PRoDTEC
Puerto Rico Diagnostic Test Evaluation Commission

Antigen Template for Laboratories [1]

This template (the "template") provides FDA's and PRoDTEC's current recommendations concerning what data and information should be submitted to FDA in support of a pre-EUA/EUA submission for a SARS-CoV-2 antigen test. As outlined in Section V.C. of the FDA guidance document: *Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency (Revised)*,[2] FDA and PRoDTEC's 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, Clinical Agreement Study
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 laboratory (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.
- 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

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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, stool, and serum, etc.]*** from patients who are 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 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 may be adapted according to the specific emergency situation addressed by the device.**

[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]*** from individuals who are 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 moderate and high complexity tests, or by similarly qualified non-U.S. laboratories] and as applicable, Point of Care (POC) testing].***

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 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 note that if applicable, an in silico analysis against available reference protein sequences for different strains of the target pathogen is requested as part of the cross-reactivity evaluation (Section J).**

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.

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) A 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) A 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 used ***[describe use – please also specify frequency of use]***.

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 results.]**

2) **Examination and Interpretation of Patient Specimen Results: [Describe when clinical specimen test results should be assessed and outline the criteria for test validity.]**

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, provide 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. 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 swabs, 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 19/20 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, nasopharyngeal (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 nasopharyngeal (NP) swabs with and without VTM to be the most challenging upper respiratory matrix and sputum to be the most challenging lower respiratory matrix. 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 material used in-activated virus (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 describe 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. If you are claiming a non-respiratory matrix (e.g., blood, stool, etc.), please contact FDA to discuss the list of organisms recommended for cross-reactivity testing.**

Recommended List of Organisms

Other high priority pathogens from the same genetic family	High priority organisms likely in the circulating area
Wet-testing	
Human coronavirus 229E	Adenovirus (e.g. C1 Ad. 71)
Human coronavirus OC43	Human Metapneumovirus (hMPV)
Human coronavirus NL63	Parainfluenza virus 1-4
	Influenza A & B
	Enterovirus
	Respiratory syncytial virus
	Rhinovirus
	Haemophilus influenzae
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Candida albicans
	Pooled human nasal wash – *representative of normal respiratory microbial flora*
	Bordetella pertussis
	Mycoplasma pneumoniae
	Chlamydia pneumoniae
	Legionella pneumophila
In-silico (protein blast)	
SARS-coronavirus	*Mycobacterium tuberculosis*
MERS-coronavirus	*Pneumocystis jirovecii* (PJP)
Human coronavirus HKU1	

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. Microbial interference 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. Please 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 perfomed 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 see https://www.accessdata.fda.gov/cdrh_docs/reviews/K112177.pdf for examples of substances to test. Please contact FDA to discuss the appropriate study designs.*

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. Please 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) ***Matrix Equivalency (if applicable)**: A matrix equivalency study may be used to support certain specimen types that were not evaluated during the clinical study. Please note that LoD and clinical studies are required for all claimed matrices. The matrix in which the clinical studies are conducted is the comparator. All other matrices are to be shown to be equivalent to the comparator matrix. Please consult with FDA concerning an appropriate study design prior to initiating any studies.*

7) ***Specimen Stability**: A specimen stability study may be needed to support specimen stability claims if storage and transport of specimens is recommended in the instructions for use. Additional stability studies may be needed if freezing/thawing is expected.*

8) ***Studies to support Point of Care claim, as applicable: [If the device is intended for near patient testing or Point of Care (POC), 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 near 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.).]

9) **Clinical Evaluation: Use of natural clinical specimens is needed for the clinical evaluation. Use of contrived clinical specimens is not acceptable. You should confirm the performance of your assay with a series of clinical specimens by testing a minimum of 30 positive specimens and 30 negative specimens in a randomized blinded fashion. We recommend only using a high sensitivity EUA RT-PCR test which uses a chemical lysis step followed by solid phase extraction of nucleic acid (e.g., silica bead extraction) as the comparator method. Specimens may be prospective or retrospectively collected. If you intend to seek a claim for saliva or oral fluid, you should test at least 30 positive specimens with paired 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. If the device is anticipated for near-patient testing, please provide any relevant information regarding user training.***
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 line data in an Excel file as part of the EUA submission for each specimen (please indicate for each specimen if it was prospective, retrospective or contrived and the clinical matrix it represents); the data should include the signal values if applicable and available (for the specimen and the controls) and call/result for your device and, in the case of the natural clinical specimens, the comparator method results should be included.] ***For clinical specimens collected to support the EUA request, you should adhere to all applicable rules of human subject protection, including IRB approval.***

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

J. UNMET NEED ADDRESSED BY THE PRODUCT

This section will be completed by FDA.

K. APPROVED/CLEARED ALTERNATIVE PRODUCTS

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

L. BENEFITS AND RISKS:

This section will be completed by FDA.

M. 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. During review, FDA will make available Fact Sheet templates.

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

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

P. RECORD KEEPING AND REPORTING INFORMATION TO FDA:

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