Xpert® Mpox EUA*

Verification Procedure Guide

Disclaimer: This example of a verification procedure was developed by Cepheid Medical/Scientific Affairs to provide assistance to customers who are performing verification studies for the Xpert Mpox test. The guide content has been developed to meet ISO 15189 requirements but may be modified or expanded to meet institution or local requirements. Governing bodies such as regulatory agencies, and national or local organizations may require test method verification but often do not describe specific elements of the testing procedure. It is up to the Laboratory Director or designee to establish an appropriate procedure to fulfill requirements. This includes establishing sample size, the number of replicates, the number of days of testing, and the number of operators performing the testing. Review of regulatory requirements, laboratory accepted standards, and manufacturer's guidelines, such as the test procedure described in this document, are useful resources for creating a custom verification procedure that meets the needs of the laboratory.

1 Objective

The objective of this example procedure is to facilitate verification studies of the Xpert Mpox test during the introduction of the test into the laboratory. This example of a verification procedure describes precision and accuracy testing, using either known clinical specimens or monkeypox virus controls from Zeptometrix® (refer to instructions for use for more information). Accuracy and precision testing may be performed using the same samples. It is the responsibility of the Laboratory Director or designee to determine the optimal test method verification procedure for adherence to their specific laboratory accreditation rules or national and local laws. This includes establishing sample size, the number of replicates, the number of days of testing, and the number of operators performing the testing.

2 External Control Options Available for Verification Studies

The following control materials are available from ZeptoMetrix® (Buffalo, NY):

- ZeptoMetrix External Positive Control, Catalog# NATMPXVPOS-6C
- ZeptoMetrix External Negative Control, Catalog# NATMPXVNEG-6C

3 Biosafety Information and Precautions

3.1. The ZeptoMetrix control materials are formulated with purified, intact viral particles (positive control) and human A549 cells (negative control). The virus particles have been chemically modified to render them non-infectious and refrigerator stable. However, the reference material should be handled as infectious materials using standard precautions and in accordance with good laboratory practices to avoid personal exposure and contamination of laboratory equipment and reagents that could cause false positive results.

3.2. It is expected that all patient samples and controls will be handled using personal protective equipment (PPE) including, but not limited to, eye protection, a lab coat, and disposable gloves. Hands should be washed thoroughly after removing PPE.

3.3. To avoid sample-to-sample contamination, change gloves after loading each cartridge and before touching the next sample.

3.4. Store verification materials at appropriate temperatures per the manufacturer's storage requirements.

IVD. *In Vitro* Diagnostic Medical Device. May not be available in all countries.

* For use under an Emergency Use Authorization in the United States

4 Testing

The Laboratory Director or designee creates a testing plan, i.e., instructions, for the operators to execute that will confirm the accuracy and precision of the test. Accuracy is described as the ability of a test to consistently produce the correct result. Precision describes the ability of a test to consistently produce the correct result repeatedly. Accuracy and precision can be confirmed at the same time in the verification procedure. Accuracy is determined by testing of the material to see if the expected result is obtained. To confirm precision/repeatability, multiple tests of the same sample are performed. This is normally done by testing a sample in duplicate or triplicate. Precision is determined for each sample type, Positive and Negative. A section of the testing plan may also include directions for determining inter-operator variation. An example test plan outline is provided below. A sample results table form is provided below for use in documenting the test results.

Testing of external controls and samples for verification studies should be performed according to the test's Instructions for Use.

5 Results Reporting & Interpretation

Note: Depending on the test system and the laboratory's testing volume, the actual number of specimens needed for the verification study may vary. The Laboratory Director or designee is responsible for determining the appropriate number of samples to ensure confidence in the performance of the new test. Increasing the sample number improves the quality of the data, although limitations in laboratory resources sometimes limit the ability to use larger sample sizes.

5.1. Overall acceptable agreement is achieved when the expected result and the operator's results are identical.

5.2 It is the responsibility of the Laboratory Director or designee to evaluate the results and determine what percentage of agreement constitutes an acceptable verification of test performance. Agreement in the range of 95% or higher is generally considered to be appropriate.

5.3 When a sample result does not agree with the expected result, repeat the test. Provide a comment in the area provided below the results table, noting that repeat testing occurred.

5.4 If the sample repeat does not agree with the expected result, investigate and determine root cause. Document the investigation in the area provided below the results table.

5.5 If agreement issues continue, consult with the Laboratory Director or designee before proceeding.

6 References

6.1. Medical laboratories — Requirements for Quality and Competence ISO 15189:2012. Accessed February 2023. https://www.iso.org/fr/standard/56115.html

6.2 CAP Commission on Laboratory Accreditation, All Common Checklist. 2021. https://documents.cap.org/documents/cap-laboratory-accreditation-checklist-order-form.pdf. Accessed February 2023.

6.3 Cepheid Xpert Mpox Instructions for Use. (EUA) 302-9629 and 301-9630.

- GxDx/Infinity systems 302-9629
- Xpert® **Xpress** systems 301-9630

Example Test Plan

Verification of the Cepheid Xpert® Mpox Test

Date of Plan: _____

Laboratory Director or Designee: _____

Description:

This testing is being performed to confirm the accuracy and precision of the Cepheid Xpert Mpox test before introduction into the laboratory.

Details of the Procedure:

1. There will be ____ (*enter number determined here*) samples tested
2. ____ (*enter number determined here*) samples will also be tested in ____ (*enter duplicate or triplicate or other number of repeats here*).
3. The testing will be performed over ____ days by ____ operators
4. Materials and Equipment Needed:
    a. (___) Cepheid GeneXpert® System(s)
    b. (___) Xpert Mpox cartridges
    c. (___) External Controls (*list type and number needed*)
    d. 3 ml VTM/UTM tubes for dilution of positive control material (if using Zeptometrix product)
5. External Quality Control Materials:

    ZeptoMetrix External Positive Control, Catalog# NATMPXVPOS-6C
    ZeptoMetrix External Negative Control, Catalog# NATMPXVNEG-6C

    Quality Control testing should be performed prior to initiating verification studies.

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

    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 with a new vial.

    If the expected results for the external control materials are not obtained upon repeat, contact Cepheid Technical Support.
6. Results Reporting
    a. Results are reported by the operator on the (*title of report form here*). Results are evaluated and approved by the Laboratory Director or designee before the new test is introduced.

Results Reporting Day 1

Day 1 Quality Control Results

	MPXV Clade II Detected	Non-variola OPXV NOT DETECTED	PASS/FAIL
ZeptoMetrix External Positive Control			
ZeptoMetrix External Negative Control			

Example Results Table

	EXPECTED RESULT FOR SAMPLE	OPERATOR 1 RESULT	OPERATOR 2 (*OPTIONAL*) RESULT	AGREEMENT? (Y/N)	RESULT (Pass/Fail)
DAY 1					
Sample 1					
Sample 1 *retested for repeatability*					
Sample 2					
Sample 2 *retested for repeatability*					
Sample 3					
Sample 4					
Sample 5					
Sample 6					
Sample 7					
Sample 8					
Sample 9					
Sample 10					
Sample 11					
Sample 12					
Sample 13					
Sample 14					
Sample 15					
Sample 16					
Sample 17					
Sample 18					
Sample 19					
Sample 20					
Any additional samples as determined by Lab Director, add below					

Results Reporting Day 2
(Optional —as requested by Laboratory Director or designee)

Day 2 Quality Control Results

	MPXV Clade II Detected	Non-variola OPXV NOT DETECTED	PASS/FAIL
ZeptoMetrix External Positive Control			
ZeptoMetrix External Negative Control			

Example Results Table

	EXPECTED RESULT FOR SAMPLE	OPERATOR 1 RESULT	OPERATOR 2 (*OPTIONAL*) RESULT	AGREEMENT? (Y/N)	RESULT (Pass/Fail)
DAY 2 (Laboratory Director or designee's decision)					
Sample 1					
Sample 1 *retested for repeatability*					
Sample 2					
Sample 2 *retested for repeatability*					
Sample 3					
Sample 4					
Sample 5					
Sample 6					
Sample 7					
Sample 8					
Sample 9					
Sample 10					
Sample 11					
Sample 12					
Sample 13					
Sample 14					
Sample 15					
Sample 16					
Sample 17					
Sample 18					
Sample 19					
Sample 20					
Any additional samples as determined by Lab Director, add below					

Were there any unexpected results or failures during testing? If so, describe below:

Can the root cause of the failure(s) be determined?

Was retesting performed? If so describe results here:

Accuracy confirmed? Yes ☐ No ☐

Accuracy = The expected result was obtained for all samples tested

Precision confirmed? Yes ☐ No ☐

Precision = Repeated measurements of the same verification material sample result in the same value

Comments:

Performance Verification Approved by Laboratory Director or Designee:

I have reviewed the verification data for accuracy and precision for the Cepheid Xpert Mpox and the performance of the method is considered acceptable for patient testing.

Signature *Title* *Date*

Retain this record per regulatory and/or laboratory guidelines.

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