Mpox disease Emergency Use Listing Procedure (EUL) for IVDs

Product: Monkeypox Virus (MPXV) Fast Real Time PCR Kit

EUL Number: MPXV-13195-202-00
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 Monkeypox Virus (MPXV) Fast Real Time PCR Kit, with product codes JC70203-1NW-25T and JC70203-1NW-50T, Rest-of-World regulatory version, manufactured by Jiangsu Bioperfectus Technologies Co., Ltd, located at 3F, Bldg G19, N0.1 Medical City Avenue Taizhou City, Jiangsu Province, People's Republic of China, was listed as eligible for WHO procurement on 3 December 2025.

Intended use:

According to the claim of intended use from Jiangsu Bioperfectus Technologies Co., Ltd, *"The Monkeypox Virus (MPXV) Fast Real Time PCR Kit is an In Vitro Diagnostic (IVD) reagent applying on fluorescent PCR technology and aiming at qualitatively detect Monkeypox virus (mpox, clade I/II) specific genes: F3L gene and B7R gene from human lesion swab specimen from individuals suspected of monkeypox infection by their healthcare provider (i.e., hospitals and clincs).*
Human skin lesion swab specimen include the swab of skin exudate in Universal transport media (UTM).
The Monkeypox virus (clade I/II) DNA is generally detectable in human skin exudate specimen 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.

The product is intended for use on populations suspected to have Monkeypox virus (clade I/II) infection, as an aid in the diagnosis of Monkeypox virus infection by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR.
The product is capable of detection but not differentiation of Monkeypox virus clade I and clade II."

Validated specimen type:
Human skin lesion swab specimens using the Copan Regular Polyester Swab (CLASSIQSwabs 1U054S01) immediately placed in Copan Universal Transport Media (UTM Universal Transport Medium 3C059N).

Test kit contents:

Kit components	Quantity	Product code JC70203-1NW-25T	Product code JC70203-1NW-50T
PCR Reaction Mix	1	250 µL	500 µL
Monkeypox Virus Detection Mix	1	125 µL	250 µL
Positive control	1	500µL	500µL
Negative control	1	500µL	500µL

Items required but not provided:

Extraction reagents

Manufacturer	Nucleic Acid Isolation Kit	Cat. No.
Bioperfectus Technologies	Viral Nucleic Acid Rapid Extraction Kit (Magnetic Bead Method)	SDKF60101
	Viral Nucleic Acid Extraction Kit (Silica-Based Spin Column)	SDK60102
	Viral Nucleic Acid Extraction Kit (Magnetic Bead Method)	SDK60104
Qiagen	QIAamp DNA Mini Kit (50)	51304
	QIAamp DNA Mini Kit (250)	51306

Instrument and consumables

Validated real-time PCR systems:

Real-Time PCR System	Software
Bio-Rad CFX96 Touch	CFX Maestro 2.2
Applied Biosystems QuantStudio 5	QuantStudio Design & Analysis Software 1.2.0
Applied Biosystems 7500	7500 Software v2.3
Applied Biosystems ViiA 7	QuantStudio Software v1.3
QIAGEN Rotor-Gene Q	Rotor-Gene Q Software v2.3.5
Analytik Jena qTOWER³	qPCRsoft V3.1
Applied Biosystems QuantStudio 7	QuantStudioSoftware v1.3
Roche LightCycler 480 II	LightCycler 480 Software v1.5.0
Bioperfectus STC-96A/96A PLUS	SLAN 8.2.2

Validated Nucleic acid extraction systems:

Extraction System	Software
Bioperfectus SSNP-2000B (32 channels)	V1.0.0.0
Bioperfectus SSNP-3000A (64 channels)	V1.0.0.0
Bioperfectus SSNP-9600A (96 channels)	V1.0.0.0
Bioperfectus SMPE-960 (96 channels)	V1.0.5.0
Bioperfectus SAW-96 (96 channels)	V1.0.3.0
Bioperfectus SAW-48 (48 channels)	V1.0.2.0

- Vortex mixer
- Centrifuge
- Calibrated adjustable pipettes (10µL, 100µL, 200µL, 1000µL)
- Calibrated adjustable multi-channel pipette (5-50µL)
- 1.5 mL centrifuge tube shelf
- Magnetic grate for 1.5 mL centrifuge tube
- Specimen collection: Copan Regular Polyester Swab (CLASSIQSwabs 1U054S01)
- Specimen preservation fluid (Universal transfer medium):Copan UTM® Universal Transport Medium(UTM 3C059N)
- RNase-free Water
- 10% sodium hypochlorite or Pasteurized disinfectant
- Disposable particle-free gloves and operating gown
- Pipette tips with filter
- 1.5 mL centrifuge tube (No DNase/RNase)
- 0.2 mL PCR plate (Applied Biosystems)
- 0.2 mL PCR tube (Applied Biosystems)
- 8-well PCR tube strips or 96-well reaction plate

- Biological safety cabinet or PCR hood

Storage:
The test kit must be stored at -20±5 ℃.

Shelf-life upon manufacture[1]:
The shelf life is currently assigned a 12-month dating period.

Product dossier assessment

Jiangsu Bioperfectus Technologies Co., Ltd submitted the product dossier for the Monkeypox Virus (MPXV) Fast Real Time PCR Kit alignment with the Instructions and requirements for Emergency Use Listing (EUL) Submission: In vitro diagnostics detecting Monkeypox virus nucleic acid (PQDx_457). 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, Jiangsu Bioperfectus Technologies Co., Ltd 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, Jiangsu Bioperfectus Technologies Co., Ltd 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 conclusion of the quality management system assessment 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] The assigned device shelf-life is based on stability data generated from the date of manufacture. The finished goods shelf-life, calculated from the date of packaging completion, may be shorter depending on the time elapsed between manufacture and final packaging of the device.

1. Notification to WHO of any planned changes to a prequalified product, in accordance with "*Reportable changes to WHO prequalified and emergency use listed in vitro diagnostics*"[2]; 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)*[3].

Jiangsu Bioperfectus Technologies Co., Ltd 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 Monkeypox Virus (MPXV) Fast Real Time PCR Kit, with product codes JC70203-1NW-25T and JC70203-1NW-50T, manufactured by Jiangsu Bioperfectus Technologies Co., Ltd, is eligible for WHO procurement for 12 months from the day of listing. The assay detects nucleic acid from monkeypox virus, including clades I and 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, Jiangsu Bioperfectus Technologies Co., Ltd must engage in post-market surveillance activities to ensure that the product continues to meet safety, quality, and performance requirements. Jiangsu Bioperfectus Technologies Co., Ltd is required to notify WHO of any serious reportable 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.

Labelling review

The labelling submitted for the Monkeypox Virus (MPXV) Fast Real Time PCR Kit was reviewed by WHO staff and external technical experts appointed by WHO. The review evaluated the labelling for clarity and consistency with the information submitted in the product dossier, alignment with international guidance and standards, and suitability for the intended users and settings in WHO Member States, including low- and middle-income countries.

[2] https://iris.who.int/handle/10665/381373

[3] https://iris.who.int/handle/10665/337551

The table below provides traceability of the labelling documents reviewed during the assessment, including document titles, version numbers, approval dates, and control identifiers.

Controlled Labelling References

Document Type	Document Title	Version / Revision	Date Approved	Controlled Document No.
Outer box artwork	Labels-QR046-005-2 V1.0	v1.0	21 Nov 2025	QR046-005-2
Pouch / Device label	Labels-QR046-005-2 V1.0	v1.0	21 Nov 2025	QR046-005-2
Reagent bottle labels	Labels-QR046-005-2 V1.0	v1.0	21 Nov 2025	QR046-005-2
Instructions for Use (IFU)	Instruction for Use-QR0416-005-1 V1.3	v1.3	26 Nov 2025	QR046-005-1

Labels

1. JC70203-1NW-25T

1.1. Outer Package

1.2. Main Kit Label-1

55mm

30mm

Monkeypox Virus (MPXV) Fast Real Time PCR Kit

REF JC70203-1NW-25T Σ 25 Consult IFU

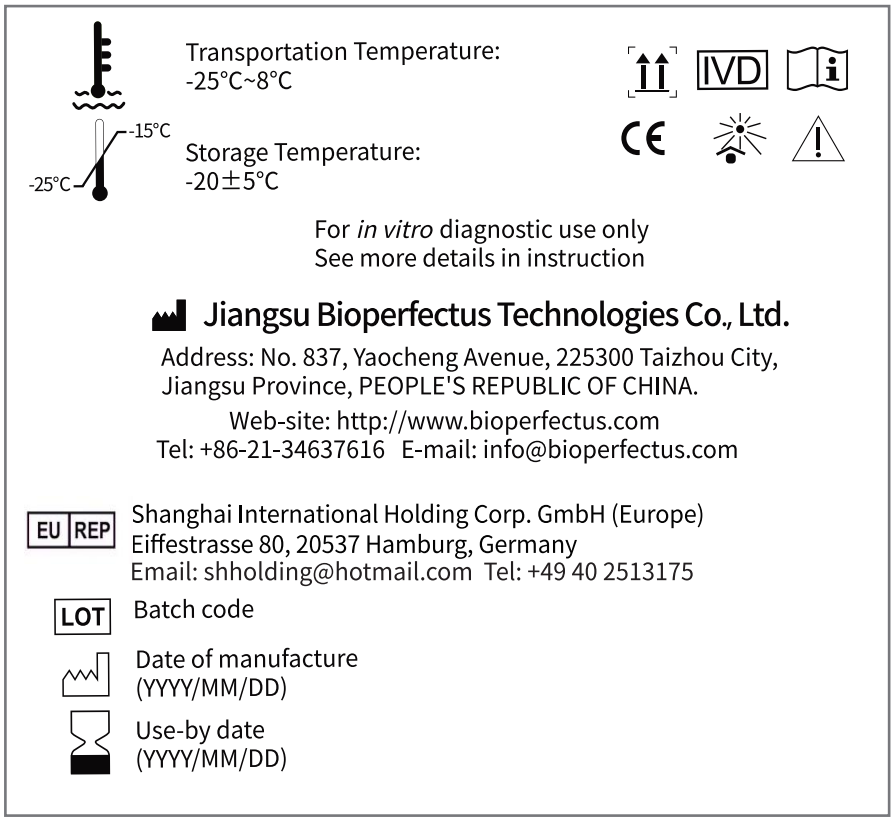
Kit Components

PCR Reaction Mix	250μL × 1
Monkeypox Vius Detection Mix	125μL × 1
Positive Control	500μL × 1
Blank Control	500μL × 1

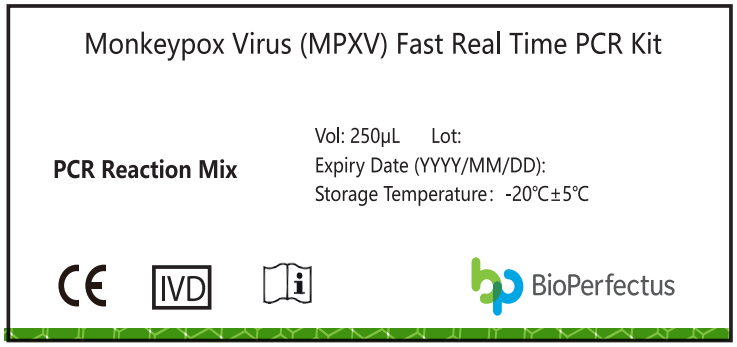
1.3. Main Kit Label-**2**

60mm

54mm



1.4. Tube Label



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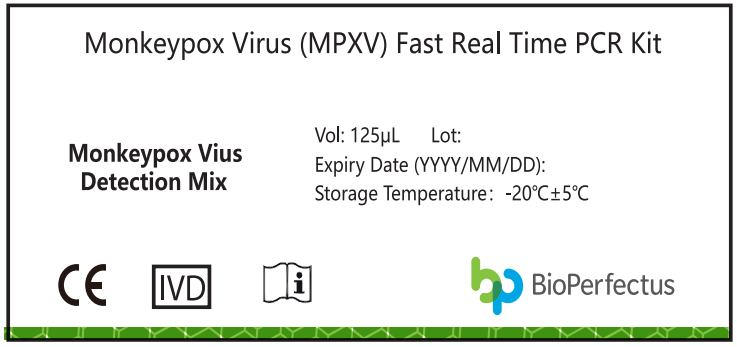

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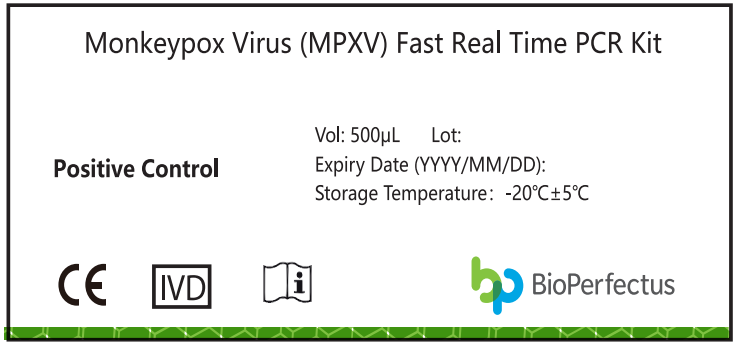

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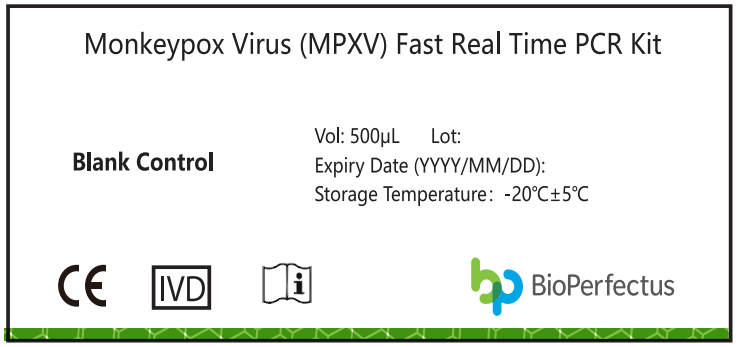

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2. JC70203-1NW-50T

2.1. Outer Package

2.2. Main Kit Label-1

55mm

30mm

Monkeypox Virus (MPXV) Fast Real Time PCR Kit

REF JC70203-1NW-50T Σ 50 Consult IFU

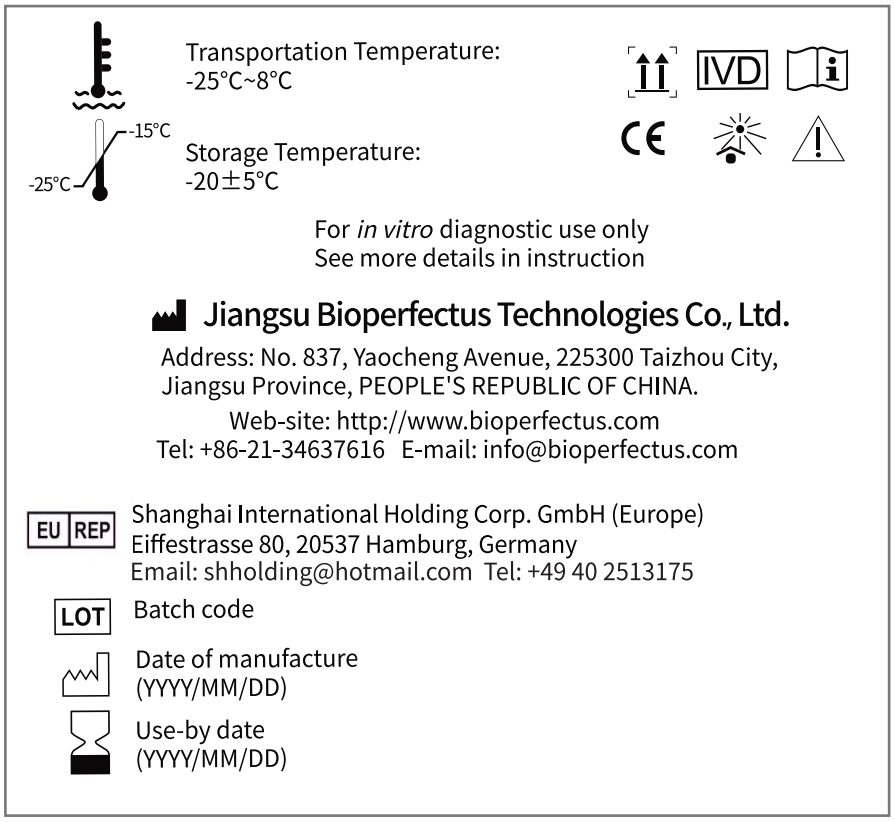
Kit Components

PCR Reaction Mix	500μL × 1
Monkeypox Vius Detection Mix	250μL × 1
Positive Control	500μL × 1
Blank Control	500μL × 1

2.3. Main Kit Label-2

60mm

54mm



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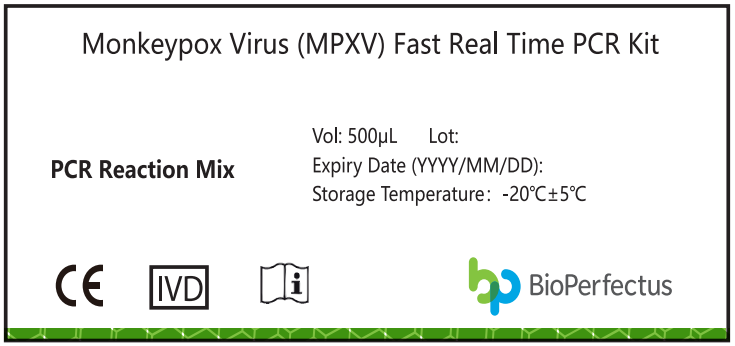

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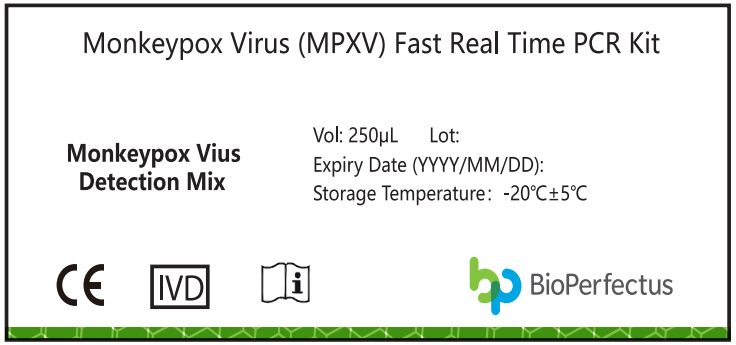

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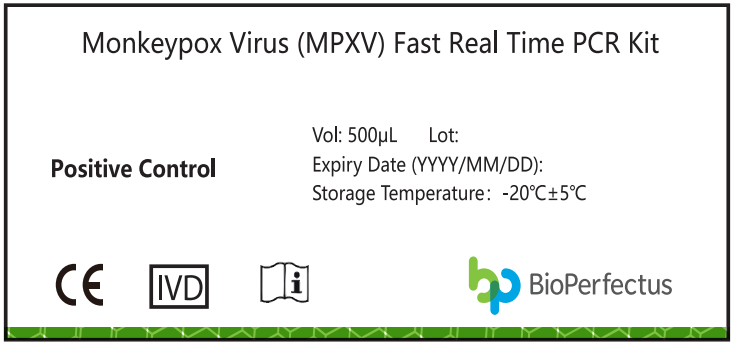

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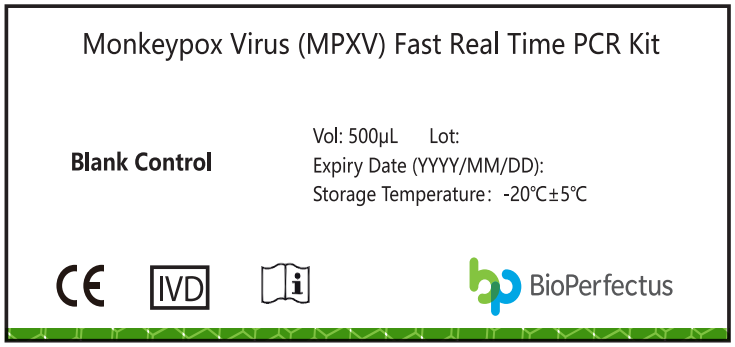

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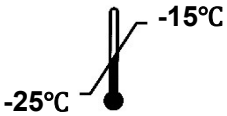
Instructions for Use[4]

[4] 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.

Monkeypox Virus (MPXV) Fast Real Time PCR Kit

IVD

CE



INSTRUCTIONS FOR USE

Version: 1.3
Issue Date: Nov. 26th ,2025

Cat No. JC70203-1NW-25T
JC70203-1NW-50T

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Contents

1. Intended Use

The Monkeypox Virus (MPXV) Fast Real Time PCR Kit is an In Vitro Diagnostic (IVD) reagent applying on fluorescent PCR technology and aiming at qualitatively detect Monkeypox virus (mpox, clade I/II) specific genes: F3L gene and B7R gene from human lesion swab specimen from individuals suspected of monkeypox infection by their healthcare provider (i.e., hospitals and clinics).
Human skin lesion swab specimen include the swab of skin exudate in Universal transport media (UTM).

The Monkeypox virus (clade I/II) DNA is generally detectable in human skin exudate specimen 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.
The product is intended for use on populations suspected to have Monkeypox virus (clade I/II) infection, as an aid in the diagnosis of Monkeypox virus infection by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR.
The product is capable of detection but not differentiation of Monkeypox virus clade I and clade II.

2. Background

The Monkeypox virus is a double-stranded DNA virus belonging to the genus Orthopoxvirus in the family Poxviridae, the same family as the variola virus [1],[2]. The virus can be cultured in Vero cells and produces characteristic cytopathic effects consistent with orthopoxvirus replication [3]. Humans are infected with the monkeypox virus mainly through bites or scratches from infected animals or via direct contact with blood, body fluids, or lesion exudate of infected animals; primary transmission is zoonotic, with occasional human-to-human spread through close physical contact or respiratory droplets [4]-[6].

The main clinical manifestations include high fever, headache, back pain, general malaise, cough, and lymphadenopathy, with generalized rashes similar to those produced by variola virus and, occasionally, abdominal pain [7]-[9]. Monkeypox is generally a self-limiting illness, with symptoms typically lasting 2–4 weeks [7],[10]. It is primarily transmitted through close contact with body fluids, lesion material, and, less commonly, blood [4]-[6]. The case fatality rate ranges from 0–11% in the general population in endemic regions, with approximately 3–6% mortality reported in children [11]-[13].

PCR technology is the primary laboratory method used to detect monkeypox virus DNA from skin lesion specimens, providing a rapid and reliable clinical

diagnostic approach [1],[14],[15]. The virus can also be isolated from skin lesions via electron microscopy or cell culture, enabling virological confirmation [16]-[18]. In addition, fluorescent antibody assays and radioimmunoassays may be used to detect monkeypox-specific antibodies in serum, although these serological methods are generally reserved for epidemiological investigations [19],[20].

3. Technical principle

The oligonucleotide primers and a probe for specific detection of monkeypox virus (MPXV) are selected from the region of F3L and B7R genes of the monkeypox viral genome. The F3L gene specific probe is labeled with FAM, and the B7R gene specific probe is labeled with VIC. In addition, the kit also contains primers and a probe (labeled with Cy5) for the human RNase P gene as an endogenous internal control for specimen integrity, nucleic acid isolation, amplification, and detection. DNA extracted and purified from the human skin lesion specimens is amplified using target specific primers in the present of target specific probes. Probes consist of a reporter dye at the 5' end and quenching dye at the 3' end. The fluorescent signals emitted from the reporter dye are absorbed by the quencher. During PCR amplification, probes hybridized to amplified templates are degraded by the Taq DNA polymerase with 5'-3' exonuclease activity, thereby separating the reporter dye and quencher and generating fluorescent signals that increase with each cycle. The PCR instrument automatically draws a real-time amplification curve for each optical channel based on the signal change and calculates cycle threshold (Ct) values (the point at which fluorescence is detectable above background) that are interpreted by the operator to determine the presence/absence of the monkeypox virus.

4. Materials provided

Components	Quantity	Volume JC70203-1NW-25T	Volume JC70203-1NW-50T	Ingredients
PCR Reaction Mix	1	250 µL	500 µL	Tris Hydroxy Methyl Aminomethane, Nucleotides mix, DNA polymerase
Monkeypox Virus Detection Mix	1	125 µL	250 µL	Primers and probes for Monkeypox virus and RNase P
Positive Control	1	500 µL	500 µL	virus-like particles of Monkeypox virus and RNase P
Blank Control	1	500 µL	500 µL	RNase-free Water

5. Materials required but not provided

Extraction reagent

Manufacturer	Nucleic Acid Isolation Kit	Cat. No.

Bioperfectus Technologies	Viral Nucleic Acid Rapid Extraction Kit (Magnetic Bead Method)	SDKF60101
	Viral Nucleic Acid Extraction Kit (Silica-Based Spin Column)	SDK60102
	Viral Nucleic Acid Extraction Kit (Magnetic Bead Method)	SDK60104
QIAGEN	QIAamp DNA Mini Kit (50)	51304
	QIAamp DNA Mini Kit (250)	51306

Instrument and consumables

- Appropriate real-time PCR system:

Real-Time PCR System	Software
Bio-Rad CFX96™ Touch	CFX Maestro 2.2
Applied Biosystems™ QuantStudio™ 5	QuantStudio™ Design & Analysis Software 1.2.0
Applied Biosystems 7500	7500 Software v2.3
Applied Biosystems ViiA™ 7	QuantStudio™ Software v1.3
QIAGEN Rotor-Gene Q	Rotor-Gene Q Software v2.3.5
Analytik Jena qTOWER³	qPCRsoft V3.1
Applied Biosystems QuantStudio™ 7	QuantStudio™ Software v1.3
Roche LightCycler® 480 II	LightCycler® 480 Software v1.5.0
Bioperfectus STC-96A/96A PLUS	SLAN 8.2.2

- Appropriate Nucleic acid extraction system:

Extraction System	Software
Bioperfectus SSNP-2000B (32 channels)	V1.0.0.0
Bioperfectus SSNP-3000A (64 channels)	V1.0.0.0
Bioperfectus SSNP-9600A (96 channels)	V1.0.0.0
Bioperfectus SMPE-960 (96 channels)	V1.0.5.0
Bioperfectus SAW-96 (96 channels)	V1.0.3.0
Bioperfectus SAW-48 (48 channels)	V1.0.2.0

- Vortexmixer
- Centrifuge
- Calibrated adjustable pipettes (10μL, 100μL, 200μL, 1000μL)
- Calibrated adjustable multi-channel pipette (5-50μL)
- 1.5 mL centrifuge tube shelf
- Magnetic grate for 1.5 mL centrifuge tube
- Specimen collection: Copan Regular Polyester Swab (CLASSIQSwabs™ 1U054S01)
- Specimen preservation fluid (Universal transfer medium): Copan UTM® Universal Transport Medium™ (UTM® 3C059N)
- RNase-free Water
- 10% sodium hypochlorite or Pasteurized disinfectant
- Disposable particle-free gloves and operating gown
- Pipette tips with filter
- 1.5 mL centrifuge tube (No DNase/RNase)
- 0.2 mL PCR plate (Applied Biosystems)
- 0.2 mL PCR tube (Applied Biosystems)

- 8-well PCR tube strips or 96-well reaction plate
- Biological safety cabinet or PCR hood

6. Warning and precautions

- For professional use only.
- For in vitro diagnostic use only.
- Operator should be well trained on real-time PCR techniques.
- Nucleic acid extraction should be manually carried out in biosafety cabinet or by automatic nucleic acid extraction system.
- Mpox infection may be contracted during the specimen processing stage and testing should be performed in appropriately equipped laboratories.
- Wear personal protective equipment (PPE), including (but not limited to) disposable clean powder-free gloves, mask and goggles.
- Working zones in laboratory should be strictly separated. Use separated and segregated working areas for (i) Reagent preparation, (ii)Specimen preparation and (iii) Amplification. The workflow in the laboratory should proceed in unidirectional manner. The experiment processes shall comply with the Good Clinical Laboratory Practice (GCLP) for Molecular Based Tests Used in Diagnostic Laboratories.https://cdn.intechopen.com/pdfs-wm/23728.pdf
- Positive control handling procedures to prevent cross-contamination:
  - The positive control contains a high concentration of target nucleic acid. Handle with care to avoid cross-contamination.
  - Always add the positive control after all clinical samples and negative controls have been prepared.
  - Change pipette tips between samples and controls, and discard after each use.
  - Change gloves immediately after handling the positive control.
  - Maintain unidirectional workflow (sample preparation → reaction setup → amplification/detection). Do not return to earlier steps once the positive control has been opened.
  - Dispose of all waste (tubes, tips, gloves) that has come into contact with the positive control as biohazard material.
- Clean work benches, pipettes and centrifuge by using 10% sodium hypochlorite and 75% ethanol.
- Work benches should be cleaned immediately after use. Amplicon contamination should be avoided according to

https://www.cdc.gov/mpox/hcp/laboratories/biosafety.html

- Use applicable real-time PCR instrument and nucleic acid extraction system to ensure optimal test performance.
- Use reagents before expiry date. Don’t replace or interchange reagents from different batches or manufactures.
- Discard specimens and assay waste according to your local safety regulations.

7. Kit storage, procession and reliability

- Store the kits and reagents at -20±5℃.
- Keep Monkeypox Virus Detection Mix away from light.

- Properly thaw and mix before reagent preparation.
- Avoid repeatedly freeze-thaw more than five times.
- Always check expiry date before use and do not use expired reagent.
- Manufacturing date and expiry date: see outer packing box.

8. Specimen type

Human skin lesion swab specimen can be collected using the Copan Regular Polyester Swab (CLASSIQSwabs™ 1U054S01) immediately placed in Copan Universal Transport Media (UTM® Universal Transport Medium™ 3C059N).

9. Specimen collection, transportation and storage

➢ Sampling

According to *Diagnostic testing for the monkeypox virus (MPXV): interim guidance, 10 May 2024*:

- Appropriate PPE should be worn when collecting the skin lesion specimen from a person with suspected or confirmed mpox.
- Select active lesions, and clean lesion area with sterile saline if necessary.
- Vigorously swab the base of the lesion to collect exudate using Copan Regular Polyester Swab (CLASSIQSwabs™ 1U054S01) after removing crust to ensure adequate viral material is collected.
- Immediately Insert the collected sample in 3 mL of Copan Universal Transprt Media (UTM® Universal Transport Medium™ 3C059N).
- Collect 2-3 lesion exudate from different body areas in a single tube.

➢ Packaging

According to *Diagnostic testing for the monkeypox virus (MPXV): interim guidance, 10 May 2024*:

- Skin lesion swab should be stored refrigerated (2-8℃) or frozen (-20℃ or lower) within an hour of collection and transported to laboratory as soon as possible after collection.
- Collected specimen being transported should have appropriate triple packaging, labelling, and documentation.

➢ Storage

- Specimen preserves at 22-25°C for no more than 3 days after received.
- Specimen preserves at 2-8°C for no more than 5 days after received.
- Specimen preserves at -80°C or colder for no more than 3 days after received.
- Avoid repeat freeze/thaw of specimen for more than 7 times.

10. Specimen transportation

Correct handling and storage of specimens during transportation is essential for accurate diagnostic testing. Specimen should be sent to laboratory as soon as possible and using refrigerated preservation if long-distance transportation is inevitable.

- Transport all specimens as soon as possible to qualified laboratories after collection.
- Transport of specimens should comply with any applicable national and/or international regulations, including the UN Model Regulations and any other applicable regulations depending on the mode of transport being used.
- For international transport, specimens from suspected, probable or confirmed mpox cases, excluding viral isolates and cultures– should be transported in principle as Category B, UN3373 “infectious substance, affecting humans”, unless national regulations specify otherwise.
- Please inform receiving laboratory before sending specimens.

11. Reagent preparation

1) Preserve kit at -20±5°C after receipt.
2) Attentions: Use reagent at clean environment and preserve reagent at -20±5°C away from direct light. Avoid freeze-thaw cycles for more than 5 times.
3) Properly dissolve and mix before use.
4) Process the blank control and positive control carefully at specimen processing zone to avoid contamination. Avoid freeze-thaw cycles for more than 5 times.

12. Reagent setup (in reagent preparation area)

Master Mix and reaction well setting

Note: Setting of reaction wells varies with sample quantity. Each run should contain blank and positive controls.

1) Thaw the PCR Reaction Mix and Monkeypox Virus Detection Mix at room temperature, oscillate and mix thoroughly in reagent preparation zone.
2) Vortex PCR Reaction Mix and Monkeypox Virus Detection Mix for 5 seconds before use.
3) Centrifuge at 5000rpm for 10s.
4) Calculate the number of tests to be prepared (n = the number of specimens + 2 control tubes). The reaction system for each test is prepared as follows:

Components	Volume
PCR Reaction Mix	N×10.0μL
Monkeypox Virus Detection Mix	N×5.0μL
Total Volume (Master Mix)	**N×15.0μL**

5) Calculate the amount of each reagent used above, add to an appropriate volume of centrifuge tube.
6) Mix thoroughly and centrifuge at 5000 rpm for 5 seconds.
7) Place PCR reaction tube or plate into 96-well shelf.
8) Dispense 15 μL of master mix into each reaction well.
9) Tightly close the PCR reaction well/plate and transfer to the nucleic acid processing zone.

13. Nucleic acid extraction (in specimen preparation area)

Instruments preparation

Clean all work benches, pipettes, centrifuge and other instruments using 5% sodium hypochlorite and 75% ethanol.

Nucleic acid extraction

Performance of Monkeypox Virus (MPXV) Fast Real Time PCR Kit depends on the quantity and purity of nucleic acid extraction. Below listed extraction kits have been validated and could be used for Monkeypox virus extraction.

Bioperfectus Viral Nucleic Acid Isolation Kit

SDK60102, spin column
SDK60104, magnetic beads (auto)
SDKF60101, magnetic beads (auto)
Qiagen QIAamp Viral RNA Mini Kit 51304/51306, spin column
Extraction follows instructions from kit manufacturer. Blank and positive controls shall fully involve nucleic acid extraction process.

Add extraction sample eluate

1) Tenderly vortex mix centrifuge tube containing extraction sample eluate for 5 seconds.
2) Centrifuge at 5000 rpm for 5 seconds to sediment purified DNA.
3) Add 10μL of extraction sample eluate to each well.
4) Close the cap after adding 8 wells.
5) Continue the work to avoid contamination.
6) Wear gloves to avoid contamination.
7) Close all caps and then add extracted quality controls.

Add quality control

1) Add 10 μL blank control to reaction well and close the cap.
2) Add 10 μL positive control to reaction well and close the cap.
Note: please follow the sequence from 1 to 8 if using 8-tube strips.
3) Centrifuge the 8-tube strip at 5000 rpm for 5 s and then place back to the shelf.
4) Note: If using a PCR 96-well plate, centrifuge for 30 s at 1000 rpm.

14. Amplification and detection (in amplification area)

5) Set the thermocycling profile on validated real-time PCR platform: Applied Biosystems 7500, ViiA™ 7, QuantStudio™ 5, Bio-Rad CFX96™, QIAGEN Rotor-Gene Q, Analytik Jena qTOWER3, Quant Studio™ 6/7, Roche LightCycler®480, Bioperfectus STC-96A/96A PLUS, following the parameters provided in the subsequent section.

14.1. Initial Setup

Instrument	Steps

Applied Biosystems 7500	a. Choose "Design Wizard" b. Go to Section 1A: Experiment Properties c. Choose "7500 (96 wells)" d. Choose "Quantitation" e. Go to Section 1B: Methods & Materials f. Choose "Standard Curve" g. Choose "TaqMan® Reagents" h. Choose "Standard" i. Choose "cDNA"
QuantStudio™ 5	a. Choose "Create New Experiment" b. Choose Instrument type: QuantStudio™ 5 c. Choose Block type "96-Well 0.2-mL Block" d. Choose Experiment type "Standard Curve" e. Choose Chemistry "TaqMan® Reagents" f. Choose Run mode "Fast"
ViiA™ 7 and Quant Studio™ 6/7	a. Choose "Create New Experiment" b. Choose Instrument type: ViiA™ 7 or Quant Studio™ 6/7 c. Choose Block type "96-Well 0.2-mL Block" d. Choose Experiment type "Standard Curve" e. Choose Chemistry "TaqMan® Reagents" f. Run mode "Standard"
Bio-Rad CFX96™	a. Choose "PrimePCR" b. Choose "Probe"
Rotor-Gene Q	a. Choose "Advanced" b. Choose "Empty Run" c. Choose Rotor Type d. Tick "Locking Ring Attached" e. Click "Next" f. Input Reaction Volume: 25 µL g. Click "Next"
Roche LightCycler®480	a. Choose "New Experiment" b. Go to "Tools" Icon c. Choose "Detection Format" d. Choose "New" e. Choose the right four filter combinations in the "Filter Combination Selection" f. Enter a "Name" and the correct values for "Quant Factor" and "Max. Integration Time" g. The new detection format is automatically saved after closing

Bioperfectus STC-96A/96A PLUS	a. Choose "Project" b. Click "Create" c. Choose "General" d. Enter Project name e. Choose Project type "Qualitative/Absolute Quantitative" f. Input Reaction volume: 25 µL
Analytik Jena qTOWER3	a. Choose "File" b. Choose "New Project" c. Enter "Project Name" d. Click "New Experiment" e. Choose "Absolute Quantification" f. Go to "Settings" g. Choose "Plate" h. Select "96-well"

14.2. Selection of Channels

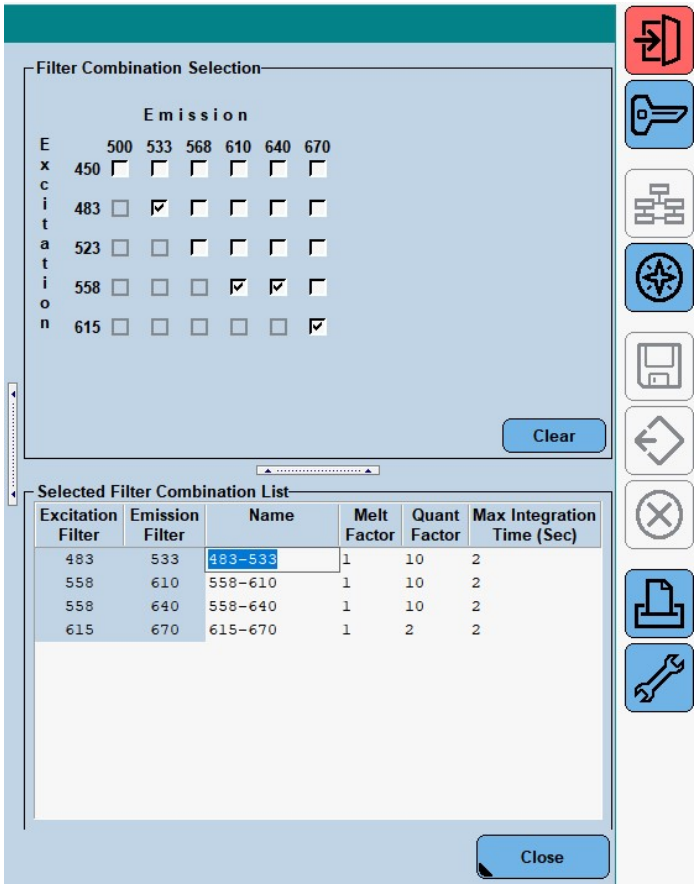
a. For Applied Biosystems 7500, ViiA™ 7, QuantStudio™ 5, Analytik Jena qTOWER3, Quant Studio™ 6/7 and Bioperfectus STC-96A/96A PLUS, assign the target F3L as “FAM”, the target B7R as “VIC/HEX” and the RNase P (Internal control) as “Cy5”< respectively.

b. For Bio-Rad CFX 384/96, select all channel.

c. For Roche LightCycler 480, in “Filter Combination Selection” select following combination:

d. For Rotor-Gene Q: Use default channel selection.

Instrument Model	F3L Assignment	B7R Assignment	RNase P (Internal Control) Assignment
Applied Biosystems 7500, ViiA™ 7, QuantStudio™ 5, Analytik Jena qTOWER3, Quant Studio™ 6/7, Bioperfectus STC-96A/96A PLUS	FAM	VIC/HEX	Cy5
Bio-Rad CFX 96	FAM	HEX	Cy5
Roche LightCycler 480	Select filter combination in "Filter Combination Selection" based on targets showed below:		

	
Rotor-Gene Q	Use default channel selection

14.3. Setup PCR Amplification Protocol

14.3.1. The following amplification protocol was developed for the use of the Applied Biosystems 7500, ViiA™ 7, QuantStudio™ 5, Bio-Rad CFX96™, QIAGEN Rotor-Gene Q, Analytik Jena qTOWER3, QuantStudio™ 6/7, Roche LightCycler® 480, Bioperfectus STC-96A/96A PLUS.

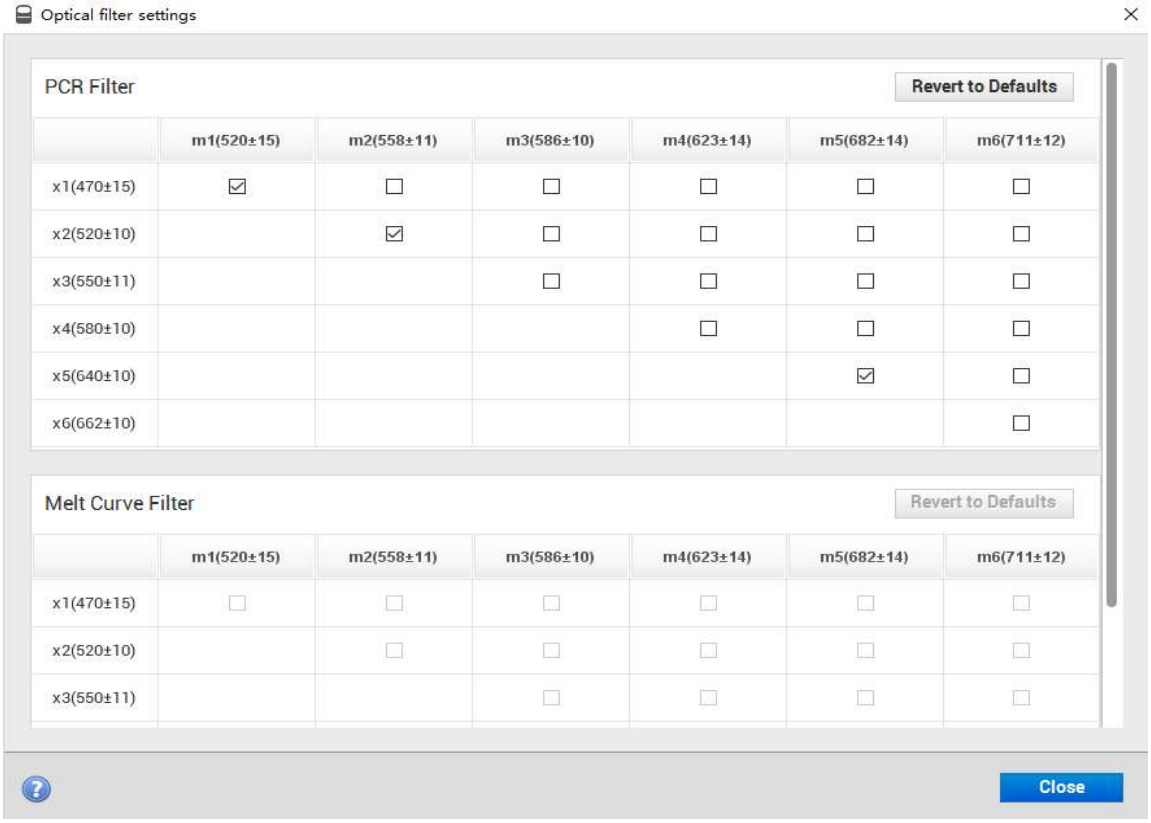
Step		Temperature	Time	Cycles
1	UDG enzyme processing	37°C	2 min	1 cycle
2	Pre-denaturation	95°C	5 min	1 cycle
3	Denaturation	95°C	10 sec	45 cycles
	Annealing, extension, and fluorescence signal collection	58°C	30sec	

14.3.2. The following amplification protocol was developed for the use of the QuantStudio™ 5*, Bio-Rad CFX96™ and Analytik Jena qTOWER3.

Step	Temperature	Time	Cycles	Rate of Temperature Increase/Decrease

1	UDG enzyme processing	37°C	2 min	1 cycle	3.19°C/s
2	Pre-denaturation	95°C	10sec	1 cycle	3.19°C/s
3	Denaturation	95°C	1sec	42 cycles	3.19°C/s
	Annealing, extension, and fluorescence signal collection	60°C	10sec		2.45°C/s

***Note**: For QuantStudio™ 5, before protocol setting, select “Action” in “Experiment Method” interface, and choose “Optical filter settings”. Select PCR filter "x1 (470±15) & m1 (520±15), x2 (520±10) & m2 (558±11), x5 (640±10) & m5 (682±14)" showed below:



14.4. Start the Amplification

14.5. Analysis

14.5.1. Threshold

The assay uses the Instrument’s automatic threshold determination, which sets the threshold above baseline variation and within the exponential portion of positive amplification curves. The auto-threshold algorithm was validated to ensure consistent Ct calling, and each run is accepted only when controls perform within specification.

14.5.2. Ct Values for Quality Control

- Quality control should be enforced according to local regulations, certificate requirements or laboratory standard quality control process.
- Quality control is used to monitor reagent and results analysis.
- Check positive and blank controls before using each new batch of kits.
- Add blank and positive control to each nucleic acid extraction and purification.
  Each nucleic acid testing should include positive and blank controls.
  Human RNase P gene should be monitored to guarantee specimen quality and extraction process.
- Expected performance of Monkeypox Virus (MPXV) Fast Real Time PCR Kit quality control as follow:

Channels / Controls	Threshold cycle (Ct) value		
	FAM(F3L)	VIC(B7R)	CY5(RNase P)
Blank Control	UNDET	UNDET	UNDET
Positive Control	Ct≤30.0	Ct≤30.0	Ct≤30.0

Internal control RNase P gene monitors sampling and extraction processes and potential contamination of extraction process.
If any of above controls shows incorrect Ct value, it means that the testing is incorrect or reagent/instrument has problems. Repeating the test is necessary.

14.5.3. Cut-off Value for Samples

Analysis of the results for each sample should be based on the Cut-off Ct values below:

Channel（Target）	Cut-off Value	Results
FAM(F3L)	≤ 40.0	+
FAM(F3L)	> 40.0	-
VIC(B7R)	≤ 40.0	+
VIC(B7R)	> 40.0	-
CY5(RNase P)	≤ 37.0	+
CY5(RNase P)	> 37.0	-

15. Results interpretation and reporting

Below table lists expected results of Monkeypox Virus (MPXV) Fast Real Time PCR Kit. If these criteria are not satisfied, repeat the test.

FAM(F3L)	VIC(B7R)	CY5 (RNase P)	Results	Report
+	+	±	Detected Monkeypox Virus	Report positive
-	-	+	Undetected	Report negative
+	-	±	Detected suspected	Report suspected
-	+	±		
-	-	-	Invalid	Invalid

Note:

1) For Monkeypox Virus: FAM and VIC Ct value ≤40 is considered positive (+); Ct value > 40 is considered negative (-).
2) For RNase P: CY5 Ct value ≤37.0 is considered positive (+); Ct value > 37.0 is considered negative (-).
3) For suspected result, Ct values ≤ 40 for one of the FAM and VIC channels, the test should be repeated. If the retest result is still positive for single or double channel, the test is judged to be positive for monkeypox virus.
4) For invalid results, the test should be repeated. This means re-collection of the sample, nucleic acid extraction and testing.

16. Limitations

- All operator, data analysis staff and results reporting staff should be trained and proven to have competence for doing test and explaining results independently. User of the kit is limited to staff who has successfully passed the training.
- Negative results can neither straightforwardly exclude Monkeypox Virus infection, nor the only decision-making evidence for treatment and patient management. Optimized specimen type and time of peek virus value induced by Monkeypox Virus infection has not been finalized, and therefore multiple specimen collection (type and time) is necessary.
- Improper specimen collection, transportation and processing may lead to false negative results. Inhibitors or insufficient viral load may also lead false negative results.
- Improper handling of specimens, reagents, or test materials may lead to contamination and result in false-positive results. Strict adherence to laboratory contamination-control practices is required to ensure test reliability.
- This assay has been validated only for the specimen types listed in this Instruction for Use. Use of unvalidated specimen types may lead to inaccurate, unreliable, or invalid results and should be avoided.
- Predictive positive and negative rate is based on prevalence rate. Prevalence thus impacts the positive predictive value (PPV) and negative predictive value (NPV) of tests. As the prevalence increases, the PPV also increases but the NPV decreases. Similarly, as the prevalence decreases the PPV decreases while the NPV increases.
- Monkeypox Virus maybe undetectable if target genes of the virus mutate.
- Inhibitors and other interferences may lead false negative results.
- Performance has not been validated for treatment monitoring.
- Detection using the kit cannot exclude other bacteria or pathogen induced diseases.

17. Performance Characteristics

17.1. Analytical performance

Limit of detection (LoD)

To estimate the limit of detection (LoD), Monkeypox virus positive clinical sample was diluted to 5 concentrations (7×10^5 copies/mL, 7×10^4 copies/mL, 7×10^3 copies/mL, 7×10^2 copies/mL, 7×10^1 copies/mL) and tested 3 times

each using one batch of reagents, the lowest concentration at which all were detected was initially determined as the limit of detection (Cy).
Monkeypox virus positive clinical sample was subjected to a 2-fold gradient dilution to 2Cy, Cy, Cy/2, Cy/4 and Cy/8, and each sample was tested 20 times using the same batch of reagents and the lowest concentration with a positive detection rate greater than 95% was initially determined as the lowest detection limit concentration.

Table 1. The initial establishment of LoD

Concentration	Detection times	Positive rate
7×10^5 copies/mL	3	100% (3/3)
7×10^4 copies/mL	3	100% (3/3)
7×10^3 copies/mL	3	100% (3/3)
7×10^2 copies/mL	3	100% (3/3)
7×10^1 copies/mL	3	33.3% (1/3)

Table 2. The result of estimation of LoD

Concentration	Detection times	Positive rate
1.4×10^3 copies/mL	20	100% (20/20)
7×10^2 copies/mL	20	100% (20/20)
3.5×10^2 copies/mL	20	100% (20/20)
1.75×10^2 copies/mL	20	95% (19/20)
8.75×10^1 copies/mL	20	55% (11/20)

To determine the LoD, Monkeypox virus positive clinical sample (1×10^7 copies/mL) was diluted to a concentration of Lod for monkeypox virus. Dilution was extracted and the elution volume was 80μL. Dilution was tested 20 times by three lots of reagents. The lowest concentration that has positive detection for all three replicates is the potential preliminary LOD and positive detection rate should be no less than 95%.

Table 3. The result of the final LoD

Sample	Concentration	Detection times	Positive rate		
			Lot 1	Lot 2	Lot 3
Simulated sample	1.75×10^2 copies/mL	20	100% (20/20)	95% (19/20)	95% (19/20)

The limit of detection (LoD) for the kit is 175 copies/mL.

Cross-reactivity (*In Silico Analysis*)

An *in silico* cross-reactivity analysis was conducted by aligning the MPXV_F3L and B7R target primers and probe sequence against available pathogens listed by the WHO sequences as of January, 2024 in the NCBI complete genome database. Basic local alignment search tool (BLAST) searches were performed

using the MPXV_F3L and MPXV_B7R primer and probe sets against the NCBI database.
A match was counted when primer/probe coverage and sequence identity was ⩾80%. Cross-reaction or interference would not occur when homologies exist on the same sense strand, provided they are separated by at least 10,000 base pairs and feature multiple mismatches at the 3’-end of the forward primer. Such cross-reaction and interference remain highly unlikely.

Table 4. Sequence homology between MPXV-F3L primers/probe and other microorganisms by *in silico* analysis

Category	Organism	Tax ID	Genus	Sequences with ≥80% match to at least one primer	Sequences with ≥80% match to both primers	Sequences with ≥80% match to probe	Sequences with ≥80% match to both primers and the probe
Bacteria	Acinetobacter calcoaceticus	471	*Acinetobacter*	0	0	0	0
Bacteria	Bacteroides fragilis	817	*Bacteroides*	0	0	0	0
Bacteria	Chlamydia trachomatis	813	*Chlamydia*	0	0	0	0
Bacteria	Corynebacterium diphtheriae	1301	*Corynebacterium*	0	0	0	0
Bacteria	Enterococcus faecalis	1352	*Enterococcus*	0	0	0	0
Bacteria	Escherichia coli	562	*Escherichia*	0	0	0	0
Bacteria	Homo sapiens	9606	*Homo*	4	0	0	0
Bacteria	Human papillomavirus (HPV)	10566	*Human papillomavirus*	0	0	0	0
Bacteria	Human herpesvirus 1 (HSV−1)	10298	*Simplexvirus*	0	0	0	0
Bacteria	Human herpesvirus 2 (HSV−2)	10310	*Simplexvirus*	12	0	0	0
Bacteria	Lactobacillus spp.	1578	*Lactobacillus*	0	0	0	0
Bacteria	Mycoplasma pneumoniae	2095	*Mycoplasma*	0	0	0	0
Bacteria	Neisseria gonorrhoeae	485	*Neisseria*	0	0	0	0
Bacteria	Pseudomonas aeruginosa	287	*Pseudomonas*	0	0	0	0
Bacteria	Staphylococcus aureus	1280	*Staphylococcus*	0	0	0	0
Bacteria	Staphylococcus epidermidis	112429	*Staphylococcus*	0	0	0	0
Bacteria	Streptococcus agalactiae	1311	*Streptococcus*	0	0	0	0
Bacteria	Streptococcus Group C/G	2179	*Streptococcus*	0	0	0	0
Bacteria	Streptococcus mitis	28037	*Streptococcus*	0	0	0	0

Fungi	Streptococcus pyogenes	1314	*Streptococcus*	0	0	0	0
Fungi	Treponema pallidum	771	*Treponema*	0	0	0	0
Parasite	Buffalopox virus	32605	*Orthopoxvirus*	12	0	0	0
Human	Camelpox virus	10244	*Orthopoxvirus*	2	0	0	0
Virus	Candida albicans	5476	*Candida*	0	0	0	0
Virus	Corynebacterium jeikeium	38289	*Corynebacterium*	0	0	0	0
Virus	Cowpox virus	10257	*Orthopoxvirus*	36	5	7	5
Virus	Ectromelia virus	10260	*Orthopoxvirus*	5	0	0	0
Virus	Molluscum contagiosum virus	10279	*Molluscipoxvirus*	0	0	0	0
Virus	Mycoplasma genitalium	2097	*Mycoplasma*	0	0	0	0
Virus	Trichophyton rubrum	4990	*Trichophyton*	0	0	0	0
Virus	Vaccinia virus	10245	*Orthopoxvirus*	2	0	0	0
Virus	Varicella–zoster virus	10335	*Varicellovirus*	0	0	0	0
Virus	Variola virus	10254	*Orthopoxvirus*	78	0	0	0
Virus	Trichomonas vaginalis	5722	*Trichomonas*	0	0	0	0

Table 5. Sequence homology between MPXV-B7R primers/probe and other microorganisms by *in silico* analysis

Category	Organism	Tax ID	Genus	Sequences with ≥80% match to at least one primer	Sequences with ≥80% match to both primers	Sequences with ≥80% match to probe	Sequences with ≥80% match to both primers and the probe
Bacteria	Acinetobacter calcoaceticus	471	*Acinetobacter*	0	0	0	0
Bacteria	Bacteroides fragilis	817	*Bacteroides*	0	0	0	0
Bacteria	Chlamydia trachomatis	813	*Chlamydia*	0	0	0	0
Bacteria	Corynebacterium diphtheriae	1301	*Corynebacterium*	0	0	0	0
Bacteria	Enterococcus faecalis	1352	*Enterococcus*	172	0	0	0
Bacteria	Escherichia coli	562	*Escherichia*	3	0	0	0
Bacteria	Homo sapiens	9606	*Homo*	11	3 (primers are>10,000 ,000 away)	1 (On different strain with primers)	0
Bacteria	Human papillomavirus (HPV)	10566	*Human papillomavirus*	0	0	0	0
Bacteria	Human herpesvirus 1 (HSV–1)	10298	*Simplexvirus*	0	0	0	0
Bacteria	Human herpesvirus 2 (HSV–2)	10310	*Simplexvirus*	0	0	0	0

Bacteria	Lactobacillus spp.	1578	*Lactobacillus*	0	0	0	0
Bacteria	Mycoplasma pneumoniae	2095	*Mycoplasma*	0	0	0	0
Bacteria	Neisseria gonorrhoeae	485	*Neisseria*	0	0	0	0
Bacteria	Pseudomonas aeruginosa	287	*Pseudomonas*	0	0	0	0
Bacteria	Staphylococcus aureus	1280	*Staphylococcus*	163	0	0	0
Bacteria	Staphylococcus epidermidis	112429	*Staphylococcus*	1	0	0	0
Bacteria	Streptococcus agalactiae	1311	*Streptococcus*	0	0	0	0
Bacteria	Streptococcus Group C/G	2179	*Streptococcus*	0	0	0	0
Bacteria	Streptococcus mitis	28037	*Streptococcus*	0	0	0	0
Fungi	Streptococcus pyogenes	1314	*Streptococcus*	0	0	0	0
Fungi	Treponema pallidum	771	*Treponema*	0	0	0	0
Parasite	Buffalopox virus	32605	*Orthopoxvirus*	12	0	0	0
Human	Camelpox virus	10244	*Orthopoxvirus*	2	0	0	0
Virus	Candida albicans	5476	*Candida*	0	0	0	0
Virus	Corynebacterium jeikeium	38289	*Corynebacterium*	0	0	0	0
Virus	Cowpox virus	10257	*Orthopoxvirus*	92	36	0	0
Virus	Ectromelia virus	10260	*Orthopoxvirus*	5	5	0	0
Virus	Molluscum contagiosum virus	10279	*Molluscipoxvirus*	0	0	0	0
Virus	Mycoplasma genitalium	2097	*Mycoplasma*	0	0	0	0
Virus	Trichophyton rubrum	4990	*Trichophyton*	0	0	0	0
Virus	Vaccinia virus	10245	*Orthopoxvirus*	2	0	0	0
Virus	Varicella–zoster virus	10335	*Varicellovirus*	0	0	0	0
Virus	Variola virus	10254	*Orthopoxvirus*	78	78	0	0
Virus	Trichomonas vaginalis	5722	*Trichomonas*	1	0	0	0

The results of in-silico analysis indicate that except Cowpox virus, Vaccinia virus, Variola virus, Ectromelia virus, Camelpox virus, Staphylococcus aureus, Buffalopox virus and Enterococcus faecalis, both of the primers and probe set for the F3L and B7R gene targets are Monkeypox virus specific, no cross-reactivity and interference were predicted for all other microorganisms including the other non-variola orthopoxvirus.

Cross-reactivity (Microbial Interference Study)

To confirm the in-silico analysis predication, we tested the contrived Cowpox virus, Vaccinia virus, Variola virus, Ectromelia virus, Camelpox virus, Staphylococcus aureus, Buffalopox virus and Enterococcus faecalis which are show high homology with at least one primer sequence of the kit, with concentration higher than 10^5 TCID50/mL or equivalent concentration with or without the presence of 3×LoD MPXV.

Table 6. Microbe Information

Virus	Source	Concentration
Cowpox virus	ATCC	2.5×10^7 TCID50/mL
Vaccinia virus	ATCC	1.6×10^6 TCID50/mL
Variola virus	Plasmid	1×10^8 Copies/mL
Ectromelia virus	Plasmid	1.8×10^7 Copies/mL
Camelpox virus	Plasmid	5.2×10^7 Copies/mL
Staphylococcus aureus	Plasmid	1×10^8 Copies/mL
Buffalopox virus	Plasmid	1×10^8 Copies/mL
Enterococcus faecalis	Plasmid	1.2×10^7 Copies/mL

All contrived interference microorganism samples tested negative without addition of monkeypox virus, whereases all samples with 3×LOD monkeypox virus added were tested positive.

Table 7. Results for Microbial Interference Study

Pathogen	Concentration	Result	
		Without MPXV	With MPXV (1200 copies/mL)
Cowpox virus	2.5×10^5 TCID50/mL	Negative	Positive
Vaccinia virus	1.6×10^5 PFU/mL	Negative	Positive
Variola virus	1×10^5 Copies/mL	Negative	Positive
Ectromelia virus	1.8×10^7 Copies/mL	Negative	Positive
Camelpox virus	5.2×10^7 Copies/mL	Negative	Positive
Staphylococcus aureus	1×10^8 Copies/mL	Negative	Positive
Buffalopox virus	1×10^8 Copies/mL	Negative	Positive
Enterococcus faecalis	1.2×10^7 Copies/mL	Negative	Positive

The results confirm that Cowpox virus, Vaccinia virus, Variola virus, Ectromelia virus, Camelpox virus, Staphylococcus aureus, Buffalopox virus and Enterococcus faecalis don't interact with the primer and probe sets of the assay. Therefore, this assay is highly specific for the Monkeypox virus as expected.

Interfering substance

The following interference substances showed no interference to the kit.

No.	Interfering Substances	Concentration
1	Abreva (Docosanol containing cold sore treatment)	7% w/v (0.07g/mL)
2	Acyclovir	3.5 mg/mL
3	Albumin	2.2 mg/mL
4	Blood/EDTA	5% v/v
5	Mucin	60ug/mL

6	Hydrocortisone cream*	~7% w/v
7	Benadryl cream/ointment	7% w/v
8	Carmex* (Petrolatum containing lip/skin care)	~7% w/v
9	Casein	7mg/mL
10	Lanacane (Benzocaine containing local anesthetic)	3.5% w/v
11	KY Jelly (Lubricant)	7% v/v
12	Douche	7%
13	Neosporin*	7% w/v
14	Female urine	7-10% v/v (7%)
15	Male urine	7-10% v/v (7%)
16	Feces	0.22% w/v (2.2 mg/mL)
17	Seminal fluid	2-7% (7%)
18	Zinc Oxide ointment	7%
19	Vagisil Cream (Lidocaine containing cream)	1%
20	Cornstarch	2.5mg/mL

Precision(repeatability and reproducibility)
The in-house precision references were tested 10 replicates, variation coefficient (CV, %) of Ct value were not more than 5%.

17.2. Clinical Study
The sample size for the Altona vs. Bioperfectus statistical test results was 386. The kappa-value for Altona vs. Bioperfectus amounts to 0.98959. These results are both higher than the acceptability criteria, thus resulting in a sufficient correlation between the golden standard Altona and the Bioperfectus kit. The sensitivity (PPA) and specificity (NPA) of the Altona vs. Bioperfectus are 100% (95% CI: 98.16%~100.00%) and 98% (95% CI: 96.06% – 99.70%), respectively. The total percentage agreement is 99.48% (95% CI: 98.13% – 99.86%), while the positive predictive value is 99.03% and the negative predictive value is 100%. The positive likelihood ratio for Bioperfectus is 90.5, while the negative likelihood ratio is 0. It can thus be concluded that the acceptability criteria for the golden standard (Altona) vs. Bioperfectus were met for the qualitative analysis.

Bioperfectus	Altona		Total
	Positive	Negative	
Positive	205	2	207
Negative	0	179	179
Total	205	181	386

Positive percentage agreement (PPA)	100% (95% CI: 98.16%~100.00%)
Negative percentage agreement (NPA)	98.90% (95% CI: 96.06%–99.70%)
Total percent agreement	99.48% (95% CI: 98.13%–99.86%)
Positive predictive value	0.990338164
Negative predictive value	1
Positive likelihood ratio	90.5
Negative likelihood ratio	0
PA	0.994818653
Pe	0.502255094
Kappa	0.989590356

18. Appendix

Index of Symbols

	In vitro diagnostic medical device		Date of manufacture
	Contains sufficient for <n> tests		Use-by data
	Consult IFU		Lot code
	Temperature limit		Manufacturer
	Catalogue number		Authorized representatives in the European Community
 Transportation Temperature	The symbol indicates that the product must be transported within the specified temperature range. Requirements：The specific temperature range is indicated beside the symbol.		

19. Contact and Support

For more information about Jiangsu Bioperfectus Technologies Co, Ltd., please visit our website at: http://www.bioperfectus.com or contact at E-mail: info@bioperfectus.com.

For detailed programming instructions regarding the use of the Bioperfectus Technologies Real Time PCR Kits on specific Real Time PCR instruments please contact our Technical Support at E-mail:support@bioperfectus.com.

20. References

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Revision

Revision DD/MM/YYYY	Change description
1.0 15/07/2022	First Publishing.
1.1 24/11/2022	Update manufacture address and logo.
1.2 23/09/2025	Update guidance and procedural steps to minimize the risk of cross-contamination associated with the use of the positive control in Section 6.
1.3 26/11/2025	Update and restructure the IFU to ensure full compliance with WHO Emergency Use Listing (EUL) requirements.