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Performance evaluation of the Viasure PCR assay for the diagnosis of monkeypox: A multicentre study

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ABSTRACT

Background: Monkeypox virus (MPXV) is the causative agent of the 2022 monkeypox global outbreak. Rapid detection of MPXV infection is essential to inform patient management and public health response. Currently, there is a lack of established real-time PCR assays to support a rapid diagnosis of monkeypox.
Objectives: To evaluate the performance characteristics of the Viasure MPXV PCR assay in three London teaching hospitals.
Study design: Prospectively collected paired patient swabs from matched or unmatched anatomical sites were evaluated by the reference laboratory and Viasure MPXV PCR assays. A subset of samples were also tested for HSV, VZV, and/or *Treponema pallidum* DNA.
Results: 217 paired samples were evaluated. 91.2% of the paired swabs generated concordant results whilst 8.8% generated discordant results. The accuracy, diagnostic sensitivity, diagnostic specificity, positive predictive value, negative predictive value, likelihood ratio positive, and likelihood ratio negative of the Viasure PCR assay across the hospitals were 93.2 – 96.3%, 90.0 – 100%, 88.2 – 100%, 94.9 – 100%, 87.9 – 100%, 8.50 – 14.41, and 0.00 – 0.10 respectively. MPXV co-infections with HSV were detected in two patients. Five patients were negative for monkeypox but positive for herpes or chickenpox.
Conclusions: The Viasure MPXV PCR assay demonstrated excellent performance characteristics, was easy to use, and is fit for routine diagnostic purpose. Where implemented, the assay would allow rapid and accurate laboratory diagnosis of MPXV infections and support a timely management of monkeypox. To reduce the risk of false negative detections, vesicular lesions from any anatomical site should be preferentially and optimally sampled.

1. Background

Monkeypox virus (MPXV), an Orthopoxvirus of the *Poxviridae* family, is the causative agent of the 2022 monkeypox global outbreak [1]. In an ongoing epidemic, a rapid detection of monkeypox virus infection is essential to inform patient management, infection control and public health response. Currently, there is a lack of established PCR assays that are relatively easy to be implemented by routine diagnostic laboratories to support a rapid diagnosis of MPXV infections. To support a rapid detection, CerTest Biotec (Zaragoza, Spain) recently introduced the Viasure MPXV PCR assay which can generate up to 96 results in approximately 105 mins [2]. The lyophilised reagents enable storage between 2–40 °C for up to 2 years and only require reconstitution with a provided buffer prior to PCR setup. The ease of handling makes the assay attractive for use by routine diagnostic laboratories. The aim of the current study was to evaluate the performance characteristics of the

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Viasure MPXV PCR assay in three London teaching hospitals.

2. Study design

2.1. Collection and pre-analytical processing of samples

Paired patient swabs from matched (e.g. throat/throat) or unmatched (e.g. chest/leg) anatomical sites collected in viral transport media (Σ-Virocult®, MWE, UK) and submitted for the investigation of monkeypox at three London hospital Trusts were evaluated (Imperial College Healthcare NHS Trust (hospital 1), St George's University Hospitals NHS Foundation Trust (hospital 2), and Guy's and St Thomas' NHS Foundation Trust (hospital 3)). One sample was tested by the reference PCR assay at the national reference laboratory (Rare and Imported Pathogens Laboratory (RIPL), UK) and the other was tested by the Viasure MPXV PCR assay (CerTest Biotec, Spain) at the respective hospital pathology laboratory (North West London Pathology (NWLP) at hospital 1, South West London Pathology (SWLP) at hospital 2, and Synnovis at hospital 3). Discordant MPXV PCR results were resolved by a laboratory-developed test (LDT) (NWLP), or repeat testing at RIPL (SWLP and Synnovis). A subset of samples tested at SWLP were also tested for HSV-1, HSV-2, VZV, and/or *Treponema pallidum* DNA as part of the clinical investigation of a rash illness. The protocol for the pre-analytical processing of samples is summarised in Table 1.

2.2. RIPL PCR assay

The RIPL MPXV RT-PCR assay was adapted from published literature [3]. The assay targets the G2R region common to all MPXV sequences. Samples were extracted on either the EZ1 Advanced XL (Qiagen, Germany) or MagNA Pure 96 (Roche Diagnostics, UK) instruments. MS2 phage was used as extraction and amplification control. PCR thermocycling was performed on the ABI ViiA 7 system.

2.3. NWLP LDT

The NWLP MPXV PCR assay was adapted from published literature [3,4]. The assay targets the G2R_WA and E9L-NVAR sequences of MPXV. Samples were extracted using the 200 µl input and 60 µl output protocol with the Virus Mini Kit v2.0 on the EZ1 Advanced XL (Qiagen, Germany). PCR thermocycling was performed with QuantiFast® Pathogen PCR +IC kit (Qiagen, Germany) according to the manufacturer's instructions on the ABI 7500 Fast system.

2.4. Viasure MPXV PCR assay

The Viasure MPXV PCR assay was performed according to the manufacturer's instructions [2]. Briefly, the lyophilised and ready-to-use PCR mastermix was reconstituted with the provided buffer prior to dispense (15 µl) into 96-well PCR plates or Rotor-Gene PCR tubes. For each sample, 5 µl extract was subsequently added to the well or tube. The hands-on time required for setting up 20 samples was 5 mins. PCR thermocycling was performed on the ABI 7500 Fast system (NWLP and Synnovis) or Rotor-Gene Q (SWLP). The assay targets the G2R_G and F3L genes of MPXV and uses the haemoglobin-β gene as extraction, amplification and sample adequacy control.

Table 1
Sample inactivation and extraction protocols for the Viasure PCR assay.

Protocol	Volume (µl)	NWLP (Hospital 1)	SWLP (Hospital 2)	Synnovis (Hospital 3)
Inactivation	Sample	125	200	200
	Lysis buffer (LB)	125 (External LB, Roche)	200 (NucliSENS® LB, bioMerieux)	430 (ATL/ACL LB, Qiagen)
	Heating	Not performed	Not performed	68 °C (15 mins)
Extraction	Input	200	400	630
	Output	60	60	60
	Instrument	EZ1 Advanced XL (Qiagen)	EZ1 Advanced XL (Qiagen)	QIAsymphony SP (Qiagen)

2.5. Data analysis

Data analysis was performed using MedCalc Software (MedCalc Software Ltd, Belgium). Accuracy, diagnostic sensitivity, diagnostic specificity, positive predictive value (PPV), negative predictive value (NPV), likelihood ratio positive (LR+), and likelihood ratio negative (LR-) of the Viasure PCR assay with 95% confidence interval (95% CI) were computed.

3. Results

In total, 217 paired clinical samples were evaluated by the RIPL and Viasure MPXV PCR assays (Fig. 1). Most patients presented at sexual health clinics and had a recent contact with monkeypox. 91.2% (198/217) of the paired swabs generated concordant results whilst 8.8% (19/217) generated discordant results. 72.2% (143/198) and 27.8% (55/198) of the concordant results were obtained from paired swabs collected from matched and unmatched anatomical sites respectively. Of the discordant results, 31.6% (6/19) were obtained from paired swabs collected from matched anatomical sites whilst 68.4% (13/19) were obtained from unmatched sites. MPXV co-infections with HSV-1 ($n = 1$) and HSV-1 and HSV-2 ($n = 1$) were detected in two patients that had additional testing at SWLP. In addition, five patients were negative for monkeypox but positive for herpes (HSV-2, $n = 2$) or chickenpox ($n = 3$).

Overall, the performance of the Viasure MPXV PCR assay was satisfactory (Table 2). The assay demonstrated good accuracy (88.6 – 94.7%), diagnostic sensitivity (89.7 – 100%), PPV (87.2 – 96.3%), and NPV (87.9 – 100%). The LR+ (4.00 – 41.24) indicated that the Viasure assay may be useful to rule in (i.e. confirm) a MPXV infection when the result is detected, and the LR- (0.00 – 0.11) may be sufficiently small to rule out an infection when the result is not detected. The assay exhibited a lower diagnostic specificity of 82.9% (95% CI 66.4 – 93.4%) and 75.0% (95% CI 50.9 – 91.3%) at NWLP and Synnovis respectively due to the testing of paired swabs predominantly collected from unmatched anatomical sites (Table 3). The paired swabs collected from matched anatomical sites i.e., throat/throat (sample 7) at NWLP and lesion/lesion and skin/skin (sample 17 and 18) at Synnovis, were two different swabs. Discordant testing of all swabs (where available and depending on which swab was tested) confirmed the primary testing results by the RIPL or Viasure assay. If the discordant testing results (Table 3) were included in the analysis, the accuracy, diagnostic sensitivity, diagnostic specificity and PPV of the Viasure MPXV PCR assay improved to 93.2 – 96.3%, 90.0 – 100%, 88.2 – 100%, and 94.9 – 100% respectively, and as a result, improves the LR+ (8.50 – 14.41) and LR- (0.00 – 0.10) of the

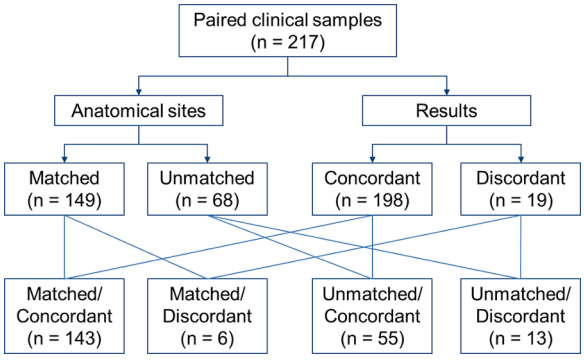

Fig. 1. Paired swab samples evaluated by the RIPL and Viasure PCR assays.

Table 2
Performance characteristics of the Viasure MPXV PCR assay at NWLP, SWLP and Synnovis laboratories.

		NWLP (Hospital 1) RIPL (reference assay for all laboratories)		SWLP (Hospital 2)		Synnovis (Hospital 3)	
		Detected	Not detected	Detected	Not detected	Detected	Not detected
Viasure	Detected	49	6**	26	1****	34	5*****
	Not detected	4*	29	3***	45	0	15
Accuracy (95%CI)		88.6 (80.1 – 94.4)		94.7 (86.9 – 98.5)		90.7 (79.7 – 96.9)	
Diag. Sensitivity (95%CI)		92.5 (81.8 – 97.9)		89.7 (72.7 – 97.8)		100.0 (89.7 – 100.0)	
Diag. Specificity (95%CI)		82.9 (66.4 – 93.4)		97.8 (88.5 – 99.9)		75.0 (50.9 – 91.3)	
PPV (95% CI)		89.1 (79.7 – 94.4)		96.3 (78.8 – 99.5)		87.2 (76.1 – 93.6)	
NPV (95% CI)		87.9 (73.6 – 95.0)		93.8 (83.7 – 97.8)		100.0	
LR+ (95% CI)		5.39 (2.59 – 11.22)		41.24 (5.91 – 287.7)		4.00 (1.87 – 8.55)	
LR- (95% CI)		0.09 (0.04 – 0.24)		0.11 (0.04 – 0.31)		0.00	

NWLP, North West London Pathology; SWLP, South West London Pathology; RIPL, Rare and Imported Pathogens Laboratory; PPV, positive predictive value; NPV, negative predictive value; LR+, likelihood ratio positive; LR-, likelihood ratio negative; CI, confidence interval.

* Sample 1 – 4 in Table 3.
** Sample 5 – 10 in Table 3.
*** Sample 11 – 13 in Table 3.
**** Sample 14 in Table 3.
***** Sample 15 – 19 in Table 3.

Table 3
Discordant PCR results.

Laboratory	Sample No	Assay							
		Primary testing				Discordant testing			
		RIPL		Viasure		LDT		RIPL	
		Result	Site	Result (Ct)	Site	Result (Ct)	Site	Result (Ct)	Site
NWLP	1	D	Lesion	ND	Throat	ND	Throat	–	–
	2	D	Penile	ND	Throat	D (39.0)	Penile	–	–
	3	D	Swab	ND	Throat	ND	Throat	–	–
	4	D	Swab	ND	Skin	ND	Skin	–	–
	5	ND	Lesion	D (33.9)	Throat	Inhibitory	Lesion	–	–
	6	ND	Skin	D (29.0)	Throat	–	–	–	–
	7	ND	Throat	D (23.3)	Throat	D (28.8)	Throat	–	–
	8	ND	Skin	D (27.9)	Perianal	D (32.9)	Perianal	–	–
	9	ND	Hand	D (14.1)	Perianal	D (18.8)	Perianal	–	–
	10	ND	Swab	D (16.3)	Perianal	D (23.0)	Perianal	–	–
SWLP	11	D	Skin	ND	Throat	–	–	–	–
	12	D	Groin	ND	Throat	–	–	–	–
	13	D	Swab	ND	Throat	–	–	–	–
	14	ND	Throat	D (14.3)	Lesion	–	–	D	Lesion
Synnovis	15	ND	Chest	D (19.5)	Leg	–	–	D (21.4)	Leg
	16	ND	Groin	D (29.0)	Ear	–	–	D (30.9)	Ear
	17	ND	Lesion	D (14.5)	Lesion	–	–	D (19.1)	Lesion
	18	ND	Skin	D (38.1)	Skin	–	–	–	–
	19	ND	Perianal	D (38.0)	Lesion	–	–	–	–

RIPL, Rare and Imported Pathogens Laboratory; LDT, laboratory developed test; Ct, cycle threshold; NWLP, North West London Pathology; SWLP, South West London Pathology; D, detected; ND, not detected; -, Not performed.

Viasure assay.

4. Discussion

The response to the ongoing epidemic of monkeypox necessitates the availability of diagnostics that enable rapid detection of MPXV from clinical samples. We found the commercially available Viasure MPXV PCR assay demonstrated excellent accuracy, diagnostic sensitivity and diagnostic specificity. The assay requires minimal user input and hands-on time, and is suitable for routine diagnostic laboratory investigations of monkeypox.

As MPXV shedding in bodily fluids may be low in the early and late phases of infection, and at certain anatomical sites relative to skin lesions [5], optimal swab sampling is essential to reduce the risk of false negative results and improve the accuracy of the Viasure assay. We found vesicular lesions from any anatomical site, if present, should be preferentially sampled. In addition, to improve the sensitivity of the assay, testing swabs collected from different anatomical sites may be warranted.

In the context of detecting MPXV in a predominantly at-risk population that may be co-infected with other organisms, or presents with an undifferentiated rash disease, an assay with a high specificity or LR+ that can rule in a MPXV infection is necessary. *In-silico* analysis [6] and our analytical specificity (data not shown) and diagnostic specificity data have demonstrated that the Viasure assay is specific, and provides a clinically useful LR+ that would support clinicians in their diagnosis of MPXV infections. To reduce the risk of false positive results caused by contamination from high titre MPXV samples, the incorporation of negative controls at various testing steps is suggested.

In conclusion, the Viasure MPXV PCR assay demonstrated excellent performance characteristics and is fit for routine diagnostic purpose. If implemented, the assay would allow a rapid and accurate laboratory diagnosis of MPXV infections, and facilitate a timely management of monkeypox.

Ethical approval

Not required.

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The Viasure MPXV PCR assay reagents were supplied free of charge by CerTest Biotec. CerTest Biotec had no role in the study design, data collection and analysis, result interpretation, writing of the manuscript and the decision to submit the article for publication.

CRediT authorship contribution statement

Ngee Keong Tan: Conceptualization, Methodology, Validation, Formal analysis, Data curation, Writing – original draft, Visualization. **Cindy P. Madona:** Investigation, Writing – review & editing. **Joshua F. Taylor:** Investigation, Data curation, Writing – review & editing. **Lynda Hadjilah Fourali:** Investigation, Data curation, Writing – review & editing. **Jasveen K. Sehmi:** Investigation, Data curation, Writing – review & editing. **Madeline J. Stone:** Investigation, Writing – review & editing. **Marcus J. Pond:** Conceptualization, Methodology, Investigation, Data curation, Writing – review & editing, Supervision. **Penelope R. Cliff:** Conceptualization, Methodology, Writing – review & editing, Supervision. **Cassie F. Pope:** Conceptualization, Methodology, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: JFT received conference registration expense from Pro-Lab Diagnostics to attend the 2022 Federation of Infection Societies Conference. All other authors have no relevant financial or non-financial competing interests to declare.

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