Mpox disease Emergency Use Listing Procedure (EUL) for IVDs
Product: Monkeypox Virus Real-Time PCR Diagnostic Kit (NAT-17WE)
EUL Number: MPXV-13345-14133-00
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 Real-Time PCR Diagnostic Kit (NAT-17WE), with product codes NAT-17WE (L), NAT-17WE(M), and NAT-17WE(S), Rest-of-World regulatory version manufactured by HA TECH PTY LTD, located at Unit 2, 3 Packard Avenue, Castle Hill, NSW 2154, Australia, was listed as eligible for WHO procurement on 17 October 2025.

Intended use:

According to the claim of intended use from HA TECH PTY LTD., *"The Monkeypox (Mpox) Virus Real-Time PCR Diagnostic Kit (NAT-17) is a real-time polymerase chain reaction (PCR) test intended for the qualitative detection of Clade-Specific DNA from Mpox virus in individuals suspected of Mpox by their healthcare provider, aiding the diagnostic of Mpox virus infection. The Mpox Virus Real-Time PCR Diagnostic Kit (NAT-17) is for use with samples such as skin swabs, lesion and crusts stored in viral transport media. DNA extract solely from whole blood alone is not a suitable specimen for Mpox virus diagnostic testing as the viremic phase may have already passed at the time of rash onset.*

Positive results are indicative of the presence of either Mpox Clade Ia, Ib, or 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 Mpox Virus 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 Mpox Virus Real-Time PCR Diagnostic Kit (NAT-17) is intended for manual use by qualified clinical laboratory personnel specifically trained in the techniques of PCR and in vitro diagnostic procedures.

For in vitro diagnostic use."

Validated specimen type:
Human skin, lesion and crusts swabs collected using polyester, nylon, or Dacron swabs with plastic, wood, or thin aluminium (wire) shafts.

Test kit contents:

Type of reagent	*NAT-17WE (L)*	*NAT-17WE (M)*	*NAT-17WE (S)*
NAR01 PCR Mastermix	1 x 1000 µL	1 x 500 µL	1 x 250 µL
NAR24 PCR Multiple Mix	1 x 500 µL	1 x 250 µL	1 x 125 µL
NAR25 PCR positive control	1 x 50 µL	1 x 40 µL	1 x 30 µL
NAR05 PCR negative control	1 x 1000 µL	1 x 5000 µL	1 x 250 µL

Items required but not provided:

Equipment required

1. Real-Time PCR Thermal Cyclers with FAM, HEX, ROX and Cy5 Channels, or equivalent. The PCR instruments that are validated with the assay: Bio-Rad CFX96 (CFX Maestro Software 2.3), Bio Molecular Systems Mic qPCR (Mic V-IVD v1.0.2) and QuantStudio 5 Dx Real-Time PCR (Design & Analysis 2.6.0).
2. Viral DNA/ RNA Extraction system, or equivalent. The extraction system that is validated with the assay: KingFisher Flex Purification System (ThermoFisher Scientific).
3. Class II Biological Safety Cabinet (BSC)
4. Centrifuge
5. Vortex
6. Micropipettes
7. Refrigerator (2-8 ℃) and Freezer (preferably ≤-20 ℃)

Other equipment and consumables required but not provided:

a) Sample collection and nucleic acid extraction materials

1. Sterile, synthetic swabs (including but not limited to polyester, nylon, or Dacron) with plastic, wood, or thin aluminium (wire) shafts.
2. Sterile collection tubes.
3. DNA Extraction reagents.

E.g. ThermoFisher MagMAX Prime Viral/Pathogen NA Isolation Kit (Cat. A58145).

b. Other reagents and consumables required but not included with the test:

1. Nuclease free water, anhydrous ethanol, anhydrous isopropanol.
2. 96-well plates or PCR tubes, pipette tips with filters, and microcentrifuge tubes
3. Liquid waste container, solid waste bag and container.
4. Double-layer latex gloves, waterproof boot covers, protective clothing, safety

glasses, and masks with high filtration efficiency.

Storage:
The test kit must be stored at 20 ± 5 °C, protected from light.

Shelf-life upon manufacture:
The shelf life is currently assigned a 6-month dating period.

Warnings/limitations:

Please refer to the Instructions for Use attached to this public assessment report.

Product dossier assessment

HA TECH PTY LTD. submitted the product dossier for the Monkeypox Virus Real-Time PCR Diagnostic Kit (NAT-17WE) in 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, HA TECH PTY 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, HA TECH PTY 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

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

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].

HA TECH PTY 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 Real-Time PCR Diagnostic Kit (NAT-17WE), with product codes NAT-17WE (L), NAT-17WE(M), and NAT-17WE(S), manufactured by HA TECH PTY LTD., is eligible for WHO procurement for 12 months from the day of listing. The assay detects nucleic acid from monkeypox virus, including clades Ia, Ib, 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, HA TECH PTY LTD must engage in post-market surveillance activities to ensure that the product continues to meet safety, quality, and performance requirements. HA TECH PTY 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 Monkeypox Virus Real-Time PCR Diagnostic Kit (NAT-17WE) 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.

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

Controlled Labelling References

Document Type	Document Title	Version / Revision	Date Approved	Controlled Document No.
Outer box artwork	[NAT-17WE Product Labelling - Artwork Proof]	[v 1.0]	[02 Oct 2025]	[CC-68, File Note_NAT-17-04Oct25]
Pouch / Device label (L)	[N17WEB.01 & N17WEAL.01]	[v 1.0]	[02 Oct 2025]	[CC-68, File Note_NAT-17-04Oct25]
Pouch / Device label (M)	[N17WEB.01 & N17WEAM.01]	[v 1.0]	[02 Oct 2025]	[CC-68, File Note_NAT-17-04Oct25]
Pouch / Device label (S)	[N17WEB.01 & N17WEAS.01]	[v 1.0]	[02 Oct 2025]	[CC-68, File Note_NAT-17-04Oct25]
Reagent bottle labels	[NAR-05.01, NAR-01.01, NAR-24.01, NAR-25.01]	[v 1.0]	[02 Oct 2025]	[CC-68, File Note_NAT-17-04Oct25]
Instructions for Use (IFU)	[IFU-39WE]	[v2.0.X]	[04 Oct 2025]	[CC-68, File Note_NAT-17-04Oct25]
Product specification sheet	[PI-N17WE]	[v 1.0]	[02 Oct 2025]	[CC-68, File Note_NAT-17-04Oct25]

Labels

Pouch Back Label 100mm

Mpox Virus Real-Time PCR Diagnostic Kit (NAT-17WE)

LOT NAT-17WE-250801

2025 - 08 - 07

2027 - 08 - 07

98mm

HA Tech Pty Ltd
Unit 2 / 3 Packard Avenue
Castle Hill
NSW 2154
Australia
ha-tech-ltd.com

N17WEB.01

*NB: UDI-DI numbers (01) - are complimented with example test batch, manufacture date & expiry date to demonstrate SG1 data matrix

Pouch Front Label (L) 96 tests* 100mm

HA TECH
BioPharma & Diagnostics

Mpox Virus Real-Time PCR Diagnostic Kit (NAT-17WE)

Kit contains sufficient reagents for 96 tests (20µL reaction volume)

Master Mix (NAR-01), 2x	1 x 1000µL
Multiplex Mix (NAR-24), 4x	1 x 500µL
Positive Control (NAR-25), 4x	1 x 50µL
Negative Control (NAR-05)	1 x 1000µL

98mm

REF NAT-17WE(L)

-15°C
-25°C

(01)9359778000158
(10)NAT-17WE-250801
(11)070825
(17)070827

AUSTRALIAN MADE AND OWNED

N17WEAL.01

Pouch Front Label (M) 48 tests* 100mm

HFR TECH
BioPharma & Diagnostics

Mpox Virus Real-Time PCR Diagnostic Kit (NAT-17WE)

Kit contains sufficient reagents for 48 tests (20µL reaction volume)

Master Mix (NAR-01), 2x	1 x 500µL
Multiplex Mix (NAR-24), 4x	1 x 250µL
Positive Control (NAR-25), 4x	1 x 450µL
Negative Control (NAR-05)	1 x 500µL

98mm

REF NAT-17WE(M)

-15°C
-25°C

(01)9359778000400
(10)NAT-17WE-250801
(11)070825
(17) 070827

AUSTRALIAN MADE AND OWNED

N17WEAM.01

Pouch Front Label (S) 24 tests* 100mm

HFR TECH
BioPharma & Diagnostics

Mpox Virus Real-Time PCR Diagnostic Kit (NAT-17WE)

Kit contains sufficient reagents for 24 tests (20µL reaction volume)

Master Mix (NAR-01), 2x	1 x 250µL
Multiplex Mix (NAR-24), 4x	1 x 125µL
Positive Control (NAR-25), 4x	1 x 30µL
Negative Control (NAR-05)	1 x 250µL

98mm

REF NAT-17WE(S)

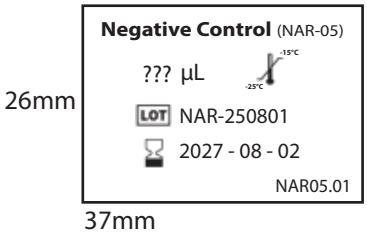
-15°C
-25°C

(01)9359778000394
(10)NAT-17WE-250801
(11)070825
(17)070827

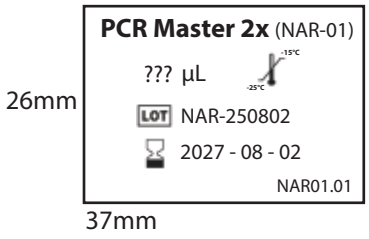
AUSTRALIAN MADE AND OWNED

N17WEAS.01

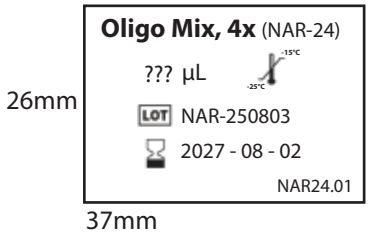
NAR-05.01 Negative Control FZV Label



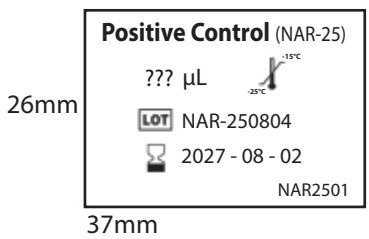
NAR-01.01 PCR Master Mix FZV Label



NAR-24.01 Oligo Mix FZV Label



NAR-25.01 Positive Control FZV Label



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.

Package Insert

NAT-17WE
Monkeypox Virus Real-Time PCR Diagnostic Kit (NAT-17WE)
Detection of Monkeypox (Mpox) Clades Ia, Ib, and II (a and b)

EN

Product size

96 test/Kit REF NAT-17WE(L)

48 test/Kit REF NAT-17WE(M)

24 test/ Kit REF NAT-17WE(S)

Accessory Products-
Analysis software
LUMEA™ – CFX 96
LUMEA™ – Quant Studio 5
LUMEA™ – MIC IVD

FOR PROFESSIONAL USE ONLY

Intended use

The HA TECH Mpox Virus PCR Diagnostic Kit is a real time PCR test (NAT-17WE) designed for the qualitative and specific detection of DNA from Mpox Clades Ia, Ib, and II (a and b) in human.
NAT-17WE kit is aid in the diagnosis of Mpox virus in human specimens such as lesion swab, exudate and crusts in VTM/UTM following CDC guidelines. Specimens that do not contain enough human DNA/RNA may lead to inconclusive PCR test results wherein re-testing is recommended, including nucleic acid extraction of the samples should be performed according to the corresponding requirements and procedures of the selected Viral DNA extraction kits.
NAT-17WE kit is intended for use by qualified clinical laboratory or hospitals reference laboratories personnel specifically trained in the techniques of PCR and in vitro diagnostic procedures.

2. Kit components

Table 1. Kit components of NAT-17WE [REF NAT-17WE (L)]

Cap colour	Components	Description	Quantity
Blue	NAR-01_PCR_Master Mix	Master mix including dNTPs, DNA polymerase and buffer	1 x 1000 µL
Brown	NAR-24_PCR_Multiplex Mix	Oligo mix including primers and probes for Mpox	1 x 500 µL
Green	NAR-25_PCR_Positive Control	Positive control including gene fragments for Mpox	1 x 50 µL
White	NAR-05_PCR_Negative Control	Nuclease Free Water	1 x 1000 µL

Table 2. Kit components of NAT-17WE [REF NAT-17WE (M)]

Cap colour	Components	Description	Quantity
Blue	NAR-01_PCR_Master Mix	Master mix including dNTPs, DNA polymerase and buffer	1 x 500 µL
Brown	NAR-24_PCR_Multiplex Mix	Oligo mix including primers and probes for Mpox	1 x 250 µL
Green	NAR-25_PCR_Positive Control	Positive control including gene fragments for Mpox	1 x 40 µL
White	NAR-05_PCR_Negative Control	Nuclease Free Water	1 x 500 µL

Table 3. Kit components of NAT-17WE [REF NAT-17WE (S)]

Cap colour	Components	Description	Quantity
Blue	NAR-01_PCR_Master Mix	Master mix including dNTPs, DNA polymerase and buffer	1 x 250 µL
Brown	NAR-24_PCR_Multiplex Mix	Oligo mix including primers and probes for Mpox	1 x 125 µL
Green	NAR-25_PCR_Positive Control	Positive control including gene fragments for Mpox	1 x 30 µL
White	NAR-05_PCR_Negative Control	Nuclease Free Water	1 x 250 µL

3. Instructions

Please refer to HA TECH NAT-17- Assay Manual (Reference No. IFU-39WE) for the detailed protocol. This document can be accessed by contacting info@ha-tech-ltd.com, or call HA-TECH Services at +61 (0) 431 581 133.

4. Shipping, Storage and Shelf-life

- All reagents should be stored at -20±5°C with protection from light. Avoid more than 6 freeze-thaw cycles.
- Expiration date is 24 months after production date. Do not use past expiry date.
- In-use stability has been validated for 2 weeks after opening of the reagent vials at 2-8 °C, can be stored at -20±5°C for up to 3 months.
- Any serious incident shall be reported to HA TECH by contacting info@ha-tech-ltd.com
- Shipping temperature below 8°C (with ice pack) for a maximum of 3 days for local shipment only. For international shipments, the product should be shipped at -20±5°C or use dry ice shipment.

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NAT-17WE

Monkeypox Virus Real-Time PCR Diagnostic Kit (NAT-17WE)

Detection of *M*onkeypox (Mpox) virus

EN

Product size

96 test/Kit REF NAT-17WE(L)

48 test/Kit REF NAT-17WE(M)

24 test/ Kit REF NAT-17WE(S)

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FOR PROFESSIONAL USE ONLY

Product
Monkeypox Virus Real-Time PCR Diagnostic Kit (NAT-17WE)

In this document, NAT-17 represents NAT-17WE. Both designations refer to the same product; the difference in nomenclature/ product code reflects labelling requirements of different regulatory authorities.

Size

24 test/Kit, 48 test/Kit, 96 test/Kit

Intended Use

The Monkeypox (Mpox) Virus Real-Time PCR Diagnostic Kit (NAT-17) is a real-time polymerase chain reaction (PCR) test intended for the qualitative detection of Clade-Specific DNA from Mpox virus in individuals suspected of Mpox by their healthcare provider, aiding the diagnostic of Mpox virus infection. The Mpox Virus Real-Time PCR Diagnostic Kit (NAT-17) is for use with samples such as skin swabs, lesion and crusts stored in viral transport media. DNA extract solely from whole blood alone is not a suitable specimen for Mpox virus diagnostic testing as the viremic phase may have already passed at the time of rash onset.

Positive results are indicative of the presence of either Mpox Clade Ia, Ib, or 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 Mpox Virus 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 Mpox Virus Real-Time PCR Diagnostic Kit (NAT-17) is intended for manual use by qualified clinical laboratory personnel specifically trained in the techniques of PCR and in vitro diagnostic procedures.

For *in vitro* diagnostic use.

Overview

Pathogen characteristics

Mpox is a rare disease that is caused by infection with Mpox Virus. Mpox Virus belongs to the *Orthopoxvirus* genus in the family *Poxviridae* and has a double-stranded DNA genome *(Benvenuto et al. 2022).* The *Orthopoxvirus* genus also includes variola virus (which causes smallpox), vaccinia virus (used in the smallpox vaccine) and other pox viruses such as cowpox virus.

There are two clades of Mpox virus: Clade I (previously Congo Basin/Central African) and Clade II (previously West African). The subclade Clade Ib comprises largely of the circulating variants of the 2024 global outbreak.

Symptomatology

Mpox symptoms are similar to those seen in the past in smallpox patients, although less severe, and can include a flu-like prodrome followed by a rash. Prodromal symptoms might not develop or can occur concurrently with or after rash onset, and may include fever, headache, muscle aches, swollen lymph nodes and fatigue.

The rash is often very painful and goes through different stages before healing completely. It often starts in a mucosal area, including the mouth, genital or rectal areas, and may remain in a limited area or become more widespread to the face, torso or extremities (including palms or soles). The initial rash has also been documented in other non-mucosal locations. Mpox rashes follow the classical progression from macules to papules, then vesicles and pustules until they crust over to become scabs. Patients may not experience the entire constellation of these symptoms.

The incubation period for Mpox is usually 7-14 days of exposure to the virus (but can range from 5-21). If someone has flu-like symptoms, they will usually develop a rash 1-4 days after onset of symptoms. Mpox can spread from the time symptoms start until the rash has fully healed and a fresh layer of skin has formed. The illness typically lasts 2-4 weeks and treatment is aimed at relieving symptoms with simple pain medicines and staying hydrated *(World Health Organization 2024).*

Transmission

Mpox spreads in different ways. The virus can spread from person-to-person through direct contact with the infectious rash, scabs, or body fluids. It also can be spread by respiratory secretions during prolonged face-to-face contact, or during intimate physical contact, such as kissing, cuddling, or sex. In addition, pregnant people can spread the virus to their foetus through the placenta.

Touching items (such as clothing or linens) that previously touched the infectious rash or body fluids is another way Mpox spreads. It's also possible for people to get Mpox from infected animals, either by being scratched or bitten by the animal or by eating meat or using products from an infected animal *(World Health Organization 2024)*.

Principles of the Assay

The HA TECH Mpox Virus PCR Diagnostic Kit is a real time PCR test designed for the qualitative and specific detection of DNA from Mpox Clades Ia, Ib, and II (a and b) in human biological samples including skin swabs and crusts stored in viral transport media (Ghate et al. 2023). The real-time PCR test is intended for use by qualified clinical laboratory personnel specifically trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.

The primers and probes in this assay are designed based on conserved regions from published Mpox Virus whole genome sequences on National Center for Biotechnology Information (NCBI). The kit comprises primers and probes targeting regions specific to subclades Ia and Ib, and for clade II. Both Clade I primer sets are directed at the C3L gene, with Clade Ib primers exploiting a deletion unique to this subclade. The Clade Ib primers span the deleted region, while the Clade Ia primers target the undeleted variant of the C3L gene. Additionally, primers targeting the OPG210 gene, along with a Centers for Disease Control and Prevention (CDC)-recommended set for the OPG002 gene, are integrated into the same fluorescence channel and will act as a pan-clade Mpox detector *(Li et al., 2010).* Therefore, detection of Mpox DNA using the non-clade-specific primers, coupled with negative results in the clade-specific assays, would suggest Clade II. The human RNaseP gene serves as an internal control, ensuring both sample integrity and PCR amplification validity.

Hydrolysis probes consist of a reporter fluorophore at the 5′ end and quencher at the 3′ end. Each dual-labelled probe can hybridize specifically with a part of the gene sequence target. The fluorescent signals emitted from reporter fluorophores are absorbed by the quenchers so intact probes emit a low signal. During amplification, the 5′ fluorophore from the probe will be cleaved by Taq polymerase via its 5′-3′ exonuclease activity and released from the 3′ quencher group, generating increased fluorescent signals. This fluorescence can be measured by a qPCR instrument which will then automatically draw a real-time amplification curve based on the signal change, realising the qualitative detection of Mpox.

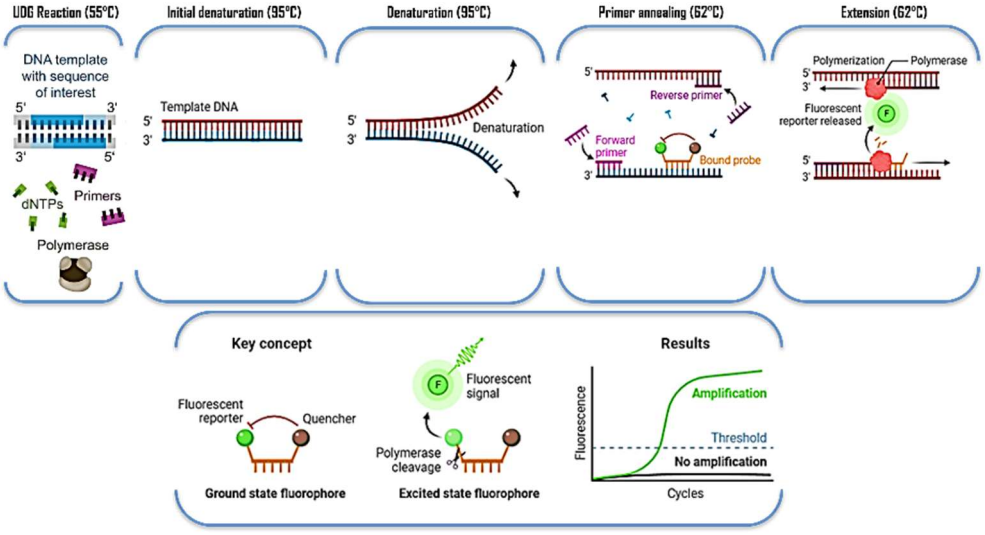

Figure 1. Schematic diagram of principles assay of PCR

Main components

There are three kit sizes available: 24 tests, 48 tests and 96 tests. Mpox Virus Real-Time PCR Diagnostic Kit (NAT-17) is composed for the following Real-Time PCR reagents and positive and negative controls. Table 1 presents kit components of NAT-17 with labelled colour code that links components to the corresponding colour of their caps.

Table 1. Kit Components

Table 1a. Kit components of NAT-17 [REF NAT-17WE (L)]			
Cap colour	Components	Description	Quantity
Blue	NAR-01_PCR_Master Mix	Master mix including dNTPs, DNA polymerase and buffer	1 x 1000 µL
Brown	NAR-24_PCR_Multiplex Mix	Oligo mix including primers and probes for Mpox	1 x 500 µL
Green	NAR-25_PCR_Positive Control	Positive control including gene fragments for Mpox	1 x 50 µL
White	NAR-05_PCR_Negative Control	Nuclease Free Water	1 x 1000 µL

Table 1b. Kit components of NAT-17 [REF NAT-17WE (M)]			
Cap colour	Components	Description	Quantity
Blue	NAR-01_PCR_Master Mix	Master mix including dNTPs, DNA polymerase and buffer	1 x 500 µL
Brown	NAR-24_PCR_Multiplex Mix	Oligo mix including primers and probes for Mpox	1 x 250 µL
Green	NAR-25_PCR_Positive Control	Positive control including gene fragments for Mpox	1 x 40 µL
White	NAR-05_PCR_Negative Control	Nuclease Free Water	1 x 500 µL

Table 1c. Kit components of NAT-17 [REF NAT-17WE (S)]			
Cap colour	Components	Description	Quantity
Blue	NAR-01_PCR_Master Mix	Master mix including dNTPs, DNA polymerase and buffer	1 x 250 µL
Brown	NAR-24_PCR_Multiplex Mix	Oligo mix including primers and probes for Mpox	1 x 125 µL
Green	NAR-25_PCR_Positive Control	Positive control including gene fragments for Mpox	1 x 30 µL
White	NAR-05_PCR_Negative Control	Nuclease Free Water	1 x 250 µL

Fluorescence detection is accomplished using FAM, HEX, ROX and Cy5 filters. The assay amplifies target regions within the C3L, OPG210/B21R, and OPG002 genes, with fluorescence detection using specific filters for each target. The non-clade-specific Mpox DNA is detected via the OPG210/OPG002 primer sets using a FAM filter. Clade Ia is detected by primers targeting the undeleted C3L gene with ROX fluorescence, while Clade Ib detection, spanning the deleted C3L region, uses HEX fluorescence. An internal control targeting the human RNaseP gene is integrated to monitor nucleic acid extraction, PCR inhibition, and operational errors, with amplification detected using a Cy5 filter.

Table 2. Kit Target genes fluorophores

Target gene	**Fluorophore (λ_{emm})**
Mpox – *OPG210* gene, *OPG002* gene	FAM (520 nm)
Clade Ib – *C3L* gene (Clade Ib specific region)	HEX (556 nm)
Clade Ia – *C3L* gene (Clade Ia specific region)	ROX (602 nm)
Internal control – *RNaseP*	Cy5 (670 nm)

Equipment required

1. Real-Time PCR Thermal Cyclers with FAM, HEX, ROX and Cy5 Channels, or equivalent.
   The PCR instruments that are validated with the assay: Bio-Rad CFX96 (CFX Maestro Software 2.3), Bio Molecular Systems Mic qPCR (Mic V-IVD v1.0.2) and QuantStudio™ 5 Dx Real-Time PCR (Design & Analysis 2.6.0).
2. Viral DNA/ RNA Extraction system, or equivalent.
   The extraction system that is validated with the assay: *KingFisher™ Flex Purification System (ThermoFisher Scientific™)*
3. Class II Biological Safety Cabinet (BSC)
4. Centrifuge
5. Vortex
6. Micropipettes
7. Refrigerator (2-8 °C) and Freezer (preferably ≤-20°C)

Components required but not provided

a. Sample collection and nucleic acid extraction materials
    1. Sterile, synthetic swabs (including but not limited to polyester, nylon, or Dacron) with plastic, wood, or thin aluminium (wire) shafts.
    2. Sterile collection tubes.
    3. DNA Extraction reagents

    E.g. ThermoFisher MagMAX™ Prime Viral/Pathogen NA Isolation Kit (Cat. A58145).

b. Other reagents and consumables required but not included with the test:
    1. Nuclease free water, anhydrous ethanol, anhydrous isopropanol
    2. 96 well plates or PCR tubes, pipette tips with filters, microcentrifuge tubes
    3. Liquid waste container, solid waste bag and container
    4. Double-layer latex gloves, waterproof boot covers, protective clothing, safety glasses, and masks with high filtration efficiency.

Storage and Shelf life

Shipping temperature below 8°C (with ice pack) for a maximum of 3 days for local shipment only. For international shipments, the product should be shipped at -20±5°C or use dry ice shipment.

- All reagents should be stored at -20±5°C with protection from light. Avoid multiple freeze-thaw cycles.
- Expiration date is 24 months after production date.
- In-use stability has been validated for 2 weeks after opening of the reagent vials at 2-8 °C, can be stored at -20±5°C for up to 3 months.
- Avoid more than 6 freeze-thaw cycles.
- On-board stability has been validated for up to 1hr holding time at 2-8°C and room temperature after addition of sample to reagents.

Sample requirement:

Sample:	Viral DNA (extracted from swabs of lesion surface, exudate, or lesion crusts).
Shipping:	Sealed foam box with dry shipper. Samples shall be transported at low temperature (-20±5°C) in accordance with biosafety regulations.
Sample Stability:	Swab specimens & lesion crust(s) in UTM can be tested within 24 hours of storage in fridge (2-8°C) and that are stored frozen (-20°C or lower) can be tested up to 30 days from collection whereas sample stored below (-70°C) can be tested for up to 6 months.

Warnings and Precautions

- Avoid using the kit if it was not transported under recommended shipping conditions. The performance of the kit may be compromised when transported outside of recommended conditions.
- Inappropriate sample collection, transfer, storage, and operation may lead to inaccurate test results. Before using the kit, check tubes for leakage or damage.
- DNA extraction should be carried out as soon as possible after sample collection to avoid degradation. If it cannot be carried out immediately, samples should be stored in accordance with CDC guidelines.

- The contamination of laboratory environment and reagents, or cross contamination during specimen treatment, including incorrect pipetting technique, may lead to false positive results. Decontaminate work surfaces and equipment with appropriate disinfectants and follow the manufacturer's recommendations.
- Ensure that all equipment and software are routinely calibrated and operated according to the manufacturer's instructions, as improper calibration or incorrect operation may result in invalid or inaccurate results.
- Lesion specimens are known to have the highest quantity of Mpox virus. When possible, vaccinated (i.e., smallpox vaccination within the past 3 years) staff should perform laboratory work that involves handling lesion specimens that are being processed for Mpox virus testing. When only non-immunized staff are available, additional personal protective equipment and practices should be used to further reduce the risk for exposures, including testing specimens in BSL-2 containment with BSL-3 practices.
- Sample preparation should be performed in a certified class II biological safety cabinet. Handle all specimens as if infectious using safe laboratory procedures. During testing, appropriate PPE shall be worn including disposable gloves which should be replaced frequently to avoid cross contamination between samples, refer to CDC guideline. Avoid touching exposed skin and tube openings. The operation of sample and waste shall meet the requirements of relevant laws and regulations.
- If exposure to skin or mucous membranes occurs, immediately wash the area with large amounts of water. Seek medical advice immediately.
- In the event of spills or accidental exposure, immediately decontaminate the affected area with a validated disinfectant (e.g., freshly prepared 0.5% sodium hypochlorite or 70% ethanol), dispose of contaminated materials according to institutional, WHO and CDC guidelines, and report the incident to the laboratory supervisor.
- Do not interchange vial or bottle caps, as cross-contamination may occur. Reagents supplied are formulated specifically for use with this kit. Make no substitutions to ensure optimal performance of the kit. Further dilution of the reagents or alteration of incubation time and temperature may result in erroneous or discordant data. Do not use components beyond the expiration the date printed on the kit boxes. Do not mix reagents from different lots.

Method

1. Sample preparation

Mpox sample collection follows CDC guidelines, where brief step-by-step instructions are outlined below *(Centers for Disease Control and Prevention 2024).*

1) Wear appropriate PPE before starting specimen collection.
2) Use only sterile synthetic swabs (polyester, nylon, or Dacron with plastic, wood, or thin aluminium shafts). Do not use cotton swabs.
3) Identify skin lesions (vesicle, exudate, or crust). Select 2–3 lesions from different body locations or with differing appearances.
4) Collect two swabs per lesion:
    - Vigorously swab the surface, exudate, or crust.
    - Avoid contaminating gloves or surrounding surfaces.
    - Do not unroof, aspirate, or use sharp instruments.
5) Separate specimens: Place swabs from each lesion, crust, or exudate into individual sterile containers.
6) Specimen type: Wet swabs are submerged in viral transport media (VTM) where dry crusts are separated individually into a dry and sterile container.
7) Do not use media intended for bacterial preservation (may inhibit PCR).
8) Prepare containers:
    - Break shaft if necessary to fit into sterile container (avoid glass).
    - Secure lid tightly.
9) Remove gloves and perform hand hygiene. Don new gloves before handling next specimen.

For children, specimen collection should be performed with additional assistance to ensure safety and to minimise discomfort. When collecting from different lesion types, vesicles or pustules should be swabbed on the surface or exudate, while crusted lesions may be sampled by swabbing or collecting a portion of the crust. Whenever possible, specimens should be obtained from lesions at different stages of development to improve diagnostic yield. Specimens that do not contain enough human DNA may lead to inconclusive PCR test results wherein re-testing is recommended. More viral DNA can be found in crusts when present.

Nucleic acid extraction of the samples should be performed according to the corresponding requirements and procedures of the selected viral DNA extraction kits. The extracted DNA can be directly used for detection. If the extracted DNA is not used for detection immediately, store at -20°C, avoiding repeated freeze-thaw. The extraction protocols validated with the test:

Table 3. Extraction Protocols validated with the Test

Extraction Method	Sample Volume (µL)	Protocol	Elution Volume (µL)
KingFisher (Prime_FLX_V1)	200	MagMAX™ Prime Viral/Pathogen NA Isolation Kit (Cat. A58145)	60

2. Reaction master-mix preparation

The PCR Master Mix contains PCR buffer, dNTPs and polymerase enzyme (NAR-01) and a mix of primers and probes specific for Mpox Virus and for *RNaseP* gene (NAR-24).

Thaw reagents at room temperature, then place on ice. After thawing completely, briefly mix each component by either inversion, pipetting or gentle vortexing to ensure homogeneity prior to use. Spin down each component at 1000 rpm for 15 sec, and then place on ice in a sterile hood.

Calculate the number of required reactions: n = number-of-samples + positive control + negative control + reagent excess to ensure there is enough for each tube. The inclusion of a negative and positive control is required for each run. Determine the total volume for the appropriate number of reactions and prepare the reaction master-mix with all components, except DNA template, according to the table below. Prepare the reaction master-mix by adding the required volume of each component into a RNase-free microcentrifuge tube on ice. Mix thoroughly but gently by pipetting or vortexing. Collect liquid to the bottom of the tube by brief centrifugation.

Table 4. Master Mix components and volume required per reaction

Components	Vol (µl) per reaction
HA TECH_PCR_Master Mix_1 (NAR-01)	10
HA TECH_Mpox_PCR_Multiplex Mix (NAR-24)	5

Aliquot 15µl of the reaction master-mix into PCR tubes or wells of the appropriate PCR-plate on ice. For best results, ensure accurate and consistent pipetting volumes and minimize bubbles.

3. Sample loading

Add 5µL of each extracted sample, PC and NTC (RNase-free water) into the tubes containing 15µl of the Reaction master-mix while on ice. Change tips for each sample to avoid contamination. Change gloves often and when necessary to avoid contamination. The total volume is 20µL. Seal the reaction tubes/plate tightly and centrifuge briefly at low speed to collect all reagents at the bottom of the tube and remove bubbles.

4. Real-Time PCR protocol

Place the PCR tube into the qPCR System, and follow the thermocycling protocol below:

Table 5. RT-PCR Run Profile

	Step	Temperature (°C)	Time (min:sec)
Hold	Initial Denaturation	95	2:00
Cycling x45	Denaturation	95	0:10
	Extension	57	0:30

Fluorescence data: Use the FAM, HEX, ROX, and Cy5 channels, or equivalent, and the signal should be collected at the 57°C extension step. Approximate running time of NAT-17 PCR is 70 minutes depending on the operator and machine.

PCR Controls

Positive Control (PC) is used to confirm test validity, and functions as the validation control for PCR amplification and the target gene detection. The PC consists of synthetic DNA with the amplicon region. The PC must be included in each assay.

No-Template-Control/negative control (NTC) is used as a PCR control to confirm test validity, and the absence of any contaminants during testing. The NTC is prepared using Nuclease-free water and must be included in each assay. No signal should be detected with the NTC.

The *RNaseP* assay is used as an internal control (IC) to confirm sufficient patient sample collection, extraction, and PCR amplification.

Data Analysis

1. Quality Control

No template control and positive control provide the calibration for the kit and shall be determined for each assay. The result is valid if all the below criteria are met (see Table below). Otherwise, the test is invalid, in which case, instrument error, reagent contamination, amplification conditions, etc. should be checked, and the entire run that includes PC & NTC samples need to be repeated.

Table 6. Ct values Criteria for PC and NTC

Channel / Control	**FAM**	**HEX**	**ROX**	***RNase P* Cy5**
PC (NAR-25)	Ct ≤ 38	Ct ≤ 38	Ct ≤ 38	NA
NTC (NAR-05)	No Ct or Ct > 38	No Ct or Ct > 38	No Ct or Ct > 38	No Ct or Ct > 38

2. Interpreting Test Results

A NAT-17 assay can be interpreted as positive (+) if Ct ≤ 38 and negative (-) if Ct >38 or there is no Ct. Refer to Table 7.

Table 7. Results Interpretation (Ct values)

Result type	FAM	HEX	ROX	(Cy5)	Interpretation	Result
1	-/Ct >38	-/Ct >38	-/Ct >38	+Ct ≤ 38	Negative	All target results are valid. Mpox DNA is not detected.
2	+Ct ≤ 38	-/Ct >38	-/Ct >38	+Ct ≤ 38	Positive	All target results are valid. Mpox **Clade II** DNA is detected.
3	+Ct ≤ 38	+Ct ≤ 38	-/Ct >38	+Ct ≤ 38	Positive	All target results are valid. Mpox **Clade Ib** DNA is detected.
4	+Ct ≤ 38	-/Ct >38	+Ct ≤ 38	+Ct ≤ 38	Positive	All target results are valid. Mpox **Clade Ia** DNA is detected.
5	+Ct ≤ 38	-/Ct >38	-/Ct >38	-/Ct >38	Presumptive Positive	Mpox **Clade II** DNA is detected*
6	+Ct ≤ 38	+Ct ≤ 38	-/Ct >38	-/Ct >38	Presumptive Positive	Mpox **Clade Ib** DNA is detected*
7	-/Ct >38	+Ct ≤ 38	-/Ct >38	-/Ct >38	Presumptive Positive	Mpox **Clade Ib** DNA is detected*
8	+Ct ≤ 38	-/Ct >38	+Ct ≤ 38	-/Ct >38	Presumptive Positive	Mpox **Clade Ia** DNA is detected*
9	-/Ct >38	-/Ct >38	+Ct ≤ 38	-/Ct >38	Presumptive Positive	Mpox **Clade Ia** DNA is detected*
10	-/Ct >38	-/Ct >38	-/Ct >38	-/Ct ≥ 38	Invalid	Results are invalid. Repeat the test. If the result is still invalid, a new specimen should be obtained.

*38 is the cut-off value for NAT-17, Ct values less than or equal to 38 are considered positive whereas Ct values larger than 38 (e.g. 38.01) are considered negative.
*If the OPG210/OPG002 (FAM) gene and/or C3L gene (HEX or ROX) is positive but RNaseP (Cy5) is negative, it is recommended to repeat the test with new specimen, RNaseP is negative possibly due to the inadequate sample collection. In the case that the ROX channel shows a positive result (Ct < 38), without being accompanied by a positive result in the FAM channel, the test should be repeated. If the same result is obtained, then the sample should be assumed as negative for Mpox. This may be due to cross-reactivity of the Clade Ia-specific primer set with either Cowpox, Buffalopox, Camelpox, Mousepox, Vaccinia Virus, or Variola Virus.

A sample can be interpreted as negative only if the analysis of the RNase P (Cy5 channel) indicates that amplification has occurred in the reaction tube but no signal from the target reporter genes has been detected.

A positive signal for the Cy5 channel (RNaseP control) indicates that all processing steps performed were successful. If the Ct value of Cy5 channel is >38, it may indicate insufficient specimen collection, the extracted nucleic acid has degraded, certain inhibitors were present in the reaction or competitive assay inhibition if there is a high viral load of Mpox Virus also present. A negative signal for the targets and a negative signal or Ct>38 for Cy5 channel, invalidates all negative results in the analysis of that sample. Repeat the assay if an invalid result is registered.

Missing amplification of individual targets may be due to:

a) a sample at concentrations near or below the limit of detection of the test,
b) a mutation in the corresponding target region, or
c) other factors such as PCR inhibitors in the sample.

Assay limitations

- The assay design is impacted by the accuracy of the publicly available Mpox genome sequences. Genomic mutations on primer or probe binding can result in missed amplification.
- Assay performance may be affected by various uncontrolled factors such as sample quality, various sample extraction methods, sample cross-contamination, and data analysis variation.
- The limit of detection (LoD) is determined based on an equal or greater than 95% confidence of detection. When Mpox DNA is present at or above the LoD concentration in the test specimen, there will be a low probability that Mpox is not detected. When Mpox is below the LoD concentration, there will also be a chance that Mpox will be detected.
- The instruments and assay procedures reduce the risk of contamination by amplification products. However, nucleic acid contamination from positive controls or specimens must be controlled by good laboratory practice.
- Optimal performance of this test requires appropriate specimen collection and handling. Detection of Mpox DNA may be affected by sample collection methods (eg, if a specimen is improperly collected, transported or handled), patient factor (eg, presence, type, and duration of symptoms), and/or stage of infection (eg, if collected too early or too late in the course of illness).
- Failure to follow safety and handling instructions, including use of required PPE, spill management procedures, and good laboratory practices, may result in exposure risk or invalid Mpox PCR test results.
- The assay is indicated for testing of swab specimens. Performance for other specimen types has not been established.
- False-negative results may arise from degradation of the Mpox DNA during storage and transport of the specimens.

- According to Interference Substances study of NAT-17, topical lesion treatments, such as Acyclovir and Docosanol (Abreva), may inhibit amplification of the RNaseP endogenous control, potentially invalidating negative results. If all Mpox targets are negative and RNaseP is undetected (or has a Ct value >38), the test must be repeated. If the issue persists, a new sample should be collected without prior application of topical products; if such products have been applied, all visible residues should be thoroughly removed before sampling.

Troubleshooting guide

Table 8. Troubleshooting guide

Issue	Possible Cause	Recommended Action
No amplification (Ct > 38) in FAM, HEX & ROX channels for PC	Reagent degradation, incorrect reagent preparation, instrument error	Verify reagent storage conditions and repeat entire run.
Amplification of NTC (Ct ≤ 38) in all channels	Contamination during pipetting or reagent preparation	Repeat run with fresh reagents; decontaminate workspace and equipment; review pipetting technique.
OPG210/OPG002 (FAM) gene and/or C3L gene (HEX or ROX) is positive but RNaseP (Cy5) is negative (Ct > 38)	Inadequate sample collection, sample degradation, improper extraction process.	Repeat the test with new specimen.
ROX channel shows a positive result (Ct < 38), without being accompanied by a positive result in the FAM channel	Inadequate sample collection, improper reagent preparation and/or potential cross-reactivity.	Entire run should be repeated. If same results is obtained, sample is interpreted as negative due to cross-reactivity.
Sample shows no amplification (Ct > 38) in all channels (FAM, HEX, ROX & Cy5)	Potential cross-interference with topical lesion treatment products (e.g. Acyclovir & Abreva). Inadequate sample collection, sample degradation, improper extraction process.	Repeat the test. If issue persists, collect a new sample without topical products, or ensure any residues are removed before sampling.

Note: If issues persist after following the troubleshooting steps, discontinue use of the current reagents and contact the HA TECH's technical support for further assistance.

Performance characteristics

Limit of Detection (LoD):

LoD studies determined the minimum concentration of Mpox virus DNA for each primer set that the NAT-17 assay can reliably detect under standard operating conditions. LoD NAT-17 was established as the lowest concentration of target nucleic acid consistently detected (≥95% of replicates) - LoD95%.

Mpox Pan-Clade Detector (*OPG210* gene and *OPG002* gene)

NAT-17 detected 19 out of the 20 replicates, satisfying the 95% detection rate, determining the LoD for pan-clade detector primer set as 0.039 copies/µL.

Table 9. Confirmatory LoD for *OPG210* and *OPG002*

Replicate Number	FAM	HEX	ROX	Cy5
1	35.83	ND	ND	34.78
2	35.51	ND	ND	33.79
3	35.86	ND	ND	35.35
4	35.72	ND	ND	33.61
5	35.45	ND	ND	33.46
6	35.54	ND	ND	33.98
7	34.33	ND	ND	34.43
8	35.34	ND	ND	33.80
9	35.01	ND	ND	33.79
10	38.24	ND	ND	34.10
11	36.78	ND	ND	34.04
12	35.74	ND	ND	35.35
13	35.38	ND	ND	36.61
14	35.55	ND	ND	33.57
15	35.08	ND	ND	34.02
16	34.76	ND	ND	33.70
17	37.03	ND	ND	34.16
18	35.17	ND	ND	34.55
19	34.59	ND	ND	34.42
20	35.02	ND	ND	34.49
Mean	35.60	N/A	N/A	34.30
Std.	0.89	N/A	N/A	0.76
%CV	2.51	N/A	N/A	2.22

Clade Ib Primer Set (*C3L* gene)

NAT-17 detected 19 out of the 20 replicates, satisfying the 95% detection rate, confirming the Clade Ib primer set LoD as 0.30 copies/µL.

Table 10. Confirmatory LoD for *Clade Ib C3L gene* at 0.30 cp/µL

Replicate Number	FAM	HEX	ROX	Cy5
1	35.62	34.57	ND	ND
2	39.20	33.15	ND	ND
3	35.17	35.95	ND	ND
4	35.48	34.93	ND	ND
5	36.00	34.20	ND	ND
6	36.74	33.68	ND	ND
7	36.33	35.00	ND	ND
8	36.82	ND	ND	ND

9	38.29	37.80	ND	ND
10	36.49	33.94	ND	ND
11	36.73	36.16	ND	ND
12	35.84	37.20	ND	ND
13	35.20	36.36	ND	ND
14	36.51	33.57	ND	ND
15	35.54	33.41	ND	ND
16	ND	33.54	ND	ND
17	39.93	35.35	ND	ND
18	35.72	34.38	ND	ND
19	35.65	35.29	ND	ND
20	36.33	33.93	ND	ND
Mean	N/A	N/A	34.38	N/A
Std.	N/A	N/A	1.37	N/A
%CV	N/A	N/A	3.98	N/A

Clade Ia Primer Set (*C3L* gene)

NAT-17 detected all 20 replicates, satisfying the 95% detection rate, confirming the Clade Ia primer set LoD as 1.25 copies/µL.

Table 11. Confirmatory LoD for *Clade Ia C3L gene* at 1.25 cp/µL

Replicate Number	FAM	HEX	ROX	Cy5
1	ND	ND	33.76	ND
2	ND	ND	33.99	ND
3	ND	ND	34.82	ND
4	ND	ND	35.94	ND
5	ND	ND	36.25	ND
6	ND	ND	36.18	ND
7	ND	ND	33.57	ND
8	ND	ND	34.10	ND
9	ND	ND	34.32	ND
10	ND	ND	33.16	ND
11	ND	ND	32.59	ND
12	ND	ND	36.11	ND
13	ND	ND	33.32	ND
14	ND	ND	36.18	ND
15	ND	ND	32.74	ND
16	ND	ND	36.41	ND
17	ND	ND	33.53	ND
18	ND	ND	34.99	ND
19	ND	ND	32.52	ND
20	ND	ND	33.18	ND
Mean	N/A	N/A	34.38	N/A
Std.	N/A	N/A	1.37	N/A
%CV	N/A	N/A	3.98	N/A

The established LoD values for the panel are shown below:

Table 12. Summary table for NAT-17 LoD

Target gene	Fluorophore	LoD concentration
Mpox (*OPG210* gene and *OPG002* gene)	FAM (520 nm)	0.039 copies/µL
Clade Ib – C3L gene (Clade Ib specific region)	HEX (556 nm)	0.30 copies/µL
Clade Ia – C3L gene (Clade Ia specific region)	ROX (602 nm)	1.25 copies/µL

Analytical Reactivity (Inclusivity)

The inclusivity study for the HA TECH Monkeypox Virus Real-Time PCR Diagnostic Kit (NAT-17) evaluated the assay's ability to detect Mpox virus DNA across a wide range of genetic variations within known Mpox clades (Clade Ia, Clade Ib, and Clade II). This study utilised in-silico analysis and experimental testing to ensure comprehensive coverage of target sequences. This study demonstrated that all primer sets were inclusive of all available sequences from GISAID for their respective target. In-silico analyses revealed several mismatches in the pan-clade detector. The mismatches with the highest potential to effect NAT-17 detection were in MPXV_Li_Generic_R found in Clade Ib and IIb populations, and MPXV_Li_Generic_F found in Clade IIa and IIb populations. Following this result, theoretical changes in melting temperature and binding percentage (at NAT-17 annealing temperature) were predicted. To confirm whether the mismatch in the 17.G -> A mismatch in MPXV_Li_Generic_R, the 4.A -> G mismatch in the MPX1.1R, and the 6.A -> G mismatch in MPXV_Li_Generic_F have an effect on NAT-17 detection, plasmids with these mismatches were constructed and tested with NAT-17.

Table 13. Assessment of Mismatches in the Pan-Clade Detector

Target	Concentration (cp/µL)	NAT-17 Ct
NAT-17 Positive Control (NAR-25)	10^6	17.72
	10^5	21.24
N17CDC_P123	10^6	18.04
	10^5	21.47
N17HAT_P126	10^6	18.06
	10^5	21.25

There was no detectable difference between these sequences and the non-mismatch sequences. The plasmid containing the 17.G -> A mismatch was slightly out of the ±1 Ct range for the concentration of 10^5, however with the 10^6 copies/µL concentration being within range, it is likely that this was merely due to handling inconsistencies.

Further tests were performed with whole genome samples of Clade Ib and IIb, and a plasmid sequence representing the Clade Ia sequence in the MPX1.1 amplicon region were also tested at low concentrations using NAT-17. All samples predictably amplified.

Cross-Reactivity

The cross-reactivity study for the HA TECH Monkeypox Virus Real-Time PCR Diagnostic Kit (NAT-17) evaluated the potential for non-specific amplification by the kit's primers and probes. This included an initial in-silico homology assessment using NCBI BLASTn and confirmatory testing for sequences with significant homology. Results have shown that our primer sets for Clade Ib and the pan-clade detector are not cross-reactive with any tested organisms, with the pan-clade detector being cleared of cross-reactivity with cowpox through confirmatory qPCR testing. Clade Ia was confirmed as being cross-reactive with cowpox and is suspected of being cross-reactive with Buffalopox, Camelpox, Vaccinia virus, and Variola virus. However, our pan-clade detector is

specific for all lineages of Mpox and thus acts a control for the presence of Mpox. In the case that Clade Ia shows amplification without amplification in the pan-clade detector, the result will be deemed invalid according to the *IFU-39-NAT-17 Quick Assay Manual (1.8).pdf*.

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Table 14. In-Silico cross-reactivity results

Pathogen	Mpox Pan-Clade						Mpox Clade Ia			Mpox Clade Ib		
	F1	P1	R1	F2	P2	R2	F1	P1	R1	F1	P1	R1
Acinetobacter baumannii	No	No	No	No	No	No	No	No	No	No	No	No
Acinetobacter calcoaceticus	No	No	No	No	No	No	No	No	No	No	No	No
Acinetobacter lwoffi	No	No	No	No	No	No	No	No	No	No	No	No
Actinomyces israelii	No	No	No	No	No	No	No	No	No	No	No	No
Adenovirus 1	No	No	No	No	No	No	No	No	No	No	No	No
Adenovirus 3	No	No	No	No	No	No	No	No	No	No	No	No
Adenovirus 31	No	No	No	No	No	No	No	No	No	No	No	No
Atopobium vaginae	No	No	No	No	No	No	No	No	No	No	No	No
Bacteroides fragilis	No	No	No	No	No	No	No	No	No	No	No	No
BK Virus	No	No	No	No	No	No	No	No	No	No	No	No
Bordetella hinzii	No	No	No	No	No	No	No	No	No	No	No	No
Bordetella parapertussis	No	No	No	No	No	No	No	No	No	No	No	No
Bordetella pertussis	No	No	No	No	No	No	No	No	No	No	No	No
Borrelia burgdorferi	No	No	No	No	No	No	No	No	No	No	No	No
Bovine papular stomatitis virus (BPSV)	No	No	No	No	No	No	No	No	No	No	No	No
Buffalopox virus	No	No	No	92%	No	No	100%	89%	89%	92%	No	No
Camelpox	96%	No	No	92%	No	96%	100%	89%	89%	92%	No	88%
Campylobacter jejuni	No	No	No	No	No	No	No	No	No	No	No	No
Candida albicans	No	No	No	No	No	No	No	No	No	No	No	No
Candida auris	No	No	No	No	No	No	No	No	No	No	No	No
Candida glabrata	No	No	No	No	No	No	No	No	No	No	No	No
Candida krusei	No	No	No	No	No	No	No	No	No	No	No	No
Candida parapsilosis	No	No	No	No	No	No	No	No	No	No	No	No
Candida tropicalis	No	No	No	No	No	No	No	No	No	No	No	No
Chlamydia pneumoniae	No	No	No	No	No	No	No	No	No	No	No	No
Chlamydia trachomatis	No	No	No	No	No	No	No	No	No	No	No	No
Clostridium difficile	No	No	No	No	No	No	No	No	No	No	No	No
Clostridium perfringens	No	No	No	No	No	No	No	No	No	No	No	No
Coronavirus	No	No	No	No	No	No	No	No	No	No	No	No
Corynebacterium diphtheriae	No	No	No	No	No	No	No	No	No	No	No	No
Corynebacterium genitalium	No	No	No	No	No	No	No	No	No	No	No	No
Corynebacterium jeikeium	No	No	No	No	No	No	No	No	No	No	No	No
Cowpox	100%	100%	86%	96%	No	96%	100%	89%	89%	92%	No	88%
Coxsackievirus Type A16	No	No	No	No	No	No	No	No	No	No	No	No
Coxsackievirus Type A9	No	No	No	No	No	No	No	No	No	No	No	No

Coxsackievirus Type B5	No	No	No	No	No	No	No	No	No	No	No	No
Cryptococcus neoformans	No	No	No	No	No	No	No	No	No	No	No	No
Cutibacterium acnes	No	No	No	No	No	No	No	No	No	No	No	No
Cytomegalovirus	No	No	No	No	No	No	No	No	No	No	No	No
Dientamoeba fragilis	No	No	No	No	No	No	No	No	No	No	No	No
Echovirus Type 06	No	No	No	No	No	No	No	No	No	No	No	No
Echovirus Type 11	No	No	No	No	No	No	No	No	No	No	No	No
Echovirus Type 14	No	No	No	No	No	No	No	No	No	No	No	No
Echovirus Type 30	No	No	No	No	No	No	No	No	No	No	No	No
Ectromelia virus (Mousepox)	92%	No	83%	96%	No	No	100%	89%	89%	92%	No	88%
Enterobacter aerogenes	No	No	No	No	No	No	No	No	No	No	No	No
Enterobacter cloacae	No	No	No	No	No	No	No	No	No	No	No	No
Enterococcus faecalis	No	No	No	No	No	No	No	No	No	No	No	No
Enterococcus faecium	No	No	No	No	No	No	No	No	No	No	No	No
Epstein Barr Virus	No	No	No	No	No	No	No	No	No	No	No	No
Escherchia coli	No	No	No	No	No	No	No	No	No	No	No	No
Fusobacterium nucleatum	No	No	No	No	No	No	No	No	No	No	No	No
Gardnerella vaginalis	No	No	No	No	No	No	No	No	No	No	No	No
Haemophilus ducreyi	No	No	No	No	No	No	No	No	No	No	No	No
Haemophilus influenzae	No	No	No	No	No	No	No	No	No	No	No	No
HIV-1	No	No	No	No	No	No	No	No	No	No	No	No
Homo sapiens	No	No	No	No	No	No	No	No	No	No	No	No
HSV-1	No	No	No	No	No	No	No	No	No	No	No	No
HSV-2	No	No	No	No	No	No	No	No	No	No	No	No
Human papilloma virus (HPV)	No	No	No	No	No	No	No	No	No	No	No	No
Influenza A	No	No	No	No	No	No	No	No	No	No	No	No
Influenza B	No	No	No	No	No	No	No	No	No	No	No	No
Klebsiella aerogenes	No	No	No	No	No	No	No	No	No	No	No	No
Klebsiella oxytoca	No	No	No	No	No	No	No	No	No	No	No	No
Klebsiella pneumoniae	No	No	No	No	No	No	No	No	No	No	No	No
Lactobacillus acidophilus	No	No	No	No	No	No	No	No	No	No	No	No
Lactobacillus jensenii	No	No	No	No	No	No	No	No	No	No	No	No
Lactobacillus vaginalis	No	No	No	No	No	No	No	No	No	No	No	No
Legionella pneumophila	No	No	No	No	No	No	No	No	No	No	No	No
Listeria monocytogenes	No	No	No	No	No	No	No	No	No	No	No	No
Measles morbillivirus	No	No	No	No	No	No	No	No	No	No	No	No
Metapneumovirus 8	No	No	No	No	No	No	No	No	No	No	No	No
Mobiluncus curtisii	No	No	No	No	No	No	No	No	No	No	No	No
Mobiluncus mulieris	No	No	No	No	No	No	No	No	No	No	No	No
Molluscum Contagiosum Virus	No	No	No	No	No	No	No	No	No	No	No	No
Moraxella catarrhalis	No	No	No	No	No	No	No	No	No	No	No	No
Mumps orthorubulavirus	No	No	No	No	No	No	No	No	No	No	No	No

Mycoplasma genitalium	No	No	No	No	No	No	No	No	No	No	No	No
Mycoplasma hominis	No	No	No	No	No	No	No	No	No	No	No	No
Mycoplasma orale	No	No	No	No	No	No	No	No	No	No	No	No
Mycoplasma pneumoniae	No	No	No	No	No	No	No	No	No	No	No	No
Neisseria gonorrhoeae	No	No	No	No	No	No	No	No	No	No	No	No
Neisseria meningitidis	No	No	No	No	No	No	No	No	No	No	No	No
Orf virus (ORFV)	No	No	No	No	No	No	No	No	No	No	No	No
Parainfluenza 1	No	No	No	No	No	No	No	No	No	No	No	No
Parainfluenza 2	No	No	No	No	No	No	No	No	No	No	No	No
Parainfluenza 3	No	No	No	No	No	No	No	No	No	No	No	No
Parainfluenza 4	No	No	No	No	No	No	No	No	No	No	No	No
Parechovirus Type 1	No	No	No	No	No	No	No	No	No	No	No	No
Pentatrichomonas hominis	No	No	No	No	No	No	No	No	No	No	No	No
Peptostreptococcus anaerobius	No	No	No	No	No	No	No	No	No	No	No	No
Phytophthora mirabilis	No	No	No	No	No	No	No	No	No	No	No	No
Prevotella bivia	No	No	No	No	No	No	No	No	No	No	No	No
Propionibacterium acnes	No	No	No	No	No	No	No	No	No	No	No	No
Proteus mirabilis	No	No	No	No	No	No	No	No	No	No	No	No
Pseudocowpox virus (PCPV)	No	No	No	No	No	No	No	No	No	No	No	No
Pseudomonas aeruginosa	No	No	No	No	No	No	No	No	No	No	No	No
Rabbitpox virus	No	No	No	92%	No	No	100%	No	No	92%	No	No
Raccoonpox virus	No	92%	No	No	No	No	91%	No	No	92%	No	No
Red deer parapoxvirus (PVNZ)	No	No	No	No	No	No	No	No	No	No	No	No
Rhinovirus 1A	No	No	No	No	No	No	No	No	No	No	No	No
RSV A	No	No	No	No	No	No	No	No	No	No	No	No
RSV A2	No	No	No	No	No	No	No	No	No	No	No	No
Rubella Virus	No	No	No	No	No	No	No	No	No	No	No	No
Salmonella enterica serovar Typhimurium	No	No	No	No	No	No	No	No	No	No	No	No
SARS-CoV-2	No	No	No	No	No	No	No	No	No	No	No	No
Serratia marcescens	No	No	No	No	No	No	No	No	No	No	No	No
St. Louis Encephalitis Virus	No	No	No	No	No	No	No	No	No	No	No	No
Staphylococcus aureus	No	No	No	No	No	No	No	No	No	No	No	No
Staphylococcus epidermidis	No	No	No	No	No	No	No	No	No	No	No	No
Staphylococcus lugdunensis	No	No	No	No	No	No	No	No	No	No	No	No
Staphylococcus saprophyticus	No	No	No	No	No	No	No	No	No	No	No	No
Stenotrophomonas maltophilia	No	No	No	No	No	No	No	No	No	No	No	No
Streptococcus agalactiae	No	No	No	No	No	No	No	No	No	No	No	No
Streptococcus anginosus	No	No	No	No	No	No	No	No	No	No	No	No
Streptococcus canis	No	No	No	No	No	No	No	No	No	No	No	No
Streptococcus dysgalactiae	No	No	No	No	No	No	No	No	No	No	No	No
Streptococcus equi	No	No	No	No	No	No	No	No	No	No	No	No

Streptococcus equisimilis	No	No	No	No	No	No	No	No	No	No	No	No
Streptococcus mitis	No	No	No	No	No	No	No	No	No	No	No	No
Streptococcus pneumoniae	No	No	No	No	No	No	No	No	No	No	No	No
Streptococcus pyogenes	No	No	No	No	No	No	No	No	No	No	No	No
Streptococcus salivarius	No	No	No	No	No	No	No	No	No	No	No	No
Streptococcus zooepidemicus	No	No	No	No	No	No	No	No	No	No	No	No
Toxoplasma gondii	No	No	No	No	No	No	No	No	No	No	No	No
Treponema pallidum	No	No	No	No	No	No	No	No	No	No	No	No
Trichomonas tenax	No	No	No	No	No	No	No	No	No	No	No	No
Trichomonas vaginalis	No	No	No	No	No	No	No	No	No	No	No	No
Trichophyton rubrum	No	No	No	No	No	No	No	No	No	No	No	No
Ureaplasma urealyticum	No	No	No	No	No	No	No	No	No	No	No	No
Vaccinia virus	No	No	No	92%	No	96%	100%	89%	89%	92%	No	88%
Varicella-Zoster Virus (Chickenpox)	No	No	No	No	No	No	No	No	No	No	No	No
Variola virus	96%	No	No	92%	No	100%	100%	89%	89%	92%	No	88%

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Table 15. Cross-Reactivity qPCR Results

Sample	Pan-Clade (FAM)	Clade Ib (HEX)	Clade Ia (ROX)	RNAseP (Cy5)
Cowpox virus	ND	ND	25.50	41.60
(Strain: Brighton)	ND	ND	26.29	ND
ATCVR302D	ND	ND	26.66	ND
	21.18	23.02	18.85	21.54
NAR-25	21.28	22.98	19.02	21.62
	21.25	22.96	18.96	21.60
	ND	ND	ND	ND
NTC	ND	ND	ND	ND
	ND	ND	ND	ND

Table 16. Cross-Reactivity Cowpox Titration qPCR Results

Concentration (cp/µL)	Pan-Clade (FAM)	Clade Ib (HEX)	Clade Ia (ROX)	RNAseP (Cy5)
	ND	ND	26.50	34.60
3320	ND	ND	26.98	ND
	ND	ND	27.85	ND
	ND	ND	30.65	ND
664	ND	ND	31.41	ND
	ND	ND	31.97	ND
	ND	ND	ND	ND
332	ND	ND	ND	ND
	ND	ND	ND	ND
	21.22	23.14	21.27	20.04
NAR-25	21.24	23.26	21.41	20.05
	21.21	22.34	21.27	20.04
	ND	ND	ND	ND
NTC	ND	ND	ND	ND
	ND	ND	ND	ND

Microbial Interference

Microbial interference studies evaluates whether the presence of non-target microorganisms could cause false-negative results or otherwise interfere with the NAT-17 assay's ability to accurately detect Mpox virus DNA. Cowpox virus was identified as the only microorganism requiring further investigation based on in-silico analyses showing significant homology (≥80%) with the Clade Ia and our pan-clade detector.

In this study, qPCR was performed with spiked Cowpox DNA & Monkeypox DNA with skin swab using NAT-17 to identify the highest concentration at which false negatives are still observed. No interference was found for the Mpox Pan-Clade Detector with Cowpox at a concentration of 3.32×10^3 cp/µL, with amplification being observed in all three replicates. However, the Clade Ia primer set showed no amplification of Mpox Clade Ia plasmid DNA in the presence of Cowpox genomic DNA at 3.32×10^3 cp/µL. No amplification was observed for any of the three replicates. This indicates that the presence of cowpox DNA acts as an interferent for the NAT-17 with respect to the Clade Ia primer set.

Table 17. Cowpox Microbial Interference qPCR Results

Sample	FAM Ct	HEX Ct	ROX Ct	Cy5 Ct	Result (+/-)
Clade II Clinical Sample	34.54	ND	ND	ND	+
	34.35	ND	ND	ND	+
	34.09	ND	ND	ND	+
Clade Ib Plasmid	ND	33.69	ND	ND	+
	ND	34.85	ND	ND	+
	ND	36.32	ND	ND	+
Clade Ia Plasmid	ND	ND	ND	ND	-
	ND	ND	ND	ND	-
	ND	ND	ND	ND	-
NAR-25	19.52	19.89	19.00	19.83	+
	19.46	19.45	18.86	19.79	+
	19.46	19.67	18.81	19.72	+

Following these results, a titration study was performed in order to determine the minimum concentration of cowpox that would still elicit interference in Mpox Clade Ia detection. Through these studies, an interference boundary between 332 and 166 cp/µL was identified.

Table 18. Cowpox Microbial Interference qPCR Results

Cowpox Concentration (cp/µL)	FAM Ct	HEX Ct	ROX Ct	Cy5 Ct	Result (+/-)
664	ND	ND	ND	ND	-
	ND	ND	ND	ND	-
	ND	ND	29.08	ND	+
332	ND	ND	ND	ND	-
	ND	ND	ND	ND	-
	ND	ND	ND	ND	-
166	ND	ND	31.89	ND	+
	ND	ND	32.86	ND	+
	ND	ND	32.56	ND	+
83	ND	ND	32.81	ND	+
	ND	ND	32.68	ND	+
	ND	ND	32.08	ND	+
41.5	ND	ND	32.48	ND	+
	ND	ND	32.26	ND	+
	ND	ND	31.85	ND	+
No Cowpox	ND	ND	33.04	ND	+
	ND	ND	32.31	ND	+
	ND	ND	32.83	ND	+
NAR-25	24.07	24.31	23.14	23.14	+
	24.14	24.22	23.18	23.14	+
	24.14	24.22	23.03	23.13	+

Interfering Substances

Interfering substances studies determine whether any substances commonly found adjacent to Mpox skin lesions may interfere with the DNA extraction or qPCR process. To assess the impact of these substances on NAT-17, they will be spiked into both contrived positive and negative Mpox samples. An

interfering substances study has been performed for the pan-clade detector and the Clade Ib primer set, however, due to low DNA extraction efficiency for the Clade Ia plasmid sample, this study could not be performed for the Clade Ia primer set at this time.

Results for both our Pan-Clade Detector and Clade Ib primer set suggest that all tested substances do not have an inhibitory effect on the extraction procedure and/or PCR for one or both molecular targets. No false positives were observed in any channel for the negative skin swab matrix samples when in the presence of the tested substances. Additionally, the negative skin swab matrix samples showed that RNaseP was robust against all substances except for Acyclovir and Abrevea, where amplification was only observed in 1 out of the 3 replicates.

Table 19. Pan-Clade Detector Contrived Low Positive Samples with Potentially Interfering Substances

	3 x LoD Sample				
Substance	**FAM Ct**	**HEX Ct**	**ROX Ct**	**Cy5 Ct**	**Result (+/-)**
Vagisil	32.35	ND	ND	36.23	+
	32.35	ND	ND	ND	+
	31.95	ND	ND	36.66	+
SOOV IT	35.01	ND	ND	ND	+
	33.38	ND	ND	ND	+
	35.56	ND	ND	ND	+
Proctosedyl	32.78	ND	ND	ND	+
	33.11	ND	ND	ND	+
	32.53	ND	ND	ND	+
Carmex	35.31	ND	ND	36.08	+
	34.34	ND	ND	ND	+
	34.85	ND	ND	ND	+
Douche	35.26	ND	ND	ND	+
	36.79	ND	ND	36.85	+
	36.08	ND	ND	38.01	+
KY Jelly	35.96	ND	ND	ND	+
	34.99	ND	ND	35.45	+
	34.90	ND	ND	ND	+
Respiratory Droplets	34.03	ND	ND	31.86	+
	35.17	ND	ND	32.03	+
	34.23	ND	ND	32.60	+
Blood	35.36	ND	ND	29.52	+
	34.91	ND	ND	29.37	+
	34.08	ND	ND	29.29	+
Seminal Fluid	33.80	ND	ND	26.60	+
	34.03	ND	ND	26.57	+
	33.65	ND	ND	26.56	+
Faeces	34.79	ND	ND	ND	+
	34.96	ND	ND	36.98	+
	34.97	ND	ND	36.70	+
Male Urine	33.32	ND	ND	ND	+
	33.39	ND	ND	ND	+
	33.56	ND	ND	36.61	+
Female Urine	33.96	ND	ND	ND	+
	34.30	ND	ND	ND	+
	34.13	ND	ND	ND	+
Mucin	34.20	ND	ND	38.62	+

	33.25	ND	ND	40.09	+
	33.23	ND	ND	ND	+
Albumin	33.58	ND	ND	37.33	+
	34.02	ND	ND	36.72	+
	34.00	ND	ND	ND	+
Casein	34.52	ND	ND	ND	+
	35.30	ND	ND	ND	+
	34.66	ND	ND	ND	+
Cornstarch	33.99	ND	ND	ND	+
	33.29	ND	ND	38.58	+
	33.26	ND	ND	41.04	+
Zinc Ointment	33.04	ND	ND	ND	+
	33.36	ND	ND	ND	+
	33.14	ND	ND	ND	+
Apohealth Cold Sore Cream	36.18	ND	ND	ND	+
	35.47	ND	ND	37.22	+
	34.75	ND	ND	35.39	+
Abrevea	35.70	ND	ND	ND	+
	35.58	ND	ND	ND	+
	35.37	ND	ND	35.66	+
Neosporin	35.70	ND	ND	ND	+
	35.58	ND	ND	ND	+
	35.37	ND	ND	35.66	+
Dermaid	33.25	ND	ND	ND	+
	33.46	ND	ND	ND	+
	33.18	ND	ND	ND	+
Lanacane	34.03	ND	ND	ND	+
	35.17	ND	ND	ND	+
	34.23	ND	ND	ND	+
Flixonase	33.48	ND	ND	34.63	+
	34.46	ND	ND	ND	+
	34.08	ND	ND	ND	+
Apohealth Budesonide Hayfever	32.66	ND	ND	ND	+
	33.10	ND	ND	ND	+
	32.58	ND	ND	36.40	+
Nasonex Allergy	32.26	ND	ND	35.02	+
	32.60	ND	ND	35.72	+
	32.09	ND	ND	ND	+
Apohealth Decongestant Nasal Spray	31.15	ND	ND	35.44	+
	31.15	ND	ND	36.67	+
	31.79	ND	ND	35.38	+
Vicks VapoDrops (with tumeric)	35.88	ND	ND	ND	+
	35.05	ND	ND	ND	+
	34.75	ND	ND	ND	+
Vaseline	32.76	ND	ND	ND	+
	32.98	ND	ND	ND	+
	33.37	ND	ND	35.40	+
Difflam	35.48	ND	ND	ND	+
	34.69	ND	ND	ND	+
	35.09	ND	ND	32.55	+

*ND for not detected

Table 20. Clade Ib Primer Set Contrived Low Positive Samples with Potentially Interfering Substances

	3 x LoD Sample				
Substance	**FAM Ct**	**HEX Ct**	**ROX Ct**	**Cy5 Ct**	**Result (+/-)**
Vagisil	35.45	32.29	ND	ND	+
	34.10	32.93	ND	ND	+
	34.80	32.35	ND	ND	+
SOOV IT	36.03	36.54	ND	ND	+
	35.35	37.93	ND	ND	+
	35.70	35.84	ND	ND	+
Proctosedyl	36.15	36.30	ND	37.04	+
	36.05	35.00	ND	36.06	+
	37.60	36.44	ND	ND	+
Carmex	35.90	36.90	ND	35.99	+
	35.45	33.56	ND	36.00	+
	36.28	35.31	ND	ND	+
Douche	36.89	35.69	ND	ND	+
	36.13	36.08	ND	ND	+
	37.06	35.88	ND	ND	+
KY Jelly	33.65	31.51	ND	37.18	+
	34.90	32.20	ND	ND	+
	35.69	31.58	ND	ND	+
Respiratory Droplets	33.71	31.09	ND	31.11	+
	33.28	31.73	ND	30.96	+
	34.27	31.39	ND	31.00	+
Blood	34.16	34.12	ND	23.04	+
	34.75	34.90	ND	23.00	+
	34.38	34.62	ND	22.99	+
Seminal Fluid	34.33	33.92	ND	22.38	+
	34.34	34.60	ND	22.34	+
	34.37	34.07	ND	22.28	+
Faeces	33.67	33.24	ND	35.00	+
	33.75	34.43	ND	34.69	+
	34.04	33.89	ND	35.03	+
Male Urine	32.53	34.51	ND	ND	+
	33.37	34.11	ND	ND	+
	32.98	33.45	ND	ND	+
Female Urine	35.37	34.42	ND	ND	+
	35.32	34.16	ND	ND	+
	35.83	35.10	ND	ND	+
Mucin	34.15	33.82	ND	ND	+
	33.89	33.70	ND	ND	+
	33.85	32.52	ND	ND	+
Albumin	34.09	34.73	ND	ND	+
	34.40	35.05	ND	ND	+
	33.88	34.20	ND	36.28	+
Casein	36.08	33.65	ND	ND	+
	34.85	35.73	ND	36.02	+
	35.42	36.41	ND	ND	+
Cornstarch	35.88	34.16	ND	ND	+
	34.89	34.73	ND	ND	+
	36.38	35.23	ND	ND	+
Zinc Ointment	35.49	33.63	ND	36.83	+

	36.07	33.40	ND	ND	+
	35.12	34.21	ND	36.04	+
Apohealth Cold Sore Cream	35.47	35.30	ND	ND	+
	35.47	34.56	ND	ND	+
	35.40	35.20	ND	ND	+
Abrevea	36.03	33.14	ND	36.32	+
	35.13	32.05	ND	ND	+
	34.33	32.52	ND	ND	+
Neosporin	34.38	33.52	ND	ND	+
	34.82	32.97	ND	36.01	+
	33.98	33.28	ND	ND	+
Dermaid	35.64	34.83	ND	ND	+
	35.21	36.65	ND	ND	+
	34.82	33.25	ND	36.22	+
Lanacane	35.34	33.46	ND	ND	+
	35.45	33.22	ND	ND	+
	34.67	33.66	ND	ND	+
Flixonase	33.73	30.93	ND	ND	+
	33.03	31.26	ND	35.86	+
	34.34	32.03	ND	ND	+
Apohealth Budesonide Hayfever	34.55	33.15	ND	ND	+
	35.06	32.58	ND	ND	+
	34.31	33.47	ND	ND	+
Nasonex Allergy	35.36	34.09	ND	37.11	+
	35.44	32.27	ND	ND	+
	35.16	33.07	ND	ND	+
Apohealth Decongestant Nasal Spray	36.27	35.04	ND	ND	+
	36.03	34.20	ND	ND	+
	35.49	32.88	ND	ND	+
Vicks VapoDrops (with tumeric)	35.45	33.06	ND	ND	+
	35.23	33.44	ND	ND	+
	36.40	33.00	ND	36.67	+
Vaseline	36.43	36.09	ND	36.96	+
	36.14	34.56	ND	34.77	+
	36.31	33.94	ND	ND	+
Difflam	35.92	32.21	ND	36.89	+
	36.44	31.28	ND	ND	+
	34.41	31.35	ND	ND	+

*ND for not detected

Table 21. Negative Matrix Samples with Potentially Interfering Substances

	3 x LoD Sample				
Substance	**FAM Ct**	**HEX Ct**	**ROX Ct**	**Cy5 Ct**	**Result (+/-)**
Vagisil	ND	ND	ND	33.42	+
	ND	ND	ND	33.79	+
	ND	ND	ND	33.92	+
SOOV IT	ND	ND	ND	33.18	+
	ND	ND	ND	34.11	+
	ND	ND	ND	34.39	+
Proctosedyl	ND	ND	ND	34.92	+
	ND	ND	ND	32.71	+
	ND	ND	ND	34.68	+

Carmex	ND	ND	ND	33.99	+
	ND	ND	ND	34.14	+
	ND	ND	ND	34.06	+
Douche	ND	ND	ND	34.65	+
	ND	ND	ND	33.18	+
	ND	ND	ND	34.63	+
KY Jelly	ND	ND	ND	33.63	+
	ND	ND	ND	34.68	+
	ND	ND	ND	33.86	+
Respiratory Droplets	ND	ND	ND	28.18	+
	ND	ND	ND	28.10	+
	ND	ND	ND	27.96	+
Blood	ND	ND	ND	27.46	+
	ND	ND	ND	28.40	+
	ND	ND	ND	27.95	+
Seminal Fluid	ND	ND	ND	24.81	+
	ND	ND	ND	25.03	+
	ND	ND	ND	25.08	+
Faeces	ND	ND	ND	32.98	+
	ND	ND	ND	33.42	+
	ND	ND	ND	32.80	+
Male Urine	ND	ND	ND	33.02	+
	ND	ND	ND	33.68	+
	ND	ND	ND	33.33	+
Female Urine	ND	ND	ND	34.18	+
	ND	ND	ND	34.12	+
	ND	ND	ND	33.76	+
Mucin	ND	ND	ND	35.17	+
	ND	ND	ND	34.20	+
	ND	ND	ND	33.35	+
Albumin	ND	ND	ND	33.68	+
	ND	ND	ND	33.46	+
	ND	ND	ND	33.50	+
Casein	ND	ND	ND	33.25	+
	ND	ND	ND	32.76	+
	ND	ND	ND	34.19	+
Cornstarch	ND	ND	ND	34.30	+
	ND	ND	ND	33.35	+
	ND	ND	ND	33.38	+
Zinc Ointment	ND	ND	ND	33.91	+
	ND	ND	ND	33.84	+
	ND	ND	ND	34.18	+
Apohealth Cold Sore Cream	ND	ND	ND	ND	+
	ND	ND	ND	35.76	+
	ND	ND	ND	ND	+
Abrevea	ND	ND	ND	ND	+
	ND	ND	ND	35.76	+
	ND	ND	ND	ND	+
Neosporin	ND	ND	ND	33.72	+
	ND	ND	ND	33.48	+
	ND	ND	ND	33.49	+
Dermaid	ND	ND	ND	35.33	+
	ND	ND	ND	34.36	+

	ND	ND	ND	34.09	+
Lanacane	ND	ND	ND	33.33	+
	ND	ND	ND	33.25	+
	ND	ND	ND	33.53	+
Flixonase	ND	ND	ND	33.54	+
	ND	ND	ND	34.37	+
	ND	ND	ND	34.00	+
Apohealth Budesonide Hayfever	ND	ND	ND	33.29	+
	ND	ND	ND	33.88	+
	ND	ND	ND	34.14	+
Nasonex Allergy	ND	ND	ND	33.06	+
	ND	ND	ND	33.36	+
	ND	ND	ND	33.44	+
Apohealth Decongestant Nasal Spray	ND	ND	ND	34.16	+
	ND	ND	ND	34.20	+
	ND	ND	ND	35.19	+
Vicks VapoDrops (with tumeric)	ND	ND	ND	33.51	+
	ND	ND	ND	34.19	+
	ND	ND	ND	34.15	+
Vaseline	ND	ND	ND	34.56	+
	ND	ND	ND	34.30	+
	ND	ND	ND	36.39	+
Difflam	ND	ND	ND	36.39	+
	ND	ND	ND	35.37	+
	ND	ND	ND	35.78	+

*ND for not detected

Accuracy: Clinical Evaluation

The objective of the clinical evidence studies was to determine the clinical accuracy of our primer sets in the context of another comparator assay. The comparator assay is used as a gold standard to determine whether a particular sample is a true positive or negative sample. As such each sample will be run using the comparator kit and NAT-17 to calculate the positive percent agreement (PPA) and negative percent agreement (NPA) to provide valuable insights into accuracy of NAT-17 as well as analyse the threshold cut-off that maximised specificity and selectivity.

Mpox Pan-Clade Detector (*OPG210* gene and *OPG002* gene)

The clinical accuracy of NAT-17 to detect Clade II was studied using Clade IIb clinical samples from our clinical collaborator. Testing of these samples revealed that the pan-clade detector was highly accurate in discerning between positive and negative Mpox samples. Using the MONKEYPOX CDC primers/probe set as a comparator assay showed that NAT-17 obtained 100% agreement in both positive percent agreement (PPA) and negative percent agreement (NPA), highlighting the accuracy and robustness of this primer set in detecting Mpox DNA.

Table 22. NAT-17 Pan-Clade Clinical Evidence – Positive Samples

Sample Name	FAM	HEX	ROX	Cy5
MCS-P6	33.82	ND	ND	ND
MCS-P7	29.82	ND	ND	35.61
MCS-P8	35.10	ND	ND	36.35

MCS-P9	30.98	ND	ND	36.58
MCS-P31	28.40	ND	ND	34.29
MCS-P33	28.21	ND	ND	36.89
MCS-P34	30.71	ND	ND	35.27
MCS-P35	36.97	ND	ND	32.67
MCS-P38	34.78	ND	ND	ND
MCS-P40	35.09	ND	ND	34.28
MCS-P51	27.97	ND	ND	38.13
MCS-P52	33.09	ND	ND	ND
MCS-P53	28.82	ND	ND	39.36
MCS-P54	36.21	ND	ND	35.03
MCS-P56	30.87	ND	ND	ND
MCS-P60	35.05	ND	ND	ND
MCS-P61	29.94	ND	ND	39.02
MCS-P73	31.18	ND	ND	37.79
MCS-P74	36.33	ND	ND	31.12
MCS-P75	25.96	ND	ND	ND
MCS-P78	30.34	ND	ND	39.45
MCS-P82	28.16	ND	ND	36.54
MCS-P84	31.29	ND	ND	40.22
MCS-P87	35.69	ND	ND	34.63
MCS-P100	29.29	ND	ND	38.94
MCS-P101	26.68	ND	ND	34.31
MCS-P816	27.14	ND	ND	35.31

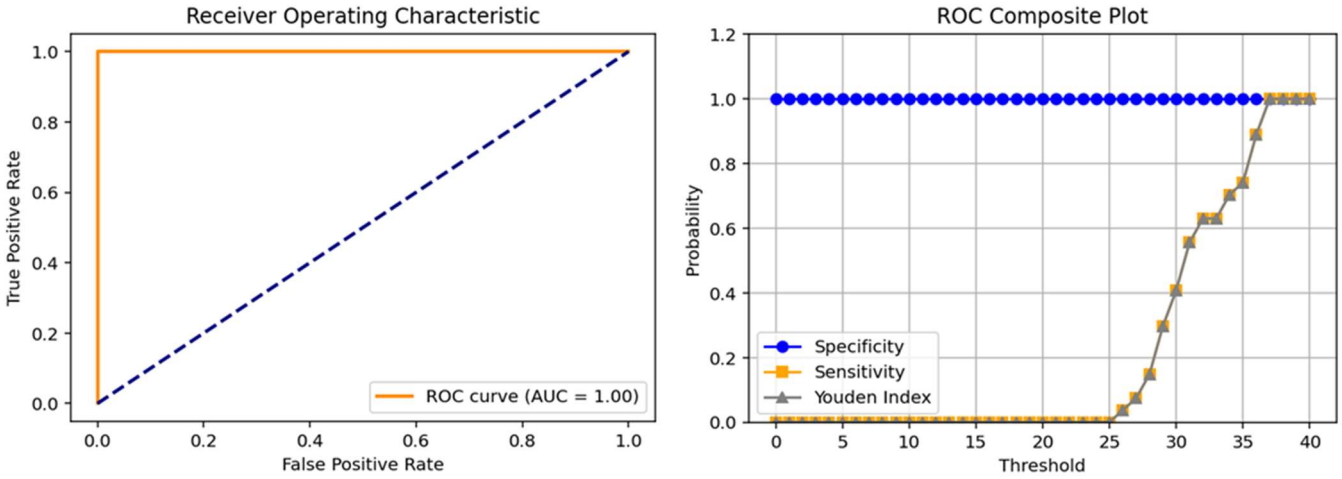
Table 23. CDC Primer Set Pan-Clade Clinical Evidence – Positive Samples

Sample Name	FAM	Cy5
MCS-P6	35.93	ND
MCS-P7	30.34	37.21
MCS-P8	37.70	40.52
MCS-P9	32.64	ND
MCS-P31	30.03	35.92
MCS-P33	29.23	37.81
MCS-P34	30.22	ND
MCS-P35	34.57	35.24
MCS-P38	37.55	ND
MCS-P40	37.40	ND
MCS-P51	36.78	ND
MCS-P52	34.43	ND
MCS-P53	32.20	37.20
MCS-P54	35.79	38.56
MCS-P56	34.19	37.46
MCS-P60	36.26	38.77
MCS-P61	29.80	33.09
MCS-P73	31.31	35.89
MCS-P74	36.58	28.68
MCS-P75	30.43	ND
MCS-P78	36.16	36.73
MCS-P82	32.43	38.49
MCS-P84	35.93	ND
MCS-P87	32.61	30.13
MCS-P100	32.57	35.45
MCS-P101	30.53	36.54

MCS-P816	29.29	37.67

ROC curve analyses showed that 36.97 was the optimal cut-off value, however, due to lack of any false positive or false negative results, this value is merely the highest recorded Ct value generated by NAT-17 in the clinical study. Based on results obtained in the LoD study for this primer set, we have seen Mpox DNA at Ct values above this threshold and thus will implement a Ct cut-off value of 38.

Figure 1. NAT-17 Clade II Clinical Evidence – Negative Samples



Mpox Clade Ib (C3L gene) Contrived Study

The clinical accuracy of NAT-17 to detect Clade Ib was studied using contrived Clade Ib samples from a whole genome DNA sample of Clade Ib. Testing of these samples revealed that the pan-clade detector and Clade Ib primer set was highly accurate in discerning between positive and negative Mpox samples. Using the MONKEYPOX CDC primers/probe set as a comparator assay showed that NAT-17 obtained 100% agreement in both positive percent agreement (PPA) and negative percent agreement (NPA), highlighting the accuracy and robustness of this primer set in detecting Mpox DNA.

Table 24. NAT-17 Clade Ib Primer Set Clinical Evidence – Positive Samples

Sample Name	FAM	HEX	ROX	Cy5
MIB-01	32.19	26.78	ND	29.03
MIB-02	32.12	26.80	ND	28.75
MIB-03	32.22	26.48	ND	28.92
MIB-04	32.02	26.41	ND	28.72
MIB-05	32.12	26.72	ND	28.89
MIB-06	32.49	27.11	ND	29.05
MIB-07	32.67	27.06	ND	29.67
MIB-08	32.88	27.09	ND	29.04
MIB-09	32.81	27.09	ND	29.44
MIB-10	32.64	27.14	ND	29.20
MIB-11	33.37	27.73	ND	30.06
MIB-12	33.45	27.74	ND	29.63
MIB-13	33.37	27.64	ND	29.98
MIB-14	33.40	27.86	ND	30.15
MIB-15	33.37	27.52	ND	30.48
MIB-16	33.53	27.85	ND	29.96
MIB-17	33.21	27.87	ND	30.11

MIB-18	34.47	28.54	ND	32.05
MIB-19	34.37	28.20	ND	31.48
MIB-20	34.54	28.92	ND	30.41
MIB-21	34.50	29.02	ND	30.80
MIB-22	31.88	31.18	ND	33.41
MIB-23	32.21	31.21	ND	34.18
MIB-24	31.68	31.32	ND	34.13
MIB-25	32.72	31.93	ND	34.44
MIB-26	32.35	31.91	ND	34.87
MIB-27	32.42	32.70	ND	33.87
MIB-28	33.09	31.55	ND	33.88
MIB-29	32.89	31.61	ND	33.83
MIB-30	33.16	32.18	ND	35.68
MIB-31	32.73	32.07	ND	34.94
MIB-32	33.10	31.54	ND	35.37
MIB-33	34.44	33.06	ND	35.93
MIB-34	35.25	34.05	ND	35.96
MIB-35	34.46	34.25	ND	36.02
MIB-36	35.08	33.31	ND	35.29
MIB-37	34.32	34.04	ND	35.17
MIB-38	33.52	34.75	ND	35.67
MIB-39	34.23	36.31	ND	35.46
MIB-40	33.98	33.08	ND	36.03
MIB-41	34.18	33.64	ND	36.03
MIB-42	35.48	34.70	ND	35.85

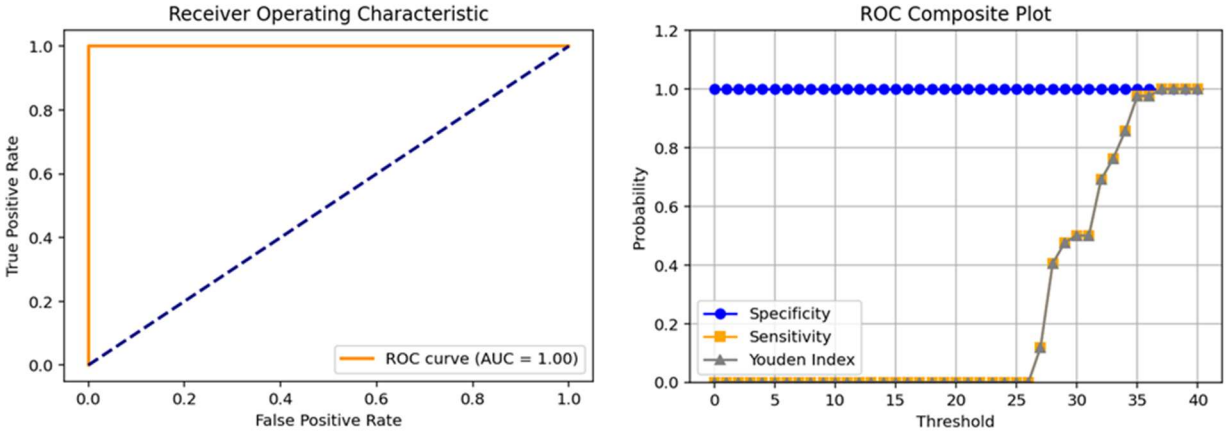
Table 25. CDC Primer Set Clade Ib Primer Set Clinical Evidence – Positive Samples

Sample Name	FAM	Cy5
MIB-01	23.08	27.03
MIB-02	22.74	26.00
MIB-03	22.83	26.59
MIB-04	22.88	25.81
MIB-05	23.07	26.39
MIB-06	23.47	27.11
MIB-07	23.39	26.37
MIB-08	23.50	26.84
MIB-09	23.26	27.12
MIB-10	23.28	27.57
MIB-11	24.34	27.15
MIB-12	24.08	26.40
MIB-13	24.24	27.15
MIB-14	24.03	27.63
MIB-15	24.16	ND
MIB-16	24.30	27.22
MIB-17	24.53	27.11
MIB-18	25.64	27.92
MIB-19	25.39	27.23
MIB-20	25.25	ND
MIB-21	25.26	27.45
MIB-22	25.19	27.22
MIB-23	25.61	ND
MIB-24	25.17	27.86
MIB-25	25.81	ND

MIB-26	26.25	ND
MIB-27	25.90	ND
MIB-28	26.11	ND
MIB-29	25.77	28.27
MIB-30	25.80	28.24
MIB-31	26.15	ND
MIB-32	26.06	ND
MIB-33	27.41	ND
MIB-34	27.27	ND
MIB-35	27.94	ND
MIB-36	27.39	ND
MIB-37	27.92	28.37
MIB-38	27.13	28.35
MIB-39	28.01	ND
MIB-40	27.67	ND
MIB-41	27.92	ND
MIB-42	27.54	28.40

ROC analysis showed that 36.31 was the optimal cut-off value, however, due to lack of any false positive or false negative results, this value is merely the highest recorded Ct value generated by NAT-17 in the clinical study. Based on results obtained in the LoD study for this primer set, we have seen Mpox DNA at Ct values above this threshold and thus will implement a Ct cut-off value of 38.

Figure 2. NAT-17 Clade Ib Clinical Evidence



Mpox Clade Ia (C3L gene) Contrived Study

Due to the lack of Clade Ia clinical samples, contrived positive samples were created using plasmids containing the amplicon sequence of the respective primer sets. The use of these plasmid-containing samples was able to show that amplification was observed at low concentrations of the plasmid.

Table 26. NAT-17 Clade Ia Clinical Evidence – Contrived Positive Samples

Sample Name	FAM	HEX	ROX	Cy5
Cla-P1	ND	ND	34.62	ND
Cla-P2	ND	ND	36.23	39.78
Cla-P3	ND	ND	36.06	ND
Cla-P4	ND	ND	35.86	ND
Cla-P5	ND	ND	36.33	ND
Cla-P6	ND	ND	36.20	40.46

Cla-P7	ND	ND	34.44	ND
Cla-P8	ND	ND	35.60	ND
Cla-P9	ND	ND	34.93	ND
Cla-P10	ND	ND	35.99	ND
Cla-P11	ND	ND	36.00	ND
Cla-P12	ND	ND	36.33	ND
Cla-P13	ND	ND	34.55	ND
Cla-P14	ND	ND	35.33	ND
Cla-P15	ND	ND	35.65	39.59
Cla-P16	ND	ND	35.57	ND
Cla-P17	ND	ND	35.10	ND
Cla-P18	ND	ND	35.88	ND
Cla-P19	ND	ND	34.74	ND
Cla-P20	ND	ND	35.48	ND
Cla-P21	ND	ND	36.15	ND
Cla-P22	ND	ND	36.17	39.09
Cla-P23	ND	ND	36.49	ND
Cla-P24	ND	ND	36.66	ND
Cla-P25	ND	ND	35.24	ND
Cla-P26	ND	ND	35.80	ND
Cla-P27	ND	ND	36.07	ND
Cla-P28	ND	ND	35.95	ND
Cla-P29	ND	ND	35.83	ND
Cla-P30	ND	ND	36.52	ND

Negative Specimen Matrix Samples

Table 27. Negative Matrix Samples NAT-17 and CDC Primer Set

Sample Name	NAT-17 FAM Ct	NAT-17 HEX	NAT-17 ROX Ct	NAT-17 Cy5 Ct	CDC FAM	CDC Cy5
N1	ND	ND	ND	33.22	ND	33.53
N2	ND	ND	ND	34.97	ND	36.61
N3	ND	ND	ND	30.20	ND	29.30
N4	ND	ND	ND	34.73	ND	34.31
N5	ND	ND	ND	30.32	ND	29.28
N6	ND	ND	ND	34.65	ND	33.88
N7	ND	ND	ND	30.21	ND	29.51
N8	ND	ND	ND	30.22	ND	30.01
N9	ND	ND	ND	30.27	ND	29.33
N10	ND	ND	ND	29.82	ND	29.59
N11	ND	ND	ND	29.96	ND	29.79
N12	ND	ND	ND	30.45	ND	29.37
N13	ND	ND	ND	30.32	ND	29.14
N14	ND	ND	ND	30.31	ND	29.56
N15	ND	ND	ND	30.19	ND	29.17
N16	ND	ND	ND	30.33	ND	29.62
N17	ND	ND	ND	34.85	ND	35.85
N18	ND	ND	ND	33.15	ND	33.57
N19	ND	ND	ND	35.05	ND	34.85
N20	ND	ND	ND	30.48	ND	29.43
N21	ND	ND	ND	30.10	ND	29.20
N22	ND	ND	ND	29.89	ND	28.82
N23	ND	ND	ND	30.27	ND	29.14

N24	ND	ND	ND	30.28	ND	29.73
N25	ND	ND	ND	30.16	ND	29.24
N26	ND	ND	ND	37.20	ND	35.87
N27	ND	ND	ND	35.45	ND	37.92
N28	ND	ND	ND	30.22	ND	29.81
N29	ND	ND	ND	30.17	ND	29.22
N30	ND	ND	ND	29.89	ND	29.43
N31	ND	ND	ND	34.93	ND	34.75
N32	ND	ND	ND	35.17	ND	34.17
N33	ND	ND	ND	35.55	ND	35.25
N34	ND	ND	ND	37.29	ND	35.18
N35	ND	ND	ND	36.28	ND	35.58
N36	ND	ND	ND	30.21	ND	29.23
N37	ND	ND	ND	33.83	ND	34.14
N38	ND	ND	ND	34.84	ND	35.43
N39	ND	ND	ND	33.20	ND	33.18
N40	ND	ND	ND	31.70	ND	31.55
N41	ND	ND	ND	34.38	ND	34.63
N42	ND	ND	ND	30.45	ND	29.47
N43	ND	ND	ND	35.73	ND	34.45
N44	ND	ND	ND	30.36	ND	29.96
N45	ND	ND	ND	28.70	ND	28.70
N46	ND	ND	ND	34.97	ND	34.97
N47	ND	ND	ND	30.34	ND	30.34
N48	ND	ND	ND	29.36	ND	29.36
N49	ND	ND	ND	30.30	ND	30.30
N50	ND	ND	ND	32.96	ND	32.96
N51	ND	ND	ND	30.36	ND	30.36
N52	ND	ND	ND	30.30	ND	30.30
N53	ND	ND	ND	30.29	ND	30.29
N54	ND	ND	ND	36.81	ND	36.81
N55	ND	ND	ND	30.11	ND	30.11
N56	ND	ND	ND	39.54	ND	39.54
N57	ND	ND	ND	30.30	ND	30.30
N58	ND	ND	ND	36.61	ND	36.61
N59	ND	ND	ND	37.02	ND	37.02
N60	ND	ND	ND	30.36	ND	30.36
N61	ND	ND	ND	30.33	ND	30.33
N62	ND	ND	ND	38.49	ND	38.49
N63	ND	ND	ND	36.88	ND	36.88
N64	ND	ND	ND	35.39	ND	35.39
N65	ND	ND	ND	30.18	ND	30.18
N66	ND	ND	ND	30.28	ND	30.28
N67	ND	ND	ND	29.83	ND	29.83
N68	ND	ND	ND	36.87	ND	36.87
N69	ND	ND	ND	37.33	ND	37.33
N70	ND	ND	ND	30.21	ND	30.21
N71	ND	ND	ND	29.92	ND	29.92
N72	ND	ND	ND	30.22	ND	30.22
N73	ND	ND	ND	35.18	ND	35.18
N74	ND	ND	ND	30.27	ND	30.27
N75	ND	ND	ND	30.16	ND	30.16
N76	ND	ND	ND	36.25	ND	36.25
N77	ND	ND	ND	34.41	ND	34.41

N78	ND	ND	ND	37.48	ND	37.48
N79	ND	ND	ND	36.56	ND	36.56
N80	ND	ND	ND	35.52	ND	35.52
N81	ND	ND	ND	30.37	ND	30.37
N82	ND	ND	ND	30.28	ND	30.28
N83	ND	ND	ND	30.37	ND	30.37
N84	ND	ND	ND	37.43	ND	37.43
N85	ND	ND	ND	36.08	ND	36.08
N86	ND	ND	ND	36.93	ND	36.93
N87	ND	ND	ND	29.70	ND	29.70
N88	ND	ND	ND	34.26	ND	34.26
N89	ND	ND	ND	30.02	ND	30.02
N90	ND	ND	ND	30.11	ND	30.11
N91	ND	ND	ND	26.77	ND	26.77
N92	ND	ND	ND	34.43	ND	34.43
N93	ND	ND	ND	26.59	ND	26.59
N94	ND	ND	ND	26.59	ND	26.59
N95	ND	ND	ND	24.74	ND	24.74
N96	ND	ND	ND	33.75	ND	33.75
N97	ND	ND	ND	34.72	ND	34.72
N98	ND	ND	ND	24.78	ND	24.78
N99	ND	ND	ND	32.22	ND	32.22
N100	ND	ND	ND	24.84	ND	24.84
N101	ND	ND	ND	31.09	ND	31.09
N102	ND	ND	ND	32.45	ND	32.45
N103	ND	ND	ND	26.62	ND	26.62
N104	ND	ND	ND	26.80	ND	26.80

The clinical evidence studies demonstrated the accuracy of the HA TECH Monkeypox Virus Real-Time PCR Diagnostic Kit (NAT-17) in detecting Mpox DNA from clinical samples. In particular, the pan-clade detector was able to correctly identify all positive and negative clinical specimens according to the comparator assay. This resulted in a PPA and NPA of 100%. Through ROC curve analysis, the Ct cut-off of 38 was determined for all channels of NAT-17.

Precision Studies

Precision studies evaluate the repeatability and reproducibility of a monkeypox DNA assay using purified genomic Monkeypox DNA control material. Two concentrations were tested—a medium positive at 0.39 copies/µL (10x LoD) and a low positive at 0.115 copies/µL (~3x LoD)—across five replicates. Experiments were conducted over multiple days, using three different lots and two operators. Intra-assay (repeatability) and inter-assay (reproducibility) precision were determined by analyzing Ct values and calculating the coefficient of variation (CV%). All data met UK Health Security Agency criteria, confirming the assay's consistency and reliability across various conditions.

Inter-assay Precision (Repeatability)

Five replicate values of genomic Monkeypox DNA (0.39 copies/µL and 0.115 copies/µL) were tested in one assay by two different operators. The CV percentages remain well below 5%, indicating high degree of repeatability.

Table 28: Summary Table of Intra-assay Precision between Operators

Concentration	Between Operators Mean (Ct)	Between Operators SD	Between Operators CV%
0.39 copies/µL	26.19	0.32	1.22%
0.115 copies/µL	27.61	0.85	3.09%

Inter-assay Precision (Reproducibility)

Five replicate values of two different Monkeypox Control concentrations (0.39 copies/µL and 0.115 copies/µL) were tested on three different days with the same kit. CV percentages across the three days were well below 5% for both tested concentrations, indicating high degree of reproducibility.

Table 29: Summary Table of Inter-assay Precision (between days)

Concentration	Between Days Mean (Ct)	Between Days SD	Between Days CV (%)
100 copies/µL	25.81	0.51	1.97
32 copies/µL	27.09	0.31	1.16

Five replicate values of two different Monkeypox Control concentrations (0.39 and 0.115 copies/µL) were tested with three different lots. The CV percentages across the three days were below 5%, demonstrating the reproducibility of NAT-17.

Table 30: Summary Table of Inter-assay Precision (between lots/batches)

Concentration	Between Lot Mean (Ct)	Between Lot SD	Between Lot CV%
0.39 copies/µL	26.67	0.81	3.03
0.115 copies/µL	27.96	1.09	3.90

The reproducibility and repeatability data show that the Coefficient of Variation between operators, between days and between batches were well below the 5% in all tested cases. This demonstrates that the assay performs consistently and is precise at low positive and medium positive concentrations of sample.

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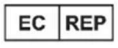
Symbol key

Manufacturer



Authorized representative in the European Community/European Union

Date of manufacture

Use-by date

Batch code

Catalogue number

Temperature limit

Consult instructions for use or consult electronic instructions for use