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Diagnostic accuracy of Truenat® MPX assay in comparison to Non-variola Orthopoxvirus RT PCR for diagnosis of mpox disease in Uganda

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Article

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Keywords: Diagnostic accuracy, mpox, Truenat MPX, Non-Variola Orthopoxvirus RT PCR, skin lesions.

Short title: Diagnostic accuracy study of the Truenat MPX assay

Abstract

There have been intensified efforts towards low-complexity molecular diagnostics to respond to the mpox epidemic. This study evaluated the performance of the Truenat MPX (Molbio Diagnostics Limited, India) assay for the detection of mpox in Uganda, using the Non-variola Orthopoxvirus RT-PCR as the comparator assay.

We conducted a laboratory-based cross-sectional study using Non-variola Orthopoxvirus, which targets both MPXV Clade I and II. Participants' samples from patients suspected of having mpox in all four regions of Uganda were utilised.

Among the 150 participants in this study, 49 were mpox-positive, and 101 were mpox-negative by Non-variola Orthopoxvirus RT-PCR. 77 (51.3%) participants were male, and 72 (48.3%) were female, with a median age of 22 years (19-26). Samples used mainly skin lesions, oropharyngeal swabs and oral swabs. The Truenat MPX assay sensitivity was 96% (47/49, n/N 95% CI 86.3-98.9), and the specificity was 99% (100/101 n/N, 95% CI 94.6-99.8). 1.3% (2/150, n/N 95% CI 0.4–4.7) invalid results

The Truenat MPX assay offers high sensitivity and specificity for MPXV detection, with differentiation from other orthopoxviruses, rapid turnaround time, ease of use in integrated workflows, compatibility with multiple specimen types, and suitability for low-resource settings.

Introduction (306)

Mpox is a zoonotic disease caused by the Monkeypox virus (MPXV), a member of the family Poxviridae and the genus Orthopoxvirus, which is related to variola, cowpox, vaccinia, and other viruses. There are two distinct clades of the virus: clade I (with subclades Ia and Ib) and clade II (with subclades IIa and IIb), both first isolated from lesions observed in captive monkeys in Copenhagen, Denmark (2). The Human mpox case was first identified in the Democratic Republic of Congo (DRC) in 1970. MPXV causes a disease in humans that resembles smallpox but has a lower case-fatality rate (3). The disease is endemic in the rainforests of central and western Africa, where squirrels and monkeys have been suggested to play a role in the virus's life cycle. Animal antibody surveys in the DRC suggested that squirrels play a major role as a reservoir of the virus and that humans are sporadically infected(4). Human-to-Human transmission occurs with an incubation period of 12 days. In subsequent decades, sporadic outbreaks were reported in Africa, with mortality rates ranging between 1-2%. Human infection with mpox occurred in the United States in 2003, when imported animals from Africa infected pet prairie dogs (5). In September 2018, in the United Kingdom, two

imported cases of mpox were detected in persons from Nigeria; a healthcare worker acquired nosocomial infection from one of those patients, suggesting that the disease must be regarded as an important re-emerging infectious disease. The number of reported cases each year has increased steadily over that period (6).

On August 14, 2024, the World Health Organisation (WHO) declared mpox a Public Health Emergency of International Concern (PHEIC), following a similar declaration by the Africa Centres for Disease Control and Prevention on August 13 (7, 8). These declarations emphasized the severity of the outbreak and the urgent need for coordinated global action to control the disease. This marked the second time in two years that mpox was reclassified to the highest response level. Globally, between Jan 2025 and March 2026, 58214 confirmed mpox cases were reported in 100 countries, and 238 of these cases resulted in death, for a 0.4% mortality rate (9).

According to the WHO multi-country external situation mpox report, 1235 mpox cases were confirmed in March 2026, of which 70.4% were reported from the WHO African region (9). This highlights a critical need for new decentralized testing, which is cheaper, can be used on already available platforms, and has higher diagnostic accuracy to swiftly identify and contain mmpox, especially in Africa, to prevent further transmission and fatalities (10).

Methods (651)

Ethical Approval Statement

This study was conducted in accordance with the ICH-GCP guidelines. Ethics approval was obtained from the Makerere University School of Biomedical Sciences Research Ethics Committee (SBS-2025-82) and the Uganda National Council of Science and Technology (HS5956ES).

Informed Consent Statement

We obtained a waiver of informed consent from the Makerere University School of Biomedical Sciences Research Ethics Committee (SBS-REC).

Study population

The study utilized biobanked specimens from the Central Emergency Response and Surveillance Laboratory (CERSL) collected from patients in Uganda with presumptive mpox disease between May and August 2025. Specimens utilized in this evaluation were skin lesions, oropharyngeal and oral swabs collected in viral transport medium (VTM), stored at 2-8°C

before testing using the Non-variola Orthopoxvirus RT PCR assay, which has been validated by CDC (11), and stored at -80°C for long-term storage for the Molbio Truenat MPX assay testing.

Truenat MPX assay procedure: Specimens were thawed at room temperature, and 0.5 mL of the sample was transferred to the Lysis Buffer Tube and mixed by pipetting with a single-use Pasteur pipette. The contents from the lysis buffer tube were dispensed into the sample chamber of the Trueprep® AUTO V2 Universal Cartridge and loaded into the Trueprep AUTO for sample nucleic acid purification. A purified sample was collected using a Pasteur pipette and dispensed into the Elute Collection Tube. The Truenat MPX PCR microchip was removed from the pouch and loaded into the Truelab® Duo Real Time micro-PCR analyser. The microtube from the same pouch containing freeze-dried PCR reagents was placed on the microtube stand. Using a 6µL fixed volume pipette, 6µL of the elute solution was dispensed into the freeze dried PCR reagents microtube and allowed to stand for 30-60 seconds to get a clear solution from which 6µL of this clear solution was pipetted out using the same pipette and tip and was dispensed into the center of the white reaction well of the Truenat MPX chip and the PCR reaction started on Truelab Duo PCR analyzer.

Truenat MPX assay results and interpretation: The Truelab Duo PCR analyzer screen displayed OPOX and MPOX DETECTED for a positive result or NOT DETECTED for a negative result. The screen also displayed viral load as HIGH (Ct<20), MEDIUM (20 Ct <25), LOW (25≤Ct<30), or VERY LOW (Ct≥30) for positive samples. The screen also displayed results as valid or invalid.

DNA extraction for mpox samples; DNA was extracted from the specimens using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol. Before extraction, VTM containing lesion swabs were vortexed thoroughly for 10–15 seconds to ensure homogeneity of viral particles and associated cellular material within the transport medium. 200 µL of the specimen suspension was transferred into a sterile 1.5 mL microcentrifuge tube containing 20 µL of QIAGEN Protease (proteinase K). Subsequently, 200 µL of Buffer AL was added, and the mixture was vortexed for 15 seconds to achieve complete homogenization. The lysate was incubated at 56 °C for 10 minutes to facilitate protein digestion and viral particle lysis, thereby maximizing nucleic acid release. After incubation, the tube was briefly centrifuged to remove condensation from the lid, and 200 µL of absolute ethanol was added. The mixture was vortexed for 15 seconds to promote efficient nucleic acid binding conditions. The entire lysate was carefully transferred to a QIAamp Mini spin column placed in a 2 mL collection tube, and the column was centrifuged at 6000 × g for 1 minute to allow

DNA to adsorb onto the silica membrane. The spin column was subsequently washed with 500 µL Buffer AW1, centrifuged at 6000 × g for 1 minute, then washed again with 500 µL Buffer AW2 and centrifuged at 20,000 × g for 3 minutes to remove residual contaminants and inhibitors. To eliminate carryover from the wash buffer, the spin column was transferred to a new collection tube and centrifuged at full speed for an additional 1 minute. The column was then placed into a clean 1.5 mL microcentrifuge tube, and DNA was eluted by adding 200 µL Buffer AE directly onto the membrane. After incubation at room temperature for 5 minutes to enhance elution efficiency, the column was centrifuged at 6000 × g for 1 minute. The eluted DNA was immediately stored at −20 °C until downstream molecular analysis.

Non-Variola Orthopoxvirus Real-Time PCR: The assay targets conserved genomic regions specific to Orthopoxviruses. Amplification reactions were prepared in a final volume of 25 µL using a commercially validated real-time PCR master mix (Quanta Biosciences, USA), containing optimized concentrations of DNA polymerase, reaction buffer, deoxynucleotide triphosphates, and magnesium chloride. Each reaction mixture consisted of 20 µL of master mix supplemented with Non-variola Orthopoxviru**s** -specific forward and reverse primers and a fluorescent-labelled hydrolysis probe targeting the Orthopoxvirus genome, and 5 µL of extracted DNA template. All reactions were prepared in a dedicated clean area to prevent contamination. In parallel, amplification of the human RNase P gene was performed for each specimen as an internal control to verify nucleic acid extraction efficiency, specimen adequacy, and absence of PCR inhibition. Each PCR run included a positive control containing Orthopoxvirus DNA to confirm assay performance and a no-template control containing nuclease-free water to monitor contamination. Real-time PCR amplification was performed using a calibrated real-time PCR instrument under the following cycling conditions: an initial denaturation step at 95 °C for 8 minutes to activate the DNA polymerase and denature template DNA, followed by 40 amplification cycles consisting of denaturation at 95 °C for 5 seconds and annealing/extension at 60 °C for 30 seconds. Fluorescence signals were measured at the end of each annealing/extension step using appropriate detection channels. Results were interpreted based on cycle threshold (Ct) values. A confirmed mpox case is a person with laboratory-confirmed mpox virus infection, as detected by real-time polymerase chain reaction (PCR) and/or sequencing of unique viral DNA sequences. A suspected or probable case for which laboratory testing of lesion fluid, skin specimens, or crusts by PCR and/or sequencing is negative for mpox virus (9). Specimens showing absence of amplification for both targets were considered invalid and were subjected to repeat extraction and testing.

Invalid and discordant results were repeated, and valid results and repeat-testing results in cases of discordance were then used in the analysis for diagnostic accuracy. Fresh biobanked samples were utilized for the repeat testing process, starting from the sample preparation stage as stipulated in the kit Instructions for Use (IFU).

Statistical analysis

Data were analyzed for proportions and frequencies. The sensitivity of the Truenat MPX assay was calculated as the proportion of positives compared to those positive by the Non-variola Orthopoxvirus as the comparator assay. The specificity of the Truenat MPX assay was calculated as the proportion of negative results compared to those negative in the Non-variola Orthopoxvirus comparator assay. Likewise, the positive predictive value (PPV) was calculated as the number of positives by the Truenat MPX assay divided by the total number of positives, and the Negative predictive value (NPV) as the number of negatives by the Truenat MPX assay divided by the total number of negatives. All results were reported at 95% confidence interval (95% CI) using Wilson's score method.

Results (394)

Between 24th July and 27th September 2025, 150 biobanked patient samples with existing standard-of-care Non-variola Orthopoxvirus test results were selected and tested using the Truenat MPX assay. 50 were positive, and 100 were negative for mpox by the SoC Non-variola Orthopoxvirus test. However, during resolution of discordant results, Non-variola Orthopoxvirus repeat testing identified one of the positives as a negative mpox case, thereby decreasing the positive cases from 50 to 49, while the negative cases increased from 100 to 101. The median age of participants was 22 years (IQR 19-26 years), of whom 51.7% (n/N, 77/149) were male, and 48.3% (n/N, 72/149) were female. Sample types collected from participants included 72% (n/N, 108/150) skin lesions, 24% (n/N, 36/150) oropharyngeal swabs, and 4% (n/N, 6/150) oral swabs (Table 1).

The sensitivity and specificity of the Truenat MPX assay for detection of mpox was 96% (47/49, n/N 95% CI 86.3–98.9) and 99% (100/101, n/N 95% CI 94.6-99.8), respectively, as shown in Table 2.

Invalid results were 1.3% (2/150, n/N 95% CI 0.37–4.71). We obtained three discordant samples: two were positive for Non-variola Orthopoxvirus but negative on the Truenat MPX assay, whereas one was negative for Non-variola Orthopoxvirus but positive on the Truenat MPX assay. The Ct values for discordants were: Non-variola Orthopoxvirus positive but Truenat MPX assay negative (Ct values were 32.49 and 33.5); and Non-variola Orthopoxvirus

negative but Truenat MPX assay positive (Ct value was 32.67). There was no significant difference in cycle threshold (Ct) values between the Non-variola Orthopoxvirus and Truenat MPX assays (see Figure 1).

Discussion (736)

In this diagnostic evaluation study, we have documented that Truenat MPX assay is a highly sensitive and specific decentralized molecular assay for mpox. Using samples tested with the Non-variola Orthopoxvirus as a SoC test for mpox in Uganda, the Truenat MPX assay achieved a sensitivity of 96% (47/49, n/N 95% CI 86.3–98.9) and specificity of 99% (100/101, n/N 95% CI 94.6-99.8). The level of invalid results was acceptable at 1.3% (95% CI 0.37–4.71), and the same sample yielded valid results on repeat testing. The documented findings from this evaluation meet the WHO Target Product Profile 1 (TTP 1) for molecular methods used for mpox diagnosis in prospective and retrospective clinical samples: a minimal invalid/error rate of ≤ 5% and sensitivity of ≥ 95%, with a preferred specificity of ≥ 99% (12). Assay performance between sample types is not significant due to unequal sample sizes.

The test can be performed on already available Truenat equipment and reagents for nucleic acid extraction, utilized for other pathogens like *Mycobacterium tuberculosis.*

The Truenat MPX assay, which is designed for the diagnosis of MPXV in lesion material, and in the absence of lesion material, mucosal swabs has demonstrated similar findings as studies done earlier in DRC, which utilized the same sample types; crusts and vesicles to compare the MPX-generic real-time PCR assay as a comparator with the GeneXpert MPX/OPX assay and demonstrated sensitivity of 98.8% (83/84, n/N), specificity of 100% (65/65, n/N). We found comparable diagnostic accuracy with CDC MPX/OPX GeneXpert and INRB MPX/OPX GeneXpert (12).

The Truenat platform, which is a Low Complexity-Nucleic Acid Amplification Test (LC-NAAT), though its intended use can support nPOC testing, is endorsed by the World Health Organization (WHO) for the diagnosis of tuberculosis and detection of resistance to rifampicin. Truenat platform has been fully evaluated for robustness, feasibility, and accuracy in field settings (13); however, its ability to offer detection of multiple pathogens has been rarely validated. The world is experiencing funding turbulence following US government stop-work orders, which have affected several funding mechanisms. It is indeed prudent to maximally utilize the already tested diagnostic platforms in an integrated disease program mechanism. Such a strategy will not only be cost-effective but also efficient in the face of funding challenges.

The Truenat MPX test is one of the several disease diagnostic capabilities of the Truenat platform, and our study shows that the platform can achieve more, including the detection of epidemic-prone pathogens, which facilitates efforts towards epidemic preparedness.

Limitations

The point estimate for sensitivity observed in this investigation is less than the preferred World Health Organization Target Product Profile (WHO TPP) of ≥ 97% but higher than the minimal WHO TPP of ≥95%, for mpox diagnosis (12). This can be attributed to the small sample size evaluated (95% CIs overlap with the preferred TPP target) and the utilization of biobanked samples, as evidenced by NPV and PPV values of 98.0% and 97.9%, respectively (Table 2), which means that borderline positive samples on Non-variola Orthopoxvirus RT PCR assay could have been missed on Truenat MPX due to sample stability issues in storage or upon freeze/thaw procedures. However, the overall Ct values for the Non-variola Orthopoxvirus RT PCR and Truenat MPX assays show strong agreement (Figure 1), suggesting that, despite the use of biobanked samples, deterioration did not significantly affect the diagnostic accuracy of the Truenat MPX assay. Truenat MPX consistently shows lower Ct values, which depict better amplification efficiency for OPOX and MPOX targets.

This investigation could not capture some demographic information like gender, HIV status, other STIs, occupation, interaction with animals, and many others, which other studies have demonstrated to be risk factors of mpox (14-16). However, this is one of the few studies on the diagnostic tests in the pipeline for detecting the mpox virus.

In conclusion, the Truenat MPX Test is a highly sensitive and specific LC-NAAT assay for mpox. Given the expanded use of the Truenat platforms for multi-disease testing, this test offers a rapid, easy-to-use, and cost-effective strategy for integrated diagnostics to support mpox epidemic control and research.

Data sharing

All data supporting the findings of this study are available within the paper and its Supplementary Information.

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Author contributions

WS and MJ conceptualized the study. WS was responsible for the design of the methodology. WS, JS, RK were responsible for data curation. Formal analysis was done by WS and JS, AN and WS were responsible for supervision. Validation was performed by JS, DS, VN, SS and RK. JS and WS led the writing of the original draft. All authors contributed to the review and editing of the final manuscript.

Declaration of interests

The authors declare that this research project was conducted in the absence of any known commercial or financial relationships that could be interpreted as a potential conflict of interest

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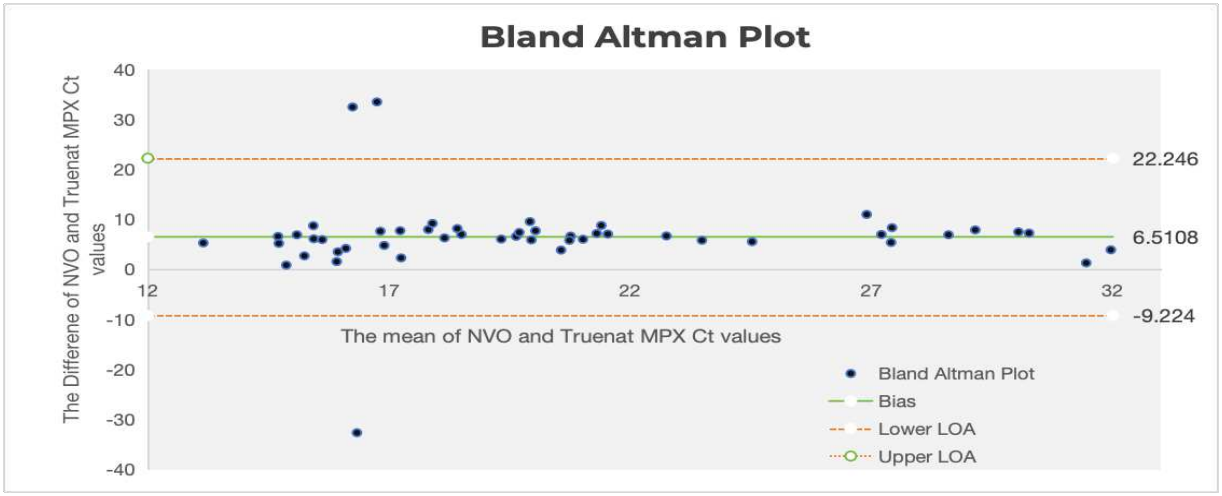
Generative AI statement

The author (s) declare that no AI was used in the creation of this manuscript.

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Figure 1: A Bland Altman plot comparing Ct values of Truenat MPX and Non-variola Orthopoxvirus.



Ct = Cycle threshold NVO = Non-Variola Orthopoxvirus RT PCR

LOA = Limits of Agreement

Table 1: Demographic and clinical characteristics

Parameter	n/N	Percentage (%)
Sex*		
Male	77/149	51.7
Female	72/149	48.3
Median age (IQR)	22	19-26
Sample types		
Skin lesion	108/150	72
Oropharyngeal swab	36/150	24
Oral swab	6/150	4
Non-variola Orthopoxvirus results		
Positive	49/150	32.7
Negative	101/150	67.3
Truenat MPX results		
Positive	47/150	31.3
Negative	103/150	68.7
Invalid	2/150	1.3
Error	0	0

* = one person's gender was undocumented

IQR = interquartile range

Table 2: Diagnostic accuracy of Truenat MPX assay using Non-variola Orthopoxvirus RT PCR as comparator assay.

Parameter	n/N	Percentage (%)	Confidence Interval (95%)
Sensitivity	47/49	95.9	86.3-98.9
Specificity	100/101	99.0	94.6-99.8
Negative predictive values (NPV)	100/102	98.0	93.1-99.5
Positive predictive values (PPV)	47/48	97.9	89.1-99.6

n= number, N= Total sample size

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryfile.xlsx