**Appendix A: Serology Template for Manufacturers**

This template (the “template”) provides FDA’s current recommendations concerning what data and information should be submitted to FDA in support of a pre-EUA/EUA submission for a SARS-CoV-2 antibody test. As outlined in Section V.C. of this guidance, 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 recommended information to FDA, but alternative approaches can be used. For more information about EUAs in general, please see the FDA Guidance document: [Emergency Use Authorization of Medical Products and Related Authorities](https://www.fda.gov/media/97321/download).

**GENERAL INFORMATION ABOUT THIS TEMPLATE**

* Text highlighted in yellow ***[Text]*** should be completed by the test manufacturer (sponsor) as applicable to their specific test.Text in **bold** outlines the Food and Drug Administration’s (FDA) additional recommendations for the sponsors’ consideration when completing the suggested information in each section.
* Please be reminded that tests for the detection of antibodies against SARS-CoV-2 must not be distributed and/or used for clinical diagnoses.
* This is an EUA interactive review template for Pre-EUA/EUA submissions. The template is subject to change as we learn more about the COVID-19 disease and its risk-benefit profile.
* A test authorized under an EUA is only authorized for emergency use while the EUA is in effect.
* The EUA is not a pathway to permanent marketing of your device. Therefore, we strongly recommend that you consider, in addition to an EUA, a traditional premarket submission for your IVD so that your device can still be legally marketed after termination of the public health emergency declaration. We recommend that you identify as soon as possible in the Pre-EUA review process any consideration of moving your product forward towards De Novo/510(k) clearance.

**EXAMPLE TEMPLATE:**

1. **PURPOSE FOR SUBMISSION**

Emergency Use Authorization (EUA) request for distribution of the ***[test name]*** in ***[indicate labs e.g., U.S. laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform moderate complexity and high complexity tests, and U.S. laboratories certified under CLIA to perform high complexity tests, and as applicable, for near patient testing, or point of care use]***, for the detection of ***[specify types of antibodies e.g., IgM, IgG, total]*** antibodies to SARS-CoV-2 in ***[specify matrices]*** from individuals with current or prior COVID-19 infection.

1. **MEASURAND**

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

1. **APPLICANT**

**[*Official name, address and contact information of applicant]***

1. **PROPRIETARY AND ESTABLISHED NAMES**

Proprietary Name: ***[test name]***

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

1. **REGULATORY INFORMATION**

***Approval/Clearance Status:***

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

***Product Code:***

QKO

1. **PROPOSED INTENDED USE**
2. ***Intended Use:***

**The proposed IU will be finalized based on the data provided at the time of authorization.**

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. Testing is limited to ***[laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C 263a, to perform moderate or high complexity tests and as applicable, Point of Care (POC) testing].***

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. ***[For lateral flow devices: Due to the risk of false positive results, confirmation of positive results should be considered using second, different [as appropriate, IgG or IgM] assay].***

The ***[test name]*** is only for use under the Food and Drug Administration’s Emergency Use Authorization.

1. ***Special Conditions for Use Statements:***

For prescription use only

For *in vitro* diagnostic use only

For Emergency Use Authorization only

1. ***Special Instrument Requirements:***

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

1. **DEVICE DESCRIPTION AND TEST PRINCIPLE**
2. ***Product Overview/Test Principle:***

***[Describe the technology of the test and how this technology works to identify measurand (i.e., the test principle), 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]***

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

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

Controls that will be provided with the test kit include:

1. An external positive control for each antibody class claimed (e.g., IgG, IgM) is needed to ***[describe need]*** and is used ***[describe use – please specify the concentration of the positive control relative to the cut-off of your test (note that ideally the positive control concentration should be such that it is close to the cut-off of your test) and specify frequency of use.]***
2. 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.]***
3. 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 develop and market 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]***.

1. **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 describe how results are to be interpreted. If applicable, clearly indicate how to interpret numeric test values as positive or negative for the presence of antibodies against SARS-CoV-2. Indicate how to identify indeterminate/equivocal results (if applicable) and how the user should resolve them. Also describe if and when repeat testing may be required.***

***If your test is a lateral flow, please describe the results interpretation for each of the test lines.*** ***You could consider reporting the results in the form of a table as shown below for a test that detects and differentiates between IgG and IgM:***

**Table 1. Interpretation of Results**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **C Line** | **M****Line** | **G****Line** | **Test Result Interpretation** |
| 1 | not present | Any | Any | Invalid Test. The specimen must be retested with another device |
| 2 | + | - | - | Valid Test, Negative for antibodies for SARS-CoV-2 |
| 3 | + | + | - | Valid Test, IgM positive for antibodies for SARS-CoV-2 |
| 4 | + | + | + | Valid Test, IgM and IgG positive for antibodies for SARS-Cov-2 |
| 5 | + | - | + | Valid Test, IgG positive for antibodies for SARS-CoV-2 |

***If you have a lateral flow device, please include a schematic/picture showing the location of the sample well, buffer well, and control and test lines]***

1. **PRODUCT MANUFACTURING**
2. ***Overview of Manufacturing and Distribution***

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

The current manufacturing capabilities include the ability to manufacture approximately ***[insert the approximate number of units/products that can currently be manufactured per week at the manufacturing facility]*** products per week, however in the event of a surge in demand this could be increased to ***[please insert the approximate maximum number of units/products that could potentially be manufactured per week at the manufacturing facility if there was a surge in demand]*** product per week within a ***[please specify in weeks/months the expected timeframe required to increase product production if required]***timeframe.

1. **Component Included with the Test:** Components manufactured ***by [manufacturer’s name and FDA registration number (if applicable)]***and supplied with the test include:

***[List all components and reagents provided for your test, including volumes, concentrations, quantities, etc.]***

**Example: Table Kit Components**

|  |  |
| --- | --- |
| **Kit components (example)** | **Manufacturer** |
| Test Cassette with test strip |   |
| Negative control  |   |
| Positive control  |   |
| Sample buffer (bottle)  |   |
| Transfer pipette |  |
| Lancet (for fingerstick only) |  |
| Instructions for Use leaflet |  |
| Packing materials |  |

1. ***Components required but not included with the test:***

***[List all components (e.g., timer, analyzer/reader) 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].***

1. The ***[test name]*** has been validated using only the components referenced above. The ***[test name]*** was developed using ***[briefly describe the capture antigens and antibodies used in the test, how they were designed and purified (e.g., are monoclonal antibodies used, are they manufactured in house or purchased commercially, what species they derive from, what epitope is targeted by the antibodies used in the assay, and if commercial products is there a certificate of analysis, etc.).***
2. ***Testing Capabilities: [Briefly describe current sample throughput capacity, total time required to perform the test (from clinical specimen collection to result), and number of tests that can be performed. Please provide the number of kits you can manufacture per day/week.]***

1. ***Distribution Plan***

***[Describe if you will partner with other companies for the distribution of the device]***

1. ***Reagent Stability***

***[Describe the information that supports the stability claims for your device. Please indicate if the test is already in use in other parts of the world.]***

1. **PERFORMANCE EVALUATION**
2. ***Analytical Sensitivity and Specificity***
3. *Reactivity/lnclusivity:*

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).

1. *Cross-Reactivity:*

**If a large number of known negative samples (e.g., ≥75 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 >98% 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. Please include in your description the number of samples tested and how samples were prepared.]***

**If testing of the cross-reactants is needed to demonstrate cross-reactivity of 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.**

**Table 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.]***

1. ***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.]***

***[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.]***

**Approaches to evaluate class specificity depend on the assay format. If you have well-characterized the anti-IgG and anti-IgM reagents, used in your test class specificity testing may not be needed. In this case, please describe how the reagents were characterized and 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.**

***[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:**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| SampleID | Replicates | Result NO DTT Treatment(IgM/IgG) | ResultDTT 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 |

1. ***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 antibody positive samples for each immunoglobulin claimed and 75 antibody negative samples from patients tested for SARS-CoV-2 and confirmed as negative and the data should demonstrate a minimum overall 90.0% positive percent agreement and overall 95.0% negative percent agreement, and for tests that report specifically IgM and IgG results, a minimum positive percent agreement for IgM of 70% and a minimum positive percent agreement for IgG of 90%. Point estimates not lower than 93% for combined NPA, not lower than 90% for combined PPA, and for tests that specifically report IgG or IgG and IgM, PPA for IgG not lower than 87%, and PPA for IgM not lower than 67% may be acceptable if a larger number of 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 antibody positive and 75 antibody negative samples.**

**For visually read tests, blinding and randomization should be included in the experimental design**

**If a claim for fingerstick is desired, we believe evaluating a minimum of 30 positive and 30 negative fingerstick whole blood samples may be acceptable to demonstrate clinical performance in fingerstick samples. If a claim for near patient testing, or point-of-care (POC) is desired, please see the section specific to POC below.**

***[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, using 2 x 2 tables as follows:***

|  |  |
| --- | --- |
|  | ***Comparator method/Clinical truth*** |
| ***Your Device*** |  | ***Positive***  | ***Negative*** |
| ***Positive*** | ***A*** | ***B*** |
| ***Negative*** | ***C*** | ***D*** |

***Percent Positive Agreement = A/(A + C) or***

***True Positives/(True Positives + False Negatives)***

***Negative Percent Agreement = D/(B + D) or***

 ***True Negatives/(True Negatives + False positives)***

***If you claim that your test can differentiate between IgG and IgM, PPA and NPA for IgG and IgM separately should be calculated separately.]***

1. ***Matrix Equivalency***

***[Please describe the protocol and provide the 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.]* Please note: Fingerstick whole blood is not considered to be the same sample type as venipuncture whole blood and clinical agreement against PCR should be evaluated (please see Section J.3 above).**

**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 (e.g., for lateral flow tests, faint test line), and moderate positive (e.g., for lateral flow tests, strong test line) 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 visually read tests, blinding and randomization are important considerations for the experimental design.**

**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.**

1. ***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).]***

1. **UNMET NEED ADDRESSED BY THE PRODUCT**

**This section will be completed by FDA**.

1. **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.

1. **RISKS AND BENEFITS:**

**This section will be completed by FDA.**

1. **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.***

1. **INSTRUCTIONS FOR USE/ PROPOSED LABELING/PACKAGE INSERT:**

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

1. **RECORD KEEPING AND REPORTING INFORMATION TO FDA:**

***[Manufacturer Name]*** will track adverse events and report to FDA under 21 CFR Part 803. A website is available to report on adverse events, and this website is referenced in the Fact Sheet for Health Care providers as well as through the ***[Manufacturer Name]*** Product Support website:[[link to Manufacturer’s website]](http://www.cellex.us/)*[.](http://www.cellex.us/)*Each report of an adverse event will be processed according to [Manufacturer Name]’s Non-Conformance Reporting Requirements, and Medical Device Reports will be filed with the FDA as required. Through a process of inventory control, [Manufacturer Name]will also maintain records of device usage/purchase. [Manufacturer Name] will collect information on the performance of the test, and report to FDA any suspected occurrence of false positive or false negative results of which [Manufacturer Name]becomes aware. [Manufacturer Name] will maintain records associated with this EUA and ensure these records are maintained until notified by FDA. Such records will be made available to FDA for inspection upon request.