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Contains Nonbinding Recommendations

Summary Template for Developers of Molecular 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 a non-variola *Orthopoxvirus* or monkeypox virus molecular diagnostic test. FDA generally recommends that the following validation studies be conducted for such molecular diagnostic tests: limit of detection (LOD), inclusivity, cross-reactivity, sample stability, and clinical evaluation.

As described in the FDA guidance document Policy for Monkeypox Tests to Address the Public Health Emergency[1] FDA is providing recommendations in this template, one other EUA template, and other templates that may be developed 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 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.[2]

FDA encourages including a highly conserved monkeypox virus target (i.e., a target in a portion of the genetic code not restricted to a specific monkeypox virus variant) or non-variola Orthopoxvirus target as part of a multiple target test which may improve performance with new genetic variants; however, the number of targets in the test should be appropriate to provide resilience (i.e., a reduction of the risk that viral mutation will impact test performance) and most efficiently leverage developer and laboratory resources.

Test developers interested in requesting an EUA may submit a pre-EUA (if not all validation studies are completed) to begin discussions with the FDA or may submit an EUA request (if the validation studies are completed) to MPXDx@fda.hhs.gov.

[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/emergency-use-authorization-medical-products-and-related-authorities

Emergency Use Authorization (EUA) Request Template

Molecular 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 nucleic acid sequences from the genome of Please specify the targeted gene(s) of the pathogen
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:** ☐ RT-PCR ☐ LAMP ☐ Chemiluminescent ☐ Sequencing ☐ CRISPR ☐ TMA ☐ MALDI-TOF ☐ 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

*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. **Primers/Probes: Please list any primer and probe sets, including a description of the targets and nucleic acid sequences they detect.**
6. **Test Steps:** Please describe, in order, the test steps required to perform the test, including instrument(s)
7. **Controls Required[3]:**

   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

   ☐ Extraction[4]: 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 (e.g., sample adequacy); if external, include supplier and catalog #.

   Required to: Describe need

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

[3] 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.
[4] If the positive control is taken through the entire sample processing procedure, including the extraction, then a separate extraction control is generally not needed.

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 refer to Appendix A of the template for additional information regarding multiplex panels and Appendix B of the template for additional information regarding tests with multiple instruments or extraction methods.

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)**

   You should provide in silico analysis to demonstrate inclusivity of your assay.

   FDA encourages including a highly conserved monkeypox virus target (i.e., a target in a portion of the genetic code not restricted to a specific monkeypox virus variant) or non-variola Orthopoxvirus target as part of a multiple target test which may improve performance with new genetic variants; however, the number of targets in the test should be appropriate to provide resilience (i.e., a reduction of the risk that viral mutation will impact test performance) and most efficiently leverage developer and laboratory resources.

   Inclusivity analysis establishes the extent to which variation in the monkeypox virus or other non-variola *Orthopoxvirus* genome, as applicable, may impact sensitivity of test performance. Developers should document the methodology and results of an in silico inclusivity analysis that establishes the extent to which variation in the monkeypox virus or other non-variola *Orthopoxvirus* genome may impact sensitivity of test performance 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 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 a clinical sample. It is acceptable to conduct an in silico analysis of published genome sequences using the assay's primers and probes. 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 in silico analysis reveals ≥ 80% homology between the cross-reactivity microorganisms and your test primers/ probe(s) set(s), we recommend that you either perform (1) a microbial interference study with monkeypox virus or other non-variola *Orthopoxvirus*, as applicable, and the microorganisms that your test primers/ probe(s) have homology to, or, as an alternative to the microbial interference study, (2) you may provide justification as to why (e.g., amount of primer(s)/ probe(s) included in your master mix) the performance of your test would not be impacted by the presence of a causative agent of a clinically significant co-infection, or (3) explain why the in silico results are clinically irrelevant (e.g., low prevalence, etc.). 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**

   If the candidate test uses extraction methods not previously reviewed by FDA as part of a premarket submission or the candidate test does not use an extraction procedure, we recommend testing for potential interferents. For more information on FDA's recommendations regarding endogenous/exogenous interference substances studies, please refer to Section C.5 of the template.

6. **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.6 of the template.

7. **Clinical Evaluation**

   FDA recommends conducting prospective, blinded, randomized clinical agreement study with at least 30 positive samples and 30 negative natural clinical samples (prospective, retrospective, or leftover) from patients suspected of monkeypox by their healthcare provider. If no prospective or retrospective specimens are available at the time of your EUA/pre-EUA request for a monkeypox or non-variola *Orthopoxvirus* test, FDA may consider a fully contrived clinical evaluation, with each contrived clinical specimen prepared using a unique natural clinical specimen matrix.

   Candidate tests should demonstrate a minimum of 95% positive and negative agreement for all specimen types requested as compared to an EUA authorized test or FDA-cleared test for monkeypox virus or other non-variola *Orthopoxvirus* when using natural clinical samples. For more information on FDA's recommendation for the clinical evaluation for patients suspected of monkeypox, please refer to section C.7 of the template.

8. **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) supplemental POC samples, and (3) POC flex studies. For more details, please refer to section C.8 of the template.