility:</p> <ul style="list-style-type: none"> • Provide a brief experimental description and data demonstrating intra-assay reproducibility.
	<p>Guidance: At least 2 authentic clinical samples or spiked clinical samples should be run in triplicate. If spiked, the three samples should include at least one concentration at or near the limit of detection. Also, if different instruments, platforms, models or technicians will be used to perform the assay, demonstrate the assay's consistency across these variables.</p>

	<p>Accuracy Verification:</p> <ul style="list-style-type: none"> • Provide a description of the validation study design. Accuracy should be verified by conducting a randomized, blinded validation study where the assay results are compared to those of an FDA-approved assay, or results of spiked clinical samples that are compared to predicted results based on the spiking values. • Provide data from <i>at least</i> 20 positive samples and 10 negative samples for each specimen type together with controls used in the assay. Preferably present the results in tables (2 x 2), showing the qualitative results compared to results obtained by the comparison method. The samples can be spiked samples or actual clinical specimens. At least 10 of the positive samples should be 1x-2x the method's LOD. All other specimens should span the assay's testing range. For quantitative assays, please provide data across the full range of concentrations likely to be encountered in clinical samples. • For each assay, provide the number and type of specimens tested, including information on the subtypes, genotypes, etc. tested in the assay. If multiple clinically relevant sub-types or genotypes are available for the organism being assayed, please include an example of each available type, or a representative range of subtypes. • Provide a brief summary of the results, including an explanation of any discrepant results and how the discrepancy was resolved. • Submit one representative example of test results (one high-quality original printout of an actual test run), a condensed summary of the raw data (such as Ct values), and a complete description of how all results were interpreted. • Molecular amplification assays for microbial detection that are not probe-based, such as SYBR Green or real-time PCR with melting curve analysis, data must be submitted from a secondary confirmatory method. These assays can be used as screening assays but must be confirmed with an alternative method, i.e., a probe-based, hybridization-based, or sequence-based method. In the absence of such confirmation, positive results are considered presumptive, and this should be clearly indicated on the report. (Also see Specificity validation guidance above). • The acceptance criteria for the performance must be at least 95% agreement.
	<p>Specimen Stability: If the specimen source, testing time-frames, or storage criteria are altered from what is defined by the CDC, the applicable additional study will be required.</p> <ul style="list-style-type: none"> • Analyte and specimen matrix stability • Specimen transport conditions • Storage time and temperature <p>A minimum of 10 altered specimens will need to be analyzed for Specificity, Sensitivity Inter-assay Reproducibility, Intra-Assay Reproducibility and Accuracy as defined in section A.3.</p>
<p>3. Reference materials</p>	
	<p>A list of relevant literature references that describe the scientific basis and clinical validity of the assay. Provide copies of only primary references.</p>
	<p>Applicable package inserts</p>

Once the package has been reviewed, the laboratory will receive either an Emergency Use Authorization letter or a letter requesting additional information. In order to facilitate our response to resubmissions, we request that these be as clear and well organized as possible. Please provide a point-by-point response to the reviewers' comments, as well as the appropriate supporting documentation. If revisions to the procedure manuals were requested, please submit entire new versions, and clearly indicate where the changes were made.

April 1, 2020