Molecular Diagnostic Template for Manufacturers [1]

This template (the "template") provides FDA's current recommendations concerning what data and information should be submitted to FDA in support of a pre-EUA/EUA submission for a molecular diagnostic for SARS-CoV-2. 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

- Text highlighted in yellow ***[Text]*** should be completed by the test manufacturer (sponsor) as applicable to their specific test. Text in **bold** outlines the Food and Drug Administration's (FDA) additional recommendations for the sponsors' consideration when completing the suggested information in each section.
- This template is intended for testing with respiratory specimens; if you are considering non-respiratory specimens (e.g., blood, stool, etc.), please contact FDA at CDRH-EUA-Templates (CDRH-EUA-Templates@fda.hhs.gov) to discuss your validation strategy.
- 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.

---

[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 distribution and/or use of the ***[test name]*** to ***[indicate labs, if applicable]*** for the *in vitro* qualitative detection of RNA from the SARS-CoV-2 in ***[add all claimed specimen types, e.g., nasopharyngeal/ oropharyngeal swabs, sputa, BAL, etc.]*** from patients suspected of COVID-19 by a healthcare provider. 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. Performance is unknown in asymptomatic patients.

B. MEASURAND

Specific nucleic acid sequences from the genome of the SARS-CoV-2 **[*please specify the targeted gene(s) of the pathogen*]**.

C. APPLICANT

[Official name, address and contact information of applicant]

D. PROPRIETARY AND ESTABLISHED NAMES

Proprietary Name - ***[test name]***
Established Name - ***[test name]***

E. REGULATORY INFORMATION

Approval/Clearance Status:

The ***[test name]*** test is not cleared, CLIA waived, approved, or subject to an approved investigational device exemption.

Product Code:

QJR

F. PROPOSED INTENDED USE

1) <u>Intended Use:</u>

The proposed IU will be finalized based on the data and recommendations from Public Health authorities at the time of authorization – example text is provided below for a qualitative molecular test that detects organism RNA but may be adapted according to the specific emergency situation addressed by the device.

[***Test name***] is a [***specify test technology such as, real-time RT-PCR test***] intended for the [***presumptive***] qualitative detection of RNA from the SARS-CoV-2 in [***describe all the specimen types, 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 [***laboratories - certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests, or by similarly qualified non-U.S. laboratories***].

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 ***[test name]*** is intended for use by **[*include intended user, e.g., qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures*]**. The ***[test name]*** is only for use under the Food and Drug Administration's Emergency Use Authorization.

2) Special Conditions for Use Statements:

For Emergency Use Authorization (EUA) only
For prescription use only
For in vitro diagnostic use only

3) Special Instrument Requirements:

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

G. 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 that for new investigative technologies FDA may request additional detailed information so we can adequately assess the risks and benefits associated with the device.

1) ***Product Overview/Test Principle:***

Describe the technology of the test and how this technology works to identify the measurand, the instruments employed/required to perform the test from sample collection to result (include all claimed extraction and PCR detection instruments), and the specimen types for which you claim to have specific performance characteristics as described below. If applicable, list all primer and probe sets and briefly describe what they detect. Please include the nucleic acid sequences for all primers and probes used in the test. Please indicate if the test uses biotin-Streptavidin/avidin chemistry in any of the steps for coupling reagents. Please note that an alignment with available reference genomes for different strains of the target pathogen is requested as part of the inclusivity evaluation (Section J).

The ***[test name]*** 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 ***[list all the specimens]*** from patients suspected of COVID-19 by their healthcare provider.

2) ***Description of Test Steps:***

List and describe in detail all the steps of the test sequentially from specimen collection to detection.

Nucleic acids are isolated and purified from ***[specimens]*** using ***[please describe the method(s) of extraction (please specify the specimen input volume for extraction and/or test, the nucleic acid elution volume and whether isolation/purification is manual and/or automated)].*** The purified nucleic acid is reverse transcribed using ***[enzyme mix/kits – please specify the input volume of purified nucleic acid added to the rRT-PCR reaction mix]*** into cDNA which is then subsequently amplified in ***[please describe the instrument(s) and enzyme mix]***. In the process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by ***[please describe the detection instrument(s)]***.

3) ***Control Material(s) to be Used with [test name]:***

List all control materials (provided with the test kit and/or required but not provided with the test kit and describe what they are, how they are expected to work, where in the testing process they are used, and the frequency of use. If a control is commercially available, provide supplier's name and catalog number or other identifier; if your device relies on external controls that are manufactured by a third party please note that these controls should also be validated within your analytical and clinical studies described below in Section J.

Controls that will be provided with the test kit include:

a) A "no template" (negative) control is needed to ***[describe need]*** and is used ***[describe use – please also specify frequency of use]***

b) A positive template control is needed to ***[describe need]*** and is used ***[describe use – please specify the concentration of the positive control relative to the LoD of your test (note that ideally the positive control concentration should be such that it is close to the LoD of your test) and also specify frequency of use]***

c) An extraction control ***[describe control]*** is needed to ***[describe need]*** and is used ***[describe use – please also specify frequency of use].*** **Please note that if the no template control and positive control, are taken through the entire sample processing procedure, including the extraction, then a separate extraction control is not required**.

d) An internal control ***[describe control]*** is needed to ***[describe need]*** and is used ***[describe use]***.

Controls that are required but not provided with the test kit include ***[describe control – provide recommended sources of the control materials – either a separate control kit for purchase that you the applicant develops or a control material that can be purchased from a third party].*** This/these control(s) is/are needed to ***[describe need]*** and is used ***[describe use – please also specify frequency of use]***.

Please note that any control recommended to be used with your device (provided with the kit or not) should be validated in the context of your analytical and clinical study (i.e., you will need to run these controls as part of your studies). In instances where control material is not readily available through 3rd party vendors (which is often the case at the beginning of an outbreak) FDA may request that you include suitable control material with your device. Please note that external control materials are considered particularly important when GMP requirements are waived and reagent stability studies are limited.

H. INTERPRETATION OF RESULTS

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted. ***Please describe if a Ct cutoff is used as part of your testing algorithm and/or if the end user is required to review fluorescent curves for weakly positive samples before final interpretation. Although not typical for molecular-based tests, if the test result involves the use of an algorithm/calculation, for example a ratio value,***

when determining the final patient test result, please include a detailed description and any additional calibration materials that may be required.

1) ***[Test name] Controls – Positive, Negative and Internal***

   Describe in detail the expected results generated, including acceptance criteria, for all the controls described in detail in Section G above. Describe the measured values (if applicable) for valid and invalid controls and outline the recommended actions the laboratory should take in the event of an invalid control result.

2) ***Examination and Interpretation of Patient Specimen Results:***

   Describe when clinical specimen test results should be assessed and outline the criteria for test validity. Example text: Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.

   Clearly indicate how to interpret numeric test values (if applicable) as positive or negative for presence of the SARS-CoV-2***. Indicate if the end user is required to review fluorescent curves for weakly positive samples before final interpretation and how to identify indeterminate/inconclusive results (if they exist) results and how the user should resolve them, e.g. if repeat testing may be required.***

   When applicable, provide a table clearly describing the possible combinations of test result values for each primer/probe set, and how they should be combined into a final interpretation of the result for your test. If the test produces result that will be used as part of a CDC recommended testing algorithm, please indicate what follow-up testing/ process should be conducted, if applicable.

I. PRODUCT MANUFACTURING

1) ***Overview of Manufacturing and Distribution:***

   The product will be manufactured at [***manufacturer's name and FDA registration number (if applicable) or laboratory name if an LDT***] by [***manufacturer name, or laboratory name if an LDT***] personnel consistent with practices for the production of **[*types of devices*]** based on [***type of quality system****]. Material manufactured by [***manufacturer's name, or laboratory name if an LDT***] may be bottled and kitted by [***packager name***] manufacturing facility.

   The current manufacturing capabilities include the ability to manufacture approximately **[*please insert the approximate number of units/products that can currently be manufactured per week at the manufacturing facility*]** products per week, however in the event of a surge in demand this could be increased to **[*please insert the approximate*

***maximum number of units/products that could potentially be manufactured per week at the manufacturing facility if there was a surge in demand*]** product per week within a **[*please specify in weeks/months the expected timeframe required to increase product production if required*]** timeframe.

The product will be distributed by **[*please describe the distribution plan for the product and list all current distributors*]**.

2) ***Components Included with the Test***

Components manufactured by [***manufacturer's name and FDA registration number (if applicable)***] and supplied with the test include:
List all components and reagents provided for your test, including a description of the primers and probes, volumes, concentrations, quantities, buffer components, etc.

3) ***Components Required But Not Included with the Test***

Components required but not included with the test:
List all components and reagents not included with the test that must be supplied by the user to perform the test, with specific supplier names and catalog numbers or other identifiers for obtaining these components and reagents. Please include here all specific consumables that were validated for use with your device, that are not interchangeable with other products and that are needed to guarantee device performance as established in the EUA validation studies listed in Section J below.

4) ***Testing Capabilities***

Briefly describe current sample throughput capacity, total time required to perform the test (from clinical specimen collection, specimen transport to result), and number of tests that can be performed per instrument run and per day.

5) ***Reagent Stability:***

Briefly describe stability test plan for reagents and include accelerated stability information, if available. Please note that reagent stability studies do not need to be completed at the time of EUA issuance, however the study design should be agreed upon during interactive review and the stability studies started immediately following authorization, if not before. You should consider the following recommendations when designing your stability study:

- For EUAs you may follow the current FDA recognized CLSI Standard EP25 – Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline when evaluating the suitability of stability study designs. If you are planning to pursue a De Novo/510(k) for your device we recommend discussing in more detail your stability design to facilitate potential use of the EUA data in your regular premarket submission.

- We recommend testing a known positive diluted patient sample at 3-5x LoD rather than positive control material to establish reagent stability.
- If you are claiming multiple clinical specimen types in which similar LoDs are determined, you should use the most challenging clinical matrix for this study.
- We typically recommend your stability study design includes the evaluation of at least 5 replicates. You should also evaluate, if available, 3 different lots of reagents.
- You should design your study to provide data for a timeframe that is about 10% longer than the one to be claimed – for example; a claim of 18 months should be supported by stability data out to 20 months and a claim of 7 days should include stability data out to 8 days.
- FDA considers 15-30°C to represent room temperature conditions. Ideally you should evaluate stability at both 15°C and 30°C, however, for the purposes of the EUA evaluation at 30°C is acceptable as the worse-case scenario.
- Shelf-Life Stability- Unopened kit:
    - You should evaluate real-time kit stability studies with unopened kits stored at the claimed storage temperature for your test.
    - Accelerated stability evaluations for unopened kits is acceptable for EUA submissions while the real-time studies are on-going. However, please note real-time stability data is required to support regular pre-market submissions and for the final claim of an EUA.
- Shipping Stability - Unopened kit: Study should evaluate the anticipated handling and shipping times and temperatures expected for unopened kits.
- In-use/Opened Kit Stability: Depending on your device your stability study design should also support in-use stability of the kit reagents once the kit has been opened, e.g., storage at 2-8 ºC for 7 days. This includes on board stability once reagents have been placed on the instrument (if applicable).
- Inverted stability (if applicable): Study should support inverted stability for of kits.
- Freeze-thaw Stability: If you recommend aliquoting the reagents to meet the end-users needs following the initial thaw this recommendation should be supported by a freeze-thaw stability study, including the specific number of allowed freeze-thaw cycles.
- FDA analysis recommendations for real time stability studies are as follows:
    - Baseline of the study (t=0 of stability study) should not exceed a month from bottling

- Clear baselines should be described (e.g., a month from bottling) for each stability claim under each study
- Claims should be determined based on regression analysis. Any %change (%shift) from time zero (baseline) should be calculated between the target claim and the zero-time as (Ttest-Tbaseline)/ Tbaseline*100 with 95%CI using the regression equation obtained from plotting the mean values. When formulating your acceptance criteria for evaluating the shift from baseline you should consider the reproducibility of your device. However, generally, that the shift at the target claim due to storage cannot exceed 10-15%. The target stability is the next to last tested point that was within +/- 10% of time zero.
- Acceptance criterion may be different, depending on the test samples analyte concentration distribution in the intended use population and the risk, in other words, the impact of false results to public health.

J. PERFORMANCE EVALUATION

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

1) ***Limit of Detection (LoD) - Analytical Sensitivity:***

You should determine the LoD of the device utilizing the entire test system from sample preparation to detection. 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, FDA believes that viral genomic RNA is the next best material to use to generated contrived samples for testing. As positive natural clinical specimens are increasingly becoming available, a known positive clinical specimen as determined by an EUA test can also be used in generating dilutions in artificial or real clinical matrix for LoD determination. FDA recommends that preliminary LoD be determined by testing a 2-3 fold dilution series of three replicates per concentration. The final LoD concentration should be confirmed by testing 20 extraction replicates. FDA defines LoD as the lowest concentration at which 19/20 replicates are positive. If multiple clinical matrices are intended for clinical testing, you should submit to FDA the results from one representative matrix of each claimed clinical matrix type. 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, FDA recommends that you follow the most current version of the CLSI standard, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures (CLSI EP17).
[Please describe your LoD study, the specific material used (e.g., live or in-activated viral stocks, viral RNA), 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 your Inclusivity study and confirm that there was 100% detection of all SARS-CoV-2 strains.]

3) ***Cross-reactivity (Analytical Specificity):***

Cross-reactivity studies are performed to demonstrate that the test does not react with related pathogens, high prevalence disease agents and normal or pathogenic flora that are reasonably likely to be encountered in the clinical specimen. The recommended list of organisms to be analyzed in silico and by wet testing is provided in the table below. For wet testing, concentrations of 10^6 CFU/ml or higher for bacteria and 10^5 pfu/ml or higher for viruses is recommended. In silico analyses alone may be acceptable for organisms that are difficult to obtain. 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.

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 the circulating area
Human coronavirus 229E	Adenovirus (e.g. C1 Ad. 71)
Human coronavirus OC43	Human Metapneumovirus (hMPV)
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

Microbial Interference Studies: ***If in silico analysis reveals ≥ 80% homology between the cross-reactivity microorganisms and your test primers/ probe(s), we recommend that you either perform (1) a microbial interference study with SARS-CoV-2 and the microorganisms that your test primers/ probe(s) have homology to, or (2) as an alternative to the microbial interference study, you may provide justification as to why (e.g., amount of primer(s)/ probe(s) included in your master mix) the performance of your test would not be impacted by the presence of a causative agent of a clinically significant co-infection, or (3) explain why the in silico results are clinically irrelevant (e.g., low prevalence of MERS-CoV, etc.). Competitive microbial interference testing should be conducted for multiplex panels. The study should assess the effects of clinically relevant co-infections by testing selected microorganisms commonly found in***

respiratory tract in the presence of SARS-CoV-2 at low concentration. The interference should be evaluated testing at a minimum 3 replicates of each sample spiked at a low (≤3x LoD) SARS-CoV-2 concentration and a high interferent level (preferably microorganisms), to represent the worst-case scenario. The interferent microorganisms can be tested individually or as a pool (of four or five) in the presence of low concentration of SARS-CoV-2. Each microorganism of a pool should be tested individually, if that pool shows interference. If you plan to claim both upper and lower respiratory clinical specimens, the study should be performed in the most challenging specimen matrix, i.e., sputum. If interference is observed at the level tested, an additional titration study should be performed to determine the highest microorganism interferent level your test can tolerate.

Endogenous Interference Substances Studies: ***The extent of testing for endogenous interference substances depends on the matrix that is claimed for the device as well as on the technology of the device, e.g., if a nucleic acid extraction procedure is performed prior to testing or not. If your test uses extraction methods not previous reviewed by FDA as part of premarket submission or the test does not use an extraction procedure, we recommend testing of potential interferents. Please contact FDA to discuss the appropriate study designs.***

4) ***<u>Clinical Evaluation:</u>***

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 the 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) prospective samples from the individuals suspected of COVID-19 by their healthcare provider, (2) archived/retrospective respiratory samples collected from patients with signs and symptoms of respiratory infection, and (3) other subjects that are expected to be negative for SARS-CoV-2, such as specimens collected prior to COVID-19 pandemic in the US.***

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 performance. Please provide detailed information regarding the type of collection device and transport medium you propose to validate 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 discussion additional information that may be needed to support at-home sample collection and transport, please contact FDA at CDRH-EUA-Templates@fda.hhs.gov.

Sample stability

Please provide samples stability information, including the study design and results if the sample is shipped to a testing site from a location other than healthcare settings, e.g. samples collected at home.

Multiplex Panels Under EUA:

An emergency declaration by the HHS Secretary is typically specific for a pathogen/ disease (i.e., there is a publicly declared health emergency involving a particular etiologic agent). Therefore, the EUA pathway is only applicable for testing patients with signs/symptoms/risk of exposure for that single agent in a given emergency. FDA has occasionally allowed limited additional target pathogens to be claimed for EUA devices, but panel members should be

relevant to the event/disease outbreak subject of the specific emergency declaration. When determining whether to issue an EUA for a multiplex panel FDA takes into consideration the utility of the test (multiplex pathogen detection as an aid in differential diagnosis), clearance/ approval status of IVDs for the other panel members, whether the proposed Intended Use fits within the HHS emergency declaration and the how the multiplex panel would fit into current public health authority patient testing algorithm recommendations. We recommend you contact FDA for specific feedback if you plan to claim multiple target analytes as part of your EUA test. If it is determined that a multiplex test is beneficial to the emergency response, analytical and clinical evaluations for each of the target analytes should be provided.

Claiming Multiple Instruments and/or Extraction Methods:

FDA recommends the following analytical and clinical validation for use of multiple instruments and/or extraction methods where the elution volumes from the extraction methods and PCR volumes on the different RT-PCR instruments are identical.

- Limit of Detection (LoD): These studies should be repeated for each clinical matrix claimed in the Intended Use. Pick one RT-PCR instrument and determine the tentative LoD (using 5 replicates in 10-fold dilution) followed by the confirmatory LoD (20 replicates spiked at tentative LoD) for each extraction method on the chosen instrument. Note: If you detect 20/20 replicates in your confirmatory LOD study you should test the next lower concentration, using a 3-fold dilution, until you achieve a hit rate of <20/20.
    - If the different extraction methods yield the same LoD (≤3xLOD) on the RT-PCR instrument chosen for initial testing, pick one extraction method for further LoD determination on the remaining RT-PCR instruments and follow the recommendations below.
    - If the extraction methods do not yield the same LoD on the chosen RT-PCR instrument, please choose the extraction method with the worst LoD for further comparison of the LoD on all RT-PCR instruments.

  For all other RT-PCR instruments you should use the following adaptive LoD study design:
    - Please perform a refined tentative LoD study with 5 replicates at 0.5x, 1x, and 1.5 to 2x LoD. If you detect 4/5 replicates as positive at all the tested levels, you need to include the next higher concentration (i.e., 3x LoD). If you obtain 5/5 replicates at 0.5x LoD, you need to test the next lower concentration (i.e., 0.25x LoD). You will test in this manner until you find the lowest concentration that gives you 5/5 positive results for the tested RT-PCR instrument. This concentration should be used for a confirmatory LoD study for the given RT-PCR instrument using 20 replicates.

Final reported LoD: Please list all RT-PCR instruments with their respective LoDs if different LoDs are obtained. LoDs are considered comparable if they are between 1-3xLoD. These studies should be repeated for each clinical matrix claimed in the Intended Use.

- Interference Substances Studies (if applicable): FDA recommends evaluating interfering substances with the extraction method and RT-PCR instrument combination that has the worst overall LoD.

- Inclusivity Testing: FDA recommends evaluating inclusivity with the extraction method and RT-PCR instrument combination that has the worst overall LoD.

- Exclusivity Testing: FDA recommends evaluating exclusivity with any extraction/ instrument combination.

- Clinical study: If an LoD study confirms equivalency for all RT-PCR instruments (between 2-3xLoD), then the clinical study may be conducted with any RT-PCR instrument. If one or more RT-PCR instruments have different LoDs, we recommend conducting the clinical study with the extraction method / RT-PCR instrument combination with the worst LoD.

Note, if there are differences in the extraction input volume, extraction elution volume and PCR input volume (extracted nucleic acid) then the LoD should be confirmed for each.

K. UNMET NEED ADDRESSED BY THE PRODUCT

This section will be completed by FDA.

L. APPROVED/CLEARED ALTERNATIVE PRODUCTS

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

M. BENEFITS AND RISKS:

This section will be completed by FDA.

N. 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.***

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

Include Instructions for Use, Box Labels, Vial Labels and any other proposed labeling.

P. RECORD KEEPING AND REPORTING INFORMATION TO FDA:

[***Manufacturer name***] 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 as well as through the [***Manufacturer name***] Product Support website: [***Include link to Website***]. Each report of an adverse event will be processed according to [***Manufacturer name]***'s Non-Conformance Reporting Requirements, and Medical Device Reports will be filed with the FDA as required. Through a process of inventory control, [***Manufacturer name]*** will also maintain records of device usage/purchase. [***Manufacturer name]*** will collect information on the performance of the test, and report to FDA any suspected occurrence of false positive or false negative results of which [***Manufacturer name]*** becomes aware. [***Manufacturer name]*** 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.