# Mpox disease Emergency Use Listing Procedure (EUL) for IVDs
# Product: Xpert Mpox
# EUL Number: MPXV-12646-070-00
# Outcome: Accepted.

The EUL process is intended to expedite the availability of in vitro diagnostics needed in public health emergency situations and to assist interested UN procurement agencies and Member States in determining the acceptability of using specific products in the context of a Public Health Emergency of International Concern (PHEIC), based on an essential set of available quality, safety, and performance data. The EUL procedure includes the following:

- Quality Management Systems Review and Plan for Post-Market Surveillance: a desktop 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. This evaluation of limited scope is to verify critical analytical and performance characteristics.

The Xpert Mpox, with product code GXMPX-10, U.S. Food and Drug Administration (FDA) Emergency Use Authorization (EUA) regulatory version manufactured by Cepheid, located at 904 Caribbean Drive, Sunnyvale, CA, 94089, United States of America, was listed as eligible for WHO procurement on 25 October 2024.

**Intended use:**

According to the claim of intended use from Cepheid, Inc., *"The Xpert® Mpox is a real-time PCR test intended for the qualitative detection of DNA from monkeypox virus clade II and non-variola Orthopoxvirus DNA in human lesion swab specimens (i.e., swabs of acute pustular or vesicular rash) from individuals suspected of mpox by their healthcare provider.*
*Testing on the GeneXpert Dx and GeneXpert Infinity instruments is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high or moderate complexity tests.*
*Testing on the GeneXpert Xpress (Hub Configuration) instrument is limited to laboratories certified under CLIA that meet requirements to perform high, moderate, or waived complexity tests. Testing on the GeneXpert Xpress (Hub Configuration) instrument is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation.*
*Results are for the identification of monkeypox virus (clade II) and non-variola Orthopoxvirus DNA, which are generally detectable in human pustular or vesicular lesion specimens during the acute phase of infection. Positive results are indicative of the presence of monkeypox virus (clade II) and/or non-variola Orthopoxvirus 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*

*monkeypox virus (clade II) and/or non-variola Orthopoxvirus 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 Xpert Mpox is intended for use by trained operators who are proficient in performing a test using either GeneXpert Dx, GeneXpert Infinity and/or Gene Xpert Xpress systems.*
*The Xpert Mpox test is only for use under the Food and Drug Administration's Emergency Use Authorization."*

**Validated specimen type:**
Human lesion swabs (of acute pustular or vesicular rash from individuals suspected of mpox) collected using Nylon flocked swabs by a healthcare provider.

**Test kit contents:**

| Component | Number of tests and product code (10 T/kit, GXMPX-10) |
|---|---|
| Xpert Mpox Cartridges with Integrated Reaction Tubes | 10 |
| Disposable 300 µL Transfer Pipettes | 2 bags of 12 per kit. |
| Flyer | 1 per kit. |
| Quick Reference Instructions | 1 per kit. |

**Items required but not provided:**

- Nylon flocked swab (Copan P/N 502CS01, or equivalent)
- Viral transport medium/Universal transport medium (VTM/UTM), 3 mL (Copan P/N 3C047N or equivalent)
- GeneXpert Dx System or GeneXpert Infinity System (catalog number varies by configuration): GeneXpert Instrument, computer, barcode scanner, and operator manual.
- For GeneXpert Dx System: GeneXpert Dx software version 4.7b or higher
- For GeneXpert Infinity-80 and Infinity-48 systems : Xpertise software version 6.4b or higher
- Printer: If a printer is required, contact Cepheid Technical Support to arrange for the purchase of a recommended printer.
- External controls are available from ZeptoMetrix (Buffalo, NY).
    - External Positive Control: Catalog # NATMPXVPOS-6C
    - External Negative Control: Catalog # NATMPXVNEG-6C

**Storage:**
The reagents must be stored at 2–28 °C until the expiration date provided on the label.

**Shelf-life upon manufacture:**
The shelf-life is currently assigned 12 months dating.

**Warnings/limitations:**

Please refer to the instructions for use attached to this report.

**NOTE: The Xpert Mpox Test is specifically designed to detect Clade II of the Monkeypox virus (MPXV). The OPXV target will detect non-variola Orthopoxviruses, including monkeypox Clade I and Clade II viruses and other non-variola Orthopoxviruses. A positive result for Orthopoxvirus detected only using this test is most likely indicative of Clade I. Further confirmatory testing may be required to differentiate between Orthopoxvirus species or Clades if necessary for clinical or epidemiological purposes.**

## Product dossier assessment

Cepheid submitted a product dossier for the Xpert Mpox in alignment with the U.S. FDA's EUA for mpox disease. The risk assessment was requested to specifically meet the WHO mpox disease EUL requirements for in vitro diagnostics detecting Monkeypox virus nucleic acid. The WHO reviewed the information provided in the dossier.

The risk-benefit assessment conclusion was acceptable.

## Quality Management Systems Review

To establish eligibility for WHO procurement, Cepheid was asked to provide up-to-date information about the status of its quality management system.

Based on the WHO's review of the submitted quality management system documentation, Cepheid, Inc., provided sufficient information to fulfil the requirements described in the Instructions and requirements for EUL Submission: In vitro diagnostics detecting Monkeypox virus nucleic acid (PQDx_457).

The quality management system assessment conclusion was acceptable.

## 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 minimising the potential harm of an IVD listed for emergency use.

The following post-EUL activities are required to maintain the EUL status:

1. Notification to WHO of any planned changes to a prequalified product, in accordance with "*WHO procedure for changes to a WHO prequalified in vitro diagnostic" (document number PQDx_121*)[1]; and
2. Post-market surveillance activities, in accordance with "*WHO guidance on post-market surveillance of in vitro diagnostics" (ISBN 978 92 4 150921 3)*[2].

Cepheid is also required to submit an annual report summarising sales data and all complaints. Certain complaints and changes to the product must be notified immediately to WHO, as per the above-mentioned documents. The sales data will serve as denominator data to guide the frequency of re-inspection.
The manufacturer has committed to ensuring that post-emergency use listing safety, quality, and performance monitoring activities are in place, which are in accordance with WHO guidance on post-market surveillance of in vitro diagnostics.

## Scope and duration of procurement eligibility

The Xpert Mpox, with product code GXMPX-10, manufactured by Cepheid, is eligible for WHO procurement for 12 months from the day of listing. The assay detects the monkeypox virus DNA (clade II). This listing does not infer that the product meets WHO prequalification requirements and does not mean that the product is listed as WHO-prequalified.

As part of the ongoing requirements for listing as eligible for WHO procurement, Cepheid must engage in post-market surveillance activities to ensure that the product continues to meet safety, quality, and performance requirements. Cepheid, Inc., is required to notify WHO of any complaints, including adverse events related to the use of the product, within 10 days.

WHO reserves the right to rescind eligibility for WHO procurement if additional information on the safety, quality, and performance during post-market surveillance activities and if new data becomes available to WHO that changes the risk-benefit balance.

[1] https://iris.who.int/bitstream/handle/10665/251915/WHO-EMP-RHT-PQT-2016.01-eng.pdf;jsessionid=830C82950055325AF37A0A8302BE4623?sequence=1

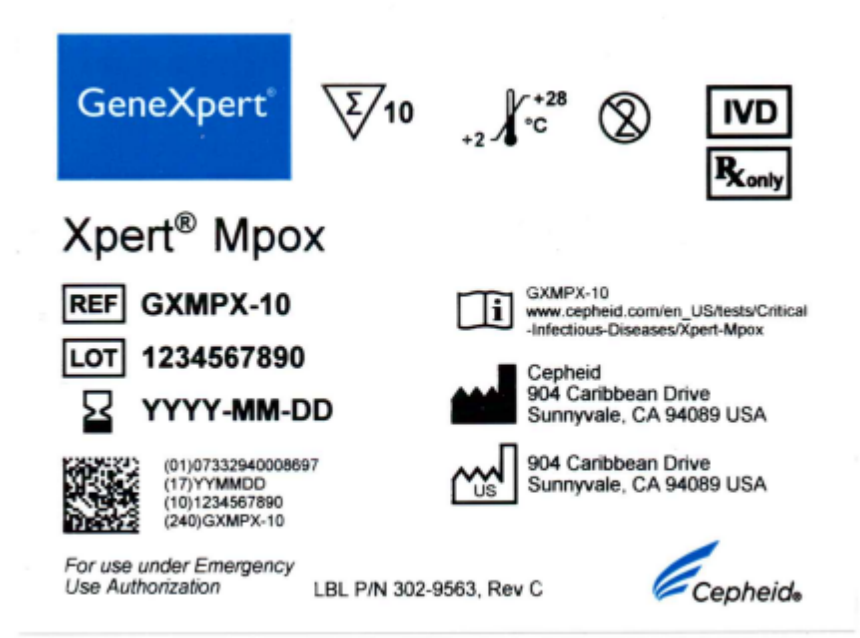
[2] https://www.who.int/publications/i/item/9789240015319

# Labelling

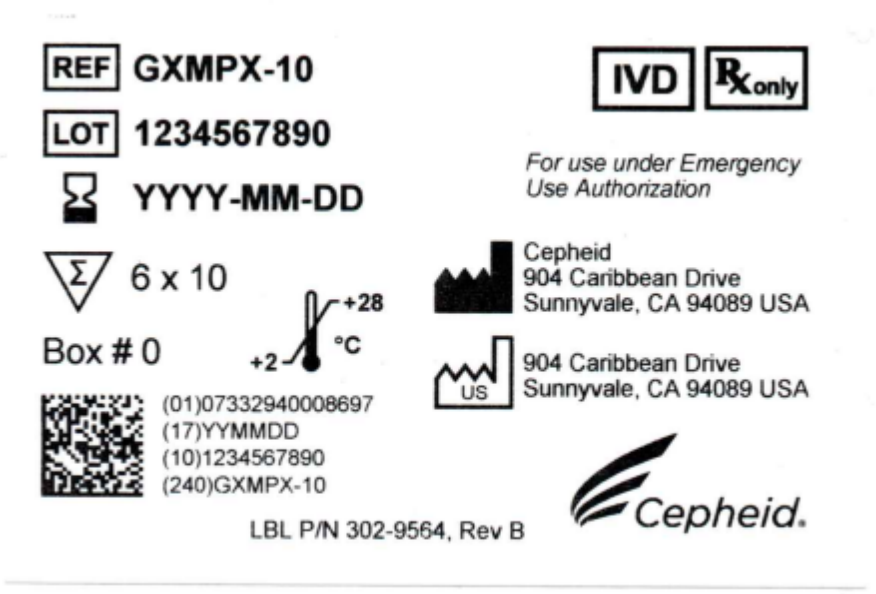
1. **Labels**
2. **Instructions for use**

## 1.0 Labels

### 1.1 Top Label

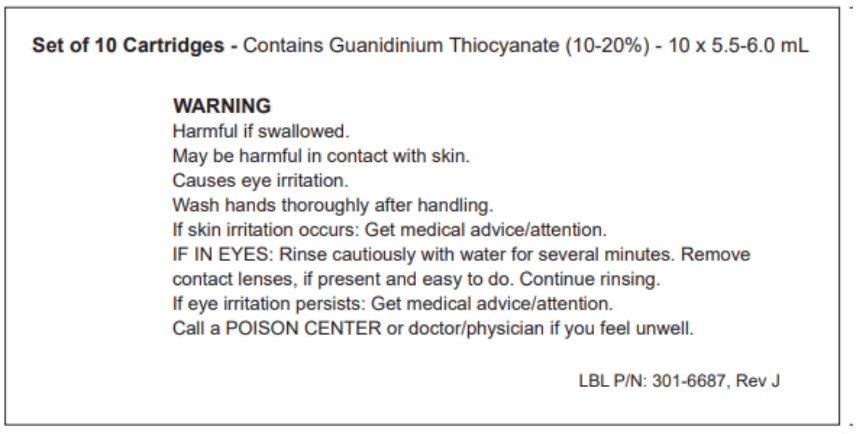


### 1.2 Top label-outer box



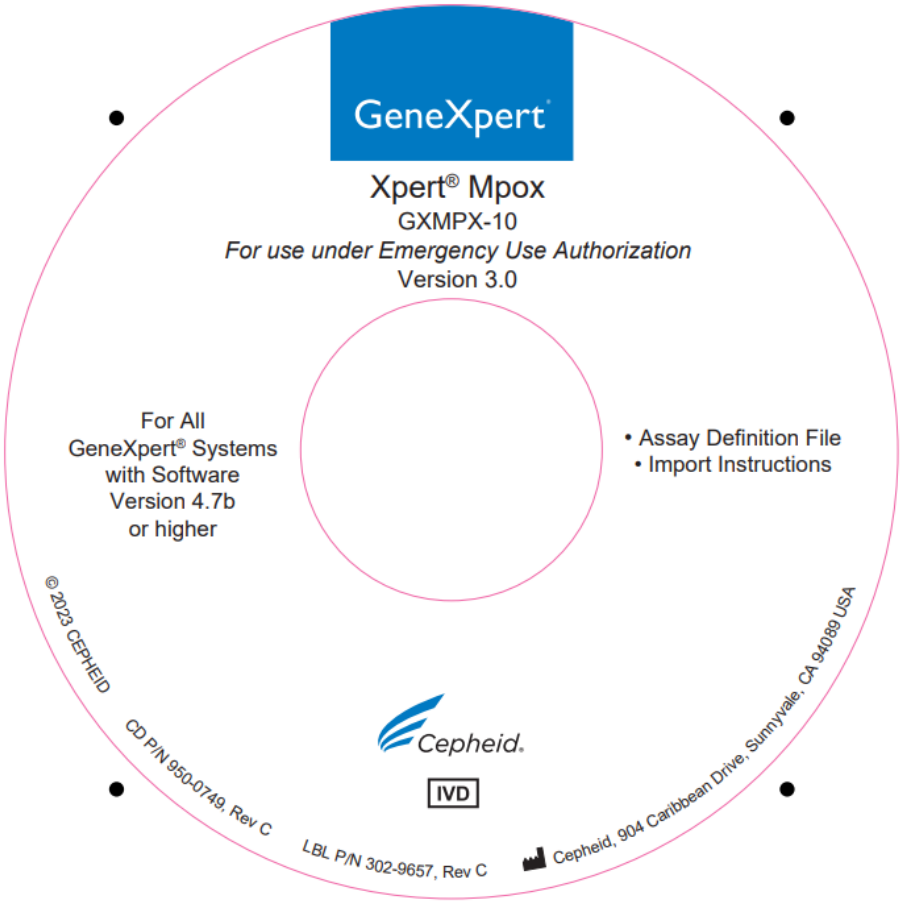

### 1.3 Side label


### 1.4 Hazard label



### 1.5 Cartridge label


### 1.6 CD label



## 2.0 Instructions for Use[3]

[3] English version of the IFU was the one that was assessed by WHO. It is the responsibility of the manufacturer to ensure correct translation into other languages.

GeneXpert
Powered By CEPHEID INNOVATION

# Xpert® Mpox

REF **GXMPX-10**

Instructions for Use

For Use Under an Emergency Use Authorization (EUA) Only

For Use with GeneXpert Dx System or GeneXpert Infinity System

**IVD**

Cepheid

*In Vitro* Diagnostic Medical Device

**302-9629, Rev. A**
**February 2023**

### Trademark, Patents, and Copyright Statements



See Section 26, Revision History for a description of changes.

# Xpert® Mpox

**For use under the Emergency Use Authorization (EUA) only.**

For *In Vitro* Diagnostic Use

IVD Rx only

## 1 Proprietary Name

Xpert® Mpox

## 2 Common or Usual Name

Xpert Mpox

## 3 Intended Use

The Xpert® Mpox is a real-time PCR test intended for the qualitative detection of DNA from monkeypox virus clade II and non-variola *Orthopoxvirus* DNA in human lesion swab specimens (i.e., swabs of acute pustular or vesicular rash) from individuals suspected of mpox by their healthcare provider.

Testing on the GeneXpert Dx and GeneXpert Infinity instruments is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high or moderate complexity tests.

Testing on the GeneXpert Xpress (Hub Configuration) instrument is limited to laboratories certified under CLIA that meet requirements to perform high, moderate, or waived complexity tests. Testing on the GeneXpert Xpress (Hub Configuration) instrument is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation.

Results are for the identification of monkeypox virus (clade II) and non-variola *Orthopoxvirus* DNA, which are generally detectable in human pustular or vesicular lesion specimens during the acute phase of infection. Positive results are indicative of the presence of monkeypox virus (clade II) and/or non-variola *Orthopoxvirus* 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 monkeypox virus (clade II) and/or non-variola *Orthopoxvirus* 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 Xpert Mpox is intended for use by trained operators who are proficient in performing a test using either GeneXpert Dx, GeneXpert Infinity and/or Gene Xpert Xpress systems.

The Xpert® Mpox test is only for use under the Food and Drug Administration's Emergency Use Authorization.

# 4 Summary and Explanation

Monkeypox virus (MPXV) is an enveloped double-stranded DNA virus that belongs to the *Orthopoxvirus* genus of the Poxviridae family. Mpox is a viral zoonotic disease capable of human-to-human transmission. The first human case of mpox was identified in 1970 in the Democratic Republic of the Congo (DRC). The disease is considered endemic to Central and West Africa.[1] Two clades of monkeypox virus have been described; monkeypox virus clade I (formerly Congo-basin) is associated with a higher rate of mortality compared to clade II (formerly West-African).[1,2]

Monkeypox virus has an incubation period ranging from 5-21 days, initial symptom onset typically last 1-5 days and consists of fever, headache, myalgia, lymphadenopathy, and fatigue before the appearance of vesiculopustular rash lasting approximately 2-3 weeks.[3] Human-to-human transmission occurs via direct contact with lesions or bodily fluids, through contaminated fomites, or through respiratory secretions.[1,3] In the spring of 2022 several cases of mpox, specifically monkeypox virus clade IIb, were identified in non- endemic areas.[1] This was followed by U.S. Department of Health and Human Services declaring mpox as a Public Health Emergency (PHE) in the United States on August 4, 2022.[4]

# 5 Principle of the Procedure

The Xpert Mpox test is an automated in vitro diagnostic test for the qualitative detection and identification of monkeypox vitus (MPXV) clade II DNA and non-variola *Orthopoxvirus* DNA. The Xpert Mpox test is performed on GeneXpert® Instrument Systems.

The GeneXpert Instrument Systems automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequences in simple or complex samples using real-time PCR tests. The systems consist of an instrument, computer, and preloaded software for running tests and viewing the results. The systems require the use of single-use disposable cartridges that hold the real-time PCR reagents and host the real-time PCR process. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the system, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*.

The Xpert Mpox test includes reagents for the detection of MPXV–clade II (MPXV target 1 and target 2) and non-variola *Orthopoxvirus* (OPXV, OPXV-E9L NVAR) targets in lesion swab specimens. A Sample Processing Control (SPC), a Sample Adequacy Control (SAC), and a Probe Check Control (PCC) are also included in the cartridge utilized by the GeneXpert instrument. The SPC is present to control for adequate processing of the sample and to monitor for the presence of potential inhibitor(s) in the real-time PCR reaction. The SPC also ensures that the real-time PCR reaction conditions (temperature and time) are appropriate for the amplification reaction and that the real-time PCR reagents are functional. The SAC reagents detect the presence of a single copy human gene and monitor whether the sample contains human DNA. The PCC verifies reagent rehydration, PCR tube filling, and confirms that all reaction components are present in the cartridge including monitoring for probe integrity and dye stability.

The Xpert Mpox test is designed for use with lesion swabs collected by a health care provider from patients suspected of having mpox.

The specimen is collected and placed into a transport tube containing 3 mL of viral transport medium or Universal Transport Medium (VTM/UTM®). The specimen is briefly mixed by rapidly inverting the collection tube 5 times. Using the supplied transfer pipette, the specimen is transferred to the sample chamber of the Xpert Mpox test cartridge. The GeneXpert cartridge is loaded onto the GeneXpert Instrument System platform, which performs hands-off, automated sample processing, and real-time PCR for detection of viral DNA.

# 6 Materials Provided

The Xpert Mpox test kit contains sufficient reagents to process 10 specimens or quality control samples. The kit contains the following:

| **Xpert Mpox Cartridges with Integrated Reaction Tubes** | **10** |
|---|---|
| Bead 1, Bead 2, Bead 3, and Bead 4 (freeze-dried) | 1 of each per cartridge |
| Lysis Reagent (Guanidinium Thiocyanate) | 1 mL per cartridge |
| Binding Reagent | 1 mL per cartridge |
| Elution Reagent | 3.0 mL per cartridge |
| Wash Reagent | 0.4 mL per cartridge |

| | |
|---|---|
| **Disposable 300 µL Transfer Pipettes** | **2 bags of 12 per kit** |
| **Flyer** | **1 per kit** |

- Instructions to locate (and import) the Assay Definition File (ADF) and documentation such as the IFU on www.cepheid.com.

| | |
|---|---|
| **Quick Reference Instructions** | **1 per kit** |
| For use with the GeneXpert Xpress System (Hub Configuration) only | |

**Note** Safety Data Sheets (SDS) are available at www.cepheid.com or www.cepheidinternational.com under the **SUPPORT** tab.

**Note** The bovine serum albumin (BSA) in the beads within this product was produced and manufactured exclusively from bovine plasma sourced in the United States. No ruminant protein or other animal protein was fed to the animals; the animals passed ante- and post-mortem testing. During processing, there was no mixing of the material with other animal materials.

# 7 Storage and Handling

- Store the Xpert Mpox test cartridges at 2–28 °C until the expiration date provided on the label.
- Do not open a cartridge until you are ready to perform testing.
- Do not use cartridges that have passed the expiration date.
- Do not use a cartridge that is wet or has leaked.

# 8 Materials Required but Not Provided

- Nylon flocked swab (Copan P/N 502CS01, or equivalent)
- Viral transport medium/Universal transport medium (VTM/UTM), 3 mL (Copan P/N 3C047N or equivalent)
- GeneXpert Dx System or GeneXpert Infinity System (catalog number varies by configuration): GeneXpert Instrument, computer, barcode scanner, and operator manual.
  - For GeneXpert Dx System: GeneXpert Dx software version 4.7b or higher
  - For GeneXpert Infinity-80 and Infinity-48 systems : Xpertise software version 6.4b or higher
- Printer: If a printer is required, contact Cepheid Technical Support to arrange for the purchase of a recommended printer.

# 9 Materials Available but Not Provided

External controls are available from ZeptoMetrix® (Buffalo, NY).

- External Positive Control: Catalog # NATMPXVPOS-6C
- External Negative Control: Catalog # NATMPXVNEG-6C

# 10 Warnings and Precautions

## 10.1 General

- For *in vitro* diagnostic use.
- For use under emergency authorization only.
- For prescription use only.
- This product has not been FDA cleared or approved but has been authorized by FDA under an EUA for use by authorized laboratories.
- This product has been authorized only for the detection of nucleic acid from monkeypox virus or other non-variola orthopoxviruses, not from any other virus or pathogens.

- 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 infection with the monkeypox virus, including in vitro diagnostics that detect and/or diagnose infection with non-variola *Orthopoxvirus*, 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.
- Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.
- Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological specimens should be treated with standard precautions.
- Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention[6] and the Clinical and Laboratory Standards Institute.[7]
- Performance characteristics of this test have been established with the specimen types listed in the Intended Use Section only. The performance of this test with other specimen types or samples has not been evaluated.
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- Do not allow Lysis Reagent, which contains guanidinium thiocyanate to contact sodium hypochlorite (bleach). This mixture can produce a highly toxic gas.
- Testing of lesion swab specimens using the Xpert Mpox test run on the GeneXpert Dx or Infinity Systems, is authorized for use or laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the regulatory requirements to perform high or moderate complexity testing
- Biological specimens, transfer devices, and used cartridges should be considered capable of transmitting infectious agents requiring standard precautions. Follow your institution’s environmental waste procedures for proper disposal of used cartridges and unused reagents. These materials may exhibit characteristics of chemical hazardous waste requiring specific disposal. If country or regional regulations do not provide clear direction on proper disposal, biological specimens and used cartridges should be disposed per WHO [World Health Organization] medical waste handling and disposal guidelines.

## 10.2 Specimens

- Specimen collection and handling procedures require specific training and guidance.
- For collection and transport of lesion swab specimens, use viral transport medium/universal transport medium (Copan P/N 3C047N, or equivalent) and nylon flocked swab (Copan P/N 502CS01, or equivalent).
- Specimens must be collected and tested before the expiration date of the viral transport medium (VTM/UTM) tube.
- Maintain proper storage conditions during specimen transport to ensure the integrity of the specimen (see Section 12). Specimen stability under shipping conditions other than those recommended has not been evaluated.
- Proper sample collection, storage, and transport are essential for correct results.

## 10.3 Test/Reagent

- The test has been validated using Cepheid GeneXpert Dx software version 4.7b or higher, and Cepheid Xpertise software version 6.4b or higher. Cepheid will validate future software versions for use with the Xpert Mpox test.
- Do not substitute Xpert Mpox test reagents with other reagents.
- Do not open the Xpert Mpox test cartridge lid except when adding sample.
- Do not use a cartridge that has been dropped after removing from the kit or shaken after the cartridge lid has been opened. Shaking or dropping the cartridge after opening the lid may yield false or non-determinate results.
- Do not place the sample ID label on the cartridge lid or on the barcode label.
- Do not use a cartridge with a damaged barcode label.
- Do not use a cartridge that has a damaged reaction tube.
- Do not use reagents beyond their expiry date.
- Each single-use Xpert Mpox test cartridge is used to process one test. Do not reuse spent cartridges.
- Each single-use disposable pipette is used to transfer one specimen. Do not reuse disposable pipettes.
- Do not use a cartridge if it appears wet or if the lid seal appears to have been broken.
- Wear clean lab coats and gloves. Change gloves between the handling of each specimen.
- In the event of a spill of specimens or controls, wear gloves and absorb the spill with paper towels. Then, thoroughly clean the contaminated area with a 1:10 dilution of freshly prepared household chlorine bleach. Final active chlorine concentration should be 0.5%. Allow a minimum of two minutes of contact time. Ensure the work area is dry before using 70% denatured ethanol to remove bleach residue. Allow surface to dry completely before proceeding. Or, follow

your institution's standard procedures for a contamination or spill event. For equipment, follow the manufacturer's recommendations for decontamination of equipment.

# 11 Chemical Hazards[8,9]

- **Signal Word:WARNING**
- **UN GHS Hazard Statements**
- Harmful if swallowed
- May be harmful in contact with skin
- Causes eye irritation
- **UN GHS Precautionary Statements**
- **Prevention**
  - Wash hands thoroughly after handling.
- **Response**
  - If skin irritation occurs: Get medical advice/attention.
  - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
  - If eye irritation persists: Get medical advice/attention.
  - Call a POISON CENTER or doctor/physician if you feel unwell.

# 12 Specimen Collection, Transport, and Storage

Proper specimen collection, storage, and transport are critical to the performance of this test. Inadequate specimen collection, improper specimen handling and/or transport may yield a false result. See Section 12.1 for lesion swab collection procedure. Lesion swab specimens can be stored at room temperature (15–30 °C) for up to 48 hours in viral transport medium (VTM/UTM) until testing is performed on the GeneXpert Instrument Systems. Alternatively, lesion swab specimens can be stored refrigerated (2–8 °C) up to seven days in viral transport medium (VTM/UTM) until testing is performed on the GeneXpert Instrument Systems.

Refer to the CDC Guidelines for Collecting and Handling Specimens for Mpox Testing.

https://www.cdc.gov/poxvirus/monkeypox/clinicians/prep-collection-specimens.html

## 12.1 Lesion Swab Collection Procedure

1. Using a sterile swab apply firm pressure to the lesion and swipe the swab back and forth at least 2-3 times before rotating the swab and repeating using the other side of the swab. If the lesion ruptures while swabbing, ensure to collect the lesion fluid (see Figure 1).

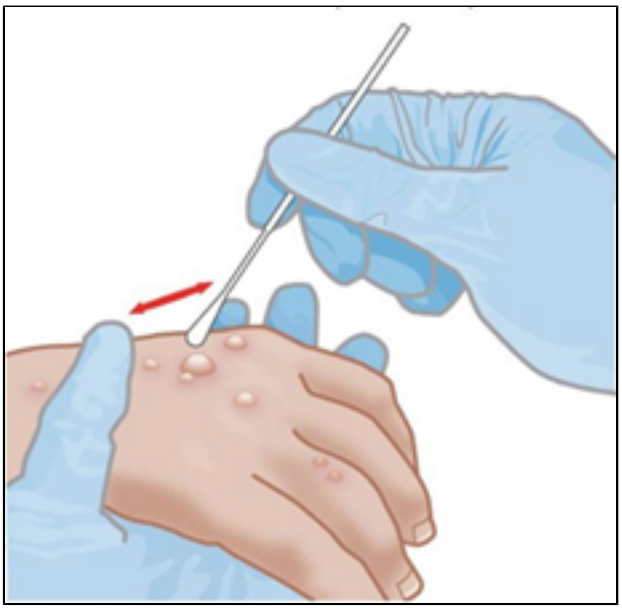


**Figure 1. Lesion Swab Collection**

2. Remove and place the swab into the tube containing 3 mL of viral transport medium (VTM/UTM). Break swab at the indicated break line and cap the specimen collection tube tightly.

# 13 Procedure

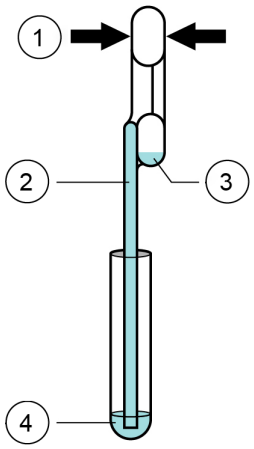
## 13.1 Preparing the Specimen and Cartridge

**Important** **Start the test within 30 minutes of adding the sample to the cartridge.**

1. Obtain the following items: Xpert Mpox test cartridge, 300 µL transfer pipette (provided), and an appropriately collected and labeled test sample.
2. Remove a cartridge from the package.
3. Inspect the cartridge for damage. If damaged, do not use it.
4. Ensure the specimen tube is tightly capped. Mix specimen by inverting the specimen tube five times. Open the cap on the specimen transport tube.
5. Open the cartridge lid by lifting the lid.
6. Remove the transfer pipette from the wrapper.

**Note** Do not place unwrapped pipette on the workbench.

7. Squeeze the top bulb of the transfer pipette completely and then place the pipette tip in the specimen transport tube (see Figure 2).




| Number | Description |
|---|---|
| 1 | Squeeze here |
| 2 | Pipette |
| 3 | Overflow Reservoir Bulb |
| 4 | Sample |

**Figure 2. Transfer Pipette**

8. Slowly release the top bulb of the pipette slowly to fill the length of the pipette before removing from the tube. After filling pipette, excess sample will be seen in the overflow reservoir bulb of the pipette (Figure 2). Check that the pipette does not contain bubbles.
9. To transfer the sample to the cartridge, squeeze the top bulb of the transfer pipette completely again to empty the contents of the pipette into the large opening (Sample Chamber) in the cartridge shown in Figure 3. Dispose of the used pipette in a biohazard container.

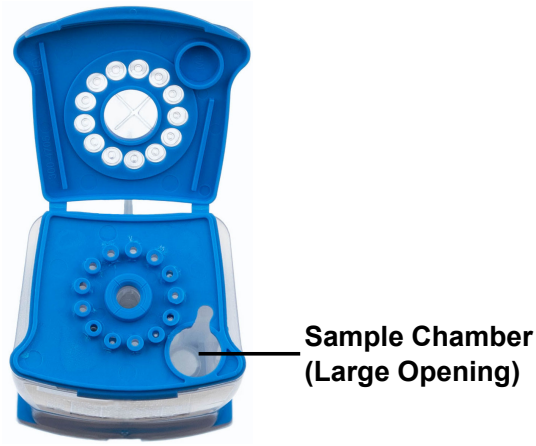



**Figure 3. Xpert Mpox Cartridge (Top View)**

**Note** Take care to dispense the entire volume of liquid into the sample chamber. False negative results may occur if insufficient sample is added to the cartridge.

10. Close the cartridge lid.

## 13.2 Running External Controls

External controls described in Section 9 are available but not provided.

**Note** The positive external control should provide a **MPXV Clade II Detected** result. If a different result is obtained, repeat the positive external control run.

**Note** The negative external control should provide a **MPXV Clade II NOT DETECTED, Non-variola OPXV NOT DETECTED** result. If a different result is obtained, repeat the external control run.

**Note** If the expected results for the external control materials are not obtained upon repeat, contact Cepheid Technical Support.

### When to Run External Controls – Positive and Negative Controls

Use external controls in accordance with local, state, and federal accrediting organizations as applicable as well as according to the frequencies indicated below.

- Each time a new shipment of Xpert Mpox kits is received, even if it is the same lot previously received.
- Each time a new operator is performing the test (i.e., operator who has not performed the test recently).
- When problems (storage, operator, instrument, or other) are suspected or identified.

**If using Positive Control #NATMPXVPOS-6C from ZeptoMetrix (Buffalo, NY), the positive control #NATMPXVPOS-6C must be diluted prior to use as follows:**

1. Label a 3 ml VTM/UTM tube “Diluted Positive Control”. Uncap the tube.
2. Using an exact volume transfer pipette provided in the kit, add one draw (300 µL) of the (undiluted) Positive Control material to the VTM/UTM tube.
3. Recap the tube tightly and mix by inverting the tube 5 times.
4. Open the cartridge lid.
5. Using a new exact volume transfer pipette provided in the kit, transfer one draw (300 µl) of the diluted Positive Control material into the large opening (Sample Chamber) in the cartridge shown in Figure 3.
6. Close cartridge lid.
7. Change gloves and clean workstation between and after running the controls.
8. Proceed to Section 14.

**If using negative control #NATMPXVNEG-6C from ZeptoMetrix (Buffalo, NY), the negative control (NATMPXVNEG-6C) is ready to use directly. No additional preparation steps are necessary.**

1. Using new exact volume transfer pipette provided in the kit, add one draw (300 µl) of the Negative Control material into the large opening (Sample Chamber) in the cartridge shown in Figure 3.
2. Close cartridge lid.
3. Proceed to Section 14.

# 14 Running the Test

- For the GeneXpert Dx System, see Section 14.1.
- For the GeneXpert Infinity System, see Section 14.2.

## 14.1 GeneXpert Dx System

### 14.1.1 Starting the Test

**Important**

**Before you start the test, make sure that:**

- The system is running the correct GeneXpert Dx software version shown in section - Materials Required but Not Provided.
- The correct assay definition file is imported into the software.

This section lists the basic steps for running the test. For detailed instructions, see the *GeneXpert Dx System Operator Manual*.

**Note** The steps you follow can be different if the system administrator changed the default workflow of the system.

1. Turn on the GeneXpert Dx System, then turn on the computer and log on. The GeneXpert software will launch automatically. If it does not, double-click the GeneXpert Dx software shortcut icon on the Windows® desktop.
2. Log on using your username and password.
3. In the **GeneXpert System** window, click **Create Test**.
   The **Create Test** window displays. The **Scan Patient ID barcode** dialog box displays.
4. Scan or type in the Patient ID. If typing the Patient ID, make sure the Patient ID is typed correctly.

The Patient ID is associated with the test results and displays in the **View Results** window and all the reports. The **Scan Sample ID barcode** dialog box displays.

5. Scan or type in the Sample ID. If typing the Sample ID, make sure the Sample ID is typed correctly.
   The Sample ID is associated with the test results and displays in the **View Results** window and all the reports. The **Scan Cartridge Barcode** dialog box displays.
6. Scan the barcode on the cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.

**Note** If the barcode on the cartridge does not scan, then repeat the test with a new cartridge. If you have scanned the cartridge barcode in the software and the assay definition file is not available, a screen displays indicating the assay definition file is not loaded on the system. If this screen displays, contact Cepheid Technical Support.

7. Click **Start Test**. In the dialog box that displays, type your password, if required.
8. Open the instrument module door with the blinking green light and load the cartridge.
9. Close the door. The test starts and the green light stops blinking.
   When the test is finished, the light turns off.
10. Wait until the system releases the door lock before opening the module door, then remove the cartridge.
11. Dispose of the used cartridges in the appropriate specimen waste containers according to your institution's standard practices.

### 14.1.2 Viewing and Printing Results

This section lists the basic steps for viewing and printing results. For more detailed instructions on how to view and print the results, see the *GeneXpert Dx System Operator Manual*.

1. Click the **View Results** icon to view results.
2. Upon completion of the test, click the **Report** button of the **View Results** window to view and/or generate a PDF report file.

## 14.2 GeneXpert Infinity System

### 14.2.1 Starting the Test

**Important**

**Before you start the test, make sure that:**

- The system is running the correct Xpertise software version shown in section - Materials Required but Not Provided.
- The correct assay definition file is imported into the software.

This section lists the basic steps for running the test. For detailed instructions, see the *GeneXpert Infinity System Operator Manual*.

**Note** The steps you follow can be different if the system administrator changed the default workflow of the system.

1. Power up the instrument. The Xpertise software will launch automatically. If it does not, double-click the Xpertise software shortcut icon on the Windows® desktop.
2. Log on to the computer, then log on to the GeneXpert Xpertise software using your user name and password.
3. In the **Xpertise Software Home** workspace, click **Orders** and in the **Orders** workspace, click **Order Test**.
   The **Order Test - Patient ID** workspace displays.
4. Scan or type in the Patient ID. If typing the Patient ID, make sure the Patient ID is typed correctly.
   The Patient ID is associated with the test results and displays in the **View Results** window and all the reports.
5. Enter any additional information required by your institution, and click the **CONTINUE** button.
   The **Order Test - Sample ID** workspace displays.
6. Scan or type in the Sample ID. If typing the Sample ID, make sure the Sample ID is typed correctly.
   The Sample ID is associated with the test results and displays in the **View Results** window and all the reports.
7. Click the **CONTINUE** button.
   The **Order Test - Assay** workspace displays.

8. Scan the barcode on the cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.

**Note** If the barcode on the cartridge does not scan, then repeat the test with a new cartridge. If you have scanned the cartridge barcode in the software and the assay definition file is not available, a screen displays indicating the assay definition file is not loaded on the system. If this screen displays, contact Cepheid Technical Support.

After the cartridge is scanned, the **Order Test - Test Information** workspace displays.

9. Verify that the information is correct, and click **Submit**. In the dialog box that displays, type your password, if required.
10. Place the cartridge on the conveyor belt.
The cartridge automatically loads, the test runs, and the used cartridge are placed into the waste container.

#### 14.2.2 Viewing and Printing Results

This section lists the basic steps for viewing and printing results. For more detailed instructions on how to view and print the results, see the *GeneXpert Infinity System Operator Manual*.

1. In the **Xpertise Software Home** workspace, click the **RESULTS** icon. The Results menu displays.
2. In the Results menu, select the **VIEW RESULTS** button. The **View Results** workspace displays showing the test results.
3. Click the **REPORT** button to view and/or generate a PDF report file.

# 15 Quality Controls

## 15.1 Internal Controls

Each test includes a Sample Processing Control (SPC), a Sample Adequacy Control (SAC), and a Probe Check Control (PCC).

- **Sample Processing Control (SPC)** —Ensures the sample was processed correctly. The SPC verifies that sample processing is adequate. Additionally, this control detects sample-associated inhibition of the real-time PCR test, ensures the PCR reaction conditions are appropriate for the amplification reaction. The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the assigned acceptance criteria.
- **Sample Adequacy Control (SAC)** —Detects human cells or human DNA present in the sample. This test includes primers and probe for the detection of a single copy human gene. The SAC signal is only to be considered when the sample is negative for both MPXV and OPXV analytes and SPC is positive. A negative SAC indicates that no human cells are present in the sample due to insufficient mixing of the sample or because of an inadequately collected sample.
- **Probe Check Control (PCC)**—Before the start of the PCR reaction, the GeneXpert System measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, and probe integrity. The PCC passes if it meets the assigned acceptance criteria.

## 15.2 External Controls

Use external controls in accordance with local, state and federal accrediting organizations as applicable. If the expected results for the external control materials are not obtained, repeat the external controls, prior to releasing patient results. If the expected results for the external control material are not obtained upon repeat, contact Cepheid Technical Support.

**Note** Cepheid recommends that all laboratories perform external QC with each new lot and shipment of reagents, at a minimum, while running the Xpert Mpox test under Emergency Use Authorization (EUA).

# 16 Interpretation of Results

The results are interpreted automatically by the GeneXpert System and are clearly shown in the **View Results** window. The Xpert Mpox test provides test results for the monkeypox virus and non-variola Orthopoxvirus targets, SAC, and SPC according to the algorithms shown in Table 1.

**Table 1. Xpert Mpox Results Algorithm**

| Result | MPXV | OPXV | SAC | SPC |
|---|---|---|---|---|
| **MPXV Clade II DETECTED** | + | +/- | +/- | +/- |
| **Non-Variola OPXV DETECTED** | - | + | +/- | +/- |
| **MPXV Clade II NOT DETECTED, Non-Variola OPXV NOT DETECTED** | - | - | + | + |
| **INVALID** | - | - | - | - |
| | - | - | - | + |
| | - | - | + | - |

See Table 2 to interpret test results statements for the Xpert Mpox test.

**Table 2. Xpert Mpox Result Interpretation**

| Result | Interpretation |
|---|---|
| **MPXV Clade II DETECTED** | Monkeypox virus Clade II DNA DETECTED<br>This specimen is **POSITIVE** for monkeypox virus clade II.<br>Laboratories should notify appropriate Federal, State, or local public health agencies of the test results in accordance with applicable laws. |
| **Non-Variola OPXV DETECTED** | Non-Variola *Orthopoxvirus* Viral DNA DETECTED.<br>This specimen is **POSITIVE** for non-variola *Orthopoxvirus* DNA.<br>Low levels of monkeypox viral DNA in a specimen may on rare occasions, result in monkeypox virus clade II DNA Not Detected and non-variola *Orthopoxvirus* DNA Detected. This result may occur in the case of low monkeypox viral DNA concentrations at levels near or below the limit of detection or may indicate another *Orthopoxvirus* infection although no other known orthopoxviruses are currently circulating in the United States.<br>If clinically indicated, consider collecting another specimen.<br>Laboratories should notify appropriate Federal, State, or local public health agencies of the test results in accordance with applicable laws. |
| **MPXV Clade II NOT DETECTED, Non-Variola OPXV NOT DETECTED** | Monkeypox Clade II Viral DNA and Non-Variola *Orthopoxvirus* Viral DNA not detected.<br>This specimen is **NEGATIVE** for monkeypox virus clade II DNA. If clinically indicated, consider collecting another specimen.<br>Laboratories should notify appropriate Federal, State, or local public health agencies of the test results in accordance with applicable laws. |
| **NO RESULT** | If the result is **NO RESULT**, repeat the test with a new cartridge. |
| **INVALID** | If the result is **INVALID** repeat the test, if the second test is **INVALID** collect a new specimen. |
| **ERROR** | The result is an instrument error or cartridge defect. Repeat the test using a new cartridge. |
| If the results of the viral targets (mpox virus clade II or non-variola *Orthopoxvirus*) are positive, the test can be considered valid regardless of the results for the SPC and SAC. | |

The Xpert Mpox test includes an Early Assay Termination (EAT) function which will provide earlier time to results in high titer specimens if the signal from the target nucleic acid reaches a predetermined threshold before the full 45 PCR cycles have been completed. When viral titers are high enough to initiate the EAT function, the SPC, SAC and/or other target amplification curves may not be seen and their results may not be reported.

# 17 Retests

## 17.1 Reasons to Repeat the Test

If any of the test results mentioned below occur, repeat the test once according to instructions in Section 17.2.

- An **ERROR** result could be due to, but not limited to, Probe Check Control failure, system component failure, or the maximum pressure limits were exceeded.
- An **INVALID** result indicated that either all targets are negative or when both the MPXV and OPXV targets are negative there is an SPC and/or SAC failure. The sample was not properly processed, PCR is inhibited, or the sample was not properly collected.
- A **NO RESULT** indicates that insufficient data was collected. For example, cartridge failed integrity test, the operator stopped a test that was in progress, or a power failure occurred.
- If an External Control fails to perform as expected, repeat the external control test and/or contact Cepheid for assistance.

## 17.2 Retest Procedure

To retest a non-determinate result (**INVALID**, **NO RESULT**, or **ERROR**), use a new cartridge.

Use the leftover sample from the original specimen transport medium tube or if running a control, use a new external control.

1. Put on a clean pair of gloves. Obtain a new Xpert Mpox test cartridge and a new transfer pipette.
2. Check that the specimen transport tube or external control tube is closed.
3. Mix the sample by rapidly inverting the specimen transport medium tube or external control tube 5 times. Open the cap on the specimen transport tube or external control tube.
4. Open the cartridge lid.
5. Using a clean transfer pipette (supplied), transfer sample (one draw) to the sample chamber with the large opening in the cartridge.
6. Close the cartridge lid.

# 18 Limitations

1. Xpert Mpox has been evaluated only for use in combination with the recommended external controls – External Positive NATMPXVPOS-6C and External Negative Control NATMPXVNEG-6C (ZeptoMetrix (Buffalo, NY), for use on the GeneXpert Dx, GeneXpert Xpress (hub configuration) and GeneXpert Infinity Systems using the procedures provided in these instructions for use only. Modifications to these procedures may alter the performance of the test.
2. Xpert Mpox has only been validated for use with specimens collected from lesions using a synthetic swab (Copan P/N 502CS01, or equivalent) which is placed into UTM or VTM. Test performance has not been validated for use with other collection media and/or specimen types. Use of other collection media and/or specimen types may lead to false positive, false negative or invalid results.
3. A specimen with a result of **MPXV Clade II NOT DETECTED, Non-Variola OPXV NOT DETECTED** does not preclude monkeypox virus infection and should not be used as the sole basis for treatment or other patient management decisions. Collection of multiple specimens (and specimens collected at different time points) from the same patient may be necessary to detect the virus.
4. Reliable results depend on proper sample collection, storage, and handling procedures.
5. Detection of monkeypox clade II viral DNA or non-variola *Orthopoxvirus* viral DNA is dependent on the number of copies present in the specimen. Detection of monkeypox clade II viral DNA or non-variola *Orthopoxvirus* viral DNA may be affected by sample collection methods (e.g., if a specimen is improperly collected, transported, or handled), patient factors (e.g., presence, type, and duration of symptoms), stage of infection (e.g., if collected too early or too late in the course of illness) and/or presence of interfering substances.

6. The performance of this test was established based on the evaluation of a limited number of clinical specimens. The clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of monkeypox virus and their prevalence, which may change over time. Results from the Xpert Mpox test should be interpreted in the context of other available laboratory and clinical data.
7. As with any molecular test, mutations within the target regions of Xpert Mpox could affect primer and/or probe binding resulting in failure to detect the presence of viral DNA.
8. Negative results do not preclude monkeypox virus clade II and/or non-variola *Orthopoxvirus* infection and should not be used as the sole basis for treatment or other patient management decisions.
9. False negative results may arise from improper sample collection, degradation of the viral DNA during shipping/storage, the presence of PCR inhibitors, and/or mutation(s) in the monkeypox virus and/or non-variola Orthopoxvirus.
10. False positive results may arise from contamination during specimen handling or preparation, or between patient specimens.
11. Results from the Xpert Mpox test should be interpreted by a trained professional in conjunction with the patient's history and clinical signs/symptoms and epidemiological risk factors.
12. Viral nucleic acid may persist in vivo, independent of virus viability. Detection of analyte target(s) does not imply that the corresponding virus(es) are infectious or are the causative agents for clinical symptoms.
13. This test has been evaluated for use with human specimen material only.
14. The test performance was established during the 2022 mpox outbreak in the US. The performance may vary depending on the prevalence and population tested.
15. This test is a qualitative test and does not provide the quantitative value of detected organism present.
16. This test has not been evaluated for patients without signs and symptoms of monkeypox virus clade II infection.
17. This test has not been evaluated for monitoring treatment of monkeypox virus clade II and/or non-variola *Orthopoxvirus* infection.
18. This test has not been evaluated for screening of blood or blood products for the presence of monkeypox virus clade II and/or non-variola *Orthopoxvirus*.
19. This test cannot rule out diseases caused by other bacterial or viral pathogens.
20. The effect of interfering substances has only been evaluated for those listed within the labeling. Interference by substances other than those described could lead to erroneous results.
21. This test has not been evaluated for immunocompromised individuals.
22. The current US outbreak of mpox is caused by monkeypox virus clade II. A positive result for non-variola *Orthopoxvirus* most likely represents the presence of monkeypox virus clade II, although there is a small possibility that this result could represent the presence of a different non-variola *Orthopoxvirus* detected by the non-variola *Orthopoxvirus* primer and probe set. If clinical concern for infection with an *Orthopoxvirus* other than monkeypox, healthcare providers should contact the jurisdictional public health authorities for guidance.
23. Based on an *in silico* analysis, some vaccinia, cowpox and camelpox virus strains have genomic sequences containing two or more mismatches with the OPXV target primers and probe (Table 9 of Inclusivity Section below), and therefore may not be detected by the Xpert Mpox test. Wet-testing of contrived low-positive samples spiked with genomic DNA from one strain of vaccinia virus demonstrated detection of the non-variola OPXV target.
24. Results from an *in silico* analysis show potential for Xpert Mpox test cross-reactivity with variola, cowpox or ectromelia viruses that could result in a false **MPXV Clade II DETECTED** result. If variola (smallpox), cowpox or ectromelia infection is suspected, the provider is advised to contact the local health department immediately for patient evaluation.
25. The impacts of specific vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs on the performance of this test have not been evaluated.
26. Detection of the Sample Adequacy Control (SAC) indicates that human nucleic acid is present and implies that human biological material was collected and successfully extracted and amplified. It does not necessarily indicate that the specimen is of appropriate quality to enable detection of monkeypox virus (clade II) and/or non-variola *Orthopoxvirus*. All MPXV and non-variola OPXV-negative specimens must have a positive Sample Adequacy Control result to be identified as valid negatives.

# 19 Conditions of Authorization

The Cepheid Xpert Mpox Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/emergency-use-authorizations-medical-devices/monkeypox-emergency-use-authorizations-medical-devices.

To assist clinical laboratories and/or Patient Care Settings using the Xpert Mpox (referred to in the Letter of Authorization as "Your Product"), the relevant Conditions of Authorization are listed below.

- Authorized laboratories[a] that receive Xpert Mpox must notify the relevant public health authorities of their intent to run your product prior to initiating testing.
- Authorized laboratories using Xpert Mpox must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories using Xpert Mpox must include with test result reports all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories using Xpert Mpox must use Xpert Mpox as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use Xpert Mpox are not permitted.
- Authorized laboratories must have a process in place to track adverse events and report to Cepheid Customer Technical Support 1-888-838 3222 and to FDA pursuant to 21 CFR Part 803.
- All operators using Xpert Mpox must be appropriately trained in performing and interpreting the results of Xpert Mpox, use appropriate personal protective equipment when handling Xpert Mpox, and use Xpert Mpox in accordance with the authorized labeling.
- Cepheid, authorized distributors, and authorized laboratories must collect information on the performance of Xpert Mpox and report any significant deviations from the established performance characteristics of Xpert Mpox of which they become aware to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA Reporting@fda.hhs.gov). In addition, authorized distributor(s) and authorized laboratories report to Cepheid (+1 888 838 3222 or techsupport@cepheid.com).
- Cepheid, authorized distributors, and authorized laboratories using Xpert Mpox must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

# 20 Performance Characteristics

## 20.1 Clinical Performance

The performance of the Xpert Mpox test was evaluated using frozen, retrospective clinical lesion swab specimens in either universal transport medium (UTM) or viral transport medium (VTM) collected from patients suspected of mpox . All of the clinical specimens were previously characterized as positive or negative for non-variola *Orthopoxvirus* using an FDA cleared real-time-PCR assay. A total of 31 positive specimens, including four low positive specimens (4/31, 12.9%), and 30 negative specimens were tested on the Xpert Mpox test in a double-blinded fashion in a point of care setting by untrained users.

Positive Percent Agreement (PPA), Negative Percent Agreement (NPA) were determined by comparing the results of the Xpert Mpox test relative to the known results from the FDA cleared real-time-PCR test.

The Xpert Mpox test demonstrated a PPA and NPA of 100.0% and 96.6% for MPXV, respectively (Table 3). The initial non-determinate rate for the Xpert Mpox test was 6.5% (4/61). On repeat testing, all four specimens yielded valid results. The final non-determinate rate for the Xpert Mpoxtest was 0.0% (0/61).

[a] The letter of authorization refers to "authorized laboratories" as follows: (1) Testing on the GeneXpert Dx and GeneXpert Infinity instruments 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 or moderate complexity tests, and (2) Testing on the GeneXpert Xpress (Hub Configuration) instruments is limited to laboratories certified under CLIA that meet requirements to perform high, moderate, or waived complexity tests. Testing is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation.

**Table 3. Xpert Mpox Test Performance Results Using Lesion Swab Specimens**

| | | FDA cleared real-time-PCR assay | | |
|---|---|---|---|---|
| | | **Positive** | **Negative** | **Total** |
| **Xpert Mpox** | **Positive** | 31 | 1 | 32 |
| | **Negative** | 0 | 29 | 29 |
| | **Total** | 31 | 30 | 61 |
| **PPA** | | 100% (95% CI: 100%-100%) | | |
| **NPA** | | 96.6% (95% CI: 96.6-96.7%) | | |

## 20.2 Xpert Mpox Test Performance Around LoD

A total of 20 contrived samples were tested in a blinded fashion by untrained users to evaluate the performance of the Xpert Mpox test near the LoD. At the same point of care site where testing of clinical specimens was conducted, contrived samples were integrated into the workflow along with clinical specimens. The 20 contrived swab samples included 10 monkeypox positive contrived swab samples at ≤2x LoD and 10 negative samples in a negative clinical buccal swab matrix. Overall agreement with expected results was 100% for monkeypox virus positive and negative samples.

# 21 Analytical Performance

## 21.1 Analytical Sensitivity (Limit of Detection)

The Limit of Detection (LoD) is the lowest concentration (genomic copies, GC/mL) per sample that can be reproducibly distinguished from negative samples with 95% confidence or the lowest concentration at which at least 19 of 20 replicates are positive. LoD studies for the Xpert Mpox test were conducted using heat-inactivated monkeypox virus (USA/MA001/2022, Lineage B.1, clade IIb) and vaccinia virus gDNA (Modified Vaccinia Virus Ankara). The concentration of the monkeypox virus stock was $4.23 \times 10^4$ GC/mL as determined by digital droplet (dd) PCR. The concentration of vaccinia virus gDNA stock was $1.82 \times 10^7$ GC/mL as provided by the manufacturer. The monkeypox virus and vaccinia virus gDNA were serially diluted in pooled negative matrix (PNM), which is made of negative human buccal swab material in universal transport media (UTM-RT Virus Specimen Collection Kit, Copan). Fifty microliters of the spiked PNM were pipetted onto a dry sterile swab, and the wetted swab was then inserted into 3 ml of UTM and tested on the Xpert Mpox test for preliminary LoD and LoD confirmation studies. The confirmed LoD values for both MPXV and OPXV are listed in Table 4.

The LoD for the **MPXV** target was confirmed to be **$2.12 \times 10^1$ GC/mL** in UTM.

The LoD for the **OPXV** target was confirmed to be **$4.23 \times 10^1$ GC/mL** for **monkeypox virus** and **$6.00 \times 10^1$ GC/mL for vaccinia virus gDNA in UTM**.

**Table 4. Xpert Mpox Test Limit of Detection (LoD)**

| **Organism** | **Xpert Mpox Test Targets (GC/mL)** | |
|---|---|---|
| | **MPXV** | **OPXV** |
| **Inactivated Monkeypox Virus (GC/mL)**[a] | $2.12 \times 10^1$ | $4.23 \times 10^1$ |
| **Vaccinia Virus gDNA (GC/mL)**[b] | - | $6.00 \times 10^1$ |

[a] For an MPXV Clade II detected result based on the Xpert Mpox test interpretation, the LoD of the Xpert Mpox test is $2.12 \times 10^1$ GC/mL

[b] For a non-variola OPXV detected result based on the Xpert Mpox test interpretation, the LoD of the Xpert Mpox test is $6.00 \times 10^1$ GC/mL

## 21.2 Analytical Specificity (Exclusivity)

An in silico analysis was performed to evaluate the Xpert Mpox test for analytical specificity (cross-reactivity). Potential cross-reactivity was assessed according to percent homology of the organisms' genomic sequences listed in Table 7 to the primers and probes in the Xpert Mpox test. The Xpert Mpox test contains two monkeypox virus clade II specific targets (MPXV target 1 and target 2), one non-variola Orthopoxvirus (OPXV) target, and SPC and SAC internal control targets.

With the exception of variola virus, vaccinia virus, cowpox virus, and ectromelia virus, in silico analysis showed that the Xpert Mpox test primers and probe sets do not show ≥80% homology to the genomic DNA sequences evaluated of the microorganisms and viruses listed in Table 6. There is potential cross-reactivity of the MPXV target 1 primers and probes with variola, vaccinia and cowpox virus and of MPXV target 2 primers and probes with ectromelia virus sequences as evidenced by the more than 80% sequence identity (Table 5).

**Table 5. Organisms with Significant Matches to the Xpert Mpox Test MPXV Target Primers and Probe Sequences**

| Xpert Mpox test targets | Species | Refseq Accession ID | Forward Primer | Probe | Reverse Primer |
|---|---|---|---|---|---|
| MPXV target 1 | Variola Virus (smallpox) | NC_001611.1 | 88% (22/25nt) | 92% (23/25nt) | 92% (23/25nt) |
| MPXV target 1 | Cowpox virus[a] | NC_003663 | 100% (25/25nt) | 88% (22/25nt) | 92% (23/25nt) |
| MPXV target 1 | Camelpox virus[a] | NC_003391 | NSM[b] | 92% (23/25nt) | 92% (23/25nt) |
| MPXV target 1 | Vaccinia virus[c] | NC_006998 | 92% (23/25nt) | 92% (23/25nt) | 92% (23/25nt) |
| MPXV target 2 | Ectromelia virus (mousepox)[d] | NC_004105 | 92% (23/25nt) | 80% (20/25nt) | 92% (23/25nt) |

[a] Based on the epidemiology of currently circulating orthopoxviruses, and lack of evidence for human-to-human transmission of cowpox and camelpox virus, a false positive result due to cowpox or camelpox infection is unlikely

[b] NSM: No significant match

[c] *In vitro* testing of vaccinia genomic DNA (gDNA) at a concentration of 1.00 x $10^6$ copies/mL showed no signal with MPXV targets

[d] The *in silico* analysis showed sufficient mismatches to the probe sequence of the MPXV target to prevent detection of ectromelia virus

In addition, the OPXV target primer and probe sets have ≥80% homology for variola virus genome The forward and reverse primers for the Xpert Mpox test OPXV target are exact matches to the locus in the variola genome, however, the probe has three mismatches (Table 6). Though the Xpert Mpox test demonstrated 91% identity to the variola locus, the results of *in vitro* testing with variola DNA conducted by Li, *et al*. demonstrated that testing with a PCR assay using the same non-variola OPXV primers/probe sequences as Xpert Mpox did not provide a positive result when tested against a wide range of variola virus samples[5].

A ≥80% homology to human genomic DNA was observed for the SAC primers and probe as they are designed to detect human genomic DNA.

**Table 6. Percent Homology of Xpert Mpox OPXV Target Primers and Probe with Variola Virus**

| Species | Refseq Accession ID | Forward Primer | Probe | Reverse Primer |
|---|---|---|---|---|
| Variola Virus (smallpox) | NC_001611.1 | 100% (23/23nt) | 91% (29/32nt) | 100% (28 / 28nt) |

Based on the epidemiology of currently circulating orthopoxviruses, a false positive result for the MPXV target (monkeypox virus clade II) due to cowpox or variola virus infection is unlikely. As with any clinical diagnostic test, results should be interpreted in the context of clinical evaluation and current epidemiology. The World Health Organization (WHO) declared

smallpox eradicated, therefore the possible variola virus cross-reactivity with the MPXV target 1 primers and probe do not pose any practical risk to the Xpert Mpox test.[10] If variola infection (smallpox) is suspected, the provider is advised to contact the local health department immediately for patient evaluation. The *in silico* analysis showed a two nucleotide deletion in the ectromelia genome sequences compared to the probe sequence of the MPXV target 2. This is predicted to be sufficient to prevent probe binding with the genome of ectromelia virus, avoiding detection of this virus. In silico exclusivity analysis results can be found in Table 7.

*In vitro* testing of vaccinia genomic DNA (gDNA) at a concentration of 1.00 x $10^6$ copies/mL showed no signal with MPXV targets while the OPXV target was positive, as expected.

**Table 7. Analytical Specificity of the Xpert Mpox Test**

| Species | Refseq Accession ID | MPXV (clade II) target I | | | MPXV (clade II) target II | | |
|---|---|---|---|---|---|---|---|
| | | Forward Primer | Probe | Reverse Primer | Forward Primer | Probe | Reverse Primer |
| Variola Virus (smallpox) | NC_001611.1 | 88% (22/25nt) | 92% (23/25nt) | 92% (23/25nt) | 84% (21/25nt) | 44% (11/25nt) | NSM |
| Herpes simplex virus (HSV-1) | NC_001806 | NSM | NSM | NSM | NSM | NSM | NSM |
| Herpes simplex virus (HSV-2) | LS480640.1 | NSM | NSM | NSM | NSM | NSM | NSM |
| Varicella-zoster virus (Chickenpox) | NC_001348.1 | NSM | NSM | NSM | NSM | NSM | NSM |
| *Staphylococcus aureus* | AP017922.1 | NSM | NSM | NSM | NSM | NSM | NSM |
| *Streptococcus pyogenes* | NZ_LS483338.1 | NSM | NSM | NSM | NSM | NSM | NSM |
| *Pseudomonas aeruginosa* | NC_002516.2 | NSM | NSM | NSM | NSM | NSM | NSM |
| *Corynebacterium jeikeium* | NC_007164.1 | NSM | NSM | NSM | NSM | NSM | NSM |
| *Escherichia coli* | NZ_CP077969.1 | NSM | NSM | NSM | NSM | NSM | NSM |
| *Bacteroides fragilis* | NZ_CP069563.1 | NSM | NSM | NSM | NSM | NSM | NSM |
| *Neisseria gonorrhoeae* | NZ_CP097846.1 | NSM | NSM | NSM | NSM | NSM | NSM |
| *Mycoplasma pneumoniae* | NZ_CP014267.1 | NSM | NSM | NSM | NSM | NSM | NSM |
| Human papilloma virus (HPV) | NC_027779.1 | NSM | NSM | NSM | NSM | NSM | NSM |
| *Treponema pallidum* | NC_016842.1 | NSM | NSM | NSM | NSM | NSM | NSM |
| Molluscum contagiosum virus | MN931752.1 | NSM | NSM | NSM | NSM | NSM | NSM |
| *Streptococcus mitis* | JVJJ01000007.1 | NSM | NSM | NSM | NSM | NSM | NSM |
| *Staphylococcus epidermidis* | JUKL01000001.1 | NSM | NSM | NSM | NSM | NSM | NSM |
| *Streptococcus agalactiae* | NZ_CP012480.1 | NSM | NSM | NSM | NSM | NSM | NSM |
| *Lactobacillus species (acidophilus)* | NZ_CP054559.1 | NSM | NSM | NSM | NSM | NSM | NSM |
| *Acinetobacter calcoaceticus* | NZ_CP020000.1 | NSM | NSM | NSM | NSM | NSM | NSM |

| Species | Refseq Accession ID | MPXV (clade II) target I | | | MPXV (clade II) target II | | |
|---|---|---|---|---|---|---|---|
| | | Forward Primer | Probe | Reverse Primer | Forward Primer | Probe | Reverse Primer |
| *Enterococcus faecalis* | NC_021023.1 | NSM | NSM | NSM | NSM | NSM | NSM |
| *Streptococcus* Group C and Group G[a] | AP012976.1 | NSM | NSM | NSM | NSM | NSM | NSM |
| *Corynebacterium diphtheriae* | NZ_CP025209.1 | NSM | NSM | NSM | NSM | NSM | NSM |
| *Chlamydia trachomatis* | NC_000117.1 | NSM | NSM | NSM | NSM | NSM | NSM |
| *Mycoplasma genitalium* | NC_000908.2 | NSM | NSM | NSM | NSM | NSM | NSM |
| Human genome[a] | Multiple accession numbers | 88% (22/25nt) chr 12 | NSM | 76% (19/25nt) | 84% (21/25nt) Chr 18 84% (21/25nt) Chr 6 80% (20/25nt) Chr 13 | 76% (19/25nt) Chr18 72% (18/25nt) Chr6 | 84% (21/25nt) Chr 5 80% (20/25nt) Chr 10 |
| *Candida albicans*[a] | Multiple accession numbers | NSM | NSM | NSM | NSM | NSM | 64% (16/25nt) |
| *Trichophyton rubrum*[a] | Multiple accession numbers | NSM | NSM | NSM | NSM | NSM | NSM |
| *Trichomonas vaginalis*[a] | Multiple accession numbers | 68% (17/25nt) | NSM | NSM | NSM | NSM | 88% (22/25nt) |
| Cowpox virus | NC_003663 | 100% (25/25nt) | 88% (22/25nt) | 92% (23/25nt) | 92% (23/25nt) | 76% (19/25) | 92% (23/25nt) |
| Camelpox virus | NC_003391 | NSM | 92% (23/25nt) | 92% (23/25nt) | NSM | 76% (19/25) | 88% (22/25nt) |
| Vaccinia virus | NC_006998 | 92% (23/25nt) | 92% (23/25nt) | 92% (23/25nt) | NSM | 76% (19/25) | 96% (24/25nt) |
| Ectromelia virus (mousepox) | NC_004105 | NSM | 88% (22/25nt) | NSM | 92% (23/25nt) | 80 (20/25nt) | 92% (23/25nt) |

[a] Multiple chromosome organisms

NSM: no significant match was reported by BLAST.

## 21.3 Analytical Reactivity (Inclusivity)

The analytical reactivities of the Xpert Mpox test MPXV and OPXV targets were evaluated *in silico* against sequences from multiple strains of monkeypox virus clade IIa and clade IIb, using comprehensive data sets from NCBI and GISAID (Table 8). According to this analysis, the two MPXV targets and the OPXV target that are present in the Xpert Mpox test are predicted to have 0 to 1 mismatch in the primer and probe sequences with all monkeypox clade II sequences analyzed. As the presence of no more than one mismatch in the primer and probe sequences is not expected to impact test performance, the Xpert Mpox test exhibited 100% inclusivity to monkeypox clade II sequences evaluated.

The analytical reactivity of the Xpert Mpox test OPXV target was evaluated against sequences from monkeypox, vaccinia, cowpox, camelpox and ectromelia viruses (Table 8). Based on the *in silico* analysis, some vaccinia, cowpox and camelpox virus strains have genomic sequences containing two or more mismatches with the OPXV target primers and probe (Table 9), and therefore may not be detected by the test.

**Table 8. Sequences Included in Inclusivity Analyses for MPXV and OPXV Targets**

| Virus | Source | Download Date | Number of unique, non-synthetic sequences analyzed |
|---|---|---|---|
| Monkeypox - clade I | GISAID | 10/15/2022 | 43 |
| Monkeypox - clade IIa | GISAID | 10/15/2022 | 2 |
| Monkeypox - clade IIb | GISAID | 10/15/2022 | 3,057 |
| Monkeypox -unassigned | NCBI | 10/15/2022 | 2,552 |
| Vaccinia | NCBI | 10/15/2022 | 87 |
| Cowpox | NCBI | 10/15/2022 | 9,287 |
| Camelpox | NCBI | 10/15/2022 | 992 |
| Ectromelia | NCBI | 10/15/2022 | 129 |

**Table 9. Percent of Organism-Specific Sequences with Two or More Mismatches with OPXV Target Primers and Probes**

| Organism | Oligonucleotide | % of sequences with 2 or more mismatches |
|---|---|---|
| Vaccinia virus | OPXV probe | 1.2% (1/86) |
| | OPXV reverse primer | 1.2% (1/86) |
| Cowpox virus[a] | OPXV probe | 56.5% (52/92) |
| | OPXV reverse primer | 7.6% (7/92) |
| Camelpox virus[a] | OPXV probe | 100.0% (9/9) |

[a] Cowpox and Camelpox viruses are not currently circulating in humans; further these viruses are not known to transmit from human-to-human.

Only the primers and probes with two or more mismatches listed.

## 21.4 Interfering Substances Study

The assay uses a well-established nucleic acid extraction method; therefore, interference from common endogenous substances is not expected. This is further supported by the results of an Interfering Substances study in which potentially interfering substances that may be present in a skin swab were evaluated for potential to impact the performance of the Xpert Mpox test. Positive and negative samples were prepared in pooled negative matrix (PNM), which is made of negative human buccal swab material in UTM, and tested in the presence of potentially interfering substances. Negative matrix samples (n=3) were tested to determine the impact of such substances in the performance of the sample processing control (SPC) and sample adequacy control (SAC). In addition, positive samples, containing monkeypox virus at 3X LoD (n = 3), were tested to determine the impact of such substances in the detection of monkeypox virus. The evaluated substances are listed in Table 10, with active ingredients and concentrations tested shown. None of the substances tested were found to impact performance of the test at the concentrations tested in study.

**Table 10. Potentially Interfering Substances Tested in the Xpert Mpox Test**

| Substance/Class | Description/Active Ingredient | Concentration Tested |
|---|---|---|
| Control | Pooled negative matrix | 100% (v/v) |
| Abrevea | Behenyl alcohol | 7% (w/v) |
| Acyclovir | Antiviral medication | 7 mg/mL |
| Albumin | Protein | ≥ 2.2 mg/mL |
| Blood/EDTA | Blood (human) | 5% (v/v) |
| Mucin | Purified Mucin protein (Bovine or porcine submaxillary gland) | 60 µg/mL |
| Hydrocortisone cream | Cortisol hormone | 5% (w/v) |
| Benadryl cream/ointment | Diphenhydramine (antihistamine) | 5% (w/v) |
| Carmex | Camphor, white petrolatum | 7% (w/v) |
| Casein | Milk protein | 7 mg/mL |
| Lanacane | Benzocaine | 3.5% (w/v) |
| KY Jelly | Glycerol (glycerine) and Hydroxyethylcellulose | 7% (w/v) |
| Douche | Benzalkonium chloride | 7% (w/v) |
| Neosporin | Bacitracin, neomycin, and polymixin b | 5% (w/v) |
| Female urine | Female urine, human | 10% (v/v) |
| Male urine | Male urine, human | 10% (v/v) |
| Feces | Feces, human | 0.22% (w/v) |
| Seminal fluid | Seminal fluid, human | 7% (v/v) |
| Zinc Oxide ointment | Zinc oxide | 7% (w/v) |
| Vagisil cream | Benzocaine and resorcinol | 1% (w/v) |
| Cornstarch | Cornstarch | 2.5 mg/mL |

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**9.** Occupational Safety and Health Standards, Hazard Communication, Toxic and Hazard Substances (March 26, 2012) (29 C.F.R., pt. 1910, subpt. Z).

**10.** World Health Organization. Smallpox Overview. https://www.who.int/health-topics/smallpox#tab=tab_1. Accessed January 15, 2023

# 23 Cepheid Headquarters Location

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# 24 Technical Assistance

### Before Contacting Us

Before contacting Cepheid Technical Support, collect the following information:

- Product name
- Lot number
- Serial number of the instrument
- Error messages (if any)
- Software version and, if applicable, Computer Service Tag Number

### United States Technical Support

Telephone: + 1 888 838 3222
Email: techsupport@cepheid.com

# 25 Table of Symbols

| Symbol | Meaning |
|---|---|
|  | Catalog number |
|  | *In vitro* diagnostic medical device |
|  | Do not reuse |
|  | Batch code |
|  | Consult instructions for use |
|  | Manufacturer |
|  | Country of manufacture |
|  | Contains sufficient for *n* tests |
|  | Control |

| Symbol | Meaning |
|---|---|
|  | Expiration date |
|  | Temperature limitation |
|  | Biological risks |
|  | Caution |
|  | Warning |
|  | For prescription use only |

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IVD

**For use under Emergency Use Authorization (EUA) Only**

# 26 Revision History

**Description of Changes**: Initial Release of 302-9629 Rev A