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Short communication

Alternative sampling specimens for the molecular detection of mpox (formerly monkeypox) virus

Jasmine Coppens [a,1], Fien Vanroye [a,1], Isabel Brosius [a], Laurens Liesenborghs [a], Saskia van Henten [a], Thibaut Vanbaelen [a], Stefanie Bracke [a], Nicole Berens-Riha [a], Irith De Baetselier [a], Chris Kenyon [a], Patrick Soentjens [a], Eric Florence [a], Johan Van Griensven [a], Kevin K. Ariën [b], Bart K.M. Jacobs [a], Dorien Van den Bossche [a], Marjan Van Esbroeck [a,2], Koen Vercauteren [a,c,*,2], ITM MPX study group

[a] Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium, Nationalestraat 155, 2000 Antwerp, Belgium
[b] Virology unit, Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium, Nationalestraat 155, 2000 Antwerp, Belgium
[c] Clinical Virology unit, Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium, Nationalestraat 155, 2000 Antwerp, Belgium

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ABSTRACT

Background: Mpox (formerly monkeypox) is a viral disease caused by the mpox virus (MPXV), endemic in Central and West Africa and currently causing a global outbreak of international concern. Much remains unknown about sample types most suited for mpox laboratory diagnosis. While it is established that high viral loads can be found in active skin lesions (currently the recommended mpox laboratory confirmation specimen type), WHO mpox testing guidelines encourage the use of oropharyngeal swabs as an additional sample type for mpox diagnosis and suggest investigating the value of other specimens like blood samples.
Objective: In this study, we verified the value of select alternative specimen types for mpox laboratory confirmation.
Methods: We included 25 patients with MPXV-confirmed skin lesions to compare diagnostic sensitivity of MPXV PCR testing on EDTA plasma and two upper respiratory specimens: oropharyngeal swabs and saliva.
Results: In our patient cohort with MPXV-confirmed skin lesions, diagnostic sensitivity of MPXV PCR was 80% in EDTA plasma, 64% in oropharyngeal swabs, and 88% in saliva. MPXV viral loads were significantly higher in saliva compared to oropharyngeal swabs and EDTA plasma.
Discussion: The WHO recommendation to collect oropharyngeal swabs as an additional specimen for mpox diagnosis might need to be revised to include saliva wherever feasible. We suggest investigating saliva as a diagnostic specimen in the absence of active skin lesions or during the phase preceding skin manifestations. Moreover, the relatively high MPXV DNA content of saliva warrants elucidating its potential role in disease transmission.

1. Background

In response to the current worldwide mpox outbreak, laboratory capacity for MPXV detection had to be scaled up rapidly worldwide. Nevertheless, most of the knowledge on diagnostic procedures for MPXV originated in central Africa, where the disease is caused by a different MPXV clade and the clinical presentation, affected population, and mode of transmission may differ substantially from the ongoing outbreak (1-5). While diagnosis of mpox is traditionally done on skin swabs, patients can also present with detectable viral DNA in saliva, oropharyngeal, and nasopharyngeal swabs, blood, seminal fluid, urine, faeces, and anal swabs (6-11).

The World Health Organization (WHO) mpox testing guidelines recommend skin lesion material as primary specimen type for mpox

* Corresponding author.
E-mail address: kvercauteren@itg.be (K. Vercauteren).
[1] These authors contributed equally to this work and share first authorship.
[2] These authors contributed equally to this work and share last authorship.

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laboratory confirmation and encourage oropharyngeal swabs as a potentially useful additional specimen (12). However, it is not clear whether MPXV preferentially binds and replicates in the pharyngeal mucosa or in the oral cavity and salivary glands and which sampling site thus has higher diagnostic yield. The WHO also mention that "EDTA blood may support detection of MPXV but may not contain the high level of virus found in lesion samples". To shed light on the usefulness of suggested additional specimens for mpox diagnosis, we assessed the diagnostic sensitivity of EDTA plasma, oropharyngeal swabs, and saliva collected from patients of whom the mpox diagnosis was confirmed on skin lesion swabs.

2. Material and methods

2.1. Sample collection

We used diagnostic samples from patients with active (early to late) skin lesions (including in the anogenital region) presenting for MPXV testing at our sexual health clinic at the Institute of Tropical Medicine (ITM), Antwerp, Belgium. We obtained swabs (Copan ESwab®, containing 1 mL of Liquid Amies medium; Murrieta, CA, USA) from (multiple) lesions (by sampling the lesion fluid and base) and the oropharynx. At least 1 ml of saliva was self-collected under supervision in an OMNIgene·ORAL® (DNA Genotek; Ontario, Canada) collection tube (that contains a proprietary viral DNA stabilizing solution), mostly after collection of the oropharyngeal swab. Blood was collected in a 9 ml EDTA K3E S-Monovette ® tube (Sarstedt, Nümbrecht, Germany) and centrifuged at 2500 g for 10 min to separate the EDTA plasma fraction. All samples were taken as patients presented for their initial diagnostic workup (thus, during the same visit and without instructions on food consumption and time of visit).

The study analyses were performed on the first 25 patients with positive MPXV PCR results on skin lesion swabs visiting the ITM sexual health clinic of whom all three alternative testing specimens (EDTA plasma, throat swab, and saliva) were available. In addition, four patients presenting at the clinic with negative MPXV PCR results on skin lesion swabs were included to verify specificity on the alternative sample types.

2.2. Laboratory diagnosis

For MPXV real-time PCR testing, DNA was either manually extracted (QIAamp DNA Mini kit, Qiagen, Hilden, Germany; using 200ul sample input and 100ul elution volume) or automatically (Maxwell®, Promega, Madison, WI, USA; using 300ul sample input and 75ul elution volume). The extraction method was kept identical within compared pairs. PCR was performed using PerfeCTa FastMix II PCR Reagents (Quantabio, Beverly, MA, USA) and MPXV generic primers/probe as described previously (13). Amplification was carried out on a QuantStudio 5 instrument (ThermoFisher Scientific, Waltham, MA, USA).

2.3. Statistical testing

Diagnostic sensitivity of alternative specimen types (EDTA plasma, oropharyngeal swab and saliva) was calculated as the number of positive results for a given specimen type divided by the total number of mpox confirmed study participants. The confidence interval of these proportions was computed with the hybrid Wilson/Brown method (Graphpad Prism 9). Sensitivities of alternative specimen types were compared using the McNemar test (Graphpad Prism online calculator). Comparison of positive MPXV PCR Ct values generated on different sample types was performed using paired t-testing or two-way ANOVA with Tukey's multiple comparisons.

3. Results

All four included patients with MPXV negative skin lesions had negative MPXV PCR testing results on the alternative specimen types. In the 25 patients with MPXV positive skin lesions, diagnostic sensitivity of plasma, oropharyngeal swab and saliva were 80% (95% CI: 0.61 to 0.91), 64% (95% CI: 0.45 to 0.80), and 88% (95% CI: 0.70 to 0.96) respectively (Table 1). Sensitivities between specimen types were not statistically significantly different.

Next, PCR Cycle threshold (Ct) values were compared between specimen types on a subset of 14 (out of the total 25) included mpox patients that had positive MPXV PCR results on all tested specimens. This analysis indicates that saliva contains the highest MPXV viral loads of the alternative specimens tested (Fig. 1). The mean MPXV PCR Ct value in the samples from the 14 patients testing positive on all tested sample types was 37.29 (95% CI: 36.04 to 38.53) in plasma, 33.88 (95% CI: 31.58 to 36.18) in oropharyngeal swabs, and 29.05 (95% CI: 26.47 to 31.63) in saliva. In comparison, the mean MPXV PCR Ct value on skin lesion swabs of these 14 mpox patients was 21.81 (95% CI: 19.04 to 24.59), which differed significantly from the matching saliva Ct value (Paired t-test P value = 0.0005).

Of note, we did not observe any difference in MPXV PCR testing between EDTA plasma and serum (both had an 80% sensitivity (95% CI: 0.61 to 0.91), and MPXV PCR Ct values were not different; supplementary figure 1).

Table 1
MPXV PCR Ct value results generated on different indicated specimen types per study participant (every row represents a single patient, $n = 25$) with indication of oral lesion presence. (nd stands for not detected)

Patient #	Plasma	Oropharyngeal swab	Saliva	Oral lesions
1	35.54	26.77	30.94	No
2	37.72	nd	41.08	No
3	34.84	39.07	36.71	Yes (lip)
4	38.87	37.94	33.85	No
5	33.44	nd	37.05	No
6	37.51	25.54	25.71	Yes (lip)
7	39.90	32.66	33.61	Yes (lip)
8	38.41	35.98	23.85	No
9	37.54	33.86	25.15	No
10	nd	nd	nd	No
11	32.74	34.98	29.13	No
12	nd	nd	nd	No
13	39.16	nd	22.76	No
14	40.58	36.39	30.23	No
15	37.27	37.02	28.23	Yes (sublingual)
16	nd	nd	37.51	No
17	36.60	36.12	35.17	No
18	36.11	nd	37.32	No
19	39.77	31.09	25.43	No
20	35.41	nd	29.90	No
21	34.77	37.61	nd	No
22	36.59	31.76	23.02	No
23	35.83	35.16	25.64	Yes (lip)
24	nd	36.14	31.03	yes (lip)
25	nd	nd	31.11	No
# positives (out of $n = 25$):	20	16	22	
Sensitivity:	0.80 (0.61 to 0.91)	0.64 (0.45 to 0.80)	0.88 (0.70 to 0.96)	
McNemar P value		vs plasma: 0.2207	vs plasma: 0.6171 vs oropharyngeal swab: 0,0771	

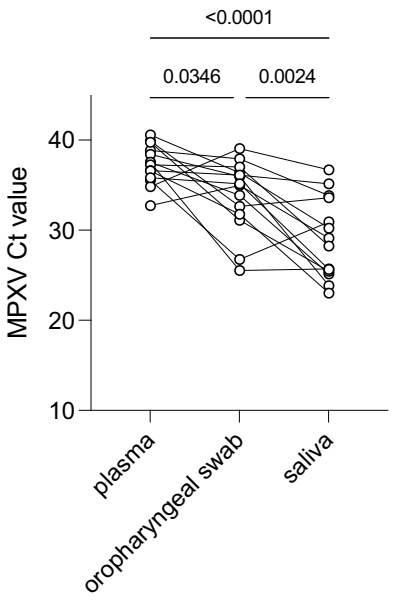

Fig. 1. Comparison of MPXV PCR Ct values generated on different indicated sample types from mpox patients testing positive on all three sample types (n = 14 out of 25 included mpox patients. All P-values denote two-way ANOVA with Tukey's multiple comparisons (the overall ANOVA P value is < 0.0001).

4. Discussion

Our study confirms recent evidence that, during acute clinical presentation of mpox with active skin lesions, plasma samples do not contain high levels of detectable virus (9).

Our data also show that the upper respiratory samples cannot replace skin lesion swabs in case of symptomatic presentation with skin lesions. For such individuals with active skin lesions, diagnostic sensitivity was 64% on oropharyngeal swabs, but reached up to 88% in saliva. Despite the low number of included mpox suspects in our study, we were able to demonstrate that saliva contained higher MPXV viral loads (using MPXV PCR Ct values as a semi-quantitative proxy) compared to oropharyngeal swabs and EDTA plasma at the exact time of patient presentation for diagnostic workup (the mean time since symptom onset was 8,5 days (95% CI: 5642 to 11,32)). Of note, the presence of oral lesions (in the mouth and at the lips; n = 6/25) was not associated with the saliva MPXV Ct value (simple logistic regression p value = 0,7825).

Our observations align with published MPXV PCR data allowing additional within-patient comparison of saliva with nasopharyngeal swabs (instead of oropharyngeal swabs tested in our report, as is recommended by WHO) (6, 7). While most of the samples in those reports were not collected at the very day of diagnosis (sampling was performed only after mpox diagnosis was confirmed (7)) these data support the presence of higher MPXV DNA content in saliva compared to a swab taken from the upper respiratory mucosa (nasopharyngeal swabs in this case) shortly after clinical presentation for skin lesions and subsequent diagnosis (supplementary figure 2).

While our data provide insights in the diagnostic value of sampling sites other than the preferred skin lesion swab specimens, they cannot be extrapolated to testing of patients presenting without skin lesions (but with other primary presentations like proctitis and lymphadenopathy). In addition, prospective studies performing longitudinal follow-up of yet asymptomatic high-risk contacts of confirmed mpox cases are needed to decipher whether and which alternative samples would have a place in testing pre-symptomatic or prodromal mpox cases. We recommend testing the value of an anorectal swab in that context based on reports on asymptomatic MPXV detection in this specimen(5, 14). An advantage of saliva in such context would be its ease of (serial) self-collection which could be helpful in some settings e.g., to lower the barrier for testing and the workload and exposure of healthcare workers.

MPXV is considered a Category A, UN2814 pathogen and is hence subjected to transportation using strict biosafety procedures (such as triple packaging) by a certified shipper. It is relevant to note that the saliva in our study was collected in a viral DNA stabilizing lysing buffer. While not specifically validated by the company for MPXV inactivation, the ease of lysis of enveloped viruses in general virus-inactivating media might also enhance biosafety during sample transportation and manipulation in the laboratory. Of note, we observed equivalence of MPXV PCR diagnostic sensitivity in an additional shorter series comparing skin swabs collected in a guanidine-based transport medium to a viral transport medium without guanidine (supplementary Figure 3), and we have now urged external requesting laboratories involved in the clinical workup of mpox suspect cases to use inactivating transport media for centralized MPXV PCR testing at our institute.

5. Conclusions

In the presence of skin lesions, lesion swabs should remain the standard specimen type for mpox laboratory confirmation by PCR. In the case where an additional testing sample is considered, our data suggest that saliva provides higher sensitivity than oropharyngeal swabs and EDTA plasma. The WHO recommendation to collect oropharyngeal swabs as an additional testing specimen to skin lesion swabs might therefore need to be revised to include saliva wherever feasible. The relatively high viral load in saliva warrants further investigation into i) the potential use of saliva as a diagnostic sample (besides oropharyngeal swabs and blood) in the absence of skin lesions, and during the pre-symptomatic phase after high-risk contact, and ii) the potential role of saliva in transmission (e.g., by droplets, kissing, using saliva as lubrification during sex).

Ethical statement

Patients were informed of the use of their samples for secondary research and asked to sign an informed consent form. Ethics approval for this research was obtained through the ITM institutional review board (file number: 1596/22).

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Authors' contributions

Conceptualisation: EF, MVE, KV, IB, LL, ITM MPX study group; Methodology and patient care: JC, FV, IB, LL, SVH, TV, SB, NB, IDB, CK, PS, EF; Formal analysis: KV, BKMJ; Writing: KV; Writing, review and editing: KV, MVE, DVDB, BKMJ, KKA, JVG, IDB, NB, SB, TV, SVH, LL, IB

Declaration of Competing Interest

None

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcv.2022.105372.

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