Mpox disease Emergency Use Listing Procedure (EUL) for IVDs
Product: Cowingene Monkeypox Virus Typing Detection Kit
EUL Number: MPXV-13599-18058-00
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 Cowingene Monkeypox Virus Typing Detection Kit, with product code ST08003W, Rest-of-World regulatory version, manufactured by Taizhou Cowingene Biotech Co., Ltd., located at No. 28, Xinlin Road, Taizhou City, People's Republic of China, was listed as eligible for WHO procurement on 30 October 2025.

Intended use:

According to the claim of intended use from Taizhou Cowingene Biotech Co., Ltd, *"This assay is a qualitative real-time PCR test to be performed on the NATBox System, Model: mini II (Taizhou Cowingene Biotech Co., Ltd.).*
This assay can test G2R gene and C3L gene for monkeypox virus, E9L gene for orthopoxvirus in human lesion materials (swabs of surface or exudate, or crusts) from individuals presenting with signs and symptoms consistent with mpox and/or with relevant epidemiological risk factors. The function of the assay is for the aid in the diagnosis of Monkeypox virus or Orthopoxvirus infections and in differentiating between MPXV Clade I, MPXV Clade II, and other Orthopoxviruses. Cowingene Monkeypox Typing Detection Kit is intended for use in a clinical laboratory by trained professionals."

Validated specimen type:
Human lesion swabs (human lesion materials (swabs of surface or exudate, or crusts) collected using flocked swabs (Yocon VTM, MT0301).

Test kit contents:

Kit components	Product code (ST08003W) (24 Tests/kit)
Cartridge	24 vials
Lysis buffer	2 tubes x 5000 µL
Positive control	1tube (500µL/tube)
Negative control	1 tube (500µL/tube)
Transfer dropper (for lysis buffer)	24 pieces (400µL)
Transfer dropper (for sample)	24 pieces (100µL)
Package insert	1

Items required but not provided:

- Yocon VTM (MT0301)

Storage:
The test kit must be stored at 2 to 8 ℃.

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

Product dossier assessment

Taizhou Cowingene Biotech Co., Ltd submitted the product dossier for the Cowingene Monkeypox Virus Typing Detection 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, TAIZHOU COWINGENE BIOTECH 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, TAIZHOU COWINGENE BIOTECH 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. 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*"[1]; and
2. Post-market surveillance activities, in accordance with "*WHO guidance on post-market surveillance of in vitro diagnostics" (ISBN 978 92 4 150921 3)*[2].

TAIZHOU COWINGENE BIOTECH 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 Cowingene Monkeypox Virus Typing Detection Kit, with product code ST08003W, manufactured by TAIZHOU COWINGENE BIOTECH 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, TAIZHOU COWINGENE BIOTECH CO., LTD must engage in post-market surveillance activities to ensure that the product continues to meet safety, quality, and performance requirements. TAIZHOU COWINGENE BIOTECH 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.

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

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

Labelling review

The labelling submitted for the Cowingene Monkeypox Virus Typing Detection 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.

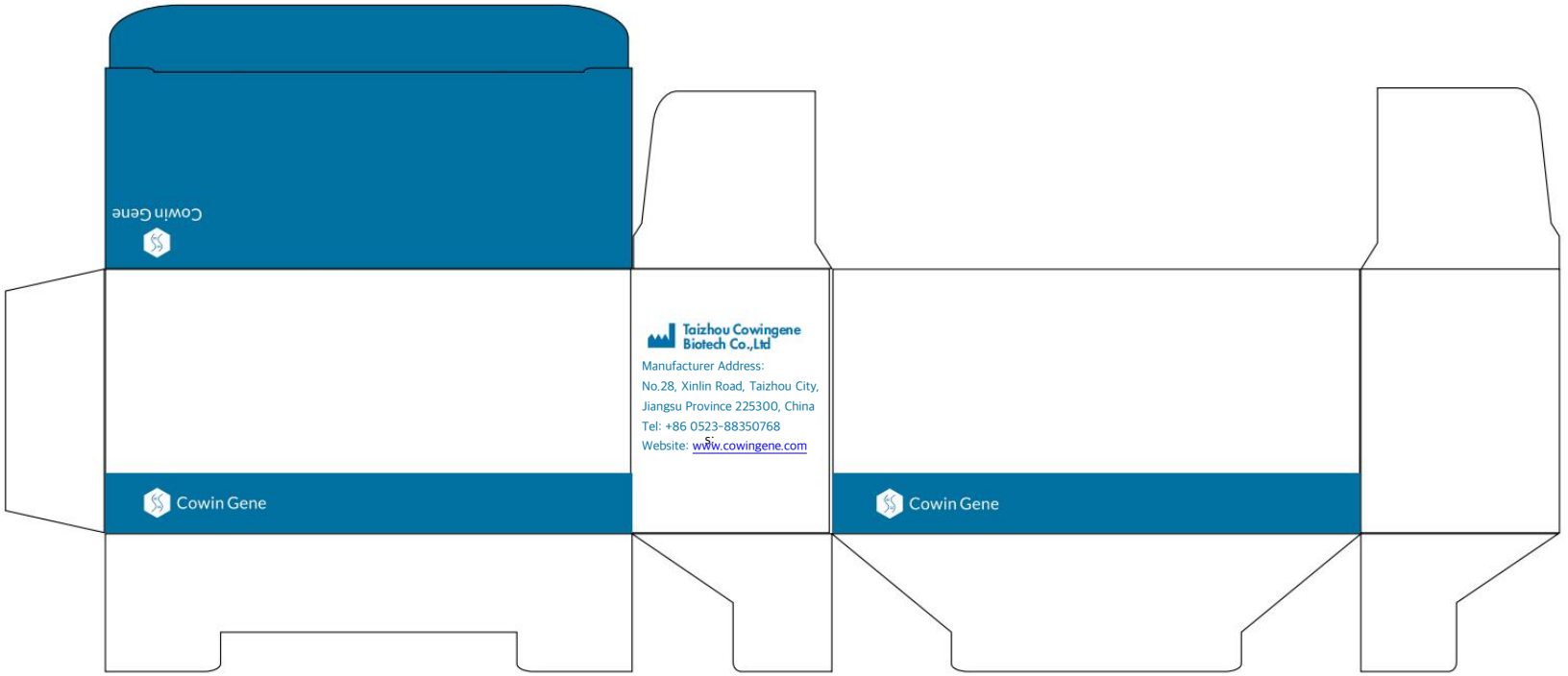
The table below provides traceability of the labelling documents reviewed during the assessment, including document titles, version numbers, approval dates, and control identifiers.

Controlled Labelling References

Document Type	Document Title	Version / Revision	Date Approved	Controlled Document No.
Outer box artwork	Label	EN1.2	2025-10-11	ST08003W-Label
Pouch / Device label	Label	EN1.2	2025-10-11	ST08003W-Label
Reagent bottle labels	Label	EN1.2	2025-10-11	ST08003W-Label
Lysis buffer	Label	EN1.2	2025-10-11	ST08003W-Label
Instructions for Use (IFU)	Instructions for use	EN1.4	2025-10-11	ST08003W-IFU

Labels

1. Kit Box


Cowin Gene
Taizhou Cowingene Biotech Co.,Ltd
Manufacturer Address:
No.28, Xinlin Road, Taizhou City,
Jiangsu Province 225300, China
Tel: +86 0523-88350768
Website: www.cowingene.com
Cowin Gene
Cowin Gene

2.Label

1.2. Label for kit 24 tests/kit

NATBox® IVD

Cowingene Monkeypox Virus Typing Detection kit

REF **ST08003W**

YYYY-MM-DD

LOT **A+YYYYMMDD+NN***

YYYY-MM-DD

NATBox® IVD

Cowingene Monkeypox Virus Typing Detection kit

Contents:	
Cartridge	24 vials
Lysis Buffer	2 tubes × 5000μL
Positive Control	1tube (500μL/tube)
Negative Control	1tube (500μL/tube)
Transfer dropper (for lysis buffer)	24 pieces (400μL)
Transfer dropper (for sample)	24 pieces (100μL)
Package Insert	1

AI-ST08003W
Version No.: EN-V1.2

⚠**[Caution]**
1. This kit is only used for in vitro diagnosis, please read the instructions for use carefully before experiment.
2. For other contents, please refer to the instructions for use.
3. For professional use only.

AI-ST08003W
Version No.: EN-V1.2

*NN: Two-digit batch sequence number for that

2.2. Labels for components:

Positive Control
REF: ST08003W
LOT:
EXP:
Store: 2-8℃
Vol. 500μL
Taizhou Cowingene Biotech Co., Ltd. EN V1.2

Negative Control
REF: ST08003W
LOT:
EXP
Store: 2-8℃
Vol. 500μL
Taizhou Cowingene Biotech Co., Ltd. EN V1.2

Lysis Buffer
REF: ST08003W
LOT:
EXP:
Store: 2-8℃
Vol. 5000μL
Taizhou Cowingene Biotech Co., Ltd. EN V1.2

Transfer dropper (for lysis buffer)
Spec. 24 pieces (400μL)
REF: ST08003W
LOT:
EXP:
Taizhou Cowingene Biotech Co., Ltd. EN V1.2

Cartridge label on the cap

Transfer dropper (for sample)
Spec. 24 pieces (100μL)
REF: ST08003W
LOT:
EXP:
Taizhou Cowingene Biotech Co., Ltd. EN V1.2

Instructions for Use[3]

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

Cowin Gene

IVD

Version: EN-V1.4

Effective Date: 2025-10-11

Cowingene Monkeypox Virus Typing Detection kit

User Manual

For In Vitro Diagnostic Use only

Taizhou Cowingene Biotech Co.,Ltd
No.28, Xinlin Road, Taizhou City, Jiangsu Province 225300, China
Tel: +86 0523-88350768
http://www.cowingene.com

Table of Contents

[Product Name]

Cowingene Monkeypox Virus Typing Detection Kit

[Package Size]

24 tests/kit (Cat. No. ST08003W)

[Intended Use]

This assay is a qualitative real-time PCR test to be performed on the NATBox System, Model: mini II (Taizhou Cowingene Biotech Co., Ltd.).

This assay can test G2R gene and C3L gene for monkeypox virus, E9L gene for orthopoxvirus in human lesion materials (swabs of surface or exudate, or crusts) from individuals presenting with signs and symptoms consistent with mpox and/or with relevant epidemiological risk factors. The function of the assay is for the aid in the diagnosis of Monkeypox virus or Orthopoxvirus infections and in differentiating between MPXV Clade I, MPXV Clade II, and other Orthopoxviruses.

Cowingene Monkeypox Typing Detection Kit is intended for use in a clinical laboratory by trained professionals.

[Background]

Orthopoxviruses (OPXV) are very large (200 nm like small bacteria), brick-shaped ds DNA viruses with a genome of ~ 200 kb. They cause febrile illnesses with prominent vesicular rash. Orthopoxviruses that are known to cause disease in humans include smallpox, vaccinia virus, cowpox, and monkeypox.[1]

Monkeypox virus (MPXV) is a zoonotic virus in the family Poxviridae, genus Orthopoxvirus. The virus causes a disease in humans that is similar to smallpox, but results in a lower case-fatality rate. With the eradication of smallpox and widespread discontinuation of smallpox vaccination, human monkeypox has re-emerged as a human health threat with major outbreaks occurring in 1996-1997 and 2022. More recently, MPXV was found to be the cause of a cluster of cases of disease in many countries. There are two known clades of MPXV: clade I, previously known as the Congo Basin clade; and clade II, previously called the West African clade.[2]

[Principles of the Procedure]

The Monkeypox Virus Typing Detection Kit is performed on the NATBox System. It is an automated in vitro diagnostic test system.

The NATBox Systems automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequences in samples. The systems require the use of

single-use cartridges that hold the PCR reagents and host the PCR process. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the system, please see the product manual of NATBox System.

The Monleypox Virus Typing Detection Kit uses PCR combined with Taqman fluorescent probes. It contains specific primers, and specific probes labeled with different fluorescent groups for the detection, which are designed to target the G2R gene for monkeypox Clade II and C3L gene for monkeypox Clade I, E9L gene for orthopoxvirus. In the process of amplification, the specific primers and probes were combined with the target sequence. During the amplification process, complete synchronization of PCR product formation and fluorescence signal accumulation is achieved based on the polymerase activity and 5'-3' exonuclease activity of the Taq DNA polymerase. Meanwhile, the human β-globin gene was set as an internal control for sample collection, nucleic acid extraction, and PCR amplification for overall quality control.

[Materials Provided]

Kit components	Reagent ingredients	Quantity per kit
Cartridge	Primers, probes, tris, dNTP, polymerase, etc.	24 vials
Lysis Buffer	Guanidine salt, tris, magnetic bead, etc.	2 tubes × 5000μL
Positive control	Recombinant plasmid containing G2R gene, C3L gene, E9L gene and β-globin gene target sequence	1tube (500μL/tube)
Negative control	Recombinant plasmid containing β-globin gene target sequence	1tube (500μL/tube)
Transfer dropper (for lysis buffer)	/	24 pieces (400μL)
Transfer dropper (for sample)	/	24 pieces (100μL)
Package insert	/	1

Note: Do not mix reagents from different batches.

[Material Required (but not provided)]

Yocon VTM (MT0301)

[Storage and Stability]

Product can be stored at 2-8℃ for 12 months. The reagent has been tested to be stable under the

simulated shipping conditions at 2-8°C for 15 days. Simulated transportation stability has not been verified for a duration longer than 15 days.

Note: For the detailed expiration date, please refer to specific product label.

[Applicable Instrument]

NATBox System, Model: mini II by Taizhou Cowingene Biotech Co., Ltd.

[Precautions]

1. This kit is intended for use by trained clinical laboratory professionals in a clinical laboratory setting.
2. Laboratory personnel must receive specific training for this assay and laboratory management shall comply with national regulations.
3. Wear appropriate personal protective equipment (PPE), including lab coats and gloves. Change gloves between handling of each specimen to prevent cross-contamination.[3]
4. Follow your institution's environmental waste procedures for proper disposal. Used cartridges and unused reagents may require handling as chemical or biohazardous waste per national/regional regulations or WHO guidelines.

[Warnings]

1. For *in vitro* diagnostic use ONLY.
2. For Professional use ONLY.
3. Do not use beyond the expiration date. Use of expired product may yield invalid results.
4. Do not mix or use reagents from different lots. Pooling reagents may lead to false results.
5. Do not use a cartridge if it is damaged, leaking, appears wet, or if the lid seal is broken. Do not reuse spent cartridges or disposable pipettes. Compromised or reused components may cause incorrect results or instrument malfunction.
6. All biological specimens, used cartridges, and transfer devices should be treated as capable of transmitting infectious agents.[4]
7. Sample types, proper sample collection, storage, and transport are critical; deviations may lead to false results.[5]
8. Do not open the cap of PCR tube after detection, thus to prevent pollutant contamination.

[Specimen]

Specimen collection

Cowingene monkeypox typing detection kit is applicable to lesion material (swabs of surface or exudate, or crusts). Sample collection and handling procedures are recommended to refer to the CDC Guidelines for Collecting and Handling Specimens for Monkeypox Virus (MPXV) Testing.

https://www.cdc.gov/monkeypox/hcp/diagnosis-testing/collecting-specimens.html

Specimen Storage

The sample to be tested should be stored at 2-8℃ for no more than 7 days, below -18℃ for no more than 6 months, and below -70℃ for a long time. The number of repeated freezing and thawing should be no more than 5 cycles.

[Assay Procedure]

Instrument Preparation

1. Turn on the power, the indicator light flashes red and green alternately. After the self-test is completed, the indicator light turns blue.
2. Open the software, search for the instrument via bluetooth automatically, then you can connect the instrument by clicking the correct serial number.
3. The indicator light flashes blue, indicating that the instrument has been successfully connected.

Cartridge Preparation

1. Take out the cartridge to room temperature (15-25°C) and equilibrate the cartridge for 5 minutes.

Sample Testing

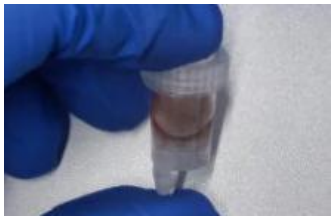
1. Before testing, if the sample is stored below -18°C, transfer it to 2~8°C until completely thawed, then proceed with the test.
2. Open the lid of the cartridge, using the Lysis Buffer Dropper (400 μL mark), add 400 μL of Lysis Buffer to the cartridge.
3. Using the Sample Dropper (100 μL mark), add 100 μL of sample to the cartridge.

Note: Ensure each dropper is used only for its designated liquid to prevent contamination.

4. Close the cartridge lid and mix well.

1. Add 400μL of lysis buffer

2. Add 100μL of sample



3. Close the lid and mix well

4. Put cartridge in instrument

Note:

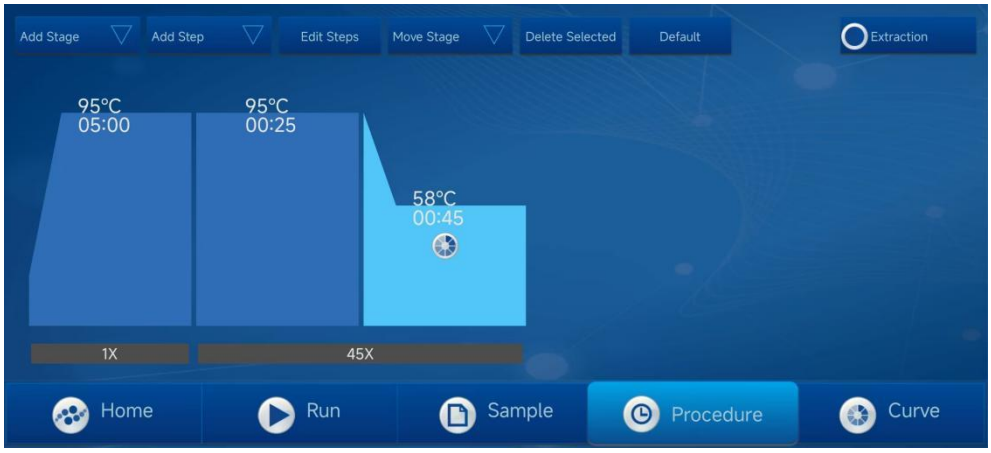
(1) After opening the caps of the lysis buffer, positive control, and negative control, these reagents can be stored at 2-8 ℃ for up to 30 days.

(2) Once the lysis buffer is added to the cartridge, the assembled cartridge may be stored at room temperature (15-25 ℃) for no more than 30 minutes.

(3) If the cartridge is not used within the specified time under either of these conditions, it shall be disposed of as medical waste.

5. Select the required module, then scan the QR code on the package.

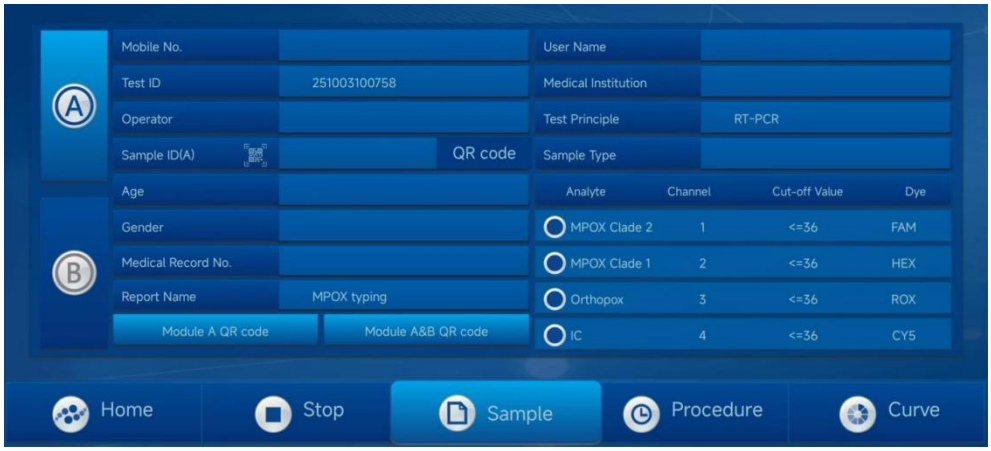
6. After the QR Code is scanned, the Protocol is identified by the software automatically.



7. Press Run to start the test.

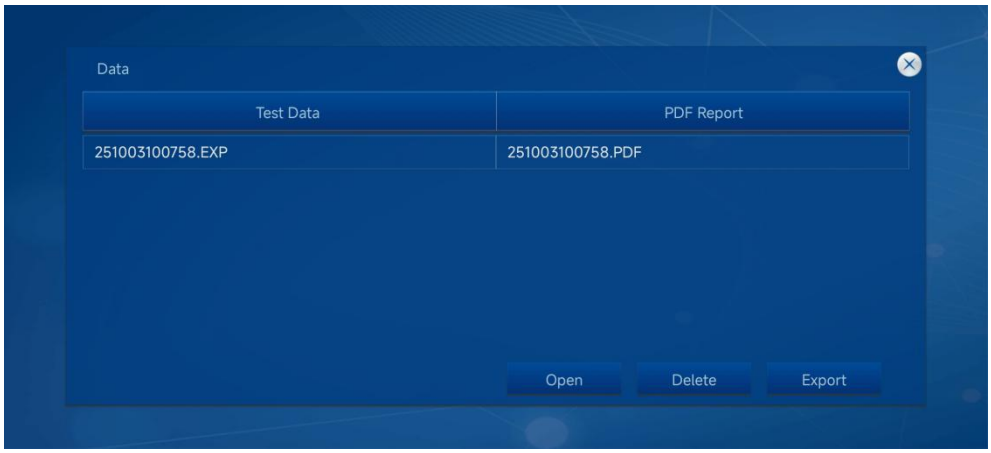
Note: In the amplification process, you can click the [Sample] button to edit the details. You can

enter the Sample ID, Negative Control or Positive Control in the blank of Samp ID to identify.



8. After the test is completed, click [Data] on the homepage

9. The results can be automatically analyzed and saved with no time limit, and can be viewed at any time.



Control Testing

It is recommended that each testing batch include one positive and one negative control. If questionable results are obtained, controls should be tested again.

1. Take out the cartridge to to room temperature (15-25°C) and equilibrate the cartridge for 5

minutes.

2. Open the lid of the cartridge, using the Lysis Buffer Dropper (400 μL mark), add 400 μL of Lysis Buffer to the cartridge.
3. Using the Sample Dropper (100 μL mark), add 100 μL of control to the cartridge.

Note: The remaining steps are identical to the sample testing procedure. The expected outcomes are detailed in Interpretation of the test results Section.

[Interpretation of the test results]

The results are interpreted by the software **automatically**.

Quality Control

This detection kit provides internal quality control and external quality control.

Internal quality control

The internal control is the human housekeeping (β-globin) gene to evaluate whether the target DNA sequence is present in the specimen or not. If the result for a specimen is target virus DNA not detected, the Ct value of the internal control must be positive (Ct≤ 36), otherwise the result of that specimen is inconclusive; if the result for a specimen is target virus DNA detected, the result of the internal control can be negative.

In case of a negative internal control result, it may be induced by improper sample collection, cartridge or instrument failure.

External quality control

The external quality control of this kit includes positive control and negative control, respectively. The results of Negative Control and Positive are interpreted by the software automatically.

Positive control constitutes of recombinant plasmid solution containing target viral sequences of G2R gene (FAM) , C3L gene (VIC) and E9L gene (ROX) as well as an internal control sequence containing of human β-globin (Cy-5). The positive control is used for monitoring the effectiveness of the cartridge and to exclude the possible influence of PCR instrument failure.

The negative control constitutes of recombinant plasmid solution containing the internal reference sequence only, and it is subsequently used for monitoring the factor of environment or reagent contamination.

Table 6 Quality control expected result

Control type	Detection Channel	Target Gene	Expected result

Positive control	FAM	G2R Gene (MPXV Clade II)	Positive
	VIC	C3L Gene (MPXV Clade I)	Positive
	ROX	E9L Gene (Orthopoxvirus)	Positive
	Cy5	β-globin (IC)	Positive
Negative control	FAM	G2R Gene (MPXV Clade II)	Negative
	VIC	C3L Gene (MPXV Clade I)	Negative
	ROX	E9L Gene (Orthopoxvirus)	Negative
	Cy5	β-globin (IC)	Positive

Examination and interpretation of patient specimen results

Table 7 Clinical sample interpretation

MPXV Clade I Positive	OPXV is positive MPXV Clade I is positive MPXV Clade II is negative IC can be positive or negative
MPXV Clade II Positive	OPXV is positive MPXV Clade I is negative MPXV Clade II is positive IC can be positive or negative
Other OPXV Positive	OPXV is positive MPXV Clade I is negative MPXV Clade II is negative IC can be positive or negative
Negative	OPXV is negative MPXV Clade I is negative

	MPXV Clade II is negative IC must be positive
Invalid	OPXV is negative MPXV Clade I is negative MPXV Clade II is negative IC is negative

Troubleshooting

Invalid Result: A retest is required. If the retest result is also invalid, a new sample must be collected (re-sampling).

No Result: This status applies in cases of process interruption (e.g., power failure or instrument malfunction). A retest should be performed. If the retest fails again, please contact Cowingene Service.

Abnormal External Control Results: For example, if the negative control yields a "positive" result, it indicates potential contamination. In this case, all testing must be halted immediately, and the workspace must be decontaminated.

[Limitations]

1. The test results of this kit are for clinical reference only and should not be used as the sole basis for diagnosis. Clinical decisions should be made in conjunction with the patient's symptoms/signs, medical history, other laboratory test results, and treatment response.

2. Potential causes of false negative results include, but are not limited to:

   a. Improper sample collection, transport, or processing.

   b. Low viral load in the specimen.

   c. Sequence variations or mutations in the target region.

   d. Sampling timing for monkeypox virus detection; therefore, sampling from multiple sites or at multiple time points may reduce the risk of false negatives.

   e. Interference from endogenous substances or exogenous substances.

   f. Presence of PCR inhibitors in the specimen.

3. The internal control (IC) is included to monitor extraction efficiency and PCR inhibition. Failure of the IC may indicate poor specimen quality, inadequate nucleic acid extraction, or the presence of PCR inhibitors. In cases of high-positive specimens, partial or full suppression of the IC signal may occur and does not necessarily invalidate the result.

4. Deviations from recommended storage conditions or handling procedures-such as exceeding specified storage temperatures or durations, or subjecting specimens to multiple freeze-thaw

cycles–may adversely affect test performance.

5. The use of specimen types not validated for this assay, as well as pipetting errors or other operator-related inaccuracies, may compromise result reliability.

[Analytical Performance Characteristics]

1. Limit of Detection (Analytical Sensitivity)

Virus	LoD
Monkeypox virus Clade II	100 copies/mL
Monkeypox virus Clade I	100 copies/mL
Orthopoxvirus	100 copies/mL

2. Analytical Specificity

2.1. Inclusivity: In silico analysis for targets

Table 8 Bioinformatics Analysis of C3L Gene (Monkeypox virus Clade I)

Subtype	Mutation on forward primer	Mutation on probe	Mutation on reverse primer
Monkeypox virus clade Ia	×	×	×
Monkeypox virus clade Ib	×	×	×

No discernible discrepancies were identified following a in silico study.

Table 9 Bioinformatics Analysis of G2R Gene (Monkeypox virus Clade II)

	Mutation on forward primer	Mutation on probe	Mutation on reverse primer
Monkeypox virus clade IIb	×	×	×

No discernible discrepancies were identified following a in silico study.

Table 10 Bioinformatics Analysis of E9L Gene (Orthopoxvirus)

	GenBank	Forward primer	Probe	Reverse primer
Vaccinia virus	NC_006998	100%	100%	100%
Cowpox virus	X94355.2	100%	100%	100%
Ectromelia virus	NC_004105.1	100%	97%	100%
Camelpox virus	MK910851.1	100%	93%	100%
Buffalopox	MG599038.1	100%	100%	95%

The sequence alignment results between the E9L primer-probe region used in this kit and the corresponding regions of non-variola orthopoxviruses are as follows: the probe region shows >90%

homology with the probe regions of non-variola orthopoxviruses, thus demonstrating its ability to amplify all non-variola orthopoxviruses.

2.2. Exclusivity: In silico analysis for cross-reactivity

Table 11 Bioinformatics Analysis of C3L Gene (Monkeypox virus Clade I)

	GenBank	Forward primer	Probe	Reverse primer
Variola virus	PP405598	100%	90%	100%
Vaccinia virus	NC_006998	100%	90%	100%
Cowpox virus	X94355.2	91%	90%	100%
Ectromelia virus	NC_004105.1	95%	75%	100%
Camelpox virus	MK910851.1	100%	90%	100%
Buffalopox	MG599038.1	100%	90%	100%
Parapox virus	HM133903.1	<80%	<80%	<80%
Molluscum contagiosum virus	OQ401160.2	<80%	<80%	<80%
Herpes Simplex1/2	GCA_027935665.1	<80%	<80%	<80%
Varicella-zoster virus	DQ457052.1	<80%	<80%	<80%
Streptococcus mitis	GCF_001281025.1	<80%	<80%	<80%
Staphylococcus aureus	BX571856.1	<80%	<80%	<80%
Staphylococcus epidermidis	JQ312423.1	<80%	<80%	<80%
Streptococcus pyogenes	AE009949.1	<80%	<80%	<80%
Streptococcus agalactiae	GCA_001552035.1	<80%	<80%	<80%
Pseudomonas aeruginosa	CP007224.1	<80%	<80%	<80%
Trichophyton rubrum	GCA_910591905.1	<80%	<80%	<80%
Corynebacterium jeikeium	GCA_028609885.1	<80%	<80%	<80%
Candida albicans	GCA_041438225.1	<80%	<80%	<80%
Human genomic DNA	GCA_046332035.1	<80%	<80%	<80%
Lactobacillus species	GCA_018852615.3	<80%	<80%	<80%
Escherichia coli	GCA_002853715.1	<80%	<80%	<80%
Acinetobacter calcoaceticus	GCA_900444805.1	<80%	<80%	<80%
Bacteroides fragilis	GCA_000025985.1	<80%	<80%	<80%

Enterococcus faecalis	GCA_000393015.1	<80%	<80%	<80%
Streptococcus Group C		<80%	<80%	<80%
Streptococcus Group G		<80%	<80%	<80%
Corynebacterium diptheriae	GCA_000011325.1	<80%	<80%	<80%
Neisseria gonorrhoeae	GCA_013030075.1	<80%	<80%	<80%
Chlamydia trachomatis	GCA_004135145.1	<80%	<80%	<80%
Mycoplasma pneumoniae	GCA_900660465.1	<80%	<80%	<80%
Mycoplasma genitalium	GCA_040556925.1	<80%	<80%	<80%
Human Papillomavirus	GCA_027935665.1	<80%	<80%	<80%
Trichomonas vaginalis	GCA_002891335.1	<80%	<80%	<80%
Treponema pallidum	GCA_000008605.1	<80%	<80%	<80%

Table 12 Bioinformatics Analysis of G2R Gene (Monkeypox virus Clade II)

	GenBank	Forward primer	Probe	Reverse primer
Variola virus	PP405598	95%	80%	91%
Vaccinia virus	NC_006998	95%	76%	95%
Cowpox virus	X94355.2	90%	76%	95%
Ectromelia virus	NC_004105.1	<80%	<80%	<80%
Camelpox virus	MK910851.1	90%	84%	100%
Buffalopox	MG599038.1	95%	76%	100%
Parapox virus	HM133903.1	<80%	<80%	<80%
Molluscum contagiosum virus	OQ401160.2	<80%	<80%	<80%
Herpes Simplex1/2	GCA_027935665.1	<80%	<80%	<80%
Varicella-zoster virus	DQ457052.1	<80%	<80%	<80%
Streptococcus mitis	GCF_001281025.1	<80%	<80%	<80%
Staphylococcus aureus	BX571856.1	<80%	<80%	<80%
Staphylococcus epidermidis	JQ312423.1	<80%	<80%	<80%
Streptococcus pyogenes	AE009949.1	<80%	<80%	<80%
Streptococcus agalactiae	GCA_001552035.1	<80%	<80%	<80%
Pseudomonas aeruginosa	CP007224.1	<80%	<80%	<80%

Trichophyton rubrum	GCA_910591905.1	<80%	<80%	<80%
Corynebacterium jeikeium	GCA_028609885.1	<80%	<80%	<80%
Candida albicans	GCA_041438225.1	<80%	<80%	<80%
Human genomic DNA	GCA_046332035.1	<80%	<80%	<80%
Lactobacillus species	GCA_018852615.3	<80%	<80%	<80%
Escherichia coli	GCA_002853715.1	<80%	<80%	<80%
Acinetobacter calcoaceticus	GCA_900444805.1	<80%	<80%	<80%
Bacteroides fragilis	GCA_000025985.1	<80%	<80%	<80%
Enterococcus faecalis	GCA_000393015.1	<80%	<80%	<80%
Streptococcus Group C		<80%	<80%	<80%
Streptococcus Group G		<80%	<80%	<80%
Corynebacterium diptheriae	GCA_000011325.1	<80%	<80%	<80%
Neisseria gonorrhoeae	GCA_013030075.1	<80%	<80%	<80%
Chlamydia trachomatis	GCA_004135145.1	<80%	<80%	<80%
Mycoplasma pneumoniae	GCA_900660465.1	<80%	<80%	<80%
Mycoplasma genitalium	GCA_040556925.1	<80%	<80%	<80%
Human Papillomavirus	GCA_027935665.1	<80%	<80%	<80%
Trichomonas vaginalis	GCA_002891335.1	<80%	<80%	<80%
Treponema pallidum	GCA_000008605.1	<80%	<80%	<80%

Table 13. Bioinformatics Analysis of E9L Gene (Orthopoxvirus)

	GenBank	Forward primer	Probe	Reverse primer
Parapox virus	HM133903.1	<80%	<80%	<80%
Molluscum contagiosum virus	OQ401160.2	<80%	<80%	<80%
Herpes Simplex1/2	GCA_027935665.1	<80%	<80%	<80%
Varicella-zoster virus	DQ457052.1	<80%	<80%	<80%
Streptococcus mitis	GCF_001281025.1	<80%	<80%	<80%
Staphylococcus aureus	BX571856.1	<80%	<80%	<80%
Staphylococcus epidermidis	JQ312423.1	<80%	<80%	<80%

Streptococcus pyogenes	AE009949.1	<80%	<80%	<80%
Streptococcus agalactiae	GCA_001552035.1	<80%	<80%	<80%
Pseudomonas aeruginosa	CP007224.1	<80%	<80%	<80%
Trichophyton rubrum	GCA_910591905.1	<80%	<80%	<80%
Corynebacterium jeikeium	GCA_028609885.1	<80%	<80%	<80%
Candida albicans	GCA_041438225.1	<80%	<80%	<80%
Human genomic DNA	GCA_046332035.1	<80%	<80%	<80%
Lactobacillus species	GCA_018852615.3	<80%	<80%	<80%
Escherichia coli	GCA_002853715.1	<80%	<80%	<80%
Acinetobacter calcoaceticus	GCA_900444805.1	<80%	<80%	<80%
Bacteroides fragilis	GCA_000025985.1	<80%	<80%	<80%
Enterococcus faecalis	GCA_000393015.1	<80%	<80%	<80%
Streptococcus Group C		<80%	<80%	<80%
Streptococcus Group G		<80%	<80%	<80%
Corynebacterium diptheriae	GCA_000011325.1	<80%	<80%	<80%
Neisseria gonorrhoeae	GCA_013030075.1	<80%	<80%	<80%
Chlamydia trachomatis	GCA_004135145.1	<80%	<80%	<80%
Mycoplasma pneumoniae	GCA_900660465.1	<80%	<80%	<80%
Mycoplasma genitalium	GCA_040556925.1	<80%	<80%	<80%
Human Papillomavirus	GCA_027935665.1	<80%	<80%	<80%
Trichomonas vaginalis	GCA_002891335.1	<80%	<80%	<80%
Treponema pallidum	GCA_000008605.1	<80%	<80%	<80%

2.3. Interfering substances

It was evaluated that whether the adding of albumin, purified mucin, blood, douche, urine, vagisil cream, feces and seminal fluid with a certain concentration in the simulated sample matrix would affect the test results or not, and the statistical results are as follows:

Table 14 Interfering substances test results

Interfering Substances	Concentration of Interfering Substances	Result
Acyclovir	7mg/mL	Unaffected

Albumin	≥2.2mg/mL	Unaffected
Benadryl cream/ointment*	5%(w/v)	Unaffected
Benzocaine containing local anesthetic	5% (v/v)	Unaffected
Blood/EDTA	5%(v/v)	Unaffected
Casein	25mg/mL	Unaffected
Cornstarch	2.5mg/mL	Unaffected
Docosanol containing cold sore treatment	5% (v/v)	Unaffected
Douche	7%(w/v)	Unaffected
Feces	0.22%(w/v)	Unaffected
Female urine	10% (v/v)	Unaffected
Hydrocortisone cream	7%(w/v)	Unaffected
Lidocaine containing cream	7%(w/v)	Unaffected
Lubricant	7%(w/v)	Unaffected
Male urine	10% (v/v)	Unaffected
Mucin	60 μg/mL	Unaffected
Neosporin	5% (w/v)	Unaffected
Petrolatum containing lip	7%(w/v)	Unaffected
Seminal fluid	7% (v/v)	Unaffected
Zinc Oxide ointment	7% (w/v)	Unaffected

[Clinical Evaluation]

1. Natural Samples

1.1. Compared with PCR Kit:

Table 15 Results of Monkeypox Clade II

Investigational Kit	Comparator		
	Positive (+)	Negative (-)	Total
Positive (+)	80	1	81
Negative (-)	0	128	128
Total	80	129	209
PPA	100.00%	95%CI (95.42%-100.00%)	
NPA	99.22%	95%CI (95.74%-99.86%)	
OPA	99.52%	95%CI (97.34%-99.92%)	
Kappa	0.990		

1.2. Compared with Sanger Sequencing:

Table 16 Results of Monkeypox Clade II

Investigational Kit	Sequencing		
	Positive (+)	Negative (-)	Total
Positive (+)	81	0	81
Negative (-)	0	128	128
Total	81	128	209
PPA	100.00%	95%CI (95.47%-100.00%)	
NPA	100.00%	95%CI (97.09%-100.00%)	
OPA	100.00%	95%CI (98.20%-100.00%)	
Kappa	1		

2. Contrived Samples

Table 17 Test Results of Contrived Monkeypox Clade I Positive Samples

Concentration	Positive Percent Agreement (PPA)
100 Copies/mL	100% (15/15)
300 Copies/mL	100% (5/5)
500 Copies/mL	100% (5/5)
700 Copies/mL	100% (5/5)

[Reference]

1. World Health Organization. WHO Monkeypox Fact Sheet. https://www.who.int/news-room/fact-sheets/detail/mpox Accessed January 17, 2023.
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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.
4. 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.
5. Clinical and Laboratory Standards Institute (CLSI). Validation and Verification of Multiplex Nucleic Acid Assays. 2nd ed. CLSI guideline MM17. Wayne, PA: CLSI. 2018.

[Contact Details]

Registrant / Manufacturer's Name: Taizhou Cowingene Biotech Co., Ltd

Address: No.28, Xinlin Road, Taizhou City, Jiangsu Province 225300, China

Website: www.cowingene.com

Name of after-sales service unit: Taizhou Cowingene Biotech Co., Ltd

Contact: +86 15996019208

[Language edition]

For the requirements of Instruction for Use in other languages, please contact Cowingene.

[Release date of the user manual]

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[Definition of Symbols]

No.	Symbols	Explanation of Symbols
1		Manufacturer

2		In Vitro Diagnostic Medical Device
3		Use By
4		Batch code
5		Reference number
6		Date of manufacture
7		Temperature limitation
8		SUFFICIENT FOR <N> TESTS
9		Consult instructions for use
10		KEEP AWAY FROM SUNLIGHT
11		KEEP DRY