PRoDTEC
Puerto Rico Diagnostic Test Evaluation Commission

Serology Template for Laboratories [1]

This template (the "template") includes FDA's and PRoDTEC's current recommendations for laboratories concerning what data and information they should submit to support an EUA request for a SARS-CoV-2 antibody test developed for use in a single CLIA certified high-complexity laboratory. 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 recommends that the following validation studies be conducted for a SARS-CoV-2 serological assay: Cross-reactivity/Analytical Specificity, Class Specificity, and 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

- This EUA review template (EUA template) is only intended for use by CLIA certified high-complexity laboratories who intend to submit a pre-EUA or EUA to FDA for a SARS-CoV-2 antibody test.
- Text highlighted in yellow ***[Text]*** should be completed by the laboratory (sponsor) as applicable to their specific test. Text in **bold** outlines the Food and Drug Administration's (FDA) recommendations for the sponsors' consideration when providing the suggested information in a specific section.
- Please be reminded that tests for the detection of antibodies against SARS-CoV-2 must not be distributed and/or used for clinical diagnoses.
- 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 OF SUBMISSION

Emergency Use Authorization (EUA) request for ***[test name]*** to be performed for the detection of ***[specify types of antibodies e.g., IgG, IgG/IgM or total]*** antibodies to SARS-CoV-2 in ***[specify matrices]*** from individuals with current or prior COVID-19 infection. The test will be performed in CLIA certified high-complexity laboratories. Additional testing and confirmation procedures should be performed in consultation with public health and/or other authorities to whom reporting is required. Positive results should also be reported in accordance with local, state, and federal regulations.

B. MEASURAND

[Specify what the test detects and whether it can differentiate between IgM and IgG or if the test detects total antibody without differentiation]

C. LABORATORY/SPONSOR

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

D. REGULATORY INFORMATION

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

E. PROPOSED INTENDED USE

1) Intended Use (IU):

The proposed IU will be finalized based on the data provided at the time of authorization. An example IU is provided below.

The ***[test name]*** is a ***[specify technology e.g., Enzyme-Linked Immunosorbent Assay (ELISA)]*** intended for qualitative ***[or semi-quantitative or quantitative tests, if appropriate validation data is provided. Performance evaluations beyond what is currently described in the template may be necessary]*** detection of ***[specify the antibody class or classes that are being detected, or indicate whether the test only detects total antibodies]*** antibodies to SARS-CoV-2 in human ***[specify matrices including anticoagulants]***. The ***[test name]*** is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. At this time, it is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity. The ***[test name]*** should not be used to diagnose acute SARS-CoV-2 infection. Testing is limited to ***[Name of Clinical Laboratory(s)]*** that are Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a certified high-complexity laboratories.

Results are for the detection of SARS CoV-2 antibodies. ***[Specify antibodies detected]*** antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, although the duration of time antibodies are present post-infection is not well characterized. Individuals may have detectable virus present for several weeks following seroconversion.

Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

[As applicable, the sensitivity of [test name] early after infection is unknown.] Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.

False positive results for ***[test name]*** may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

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) Instruments Used:

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

F. DEVICE DESCRIPTION AND TEST PRINCIPLE

1) Product Overview/Test Principle:

[Briefly describe the technology of the test and how this technology identifies the measurand (i.e., the test principle), and the instruments/reader employed/required to perform the test from sample collection to result and the specimen types for which you claim to have performance characteristics as described below.]

The ***[test name]*** uses the following: ***[List the antigen(s) and antibodies used in the assay to detect the antibodies in human specimens]***

2) Description of Test Steps: *[Describe in order the steps of the test from specimen collection to result output.]*

3) Control Material

[Please describe the assay controls to be performed in the laboratory, including the positive control for each antibody class the test is intended to detect, the negative control, and any other necessary controls. Please also describe the frequency with which controls will be performed.]

G. INTERPRETATION OF RESULTS

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 as positive or negative for the presence of antibodies against SARS-CoV-2. If applicable, indicate how to identify

indeterminate/equivocal results and how the user should resolve them. Also describe if and when repeat testing may be required.]

H. PERFORMANCE EVALUATION

1) Analytical Sensitivity and Specificity

a) Reactivity/Inclusivity:

Although mutations in the SARS-CoV-2 genome have been identified as the virus has spread, no serologically unique strains have been described relative to the originally isolated virus (this research is exceptionally limited at present).

b) Cross-Reactivity:

If a large number of known negative samples (e.g., ≥75 unique samples collected in the US prior to December 2019) are tested from a population with a high prevalence of vaccination against, and/or infection with, the following viruses, and specificity >95% is observed, cross-reactivity testing for the following viruses would not be expected at this time:

anti-influenza A (IgG and IgM)
anti-influenza B (IgG and IgM)
anti-HCV (IgG and IgM)
anti-HBV (IgG and IgM)
anti-Haemophilus influenzae (IgG and IgM)
anti-229E (alpha coronavirus)
anti-NL63 (alpha coronavirus)
anti-OC43 (beta coronavirus)
anti-HKU1 (beta coronavirus)
ANA
anti-respiratory syncytial virus (IgG and IgM)
anti-HIV

[If a large number of known negative samples are not evaluated, or lower than 95% specificity is observed, describe the cross-reactivity testing performed to evaluate the cross-reactants in the table above.]

If testing of the cross-reactants on above is needed to demonstrate cross-reactivity for the test, FDA believes testing a minimum of 5 individual samples for each disease/infectious agent/antibody class listed above may be acceptable.

If natural specimens are used, it is important to assess cross reactivity using sera from patients with the underlying diseases in the acute or convalescent stages of infection in order to obtain high levels of IgM or IgG for the underlying condition. If spiked samples with the IgM or IgG antibodies for the underlying conditions are prepared for this study, it is important to confirm that "negative samples" are SARS-CoV-2 IgM and IgG seronegative with the candidate assay prior to spiking. Additionally, commercially available IgM or IgG antibodies for the underlying conditions panels may be acceptable if

collected prior to the COVID-19 pandemic to ensure the panels are SARS-CoV-2 antibody negative.

We recommend you present your results in the following suggested table and calculate agreement between the candidate test result and the expected result.

Cross-Reactivity: *[test name]* example table for wet tested organisms below:

Virus/Bacteria/Parasite Antibody positive	Source/ Sample type	Results*

***If applicable, please include the signal output for your test's technology.**

[If your test exhibits significant cross-reactivity that would produce false positive results for any virus evaluated, please describe a plan to address this risk.]

2) **Class Specificity:**

If your test is intended for the detection of total antibody with no differentiation between different immunoglobulins, then this study does not apply. *[In this case, please indicate that this study is not applicable.]*

[If your test is intended for the detection of total antibody with no differentiation between different immunoglobulins, then this study does not apply. Please indicate not applicable.

If your test is intended to differentiate between different immunoglobulins, describe the approach used to evaluate class specificity.]

Approaches to evaluate class specificity depend on the assay format. If you have used well-characterized the anti-IgG and anti-IgM reagents in your test, class specificity testing may not be needed. In this case, FDA recommends describing how the reagents were characterized and how such characterization supports class specificity. One recommended approach includes treating the specimen with dithiothreitol (DTT) where the final IgG result will remain unaffected and the final IgM signal will decrease or be negative. A positive control should also be included that confirms DTT activity.

[If class specificity testing is needed for your test, please describe the study, or studies, performed to demonstrate that the assay accurately detects each antibody class (e.g., IgG and IgM). This should include a description of the studies performed to evaluate the potential for human IgM to cross react and therefore produce false positive results for IgG, and the reverse, and the potential for IgM to compete with IgG and produce false negative results. Please indicate the number of samples, and the number of replicates per sample, tested. FDA believes that evaluating at least 5 samples positive for both antibody classes (IgM positive while also IgG positive), in duplicate, may be acceptable. Please provide the protocol and results, including line data, from any class specificity testing.]

FDA believes that 100% agreement with expected result would establish antibody class specificity.

If a DTT Treatment approach is followed, below is an example table for IgM and IgG:

Sample ID	Replicates	Result NO DTT Treatment (IgM/IgG)	Result DTT Treatment (IgM/IgG)	Expected result with DTT treatment (IgM/IgG)	Result Agreement
1	1	+/+	-/+	-/+	yes
	2	+/+	-/+	-/+	yes
2	1	+/+	-/+	-/+	yes
	2	+/+	-/+	-/+	yes
3	1	+/+	-/+	-/+	yes
	2	+/+	-/+	-/+	yes
4	1	+/+	-/+	-/+	yes
	2	+/+	-/+	-/+	yes
5	1	+/+	-/+	-/+	yes
	2	+/+	-/+	-/+	yes

3) **Clinical Agreement Study:**

[Please describe the clinical study used to evaluate the clinical performance of the test. Please note that the exact requirements for the clinical evaluation depend on access to COVID-19 disease clinical specimens at the time of the studies and the nature of the emergency.]

Initial clinical agreement trials typically evaluate all matrices that the sponsor intends to claim in their EUA submission.

The comparator method used to establish clinical truth for the patient at this time is a PCR based assay. Results from the comparator PCR method are obtained using specimens that have been validated for use with the comparator method. Consider collecting nasal swab samples from a patient for PCR and then follow with a fingerstick or blood draw from the same patient. *[Please identify the PCR comparator that was used. If the PCR comparator is not an EUA-authorized test, please provide Limit of Detection (LoD) and cross-reactivity validation data. If it is an EUA-authorized test, then no validation is needed.]*

Ideally, performance characteristics are established in a clinical study with prospective samples. If a prospective study is not feasible, an acceptable alternative would be to test retrospectively collected SARS-CoV-2 antibody positive specimens from patients that have been previously confirmed infected by SARS-CoV-2 RT PCR, accompanied by basic information such as the population from which the sample was drawn and the comparator method, specimen collection date, date of onset of symptoms (if present/known), and

comparator method to confirm patients as SARS-CoV-2 infected or not infected (see above).

Clinical agreement data should be provided using at least 30 unique antibody positive samples, from 30 patients, for each immunoglobulin claimed and 75 unique antibody negative samples from patients tested for SARS-CoV-2 and confirmed as negative, or 75 unique samples collected prior to December 2019, and the data should demonstrate a minimum of the following:

For tests that detect and differentiate IgM and IgG:
Overall (i.e., combined IgM/IgG) positive percent agreement (PPA) of 90%, PPA for IgM of 70%, PPA for IgG of 90% and overall (i.e., combined IgM/IgG) negative percent agreement (NPA) of 95%.

For tests that detect either total antibodies, only IgG *or* only IgM:
PPA of 90% and NPA of 95%

If a larger number of unique samples are evaluated and the lower bounds of the 95% confidence intervals are higher than would be demonstrated in a clinical agreement study with 30 unique antibody positive and 75 unique antibody negative samples, the following may be acceptable:

- **Point estimates not lower than 93% for NPA**
- **For tests that detect *either* total, only IgG or only IgM, PPA not lower than 87%**
- **For tests that report IgM *and* IgG, PPA for combined IgM/IgG not lower than 87%, PPA for IgM not lower than 67%, and PPA for IgG not lower than 87%.**

Blinding and randomization should be included in the study design.

If a claim for fingerstick is desired, we believe evaluating a minimum of 30 antibody positive and 30 antibody negative fingerstick whole blood samples may be acceptable to demonstrate clinical performance in fingerstick samples.

[Please specify how the samples were generated, collected, and sourced. Please also specify if the samples were fully prospective, mix of prospective, retrospective and/or contrived. Please specify inclusion/exclusion criteria, collection and testing sites, number of samples collected and tested, and number of operators performing the testing, as available.]

[Please clearly describe the data analysis methods used and provide the results from the study, including line data. We suggest calculating positive and negative percent agreement between the candidate device and the comparator method results separately for each claimed matrix. FDA believes stratification of the data by the number of days since symptom onset should be provided. If the time between generation of the candidate device result and symptom onset is not known, stratifying by days since PCR result may be acceptable. FDA recommends providing the results using a tabular format, examples of which are provided below.]

For Total Antibody Tests:
The following table is an example of how you may present the total antibody positive percent agreement (PPA) by time of sampling from symptoms onset (in subjects confirmed to have been previously infected with SARS-CoV-2 by a prior positive PCR results):

		Candidate Device Results		
Days from Symptom Onset	Number of Samples Tested	Total Antibody Positive results	Total Antibody PPA	95% CI
0-7 days	A	a	a/A (%)	
8-14 days	B	b	b/B (%)	
≥15 days	C	c	c/C (%)	

The following table is an example of how you may present the negative percent agreement (NPA) using PCR negative samples or samples assumed to be negative since they were collected prior to December 2019:

	Candidate Device Results	
Number of Samples Tested	Negative Results	Overall NPA (95% CI)
D	d	d/D (%)

For test that detect and distinguish IgG and IgM antibodies:
If you claim that your test can differentiate between IgG and IgM, PPA and NPA for IgG and IgM should also be calculated separately, an example of which is below.

The following tables are examples of how you may present the IgG or IgM positive percent agreement (PPA) by time of sampling from symptoms onset (in subjects confirmed to have been previously infected with SARS-CoV-2 by a prior positive PCR results):

		Candidate Device Results		
Days from Symptom Onset	Number of Samples Tested	IgG Positive results	IgG PPA	95% CI
0-7 days	A	a	a/A (%)	
8-14 days	B	b	b/B (%)	
≥15 days	C	c	c/C (%)	

		Candidate Device Results		
Days from Symptom Onset	Number of Samples Tested	IgM Positive results	IgM PPA	95% CI
0-7 days	A	a	a/A (%)	
8-14 days	B	b	b/B (%)	
≥15 days	C	c	c/C (%)	

		Candidate Device Results		
Days from Symptom Onset	Number of Samples Tested	IgG/IgM Combined Antibody Positive results	IgG/IgM Combined Antibody PPA	95% CI
0-7 days	A	a	a/A (%)	
8-14 days	B	b	b/B (%)	
≥15 days	C	c	c/C (%)	

The following table provides an example of how you may present the negative percent agreement (NPA) using PCR negative samples or samples assumed to be negative since they were collected prior to December 2019:

	Candidate Device Results	
Number of Samples Tested	IgM/IgG Negative Results	NPA (95% CI)
D	d	d/D (%)

<u>For IgG only antibody tests:</u>
If you claim that your test can detect IgG only, PPA and NPA for IgG should be calculated, an example of which is below.

The following tables are examples of how you may present the IgG positive percent agreement (PPA) by time of sampling from symptoms onset (in subjects confirmed to have been previously infected with SARS-CoV-2 by a prior positive PCR results):

		Candidate Device Results		
Days from Symptom Onset	Number of Samples Tested	IgG Positive results	IgG PPA	95% CI
0-7 days	A	a	a/A (%)	
8-14 days	B	b	b/B (%)	
≥15 days	C	c	c/C (%)	

The following table provides an example of how you may present the negative percent agreement (NPA) using PCR negative samples or samples assumed to be negative since they were collected prior to December 2019:

	Candidate Device Results	
Number of Samples Tested	IgG Negative Results	IgG NPA (95% CI)
D	d	d/D (%)

For IgM only antibody tests:
If you claim that your test can detect IgM only, PPA and NPA for IgM should be calculated, examples of which are below.

The following tables are examples of how you may present the IgM positive percent agreement (PPA) by time of sampling from symptoms onset (in subjects confirmed to have been previously infected with SARS-CoV-2 by a prior positive PCR results):

		Candidate Device Results		
Days from Symptom Onset*	Number of Samples Tested	IgM Positive results	IgM PPA	95% CI
0-7 days	A	a	a/A (%)	
8-14 days	B	b	b/B (%)	
15-30 days	C	c	c/C (%)	

*Note that the days from symptom onset for IgM only antibody tests differ from the days from symptom onset used for IgG, IgG/IgM and total antibody tests.

The following table provides and example of how you can present negative percent agreement (NPA) using PCR negative samples or samples assumed to be negative since they were collected prior to December 2019:

	Candidate Device Results	
Number of Samples Tested	IgM Negative Results	IgM NPA (95% CI)
D	d	d/D (%)

4) **Matrix Equivalency**

[Please describe the protocol and results from any matrix equivalency studies performed to support the performance of the assay in claimed sample matrices (serum, EDTA plasma, venipuncture whole blood, different anticoagulants, etc.) that were not evaluated in the initial clinical agreement study. For assays with a numerical value result, FDA recommends providing a Deming regression analysis comparing the results obtained with the comparator matrix and the other matrices.]

Matrix equivalency studies are performed to evaluate specimen matrices for which clinical agreement isn't initially assessed. In these studies, the matrix in which the clinical study(ies) are conducted is the comparator matrix/specimen type and each matrix set (whole blood, plasma, serum) comes from the same donor (i.e., paired samples).

Typically, negative, low positive, and moderate positive samples are evaluated. We believe five samples, run in duplicate for each concentration for a total of 30 results per matrix (assuming 3 concentrations were evaluated) may be acceptable. To allow for comparison, negative samples for each claimed specimen type/matrix are spiked with the same amount of analyte (SARS-CoV-2 IgG and IgM). We believe confirming samples are antibody seronegative with the candidate assay before spiking with SARS-CoV-2 IgG and IgM antibodies is important.

For these types of studies, typically, each sample is assayed with the candidate device, and the results obtained for the comparator matrix are compared to the results obtained for each additional matrix under evaluation for each subject. Positive percent agreement and negative percent agreement for each matrix with respect to the comparator matrix are calculated. We believe that at least 95% agreement across all matrices/subject may be acceptable to demonstrate that performance between the matrices can be considered equivalent.

I. UNMET NEED ADDRESSED BY THE PRODUCT

This section will be completed by FDA.

J. APPROVED/CLEARED ALTERNATIVE PRODUCTS

Currently no methods for the qualitative detection of SARS-CoV-2 IgM or IgG antibodies have been approved or cleared by FDA.

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K. RISKS AND BENEFITS:

This section will be completed by FDA.

L. 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.

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

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

N. 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.