

## Template for Developers<sup>1</sup> of Antigen Diagnostic Tests

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](#),<sup>2</sup> 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<sup>3</sup> 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](#).<sup>4</sup>

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 request submit preliminary information to FDA to indicate their intent to [MPXDx@fda.hhs.gov](mailto:MPXDx@fda.hhs.gov) as described in the FDA guidance document: [Policy for Monkeypox Tests to Address the Public Health Emergency](#).<sup>5</sup> 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<sup>6</sup>.

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](mailto:MPXDx@fda.hhs.gov).

<sup>1</sup> 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>.

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

<sup>3</sup> 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>.

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

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

<sup>6</sup> 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

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#### 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 a healthcare provider
  - Other: Please describe
9. **Notification reference number (if applicable):** Please enter number if applicable

#### FOR FDA USE:

**Regulatory Information:** Panel Code: to be completed by FDA; Review Group: to be completed by FDA;  
Product Code: to be completed by FDA

**Unmet Need Addressed:** to be completed by FDA

## II. MAIN TEMPLATE

### A. PRODUCT INFORMATION

#### 1. Proposed 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 test, proposed intended use population, testing sites, or performance characteristics.

The **[test name]** is a **[specify test technology, such as lateral flow immunoassay]** for the **[presumptive]** qualitative detection of **[protein name]** antigen from **[Orthopoxvirus/monkeypox virus]** in **[describe all the sample types that were evaluated, e.g., human skin lesion material specimens such as lesion exudate, lesion roofs or lesion crusts, etc.]** **[If your test is intended for detecting multiple pathogens, please list the specific analytes detected by your test.]** **[describe intended use population, e.g., from individuals suspected of monkeypox by their healthcare provider]**. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, that meet the requirements to perform **[insert testing complexity, e.g., moderate complexity, high complexity, or waived tests. This test is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation.]**.

Results are for the identification of **[Orthopoxvirus or monkeypox virus]** antigen. Antigen is generally detectable in **[name sample type, human pustular or vesicular rash specimens]** 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 patient 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. Negative results should be treated as presumptive, and do not rule out monkeypox virus 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 monkeypox, and confirmed with a molecular assay, if necessary, for patient management.

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

The **[test name]** is intended for use by **[include intended user, e.g., qualified, and trained clinical laboratory personnel specifically instructed and trained in vitro diagnostic procedures and healthcare personnel who are proficient in performing tests in point of care settings]**.

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

For prescription use only

For *in vitro* diagnostic use

## Contains Nonbinding Recommendations

For Emergency Use Authorization only

The proposed IU will be finalized based on, among other things, the data provided and recommendations from Public Health authorities at the time of authorization. Depending on the performance and the populations studied in the clinical evaluation, additional limitations may be recommended.

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 (non-lesion), please contact FDA at [MPXDx@fda.hhs.gov](mailto:MPXDx@fda.hhs.gov) to discuss your validation strategy.

4. **Instruments Required:** Please list all instruments, software, cameras, smart phone operating systems, other applicable instrumentation, etc.

**If your test system includes an instrument, the instrumentation manual should be submitted as part of the EUA request.**

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

Antibody Description:

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Amino Acid Description:

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Immunogen Generation Description:

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**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 list and describe in detail all of the steps of the test sequentially, from sample collection to assay report.

7. **Controls Required<sup>7</sup>:**

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<sup>7</sup> 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.

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Included with the Test Kit:

Control		Requirement	How it works	Where it is used	Frequency of use
<b>Positive</b>	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.	Describe need	Describe how the control is expected to work	Describe where the control is used	Describe frequency of use
<b>Negative</b>	Describe the control material; if external, include supplier and catalog #	Describe need	Describe how the control is expected to work	Describe where the control is used	Describe frequency of use
<b>Internal</b>	Describe the endogenous internal control material, as applicable (e.g., sample adequacy, internal); if external, include supplier and catalog #.	Describe need	Describe how the control is expected to work	Describe how the control is used	Describe frequency of use

NOT included with the Test Kit: Please describe any controls that are required, but not included with the test kit; description of the control, recommended sources of the material, the need for the control, how it works, where in the test is it used, and the frequency of use.

**Please note that any control used with your device (provided with the kit or not) should be validated in the context of your analytical and clinical studies (i.e., your studies should include use of these controls). In instances where control material is not readily available through 3<sup>rd</sup> party vendors, FDA recommends that you include suitable control material with your device. External control materials are considered particularly important when good manufacturing practice (GMP) requirements are waived, and reagent stability studies are limited.**

### B. MANUFACTURING INFORMATION

***FDA recommends that you confirm your agreement with the following statement to help facilitate authorization in the event FDA determines it is appropriate to authorize the candidate test:***

Yes  No

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*The Assay name has been validated using only the components referenced in this request and will not be changed after authorization without prior concurrence from the FDA except as described in section IV.A.3 of the Policy for Monkeypox Tests to Address the Public Health Emergency.*

- 1. Manufacturing Location:** Please list the manufacturing location name and contact information
- 2. FDA Registration Number:** FDA registration #, or N/A if not applicable
- 3. Quality System<sup>8</sup>:** e.g., 21 CFR 820 or ISO 13485
- 4. Packager:** Please include the name of the packager, if applicable (e.g., material may be bottled and kitted by [packager name])
- 5. Manufacturing and Testing Capabilities**

**Total time required to perform all steps of the test:** Please describe the total time required to perform the test (from clinical sample collection to result).

**Number of patient tests that can be performed per day (8hr shift):** Please describe current sample throughput testing capacity, the total time required to perform the test (from clinical sample collection to result), and the number of tests that can be performed per day (8-hour shift), excluding controls and calibrator, as applicable.

**Current manufacturing capacity: Number of tests manufactured per 7 days for US distribution:** Please describe the number of kits you can manufacture per day/week for distribution in the United States.

**Surge manufacturing capacity:** Please include the approximate maximum number of tests that could potentially be manufactured per week. Please include the approximate timeframe to increase to surge manufacturing capacity.

- 6. Distribution:**

US Distributors: Please list all current US distributors

- 7. Device Components:**

Components Included with the Test: List all components and other materials/information included with your test, including a description of the reagents, volumes, concentrations, quantities, buffer components, etc.

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<sup>8</sup> Under an EUA, certain sections of the 21 CFR Part 820 Quality System Regulation (QSR) may be waived for an authorized product during the duration of the EUA, but FDA recommends that test developers follow comparable practices as much as possible.

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### Example: Kit components & Other Materials/Information Table

Kit Components & Other Materials/Information	Main Reagents Composition/Matrix	Concentration/Quantity/Volume	Manufacturer
Test cassette with test strip			
Negative control			
Positive control			
Calibrators			
Sample buffer (bottle)			
Transfer pipette			
Instructions for Use leaflet			
Packing materials			
Others, as applicable			

**Components Required but NOT Included with the Test:** List all components and other materials/information (e.g., instruments, 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 the components. 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.

**Research Use Only (RUO) Test Components:** Please specify any instruments or other components of your test which are labeled as research use only (RUO), or are otherwise not labeled with the statement “For In Vitro Diagnostic Use”, or associated symbol.

Does the test use an RUO instrument that will be distributed to more than one lab?

No    Yes;

If yes and if EUA requestor is **NOT** the manufacturer of the RUO instrument, it is recommended that you provide:

- Appropriate procedures in the instructions for use, including acceptance criteria, that laboratory customers should follow to qualify the performance of the RUO instrument prior to use with your test.
- A "For Emergency Use Authorization only" label that users can affix to the instrument after it has been qualified. This can be provided as an Appendix in the assay instructions for use.
- Ensure that your test's labeling either reproduces the parts of the instrument operating manual that are relevant to run your test or references the relevant sections of the manual.

If yes and if EUA requestor **IS** the manufacturer of the RUO instrument, it is recommended that you provide:

- Qualification protocol or the ISO 13485 certificate for the site where your instrument is manufactured.
- A document mapping out the parts of your quality system that fulfill each of the following 21 CFR part 820 requirements:
  - Subpart H (Acceptance Activities, 21 CFR 820.80 and 21 CFR 820.86),

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- Subpart I (Nonconforming Product, 21 CFR 820.90), and
  - Subpart O (Statistical Techniques, 21CFR 820.250).
- A "For Emergency Use Authorization only" label that users can affix to the instrument after it has been qualified. This can be provided as an Appendix in the assay instructions for use.
- An instrument operating manual addendum with information such as the following, as an example:

For emergency use authorization only with the Assay name.

The Assay name is authorized for use under the US Food and Drug Administration (FDA) Emergency Use Authorization (EUA) with the insert name of instrument(s).for the [presumptive] qualitative detection of antigen protein from *Orthopoxvirus/monkeypox virus*. Refer to the Assay name instructions for use for additional information provide hyperlink.

This instrument operation manual addendum applies to the instruments listed in Table 1 that are authorized for use with the Assay name.

**Table 1:** Instruments Authorized for Emergency Use Only with the Assay name.

Catalog Number	Product Name

### Warnings:

1. This product has not been FDA cleared or approved; the product has been authorized by FDA as part of Assay name under an EUA for emergency use only by authorized laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C § 263a.
2. This product has been authorized only for the detection of antigen protein from Specific virus, not for any other viruses or pathogens.
3. The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of Specific virus under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated, or authorization is revoked sooner.

### 8. Software:

Does the proposed device contain software? Yes No; If No, please continue to the next Section.

Has the software been previously reviewed by FDA? Yes No;

If Yes, please include previous submission number where the software has been reviewed: Please include previous submission number, if applicable



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If No, please provide the following:

Software Level of Concern<sup>9</sup>: Major    Moderate    Minor

Software Validation<sup>10</sup>:  Validation complete     Developed per GMPs<sup>11</sup>

If software validation is not complete and the test has been designed and developed per GMPs, future submission of documentation of software validation may be a condition of authorization.

If software validation is complete, please provide all applicable test protocols and reports, including thorough functional descriptions of system software and instrumentation specifications needed to support the intended use of the test and provide evidence that specifications have been fulfilled, including:

- The inputs and outputs of the software appropriate to fulfill the system and assay requirements (e.g., System Specifications);
- All expected inputs produce the expected outputs for all functions critical for system operation (e.g., Validation); and
- The system will be provided to the customer free of defects, or defects are documented and mitigated to an acceptable risk level (e.g., Hazard Analysis).

### Example: System Specifications and Validation Table

Critical Specifications	Description of the Specification
Optical system of each instrument sent to a user has sufficient dynamic range to appropriately differentiate between positive and negative test results	<b>Evidence that the design of the system can fulfill the specification. This column should consist of system-level validation data.</b>
Software displays appropriate result during test run	
If reader stores test result, software accurately stores and retrieves test results	
System has a defined lifetime where the user can expect the system to maintain performance as stated in the label	
Others, as applicable	

### Example: Hazard Analysis Table

ID	Hazard	Adverse Effect	Severity	Potential Causes of Hazard	Risk Mitigation Measure	Risk of Experiencing the Hazard

<sup>9</sup> Please see [Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices](https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-content-premarket-submissions-software-contained-medical-devices) (at <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-content-premarket-submissions-software-contained-medical-devices>)

<sup>10</sup> Please see [General Principles of Software Validation | FDA](https://www.fda.gov/regulatory-information/search-fda-guidance-documents/general-principles-software-validation) (at Please see <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/general-principles-software-validation>)

<sup>11</sup> If this evidence is not available prior to authorization and the software and hardware have been designed and developed in a manner consistent with current GMPs (for additional information, please see the discussion of “[Quality System Regulation/Medical Device Good Manufacturing Practices](https://www.fda.gov/medical-devices/postmarket-requirements-devices/quality-system-qs-regulationmedical-device-good-manufacturing-practices),” at <https://www.fda.gov/medical-devices/postmarket-requirements-devices/quality-system-qs-regulationmedical-device-good-manufacturing-practices> the FDA website), additional software validation documentation may be incorporated into the conditions of authorization. If changes which impact assay performance or safety and effectiveness of the system are needed to address validation failures post-authorization, an EUA supplement may be required under the conditions of authorization.

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						after Mitigation
1	Invalid result	Delay in returning test result	Low	User inserts cartridge incorrectly	Labeling noting correct orientation	Low
2	False result	Wrong result returned to user	High	Incorrect alignment of test strip and optics; test strip inserted in the wrong orientation	Mechanical design of reader input slot	Moderate
3	False negative result	Wrong result returned to user	High	User reads test strip too early; incubation time not sufficient	Labeling noting correct incubation time	Moderate
4	False result	Wrong result returned to user	High	Incorrect alignment of test strip and optics; control line misinterpreted	Software interprets data from optical system identifying valid/invalid control	Moderate
5	False result	Wrong result returned to user	High	Control reaction intensity is misinterpreted	Software interprets data from optical system identifying valid/invalid control	Moderate
6	False result	Wrong result returned to user	High	Analyte reaction intensity is misinterpreted	Software interprets data from optical system identifying valid/invalid control	Moderate

Does the device software contain any external wired and/or wireless communication interfaces? (e.g., (Wired: USB, ethernet, SD, CD, RGA, etc. or Wireless: Wi-Fi, Bluetooth, RF, inductive, Cloud, etc.)

Yes No;

If Yes, please include evaluation of the cybersecurity of your system in your software validation documentation to ensure user and patient safety in the intended use environment

#### 9. Basic Safety and Essential Performance<sup>12</sup>:

Does the test have electrical components previously reviewed by FDA? Yes No;

If Yes, please include previous submission number where the electrical testing has been reviewed: Please include previous submission number, if applicable

If No, please indicate if the basic safety requirements were evaluated according to International Electrotechnical Commission (IEC) 60601-1 (Medical electrical equipment – Part 1: General requirements for basic safety and essential performance)? Yes No;

<sup>12</sup> We recommend that you consult the general requirements for basic safety, as indicated in International Electrotechnical Commission (IEC) 60601-1 (Medical electrical equipment – Part 1: General requirements for basic safety and essential performance). IEC 60601-1 is a standard that specifies the general requirements for basic safety and essential performance. IEC 60601-1 defines basic safety as freedom from unacceptable risk directly caused by physical hazards when medical electrical equipment is used under normal condition and single fault condition.

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If No, please include a summary of the standard utilized, or alternate methodologies: Please describe the alternate evaluation and testing methodologies utilized

### 10. Electromagnetic Compatibility (EMC) Testing<sup>13</sup>:

Does the test use a battery or power source previously reviewed by FDA? Yes No;

If Yes, please include previous submission number where the software has been reviewed: Please include previous submission number, if applicable

If No, please indicate if EMC testing was performed according to the International Electrotechnical Commission (IEC) 60601-1-2 Edition 4.0:2014? Yes No;

If No, please include a summary of the standard utilized, or alternate methodologies, including a test plan, test report, acceptance criteria, and risk analysis to support your approach: Please describe the alternate EMC testing methodologies utilized

### 11. Reagent Stability:

Reagent stability studies generally do not need to be completed prior to authorization; however, FDA recommends that the study design be submitted in the EUA request and that testing begin immediately following authorization, if not before. In the absence of real-time stability data, initial reagent stability claims should not exceed four to six months. Expiration dates can be extended once real-time data becomes available.

Have reagent stability studies been completed? Yes No

If Yes, please provide all applicable test protocols and reports

If No, please provide the following information:

#### Reagent Stability Test Plan:

Standards Followed for Plan Development: FDA recommends following “Clinical Laboratory Standards Institute (CLSI) Standard EP25 – Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline”

Controls Included<sup>14</sup>:  External positive  External negative

Number of Samples: FDA recommends at least one sample

Sample Preparation: FDA recommends spiking negative clinical matrix or acceptable simulated matrix at an analyte concentration of 3-5x LoD of inactivated virus, not recombinant protein

Clinical Matrix: If you are using multiple clinical sample types in which similar LoDs are determined, you should use the most challenging clinical matrix for this study

Replicates: FDA recommends at least 5 replicates

Number of Lots: FDA recommends at least 3 lots

<sup>13</sup> Please see [Electromagnetic Compatibility \(EMC\) of Medical Devices | FDA](#)

<sup>14</sup> Please note that use of the positive controls alone is not recommended for reagent stability evaluation because controls are usually formulated at a moderate positive level.

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**Stability Timeframe:** FDA recommends evaluating about 10% longer than the one to be authorized. For example, 18 months should be supported by stability data out to 20 months and 7 days should include stability data out to 8 days

**Stability Temperatures:** FDA considers 15-30°C to represent room temperature conditions. Ideally you should evaluate stability at both 15°C and 30°C; however, for the purposes of the EUA evaluation, 30°C is generally recommended as the maximum temperature

**In-use/Opened Kit Stability:** Please describe evaluation how your stability design supports in-use stability of the kit reagents once the kit has been opened, e.g., storage at 2-8°C for 7 days. This includes on board stability once reagents have been placed on the instrument (N/A if not applicable)

**Unopened Kit Shelf-Life Stability:** You should evaluate kits stored at the claimed storage temperature. Accelerated studies up to 6 months may be acceptable for authorization, with real-time studies included as a condition of authorization. Real-time studies should include a baseline <1m from production. Any %change (%shift) from time zero (baseline) should be calculated between the target claim and the zero-time as  $(T_{test} - T_{baseline}) / T_{baseline} * 100$  with 95% confidence interval (CI) using the regression equation obtained from plotting the mean values. Generally, the shift at the target claim due to storage should not exceed 10-15%. The target stability is the next to last tested point that was within +/- 10% of time zero. Acceptance criteria may differ depending on the reproducibility of the test, the distribution of analyte concentration expected in samples from the intended use population, and the risk of false results to public health.

**Unopened Kit Shipping Stability:** You should evaluate the anticipated shipping times and temperatures expected under different temperature conditions. The recommended summer profile is storage at 40°C for 8 hours and then 22°C for 4 hours and the recommended winter profile is -10°C for 8 hours and then 18°C for 4 hours.

**Freeze-thaw Stability:** If you recommend aliquoting the reagents to meet the end-users needs, following the initial thaw this recommendation should be supported by a freeze-thaw stability study, including the specific number of allowed freeze-thaw cycles. Please describe how your stability design supports freeze-thaw reagent stability (N/A if not applicable).

**Inverted Stability:** You should evaluate stability for kits if stored inverted or in the wrong orientation, if applicable

### C. 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 to adequately assess the known and potential risks and benefits associated with the candidate test.***

***All validation studies should be conducted with live or inactivated virus, not recombinant protein.***

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

## Contains Nonbinding Recommendations

LoD studies determine the lowest detectable concentration of monkeypox virus at which approximately 95% of all (true positive) replicates test positive. You should determine the LoD of the candidate test utilizing the entire test system from sample preparation and extraction to detection. FDA recommends that you follow the most current version of the CLSI EP17 "Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures" where applicable.

FDA recommends spiking quantified virus (e.g., live virus or inactivated via heat treatment, chemically modified, or irradiated) into natural clinical matrix (e.g., human skin lesion material specimens, or for dry swabs, an acceptable simulated lesion swab matrix derived from natural clinical matrix). The use of recombinant antigen is not recommended for the LoD determination. If VTM is indicated for use, you should spike swabs with virus prior to immersing into VTM. Collection media without clinical matrix or collection kits that were not used to collect a clinical sample are generally not considered real clinical matrix. It is generally not appropriate to prepare samples with your assay reagents (e.g., extraction buffer) nor is it generally appropriate to dilute clinical matrix in VTM if the test is not indicated for use with VTM. If specimen collection involves the use of a swab, we recommend that you spike your viral material onto the swab and then perform the test per your instructions for use.

FDA recommends that preliminary LoD be determined by testing a 2-3-fold dilution series of 3 replicates per concentration, and then confirmed with 20 replicates of the concentration determined to be the preliminary LoD. For purposes of this document, the preliminary LoD is the lowest concentration that gives positive results 100% of the time and the final LoD is the lowest concentration at which 19 of 20 replicates are positive. The preliminary LoD studies should include at least one concentration that does not yield 100% positive results. Replicates should be interpreted per the result interpretation of your test. If multiple clinical matrices are intended for clinical testing, you should include the results of LoD evaluation in each clinical matrix type.

If the candidate test is an *Orthopoxvirus* IVD you should perform the LoD with at least two species of orthopoxviruses, if available, including one representative monkeypox virus (clade II).

Lowest detectable concentration of virus at which approximately 95% of all (true positive) replicates test positive (LoD): Please describe

Please specify what the specimens used for evaluation are made with:

- live virus                       inactivated virus

Please include the following as attachments to your EUA Request

- Study Protocol, including a detailed, step-by-step description of sample preparation and conduction of testing, titers and strains of the monkeypox virus (and other *Orthopoxvirus*) stocks used for the LoD study and how the organism stocks were prepared and how the titers were determined (with appropriate units), the dilution factor and number of serial dilutions of the characterized monkeypox virus that were tested to determine the LoD, the starting concentration, dilution factor used to reach target concentration, the volume of negative matrix with [*Orthopoxvirus* or] monkeypox virus spiked onto each swab in your LoD study, and the type of dilutant used (e.g., Phosphate Buffered Saline (PBS), saline, etc.) to prepare each replicate in your LoD study.

## ***Contains Nonbinding Recommendations***

- Complete study line data in an Excel-compatible format; including the following individual columns: coded identifiers for all samples and replicates; the clinical matrix tested; the specific virus concentration; raw signal output (i.e., if the assay includes use of an analyzer or application to generate test results, you should include the analyzer value for each test replicate).

For any data or information not included in your attached EUA request, or, for any additional discussion required, please use the space below:

Click or tap here to enter text.

### **2. Inclusivity (Analytical Reactivity)**

Viral mutations and viral variants could result in altered immunogenicity relative to the originally isolated virus, which could impact the performance of antigen tests.

Test developers should monitor new and emerging viral mutations and variants that could impact antigen test performance on an ongoing basis. Monitoring should 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 any viral mutations and variants that are identified as prevalent and/or clinically significant as described above, you should assess whether the resulting predicted amino acid change(s) in the viral proteins are critical to your test design. If the mutations are found to be critical to your test design, such mutations and variants should be evaluated using clinical (or contrived, as available and as appropriate) samples to assess the impact of the mutation or variant on your test's performance. The aggregate impact of the mutations should not reduce the clinical performance of the test by 5% or more or decrease the clinical performance point estimates for the test below the clinical performance recommendations described in Section J(10).

FDA also monitors for viral mutations and may request testing with clinical (or contrived, as available and as appropriate) samples to assess the impact of the mutation or variant on the performance of your test.

**Monitoring Plan Strategy:** Please provide a summary of your strategy to monitor new and emerging viral mutations and variants that could impact antigen test performance on an ongoing basis; please include your assessment strategy for the impact on the performance of your assay over time.

**Mutation and/or Variant:** For mutations and variants that have been identified as prevalent and/or clinically significant as part of ongoing monitoring at the time of your EUA request, please provide information on the potential impact of the mutation(s) and variants on your test's performance or explain how the risk associated with the unknown performance of your device in samples from individuals with the variant(s) can be adequately mitigated.

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Mitigation Plan: Please describe

Critical to Test Performance:  Yes  No

For any data or information not included in your attached EUA Submission, or, for any additional discussion required, please use the space below:

Click or tap here to enter text.

### 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 the clinical sample. We recommend wet-testing the organisms listed below in negative clinical matrix in three replicates. We recommend using concentrations of  $10^6$  CFU/ml or higher for bacteria and  $10^5$  pfu/ml, or TCID<sub>50</sub>/mL (tissue culture infective dose) or higher for viruses. Please contact FDA if you are unable to obtain specific organisms to discuss potential options and labeling mitigations. *In silico* analyses may be appropriate for certain organisms that are difficult to obtain. .

Please confirm you tested all the organisms listed below (where applicable):

Yes  No

If No, please provide justification: [Provide justification here](#)

Please indicate which organisms demonstrated cross-reactivity:

- |   |   |
|---|---|
| <input type="checkbox"/> variola virus (smallpox)*              | <input type="checkbox"/> molluscum contagiosum virus        |
| <input type="checkbox"/> herpes simplex virus (HSV-1 and HSV-2) | <input type="checkbox"/> vaccinia virus*                    |
| <input type="checkbox"/> varicella-zoster virus (Chickenpox)    | <input type="checkbox"/> <i>Streptococcus mitis</i>         |
| <input type="checkbox"/> <i>Staphylococcus aureus</i>           | <input type="checkbox"/> <i>Staphylococcus epidermidis</i>  |
| <input type="checkbox"/> <i>Streptococcus pyogenes</i>          | <input type="checkbox"/> <i>Streptococcus agalactiae</i>    |
| <input type="checkbox"/> <i>Pseudomonas aeruginosa</i>          | <input type="checkbox"/> <i>Trichophyton rubrum</i>         |
| <input type="checkbox"/> <i>Corynebacterium jeikeium</i>        | <input type="checkbox"/> <i>Candida albicans</i>            |
| <input type="checkbox"/> Human Genomic DNA                      | <input type="checkbox"/> <i>Lactobacillus</i> species       |
| <input type="checkbox"/> <i>Escherichia coli</i>                | <input type="checkbox"/> <i>Acinetobacter calcoaceticus</i> |
| <input type="checkbox"/> <i>Bacteroides fragilis</i>            | <input type="checkbox"/> <i>Enterococcus faecalis</i>       |
| <input type="checkbox"/> cowpox virus*                          | <input type="checkbox"/> Ectromelia (mousepox) virus*       |
| <input type="checkbox"/> camelpox virus*                        | <input type="checkbox"/> <i>Streptococcus</i> Group C       |

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- |   |   |
|---|---|
| <input type="checkbox"/> <i>Streptococcus</i> Group G | <input type="checkbox"/> <i>Corynebacterium diphtheriae</i> |
| <input type="checkbox"/> <i>Neisseria gonorrhoeae</i> | <input type="checkbox"/> <i>Chlamydia trachomatis</i>       |
| <input type="checkbox"/> <i>Mycoplasma pneumoniae</i> | <input type="checkbox"/> <i>Mycoplasma genitalium</i>       |
| <input type="checkbox"/> Human papilloma virus (HPV)  | <input type="checkbox"/> <i>Trichomonas vaginalis</i>       |
| <input type="checkbox"/> <i>Treponema pallidum</i>    |   |

**\*not applicable for Orthopoxvirus tests**

Please include the following as attachments to your EUA Request

- study protocol, including a detailed, step-by-step description of how samples were prepared (e.g., starting concentration, dilution factor used to reach target concentration, volume of organism suspension, volume of clinical matrix, etc.) and tested with your device, the specific materials used to assess cross-reactivity and where these materials were obtained. Please include the Certificates of Analysis for each microorganism that is tested, or equivalent information (e.g., the culture protocol, lot number, manufacturing date, viral strain, a description of viral inactivation, pre-inactivation titer, and pre-inactivation sterility for viral isolates, etc.). For bacterial isolates, please also include the isolate source, method for identification, number of passages, and microbiological features.
- complete line data in an Excel-compatible format, including the analyzer value with each test replicate, if applicable.

For any data or information not included in your attached EUA request, or, for any additional discussion required, please use the space below:

Click or tap here to enter text.

#### 4. Microbial Interference Studies

Did your Analytical Specificity demonstrate cross-reactivity?  Yes  No

If yes, FDA recommends evaluating Microbial interference using at least three replicate samples spiked with a low concentration (2-3x LoD) of monkeypox virus and a high concentration of microorganisms ( $10^6$  CFU/ml or higher for bacteria and  $10^5$  pfu/ml, or TCID<sub>50</sub>/mL (tissue culture infective dose) or higher for viruses). 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 multiple clinical matrices, the study should be performed in the most challenging clinical matrix. 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. Please include the following as attachments to your EUA request:

- Study Protocol, including a detailed, step-by-step description of how samples were prepared (e.g., starting concentration, dilution factor used to reach target concentration, volume of organism



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suspension for both inactivated monkeypox virus and microbial interferent, volume of clinical matrix, etc.) and tested with your test, the specific materials used and where these materials were obtained. Please include the Certificates of Analysis for each microorganism that is tested, or equivalent information (e.g., the culture protocol, lot number, manufacturing date, viral strain, a description of viral inactivation, pre-inactivation titer, and pre-inactivation sterility for viral isolates, etc.). For bacterial isolates, please also include the isolate source, method for identification, number of passages, microbiological features, or other information.

- Complete study line data in an Excel-compatible format, including the analyzer value with each test replicate, if applicable.

For any data or information not included in your attached EUA Submission, or, for any additional discussion required, please use the space below:

Click or tap here to enter text.

### 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.

Please confirm you tested all the potential interferents for skin lesions below  Yes  No

If no, please provide justification: [Provide justification here](#)

FDA recommends that you test the following potential interferents with and without inactivated monkeypox virus at 2-3x LoD in three replicates for each substance. Please indicate those shown to interfere with the test:

- |   |  |   |
|---|--|---|
| <input type="checkbox"/> Abreva (7%)              | <input type="checkbox"/> Acyclovir (2.5-7 mg/mL) | <input type="checkbox"/> Albumin (3.3 mg/mL)      |
| <input type="checkbox"/> Blood/EDTA (5.00%)       | <input type="checkbox"/> Mucin (60 µg/mL)        | <input type="checkbox"/> Hydrocortisone cream*    |
| <input type="checkbox"/> Benadryl cream/ointment* | <input type="checkbox"/> Carmex (7%)             | <input type="checkbox"/> Casein (7 mg/mL)         |
| <input type="checkbox"/> Lanacane (3.5%)          | <input type="checkbox"/> KY Jelly (7%)           | <input type="checkbox"/> Douche (7%)              |
| <input type="checkbox"/> Neosporin*               | <input type="checkbox"/> Female urine (7-10%)    | <input type="checkbox"/> Male urine (7-10%)       |
| <input type="checkbox"/> Feces (0.22%)            | <input type="checkbox"/> Seminal fluid (2-7%)    | <input type="checkbox"/> Zinc Oxide ointment (7%) |
| <input type="checkbox"/> Vagisil Cream (1%)       | <input type="checkbox"/> Cornstarch (2.5 mg/mL)  |   |

\*please identify the concentration used and provide a rationale

For any data or information not included in your attached EUA request, or, for any additional discussion required, please use the space below:

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Click or tap here to enter text.

### 6. Biotin Interference

Does your assay use biotin/anti-biotin capture system?  Yes  No

If yes, 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. We recommend testing up to 3,500 ng/mL with and without inactivated monkeypox virus at 2-3x LoD in your most challenging clinical matrix. Please include the following as attachments to your EUA request:

- Study protocol including, a step-by-step description of how samples were prepared and tested with your device.
- Complete study line data in an Excel-compatible format, including the analyzer value with each test replicate, if applicable.

If biotin interference is observed, please provide an appropriate labeling limitation in your instructions for use.

### 7. High-dose Hook Effect

A high-dose hook effect refers to false negative results when very high levels of target are present in a tested sample. FDA recommends evaluating if a hook effect occurs by testing increasing antigen concentrations.

Does your assay have a high dose hook effect?  Yes  No

If yes, indicate the concentration at which performance begins to degrade: [provide concentration here](#)

Please include the following as attachments to your EUA request:

- Study protocol, including a detailed, step-by-step description of how you prepared and tested each replicate
- complete study line data in an Excel-compatible format, including the analyzer values with each test replicate, if applicable.

### 8. Sample Stability

Does the test include use of specimens other than dry swabs recommended by CDC?  Yes  No

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If yes, 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 50 samples as identified in the table below:

LoD Target Level	Number of Samples
3-5 times LoD	10
1-2 times LoD	30
Negative	10
<b>Total</b>	<b>50</b>

If the test is intended to be performed on the sample immediately or shortly after obtaining the sample, sample stability may be evaluated with contrived samples at 3x LoD using inactivated monkeypox virus spiked into negative clinical matrix for 2 hours at room temperature.

If the test is intended to be performed on clinical samples that have been frozen, you should evaluate both fresh and frozen samples in an equivalence study.

Please include the following as attachments to your EUA request:

- Study Protocol, including a detailed, step-by-step description of how you prepared and tested each replicate, including how you evaluated shipping from a testing site to another location
- Complete study line data in an Excel-compatible format, including the analyzer value with each test replicate, if applicable

For any data or information not included in your attached EUA Submission, or, for any additional discussion required, please use the space below:

Click or tap here to enter text.

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

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. Therefore, each brand of transport media listed in your intended use statement should be validated during your clinical and analytical validation studies and provided in your EUA request.

Once an EUA has been granted, additional brands or types of VTM can be added with a VTM equivalency study. If you plan to add VTM brands post-authorization, please provide a complete VTM equivalency protocol, including a detailed, step-by-step description of how you prepared and tested each replicate, and all study data in an Excel-compatible format, with analyzer values, if applicable. An appropriate VTM equivalency study for most types of VTM would test 5 replicates at 2x LoD, 5 replicates at 5x LoD and 10 negative replicates for three lots of each claimed VTM (60 total replicates per VTM). Each replicate

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should be prepared in negative clinical matrix. For types of VTM with known cross-reactivity issues, additional negative replicates may be requested.

### **10. Clinical Evaluation for Patients Suspected of Monkeypox**

FDA recommends a clinical agreement study with at least 30 positive and 30 negative natural clinical samples evaluated by both the candidate test and a comparator test. Candidate tests should demonstrate a minimum of 80% positive percent agreement (PPA) and 95% negative percent agreement (NPA) for all specimen types.

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  $\geq 80\%$  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.

FDA recommends using only a high sensitivity FDA-cleared or EUA-authorized RT-PCR assay which reports the cycle threshold (Ct) value and uses a chemical lysis step followed by solid phase extraction of nucleic acid (e.g., silica bead extraction) as the comparator test. Evaluations with the comparator test should be conducted per the authorized or cleared instructions for use. If any modifications are made to the authorized or cleared comparator test, we recommend discussing the proposed comparator method with FDA prior to initiating your studies.

FDA recommends a prospective, blinded, randomized study of patients who represent your intended use population (e.g., symptomatic within X days of symptom onset, etc.). If you believe your test may have appropriate performance for all individuals suspected of infection, please contact FDA to discuss the necessary supportive clinical data. As the lower sensitivity of many antigen tests may not support use in all individuals suspected of infection, the number of days post symptom onset should be captured for all clinical samples. 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. Samples from individuals that are not indicated for testing as part of the proposed intended use statement or for which insufficient descriptive information is available (such as days post symptom onset) should be excluded from the primary data analysis. 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.

When collecting samples, the standard of care sample (i.e., the sample used for clinical and not investigational purposes) should always be collected first, including when the comparator test is also the standard of care. If the comparator test is not the standard of care, swabs taken from the same lesion for the comparator test and candidate test should be randomized to ensure that bias is not introduced due to an unequal distribution of viral materials. When two distinct anatomical sites are being assessed, it is not necessary to randomize sample collection order.

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If you seek authorization for multiple lesion sample types (e.g., lesion specimens with and without transport media), each sample type should be evaluated. You may collect samples from different anatomical sites from the same patient to support authorization of multiple lesion sample types. To minimize the occurrence of discordant results due to biological variability, both samples should be collected within a short time period (e.g., within the same healthcare visit).

You may consider use of an enrichment strategy in which individuals with a known monkeypox infection status are invited to participate in your clinical evaluation study. If using an enrichment strategy, you should carefully consider how you will randomize and blind operators to the participant's infection status and minimize potential bias. Data from an enriched study design should represent the full range of viral loads, with both low and high positives samples. Please contact FDA to discuss any alternative study designs or enrichment strategies.

All clinical samples tested in your study should be evaluated in accordance with the candidate test's proposed diagnostic algorithm (i.e., tested using the procedure in the instructions for use), including retesting when appropriate. The limited volume of natural samples may preclude retesting. In instances where retesting is indicated but not performed, for the purposes of performance evaluation, initial results should be analyzed for performance and equivocal/indeterminate/inconclusive results should count against your final performance. Samples should be tested in a blinded fashion, e.g., positive, and negative samples should be presented to the end user in a blinded fashion. The end user should also be blinded to the results of any comparator method testing.

FDA recommends establishing a discordant analysis plan prior to your clinical study. Discordant samples should be tested with a second EUA authorized or cleared RT-PCR test, if available, that has also demonstrated high sensitivity from the evaluation of natural clinical specimen testing and which uses a chemical lysis step followed by solid phase extraction of nucleic acids (e.g., silica bead extraction). Results from a discrepant analysis should not be included in the calculation of NPA and PPA but may be added to the performance table as a footnote.

Studies involving clinical samples (human specimens) conducted in support of an EUA request are subject to applicable requirements for Institutional Review Board (IRB) review and approval and informed consent (see 21 CFR parts 50, 56, and 812). FDA's policy regarding informed consent requirements for certain studies using leftover, de-identified samples is outlined in the FDA guidance "Guidance on Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable."

You may submit your EUA request with results from only the first five prospective clinical samples if you have also conducted a study with retrospective clinical samples. Retrospective clinical samples should be randomized and tested blindly at your clinical study sites. All positive retrospective clinical samples should be reflective of the natural distribution of monkeypox viral loads. Approximately 12-20% of the clinical samples should be low positives, based on observations with clinical samples tested in the US during the 2022 monkeypox virus outbreak. Low positives are defined for purposes of this document as samples in which any gene target is within 3 cycle thresholds (Ct's) of the mean Ct count at the comparator test's LoD. If a retrospective study is leveraged for authorization, the completed prospective study would be a condition of authorization.

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Comparator Test Name: Please describe

Comparator test used per cleared or authorized instructions for use

Specimens:  Multiple specimen types  Fresh  Frozen

Number of natural negative specimens: Please describe

Number of natural positive specimens: Please describe

Please describe

Results Positive percent agreement (PPA): Please describe

Negative percent agreement (NPA): Please describe

Please include the following as attachments to your EUA request:

Study Protocol, including: whether the samples were fully prospective or a mix of prospective and retrospective; the names and locations of the collection and testing sites, number of samples collected at each site, and number of operators used to run your assay at each site; enrollment criteria (inclusion/exclusion criteria); a detailed description of the patient population at each site; the sample matrix(ces) tested for both the candidate test and comparator test; a detailed description of how patients are enrolled and tested; a detailed description of the methods of randomization and blinding; the technique and collection device(s), including transport media, used to obtain clinical samples; a detailed description of how samples were collected, transported, stored, and tested with the candidate test and the comparator test; and a detailed description of the order in which swabs were collected

Complete study line data in an Excel-compatible format, including the following for each sample evaluated by the candidate and comparator test: sample type; sample collection date and time; sample testing date and time; transport media type as applicable; test result (with the analyzer or reader value for the candidate test and +/- and Ct value for the comparator test); number of days post-onset of patient symptoms; and patient age and gender, if available

Detailed study design for post-authorization clinical studies, as applicable

### 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) supplemental POC samples, and (3) POC flex studies. For more details, please see each section below.

#### a. Clinical Evaluation

The clinical study design should mimic how the test will be used in clinical practice. It is expected that a test with a "POC" designation will be widely used in CLIA waived medical facilities (e.g., physician office, outpatient clinic, emergency room (ER)) where health care providers are present.

Please see section C(10), for recommendations regarding clinical validation studies. The clinical evaluation study recommendations from section C(10) are applicable to POC clinical evaluation studies. Please note that all elements of the clinical evaluation study for a candidate test intended for POC use should be conducted in an appropriate POC clinical study site (i.e., a CLIA waived site in the US). You may

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also send the samples for the cleared or EUA authorized RT-PCR comparator test to a central laboratory for testing if that is within the comparator test's authorized intended use.

### **i. Site and Test Users (Operators):**

You should select one or two non-laboratory sites in the United States (U.S.) to assure that the operators are representative of operators in the U.S., e.g., doctor's office, ER, outpatient clinic, or another area in a medical facility outside the central laboratory where samples are collected and tested in real time. This would allow evaluation of the sample collection and handling, including addition into the sample port/well of the test, both of which may be significant sources of error. Four to six operators, representing healthcare professionals, but who are not laboratory trained (e.g., nurses, nursing assistants and doctors) should participate in the study. Testing should be performed using only the Quick Reference Instructions (QRI); supplemental materials, such as a video or mobile application that can be easily accessed by the user, are encouraged to be included with the proposed candidate test but should not be used during the study to mimic the worst-case scenario.

Please provide the detailed individual replicate result data in an Excel-compatible format and protocols for each of your studies, including:

- The objective of the study
- Detailed test procedure
- Materials used
- A list of samples tested
- Results (presented in tabular format), including invalid results
- Conclusions
- Any appropriate mitigation measures (e.g., labeling changes, changes to test design, etc.)
- Operator background (e.g., education, training, experience, etc.)

As part of your EUA request, please include a table in which your study results are stratified by operator.

### **b. Performance around LoD:**

You should also conduct testing with contrived samples prepared with non-variola *Orthopoxvirus* or monkeypox viral load near the LoD of the candidate test in clinical matrix. Samples should mimic the clinical specimens applicable to the candidate test as closely as possible (e.g., direct dry swab samples). The testing should be conducted by minimally trained operators and should consist of 10 low positives (<2x LoD) and 10 negative samples per site. All contrived samples should be blinded and randomized and Each operator should test at least three low positive and three negative samples integrated into the site's workflow with the clinical samples above. These samples are intended to supplement, not replace, the clinical samples in your study.

Please provide a table in an Excel-compatible format with your study results stratified by operator.

### **c. POC Flex Studies:**

You should also conduct a thorough hazard analysis considering the main known sources of errors. Based upon your hazard analysis, you should conduct flex studies to evaluate the impact of errors, or out-of-specification conditions, on the candidate test performance. Each sample should be prepared at

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2xLoD in negative clinical matrix and should be evaluated in three replicates for each condition under evaluation. Flex studies can be conducted with trained operators at an internal testing site.

Each study should be performed using a pre-defined study protocol that includes the following:

- The objective of the study
- Detailed test procedure
- Materials used

Potential stress conditions include:

- 40°C and 95% relative humidity (RH) (mimicking hot and humid climates);
- Delay in sample testing or reading time;
- Delay and/or disturbance in operational steps;
- Sample volume variability;
- Buffer volume variability;
- Read time variability;
- Swab rehydration volume and time variability (if applicable);
- Other, as appropriate.

Please provide the following:

- Detailed, step-by-step description of how you prepared and tested each replicate
- All study data in an Excel compatible format, with analyzer values, if applicable. Data for each sample evaluated (i.e., line data) should be provided.
- Adequate mitigation(s) if erroneous results are observed during studies evaluating the robustness of the device

Refer to “Appendix A: Recommended Flex Study Design Details” of this template for more in-depth flex study designs. Alternative sources of information on flex studies that may be appropriate for the candidate test can be found on the FDA CDRH website containing **CLIA Waiver by Application Decision Summaries**<sup>15</sup>

For any data or information not included in your attached EUA request, or, for any additional discussion required, please use the space below:

Click or tap here to enter text.

### D. FACT SHEET FOR HEALTHCARE PROVIDERS AND PATIENTS

FDA may provide Fact Sheet Templates. Please check the FDA website for additional information.

<sup>15</sup> Available at <https://www.fda.gov/about-fda/cdrh-transparency/clia-waiver-application-decision-summaries>.



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### E. LABELING

Instructions for Use – at a minimum you should include the following sections in your IFU:

- Intended Use
- Summary and Explanation of the Test/Product Description
- Reagents and Materials Provided – this section should include details of the materials provided with the test including storage and handling requirements.
- Reagents and Materials Not Provided with the Test
- Instruments and Software Required
- General Warning and Precautions
- Sample Collection, Handling and Transport
- Test Procedure
- Test Results

a. Quality Control Result Interpretation

Appropriate control interpretation criteria: Please describe in detail the expected results generated, including the acceptance criteria, for all the controls described in Section A.6 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 result.

b. Patient Specimen Result Interpretation (see below)

- Please describe when clinical sample test results should be assessed and outline the criteria for test validity.
- Appropriate specimen interpretation criteria. Please describe how to interpret numeric test values (if applicable) as positive or negative for presence of *Orthopoxvirus* or monkeypox virus antigen<sup>16</sup>. If applicable, indicate how to identify indeterminate/inconclusive/equivocal results. When applicable, we recommend providing 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, if applicable. If the test produces a presumptive result, please indicate what follow-up testing/process should be conducted, i.e., sending specimens to a reference laboratory for confirmation with a molecular assay – clinical management of the patient should be based on the presumptive result and not wait for CDC confirmation.

<sup>16</sup> Note: If you propose to report a quantitative value to healthcare providers, there may be additional considerations for the analytical and clinical validation of your device. Please contact FDA to discuss any validation proposals for a quantitative or semi-quantitative assay.

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### c. Appropriate Result Reporting

Test results are to be reported to healthcare providers and relevant public health authorities in accordance with local, state, and federal requirements, using appropriate LOINC and SNOMED codes, as provided by the Centers for Disease Control and Prevention (CDC) at [How to Report Test Results | Monkeypox | Poxvirus | CDC<sup>17</sup>](#)

- Limitations
- Conditions of authorization for the laboratory
- Performance Characteristics – this will include a summary of the test analytical and clinical performance.
- Additional Information (optional)
- Symbols
- Technical Support Information
- Manufacturer and Distributor Information.
  - a. Box labels
  - b. Vial labels
  - c. Any additional proposed labeling, if applicable

## F. RECORD KEEPING AND REPORTING INFORMATION

**FDA recommends that you confirm your agreement with the following statement to help facilitate authorization in the event FDA determines it is appropriate to authorize the candidate test:** Yes No

**As allowed by Section 564(e) of the FD&C Act, FDA may require certain conditions as part of an EUA. FDA generally includes the following record keeping and reporting information requirements in the EUA.**

Test Developer 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 Test Developer name's Product Support website: Include link to Test Developer's Website Each report of an adverse event will be processed according to Test Developer name's Non-Conformance Reporting Requirements, and Medical Device Reports will be filed with the FDA as required. Through a process of inventory control, Test Developer name will also maintain records of device usage/purchase. Test Developer 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 Test Developer name will become aware. Test Developer 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.

<sup>17</sup> Available at <https://www.cdc.gov/poxvirus/monkeypox/lab-personnel/report-results.html> (last accessed on October 23, 2022). Note this website is not controlled by FDA

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### **Appendix A: Recommended Flex Study Design Details, as appropriate for the device.**

**If incorrect results are observed under the test conditions, the test developer should implement adequate mitigations to prevent reporting of erroneous results.**

**1. Reading Time:**

You should evaluate test results at multiple reading times four-fold below and three-fold above the recommended reading time for the candidate test. For example, where the recommended read time is 20 minutes, you should evaluate read times of 5, 10, 15, 20, 30, and 60 minutes, at a minimum. If incorrect results are observed, the developer should propose adequate mitigations.

**2. Specimen Volume:**

You should evaluate candidate test results at sample volumes two times below and two times above the recommended sample volume, and the maximum possible added. For example, where the recommended sample volume is 10  $\mu\text{L}$ , you should evaluate sample volumes of 5, 10, and 20  $\mu\text{L}$ , as well as the maximum sample volume. If incorrect results are observed at either 5 or 20  $\mu\text{L}$ , additional testing at 7.5 and/or 15  $\mu\text{L}$  may be needed. The amount of diluent/buffer added should be that specified in the instructions for use.

**3. Sample Diluent Volume:**

You should evaluate candidate test results at diluent/buffer volumes at two times below and two times above the recommended diluent/buffer volume specified in the instructions for use and the maximum volume. For example, where the recommended buffer/diluent volume is 2 drops, you should evaluate sample diluent volumes of 1, 2, 3, 4 drops and the whole bottle.

**4. Sample Elution:**

You should evaluate how mixing the swab in elution buffer (or other reagent) affects candidate test results. You should evaluate all extremes from not-mixing to vigorous shaking, including generating bubbles and intermediate mixing, ( i.e., swirling 1 or 2 times).

**5. Temperature and Humidity:**

You should evaluate candidate test results at temperature and humidity extremes that are likely to occur in the United States (i.e., 40°C and 95% RH to mimic a hot and humid climate, and 5°C and 5% RH to mimicking a cold and dry climates).

**6. Light:**

You should evaluate candidate test results in different lighting conditions that would be expected during use (i.e., fluorescent, Incandescent, and natural lighting mimicking the outside environment).

**7. Disturbance During Analysis:**

You should evaluate the effect on expected candidate results of moving the candidate test while the candidate test is running. This could include dropping the candidate test while it is being run, moving the candidate test to another surface, unplugging the candidate test, receiving a phone call while the mobile app is running, etc.

**8. Device Orientation:**

### ***Contains Nonbinding Recommendations***

You should evaluate unique device characteristics, as determined by a robust risk analysis. For example, if the candidate test is intended to be run upright, you should evaluate candidate test results if the candidate test is run horizontally, or vice versa.