Rx Only

# cobas® MPXV

## Qualitative assay for use on the cobas® 6800/8800 Systems

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

For in vitro diagnostic use

| | |
|---|---|
| **cobas®** MPXV | P/N: 09863338190 |
| **cobas®** MPXV Control Kit | P/N: 09863320190 |
| **cobas®** Buffer Negative Control Kit | P/N: 07002238190 |
| | P/N: 09051953190 |

# Table of contents

# Intended use

**cobas**® MPXV for use on the **cobas**® 6800/8800 Systems (**cobas**® MPXV) is a real-time PCR assay for the qualitative detection of DNA from Monkeypox virus (MPXV, clade I/II) in human lesion swab specimens (i.e., swabs of acute pustular or vesicular rash) from individuals suspected of monkeypox infection by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42.U.S.C. §263a, that meet requirements to perform moderate or high complexity tests.

Results are for the identification of monkeypox virus (clade I/II) DNA, which is 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 I/II) 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 I/II) 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. **cobas**® MPXV is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and on the use of the **cobas**® 6800/8800 Systems.

**cobas**® MPXV is only for use under the Food and Drug Administration's Emergency Use Authorization.

# Summary and explanation of the test

### Explanation of the test

**cobas**® MPXV is a qualitative nucleic acid test for use on the **cobas**® 6800 System and **cobas**® 8800 System for the detection of Monkeypox virus (MPXV) nucleic acids in individual lesion swab samples collected in Copan Universal Transport Medium System (UTM) or BD™ Universal Viral Transport System (UVT). The test utilizes human β-globin DNA as an endogenous control to assess specimen adequacy. The DNA Internal Control, used to monitor the entire sample preparation and PCR amplification process, is introduced into each specimen during sample processing. In addition, the test utilizes external controls (a low titer positive control and a negative control).

### Principles of the procedure

**cobas**® MPXV is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The **cobas**® 6800/8800 Systems consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the **cobas**® 6800/8800 System software, which assigns test results for all tests. Results can be reviewed directly on the system screen, and printed as a report.

Nucleic acid from patient samples and added Internal Control DNA (DNA IC) molecules are simultaneously extracted. Nucleic acid is released by addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured protein, cellular debris and potential PCR inhibitors, are removed with subsequent wash steps and purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature. External controls (positive and negative) are processed in the same way.

Selective amplification of target nucleic acid from the sample is achieved by the use of three sets of forward and reverse primers targeting the MPXV F3L gene, the MPXV B21R/B22R gene, and the human β-globin gene, which were selected to hybridize to highly conserved regions of the genomic nucleic acid. Selective amplification of DNA Internal Control is achieved by the use of non-competitive sequence specific forward and reverse primers which have no homology with the Monkeypox virus or human genomes. A thermostable DNA polymerase enzyme is used for both reverse-transcription and amplification.

The **cobas**® MPXV master mix contains detection probes which are specific for MPXV (targeting MPXV genes F3L and B21R/B22R, labeled with the same fluorescent dye), the human β-globin gene and Internal Control nucleic acid. The MPXV, β-globin and Internal Control detection probes are each labeled with unique fluorescent dyes that act as a reporter. Each probe also has a second dye which acts as a quencher. When not bound to the target sequence, the fluorescent signals of the intact probes are suppressed by the quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by the 5' to 3' exonuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye increases concomitantly. Each reporter dye is measured at defined wavelengths, which enables simultaneous detection and discrimination of the amplified MPXV targets, β-globin and the DNA Internal Control. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythimidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon). Any contaminating amplicons from previous PCR runs are destroyed by the AmpErase enzyme [uracil-N-glycosylase], which is included in the PCR mix, when heated in the first thermal cycling step. However, newly formed amplicons are not destroyed since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

# Reagents and materials

The materials provided for **cobas**® MPXV can be found in Table 1 and Table 2. Materials required, but not provided can be found in Table 3, Table 4, Table 7 and Table 8.

Refer to this section and the **Precautions and handling requirements** section for the hazard information for the product.

All unopened reagents and controls shall be stored as recommended in Table 5.

## cobas® MPXV reagents and controls

**Table 1 cobas**® MPXV

Store at 2-8°C
192 test cassette (P/N 09863338190)

| Kit components | Reagent ingredients | Quantity per kit 192 tests |
|---|---|---|
| **Proteinase Solution (PASE)** | Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% proteinase, glycerol<br>EUH210: Safety data sheet available on request.<br>EUH208: Contains Subtilisin from Bacillus subtilis. May produce an allergic reaction. | 22.3 mL |
| **DNA Internal Control (DNA IC)** | Tris buffer, < 0.05% EDTA, < 0.001% non-MPXV related armored DNA construct containing primer and probe specific primer sequence regions (non-infectious DNA in MS2 bacteriophage), < 0.1% sodium azide | 21.2 mL |
| **Elution Buffer (EB)** | Tris buffer, 0.2% methyl-4 hydroxybenzoate | 21.2 mL |
| **Master Mix Reagent 1 (MMX-R1)** | Manganese acetate, potassium hydroxide, < 0.1% sodium azide | 7.5 mL |
| **MPXV Master Mix Reagent 2 (MPXV MMX-R2)** | Tricine buffer, potassium acetate, < 18% dimethyl sulfoxide, glycerol, < 0.1% Tween 20, EDTA, < 0.12% dATP, dCTP, dGTP, dUTPs, < 0.01% upstream and downstream MPXV primers, < 0.01% upstream and downstream β-globin primers, < 0.01% Internal Control forward and reverse primers, < 0.01% fluorescent-labeled oligonucleotide probes specific for MPXV, β-globin and DNA Internal Control, < 0.01% oligonucleotide aptamer, < 0.1% Z05D DNA polymerase, < 0.10% AmpErase (uracil-N-glycosylase) enzyme (microbial), < 0.1% sodium azide | 9.7 mL |

**Table 2 cobas®** MPXV Control Kit

Store at 2–8°C
(P/N 09863320190)

| Kit components | Reagent ingredients | Quantity per kit |
|---|---|---|
| **MPXV Positive Control (MPXV (+)C)** | Tris buffer, < 0.05% Sodium azide, < 0.05% EDTA, < 0.003% Poly rA, < 0.01% Non-infectious plasmid DNA (microbial) containing MPXV sequence and β-globin sequence | 16 mL (16 x 1 mL) |

**Table 3 cobas®** Buffer Negative Control Kit

Store at 2-8°C
(P/N 07002238190 or 09051953190)

| Kit components | Reagent ingredients | Quantity per kit |
|---|---|---|
| **cobas® Buffer Negative Control (BUF (-) C)** | Tris buffer, < 0.1% sodium azide, EDTA, < 0.002% Poly rA RNA (synthetic) | 16 mL (16 x 1 mL) |

# cobas omni reagents for sample preparation

**Table 4 cobas omni** reagents for sample preparation

| Reagents | Reagent ingredients | Quantity per kit | Safety symbol and warning[a] |
|---|---|---|---|
| **cobas omni MGP Reagent (MGP)**<br>Store at 2–8°C<br>(P/N 06997546190) | Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide | 480 tests | Not applicable |
| **cobas omni Specimen Diluent (SPEC DIL)**<br>Store at 2–8°C<br>(P/N 06997511190) | Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide | 4 x 875 mL | Not applicable |
| **cobas omni Lysis Reagent (LYS)**<br>Store at 2–8°C<br>(P/N 06997538190) | 43% (w/w) guanidine thiocyanate[b], 5% (w/v) polydocanol[b], 2% (w/v) dithiothreitol[b], dihydro sodium citrate | 4 x 875 mL |  <br>**DANGER**<br>H302: Harmful if swallowed.<br>H314: Causes severe skin burns and eye damage.<br>H411: Toxic to aquatic life with long lasting effects.<br>EUH032: Contact with acids liberates very toxic gas.<br>EUH071: Corrosive to the respiratory tract<br>P273: Avoid release to the environment.<br>P280: Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection.<br>P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water.<br>P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor.<br>P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor.<br>P391 Collect spillage.<br>593-84-0 Guanidinium thiocyanate<br>9002-92-0 Polidocanol<br>3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol |
| **cobas omni Wash Reagent (WASH)**<br>Store at 15–30°C<br>(P/N 06997503190) | Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate | 4.2 L | Not applicable |

[a] Product safety labeling primarily follows EU GHS guidance

[b] Hazardous substance

## Reagent storage and handling requirements

Reagents shall be stored and will be handled as specified in Table 5 and Table 6.

When reagents are not loaded on the **cobas**® 6800/8800 Systems, store them at the corresponding temperature specified in Table 5.

**Table 5** Reagent storage (when reagent is not on the system)

| Reagent | Storage temperature |
|---|---|
| **cobas**® MPXV | 2–8°C |
| **cobas**® MPXV Control Kit | 2–8°C |
| **cobas**® Buffer Negative Control Kit | 2–8°C |
| **cobas omni** Lysis Reagent | 2–8°C |
| **cobas omni** MGP Reagent | 2–8°C |
| **cobas omni** Specimen Diluent | 2–8°C |
| **cobas omni** Wash Reagent | 15–30°C |

Reagents loaded onto the **cobas**® 6800/8800 Systems are stored at appropriate temperatures and their expiration is monitored by the system. The **cobas**® 6800/8800 Systems allow reagents to be used only if all of the conditions shown in Table 6 are met. The system automatically prevents use of expired reagents. Table 6 allows the user to understand the reagent handling conditions enforced by the **cobas**® 6800/8800 Systems.

**Table 6** Reagent expiry conditions enforced by the **cobas**® 6800/8800 Systems

| Reagent | Kit expiration date | Open-kit stability | Number of runs for which this kit can be used | On-board stability (cumulative time on board outside refrigerator) |
|---|---|---|---|---|
| **cobas**® MPXV | Date not passed[a] | 90 days from first usage[a] | Max 40 runs[a] | Max 40 hours[a] |
| **cobas**® MPXV Control Kit | Date not passed[a] | Not applicable[b] | Not applicable | Max 8 hours[a] |
| **cobas**® Buffer Negative Control Kit | Date not passed | Not applicable[b] | Not applicable | Max 10 hours |
| **cobas omni** Lysis Reagent | Date not passed | 30 days from loading[c] | Not applicable | Not applicable |
| **cobas omni** MGP Reagent | Date not passed | 30 days from loading[c] | Not applicable | Not applicable |
| **cobas omni** Specimen Diluent | Date not passed | 30 days from loading[c] | Not applicable | Not applicable |
| **cobas omni** Wash Reagent | Date not passed | 30 days from loading[c] | Not applicable | Not applicable |

[a] The MPXV performance has not been established for suggested use cycles and time, but is based on similar reagents used on the same system.

[b] Single use reagents

[c] Time is measured from the first time that reagent is loaded onto the **cobas**® 6800/8800 Systems.

# Additional materials required

**Table 7** Materials and consumables for use on **cobas**® 6800/8800 Systems

| Material | P/N |
|---|---|
| **cobas omni** Processing Plate | 05534917001 |
| **cobas omni** Amplification Plate | 05534941001 |
| **cobas omni** Pipette Tips | 05534925001 |
| **cobas omni** Liquid Waste Container | 07094388001 |
| **cobas omni** Lysis Reagent | 06997538190 |
| **cobas omni** MGP Reagent | 06997546190 |
| **cobas omni** Specimen Diluent | 06997511190 |
| **cobas omni** Wash Reagent | 06997503190 |
| Solid Waste Bag and Solid Waste Container<br>or<br>Solid Waste Bag With Insert and Kit Drawer | 07435967001 and 07094361001<br>or<br>08030073001 and 08387281001 |
| **cobas omni** Secondary Tubes 13x75 (optional) | 06438776001 |
| MPA RACK 13 MM NAVY BLUE 1301-1350* | 03066282001 |
| MPA RACK 16 MM LIGHT GREEN 7001-7050* | 03143449001 |
| RD5 RACK – RD Standard rack 0001-0050 LR* | 11902997001 |

* MPA and/or RD5 racks are required to use **cobas**® MPXV. Contact your local Roche representative for a detailed order list for sample racks, racks for clotted tips and rack trays accepted on the instruments.

## Instrumentation and software required

The **cobas**® 6800/8800 System software and **cobas**® MPXV analysis package must be installed on the **cobas**® 6800/8800 instrument(s). The Instrument Gateway (IG) server will be provided with the system.

**Table 8** Instrumentation

| Equipment | P/N |
|---|---|
| **cobas**® 6800 System (Option Moveable) | 05524245001 and 06379672001 |
| **cobas**® 6800 System (Fix) | 05524245001 and 06379664001 |
| **cobas**® 8800 System | 05412722001 |
| Sample Supply Module | 06301037001 |

For additional information, please refer to the **cobas**® 6800/8800 Systems – User Assistance and/or User Guide.

Note: Contact your local Roche representative for a detailed order list for sample racks, racks for clotted tips and rack trays accepted on the instruments.

# Precautions and handling requirements

## Warnings and precautions

As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.

- For in vitro diagnostic use under Emergency Use Authorization only.
- This product has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories; use by laboratories certified under CLIA to perform moderate or high complexity tests.
- This product has been authorized only for the detection of nucleic acid from monkeypox virus, not for any other viruses 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 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.
- Positive results are indicative of the presence of MPXV nucleic acids.
- Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.[1]
- All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.[2,3] Only personnel proficient in handling infectious materials and the use of **cobas**® MPXV and the **cobas**® 6800/8800 Systems should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.6% sodium or potassium hypochlorite in distilled or deionized water or follow appropriate site procedures.
- The use of sterile disposable pipettes and nuclease-free pipette tips is recommended. Use only supplied or specified required consumables to ensure optimal test performance.
- Safety Data Sheets (SDS) are available on request from your local Roche representative.
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.
- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.

## Reagent handling

- Handle all reagents, controls, and samples according to good laboratory practice in order to prevent carryover of samples or controls.
- Before use, visually inspect each reagent cassette, diluent, lysis reagent, and wash reagent to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.
- **cobas omni** Lysis Reagent contains guanidine thiocyanate, a potentially hazardous chemical. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur.
- **cobas**® MPXV test kits, **cobas**® MPXV Control Kit, **cobas**® Buffer Negative Control Kit, **cobas omni** MGP Reagent, and **cobas omni** Specimen Diluent contain sodium azide as a preservative. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry.
- Do not allow **cobas omni** Lysis Reagent, which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.
- Dispose of all materials that have come in contact with samples and reagents in accordance with country, state, and local regulations.

## Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink, or smoke in designated work areas.
- Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents. Gloves must be changed between handling samples and **cobas**® MPXV kits, **cobas**® MPXV Control kit, **cobas**® Buffer Negative Control kit and **cobas omni** reagents to prevent contamination. Avoid contaminating gloves when handling samples and controls.
- Wash hands thoroughly after handling samples and kit reagents, and after removing the gloves.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.6% sodium or potassium hypochlorite in distilled or deionized water. Follow by wiping the surface with 70% ethanol.
- If spills occur on the **cobas**® 6800/8800 instrument, follow the instructions in the **cobas**® 6800/8800 Systems – User Assistance and/or User Guide to properly clean and decontaminate the surface of instrument(s).

# Sample collection, transport, and storage

**Note: Handle all samples and controls as if they are capable of transmitting infectious agents.**

## Sample collection

- Collect lesion specimens according to standard collection technique using synthetic swabs and immediately place in 3 mL of Copan Universal Transport Medium (UTM) or BD™ Universal Viral Transport (UVT).
- Refer to the Instructions for Use of the Collection Devices for hazard information.

## Transport and storage

- Transportation of collected specimens must comply with all applicable regulations for the transport of etiologic agents.
- Transport and store samples collected in UTM or UVT as follows:
  - After collection, specimen can be stored for up to 8 hours at 2-25°C followed by up to 6 days at 2-8°C.

# Instructions for use

## Procedural notes

- Do not use **cobas**® MPXV, **cobas**® MPXV Control Kit, **cobas**® Buffer Negative Control Kit, or **cobas omni** reagents after their expiry dates.
- Do not reuse consumables. They are for one-time use only.
- Refer to the **cobas**® 6800/8800 Systems – User Assistance and/or User Guide for proper maintenance of instruments.

## Specimens collected in UTM or UVT

Specimens collected in UTM or UVT must be transferred into a secondary tube prior to processing on the **cobas**® 6800/8800 Systems. The **cobas omni** Secondary Tube is the preferred option. The UTM or UVT samples should be processed using the 'VTM' sample type selection in the user interface (UI) of the **cobas**® MPXV.

Performance of the test has only been established in lesion swabs collected in universal viral transport media tubes (Copan UTM). Copan Universal Transport Medium System (UTM) and BD™ Universal Viral Transport System (UVT) are equivalent.

***Always use caution when transferring specimens from a primary collection tube to a secondary tube.***

***Use pipettes with aerosol-barrier or positive-displacement tips to handle specimens.***

***Always use a new pipette tip for each specimen.***

***Ensure samples are equilibrated to room temperature prior to transfer into a cobas omni Secondary Tube.***

Follow the steps below to transfer patient sample from a primary collection tube into a **cobas omni** Secondary Tube:

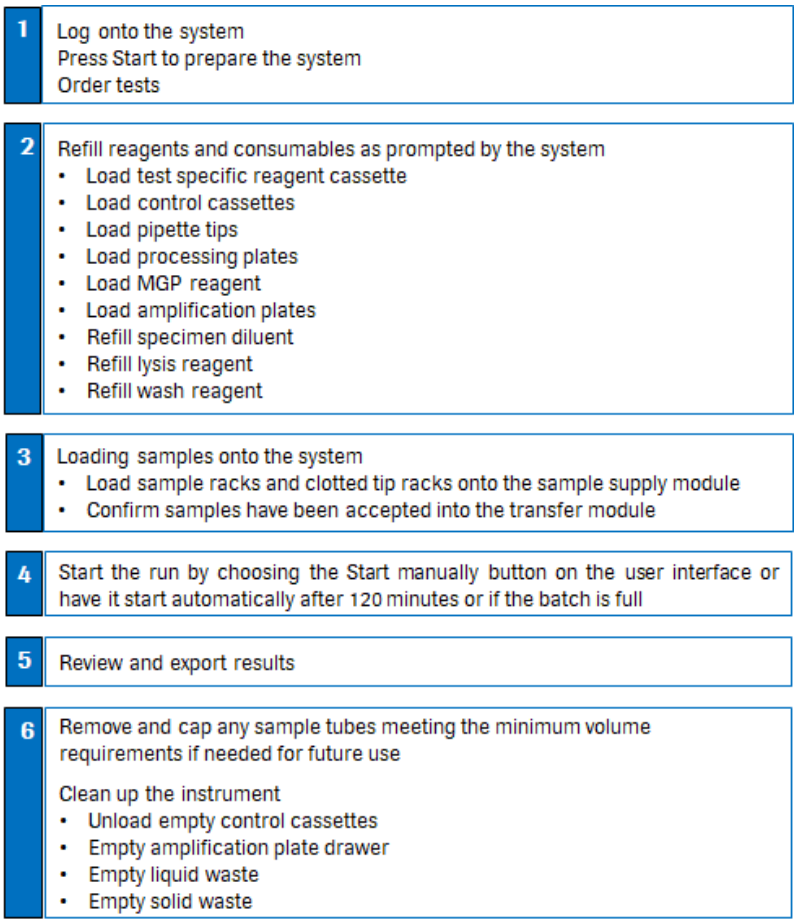
- Unscrew the primary sample tube cap.
- Lift the cap and any attached swab to allow a pipette to be inserted into the sample tube.
- Transfer 0.6 mL into the prepared barcoded secondary tube.
- Transfer secondary tube to a rack. Close the primary sample tube cap.

# Running cobas® MPXV on the cobas® 6800/8800 Systems

**cobas**® MPXV can be run with a minimum required sample volume of 0.6 mL in the **cobas omni** Secondary tube for specimens collected in UTM or UVT.

The test procedure is described in detail in the **cobas**® 6800/8800 Systems – User Assistance and/or User Guide. Figure 1 below summarizes the procedure.

**Figure 1** **cobas**® MPXV test procedure



# Results

The **cobas**® 6800/8800 Systems automatically detect MPXV DNA for each individually processed sample and control, displaying individual target results for samples as well as test validity and overall results for controls.

## Quality control and validity of results

- One negative control [(-) Ctrl] and one positive control [MPXV (+) C] are processed with each batch.
- In the **cobas**® 6800/8800 software and/or report, check for flags and their associated results to ensure the batch validity.
- All flags are described in the **cobas**® 6800/8800 Systems – User Assistance and/or User Guide.
- The batch is valid if no flags appear for any controls. If the batch is invalid, repeat testing of the entire batch.

Validation of batch results is performed automatically by the **cobas**® 6800/8800 System software based on negative and positive control performance. Validation of individual sample results is performed by the **cobas**® 6800/8800 Systems software based on Internal Control results .

## Interpretation of results

Display examples for **cobas**® MPXV are shown in Table 9.

**Table 9** Example of **cobas**® MPXV results display

| Test | Sample ID | Valid* | Flags | Sample type*** | Overall result* | Target 1 | Target 2** |
|---|---|---|---|---|---|---|---|
| MPXV | Sample_01 | NA | | VTM | NA | MPXV Negative | b-globin Negative |
| MPXV | Sample_02 | NA | Y40T | VTM | NA | Invalid | Invalid |
| MPXV | Sample_03 | NA | | VTM | NA | MPXV Negative | b-globin Positive |
| MPXV | Sample_04 | NA | | VTM | NA | MPXV Positive | b-globin Positive |
| MPXV | Sample_05 | NA | | VTM | NA | MPXV Positive | b-globin Negative |
| MPXV | Sample_06 | NA | C01H2 | VTM | NA | MPXV Positive | Invalid |
| MPXV | Sample_07 | NA | C01H1 | VTM | NA | Invalid | b-globin Positive |
| MPXV | C16142028411410110928 | Yes | | (-) Ctrl | Valid | Valid | Valid |
| MPXV | C16142028411380654036 | Yes | | MPXV (+) C | Valid | Valid | Valid |

* The "Valid" and "Overall Result" columns are not applicable to sample results for **cobas**® MPXV. Values reported in these columns are not applicable and do not impact the validity of results reported within the target result column.

** The test utilizes human β-globin DNA as an endogenous control to assess specimen adequacy.

*** "VTM" displayed under the "Sample type" column defines specific parameters for testing of swab specimens in UTM or UVT.

Refer to Table 10, **cobas**® MPXV results interpretation, for specific instructions on test results interpretation.

For a valid batch, check each individual sample for flags in the **cobas**® 6800/8800 software and/or report. The result interpretation should be as follows:

- A valid batch may include both valid and invalid sample results.
- The "Valid" and "Overall Result" columns are not applicable to sample results for **cobas**® MPXV and are marked with N/A. Values reported in these columns are not applicable and **do not** impact the validity of results reported within the target result column.
- Invalid results for one or more target combinations are possible and are reported out specifically for each channel.
- Reported target results for individual samples are valid unless indicated as "Invalid" within the target result column.
- Results of this test should only be interpreted in conjunction with information available from clinical evaluation of the patient and patient history.

Results and their corresponding interpretation for detecting MPXV are shown in Table 10.

**Table 10 cobas**® MPXV results interpretation

| Target 1 | Target 2 | Interpretation |
|---|---|---|
| **MPXV Positive** | **β-globin Positive** | MPXV Result is positive.<br>All Target Results are valid. Target signals detected for MPXV and β-globin (specimen adequacy control). |
| **MPXV Positive** | **β-globin Negative** | MPXV Result is positive.<br>All Target Results are valid.<br>Target signal detected for MPXV. Target signal not detected for β-globin (specimen adequacy control). |
| **MPXV Positive** | **β-globin Invalid** | MPXV Result is positive.<br>MPXV result is valid and signal detected.<br>β-globin (specimen adequacy control) result is invalid. |
| **MPXV Negative** | **β-globin Positive** | MPXV Result is negative.<br>All Target Results are valid.<br>Target signal not detected for MPXV. Target signal detected for β-globin (specimen adequacy control). |
| **MPXV Negative** | **β-globin Negative** | MPXV result is inconclusive.<br>All Target Results are valid.<br>Target signals not detected for MPXV and β-globin (specimen adequacy control), indicating a potential problem during sample acquisition. A new specimen should be obtained and tested to verify the MPXV result. |
| **MPXV Negative** | **β-globin Invalid** | MPXV result is inconclusive.<br>Target signal not detected for MPXV and invalid for β-globin (specimen adequacy control).<br>Original specimen should be re-tested to obtain a valid β-globin result. If the result remains invalid, and an instrument error can be excluded, a new specimen should be obtained and tested. |
| **MPXV Invalid** | **β-globin Positive** | MPXV result is invalid.<br>Original specimen should be re-tested to obtain a valid MPXV result. If the result remains invalid, and an instrument error can be excluded, a new specimen should be obtained and tested. |

| Target 1 | Target 2 | Interpretation |
|---|---|---|
| **MPXV Invalid** | **β-globin Negative** | MPXV result is invalid.<br>Target signal not detected for β-globin (specimen adequacy control), indicating a potential problem during sample acquisition.<br>Original specimen should be re-tested to obtain a valid MPXV result. If the result is still invalid and an instrument error can be excluded, a new specimen should be obtained and tested. |
| **MPXV Invalid** | **β-globin Invalid** | All target results are invalid.<br>Original specimen should be re-tested to obtain a valid MPXV result. If the result is still invalid and an instrument error can be excluded, a new specimen should be obtained and tested. |

## Procedural limitations

- **cobas**® MPXV has been evaluated only for use in combination with the **cobas**® MPXV Control Kit, **cobas**® Buffer Negative Control Kit, **cobas omni** MGP Reagent, **cobas omni** Lysis Reagent, **cobas omni** Specimen Diluent, and **cobas omni** Wash Reagent for use on the **cobas**® 6800/8800 Systems.
- **cobas**® MPXV has only been validated for use with specimen from lesions by using a synthetic swab which is placed into UTM or UVT. Assay 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 result.
- While monkeypox virus clade II is the only member of the Orthopoxvirus genus known to be circulating among humans in the US at this time, a positive result most likely represents the presence of monkeypox virus clade II, although there is a small possibility that this result could represent the presence of monkeypox virus clade I. If clinical concern for such an infection exists, healthcare providers should contact the CDC and their local public health authorities for guidance.
- A specimen with a result of "MPXV Negative" 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.
- Reliable results depend on proper sample collection, storage and handling procedures.
- β-globin amplification and detection is included in **cobas**® MPXV to differentiate valid MPXV-negative specimens from those that do not exhibit MPXV signal due to inadequate sample collection. All MPXV-negative specimens must have a positive β-globin result to be identified as valid negatives.
- Detection of MPXV DNA is dependent on the number of copies present in the specimen. Detection of MPXV 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.
- 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.
- As with any molecular test, mutations within the target regions of **cobas**® MPXV could affect primer and/or probe binding resulting in failure to detect the presence of virus.
- Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. One hundred percent agreement between the results should not be expected due to aforementioned differences between technologies. Users should follow their own specific policies/procedures.
- False negative or invalid results may occur due to interference. The Internal Control is included in **cobas**® MPXV to help identify the specimens containing substances that may interfere with nucleic acid isolation and PCR amplification. Interfering substances studies have not been performed for this assay. The assay uses conventional well-established nucleic acid extraction methods used for other similar assays. Interference from common endogenous substances is not anticipated.

- The addition of AmpErase enzyme into the **cobas**® MPXV Master Mix reagent enables selective amplification of target nucleic acids; however, good laboratory practices and careful adherence to the procedures specified in this Instructions For Use document are necessary to avoid contamination of reagents.

## Conditions of Authorization for the Laboratory

The **cobas**® MPXV 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 running **cobas**® MPXV, the relevant Conditions of Authorization are listed verbatim below, and are required to be met by laboratories performing the EUA test.

A. Authorized laboratories[1] that receive **cobas**® MPXV must notify the relevant public health authorities of their intent to run your product prior to initiating testing.
B. Authorized laboratories using **cobas**® MPXV must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
C. Authorized laboratories using **cobas**® MPXV 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.
D. Authorized laboratories using the **cobas**® MPXV must use **cobas**® MPXV 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 **cobas**® MPXV are not permitted.
E. Authorized laboratories must have a process in place to track adverse events and report to Roche Diagnostics US Customer Technical Support 1-800-526-1247 and to FDA pursuant to 21 CFR Part 803.
F. All laboratory personnel using the test must be appropriately trained in real-time PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the test in accordance with the authorized labeling.
G. RMS, its authorized distributor(s), and authorized laboratories must collect information on the performance of **cobas**® MPXV and must report any significant deviations from the established performance characteristics of **cobas**® MPXV of which they become aware to DMD/OHT7/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) In addition, authorized distributor(s) and authorized laboratories report to Roche Diagnostics US Customer Technical Support 1-800-526-1247.
H. RMS, its authorized distributor(s) and authorized laboratories using **cobas**® MPXV 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.

[1] Authorized laboratories are 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.

# Non-clinical performance evaluation

## Key performance characteristics

### Analytical sensitivity (Limit of Detection)

Limit of detection (LoD) studies determine the lowest detectable concentration of MPXV at which greater or equal to 95% of all (true positive) replicates test positive.

To determine the LoD, a heat-inactivated cultured virus representing clade IIb of an isolate from a Slovenian patient (Slovenia_MPXV-1_2022, lot number 005V-04714, 1.0E+06 $TCID_{50}$/mL before heat inactivation, assigned 5.8E+09 cp/mL with ddPCR) was serially diluted in pooled MPXV negative individual clinical specimen. A total of 7 concentration levels, with 2-fold serial dilutions between the levels, were tested with a total of 42 replicates per concentration, with an additional 42 replicates of a blank sample (pooled MPXV negative individual clinical specimen).

As shown in Table 11, the concentration level with observed hit rates greater than or equal to 95% was 57 cp/mL (0.01 $TCID_{50}$/mL). For all concentration levels, the β -globin target showed 100% positivity rate with a mean Ct 27.0. As shown in Table 12, the Probit predicted 95% hit rate was 36.5 cp/mL (6.4E-03 $TCID_{50}$/mL).

**Table 11** LoD determination

| Strain | Concentration [$TCID_{50}$/mL] | Concentration [cp/mL] | MPXV Hit rate [%] | MPXV Mean Ct* |
|---|---|---|---|---|
| Slovenia_MPXV-1_2022<br>lot number 005V-04714 | 4.00E-02 | 228.0 | 100.0 (42/42) | 33.8 |
| | 2.00E-02 | 114.0 | 100.0 (42/42) | 34.7 |
| | 1.00E-02 | 57.0 | 97.6 (41/42) | 35.9 |
| | 5.00E-03 | 28.5 | 92.9 (39/42) | 37.1 |
| | 2.50E-03 | 14.3 | 71.4 (30/42) | 37.4 |
| | 1.25E-03 | 7.1 | 33.3 (14/42) | 37.9 |
| | 6.25E-04 | 3.6 | 14.3 (6/42) | 37.9 |
| | 0.0 (blank) | 0.0 (blank) | 0.0 (0/42) | N/A |

**Table 12** Probit predicted 95% hit rate

| Strain | Probit Predicted 95% Hit Rate |
|---|---|
| Slovenia_MPXV-1_2022 | 36.5 cp/mL (6.4E-03 $TCID_{50}$/mL)<br>(95% CI in cp/mL: 27.4 – 54.2)<br>(95% CI in $TCID_{50}$/mL: 4.8E-03 – 9.5E-03) |

## Reactivity/Inclusivity

*In silico* analysis of the MPXV primer and probe binding regions in clade I, clade IIa and clade IIb MPXV genomes reported in NCBI and GISAID predicts that **cobas**® MPXV detects all analyzed MPXV isolates.

NCBI and GISAID repositories were last accessed on October 3rd, 2022 and included 31 or 33 clade I MPXV isolates and 927 or 1943 clade II MPXV isolates for NCBI or GISAID, respectively.

## Cross-reactivity

### In silico analysis

Potential cross-reactivity was assessed in silico by calculating the % homology of the genomic sequence of organisms listed in Table 13 to the primers and probes in **cobas**® MPXV. The organisms listed in Table 13 are predicted to not cross-react in **cobas**® MPXV.

**Table 13 Organisms assessed for in silico cross-reactivity analysis**

| | |
|---|---|
| *Acinetobacter calcoaceticus* (CA16) | *Lactobacillus jensenii* (DZD_CM_38_S806-bin_1) |
| *Acinetobacter johnsonii* (ANC 3681) | *Lactobacillus vaginalis* (LV515) |
| *Bacteroides fragilis* (FDAARGOS_1225) | *Micrococcus luteus* (CW.Ay) |
| Camelpox virus (M-96 from Kazakhstan) | Molluscum contagiosum virus Subtype 1 |
| *Candida albicans* (SC5314) | *Mycoplasma genitalium* (G37) |
| *Chlamydia trachomatis* (D/UW-3/CX) | *Mycoplasma pneumoniae* (NCTC10119) |
| *Corynebacterium diphteriae* (ISS 3319) | *Neisseria gonorrhoeae* (TUM19854) |
| *Corynebacterium jeikeium* (K411) | *Pseudomonas aeruginosa* (PAO1) |
| *Corynebacterium striatum* (FDAARGOS_1115) | *Staphylococcus aureus Subsp. aureus* (NCTC 8325) |
| Cowpox virus (Brighton Red) | *Staphylococcus epidermidis* (ATCC 14990) |
| Coxsackievirus A16 (12C10) | *Streptococcus agalactiae* (NGBS128) |
| *Cutibacterium acnes* (HL096PA1) | *Streptococcus mitis Streptococcus mitis* (SK629) |
| Ectromelia (mousepox) virus (Moscow) | *Streptococcus pyogenes* (NCTC12064) |
| *Enterococcus faecalis* (EnGen0336 T5) | *Streptococcus* Group C (*Streptococcus dysgalactiae* subsp. *equisimilis* 167) |
| *Escherichia coli* (K-12 substrain MG1655) | *Streptococcus* Group G (*Streptococcus dysgalactiae* subsp. *equisimilis* RE378) |
| *Haemophilus ducreyi* (VAN2) | *Treponema pallidum Subsp. pertenue* (SamoaD) |
| Herpes simplex virus 1 (17) | *Trichomonas vaginalis* (G3) |
| Herpes simplex virus 2 (HG52) | *Trichophyton rubrum* (CBS 118892) |
| Human herpesvirus 6A (Isolate U1102) | Vaccinia virus (Western Reserve) |
| Human herpesvirus 6B (Z29) | Varicella-zoster virus (Dumas) |
| Human papilloma virus (Isolate SE379) | Variola virus (India-1967, ssp. major) |
| *Lactobacillus acidophilus* (La-14) | - |

Analyzed reference strains detailed in brackets.

# Clinical performance evaluation

## Clinical performance with clinical samples

The performance of **cobas**® MPXV was evaluated at one external site using lesion swab specimens from patients suspected of Monkeypox infection by their healthcare provider. The study utilized fresh leftover de-identified specimens from routine clinical testing transported in Copan UTM between October 12, 2022 and October 18, 2022. Standard of care results from an FDA cleared method were used to identify 47 individual negative clinical samples and 30 individual positive clinical samples for evaluation with **cobas**® MPXV. The first 30 positive and first 30 negative specimens characterized by standard of care testing with an FDA-cleared comparator real-time PCR assay were selected for testing with cobas MPXV. An additional 17 standard of care negatives were also tested to ensure that that all test runs included both positive and negative specimens. Samples were stored refrigerated prior to testing with **cobas**® MPXV and were compared to the original results from the cleared method. As shown in Table 14, all samples tested negative with the comparator method were negative with **cobas**® MPXV and all samples tested positive with the comparator method were positive with **cobas**® MPXV.

Five additional positive specimens were identified and used to prepare contrived low positive specimens based on the comparator method. Specimens were serially diluted into unique negative lesion specimens and re-tested on the comparator method to identify contrived specimens with 33 < Ct < 37. The 5 contrived low positive specimens, along with the one naturally-occurring low positive (comparator Ct 35.44) were all positive by cobas® MPXV with a mean Ct of 29.7.

**Table 14** Clinical evaluation with neat lesion swab samples

| | | FDA cleared method | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| **cobas® MPXV** | **Positive** | 30 | 0 | 30 |
| | **Negative** | 0 | 47 | 47 |
| | **Total** | 30 | 47 | 77 |

| Percent Agreement | Result (%) | 95% Confidence Interval (%) |
|---|---|---|
| **PPA** | 100 (30/30) | 88.7 – 100.0 |
| **NPA** | 100 (47/47) | 92.4 – 100.0 |

# Additional information

## Key test features

| | |
|---|---|
| **Sample type** | Lesion swab samples collected in Copan UTM® System and BD™ UVT System |
| **Minimum amount of sample required** | 0.6 mL* |
| **Sample processing volume** | 0.4 mL |
| **Test duration** | Results are available within less than 3.5 hours after loading the sample on the system. |

* Dead volume of 0.2 mL is identified for the **cobas omni** Secondary tubes. Other tubes compatible with **cobas**® 6800/8800 Systems (consult User Assistance Guide) may have different dead volume and require more or less minimum volume.

# Symbols

**The following symbols are used in labeling for Roche PCR diagnostic products.**

**Table 15** Symbols used in labeling for Roche PCR diagnostics products

| | | |
|---|---|---|
| Age/DOB Age or Date of Birth | Device not for near-patient testing | QS IU/PCR QS IU per PCR reaction, use the QS International Units (IU) per PCR reaction in calculation of the results. |
| SW Ancillary Software | Device not for self-testing | |
| Assigned Range [copies/mL] Assigned Range (copies/mL) | Distributor *(Note: The applicable country/region may be designated beneath the symbol)* | SN Serial number |
| Assigned Range [IU/mL] Assigned Range (IU/mL) | Do not re-use | Site Site |
| EC REP Authorized representative in the European Community | Female | Procedure Standard Standard Procedure |
| BARCODE Barcode Data Sheet | For IVD performance evaluation only | STERILE EO Sterilized using ethylene oxide |
| LOT Batch code | GTIN Global Trade Item Number | Store in dark |
| Biological risks | Importer | Temperature limit |
| REF Catalogue number | IVD In vitro diagnostic medical device | TDF Test Definition File |
| CE CE marking of conformity, this device is in conformity with the applicable requirements for CE marking of an in vitro diagnostic medical device | LLR Lower Limit of Assigned Range | This way up |
| | Male | Procedure UltraSensitive Ultrasensitive Procedure |
| Collect Date Collect date | Manufacturer | UDI Unique Device Identifier |
| Consult instructions for use | CONTROL − Negative control | ULR Upper Limit of Assigned Range |
| Σ Contains sufficient for <*n*> tests | NON STERILE Non-sterile | Urine Fill Line Urine Fill Line |
| CONTENT Content of kit | Patient Name | Rx only For USA: Caution: Federal law restricts this device to sale by or on the order of a physician. |
| CONTROL Control | Patient number | Use-by date |
| Date of manufacture | Peel here | |
| Device for near-patient testing | CONTROL + Positive control | |
| Device for self-testing | QS copies / PCR QS copies per PCR reaction, use the QS copies per PCR reaction in calculation of the results. | |

## Technical support

For technical support (assistance) please reach out to your local affiliate:
https://www.roche.com/about/business/roche_worldwide.htm

## Manufacturer and distributors

**Table 16** Manufacturer and distributors

Roche Molecular Systems, Inc.
1080 US Highway 202 South
Branchburg, NJ 08876, USA
www.roche.com

Made in USA

Distributed by

Roche Diagnostics
9115 Hague Road
Indianapolis, IN 46250-0457, USA
(For Technical Assistance call the
Roche Response Center
toll-free: 1-800-526-1247)

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany

## Trademarks and patents

See https://diagnostics.roche.com/us/en/about-us/patents

## Copyright



## References

1. Center for Disease Control and Prevention. How to Report Results from Orthopoxvirus, Non-Variola Orthopoxvirus, and Monkeypox Virus Diagnostic Testing (page last reviewed: September 23, 2022).
2. Center for Disease Control and Prevention. Biosafety in Microbiological and Biomedical Laboratories, 5th ed. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health HHS Publication No. (CDC) 21-1112, revised December 2009.
3. Clinical and Laboratory Standards Institute (CLSI). Protection of laboratory workers from occupationally acquired infections. Approved Guideline-Fourth Edition. CLSI Document M29-A4: Wayne, PA; CLSI, 2014.

# Document revision

| Document Revision Information | |
|---|---|
| Doc Rev. 2.0<br>09/2024 | Updated to current economic operators.<br>Please contact your local Roche Representative if you have any questions. |

# cobas® MPXV

MPXV

IVD

KIT / **Cassette** LOT

Rx Only

## For USA: Emergency Use Authorization Only

### cobas® 6800/8800

SW

**cobas®** 6800/8800 MPXV ASAP version 12.1.0 or higher
**cobas®** 6800/8800 System Software Version 1.4 or higher

website: http://e-labdoc.roche.com
Product No.: 09863338190
09864547001-01 Doc Rev. 1.0

Please contact your local Roche representative at 1-800-526-1247 if you require a printed copy free of charge or need technical support to access the package insert.

This product has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories; use by laboratories certified under CLIA to perform moderate or high complexity tests.

This product has been authorized only for the detection of nucleic acid from monkeypox virus, not for any other viruses 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 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.

Roche Molecular Systems, Inc.
1080 US Highway 202 South
Branchburg, NJ 08876 USA

09864555001-02