Molecular Diagnostic Template for Laboratories[1]

This template (the "template") includes FDA's current recommendations for laboratories 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 laboratory. As outlined in Section V.A. of the FDA guidance document: *Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency (Revised)*,[2] FDA recommends that the following validation studies be conducted for a SARS-CoV-2 molecular diagnostic assay: Limit of Detection, Clinical Evaluation, Inclusivity, and Cross-reactivity. This template is intended to help manufacturers provide these validation data and other information to FDA, but alternative approaches can be used. It reflects FDA's current thinking on the topic, and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* means that something is suggested or recommended, but not required. For more information about EUAs in general, please see the FDA Guidance document: *Emergency Use Authorization of Medical Products and Related Authorities*.[3]

GENERAL INFORMATION ABOUT THIS TEMPLATE

- This EUA review template (EUA template) is only intended for use by CLIA certified high-complexity laboratories who intend to submit a pre-EUA or EUA to FDA for a SARS-CoV-2 molecular diagnostic test. Use of the template is applicable only for testing of respiratory specimens, e.g., nasopharyngeal, sputum, and BAL specimens.
- Text highlighted in yellow ***[Text]*** should be completed by the laboratory (sponsor) as applicable to their specific test. Text in **bold** outlines the Food and Drug Administration's (FDA) recommendations for the sponsors' consideration when providing the suggested information in a specific section.
- A test authorized under an EUA is only authorized for emergency use while the EUA is in effect.
- This is an EUA interactive review template for Pre-EUA/EUA submissions. We plan to update the template as appropriate as we learn more about the COVID-19 disease and gain experience with the EUA process for this test.

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[1] This template is part of the Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency (Revised) - Immediately in Effect Guidance for Clinical Laboratories, Commercial Manufacturers, and Food and Drug Administration Staff

[2] https://www.fda.gov/regulatory-information/search-fda-guidance-documents/policy-coronavirus-disease-2019-tests-during-public-health-emergency-revised

[3] https://www.fda.gov/media/97321/download

EXAMPLE TEMPLATE

A. PURPOSE FOR SUBMISSION

Emergency Use Authorization (EUA) request for use of a SARS-CoV-2 molecular diagnostic test to be performed for the in vitro qualitative detection of RNA from the SARS-CoV-2 in respiratory samples from patients as recommended for testing by public health authority guidelines. The test will be performed in CLIA certified high-complexity laboratories. Additional testing and confirmation procedures should be performed in consultation with public health and/or other authorities to whom reporting is required.

Positive results should also be reported in accordance with local, state, and federal regulations.

B. MEASURAND

Specific nucleic acid sequences from the genome of the SARS-CoV-2 ***[please specify the targeted gene(s) of the pathogen; assays with more than one target are recommended].***

C. LABORATORY/SPONSOR

[Official name, address and contact information of applicant and all locations where specimen testing will be performed]

D. REGULATORY INFORMATION

Approval/Clearance Status:
The SARS-CoV-2 assay test is not cleared, CLIA waived, approved, or subject to an approved investigational device exemption.

Product Code:
QJR

E. PROPOSED INTENDED USE

1) Intended Use:
The SARS-CoV-2 assay is a [***specify test technology such as, real-time RT-PCR test***] intended for the [***presumptive***] qualitative detection of nucleic acid from the SARS-CoV-2 in [***list respiratory specimens e.g., nasopharyngeal, nasal, and oropharyngeal swab specimens and lower respiratory tract, BAL, sputum***] from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to [***Name of Clinical Laboratory***] that is certified under

the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, ***to perform high complexity tests***.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in [***name specimen type, e.g. upper respiratory***] during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The assay is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures The assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

2) Instruments Used with Test:
The **[*test name*]** test is to be used with the **[*list all RT-PCR Instruments, software, automated extraction instruments*].**

F. DEVICE DESCRIPTION AND TEST PRINCIPLE

Example text has been added under each of the sub-headings below for a fluorescence based rRT-PCR test for detection of organism RNA. If a different test principle is used by the test for the detection of a specific analyte please modify the description accordingly to capture the salient points in each of the sub-headings below. Please note this template is intended for use only with existing, well-established technologies.

1) Product Overview/Test Principle:
The assay is a real-time reverse transcription polymerase chain reaction (rRT -PCR) test. The SARS-CoV-2 primer and probe set(s) is designed to detect RNA from the SARS-CoV-2 in respiratory specimens from patients as recommended for testing by public health authority guidelines.

2) Description of Test Steps:
[Please describe in abbreviated form the steps for performing your assay in sequential order as a numbered list, including extraction methods. This should include the names of the instruments used in your assay, e.g., ABI 7500. A copy of your laboratory procedure would be acceptable and can be appended to this form.]

3) Control Material(s) to be Used:
[Please describe the assay controls to be performed in the laboratory, including the following:

- *The positive and negative control; ideally the positive control will be used to confirm performance near the test LoD. If a template control is used, please describe in general terms the sequence used.*
- *The extraction control.*
- *The internal control, if present.*

Your description should also include the frequency that controls will be performed.]

4) Assay results and interpretation
[Please describe the results of your assay procedure, e.g., reactive (positive/detected), non-reactive (negative/non-detected), or Invalid (no result reported)]

G. PERFORMANCE EVALUATION

The following validation studies should be performed during your assay development:

1) Limit of Detection (LoD) -Analytical Sensitivity:
Laboratories should document the limit of detection (LoD) of their SARS-CoV-2 assay. It is recommended to spike inactivated virus (e.g., heat treated or irradiated virus) into real clinical matrix (e.g., BAL fluid, sputum, etc.) for LoD determination, since the inactivated virus most closely reflects live virus in a clinical sample. If you are unable to acquire inactivated virus, viral genomic RNA is acceptable to use to generated contrived samples for LoD determination.

It is recommended that 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. For example, if testing common upper respiratory tract specimens (e.g., nasopharyngeal (NP) swabs, oropharyngeal (OP), swabs, nasal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal aspirates, and nasal washes etc.), please submit results from the most challenging upper respiratory matrix. FDA considers nasopharyngeal (NP) swabs to be the most challenging upper respiratory matrix. If claiming common lower respiratory tract specimens (e.g., tracheal aspirates, sputum, etc.), please submit results from the most challenging lower respiratory matrix. FDA considers sputum to be the most challenging upper respiratory matrix. If claiming both upper and lower respiratory matrixes, submitting results from sputum samples may suffice to support both upper and lower respiratory matrices. If claiming alternative

respiratory specimens, such as saliva, oral fluid, buccal swab, etc., please submit results from testing each of the claimed uncommon respiratory specimen type. 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)

[Please describe in abbreviated form your LoD study, the specific material used (e.g., live or inactivated viral stocks, viral RNA, or in vitro transcripts), and the LoD (with appropriate units) for your assay]

2) ***Inclusivity (analytical sensitivity):***
Laboratories should document the results of an inclusivity study that demonstrates the strains of SAR-CoV-2 that can be detected by the proposed molecular assay. It is acceptable to conduct an in silico analysis of published SARS-CoV-2 sequences using the assay's primers and probes. FDA anticipates that 100% of published SAR-CoV-2 sequences will be detectable with the selected primers and probes.

[Please describe in abbreviated form your Inclusivity study and confirm that there was 100% detection of all SARS-CoV-2 strains.]

3) ***Cross-reactivity (Analytical Specificity)***
At a minimum, an in silico analysis of the assay primer and probes compared to common respiratory flora and other viral pathogens, listed in the table below, should be performed. FDA defines in silico cross-reactivity as greater than 80% homology between one of the primers/probes and any sequence present in the targeted microorganism. Laboratories should follow recognized laboratory procedures in the context of the sample types intended for testing for any additional cross-reactivity testing.

Recommended List of Organisms to be Analyzed in silico and by Wet Testing*

Other high priority pathogens from the same genetic family	High priority organisms likely in circulating areas
Human coronavirus 229E	Adenovirus (e.g. C1 Ad. 71)
Human coronavirus OC43	Human Metapneumovirus (hMPV)

Other high priority pathogens from the same genetic family	High priority organisms likely in circulating areas
Human coronavirus HKU1	**Parainfluenza virus 1-4**
Human coronavirus NL63	**Influenza A & B**
SARS-coronavirus	**Enterovirus (e.g. EV68)**
MERS-coronavirus	**Respiratory syncytial virus**
	Rhinovirus
	Chlamydia pneumoniae
	Haemophilus influenzae
	Legionella pneumophila
	Mycobacterium tuberculosis
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Bordetella pertussis
	Mycoplasma pneumoniae
	***Pneumocystis jirovecii* (PJP)**
	Pooled human nasal wash – *to represent diverse microbial flora in the human respiratory tract*
	Candida albicans
	Pseudomonas aeruginosa
	Staphylococcus epidermis
	Streptococcus salivarius

* For wet testing, concentrations of 106 CFU/ml or higher for bacteria and 105 pfu/ml or higher for viruses is recommended.

[Please describe in abbreviated form your cross-reactivity study and list the microorganisms tested, indicating whether this was performed either in in silico or wet testing. Organisms recommended for testing are listed in the table above]

4) Clinical Evaluation:

FDA recommends using natural clinical specimens in the clinical evaluation. Please refer to the following table for additional information regarding clinical study design:

Note: Clinical study recommendations listed in the table below do not apply to claims for testing asymptomatic individuals/screening and to saliva or other alternative respiratory specimen type claims.

Minimum Number of Positive Specimens	***At a minimum 30 natural (prospective or retrospective or leftover samples) positive clinical specimens should be collected from patients suspected of SARS-CoV-2 infection by theory healthcare provider in the COVID-19 disease endemic region(s).*** ***Samples can be a mixture of specimen types, if you are seeking an upper respiratory claim (e.g., nasopharyngeal (NP) swab, oropharyngeal (OP) swab, nasal swab (NS)).*** ***If you are seeking a sputum claim, and any other respiratory specimen claim except alternative respiratory specimen types (e.g., saliva), we recommend a combination of 15 NP and 15 sputum samples.*** ***Specimens collected from different anatomical sites from the same patient may be used to support claims for multiple specimen types.*** ***The use of frozen samples is acceptable.*** ***The use of samples previously tested positive by another EUA RT-PCR assay may be acceptable without additional retesting. You should indicate the source of the samples, provide results for each tested sample, indicate specimen type, and initial test date.***
Minimum Number of Negative Specimens	***In general, for EUA at a minimum 30 individual negative samples acquired from the following sources are acceptable; (1) archived/retrospective respiratory samples collected from patients with signs and symptoms of respiratory infection, and (2) other subjects that are expected to be negative for SARS-CoV-2.***

Recommended Comparator Method for percent agreement performance calculations	***Positive percent agreement should be calculated in comparison to an EUA RT-PCR test. We recommend using only a high sensitivity EUA RT-PCR assay which uses a chemical lysis step followed by solid phase extraction of nucleic acid (e.g., silica bead extraction) please see the following website for the most recent list of FDA authorized 2019-nCoV tests: https:// www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations.*** ***Negative result agreement may be calculated in comparison to an EUA RT-PCR test (prospectively collected samples) or as agreement with expected results if samples were collected from individuals known to be negative for SARS-CoV2 (e.g. collected before December 2019).*** ***The comparator assay may have the same, or different, targets as your assay.*** ***False results can be investigated using an additional EUA RT-PCR assay, and/or Sanger sequencing. The results of the discordant analysis can be footnoted in your final performance table but cannot be used to change the final performance calculations.***
Acceptance Criteria	***FDA believes 95% positive and negative agreement is acceptable clinical performance.***
Natural Clinical Specimens IRB/ Informed Consent Note	***Prospective collection of clinical specimens to support the EUA request should be done in accordance with regulations for human subject protection, including IRB approval and informed consent.*** ***Use of leftover de-identified samples may follow the policy outlined in the FDA Guidance on Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable (https://www.fda.gov/media/122648/download).***

Testing Approach Note 1	***All clinical specimens tested in your study should be evaluated in accordance with your proposed diagnostic algorithm, including retesting when appropriate. The limited volume of natural specimens may preclude retesting. In instances were retesting is indicated but not performed, for the purposed of performance evaluation, initial results will be analyzed for performance and equivocal/indeterminate/inconclusive results should count against your final performance.***
Testing Approach Note 2	***Specimens should be tested in a blinded fashion, e.g., positive and negative samples should be presented to the end user in a blinded fashion. The end user should also be blinded to the results of any comparator method testing.***

Alternative Respiratory Specimen Claims:

If you seek a claim for alternative respiratory specimens, such as saliva, oral fluid, buccal swabs, etc., you should test at least 30 paired, positive nasopharyngeal swabs and 30 of the same type of alternative respiratory specimen (e.g., all saliva). To minimize the occurrence of discordant results the samples should be collected within short time of each other and both tested using your candidate EUA assay. FDA believes ≥95% positive agreement with similar Ct values for the paired specimen types is acceptable clinical performance. Please provide detailed information regarding the type of collection device and transport medium you validated for use with your assay. Please note that some transport medium may not be compatible with assays that do not use a nucleic acid extraction step. In addition, some transport medium may not be acceptable for use for at-home collection due to the presence of hazardous chemicals. To discuss additional information that may be needed to support at-home sample collection and transport, please contact FDA at CDRH-EUA-Templates@fda.hhs.gov.

[Please describe in abbreviated form the procedure and results from clinical performance testing]

H. UNMET NEED ADDRESSED BY THE PRODUCT

This section will be completed by FDA.

I. APPROVED/CLEARED ALTERNATIVE PRODUCTS

Currently no methods for the detection of the SARS-CoV-2 have been approved/ cleared by FDA.

J. BENEFITS AND RISKS:

This section will be completed by FDA.

K. FACT SHEET FOR HEALTHCARE PROVIDERS AND PATIENTS:

Include proposed Fact Sheets for Patients and Healthcare Providers - ***see examples for authorized EUA tests on our website and templates will be made available.***

L. INSTRUCTIONS FOR USE/ PROPOSED LABELING/PACKAGE INSERT:

In lieu of a package insert or labeling please include your Laboratory SOP/protocol.

M. RECORD KEEPING AND REPORTING INFORMATION TO FDA:

The laboratory will track adverse events and report to FDA under 21 CFR Part 803. A website is available to report on adverse events, and this website is referenced in the Fact Sheet for Health Care providers. The laboratory will maintain will information on the performance of the test, and report to FDA any suspected change in performance of which they become aware. The laboratory will maintain records associated with this EUA and ensure these records are maintained until notified by FDA. Such records will be made available to FDA for inspection upon request.