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 validation studies be conducted for such molecular diagnostic tests as described below (e.g., : 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, in 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

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 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 molecular test that detects organism DNA 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, real-time PCR test]*** intended for the ***[presumptive]*** qualitative detection of DNA from [***non-variola Orthopoxvirus/monkeypox virus***] in human skin lesion material specimens ***[describe all the sample types that were evaluated, e.g., such as lesion exudate, lesion roofs or lesion crusts, etc.] [If your test is intended for testing 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 regulatory requirements to perform high complexity testing.

Results are for the identification of [***non-variola Orthopoxvirus or monkeypox virus***] DNA. The [***non-variola Orthopoxvirus or monkeypox virus***] DNA is generally detectable in ***[name sample type, such as lesion exudate, lesion roofs or lesion crusts, etc.]*** during the acute phase of infection. Positive results are indicative of the presence of [***non-variola Orthopoxvirus or monkeypox virus***] DNA; 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 obtained with this device do not preclude [***non-variola Orthopoxvirus or monkeypox virus***] infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

Laboratories within the United States and its territories are required to report test 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 the techniques of PCR and in vitro diagnostic procedures]***.

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

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:** ☐ RT-PCR ☐ LAMP ☐ TMA ☐ Chemiluminescent ☐ Sequencing ☐ CRISPR ☐ 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. Note: Validation should be performed with the instruments and/or extraction methods claimed for use with your test. Refer to "Appendix B: Multiple Instruments and/or Extraction Methods" of this template for additional recommendations.

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:

Control		**Requirement**	**How it works**	**Where it is used**	**Frequency of use**
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.	Describe need	Describe need	Describe how the control is expected to work	Describe frequency of use

[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.

Control		Requirement	How it works	Where it is used	Frequency of use
Negative	Describe the control material; if external, include supplier and catalog #	Describe need	Describe need	Describe how the control is expected to work	Describe frequency of use
Extraction	Describe the control material; if external, include supplier and catalog #	Describe need	Describe need	Describe how the control is expected to work	Describe frequency of use
Internal	Describe the endogenous internal control material (i.e., sample adequacy, internal); if external, include supplier and catalog #.	Describe need	Describe need	Describe how the control is expected to work	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.

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

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[4]:** 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])

[4] Under an EUA, certain sections of the 21 CFR Part 820 Quality System Regulation (QSR) requirements may be waived for an authorized produced during the duration of the EUA, but FDA recommends that test developers follow comparable practices as much as possible, even if such requirements are waived.

5. **Manufacturing and Testing Capabilities**

   Total time required to perform all steps of the test (e.g., extraction setup and run, detection setup, run and result analysis): Please describe

   Number of patient tests that can be performed per day (8hr shift): Please describe the number of patient tests that can be performed per day (8-hr shift) using a 1 instrument setup (e.g., 1 extraction instrument, 1 PCR instrument, etc.) and 1 trained laboratory user.

   Current manufacturing capacity: Number of tests manufactured per7 days for US distribution: Please describe

   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. Please include approximation and weeks/months

8. **Device Components:**

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

   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 the test 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 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),
- 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 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 Error: Reference source not found. Refer to the Error: Reference source not found 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 nucleic acid 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.

9. 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

If No, please provide the following:

Software Level of Concern[5]: ☐Major ☐Moderate ☐Minor

Software Validation[6]: ☐ Validation complete ☐ Developed per GMPs[7]

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);

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

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

[7] 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," 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.

☐ 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	**Evidence that the design of the system can fulfill the specification. This column should consist of system-level validation data.**
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 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

10. **Basic Safety and Essential Performance[8]:**

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;

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

11. **Electromagnetic Compatibility (EMC) Testing[9]:**

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

12. **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.

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:

[8] 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.

[9] Please see Electromagnetic Compatibility (EMC) of Medical Devices | FDA

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[10]: ☐ External positive ☐ External negative

Number of Samples: FDA recommends at least one sample

Sample Preparation: FDA recommends spiking negative clinical 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

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)

Freeze-Thaw Stability: Please describe how your stability design supports freeze-thaw reagent stability (N/A if not applicable)

Unopened 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 (Ttest-Tbaseline)/ Tbaseline*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 Shipping Stability: You should evaluate the anticipated shipping times and temperatures expected under different temperature conditions.

[10] 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.

Inverted Stability: 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. Please refer to Appendix B for additional information regarding multiplex panels and Appendix C for additional information regarding tests with multiple instruments or extraction methods.

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

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) (or genomic DNA until viral isolates of the currently circulating strain become publicly available) into natural clinical matrix (e.g., human skin lesion material specimens, or for dry swabs, an acceptable simulated matrix derived from natural clinical matrix). Live or inactivated virus is the preferred viral material for this evaluation, use of natural genomic DNA or synthetic DNA may be acceptable if access to live or inactivated virus is limited at the time of your studies. Details of your choice of natural or synthetic DNA should be discussed with FDA prior to use. Additional LoD testing of live or inactivated virus, if available, may be required as a condition of authorization. Collection media without clinical matrix are generally not considered 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, you should 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 at least 19 of 20 replicates are positive. The preliminary LoD studies should include at least one concentration that does not yield 100% positive results. The LoD range finding study should include at least one concentration that achieves 95% detectability of replicates and at least one concentration that achieves less than 95% detectability. Replicates should be interpreted per the result interpretation of your test. If multiple clinical matrices are intended for clinical testing, the LoD for each should be evaluated. If the candidate test is a non-variola *Orthopoxvirus* IVD you should perform the LoD with at least two species of non-variola orthopoxviruses or if not available, synthetic DNA may be used.

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

☐ natural genomic DNA ☐ synthetic DNA

Please include the following as attachments to your EUA Request

☐ Study Protocol, including 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 (please provide the stock concentration in copies/mL, 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 inactivated 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.

☐ 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 (applicable to studies using contrived samples); raw signal output (i.e., cycle threshold (Ct) values) and final results for each distinguishable target for both the candidate test and the comparator test (as applicable); and for both the candidate test and the comparator test, a final result for each sample/replicate based on the result interpretation algorithm of the test.

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)

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.

For tests targeting monkeypox virus, in silico analysis demonstrates inclusivity of isolates from:

☐ West African clade (clade II) ☐ Congo Basin clade (clade I) – Zaire strains in BEI

For tests targeting non-variola *Orthopoxvirus*, in silico analysis demonstrates inclusivity of:

☐ monkeypox virus ☐ cowpox virus ☐ Ectromelia (mousepox) virus

☐ camelpox virus ☐ Vaccinia virus ☐ Other* Please describe

Please provide in silico analysis against all available applicable nucleic acid sequences in well-established publicly available databases (e.g., NCBI, GISAID, etc.) and submit the result for review including the date the analysis was performed.

Please indicate the % homology for your test's primers/probes. The % homology is calculated by dividing the number of exact matches from the longest individual alignment by the total length of primer/probe being queried. If sequences with less than 100% homology with any of the primers/probes in your test are identified, please provide a risk assessment on how such mismatches may impact the performance of your test.

Test developers should also monitor new and emerging viral mutations and variants that could impact molecular 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. This may be accomplished via *in silico* analysis of published monkeypox virus sequences compared to the assay's primers and probes. 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 minimum clinical performance recommendations.

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 a 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 molecular test performance on an ongoing basis; please include your assessment strategy for the impact on the performance of your assay over time

Monitoring Access Points: Please describe where you plan to access monitoring information (e.g., sequence databases such as GISAID

Monitoring Frequency: Please describe the frequency of monitoring; FDA recommends at least monthly monitoring

Strategy to Choose Targeted Amplification Regions, and Specific Primary and Probe Regions: Please describe your proposed strategy

If any mutations and/or variants have been identified as prevalent and/or clinically significant, please provide the following information:

Mutation and/or Variant: Please describe

Mitigation Plan: Please describe

Critical to Test Performance: ☐ Yes ☐ No

Please describe; please include analysis method (e.g., in silico analysis of published sequences compared to the assay's primers and probes)

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)

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

☐ Yes ☐ No

If No, please provide justification: Provide justification here

Please indicate which microorganisms demonstrated ≥80% homology with your test primers or probe(s) set(s):

☐ variola virus (smallpox)	☐ molluscum contagiosum virus
☐ herpes simplex virus (HSV-1 and HSV-2)	☐ vaccinia virus*
☐ varicella-zoster virus (Chickenpox)	☐ *Streptococcus mitis*
☐ *Staphylococcus aureus*	☐ *Staphylococcus epidermidis*
☐ *Streptococcus pyogenes*	☐ *Streptococcus agalactiae*
☐ *Pseudomonas aeruginosa*	☐ *Trichophyton rubrum*
☐ *Corynebacterium jeikeium*	☐ *Candida albicans*
☐ Human Genomic DNA	☐ *Lactobacilllus* species
☐ *Escherichia coli*	☐ *Acinetobacter calcoaceticus*
☐ *Bacteroides fragilis*	☐ *Enterococcus faecalis*
☐ cowpox virus*	☐ Ectromelia (mousepox) virus*
☐ camelpox virus*	☐ *Streptococcus* Group C
☐ *Streptococcus* Group G	☐ *Corynebacterium diptheriae*
☐ *Neisseria gonorrhoeae*	☐ *Chlamydia trachomatis*

☐ *Mycoplasma pneumoniae* ☐ *Mycoplasma genitalium*

☐ Human papilloma virus (HPV) ☐ *Trichomonas vaginalis*

☐ *Treponema pallidum*

*not applicable for non-variola *Orthopoxvirus* tests

FDA recommends you provide your in-silico cross-reactivity data in tabular form identifying the pathogen, strain, accession # (e.g., you may pick one that represents the complete genome of the organism), and individual % homology of your test primers and probes for all targets across organisms evaluated. The % homology is calculated by dividing the number of exact matches from the longest individual alignment by the total length of primer or probe being queried.

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 Cross-Reactivity (Analytical Specificity) *in silico* analysis reveal ≥ 80% homology between microorganisms and your test primers/ probe(s) set(s)? ☐ Yes ☐ No

If Yes, FDA recommends providing one of the following (please check where applicable):

☐ Microbial interference study with the target virus and the microorganisms (using concentrations of 10^6 CFU/ml or higher for bacteria and 10^5 copies/mL or pfu/ml or higher for viruses). Please include the following as attachments to your EUA request:

☐ Study Protocol, including a 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 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, information may 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.

☐ Justification as to why the performance of your test would not be impacted by the presence of a causative agent of a clinically significant co-infection (e.g., amount of primer(s)/ probe(s) included in your master mix)

Please provide justification here, if relevant

☐ Explanation as to why the in silico results are clinically irrelevant

Please provide explanation here, if relevant

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 studies of interference substances depends on the matrix that is indicated for the candidate test as well as on the technology of the candidate test. If the candidate test uses extraction methods not previously reviewed by FDA as part of premarket submission and not covered under specific FDA enforcement discretion, or if the candidate test does not use an extraction procedure (as for example, many point-of-care tests), we recommend testing for potential interferents.

Does the test use extraction methods not previously reviewed by FDA as part of premarket submission or not use an extraction procedure? ☐ Yes ☐ No

If yes, please confirm you tested all of the potential interferents below ☐ Yes ☐ No

If no, please provide justification: Provide justification here

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

☐ Abrevea (7%)	☐ Acyclovir (2.5-7 mg/mL)	☐ Albumin (2.2 mg/mL)
☐ Blood/EDTA (5.00%)	☐ Mucin (60ug/mL)	☐ Hydrocortisone cream*
☐ Benadryl cream/ointment*	☐ Carmex (7%)	☐ Casein (7mg/mL)
☐ Lanacane (3.5%)	☐ KY Jelly (7%)	☐ Douche (7%)
☐ Neosporin*	☐ Female urine (7-10%)	☐ Male urine (7-10%)
☐ Feces (0.22%)	☐ Seminal fluid (2-7%)	☐ Zinc Oxide ointment (7%)
☐ Vagisil Cream (1%)	☐ Cornstarch (2.5mg/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:

Click or tap here to enter text.

6. Sample Stability

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

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
Total	**50**

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 virus (or genomic DNA until viral isolates of the currently circulating strain become available) 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.

7. Clinical Evaluation

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

FDA recommends using only a high sensitivity FDA-cleared or EUA-authorized RT-PCR assay which 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. The comparator test may have the same, or different, targets as the candidate test; however, different (e.g., non-overlapping) primer and probe regions are recommended.

FDA recommends use of natural clinical specimens, if available, in a prospective, blinded, randomized study of patients suspected of monkeypox by their healthcare provider. 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 anatomical area 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. Frozen samples may be used if analytical data are provided to demonstrate that preservation of samples (e.g., by freezing at ≤-70°C) does not affect the accuracy of test results compared to freshly collected samples. Samples that previously tested positive by another cleared or authorized PCR assay may be used without additional comparator testing. If fewer than 20% of positive samples are low positives per the comparator assay (i.e., Ct values within 3 Ct of the mean Ct at the LoD of the comparator test), the prospective samples should be supplemented with additional low positive samples (i.e., archived samples, samples collected from convalescent patients, etc.) such that 20% of all positive samples in the analysis have low viral load.

If you seek authorization for multiple sample types (e.g., specimens with and without VTM), each sample type should be evaluated. You may collect samples from different anatomical sites from the same patient. 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 PCR test, if available, that has also demonstrated high sensitivity 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*."[11]

If no prospective or retrospective specimens are available at the time of your submission, such as at the time of issuance of this template, a fully contrived clinical evaluation may be acceptable for initial authorization, with additional clinical testing of positive natural clinical specimens provided as a condition of authorization. Each contrived clinical specimen should be prepared using a unique natural clinical specimen matrix. Half should be prepared at the LoD and half should cover the range of the test up to and not higher than 5x LoD. If natural clinical specimens are evaluated, approximately 20% of the positive samples should have a low viral load (i.e., low positives) as measured by the comparator test (i.e., Ct values should be within 3 Ct of the mean Ct at the LoD of the comparator test).

Comparator Test Name: Please describe

☐ Comparator test used per cleared or authorized instructions for use
☐ Comparator test not cleared or authorized or modified instructions for use
☐ Not applicable for contrived specimen testing

Specimens: ☐ Multiple specimen types ☐ Fresh ☐ Frozen
Number of natural negative specimens: Please describe
Number of natural positive specimens: Please describe
Number of contrived positive specimens: Please describe

Results Positive percent agreement (PPA): Please describe
Negative percent agreement (NPA): Please describe

[11] https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-informed-consent-vitro-diagnostic-device-studies-using-leftover-human-specimens-are-not.

Please include the following as attachments to your EUA request:

- ☐ Study Protocol, including how the samples were generated, collected, and sourced; if the samples were fully prospective or a mix of prospective and retrospective; for samples known to be positive, the type and source of the samples, results, and numerical output signals such as Ct values or numerical output for each tested sample, and the initial test date; inclusion/exclusion criteria; collection and testing sites; number of samples collected and tested; number of operators performing the testing; discordant analysis plan; and any enrichment strategy used
- ☐ Complete study line data in an Excel-compatible format
- ☐ Detailed study design for post-authorization clinical study of natural specimens, if only contrived specimens were used

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 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.

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.

ii. Comparator Method: A description of an appropriate clinical comparator test is included in section C7 above.

iii. Clinical Samples:

A total of 30 prospectively collected positive (confirmed by an EUA-authorized or cleared test) and 30 negative natural clinical samples should be tested (mock clinical samples are not appropriate). 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. If obtaining positive specimens (<30) is challenging, you may collect samples at another site to ship to the testing site or use banked specimens to supplement your positive specimens. Banked samples should not be pre-selected based on Ct value and should be presented blinded (mixed with negatives) to the testing site. Ideally, the same comparator method should be used for banked and prospectively collected samples. Depending upon the number of prospective and banked positive specimens that are acquired during your study, FDA generally intends to include a condition of authorization that you conduct additional clinical testing of prospective natural clinical specimens.

iv. Clinical Performance:

A molecular POC candidate test should demonstrate positive and negative agreement of ≥ 95%. However positive agreement of ≥ 80% may be considered with appropriate limitations added to the intended use that would mitigate the risk of false negative results. For example, negative results may be considered presumptive negative if the demonstrated PPA is lower than 95%.

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 your assay in clinical matrix. Samples should mimic the clinical specimens applicable to the candidate test as closely as possible (e.g., **d**irect dry swab samples). The testing should be conducted by minimally trained operators and should consist of 10 low positives (<2 times 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 include the following as attachments to your EUA request:

☐ Study Protocol, including how the samples were generated, collected, and sourced; if the samples were fully prospective or a mix of prospective and retrospective; for samples known to be positive, the type and source of the samples, results, and numerical output signals such as Ct values or numerical output for each tested sample, and the initial test date; inclusion/exclusion

criteria; collection and testing sites; number of samples collected and tested; number of operators performing the testing; discordant analysis plan; and any enrichment strategy used

☐ Complete study line data in an Excel-compatible format

☐ Detailed study design for post-authorization clinical study of prospective natural specimens, if applicable

☐ A table in an Excel-compatible format in which your study results are 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-specifications conditions, on the candidate test performance. Each sample should be prepared at 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 descriptions 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 C: 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***[12]

[12] Available at https://www.fda.gov/about-fda/cdrh-transparency/clia-waiver-application-decision-summaries.

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.

E. LABELING

1. 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
    b. Patient Specimen Result Interpretation (see below)

    Appropriate control interpretation criteria: Please describe if a Ct (cycle threshold) cutoff is used as part of your testing algorithm and/or if the end user is required to review curves before final result interpretation. If the test result involves the use of an algorithm/calculation (e.g., a ratio value), please include a detailed description and any additional calibration materials that may be required

    - Please describe in detail the expected results generated, including acceptance criteria, for all the controls. 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.
    - 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 as positive or negative for presence of non-variola *Orthopoxvirus* or monkeypox virus, including how to identify indeterminate/inconclusive/equivocal results (if applicable). Please describe if a Ct (cycle threshold) cutoff is used as part of your testing algorithm and/or if the end user is required to review curves before final result interpretation. If the test result involves the use of an algorithm/calculation (e.g, a ratio value), please include a detailed description and any additional calibration materials that may be required. Include a table of the possible combinations of test result values for each primer/probe set and description of how they should be combined into a final interpretation of the result for your test (if applicable).

- Appropriate reporting steps: Please indicate what follow-up testing/process should be conducted If the test produces an equivocal or indeterminate result or a presumptive result (e.g., sending specimens to CDC for confirmatory testing, base clinical management of the patient on the presumptive result while awaiting CDC confirmation). All test results should 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 defined by the *Laboratory In Vitro Diagnostics (LIVD) Test Code Mapping for SARS-CoV-2 Tests*[13] provided by the Centers for Disease Control and Prevention (CDC). Core diagnostic data elements should be collected for all tests, which have been defined by the Department of Health and Human Services (HHS), along with technical specifications for implementation for lab-based and non-lab-based tests.

- 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.

2. Box labels
3. Vial labels
4. 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

[13] Available at https://www.cdc.gov/csels/dls/sars-cov-2-livd-codes.html *(last accessed on July 7, 2021). Note this website is not controlled by FDA.*

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 becomes 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.

Appendix A: Multi-Analyte Panels that Include Non-monkeypox virus/*Orthopoxvirus* analytes

To support an EUA for a multi-analyte panel candidate test that was not previously cleared by FDA, the following evaluations should be provided *for each target analyte*.

- Limit of Detection (Analytical Sensitivity)
- Cross-Reactivity / Microbial Interference
- Inclusivity / Analytical Reactivity
- Collection Media Equivalency for any sample collection media not used in your clinical study
- Interfering Substances Study (Exogenous)
- Clinical Sample Stability
- Reagent Stability testing protocol
- Carry over/Cross-Contamination
- Reproducibility and Repeatability
- If evaluations use archived frozen samples, demonstrate that preservation of samples (e.g., by freezing at ≤-70°C) does not affect the accuracy of test results compared to freshly collected samples.

Clinical performance of multi-analyte tests should be established through a prospective study. Initial authorization may be based on a study conducted at one site with archived positive and negative clinical samples. The pre-selection of archived positive samples should represent a range of viral load or Ct values including low positive samples near the candidate test cut-off. A minimum of 30 positives samples (archived if available or contrived) for each target analyte should be included.

FDA intends to request as a condition of authorization a clinical study conducted at a minimum of three sample collection sites and three testing sites, prospectively enrolling patients with general signs and symptoms of the target diseases (e.g., skin lesions/rash). For multiplex candidate tests that detect and differentiate monkeypox virus/*Orthopoxvirus* from other analytes, the post-authorization study should include a minimum of 50 positive prospectively collected and tested samples of each analyte. Until this study is completed and results reviewed by the FDA, FDA recommends including a warning/limiting statement in the instructions for use for your test indicating that results (positive and negative) for the non- monkeypox virus/*Orthopoxvirus* analytes should be confirmed with an FDA-cleared nucleic acid amplification test (NAAT) if clinically indicated.

The clinical performance of the candidate test for the non-monkeypox virus/*Orthopoxvirus* analytes (e.g., varicella-zoster virus and HSV 1/2, etc.) should be determined by comparison to an FDA-cleared or EUA-authorized PCR test with prospective clinical study data from the past 5 years. FDA recommends the following minimum performance for each analyte:

≥95% PPA (with a lower bound of the two-sided 95% confidence interval ≥85%)

≥95% NPA (with a lower bound of the two-sided 95% CI ≥90%)

We recommend that you submit a Pre-EUA with an outline of the studies that you plan to conduct to support the FDA-authorization or contact FDA at MPXDx@fda.hhs.gov for specific feedback.

Appendix B: Multiple Instruments and/or Extraction Methods

To support an EUA for a test that can be run with multiple instruments or extraction methods, the following should be considered in the performance evaluations:

- Limit of Detection (LoD) should be repeated for each PCR instrument as follows:
    - First, determine the preliminary and final LoD for one PCR instrument with each extraction method. If you detect 20/20 replicates in your final LoD study, test lower concentrations using a 3-fold dilution until you detect <20/20.
    - Select the extraction method with the least sensitive LoD for evaluation of the remaining PCR instruments. If the different extraction methods yield a similar LoD on the first PCR instrument, you can use any extraction method.
    - An adaptive LoD study design may be used for the remaining PCR instruments: Perform a refined preliminary LoD study with 5 replicates at 0.5, 1, and 1.5 to 2 times LoD. If you detect 4/5 replicates as positive at all the tested levels, include the next higher concentration (i.e., 3 times LoD). If you detect 5/5 replicates at 0.5 times LoD, test the next lower concentration (i.e., 0.25 times LoD). Test in this manner until you find the lowest concentration that gives you 5/5 positive results for each PCR instrument. This concentration should be used for a confirmatory LoD study for that PCR instrument using 20 replicates.
    - Report the LoD for each PCR instrument separately, unless they are comparable (i.e., between 1-3 times LoD).
    - Repeat studies for each clinical matrix for which the candidate test is indicated. If there are differences in the extraction input volume, extraction elution volume, and PCR input volume (extracted nucleic acid) then the LoD should be confirmed for each.
- Interfering substances should be evaluated with the extraction method and PCR instrument combination that has the least sensitive LoD.
- Inclusivity should be evaluated with the extraction method and PCR instrument combination that has the least sensitive overall LoD.
- Cross-reactivity (analytical specificity) should be evaluated with all extraction/instrument combinations.
- If one or more PCR instruments have different LoDs, the clinical study should be conducted with the extraction method / PCR instrument combination with the least sensitive LoD. If an LoD study confirms equivalency for all PCR instruments (between 2-3 times LoD), the clinical study may be conducted with any PCR instrument.

Appendix C: 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 µL, you should evaluate sample volumes of 5, 10, and 20 µL, as well as the maximum sample volume. If incorrect results are observed at either 5 or 20 µL, additional testing at 7.5 and/or 15 µL 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 mimic~~king~~ a cold and dry climate)~~s~~.

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:

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.