

Performance evaluation of the Truenat[®] MPX Test to detect Mpox in Uganda



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LabCoP EXTENDED ECHO SESSION
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Study site

Study site	Makerere University Biomedical Research Center (MAKBRC), Department of Medical Microbiology, Makerere University, Kampala, Uganda
Study Team	Dr. Willy Ssengooba, Ph.D (PI) Prof. Moses Joloba, Ph.D (CoPI) Mr. Edgar Kigozi (MAKBRC) (CoI) Mr. ABDUNOOR NYOMBI (MoH) (CoI)
Financial Support	Molbio Diagnostics Limited

Presentation outline

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- Background/Introduction
- Context and Aim
- Methods
- Results and findings
- Discussion
- Innovative contribution to policy, practice and/or research

Background

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- Mpox is a zoonotic virus in the family Poxviridae and genus *Orthopoxvirus*, first isolated from lesions seen among captive monkeys in Copenhagen, Denmark.
- Africa reported the highest number of cases (55%) in 2025, driven primarily by the MPXV Clade 1b variant, affecting several countries.
- This highlights the critical need for a low complexity rapid molecular test to **swiftly identify and contain Mpox**, to prevent further transmission and fatalities.

Current WHO Dashboard

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Mpox

Multi-country external situation report no. 65, published 30 April 2026

KEY FIGURES			
Area	Number of reported confirmed cases	Number of deaths among confirmed cases	Number of reporting countries
Global (1 Jan 2025 – 31 Mar 2026)*	58 214	238	100
Key countries in Africa (9 Mar – 19 Apr 2026)**			
Madagascar	554	2	-
Democratic Republic of the Congo	224	0	-
Guinea	52	0	-
Kenya	32	0	-
Burundi	28	0	-

* Most recent global surveillance data available.

** Countries reporting the highest number of confirmed mpox cases in the last six weeks.

Mpox Pathogenicity

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- ❑ The virus causes a disease in humans that is like smallpox but results in a lower case-fatality rate.
- ❑ Animal antibody surveys in the DRC suggested that squirrels play a reservoir role of the virus and that humans are sporadically infected.
- ❑ Human-to-human transmission occurs with an incubation period of about 12 days.

Why MPOX?

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- ❑ On August 14, 2024, the WHO declared Mpox a Public Health Emergency of International Concern (PHEIC), following a similar declaration by the Africa CDC on August 13.
- ❑ Mpox alerts emphasized the severity of the outbreak and the urgent need for coordinated global action to control the disease
- ❑ The lower mortality rate of Mpox varied by Clade but can be higher among immunocompromised individuals.
- ❑ This indicated the need for a low complexity molecular test to respond to the Mpox control urgently

Study context

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- ❑ During the epidemic, the confirmed Mpox cases were reported in almost all regions of Uganda.
- ❑ There was a need to validate available diagnostics to curb this increasing spread through early diagnosis.
- ❑ Truenat[®] MPX assay is done using the already existing Truenat platforms distributed across the country for the diagnosis of tuberculosis (in 41 HFs).
- ❑ This study offers a unique opportunity to **leverage the existing capacity for multi-disease diagnosis.**

AIMS

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□ **General Aim:**

- To evaluate the performance of the Truenat[®] MPX Test to detect Mpox in Uganda.

Specific Aims

- 1) To compare the sensitivity and specificity of the Truenat[®] MPX assay with the SoC test for Mpox in Uganda.
- 2) To assess the overall diagnostic accuracy, including the rate of false positives and negatives, of the Truenat[®] MPX assay compared to the RT-PCR standard.

Methods

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Study design	Cross sectional study
Study duration	12 months
Study Population	Samples previously collected from all individuals presenting with Mpox symptoms
Specimen Type	Vesicular lesion swabs and Oropharyngeal swabs collected from the on-going epidemiological studies
Ethical and regulatory approval	Approved by SBS-REC# 2025-82) and UNCST (#HS5956ES)

Eligibility criteria

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□ Inclusion criteria

- Samples collected from suspected Mpox individuals whose patients consented for their samples to be used for future research.



□ Exclusion criteria

- Samples with insufficient value
- Samples with biosafety risks such as broken vials
- Miss labelled or unlabeled samples

Sample size: 150 specimens (49 PCR positives and 101 PCR negatives) previously collected from Mpox suspected individuals, irrespective of gender.

Sample processing

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- Specimens were from the Central Emergency Response and Surveillance Laboratory (**CERSL**), Ministry of Health, Uganda, previously tested with the NVO RT PCR test.
- Retrieved from -80°C freezer and transported on ice packs to Mycobacteriology BSL-3 lab for testing on the Truenat platform using Truenat[®] MPX assay.
- Specimens in VTM were thawed at room temperature, and nucleic acid extraction was done using Trueprep[®] AUTO/AUTO v2.
- PCR reaction was done on the Truelab[®] Duo Real Time micro-PCR analyzer.

Sample processing

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Lab work

Clinical specimens from suspected MPOX patients were subjected to both the below assays.

Non-Variola Orthopoxvirus
Real-Time PCR assay.

Truenat[®] MPX assay.

What was done

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- ❖ 150 specimens were retrieved from CERSL (Blinded).
- ❖ Previously tested with Non-variola Orthopoxvirus (NVO: dPCR kit, Bi-Rad QX200v1.1) EUA (SoC) test **[between May and July 2025]**
- ❖ NVO RT PCR results and demographic data retrieved; age, gender, sample type, district of origin etc
- ❖ All retrieved specimens were tested using Truenat[®] MPX assay Between **24th July and 27th Sept 2025**



The data were entered in Excel and analysed for frequency, percentages, sensitivity, specificity, PPV and NPV.

Expected results

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Detection Channel			Result Interpretation
MPOX	OPOX	RNaseP	
+	+	+/-	Mpox and OPOX Positive
+	-	+/-	Mpox Positive & Negative for OPOX (This happens when it's too low titre, beyond LoD)
-	+	+/-	Negative for Mpox But OPOX Positive (This happens when other pox-family viruses are present)
-	-	+	Both Mpox OPOX are Negative
-	-	-	Invalid (Collect new swab and repeat)

Positive for both MPOX and OPOX

Truenat[®] Monkeypox						
Center	MYCOBACTERIOLOGY LAB MULAGO			Operator	JAMES	Bay 1
Profile	Monkeypox		Date	Wed 06 Aug 2025 09:23		
Lot	EMX01	Expiry Date	09-26	Sample	Skin lesion	
Patient Details						
Name	MPX-MU 017			ID	CL304378	
Age	48	Gender	Male	Referred By	Dr. Willy Ssengooba	
Result	Detected					
Control	20.11	OPOX	14.5	MPOX	12.38	
Run Status	Valid					
OPOX	DETECTED		High			
MPOX	DETECTED		High			

MPOX Positive & Negative for OPOX

Truenat[®] Monkeypox						
Center	MYCOBACTERIOLOGY LAB MULAGO			Operator	JAMES	Bay 1
Profile	Monkeypox		Date	Thu 07 Aug 2025 13:41		
Lot	EMX01	Expiry Date	09-26	Sample	Throat Swab	
Patient Details						
Name	MPX-MU 079			ID	CL307610	
Age	60	Gender	Male	Referred By	Dr. Willy Ssengooba	
Result	Detected					
Control	26.33	OPOX	ND	MPOX	32.67	
Run Status	Valid					
OPOX	NOT DETECTED					
MPOX	DETECTED Very Low					

OPOX Positive & Negative for MPOX

Truenat[®] Monkeypox

Center	MYCOBACTERIOLOGY LAB MULAGO	Operator	JAMES	Bay	1
Profile	Monkeypox	Date	Fri 08 Aug 2025 12:33		
Lot	EMX01	Expiry Date	09-26	Sample	Throat Swab

Patient Details

Name	MPX-MU 133	ID	CL307868		
Age	27	Gender	Female	Referred By	Dr. Willy Ssengooba

Result **Not Detected**

Control	31.4	OPOX	33.33	MPOX	ND
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Run Status **Valid**

OPOX **DETECTED Very Low**

MPOX **NOT DETECTED**

Both MPOX OPOX are Negative

Truenat[®] Monkeypox					
Center	MYCOBACTERIOLOGY LAB MULAGO			Operator	JAMES Bay 2
Profile	Monkeypox		Date	Fri 08 Aug 2025 12:35	
Lot	EMX01	Expiry Date	09-26	Sample	Skin lesion
Patient Details					
Name	MPX-MU 134			ID	CL307872
Age	06	Gender	Female	Referred By	Dr. Willy Ssengooba
Result	Not Detected				
Control	30.2	OPOX	ND	MPOX	ND
Run Status	Valid				
OPOX	NOT DETECTED				
MPOX	NOT DETECTED				

Invalid

Truenat[®] Monkeypox

Center MYCOBACTERIOLOGY LAB MULAGO Operator JAMES Bay 2

Profile Monkeypox Date Wed 06 Aug 2025 11:21

Lot EMX01 Expiry Date 09-26 Sample Skin lesion

Patient Details

Name MPX-MU 031 ID CL304125

Age 45 Gender Male Referred By Dr. Willy Ssengooba

Result

Control ND OPOX ND MPOX ND

Run Status Invalid

Results and Findings

Demographics and clinical characteristics

Gender*	n	%
Male	77	51.7
Female	72	48.3
Median age (IQR)	22	19-26
Sample Type		
Oral swab	6	4
Oropharyngeal Swab	36	24
Skin Lesion	108	72
NVO results		
Positive	49	32.6
Negative	101	67.3
Truenat® MPX assay results		
Positive	48	32.0
Negative	102	68.0

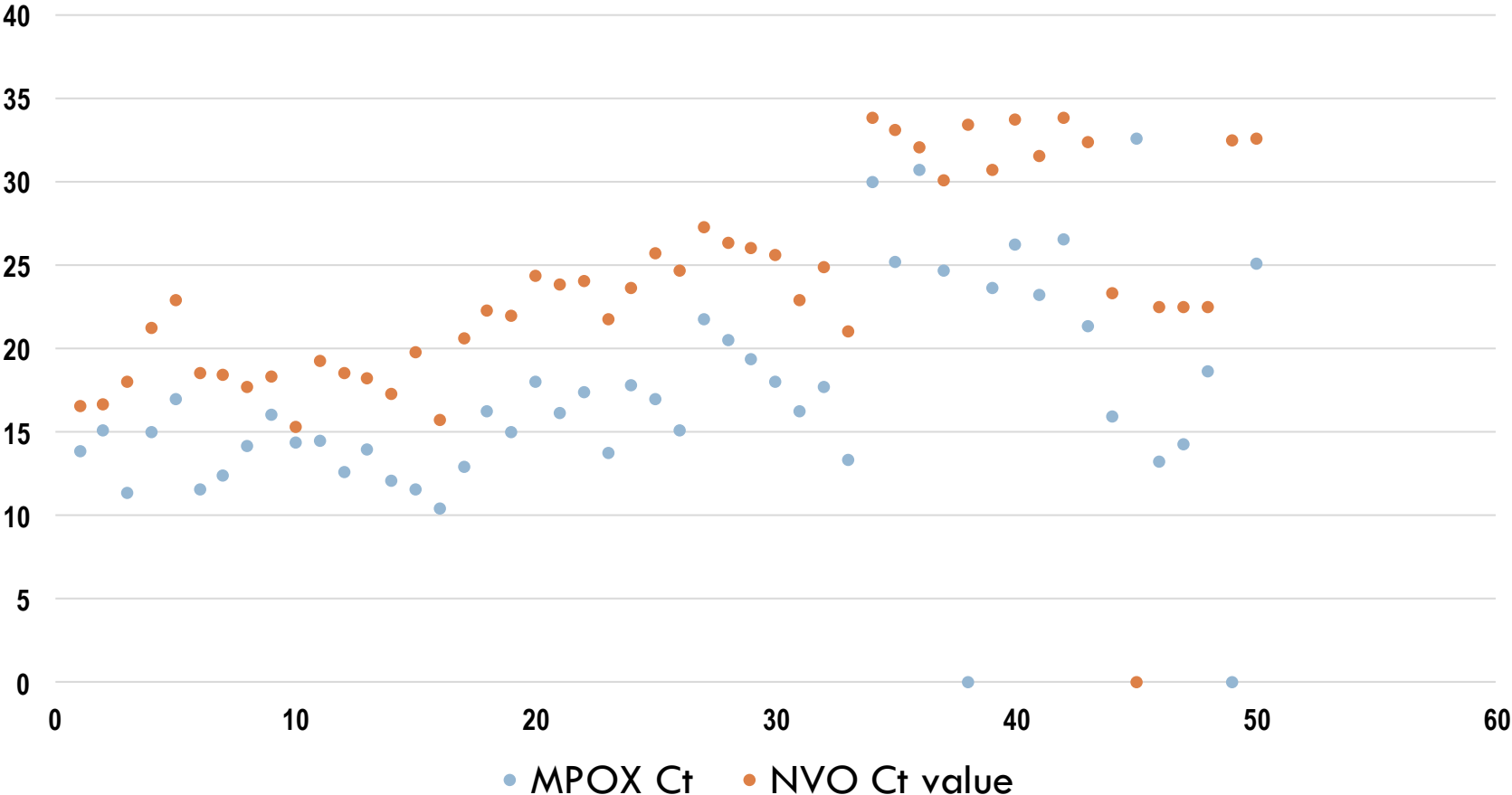
Gender* = one unknown

Diagnostic accuracy of Truenat MPX assay using NVO RT PCR as a reference comparator

Parameter	n	%	95%CI
Sensitivity	47	96.0	86.0- 99.5
Specificity	100	99.0	94.6-99.9
PPV	47	97.9	88.9-99.9
NPV	100	98.0	93.1-99.7

NVO= dPCR NVO; Mpox Assay Kit for the Bio-Rad QX200® v1.1

Comparison of Ct values on NVO and TruenatMPX



Discussion

- ✓ In this diagnostic evaluation study, Truenat® MPX assay is a highly **sensitive (96%; 86.2- 99.5)** and **specific (99% 94.5-99.9)** LC-NAAT for Mpox.
- ✓ Using samples tested with the NVO SoC test in Uganda, the level of **invalid results** was acceptable at **1.3%**, which gave valid results on repeat testing. There were only three (**0.02%**) discordances.
- ✓ The Truenat platform has been fully evaluated for robustness, feasibility, and accuracy in field settings; however, its ability to offer detection of multiple pathogens has not been fully validated.
- ✓ Optimally utilizing the already tested diagnostic platforms in an integrated disease program mechanism is imperative.

Innovative contribution to policy, practice and/or research

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- The Truenat[®] MPX Test is a highly sensitive and specific LC-NAAT test for Mpox.
- Given the extended use of the Truenat[®] platforms, mainly for TB, this test offers a rapid and effective strategy toward Mpox epidemic control and research.



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THANK YOU