Summary Template for Developers[1] of Antigen Diagnostic Tests for Monkeypox

This 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 request for an *Orthopoxvirus* or monkeypox virus antigen diagnostic test. FDA generally recommends that the following validation studies be conducted for *Orthopoxvirus* or monkeypox virus antigen diagnostic tests: limit of detection (LOD), inclusivity, cross-reactivity, microbial interference sample stability, and clinical evaluation.

As described in the FDA guidance document: *Policy for Monkeypox Tests to Address the Public Health Emergency*,[2] FDA is providing recommendations in this and other EUA templates regarding testing that should be performed to ensure appropriate analytical and clinical validity, including descriptions of appropriate comparators, for different types of tests. The EUA templates[3] are intended to help test developers provide recommended validation data and other information to FDA, but alternative approaches can be used.

This template 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.[4]

To facilitate FDA's prioritization efforts and if monitoring of the continued public health situation suggests the need for additional resources, FDA recommends that antigen test developers interested in pursuing a potential future EUA submit preliminary information to FDA to indicate their intent to submit an EUA request to MPXDx@fda.hhs.gov as described in the FDA guidance document: *Policy for Monkeypox Tests to Address the Public Health Emergency*.[5] Monkeypox virus antigen test developers should consider checking with the National Institutes of Health (NIH) Rapid Acceleration of Diagnostics (RADx) Independent Test Assessment Program (ITAP) for potential opportunities for the validation of monkeypox virus diagnostics[6].

Test developers may submit a pre-EUA (if not all validation studies are completed and/or they have questions for the agency) or may submit an EUA request (if the validation studies are completed) to MPXDx@fda.hhs.gov.

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[1] This template is part of the "Policy for Monkeypox Tests to Address the Public Health Emergency," available at https://www.fda.gov/regulatory-information/search-fda-guidance-documents/policy-monkeypox-tests-address-public-health-emergency.

[2] Available at https://www.fda.gov/regulatory-information/search-fda-guidance-documents/policy-monkeypox-tests-address-public-health-emergency.

[3] All monkeypox virus diagnostic EUA templates can be found at https://www.fda.gov/medical-devices/emergency-use-authorizations-medical-devices/monkeypox-emergency-use-authorizations-medical-devices#templates.

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

[5] Available at https://www.fda.gov/regulatory-information/search-fda-guidance-documents/policy-monkeypox-tests-address-public-health-emergency

[6] Information about NIH/RADx ITAP can be found at https://www.nibib.nih.gov/covid-19/radx-tech-program/ITAP

Emergency Use Authorization (EUA) Request Template

Antigen Diagnostic Tests for Monkeypox

I. BACKGROUND

1. **Applicant Name:** Please enter the official applicant's name
2. **Applicant Address:** Please enter the applicant's address
3. **Application Primary Correspondent:** Name; Phone Number; Email address
4. **Application Secondary Correspondent:** Name; Phone Number; Email address
5. **Assay Name:** Please enter the proprietary, abbreviated, and/or established name of the assay
6. **Measurand:** Specific antigen(s) from the *Orthopoxvirus* or monkeypox virus Please specify the targeted antigen(s).
7. **Regulatory History:** The Assay name is not cleared, CLIA waived, approved, or subject to an approved investigational device exemption.

   If the test has been previously reviewed in a pre-EUA or EUA submission, please provide the submission number, or type N/A: Previous submission number, if applicable
8. **Intended Testing Population(s)** (please check all that apply)**:**

   ☐ Patients suspected of infection by their healthcare provider

   ☐ Other: Please describe
9. **Notification reference number (if applicable):** Please enter number if applicable

II. MAIN TEMPLATE

A. PRODUCT INFORMATION

1. **Proposed Intended Use:**

   Please refer to the template for an example.
2. **Assay Technology:** ☐ Lateral Flow Immunoassay

   ☐ Other* Please describe
3. **Sample Type(s):**

   Lesion: ☐ lesion roofs ☐ lesion crusts ☐ human pustular ☐ vesicular rash

   ☐ lesion exudate ☐ Other* Please describe

   Swab transport: ☐ VTM* ☐ UTM ☐ dry ☐ Other** Please describe

   *FDA recommends against use of VTM for lateral flow antigen tests due to significant cross-reactivity observed with different brands and types of VTM for other infectious disease antigen tests.

**If you are considering other sample types, please contact FDA at MPXDx@fda.hhs.gov to discuss your validation strategy.

4. **Instruments Required:** Please list the instruments employed/required to perform the test, including software and automated extraction instruments

5. **Antibody Reagents/ Antigen Targets:**

Antibody Description:

Please provide a description of the anti-[Orthopoxvirus or monkeypox virus] antibodies used in your test, including whether t

Amino Acid Description:

Please provide a description of the specific amino acid sequence(s) (including amino acid position numbers relative to the [O

Immunogen Generation Description:

Please provide a description of how the immunogen was generated (e.g., synthetic peptide, recombinant protein, etc.).

Epitope Description: Please provide a description of the epitope(s) (if known), including whether the epitope is linear or conformational, recognized by the anti-***[Orthopoxvirus or monkeypox virus]*** antibodies used in your test to detect the ***[Orthopoxvirus or monkeypox virus]*** antigen(s).]

6. **Test Steps:** Please describe, in order, the test steps required to perform the test, including instrument(s)

7. **Controls Required[7]:**

Included with the Test Kit:

☐ Positive: Describe the control material (including concentration); if external, include supplier and catalog #. Ideally, the positive control concentration should be such that it is close to the LoD of your test.

Required to: Describe need

How it works: Describe how the control is expected to work

Where in test it is used: Describe where the control is used

Frequency of use: Describe frequency of use

☐ Negative: Describe the control material; if external, include supplier and catalog #

Required to: Describe need

How it works: Describe how the control is expected to work

Where in test it is used: Describe where the control is used

Frequency of use: Describe frequency of use

☐ Internal: Describe the internal control material, as applicable (e.g., sample adequacy, internal); if external, include supplier and catalog #.

[7] Please note that all recommended controls should be included in your analytical and clinical validation studies. If a control material is not readily available, you should include another suitable control in your validation studies.

Required to: Describe need

How it works: Describe how the control is expected to work

Where in test it is used: Describe where the control is used

Frequency of use: Describe frequency of use

B. PERFORMANCE EVALUATION

FDA generally recommends that the following validation studies be performed to support your EUA request. Please note that, particularly for new technologies, FDA may request additional studies so we can adequately assess the known and potential risks and benefits associated with the candidate test. For each validation study, you should provide a study protocol that includes a detailed, step-by-step description of how samples were prepared and how testing was conducted. You should also include complete study line data in an Excel-compatible format for all validation studies. The line data should present each replicate with the final antigen test result per the tests result interpretation. If your device includes an analyzer with a numeric output [(e.g., fluorescence, signal to cut-off ratio (S/Co)], you should include the analyzer values for each replicate.

1. **Limit of Detection (LoD) (Analytical Sensitivity)**

   You should determine the LoD of the candidate test utilizing the entire test system from sample preparation and extraction to detection. LoD studies determine the lowest detectable concentration of monkeypox virus or non-variola *Orthopoxvirus* at which approximately 95% of all (true positive) replicates test positive. For more information on FDA's recommendations regarding LoD studies, please refer to Section C.1 of the template.

2. **Inclusivity (Analytical Reactivity)**

   Test developers should monitor new and emerging viral mutations and variants that could impact antigen test performance on an ongoing basis. Monitoring should also include identifying if there are multiple credible reports indicating that a given viral variant (which may have one or more mutations) has the potential to increase virulence, increase transmission, or otherwise increase the public health risk. FDA recommends monitoring on at least a monthly basis and if requested by FDA, records of these evaluations submitted for FDA review within 48 hours of the request.

   For more information on FDA's recommendations regarding inclusivity studies, please refer to Section C.2 of the template.

3. **Cross-Reactivity (Analytical Specificity)**

   Cross-reactivity studies should be 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 a clinical sample. We recommend that the organisms are wet-tested in negative clinical matrix. *In silico* analyses may be appropriate for certain organisms that are difficult to obtain.

   For more information on FDA's recommendations regarding cross-reactivity studies, please refer to Section C.3 of the template.

4. **Microbial Interference Studies**

   If the analytical specificity study demonstrated cross-reactivity, we recommend that you perform a microbial interference study using samples prepared with a low monkeypox virus concentration and a high interferent level.. For more information on FDA's recommendations regarding microbial interference studies, please refer to section C.4 of the template.

5. **Endogenous/Exogenous Interference Substances Studies**

   The extent of testing for interference substances depends on the matrix that is indicated for the candidate test, as well as on the technology of the candidate test. We recommend you evaluate endogenous/exogenous interference substances that may be appropriate for your intended specimen type. For more information on FDA's recommendations regarding endogenous/exogenous interference substances studies, please refer to Section C.5 of the template.

6. **Biotin Interference**

   If your assay uses a biotin/anti-biotin capture system, biotin interference testing should be conducted. False negative results may occur in patients who have indicated or whose clinical status or history would indicate they are currently taking high doses of biotin. For more information on FDA's recommendations regarding biotin interference studies, please refer to Section C.6 of the template.

7. **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. For more information on FDA's recommendations regarding high-dose hook effect studies, please refer to Section C.7 of the template.

8. **Sample Stability**

   Sample stability should be performed if shipping/storage claims go beyond current CDC recommendations for dry swab specimens (Refer to https://www.cdc.gov/poxvirus/monkeypox/clinicians/prep-collection-specimens.html for additional details). Testing should be conducted to demonstrate sample stability throughout the real-world conditions in which they are collected and tested, according to your instructions for use. For more information on FDA's recommendations for sample stability studies, please refer to Section C.8 of the template.

9. **VTM/UTM Equivalency (if applicable)**

   Each brand of validated transport media should be listed in your intended use statement and validated during your clinical and analytical validation studies. FDA has observed significant cross-reactivity with different brands and types of VTM for other infectious disease antigen tests, which has resulted in erroneous patient results. Without validating and specifying each type of VTM/UTM intended for use with your device, we cannot determine if your device is fully

validated to assess the risk of erroneous results due to cross-reactivity with affected types of VTM/UTM. For more information on FDA's recommendations for sample stability studies, please refer to Section C.9 of the template.

10. Clinical Evaluation

FDA recommends conducting a prospective, blinded, randomized clinical agreement study with at least 30 positive samples and 30 negative natural clinical samples from patients who represent your intended use population (e.g., symptomatic within X days of symptom onset, etc.). We recommend that you collect demographic information on your study participants (e.g., gender, age, race, ethnicity etc.) as the appearance of rashes can vary with different skin tones. The number of negative samples may vary according to the disease prevalence at the time of your study. Evaluations with contrived clinical specimens are inadequate to support the clinical performance of an antigen test at this time.

Candidate tests should demonstrate a minimum positive percent agreement (PPA) of ≥ 80% and 95% negative percent agreement (NPA) for all sample types submitted. For less sensitive tests, you may consider leveraging a serial testing strategy and evaluate the candidate test's cumulative performance rather than its one-time test performance. If you are proposing serial testing as a mitigation for a less sensitive candidate test, you should provide data to support the cumulative clinical performance ≥ 80% PPA as well as detailed instructions for serial testing in the package insert, including the recommended testing interval, that are 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. For more information on FDA's recommendations for clinical evaluation studies, please refer to Section C.10 of the template.

11. Studies to Support Point of Care (POC) Use, as applicable

If the device is intended for POC testing, please provide a detailed study description and data to demonstrate that non-laboratory healthcare providers can perform the test accurately in the intended use environment. Your studies to support a POC claim should include the following: (1) a POC clinical evaluation including use of appropriate sites and test users, (2) near LoD POC sample evaluation, and (3) POC flex studies. For more details, please refer to section C.11 of the template.