.

Instructions and requirements for Emergency Use Listing (EUL) Submission:

In vitro diagnostics detecting Monkeypox virus nucleic acid

Emergency Use Listing of IVDs

PQDx_457 approved version; 10 September 2024

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Abbreviations

CR	Change request
EUL	Emergency Use Listing procedure
FDA	Food and Drug Administration (USA)
IFU	Instructions for Use
IS	International Standard
ISO	International Organization for Standardization
IVD	In vitro diagnostic
LoD	Limit of Detection
mpox	formerly known as Monkeypox disease
MPXV	Monkeypox virus
NAT	Nucleic Acid Test
OCR	Optical Character Recognition
OPXV	Orthopox virus
PCR	Polymerase Chain Reaction
PFU	Plaque-forming Units
PHEIC	Public Health Emergency of International Concern
POC	Point of Care
PMS	Post-market surveillance
TCID	Tissue Culture Infectious Dose
UN	United Nations
UTM	Universal Transport Medium
VTM	Viral Transport Medium

1 Introduction

Mpox (formerly known as Monkeypox disease) has been reported in the Democratic Republic of the Congo (DRC) for more than a decade, and the number of cases reported each year has increased steadily over that period. In July 2022, the multi-country outbreak of mpox was declared a Public Health Emergency of International Concern (PHEIC) as it spread rapidly via sexual contact across a range of countries where the virus had not been seen before. That PHEIC was declared over in May 2023 after there had been a sustained decline in global cases. However, last year, reported cases again increased significantly in DRC, and Monkeypox virus continues to affect people around the world. The detection and rapid spread of a new subclade (clade Ib) of Monkeypox virus in eastern DRC, its detection in neighbouring countries that had not previously reported mpox, and the potential for further spread within Africa and beyond has prompted the renewal of its classification as a PHEIC as of 14 August 2024.

The WHO Emergency Use Listing (EUL) Procedure is primarily used during a PHEIC. The EUL process is based on an essential set of available quality, safety and performance data. The EUL procedure for IVDs to detect Monkeypox virus is intended to expedite the availability of IVDs needed in PHEIC situations and, in that context, to assist interested UN procurement agencies and Member States in determining the acceptability of using specific products for time limited procurement.

The EUL procedure includes the following:

- **Quality Management Systems Review and Plan for Post-Market Surveillance**: review of the manufacturer's Quality Management System documentation and specific manufacturing documents;
- **Product Dossier Review**: assessment of the documentary evidence of safety and performance.
- **Independent laboratory evaluation**: WHO reserves the right to conduct an independent laboratory evaluation of limited scope if it is deemed necessary. The study (studies) can either be conducted during the assessment phase and/or post EUL listing.

2 Intended Audience

This document has been prepared to assist manufacturers in correctly compiling the documentary evidence for the purposes of WHO EUL review of IVDs to detect Monkeypox virus nucleic acid. It describes the required information to support WHO submissions for Monkeypox virus nucleic acid detection tests. This document should be used together with the following WHO documents:

- "Emergency Use Listing (EUL) Procedure" [1]

[1] https://extranet.who.int/pqweb/key-resources/documents/emergency-use-listing-procedure-eul

- "Invitation to manufacturers of in vitro diagnostics for Monkeypox virus to submit an application for emergency use listing by WHO"[2].
- Target product profiles for tests used for mpox (monkeypox) diagnosis[3]
- Diagnostic testing for the monkeypox virus (MPXV): interim guidance, 10 May 2024[4]

Manufacturers[5] who wish to submit the documentary evidence for an IVD should read these documents carefully and fully adopt the guidance therein to compile a successful submission. Rebranded products are outside the scope of EUL assessment and hence not accepted for assessment.

3 The Submission

3.1 Submission clarity

Manufacturers should make every effort to ensure that their product documentary evidence is clear and well-organized (see section 4.2) to help make the WHO review procedure as efficient and timely as possible.

Note: Clarification of specific data requirements will require discussion between the applicant and WHO. Applicants are strongly encouraged to contact WHO as early as possible to discuss specifics of the application.

3.2 Confidentiality

All information submitted in the product dossier is confidential. WHO assessors will treat all information to which they will gain access during the assessment, or otherwise in connection with the discharge of their responsibilities as confidential and proprietary to WHO or parties collaborating with WHO with respect to the Mpox PHEIC.

3.3 EUL submission requirements – Important guidance on documents to be submitted

All items preceded by the symbol "➢" in each section below are required to be submitted as part of the EUL submission.

The instructions and feedback WHO provides are subject to change as more is learnt about mpox/Monkeypox virus and its risk-benefit profile. Any updates will be published on the PQ IVD website[6] as they become available and applicants will be notified.

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[2] https://extranet.who.int/prequal/news/invitation-manufacturers-vitro-diagnostics-monkeypox-virus-nucleic-acid-detection-submit

[3] https://www.who.int/publications/i/item/9789240076464

[4] https://iris.who.int/handle/10665/376952

[5] For the purposes of the EUL, the following definition applies: "**Manufacturer** means any natural or legal person with responsibility for design and/or manufacture of a diagnostic with the intention of making the diagnostic available for use, under his name; whether or not such a diagnostic is designed and/or manufactured by that person himself or on his behalf by another person(s)".

[6] https://extranet.who.int/prequal/vitro-diagnostics/emergency-use-listing-procedure

4 EUL Submission Format

4.1 EUL submission format

- The EUL submission is required to be submitted electronically. Further instruction will be provided to the manufacturer by email when their application is accepted for review.

Please note: Dossiers that do not adhere to the below layout/formatting instructions (4.2 & 4.3) will be returned to the manufacturer and will not proceed to assessment.

4.2 Layout and order

WHO requires the following format for the dossier submission:

- Use the format page *1 of 2*, *2 of 2*, etc.
- Clearly divide the submission into sections, as prescribed in this document, and number all pages of each section so that they are uniquely and easily identified.
- Include a table of contents.
- Ensure that all files are identified appropriately. The names should link directly with the sections of the dossier as outlined in this document.

  The name of all files must allow the reviewer to understand the type of document and the content in addition to the file number and version. The length of the title should not be longer than 40 characters (e.g. Annex x_6.3.1.5_LOD study v1)
- Font sizes for text and tables are of a style and size (at least font size 12) that are large enough to be easily legible when provided electronically.
- For sections where information is not available, the manufacturer must provide an explanation/justification for not providing the requisite information.

Submissions should be compiled according to the WHO requirements described above. Quality management system documentation (see chapter 5) should be provided as a separate document to facilitate the efficient review. Manufacturers should contact WHO to determine if a prior regulatory authority submission is appropriate to substitute for the specific sections of the submission.

4.3 Electronic copy requirements

- A searchable PDF is the primary file format used for the electronic copy. However, you must not include any PDF that requires a password to open it.
- The file name should be descriptive of its content and meaningful to the reviewers. The name can be up to 40 characters and can have spaces, dashes (not elongated dashes), underscores, and periods. However, the name of the file must not contain any of the following special characters or it will fail the loading process:
  - tilde (~)
  - vertical bar (|)
  - asterisk (*)
  - backward slash (\)
  - apostrophe (’)
  - greater than sign (>)

- forward slash (/)
- elongated dash (–)
- colon (:)
- double quotation marks (“)
- hash sign (#)
- single quotation mark (‘)
- less than sign (<)
- various other symbols (e.g., →,*, β , α ,∞,±,™)
- question mark (?)

- All PDF files should be created directly from the source documents whenever feasible (such as sending the document to “print” and selecting to save the print prepared document as a PDF file, which should be available in a drop-down menu in the print preview box) rather than creating them by scanning. PDF documents produced by scanning paper documents are far inferior and more difficult to read to those produced directly from the source document, such as a Microsoft Word document and, thus, must be avoided.
- If submission of a scanned document is unavoidable, we request that you perform optical character recognition (OCR) so that the text is searchable and clearer. Check to see that the content has been correctly converted by: (1) highlighting an area of text and (2) searching for a word or phrase. If the word or phrase is not returned in the search, then the OCR did not recognize the text. WHO recognizes that use of OCR may not be feasible in some cases for documents with figures and images. Hence, there may be cases in which it is appropriate to have scanned documents in the electronic copy.

4.4 Language and units of measurement

- Submit all documents presented in the dossier in English (unless other arrangements have been made with WHO ***prior to*** submission of the dossier).
- Any translations of documents must be carried out by a certified translator. Provide an official document attesting to the accuracy of the translation and details on the credentials of the translator.
- All measurements units used must be expressed in the International System of Units (SI) or in WHO International Units, where applicable, unless otherwise specified.

5 Quality Management System (QMS)

IVDs submitted for the WHO EUL procedure must be manufactured under a suitable, adequate, effective, and maintained quality management system (QMS).

Assessing the manufacturer’s QMS documentation is a critical step in the reviewing of an EUL submission. Based on this assessment, the WHO decides either to continue with the review of the submission, or to request further documentation, or to terminate the application at this point.

The decision to proceed with the review process will be made only if there is sufficient objective evidence that the applicant is the manufacturer, that an adequate QMS is in place, and that the required manufacturing capacity exists.

ISO 13485:2016 *Medical devices — Quality management systems — Requirements for regulatory purposes* is widely considered the benchmark for quality management by regulatory authorities worldwide. The WHO bases its requirements on this internationally recognized standard.

The following documentation is required to be submitted for review:

If the production site/s is/are certified by MDSAP, please provide the following:

a) The latest MDSAP recertification audit report/s, including audit findings (if any) and corrective/preventive actions taken.

If the production site/s is/are not certified by MDSAP, please provide the following:

a) Documented evidence of implementation and maintenance of an adequate QMS (e.g., current ISO 13485:2016 certificate or equivalent, together with the most recent audit report from the regulatory or certification body including audit findings, if any, and corrective/preventive actions taken).
b) A copy of the quality manual, including staff organogram.
c) A list of current quality management procedures.
d) A copy of the standard operating procedures and record forms for:
    - Quality control (QC) and batch release
    - Control of design changes
    - Control of nonconforming products/goods/processes
    - Supplier evaluation and control, verification of purchased products
    - Design and development (including input, outputs, verification, and validation)
    - Complaint handling and vigilance
    - Risk management, including how risks are identified, assessed, and controlled throughout the product lifecycle
e) Minutes from the last two management review meetings.
f) Manufacturing flowchart including in-process control points and the geographical location of each step, especially for multi-site manufacturing.
g) List of critical suppliers including any outsourced processes with direct product impact [e.g. outsourced manufacturing of components (e.g. conjugated antibodies, strips, reagents), outsourced laboratory testing, packaging, printing, etc.] including details of the supplier for each process and ISO certificates of each of the critical suppliers. If ISO certificates are unavailable, please provide a copy of the supplier evaluation form.
h) Name and contact details of the responsible person at the site/s of manufacture regarding the application.
i) Full address, including latitude and longitude of the manufacturing facility(ies), including warehouse(s) and other facilities used in the manufacturing process.
j) Site/s floor plan.

k) Design lockdown date for the product under assessment and date when it was first placed on the market (or the planned timeline for placing it on the market).
l) List of all countries where the product under assessment is intended to be marketed. For manufacturers submitting to EUL, it is expected that the product under assessment be distributed globally, and particularly in low and middle-income countries.
m) If the product has ever been distributed, please detail the manufacturer's experience with the product (including research-use-only products), especially (but not limited to) number of products distributed, number of customer complaints, if any, type(s) of complaint(s) and customer feedback.
n) Details on the manufacturing output and capacity (existing inventory, current output, minimum time to provide finished product, maximum batch size, scale up capacity in percentage of current output and required time).

Note: The manufacturer's quality management system must cover all sites currently used to manufacture this product. The WHO must be notified if any new sites are added to the manufacturing process.

6 Product Dossier

The product dossier submission should include product descriptive information and documentary evidence of safety and performance. Based on the submitted documentation, a risk-based judgement will be made on whether there is a favorable benefit-risk profile.
Applicants are expected to provide the following product information:

6.1 Product information

6.1.1 Regulatory versions

Different regulatory requirements apply to different international markets for IVDs. Manufacturers who market their IVDs to multiple countries often alter some aspects of their products to comply with regional regulatory requirements and marketing needs (e.g. differences in design, information within the instructions for use (IFU), different intended use statements, different batch release procedures, different sites of manufacture, different information on package labels). If such various versions of a product exist, WHO must have a clear understanding of precisely which version of the product the manufacturer is seeking EUL.

- Identify if there are multiple regulatory versions of this product (e.g. provide a table with the different regulatory versions and associated product codes).
- If the product has multiple regulatory versions, **clearly indicate which regulatory version of the product the manufacturer is submitting for EUL assessment**.
- Ensure that for any of the documents submitted in the product dossier, that the regulatory version to which it relates is identified. Where it is not the version submitted for EUL, a justification for its inclusion in the product dossier should be provided.

6.1.2 Product description including variants (configurations) and accessories

The dossier must include product descriptive information sufficient to allow a dossier reviewer to understand the design applied to the product and how it functions. The IFU may be used to provide some of this information on the condition that it is clearly indicated in the dossier what information can be found in the IFU.

The following information is required to be provided in this section:

- Legal manufacturer.
- Product name and product code(s)/catalogue number(s).
- Overview and intended use of the IVD.
  (Note: this may be finalized based on the data and recommendations from WHO).
  - Type of IVD (e.g. polymerase chain reaction, isothermal amplification, etc.).
  - What the product detects (e.g. qualitative detection of non-variola orthopoxvirus DNA, Monkeypox virus DNA, Monkeypox virus clade I DNA, Monkeypox virus clade II DNA (including the target genes), differentiation between clades).
  - The function of the product (e.g. diagnosis of infection or aid to diagnosis).
  - The clinical indication for the IVD (i.e. specific disorder, condition or risk factor of interest that the product is intended to detect, define or differentiate).
  - Whether the product is automated or manually operated.
  - Whether the test readout is qualitative or semiquantitative.
  - The type of specimen(s) required (e.g. swab lesion material, including surface, exudate, crusts).
  - The target population (e.g. symptomatic individuals).
  - The intended user (e.g. for laboratory based molecular tests, trained laboratory professionals trained in the techniques of real time RT-PCR and IVD procedures, for (near) POC molecular tests, trained healthcare professionals, etc.).
  - The intended environment of use (laboratory, point-of-care, near POC, etc.).
- A general description of the principle of the assay method or instrument principles of operation.
- For control materials (e.g. positive, negative) to be used or provided with the assay:
  - Include a description of what they are.
  - How they are expected to work (describe their use).
  - Where in the testing process they are used.
  - The concentration of the positive control relative to the limit of detection of the test.
  - How frequently they should be used.
- If a control is commercially available, provide the supplier's name and catalogue number or other identifier.
- A description of the specimen collection and transport materials/medium that are provided with the product or descriptions of specifications recommended for use (e.g. sterile Dacron, nylons or polyester swab), with specific brand of transport media validated, as applicable).
- For instruments of automated assays: a description of the appropriate assay characteristics or dedicated assays.

- For automated assays: a description of the appropriate instrumentation characteristics or dedicated instrumentation.
- If applicable, a description of any software to be used with the product.
- If applicable, a description or complete list of the various configurations/variants of product that will be made available.
- If applicable, a description of the accessories, and other products that are intended to be used in combination with the IVD but are not provided.
- If applicable, a description of extraction kits recommended for use with the assay (including the instructions for use).

6.1.3 Testing capabilities

- Briefly describe the current specimen throughput capacity, total time required to perform the test (from clinical specimen collection to result), and number of tests that can be performed per instrument run (if applicable) and per day.

6.1.4 Risk analysis

A risk analysis shall be undertaken to identify and quantify all known or foreseeable hazards[7] for the product, taking into account such aspects as the user(s) of the device, and the technology involved. The product dossier must contain:

- A summary report of the risks identified during the risk analysis process, including, but not limited to:
    - Risk of false positive and false negative results occurring based on the technology used (e.g. through the reagents used, carry-over contamination).
    - Risk of false results based on genetic mutations or clade of the virus.
    - Risk to the patient/community arising from false positive or false negative results.
    - Risk of false results based on erroneous use of the product.
    - Indirect risks that may result from product-associated hazards, such as instability, which could lead to erroneous results.
    - User-related hazards, such as handling of infectious specimens and reagents containing infectious agents and chemicals.
- A description of how these risks have been controlled to an acceptable level.
- Measures to inform users of any residual risks.
- A conclusion with evidence that the remaining risks are acceptable when compared to the benefits. This is required to be signed by senior management.
- Evidence that the risk analysis is part of the manufacturer's risk management plan (inclusion of the relevant manufacturer’s document).

[7] Examples of possible hazards and contributing factors associated with IVDs are given in ISO 14971:2019

6.2 Product design and manufacturing information

6.2.1 Product Design

6.2.1.1 *Design overview*

- Provide information to allow a reviewer to obtain a general understanding of the design applied to the product. A schematic presentation can assist.
- Provide a flowchart of the design process including design inputs and outputs for the product for EUL.

6.2.1.2 *Formulation and composition*

- For each of the ingredients, provide formulation/composition information.
- If commercial products are used for any of the assay components, provide certificates of analysis, etc.

Please note: Only dual target multiplex assays with Internal Control are eligible for EUL assessment.

- Provide sequences for primers and probes, list of ingredients (including relevant concentrations) for buffers, master mixes, or any other critical components etc.
  Note: WHO appreciates that this information might represent proprietary information and assures that all information will be treated as strictly confidential.
- Describe the design of the internal control/procedural control, including, but not limited to, the type of control (e.g., bacteriophage MS2), whether they are spiked into each specimen (exogenous) or endogenous (house-keeping gene), the nucleic acid sequence (as appropriate), the sequence of the primer/probes used and its intended function (e.g. NA extraction control, monitor NAT inhibition, sample integrity/stability control).

  Note: an internal control (IC) must be included in the assay and at a minimum must allow control of the whole process (including nucleic acid extraction).

6.2.1.3 *Biosafety & biohazard*

In this section, the applicant is required to provide evidence demonstrating that correct use of the product is safe; and any information relating to the design, use and disposal of the product that assures safe use under conditions where the product is likely to be used.

- Provide evidence that the following aspects (as applicable) have been considered and means taken to minimize the risk and inform the user of any residual risk:
  - Specimen type.
  - Specimen collection.
  - Specimen processing.
  - Inactivation of specimen (if inactivation is claimed, evidence must be provided).
  - Safe disposal.
- Provide the specific section applicable if reference is made to the submitted risk analysis.

- If reference is made to published biosafety guidelines, include an explanation as to how these have addressed all identified risks relevant to the assay under assessment.

6.2.1.4 *Documentation of design changes*

- Provide the date of design lock-down[8].
- Have any design changes been applied to the product? If so, provide records of each design change for the product submitted including the reasons that each change was made.
- Provide references to validation/verification data to support each change.

6.3 Product performance specification and associated validation and verification studies

The manufacturer must submit, where available, evidence of relevant investigations to support the intended use.

a) For each analytical study to be submitted, the following must be provided:

- Study description, study identifier, product code, product identifier (for example, lot numbers), IFU version used, the date of initiation and the date of completion.
- Clearly defined acceptance criteria and an explanation as to how they were derived.
- A summary of the study findings including a conclusion that clarifies how the study objectives have been met.
- The study protocol and full report.
- Analytical studies should be based on the entire testing procedure using the swab supplied with the kit or specified in the IFU.
- Raw data (e.g. file extractions from the PCR instrument) may be requested to supplement the study report if required.
- For all other detection tests provide detailed information about the calibration and interpretation of the results for qualitative or quantitative operation.

- **When studies are still in progress (e.g. shelf life stability studies), the manufacturer must provide the study protocol and study plan along with anticipated dates of completion and submission to WHO.**

b) For clinical studies, the following must be provided:

- Study description, study identifier, product code, product identifier (for example, lot numbers), IFU version used, the date of initiation and the date of completion.
- Specimen type and how the specimens were collected and stored if applicable (dry vs wet swab in UTM or VTM, etc.).
- Numeric values (e.g. Ct values, CN values) where applicable, even if the result provided to the end user is qualitative.
- Clearly defined acceptance criteria and an explanation as to how they were derived.

[8] For WHO prequalification, design lock-down is the date that final documentation, including quality control and quality assurance specifications, is signed off and the finalized method is stated in the IFU.

- A summary of the study findings including a conclusion that clarifies how the study objectives have been met.
- The clinical study protocol(s), including inclusion/exclusion criteria and enrollment strategy.
- The full clinical study report, including raw data (e.g. file extractions from the PCR instrument) and results excluded from the analysis (with a justification).

6.3.1 Analytical performance

6.3.1.1 *Stability of specimen(s)*

This section contains information on the collection, storage and transport of specimens to be used:

- Identify the different specimen type that can be used with the product, including detailed information for each solution claimed in the IFU (e.g. use of different swab transport media (VTM, UTM)).
- The claimed specimen types are expected to align with the current guidelines for laboratory testing (e.g. lesion surface/roof, lesion crusts, lesion exudate)
- A specimen stability study is required to support the specimen stability claims in the IFU if the claims are outside the parameters of internationally accepted guidance[9,10]: provide studies/references in support of specimen stability claims for each specimen type.
- If in the clinical study, retrospective clinical specimens that have been frozen are tested, then it is also required to conduct fresh versus frozen studies to support use of these specimens. Where real time studies are conducted:
    - They must include storage conditions (e.g. duration at different temperatures, temperature limits, freeze/thaw cycles), transport conditions and intended use (i.e. the maximum allowable time between specimen collection and its processing or addition to the assay in the setting where testing takes place).
    - A minimum of 10 specimens containing the analyte at a low concentration (e.g. 2-3 x LoD) in the appropriate specimen type and matrix should be tested at each storage condition.

Manufacturers may submit data on later storage time points in agreement with WHO.

6.3.1.2 *Validation of specimens – matrix equivalence studies*

If a manufacturer can demonstrate equivalency between two or more matrices or specimen types, only one representative specimen type/matrix needs to be tested in the following analytical studies: section 6.3.1.8 Robustness and section 6.3.1.9 Stability of the IVD.

If the manufacturer chooses to test only one representative specimen type or matrix in these analytical studies, the following study is required to be submitted.

[9] https://iris.who.int/handle/10665/376952
[10] https://www.cdc.gov/poxvirus/mpox/clinicians/prep-collection-specimens.html

- Where the IFU includes claims for different swab types and/or different transport media a matrix equivalency study should be conducted to establish the relationship between the specimen and IVD performance.
- The following conditions should be met in the matrix equivalence study:
    - The study should include a minimum of four positive specimens: one low positive (e.g. approx. 2-3 x LoD) and three moderately positives (e.g. approx. 5-7 x LoD); and one negative specimen for each claimed specimen type/matrix.
    - Contrived specimens obtained by spiking negative clinical matrix with the appropriate amount of analyte may be used.
    - Test the five specimens in duplicate and compare the results between the matrices.
- Please note that this study aims to assess potential inhibitory effects of the matrix. LoD and clinical performance must be performed in **all claimed specimen types**, regardless of any specimen type equivalencies.

6.3.1.3 *Metrological traceability of calibrators and control material values (when reference material is available)*

- All controls and calibrators must be calibrated against the WHO International Standard once available, or equivalent reference preparations.
- If other sources of quantitated material have been used, a detailed report from the provider should be provided, e.g. for genomic, synthetic, or cloned DNA (including e.g., the GenBank accession number the sequence is based on).
- A detailed report from the supplier of the cell-culture derived virus, including the source, passage history and quantitation (PFU/mL or $TCID_{50}$/mL) must be provided. Furthermore, the virus stock should be characterized by PCR and the value in copies/ml provided.
- Calibrators and control materials used in the assay should be traceable to a validated reference material (e.g. reference material from a National Control Authority, National Standards or the WHO IS) and/or the reference method used for characterization/ value assignment should be described.

6.3.1.4 *Analytical sensitivity (limit of detection (LoD))*

- The LoD of the IVD must be determined utilizing the entire test system inclusive of specimen preparation, nucleic acid extraction, and detection.
- LoD must be determined for each claimed specimen type, irrespective of matrix equivalency.
- Live or inactivated virus is the preferred material for estimation of the LoD; genomic DNA/synthetic DNA can be used as an interim measure until current strains become publicly available. However, the manufacturer must commit to perform and document LoD studies using live or inactivated virus as soon as these become feasible. For tests that detect and / or differentiate Monkeypox virus clade I and clade II, the LoD must be estimated with both clades.
- For tests with a non-variola orthopox target, at least 2 orthopox species in addition to Monkeypox must be tested.

- The manufacturer must commit to determine the LoD using the WHO International Standard if/when it is available.

Note: It is not acceptable to use transport media or extraction buffer without clinical matrix to perform this study

Procedure:

- A tentative LoD can be established through limiting dilutions of the spiked material, followed by nucleic acid extraction, with 3-5 replicate measurements.
- Nucleic acid extraction is required to be performed on each dilution.
- Once a tentative LoD is established, the LoD should be confirmed by testing at least 20 replicate dilutions spanning the tentative LoD to obtain a more accurate estimate of the LoD (to demonstrate that the organism was detected with a minimum 95% positivity (19/20)).
- If this confirmatory study achieves a positivity of 100%, then a lower concentration needs to be tested (with 20 replicates) until <100% positivity is obtained.
- 95% LoD should be calculated from replicates of limiting dilution series using statistical tools (e. g. Probit) and must be provided per ml.
- **See Annex 1***: Bridging studies for open molecular assays for additional considerations*.

Required information:

- Titres, lineages and (if available) NCBI GenBank or GISAID accession number of the Monkeypox virus(es) used for the LoD study; a description on how the organism stocks were prepared and how the titres were determined.
- Detailed step-by step explanation of the entire testing procedure.
- The dilution factor and number of serial dilutions of the characterized Monkeypox virus that were tested to determine the LoD.
- The nucleic acid extraction/purification method, extraction platform (if applicable) and elution volume, PCR instrument and cycling conditions.

6.3.1.5 *Analytical Specificity*

a) **<u>Interfering substances</u>**

Testing of potential interfering substances is required. The evaluation is conducted to demonstrate that the potential interfering substances do not generate false positive results in known negative specimens, and do not lead to false negative results in known positive specimens.

For NAT that use conventional PCR and/or well-established extraction methods prior to testing (e.g. Boom method and column-based extraction methods) interference studies are not necessarily required.

For NATs that do not include a DNA purification procedure (see above), the following information is required:

- Refer to table 1 for evaluation of interfering substances for the ability to generate false positive and negative results.
- Indicate the interfering substances tested and concentrations used.
- Endogenous and exogenous substances should be spiked into the appropriate negative specimen at the highest levels found in individuals.
- Each endogenous and exogenous specimen must be tested unspiked and spiked with the analyte at an appropriate low concentration (e.g. approx. 2-3 x 95% LoD).
- Samples should be tested in triplicate and only one claimed specimen type and matrix is required to be included in these studies.

Table 1: Potential interfering substances

Substance	Concentration tested
Acyclovir	
Albumin	
Benadryl cream/ointment*	
Benzocaine containing local anesthetic	
Blood/EDTA	
Casein	
Cornstarch	
Docosanol containing cold sore treatment	
Douche	
Feces	
Female urine	
Hydrocortisone cream*	
Lidocaine containing cream	
Lubricant	
Male urine	
Mucin	
Neosporin*	
Petrolatum containing lip/skin care	
Seminal fluid	
Zinc Oxide ointment	

b) **<u>Cross reactivity</u>**

WHO requires testing of near-neighbour species/strains, or organisms whose infection produces symptoms similar to those observed for mpox disease, and of the normal or pathogenic microflora that may be present in specimens collected.

- Please provide in silico analysis against all applicable nucleic acid sequences (see table 2) in well-established publicly available databases (e.g. NCBI, GISAID).

In silico analysis

- The analysis should include multiple representative strains from GenBank sequence database[11] for each organism (please refer to table 2).
- The full sequence of each organism must be analysed.
- In-silico cross reactivity data should be provided in a tabular form specifying the microorganism, species/strain, accession number and individual % homology of the primers and probes for all targets of your test.
- If *in silico* analysis reveals other potential cross-reactants (i.e., ≥80% homology between one of the primers or the probe to any of the sequences of listed potential cross reactants), carefully review the alignments and determine based on the position(s) of the homologous stretches and mismatches if additional cross-reactivity and/or interference laboratory testing (please refer to microbial interference studies) is required to rule out cross-reactivity or interference of that organism that may affect the performance of your IVD.
- In these circumstances if laboratory testing is omitted you should include an explanation as to why *in silico* generated data is not clinically relevant (irrelevant isolate, location/extent of match within primer/probe, etc.), or why the performance of your test would not be impacted.

[11] https://www.ncbi.nlm.nih.gov/genbank/

Table 2: Cross-Reactivity: List of organisms to be analysed in silico (and/or tested)

Other high priority pathogens from the same virus family	*In silico analysis*	Laboratory testing – please indicate
variola virus (smallpox)	✓	
molluscum contagiosum virus	✓	
parapoxvirus (e.g. Orf virus)	✓	
vaccinia virus*	✓	
cowpox virus*	✓	
Ectromelia (mousepox) virus*	✓	
camelpox virus*	✓	
buffalopox*	✓	
Other high priority organisms		
herpes simplex virus (HSV-1 and HSV-2)	✓	
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	✓	
Streptococcus Group C	✓	
Streptococcus Group G	✓	
Corynebacterium diptheriae	✓	
Neisseria gonorrhoeae	✓	
Chlamydia trachomatis	✓	
Mycoplasma pneumoniae	✓	
Mycoplasma genitalium	✓	
Human papilloma virus (HPV)	✓	
Trichomonas vaginalis	✓	
Treponema pallidum	✓	

***Laboratory testing to evaluate cross-reactivity is not applicable for OPXV assays**

If laboratory testing is required:

- Concentrations of 10^6 CFU/ml or higher for bacteria and 10^5 PFU/ml or higher for viruses are recommended. Test specimens can be prepared by spiking cultured isolates into negative clinical matrix.
- Samples should be tested in triplicate and only one claimed specimen type is required in this study.
- Omissions from actual laboratory testing should be supported by a well-documented justification that includes a due diligence attempt to obtain the organisms (and/or purified nucleic acid).

c) **Microbial Interference Studies**

Microbial interference studies aim at demonstrating that false negatives for non-variola orthopoxvirus and/or Monkeypox virus will not occur in presence of other microorganisms.

If *in silico* analysis reveals ≥ 80% homology between the microorganisms and the test primers/probe(s), there could be interference with amplification of the target gene (even in the absence of cross-reactivity).
In this case, the following studies should be considered:

- a microbial interference study with Monkeypox virus and the microorganisms that the test primers/ probe(s) have homology to,
  1) as an alternative to the microbial interference study, you may provide justification as to why (e.g. amount of primer(s)/ probe(s) included in your master mix) the performance of your test would not be impacted by the presence of a causative agent of a clinically significant co-infection, or
  2) explain why the *in-silico* results are clinically irrelevant.
- In the case of the microbial interference study, interference should be evaluated using samples spiked at a low (2-3 x LoD) Monkeypox virus concentration and a high interferent level (either microorganisms or nucleic acids purified from them), to represent the worst-case scenario, with a minimum of 3 replicates.
- If interference is observed at the level tested, an additional titration study should be performed to determine the highest microorganism interferent level the Monkeypox virus test can tolerate.

d) **Inclusivity Studies**

For tests targeting Monkeypox virus, inclusivity must be demonstrated at a minimum using *in silico* analysis for all Monkeypox virus strains present in sequence data banks representing Monkeypox virus clade II (including Monkeypox virus clade IIb), Monkeypox virus clade Ia (formerly known as clade I) and Monkeypox virus clade Ib. Laboratory confirmation of sensitive detection is required if mismatches are obvious at positions that could impact performance of the Monkeypox virus test kit. Criteria for maximum number of mismatches and potential positions tolerated by the assay design must be defined, and studies as basis for respective conclusions submitted.

For tests targeting non-variola Orthopoxvirus, inclusivity using in silico analysis must be demonstrated for

- Monkeypox virus clade II, clade Ia, clade Ib
- Cowpox
- Buffalopox
- Ectromelia (mousepox) virus
- Camelpox
- Vaccinia virus

6.3.1.6 *Validation of the primer and probe choice*

Evidence supporting the choice of primers and probes sequences must be provided and must include:

- The target gene(s) and sequence for primers and probes.
- The rationale for selection of primers and probes and specific sequences used.
- Justification for alignments made to generate consensus sequences or best-fit modifications made to existent sequences e.g. to permit maximum homology to several strains.
- Potential genetic variations, such as mutations or deletions, present in individual Monkeypox virus strains or clades need to be evaluated to determine their impact on the assay's detection capability.

6.3.1.7 *Validation of assay procedure*

- If an endogenous internal control (housekeeping gene) is used as part of the assay design, an acceptable range of Ct values needs to be determined for each specimen type.
- If an exogenous internal control is used as part of the assay, evidence for the acceptable Ct range must be provided.

6.3.1.8 *Flex and robustness studies*

This section provides information to demonstrate that the product design is robust (e.g. insensitive to environmental and usage variation). Robustness (flex) studies are designed to challenge the system under conditions of stress to identify potential device deficiencies, including failures, and determine the robustness of the product.

- The influence of the following factors on expected results (both positive and negative) should be considered as applicable:
    - Specimen and/or reagent volume.
    - Handling contamination (e.g. from latex, powder, hand lotion, sweat, and/or soap, etc. as appropriate).
    - Operating temperature.
- The robustness of the instrumentation that is part of the IVD (both extraction and amplification) should be considered.

- For instrumentation that has already been assessed in the context of a WHO prequalified IVD, the data generated as part of the prequalification application may be used to support the EUL application.
- For new instrumentation, the following should be considered:
    - Ruggedness (including the effect of vibration from other instruments).
    - Impact of temperature, condensing humidity, dust and mold on componentry (e.g. optics).
    - Impact of power/voltage fluctuation.
- Studies investigating the impact of specimen volume should ideally be conducted **in all claimed specimen types.**
- For all other flex studies, the most common specimen type used for the clinical studies is required to be tested.
- The test panel should include one negative specimen and one low positive specimen (approx. 3 x LoD).
- Provide a summary of the evidence collected to date and a plan for further testing if such studies are not complete.

WHO acknowledges that not all studies are applicable or will have been completed when submitting to EUL. However, at a minimum, the effects of sample and/or reagent volumes should be completed prior to submission.

6.3.1.9 *Stability of the IVD*

Shelf-life, in-use stability and shipping stability information provided under this section must be consistent with the instructions for use and product labels provided within the submission.

- Clear acceptance criteria must be provided and must include a justification of an acceptable target drift as a consequence of reagent degradation over time.

a) **Shelf-life of the IVD including shipping stability**

Accelerated studies or extrapolated data from real-time data are acceptable for an initial shelf life claim provided sufficient evidence is provided to support the claim. However, it is a requirement that real time stability studies will be finalized.

Stability studies must be evaluated for the shelf life of all kit reagents and controls. All kit configurations should be tested (or provide a rationale if not). The reagents must be subjected to simulated shipping conditions prior to placing them into the shelf-life studies. The following conditions should be investigated (that reflect the environmental conditions of the countries of supply):

- Conditions to mimic extremes of conditions (temperature, humidity, pressure) products may be exposed to during transport/shipping.
- Minimum and maximum storage temperature and humidity range.
- Specimen panel: at least one negative specimen and one low positive specimen (3-5 LoD)
- Using one claimed specimen matrix spiked with inactivated virus.

- Testing should be performed in at least 5 replicates.
- Testing should be performed in at least 3 lots.
- Results of each replicate, condition, time point, concentration, and lot must be reported in numeric values (Ct, CN values).

Note: WHO acknowledges that not all studies will have been completed when submitting to EUL assessment.

- **In this case, in addition to the study protocol, a plan for the completion of the studies must be provided.**

b) **<u>In-use stability</u>**

- Provide a report on in-use stability (open pack or open vial stability).
- All labile components (e.g. buffers vials, sealed cartridges, control materials, etc.) must be evaluated.
- On-board stability must be tested for an IVD used with an instrument.
- Consideration should be given to operating temperature, humidity range and allowable freeze-thaw cycles of reagents/controls, as applicable.
- Specimen panel: at least one negative specimen and one low positive specimen (3-5 LoD).
- Using one claimed specimen matrix spiked with inactivated virus.
- Testing should be performed in at least 5 replicates.
- Results of each replicate, condition, time point, concentration, and lot must be reported in numeric values (Ct, CN values).

If a manufacturer utilizes the same instrumentation platform, buffer composition and chemistry as in a WHO prequalified IVD, provide the reference to the prequalified IVD; WHO will give due consideration to leveraging available data which was already assessed.

6.3.2 Clinical evidence

Clinical evaluation is the assessment and analysis of data generated from the clinical intended use of the product in order to verify the clinical safety and performance of the device. Clinical evidence is the combined information from the clinical data and its evaluation. A manufacturer must have clinical evidence to support any clinical claims.

- Specimens from all sections of the population for which claims are made in the IFU must be tested.
- The test should be performed by the claimed intended user in the intended testing setting.
- The clinical performance should be evaluated for **each claimed specimen type** (see footnote for exception)**.**
  Note: Clinical studies supporting additional specimen types can be submitted at a later time point as a change request.
- Small sample sizes are vulnerable to selection bias. Criteria for the selection of specimens are required to be explained (e.g. testing of consecutive patients). In

addition, archived specimens (retrospective testing) should be randomised and tested in a blinded fashion.
- For POC devices additional requirements may apply

Note: Please refer to 6.3. b) for required documentation.

a) **Comparator method**

- Percent agreement should be calculated in comparison to a comparator assay: a U.S Food and Drug Administration Emergency Use Authorization (FDA EUA) PCR test[12] or a WHO EUL listed PCR test.
- A RT-PCR test with high sensitivity, which is preceded by a chemical lysis step followed by solid-phase extraction of nucleic acid (e.g., magnetic bead extraction), should be used as the comparator method.
- The **comparator assay** must not contain the same primers/probe (sequence) as the assay under evaluation.
- Numeric values (e.g., Ct or CN values) for the assay under evaluation (including internal control results) and the comparator assay must be provided.

6.3.2.1 *Positive Percent Agreement (PPA)*

Clinical specimens

- 30 prospective positive specimens should be tested per specimen type.
- If 30 prospective specimens cannot be collected, it is acceptable to supplement with retrospectively collected Monkeypox virus positive specimens from patients.
- If archived specimens are used, the manufacturer must describe how specimens were selected and how selection bias was avoided.
- If archived (frozen) specimens are used, the impact of specimen storage must have been investigated as part of '6.3.1.1 Stability of specimen(s)'.
- Any archived specimens used in the study shall be tested in a randomized, blinded manner interspersed with an appropriate number of negative specimens.
- The same comparator test should be used for prospective and archived specimens.
- The clinical specimens must include MPXV clade 1b specimens, if not feasible see section below (contrived specimens).
- The clinical specimens must include at least 20% of weak positive specimens (low virus load) (comparator test results of CT > 35), if the specimens don't fulfill this criterion, see section below (contrived specimens).

Required information:

- The following basic information should accompany each clinical specimen:
  - The specimen type.

[12] https://www.fda.gov/medical-devices/emergency-use-authorizations-medical-devices/monkeypox-mpox-emergency-use-authorizations-medical-devices

- The specimen collection date.
- Date of onset of symptoms.
- Clinical diagnosis (if available).
- Severity of symptoms (if known).
- Tests used to identify Mpox patients.
- PCR test results (Ct values of Mpox targets and internal control).

- All efforts should be made to test positive clinical specimens from
  - Different clinical sites.
  - Different age groups if possible (children, adults, elderly).
  - Patients exhibiting the range of symptoms, including patients with mild and moderate symptoms reflecting the typical use cases.

Contrived specimens

If no weak positive clinical specimens and/or MPXV clade Ib specimens are represented in the clinical specimen study, contrived specimens can be utilized. Each contrived specimen should be prepared using a negative natural clinical specimen and spiked with virus at the concentrations indicated below.

- MPXV clade 1b study: 15 specimens at the LoD and 15 specimens covering the range of the test up to 5x LOD.
- To complement weak positive specimens: 5 specimens at LoD and 5 specimens at 2-3x LoD.

The following information is required if contrived specimens are used:

- Titres, lineages and (if available) NCBI GenBank or GISAID accession number of the Monkeypox virus(es) used for the LoD study; a description on how the organism stocks were prepared and how the titres were determined.
- Detailed step-by step explanation of the entire testing procedure.

6.3.2.2 *Negative Percent Agreement (NPA):*

- A minimum of 100 RT-PCR for Monkeypox virus (or Orthopoxvirus) negative specimens, collected from symptomatic individuals are required to be tested.

6.3.2.3 *Alternative specimen types*

If the manufacturer considers including a claim for non-lesion specimens, please contact WHO in advance.

7 Plan for Post-Market Surveillance

Post-market surveillance, including monitoring all customer feedback, detecting and acting on adverse events, product problems, non-conforming goods and processes is a critical component of minimizing potential harm of an IVD listed for emergency use. Certain adverse

events should be reported to regulatory authorities in the relevant jurisdiction(s). In the public health emergency settings this EUL procedure serves, it cannot be assumed there are sufficient resources in place to support consistent and effective post-market surveillance but manufacturers must make all efforts possible.
The manufacturer is required to ensure that should the EUL be granted, activities are in place to monitor product safety, quality and performance post-EUL. It is expected that the manufacturer monitors the emergence of variants and assesses the potential impact on product performance. It is expected that post-market surveillance activities will be in accordance with WHO guidance "Guidance for post-market surveillance and market surveillance of medical devices, including in vitro diagnostics".[13]

8 Labelling

The submission must contain a complete set of labelling associated with the product. This includes labels and Instructions for Use (IFU) as well as instrument manual (if applicable) and other instructional materials provided to the user.

8.1 Labels

- Include high quality copies of all packaging labels for the assay. This includes:
  - Outer labels (secondary packaging).
  - Component labels.
  - (if components are provided without labels, provide information in section 6.1.2).
- These labels must minimally include the following information:
  - The product name and product identification number (product code/catalogue number).
  - The name and contact details of the manufacturer, or an authorized representative of the manufacturer, on the outer package labels.
  - The name of the reagent/ingredient.
  - The expiry date.
  - An indication of any special storage and/or handling conditions that apply.
  - The warnings and precautions.
  - The lot/batch and/or serial number.
  - The information regarding particular product conditions such as product sterility.
  - The names of all included reagents in each box on the secondary package label, where possible.
- Where a component is too small to contain all the above information, it must at a minimum contain name, lot number, expiration date, volume, and storage conditions.

[13] Available on the web page https://www.who.int/publications/i/item/guidance-for-post-market-surveillance-and-market-surveillance-of-medical-devices-including-in-vitro-diagnostics

- If the product requires associated instrumentation, the above requirements also apply to the instrument.

8.2 Instructions for use (IFU)

The IFU will be reviewed for clarity, correctness, consistency with the information submitted in the dossier, and suitability for the target user group. The following must be submitted in the dossier:

- A copy of the current IFU.
- The instructions for use should comply with the Principles of Labelling for Medical Devices and IVD Medical Devices of IMDRF/GRRP WG/N52 FINAL:2019.Instrument manual

8.3 Instrument manual

- If the product requires associated instrumentation, include a copy of the instrument manual and/or associated operator manuals.

8.4 Any other instructional materials provided to the user

- Provide copies of any other instructional materials that are provided to the user.

9 Contact Information

Any inquiries regarding the EUL should be addressed to: *diagnostics@who.int*

Annex 1: Bridging studies for open molecular assays

In order to validate performance and to establish equivalent performance of **additional PCR platforms** or **nucleic extraction kits/platforms** the following study is recommended at a minimum. Additional studies may be requested for NA extraction kits or PCR platforms that are not well-established on the global market or assays intended for point-of-care use.

- Testing must be conducted in parallel with the new and original components

1) Verification of analytical sensitivity (LoD)

- Using one specimen matrix.
- 2-fold (or 3-fold) serial dilution, 3 replicates, until hit rate reaches <100%.
- Confirm LOD with 20 replicates.
-