Antigen Template for Test Developers[1]

This template (the "template") provides the Food and Drug Administration's (FDA) current recommendations concerning what data and information should be submitted to FDA in support of a pre- Emergency Use Authorization (EUA)/EUA submission for a SARS-CoV-2 antigen test. As outlined in Section V.B. 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 antigen assay: Limit of Detection/Analytical Sensitivity, Cross-reactivity/Analytical Specificity, Microbial Interference, and a Clinical Agreement Study. This template is intended to help test developers 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* mean 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 developer (sponsor) as applicable to their specific test. Text in **bold** outlines the FDA's additional recommendations for the sponsors' consideration when completing the suggested information in each 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.

[1] This template is part of the "Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency (Revised)," available at https://www.fda.gov/regulatory-information/search-fda-guidance-documents/policy-coronavirus-disease-2019-tests-during-public-health-emergency-revised.

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

[3] Available at https://www.fda.gov/regulatory-information/search-fda-guidance-documents/emergency-use-authorization-medical-products-and-related-authorities.

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 antigen from the SARS-CoV-2 in ***[add all claimed specimen types, e.g., nasopharyngeal/ oropharyngeal swabs, sputa, BAL, and serum, etc.]*** from patients who are suspected of COVID-19 by a healthcare provider ***[within the first [insert number] days of symptom onset, or for screening of individuals without symptoms or other reasons to suspect COVID-19 infection, if applicable].*** Additional testing and confirmation procedures should be performed in consultation with public health and/or other authorities to whom reporting is required. Test results should be reported in accordance with local, state, and federal regulations.

If you plan to request authorization to test specimens collected with a home specimen collection kit, please refer to the Home Specimen Collection Molecular Diagnostic Template[4] ***and include any relevant information in this request.***

B. MEASURAND

Specific antigen(s) from the SARS-CoV-2 **[*please specify the targeted antigen(s)*]**.

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: QKP

F. PROPOSED INTENDED USE

1) **Intended Use: Example text is provided below for a qualitative antigen test, but could be adapted according to the specific emergency situation addressed by the device, proposed intended use population, testing sites, or performance characteristics. Please note that if you seek authorization for testing at point of care**

[4] All templates can be accessed at https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas#covid19ivdTemplates.

(POC) sites or for asymptomatic screening, you should provide data from your clinical validation studies to support such use.

[Test name] is a ***[specify test technology, such as lateral flow immunoassay***] intended for the qualitative detection of ***[protein name]*** antigen from SARS-CoV-2 in ***[describe all the specimen types]*** ***[with specific brand of transport media, as applicable]*** from individuals who are suspected of COVID-19 by their healthcare provider [***within the first [insert number] days of symptom onset or for screening of individuals without symptoms or other reasons to suspect COVID-19 infection, if applicable]***. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform ***[moderate complexity, high complexity, or waived tests. This test is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation.].***

Results are for the identification of SARS-CoV-2 ***[protein name]*** antigen. Antigen is generally detectable in ***[specimen type]*** during the acute phase of infection. Positive results indicate the presence of viral antigens, but clinical correlation with patient history and other diagnostic information is necessary to determine 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 should be treated as presumptive, and do not rule out SARS-CoV-2 infection and should not be used as the sole basis for treatment or patient management decisions, including infection control decisions. Negative results should be considered in the context of a patient's recent exposures, history, and the presence of clinical signs and symptoms consistent with COVID-19, and confirmed with a molecular assay, if necessary, for patient management.

The ***[test name]*** is intended for use by ***[include intended user, e.g., trained clinical laboratory personnel specifically instructed and trained 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 prescription use only
For in vitro diagnostic use only
For Emergency Use Authorization only

3) **Special Instrument Requirements:**

The ***[test name]*** test is to be used with the ***[list all instruments, software requirements, other applicable instrumentation, etc.].***

G. DEVICE DESCRIPTION AND TEST PRINCIPLE

Example text has been added under each of the sub-headings. 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. 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, and the specimen types for which you claim to have specific performance characteristics, as described below. Please indicate if the test uses biotin-Streptavidin/avidin chemistry in any of the steps for coupling reagents. Please specifically state if your device is intended to be used with viral transport media and if so, provide the specific brands of transport media with which you have validated your device.]**

   The ***[test name]*** is a ***[description of technology (e.g., lateral flow, etc.)]*** test. The ***[test name]*** is designed to detect antigen from the SARS-CoV-2 in ***[list all the specimens]*** from patients who are suspected of COVID-19 by their healthcare provider ***[within the first [number of days] of symptom onset or for screening of individuals without symptoms or other reasons to suspect COVID-19 infection, if applicable]***. The ***[test name]*** is validated for use from direct specimens testing without transport media and/or specimens with ***[specific brand]*** transport media.

2) **Description of Test Steps: [List and describe in detail all of the steps of the test sequentially, from specimen collection to assay report.]**

   1. ***[Step one]***
   2. ***[Step two]***
   3. ***Etc.…]***

3) **Control Material(s) to be Used with [test name]: List all controls 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) An external positive control is needed to ***[describe need]*** and is used ***[describe use – please specify the concentration of the positive control relative to the Limit of***

Detection (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 specify frequency of use]

b) An external negative control is needed to ***[describe need]*** and is used ***[describe use – please specify the composition of the negative control and specify frequency of use]***

c) A ***[other (e.g., sample adequacy, internal, etc.)]*** control is needed to ***[describe need]*** and is used ***[describe use – please specify the composition of the control and specify frequency of 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/are 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., your studies should include use of these controls). In instances where control material is not readily available through 3rd party vendors, FDA recommends that you include suitable control material with your device. Please note that external control materials are considered particularly important when good manufacturing practice (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. ***[If the test result involves the use of an algorithm/calculation 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 Others: [Describe in detail the expected results generated, including the 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 ***[test name]*** 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 detected or not detected for presence of COVID-19 antigen. If applicable, indicate how to identify indeterminate/inconclusive/equivocal results. When applicable, we recommend providing a table clearly describing the possible combinations of test result values for each detected antigen, if applicable, and controls. Describe how they should be combined into a final interpretation of the result for your test. If the test produces an equivocal or indeterminate result, please indicate what follow-up testing/process should be conducted.]

I. PRODUCT MANUFACTURING

The ***[test name]*** has been validated using only the components referenced in this submission and will not be changed without prior concurrence from the FDA.

1) Overview of Manufacturing and Distribution:

The product will be manufactured at ***[test developer's name and FDA registration number]*** by ***[test developer name]*** personnel consistent with practices for the production of ***[types of devices]*** based on ***[type of quality system].*** Material manufactured by ***[test developer's name]*** 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]*** products per week within a ***[please specify in weeks/months the expected timeframe to increase product production, if conditions warrant]*** 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 ***[test developer's name and FDA registration number]*** and supplied with the test include:

[List all components and reagents provided for your test, including a description of the reagents (including antibodies), volumes, concentrations, quantities, buffer components, etc.]

3) **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) **Software Validation: If you are introducing a system onto the market that has not been previously reviewed by FDA, we recommend providing evidence that the software has been validated to ensure that:**
   - **The inputs and outputs of the software are appropriate to fulfill the system and assay requirements;**
   - **All expected inputs produce the expected outputs for all functions critical for system operation; and**
   - **The system will be provided to the customer free of defects, or defects will be known and mitigated.**

   If this evidence is not available prior to authorization and the software and hardware have been designed and developed in a manner consistent with current GMPs (for additional information on the Quality System Regulation/Medical Device Good Manufacturing Practices, see https://www.fda.gov/medical-devices/postmarket-requirements-devices/quality-system-qs-regulationmedical-device-good-manufacturing-practices[5]), additional software validation documentation may be incorporated into the conditions of authorization. If changes which impact assay performance or safety and effectiveness of the system are needed to address validation failures post-authorization, an EUA supplement may be required under the conditions of authorization.

   If not available prior to authorization, FDA recommends the following evaluations be performed as soon as possible post-authorization and documentation kept on file and may be incorporated as a condition of authorization:
   - **Perform electromagnetic compatibility (EMC) testing to International Electrotechnical Commission (IEC) 60601-1-2 Edition 4.0:2014;**
   - **Evaluate cybersecurity of your system to ensure user and patient safety in the intended use environment;**
   - **Complete validation of all systems and software to ensure that all functions of the system perform as labeled. For more information on system validation please see the following FDA guidance documents and resources:**

[5] Available at https://www.fda.gov/medical-devices/postmarket-requirements-devices/quality-system-qs-regulationmedical-device-good-manufacturing-practices.

a. **Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices;**[6]
b. **General Principles of Software Validation;**[7]
c. **Device Software Functions Including Medical Applications;**[8]
d. **Cybersecurity;**[9]
e. **Off-The-Shelf Software Use in Medical Devices;**[10] **and**
f. **21 CFR 820.30.**

Below are some examples of tables which could be populated with information sufficient to fulfill the three bullets above. Text in the tables is provided as an example only. In the example, the tables are completed with system-appropriate information and a thorough functional description of the system software and instrumentation needed to fulfill the intended use of the test.

System specifications and validation example

Critical specifications: Description of the specification	**Evidence that the design of the system can fulfill the specification. This column should consist of system-level validation data.**
Optical system of each instrument sent to a user has sufficient dynamic range to appropriately differentiate between positive and negative test results	
Software displays appropriate result during test run	
If reader stores test result, software accurately stores and retrieves test results	
System has a defined lifetime where the user can expect the system to maintain performance as stated in the label	
Etc…	

Hazard analysis example

ID	Hazard	Adverse Effect	Severity	Potential causes of hazard	Risk mitigation measure	Risk of experiencing the hazard

[6] Available at https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-content-premarket-submissions-software-contained-medical-devices.
[7] Available at https://www.fda.gov/regulatory-information/search-fda-guidance-documents/general-principles-software-validation.
[8] Available at https://www.fda.gov/medical-devices/digital-health-center-excellence/device-software-functions-including-mobile-medical-applications.
[9] Available at https://www.fda.gov/medical-devices/digital-health/cybersecurity.
[10] Available at https://www.fda.gov/regulatory-information/search-fda-guidance-documents/shelf-software-use-medical-devices#:~:text=Off%2Dthe%2Dshelf%20(OTS,to%20run%20device%2Dspecific%20functions.

						after mitigation
1	Invalid result	Delay in returning test result	Low	User inserts cartridge incorrectly	Labeling noting correct orientation	Low
2	False result	Wrong result returned to user	High	Incorrect alignment of test strip and optics; test strip inserted in the wrong orientation	Mechanical design of reader input slot	Moderate
3	False negative result	Wrong result returned to user	High	User reads test strip too early; incubation time not sufficient	Labeling noting correct incubation time	Moderate
4	False result	Wrong result returned to user	High	Incorrect alignment of test strip and optics; control line misinterpreted	Software interprets data from optical system identifying a valid/invalid control	Moderate
5	False result	Wrong result returned to user	High	Control reaction intensity is misinterpreted	Software interprets data from optical system identifying a valid/invalid control	Moderate
6	False result	Wrong result returned to user	High	Analyte reaction intensity is misinterpreted	Software interprets data from optical system identifying a valid/invalid control	Moderate

5) **Testing Capabilities:** ***[Briefly describe current sample throughput capacity, total time 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.]***

6) **Reagent Stability:** ***[Briefly describe stability test plan for [test name] reagents and include any 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.]***

General recommendations for reagent stability study design:

- 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 clearance or approval 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.

- Please note that use of only the positive controls is not recommended for reagent stability evaluation because controls are usually formulated at a moderate positive level. For all the stability evaluations, in addition to your external positive and negative controls and the no-template control, you should include for the EUA evaluation at least one sample, which should be prepared by spiking negative clinical matrix at an analyte concentration of 3-5xLoD.
- 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.
- Please note we typically recommend your stability study design include 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, we believe 30°C is acceptable as it represents the worse-case scenario.
- Unopened kit Shelf-Life Stability:
    a. You should evaluate real-time kit stability studies with unopened kits stored at the claimed storage temperature for your test.
    b. Accelerated stability evaluations for unopened kits can be included for EUA submissions while the real-time studies are on-going.
- Unopened kit Shipping Stability: 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°C 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 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%

confidence interval (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, 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; and

- Acceptance criterion may be different, depending on the test samples analyte concentration distribution in the intended use population and the risk of false results to public health.

J. PERFORMANCE EVALUATION

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

1) **<u>Limit of Detection (LoD) - Analytical Sensitivity</u>: 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., irradiated virus) into real clinical matrix (e.g., nasal or nasopharyngeal (NP) swabs, bronchoalveolar lavage (BAL) fluid, sputum, etc.) for LoD determination. The use of recombinant antigen is not recommended for the LoD determination. It is recommended that test developers test a 2-3 fold dilution series of 3-5 replicates per concentration, and then confirm the final concentration with 20 replicates. FDA defines LoD as the lowest concentration at which 19 of 20 replicates are positive. If multiple matrices are intended for clinical testing, test developers should submit the results from one representative of each claimed clinical matrix to FDA. For example, if testing respiratory specimens (e.g., sputum, BAL, NP swabs, etc.), please submit results from one upper respiratory matrix and one lower respiratory matrix. The most challenging matrix of the claimed matrices should be tested. FDA considers NP swabs with and without your claimed viral transport media (VTM) to be the most challenging upper respiratory matrix and sputum to be the most challenging lower respiratory matrix, as applicable. If claiming other specimen types (saliva, blood, etc.), we recommend that you establish your LoD in each matrix, with and without transport media, as applicable. 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 your LoD study, the specific viral material used to assess the LoD (e.g., irradiated virus), and the LoD (with appropriate units) for your assay. Please provide the line data for the LoD study as part of your submission.]

   LoD studies determine the lowest detectable concentration of SARS-CoV-2 at which approximately 95% of all (true positive) replicates test positive. The LoD was determined by limiting dilution studies using characterized ***[please described samples used in the study, e.g. viral stocks]***.

   [List/describe the following in this section:

- ***Titers and strains of the SARS-CoV-2 stocks used for the LoD study and how the organism stocks were prepared and how the titers were determined.***
- ***The dilution factor and number of serial dilutions of the characterized SARS-CoV-2 that were tested to determine the LoD.]***

Serial dilutions of the characterized SARS-CoV-2 were then tested in ***[number of replicates]*** replicates. The lowest concentration at which all ***[number of replicates]*** replicates were positive was treated as the tentative LoD for each test. The LoD of each test was then confirmed by testing ***[number of replicates (at least 20 recommended)]*** with concentrations at the tentative limit of detection. The final LoD of each test was determined to be the lowest concentration resulting in positive detection of ***[number of positive replicates (at least 19 out of 20 replicates)]. [Include analysis of LoD results, indicating the final LoD for each test.]***

2) **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. We recommend that the organisms in the table below are wet-tested in negative clinical matrix; please contact FDA if you are unable to obtain specific organisms to discuss potential options and labeling mitigations. If multiple matrices are claimed, the most challenging should be used for cross-reactivity testing. For wet testing, concentrations of 10^6 CFU/ml or higher for bacteria and 10^5 pfu/ml or higher for viruses is recommended.**

Recommended List of Organisms for Respiratory Specimens

Other high priority pathogens from the same genetic family	High priority organisms likely in the circulating area
Human coronavirus 229E (Wet-testing)	Adenovirus (e.g. C1 Ad. 71) (Wet-testing)
Human coronavirus OC43 (Wet-testing)	Human Metapneumovirus (hMPV) (Wet-testing)
Human coronavirus NL63 (Wet-testing)	Parainfluenza virus 1-4 (Wet-testing)
MERS-coronavirus (Wet-testing)	Influenza A & B (Wet-testing)
SARS-coronavirus (Wet-testing)	Enterovirus (Wet-testing)
Human coronavirus HKU1 (In-silico (protein blast))	Respiratory syncytial virus (Wet-testing)
	Rhinovirus (Wet-testing)
	Haemophilus influenzae (Wet-testing)
	Streptococcus pneumoniae (Wet-testing)
	Streptococcus pyogenes (Wet-testing)
	Candida albicans (Wet-testing)

	Pooled human nasal wash – *representative of normal respiratory microbial flora* (Wet-testing)
	***Bordetella pertussis* (Wet-testing)**
	***Mycoplasma pneumoniae* (Wet-testing)**
	***Chlamydia pneumoniae* (Wet-testing)**
	***Legionella pneumophila* (Wet-testing)**
	***Staphylococcus aureus* (Wet-testing)**
	***Staphylococcus epidermidis* (Wet-testing)**
	***Mycobacterium tuberculosis* (In-silico (protein blast))**
	***Pneumocystis jirovecii* (PJP) (In-silico (protein blast))**

<u>Recommended List of Organisms for Blood Specimens</u>

Other high priority pathogens from the same genetic family	High priority organisms likely in the circulating area
Human coronavirus 229E (Wet-testing)	**Cytomegalovirus (CMV) (Wet-testing)**
Human coronavirus OC43 (Wet-testing)	**Epstein-Barr Virus (EBV) (Wet-testing)**
Human coronavirus NL63 (Wet-testing)	**Varicella Zoster Virus (VZV) (Wet-testing)**
MERS-coronavirus (Wet-testing)	**Parvovirus B19 (Wet-testing)**
SARS-coronavirus (Wet-testing)	**Human Immunodeficiency Virus – 1 (HIV-1) (Wet-testing)**
Human coronavirus HKU1 (In-silico (protein blast))	**Human Immunodeficiency Virus – 2 (HIV-2) (Wet-testing)**
	Hepatitis C Virus (HCV) (Wet-testing)
	Hepatitis B Virus (HBV) (Wet-testing)
	Herpes Simplex Virus-1 (HSV-1) (Wet-testing)
	Herpes Simplex Virus-2 (HSV-2) (Wet-testing)
	***Escherichia coli* (Wet-testing)**
	***Streptococcus pneumoniae* (Wet-testing)**
	***Streptococcus pyogenes* (Wet-testing)**
	***Staphylococcus aureus* (Wet-testing)**

	Staphylococcus epidermidis **(Wet-testing)**

3) ***Microbial Interference Studies: If cross-reactivity is not observed between your assay and any of the microorganisms listed above, you should conduct a microbial interference study. A microbial interference study demonstrates that false negatives will not occur when SARS-CoV-2 is present in a specimen with other microorganisms. You should prepare contrived specimens in your most challenging claimed matrix with SARS-CoV-2 and common organisms found in that matrix. You should provide a list of common pathogens or commensal organisms for your most challenging matrix as part of your submission.***

   If applicable, microbial interference should be evaluated using samples spiked at a low (3x LoD) SARS-CoV-2 concentration and a high interferent level (preferably microorganisms), to represent the worst-case scenario, with a minimum of 3 replicates. The interferent microorganisms can be tested individually or as a pool (of 4-5); each microorganism should be tested individually if that pool shows interference. If you plan to claim both upper and lower respiratory matrices, the study should be performed in the most challenging respiratory 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.

4) ***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. For respiratory specimens, please test the following substances listed in the table below with inactivated virus at 3xLoD.***

Substance	**Concentration**
Whole Blood	4%
Mucin	0.5%
Chloraseptic (Menthol/Benzocaine)	1.5 mg/mL
Naso GEL (NeilMed)	5% v/v
CVS Nasal Drops (Phenylephrine)	15% v/v
Afrin (Oxymetazoline)	15% v/v
CVS Nasal Spray (Cromolyn)	15% v/v
Zicam	5% v/v
Homeopathic (Alkalol)	1:10 dilution
Sore Throat Phenol Spray	15% v/v
Tobramycin	4 µg/mL
Mupirocin	10 mg/mL
Fluticasone Propionate	5% v/v
Tamiflu (Oseltamivir Phosphate)	5 mg/mL

5) ***High-dose Hook Effect*: *A high-dose hook effect refers to the false negative result which can be seen when very high levels of target are present in a tested sample. We recommend you conduct studies to evaluate if a hook effect occurs by testing increasing antigen concentrations and, if applicable, indicate the concentration which begins to affect assay performance.***

6) ***Specimen Stability*: *Testing should be conducted to demonstrate specimen stability throughout the real-world conditions in which they are collected and tested, according to your instructions for use. When the test is intended to be performed on the specimen immediately or shortly after obtaining the specimen, specimen stability testing could be relatively short (i.e., 2 hours at room temperature) and conducted with contrived specimens at 3xLoD using inactivated virus. If you intend to test retrospective clinical specimens that have been frozen, you should also conduct fresh versus frozen studies to support use of these specimens.***

7) ***Clinical Evaluation*: *Use of natural clinical specimens is needed for the clinical evaluation. You should not use contrived clinical specimens as FDA believes they are inadequate to support the clinical performance of a test of this type. You should confirm the performance of your assay by testing a minimum of 30 positive specimens and 30 negative specimens in a randomized blinded fashion. We recommend only using an EUA test with high sensitivity and reverse transcription polymerase chain reaction (RT-PCR), which uses a chemical lysis step followed by solid phase extraction of nucleic acid (e.g., silica bead extraction) as the comparator method. The comparator method should be one of the more sensitive RT-PCR assays authorized by FDA. We encourage you to review the results from the FDA SARS-CoV-2 Reference Panel available here when selecting your comparator method; we strongly recommend you contact us to discuss your choice of comparator assay.***

Specimens may be prospectively or retrospectively collected. For prospective specimen collection, patients should be sequentially enrolled and tested blindly. Please contact us to discuss any proposed enrichment strategies. We believe it may be appropriate to use retrospective clinical specimens, but they should be randomized with negative specimens and tested blindly. Retrospective specimens should be reflective of the natural distribution of SARS-CoV-2 viral loads, and approximately 10-20% of the clinical specimens should be low positives (i.e., RT-PCR Ct counts >30), as has been observed in other sequentially enrolled clinical studies. Please note that FDA has observed erroneous results in association with clinical specimens stored in transport media. Therefore, if you validate your assay using 30 positive and 30 negative retrospective specimens in transport media, you should also present the results from at least five positive clinical specimens that are prospectively collected and processed directly without transport media. The remainder of the 30 positive clinical specimens

tested directly could potentially be provided post-authorization and incorporated as a condition of authorization. When collecting specimens, the standard of care specimen should be collected first. Swabs taken from the same anatomical area for the comparator and subject device (e.g., nasal swabs, oropharyngeal (OP) swabs, etc.) should be randomized to ensure that bias is not introduced due to an unequal distribution of viral materials. When two distinct anatomical sites are being assessed, FDA does not believe it is necessary to randomize specimen collection order (e.g., saliva compared to NP swabs).

If you intend to seek a claim for saliva, oral fluid, blood, or other specimen types, you should test at least 30 positive specimens with paired polymerase chain reaction (PCR) results from an NP swab.

[When you describe your clinical study please indicate/include:

1. ***Clinical study protocol, including collection and testing sites, number of samples collected, and number of operators used to run your assay***
2. ***Enrollment criteria (inclusion/exclusion criteria)***
3. ***The name of the comparator assay***
4. ***How the samples were collected or sourced***
5. ***Please describe the total number of samples tested. If the study was not a prospective study, please also list the numbers of prospective, and/or retrospective tested by each category.***
6. ***The sample matrix(ces) tested***
7. ***The technique and collection device(s), including transport media, used to obtain clinical samples. All clinical specimens tested in your study should be evaluated in accordance with your proposed diagnostic algorithm, including retesting when appropriate.***
8. ***The conditions used to collect and store specimens***

Please provide the study data in an Excel file as part of the EUA submission for each specimen. The study data should include the following:

- ***Specimen type for the antigen test***
- ***Specimen collection date and time for the antigen test***
- ***Specimen testing date and time for the antigen test***
- ***VTM type, as applicable, for the antigen test and PCR***
- ***Antigen test result with the analyzer or reader value***
- ***Specimen type for RT-PCR***
- ***Specimen collection date and time for RT-PCR test***
- ***Specimen testing date and time for RT-PCR test***
- ***Name of RT-PCR used as the comparator***
- ***RT-PCR test results (+/-)***
- ***RT-PCR test value results (Ct values)***
- ***Number of days post-onset of patient symptoms***
- ***Patient age and gender, if available***

For clinical specimens collected to support the EUA request, you must adhere to all applicable rules of human subject protection, including IRB approval consistent with 21 CFR part 50 and 21 CFR 56.103(a). 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).

Tests should demonstrate a minimum sensitivity of ≥ 80% for all sample types submitted.

Strategies for serial testing with less sensitive tests, such as 70% sensitivity, could increase overall sensitivity and be considered cumulatively rather than based on one-time testing. If you are proposing serial testing as a mitigation for a less sensitive assay, you should provide supportive evidence documenting clinical performance ≥ 80% sensitivity with serial testing. You should provide detailed instructions for conducting serial testing in your package insert, including a recommended testing interval, that is supported by your clinical data. You should also discuss how you will ensure compliance with serial testing post-authorization, such as multi-test packs, software applications, or other mitigations. Additional post-authorization studies may be necessary to assess the success of your proposed mitigations.

8) ***Studies to support a POC claim, as applicable: [If the device is intended for near patient or POC testing, please provide data to demonstrate that non-laboratory personnel can perform the test accurately in the intended use environment (i.e. a non-laboratorian healthcare provider accuracy study). Please also provide data to demonstrate robust use of your device for POC patient testing (e.g., as applicable, studies to demonstrate the impact of adding different volumes of sample, different volumes of reagents, incorrect order of sample or reagent application, etc.).]***

   a. ***POC Clinical Evaluation: A POC clinical evaluation should include 5-6 minimally trained operators at 1-2 non-laboratory (e.g., CLIA waived) sites in the United States. Each operator should test at least three positive and three negative specimens using only the test instructions and/or quick reference guide. As part of the EUA application, provide the detailed individual replicate result data and protocols for each of your studies, including:***
      - ***The objective of the study***
      - ***Detailed test procedure***
      - ***Materials used***
      - ***A list of samples tested***
      - ***Results (presented in tabular format), including invalid results***
      - ***Conclusions***
      - ***Mitigation measures, if required (e.g., labeling changes, changes to test design, etc.)***

You should also conduct testing with samples prepared with SARS-CoV-2 viral load near the LoD of your assay in clinical matrix. The testing should be conducted by minimally trained operators, and should consist of 10 low positives (<2xLoD) and 10 negative specimens per site. All contrived specimens should be blinded and randomized and each operator should test at least three low positive and three negative specimens. These specimens are intended to supplement, not replace, the clinical specimens in your study.

All testing should be conducted in a blinded fashion in which patients with an unknown SARS-CoV-2 status are presented to minimally trained operators. We recommend only using a high sensitivity EUA RT-PCR test that uses a chemical lysis step followed by solid phase extraction of nucleic acid (e.g., silica bead extraction) as the comparator method. The comparator method should be one of the more sensitive RT-PCR assays authorized by FDA. We encourage you to review the results from the FDA SARS-CoV-2 Reference Panel available here when selecting your comparator method; please contact us to discuss your choice of comparator assay. Ideally, patients should be prospectively enrolled and tested sequentially and blindly. It may be acceptable to use retrospective clinical specimens (e.g., leftover specimens in VTM), provided they are randomized with negative specimens and tested blindly. Retrospective specimens should be reflective of the natural distribution of SARS-CoV-2 viral loads, and approximately 10-20% of the clinical specimens should be low positives (i.e., RT-PCR Ct counts >30) as has been observed in other sequentially enrolled clinical studies. Please note that FDA has observed erroneous results in association with clinical specimens stored in transport media. Therefore, if you validate your assay using retrospective specimens in transport media, you should also present the results from at least five positive clinical specimens that are prospectively collected and processed directly without transport media. The remainder of the 30 positive clinical specimens tested directly can be provided post-authorization and incorporated as a condition of authorization.

When collecting specimens for the investigational device and the comparator method, the standard of care specimen should be collected first, so as not to compromise the medical care of the patient. After the standard of care specimen has been collected, swabs taken from the same area for the comparator and subject device (e.g., nasal swabs, OP swabs, etc.) should have their order of collection randomized to ensure that bias is not introduced due to an unequal distribution of viral materials. Randomizing order of collection when two distinct anatomical sites are being assessed may not be needed (e.g., saliva compared to NP swabs).

Your study data should be presented in an Excel file and include the same data elements outlined in item 7 above.

***b. POC Flex Studies**: You should also conduct a thorough hazard analysis considering the main known sources of errors. Based upon your hazard analysis, you should conduct flex studies to evaluate the impact of errors, or out-of-specifications conditions, on the assay performance. Each sample should be prepared at 2xLoD in negative clinical matrix and should be evaluated in three replicates for each condition under evaluation. Flex studies can be conducted with trained operators at an internal testing site. Each study should be performed using a pre-defined study protocol that includes the following:*

- *The objective of the study*
- *Detailed test procedure*
- *Materials used*

Potential stress conditions include:

- *40°C and 95% room humidity (RH) (mimicking hot and humid climates)*
- *Delay in sample testing or reading time*
- *Delay and/or disturbance in operational steps*
- *Sample volume variability*
- *Buffer volume variability*
- *Other, as appropriate*

Please see Appendix A for more in-depth flex study designs. Alternative sources of information on flex studies that may be appropriate for your device can be found on the FDA CDRH website containing CLIA Waiver by Application Decision Summaries.[11]

***9) Studies to support asymptomatic claim, as applicable**: If you seek authorization for screening individuals without symptoms or other reasons to suspect COVID-19, FDA recommends that you conduct a clinical study in the intended population. In the clinical study, you should compare results from your assay and a comparator assay for each patient enrolled. In addition to the clinical study recommendations listed above, you should enroll at least 20 positive asymptomatic individuals to collect unique specimens. It may also be acceptable to present the results from 10 positive specimens from asymptomatic individuals to support EUA authorization, provided data from symptomatic individuals are also submitted and analysis of cycle threshold (Ct) values demonstrates reasonably similar distribution of viral loads. The remainder of the 20 positive clinical specimens collected from unique asymptomatic individuals can be provided post-authorization and incorporated as a condition of authorization. Ideally, the specimens should be sequentially collected in a blinded fashion, and therefore, the total sample size of your study will depend on the prevalence of SARS-CoV-2 in your study population. You can also consider use of an enrichment strategy, in which individuals with a known COVID-19 infection status are invited to participate in your clinical validation study. If using an enrichment strategy, you should carefully consider*

[11] Available at https://www.fda.gov/about-fda/cdrh-transparency/clia-waiver-application-decision-summaries.

how you will randomize and blind operators to the participant's infection status and minimize potential bias. As discussed previously, FDA anticipates that any data from an enriched study design will also represent the full range of viral loads, with both low and high positives specimens included in the study data. It may also be possible to use archived specimens. Please contact FDA to discuss any alternative study designs.

10) ***<u>Studies to support multi-analyte respiratory panels under EUA</u>: To support an EUA for a multi-analyte respiratory panel, analytical and clinical evaluations for each target analyte should be provided. The validation needed to support an EUA may vary if the test platform was previously cleared by FDA, as noted below. The following analytical studies should be conducted, and data provided to the FDA for review:***

- ***Limit of Detection (Analytical Sensitivity)***
- ***Cross-Reactivity / Microbial Interference***
- ***Inclusivity / Analytical Reactivity, when different strains become available***
- ***Co-infection (Competitive Interference)***
- ***Interfering Substances Study (Endogenous and Exogenous)***
- ***Clinical Specimen Stability***
- ***Reagent Stability testing protocol***
- ***Carry over/Cross-Contamination****
- ***Reproducibility and Repeatability****
- ***Fresh vs. Frozen - If you intend to submit data collected from testing archived frozen specimens in support of your EUA, please conduct an analytical study to demonstrate that preservation of samples (e.g., by freezing at ≤-70°C) does not affect the accuracy of test results compared to freshly collected samples.***
  **** Recommended for new instruments not previously reviewed by the FDA. If the instrument was previously reviewed by FDA, please identify the submission number.***

  For multiplex devices that include Influenza A and/or Influenza B, you should establish the LoD on at least two strains of Influenza A and Influenza B. We recommend using the H12009 and H3N2 for Influenza A and the Yamagata and Victoria lineages for Influenza B.

  Clinical Performance: To evaluate the clinical performance of your multi-analyte test, a prospective clinical study should be conducted. Considering the public health needs in the current emergency, a clinical performance study in support of the EUA submission may be conducted at one site testing positive and negative clinical samples with known specimen types. Retrospective clinical specimens can be used, however, your positive samples should include results from an EUA RT-PCR device for COVID-19, or an FDA cleared RT-PCR device in which 10-20% of the specimens are low positive samples (Ct value > 30).

  If your device has not been previously FDA-cleared for influenza or other respiratory pathogens, FDA generally intends to include a condition of

authorization that you will conduct a post EUA prospective clinical study. The prospective clinical study should include a minimum of three sample collection sites and three testing sites, prospectively enrolling patients with general respiratory symptoms. You may consider conducting a prospective clinical study in the Southern Hemisphere during their typical influenza/respiratory season to increase the likelihood of obtaining a sufficient number of positive samples (e.g., for influenza at least 50 positive Flu A and 30 positive Flu B samples) in a timely fashion.

If your device has been previously FDA-cleared for influenza or other respiratory pathogens, you should confirm that the LoD for the previously cleared pathogens is unchanged due to the modification by conducting side-by-side testing of 3-5 replicates of serially diluted viruses with the original and modified versions of your device. You should also test at least 10 retrospective positive clinical specimens of each previously cleared analyte. You should perform a competitive inhibition study with clinically relevant titers of each analyte in the panel (viruses 10^5 PFU/mL, bacteria 10^6 CFU/mL).

All multiplex devices that include Influenza A and B should also verify the analytical reactivity of the device against the Centers for Disease Control and Prevention (CDC) human influenza panel to ensure adequate performance against currently circulating strains of influenza. Information on how to request this panel can be found at CDC's "Request for CDC Influenza Virus Panel" website.[12]

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:

[You should include proposed Fact Sheets for Patients and Healthcare Providers] **S*ee examples for authorized EUA tests on our website. During review, FDA will make available Fact Sheet templates.***

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

[You should include Instructions for Use, Box Labels, Vial Labels, and any other proposed labeling.]

[12] Available at https://www.fda.gov/about-fda/cdrh-transparency/clia-waiver-application-decision-summaries *(last accessed on September 22, 20209). Note this website is not controlled by FDA.*

P. RECORD KEEPING AND REPORTING INFORMATION TO FDA:

[Test Developer 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 ***[Test Developer name]*** Product Support website: ***[Include link to Test Developer's Website].*** Each report of an adverse event will be processed according to ***[Test Developer name]***'s Non-Conformance Reporting Requirements, and Medical Device Reports will be filed with the FDA as required. Through a process of inventory control, ***[Test Developer name]*** will also maintain records of device usage/purchase. ***[Test Developer 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 ***[Test Developer name]*** becomes aware. ***[Test Developer 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.

Appendix A: Sample Flex Study Design Details.

1) **Delay in Reading Time: We recommend evaluating test results at reading times four times below and three times above the recommended reading time. For example, for a test where the recommended read time is 20 minutes, reading time times would be performed to evaluate at least read times of 5, 10, 15, 20, 30, and 60 minutes.**

2) **Specimen Volume Variability: We recommend evaluating test results at specimen volumes two times below and two times above the recommended specimen volume, and the maximum possible added. For example, for a test where the recommended specimen volume is 10 µL, specimen volume testing should be performed to evaluate at least specimen volumes of 5, 10, 20 µL, and maximum volume. If incorrect results are observed at either 5 or 20 µL, additional testing at 7.5 and/or 15 uL may be needed. The diluent/buffer amount added should be that specified in the instructions for use.**

3) **Buffer Volume Variability: We recommend evaluating test results at diluent/buffer volumes at two times below and two times above the recommended diluent/buffer volume and the maximum volume. For example, for a test where the recommended buffer/diluent volume is 2 drops, sample diluent volume testing would be performed to evaluate at least sample diluent volumes of 1, 2, 3, 4 drops, and whole bottle. The sample volume added should be that specified in the instructions for use.**

4) **Temperature and Humidity: We recommend evaluating test results at temperature and humidity extremes that are likely to occur in the United States. For example, 40°C and 95% RH, mimicking hot and humid climates, and 5°C and 5% RH, mimicking cold and dry climates.**

5) **<u>Disturbance during analysis</u>: We recommend evaluating the effect on expected test results of moving the device while the test is running, such as dropping/moving the test, unplugging the test, receiving a phone call while the mobile app is running, etc.**