Saliva, Urine, and Anal Swabs as Viable Alternatives to Lesion Swabs for Molecular Diagnosis of Mpox

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BACKGROUND

Although Mpox diagnostic algorithms remain centered on lesion swabs, these are often invasive and painful, may be inaccessible in early or atypical infections, and pose logistical challenges in low-resource outbreak settings. Emerging evidence suggests multi-site viral shedding in saliva, anorectal areas, and urine; however, no robust paired evaluations or sufficiently powered studies have established formal diagnostic equivalence within conservative statistical bounds.

Research Question

Can non-invasive specimens (saliva, anal swabs, urine) perform comparably to lesion swabs for molecular Mpox diagnosis?

METHODS

Design: Cross-sectional paired diagnostic evaluation conducted during the Uganda Mpox outbreak (Feb–Mar 2025); **N = 120 suspected cases (92 confirmed, 28 alerts**)

Specimens: 4 paired specimens per participant (**480 total**): lesion swab (reference), saliva, anal swab, urine.

Testing: DNA extraction using **QIAamp Viral DNA Mini Kit**; Mpox PCR using **Bioperfectus MPXV RT-PCR** (F3L target).

Statistical analysis: Sensitivity and specificity; agreement metrics (Cohen's κ, Gwet AC1); paired comparisons (exact McNemar, Wilcoxon); agreement analysis (Bland–Altman); equivalence testing (TOST ±10%); GEE to account for within-participant correlation.

Saliva demonstrates diagnostic equivalence to lesion swabs for Mpox PCR detection.

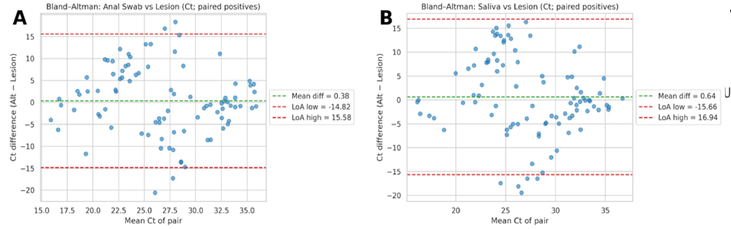
RESULTS

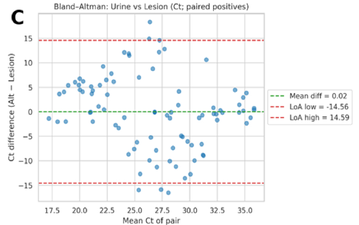
- Lesion swabs had the highest Mpox PCR positivity (83.3%) and median Ct: of 27.6, followed by saliva (81.7%) with median Ct: of 28.3.
- Anal swabs showed moderate positivity (78.3%), while urine had the lowest positivity (75.8%).
- Viral load distribution was comparable across sample types.
- No significant paired Ct differences (all p > 0.58) were observed.
- No systematic bias on Bland–Altman analysis.
- Saliva demonstrated diagnostic equivalence with Δ = −0.017 and a 90% CI fully contained within ±0.10.

Diagnostic Performance (vs Lesion)

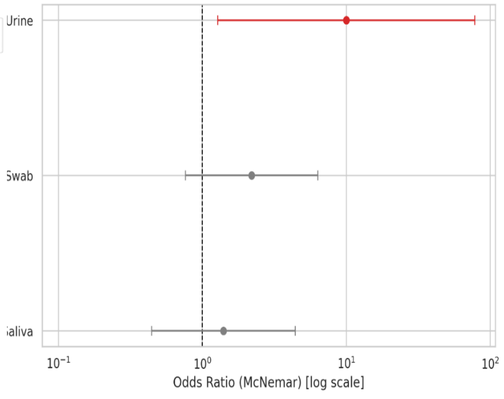
Specimen	Sensitivity	Specificity	κ	Gwet AC1	(TOST ±10%)
Saliva	93%	75%	0.65	0.86	✅ YES
Anal swab	89%	75%	0.57	0.81	❌ No
Urine	90%	95%	0.72	0.86	❌ No

- In adjusted GEE logistic regression models,:
  - Urine had lower odds of detection (OR 0.40).
  - Saliva & Anal swabs were not significantly different from the lesion swabs.

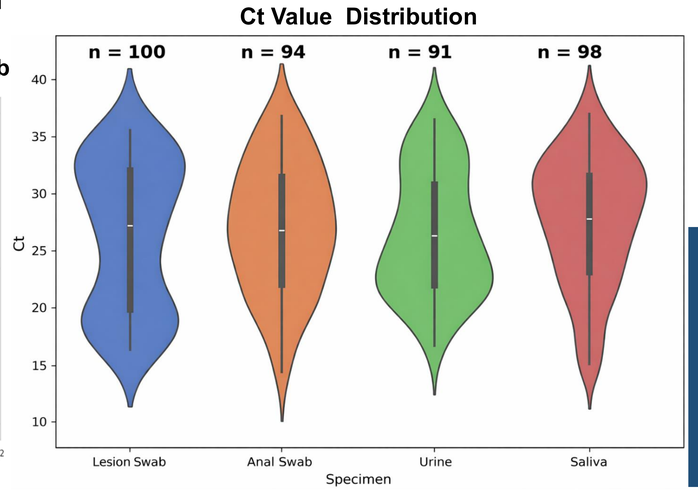




Directional discordance ORs vs lesion swab



Ct Value Distribution



CONCLUSIONS

- Lesion swabs remain the gold standard.
- **Saliva demonstrates diagnostic equivalence and high sensitivity.**
- Anal swabs show high sensitivity but narrowly miss equivalence thresholds supporting their use in contexts of anorectal exposure or symptoms.
- Urine shows high specificity but lower sensitivity; therefore, not reliable as a standalone diagnostic specimen.
- Mpox viral DNA is detectable across multiple body sites, confirming widespread viral shedding during acute infection.

PUBLIC HEALTH IMPLICATIONS

- Multi-site testing strategies improve case detection; recommended composite testing algorithms**:**
  - Lesion + Saliva
  - Lesion + Anal

Saliva provides:

- Non-invasive collection, which reduces health workers' exposure to the infectious agent.
- Scalable community and decentralized clinical testing.
- Increased patient acceptability (Patient-friendly)
- Enhances diagnostic resilience in resource-limited settings.

ADDITIONAL KEY INFORMATION

Ethics: UNHLS-2025-133

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Data available upon reasonable request

No competing interests

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PLAIN LANGUAGE SUMMARY

In Uganda's Mpox outbreak, saliva testing performed as accurately as skin lesion testing using PCR. This non-invasive approach can safely expand access to Mpox diagnosis without compromising testing accuracy.