Development and Validation of a Quantitative Synthetic Analytical Reference Material for Monkeypox Virus

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Background and Introduction

Mpox is a zoonotic viral disease caused by the human monkeypox virus (hMPXV), a double-stranded DNA virus from the genus *Orthopoxvirus* and the family *Poxviridae*. Endemic to Central and Western Africa, hMPXV has increasingly appeared in non-endemic areas, causing a smallpox-like disease in humans.

Rapid detection during early infection is crucial for containment and treatment. While culture-based detection is time-consuming and requires BSL-3 facilities, PCR-based methods offer a sensitive and rapid alternative. These assays rely on high-quality reference materials. To address this, ATCC® developed a quantitative synthetic molecular standard for hMPXV, incorporating diagnostic biomarkers from both clades of the virus. This standard uses gene sequences targeted in various assays to create a safe (BSL-1) and reliable positive analytical reference material (ARM).

The synthetic standard's functionality was tested via 15 published quantitative PCR (qPCR) assays, including those from the Centers for Disease Control and Prevention (CDC) that are specific for non-variola *Orthopoxvirus* and generic monkeypox. The data shown collectively indicate that the hMPXV synthetic molecular standard is an effectively designed ARM suitable for developing and validating molecular-based detection and quantification assays. Our findings confirm the full compatibility of this novel ARM with 15 qPCR assays. Consequently, this ARM can function as a PCR template control, providing a safe and dependable positive control material for molecular assays employed in diagnostics and surveillance.

ATCC Synthetic Molecular Standards

BSL-1	Quantitative	Five-Year Shelf Life

ATCC Catalog Number	Product Description
ATCC® VR-3270SD™	Quantitative Synthetic Monkeypox virus DNA

Applications

- Generation of a standard curve for quantitative PCR
- Positive control for qPCR assays
- Assay verification and validation studies
- Monitor assay-to-assay and lot-to-lot variation
- Molecular diagnostics assay development

Materials and Methods

Quantitative Synthetic DNA

Using a proprietary method, we designed a synthetic DNA construct for hMPXV (ATCC® VR-3270SD™). This construct comprises fragments from the following genomic regions: J2L, D14L, F3L, F8L, A27L, A29L, B6R, B7R, and N3R. Following construction, the product was authenticated via next-generation sequencing and then quantified by Droplet Digital PCR (Bio-Rad). The product concentration is between 1 x 10^5 and 1 x 10^6 copies/µL.

qPCR

Real-time PCR (qPCR) assays were performed on the CFX Opus Real-Time PCR Systems (Bio-Rad) using the Platinum Quantitative PCR SuperMix-UDG (Invitrogen) kit (catalog # 11730017). Cycling parameters and primer and probe concentrations were applied according to each corresponding publication.

Results

Clade I Assay

Clade II Assay

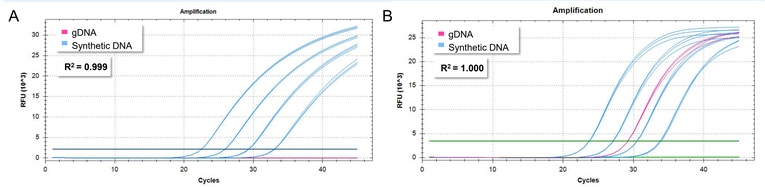

Figure 1: qPCR amplification of ATCC® VR-3270SD™ (blue) and gDNA (pink) extracted from Clade II hMPXV (ATCC® VR-3371HK™) with a (A) Clade I specific assay from Negrón, *et al.,* 2023, that targets C3L, and a (B) Clade II specific assay from Li, *et al.,* 2010, that targets G2R.

Non-Clade Specific Mpox Assays

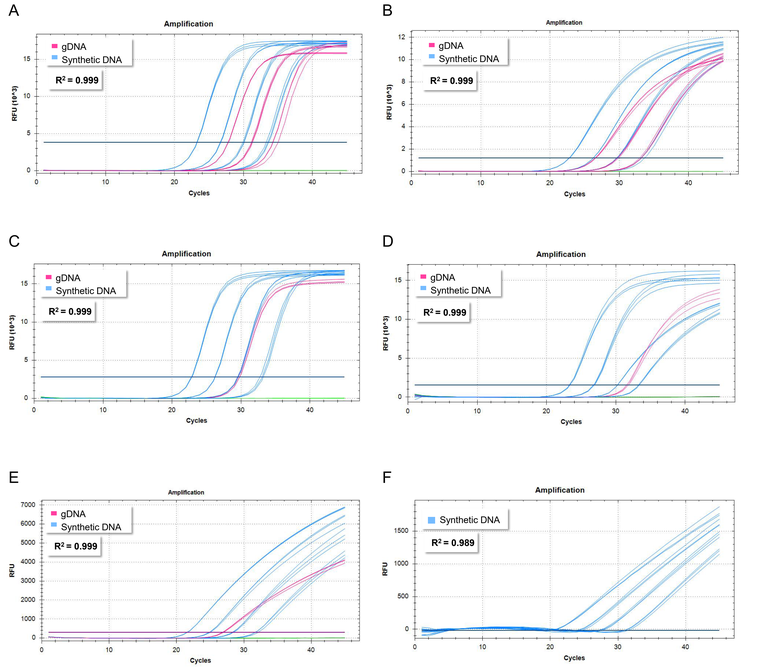

Figure 2: qPCR amplification of ATCC® VR-3270SD™ (blue) and gDNA (pink) extracted from Clade II hMPXV (ATCC® VR-3371HK™) with assays from (A) the CDC, 2022, which targets G2R; (B) Maksyutov, *et al.,* 2016, which targets F3L; (C) Kulesh, *et al.,* 2004, which targets F3L and (D) N3R; (E) Shchelkunov, *et al.,* 2011, which targets B7R; and (F) Hutson, *et al.,* 2008, which targets B6R.

Results (continued)

Generic Orthopox Assays

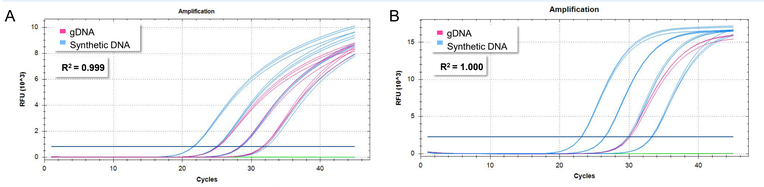

Figure 3: qPCR amplification of ATCC® VR-3270SD™ (blue) and gDNA (pink) extracted from Clade II hMPXV (ATCC® VR-3371HK™) with generic *Orthopox* detection assays from (A) the CDC, 2022, which targets E9L, and (B) Dumont, *et al.,* 2014, which targets A29L.

Table 1. Summary of Mpox diagnostic targets and experimental validation.

Specificity	Publication Source	Assay Target	Synthetic VR-3270SD™		gDNA extracted from VR-3371HK™	
			Expected	Actual	Expected	Actual
Clade I	Negrón, *et al.*, 2023	C3L	●	■	●	■
	Li, *et al.*, 2010	C3L	●	■	●	■
	Saijo, *et al.*, 2008	ATI/A27L	●	■	●	■
	Li, *et al.*, 2010	G2R	●	■	●	■
Clade II	CDC, 2022	G2R	●	■	●	■
MPOX, both clades	Maksyutov, *et al.*, 2016	F3L	●	■	●	■
	Kulesh, *et al.*, 2004	F3L	●	■	●	■
		N3R	●	■	●	■
	Hutson, *et al.*, 2008	B6R	●	■	●	■
	Shcheklkunov, *et al.*, 2011	B7R	●	■	●	■
	Neubauer, *et al.*, 1998	ATI ORF	●	■	●	■
Generic Orthopox	Dumont, *et al.*, 2014	IMV Surface Protein/A29L	●	■	●	■
	CDC, 2022	E9L	●	■	●	■

Conclusions

- Our data demonstrate that the ATCC quantitative synthetic hMPXV DNA can be used as a reliable analytical reference material for assay development, verification, and validation.
- The product can be used to determine the viral load of unknown Mpox samples through the generation of a standard curve.
- The ARM is compatible with numerous published assays and serves as a well-characterized control for viral detection and quantification.

Order the Synthetic hMPXV DNA

References

- CDC Test Procedure: Monkeypox virus Generic Real-Time PCR Test, Doc. No. CDC-007-00217, Rev. No. 2
- CDC Test Procedure: Non-variola *Orthopoxvirus* Generic Real-Time PCR Test, Doc. No. CDC-007-00215, Rev. No. 2
- Dumont C, et al. PLoS One 9(5): e96930, 2014.
- Hutson CL, et al. J Gen Virol 90(2): 323-333, 2009.
- Kulesh DA, et al. Lab Invest 84(9): 1200-1208, 2004.
- Li Y, et al. J Virol Methods 169(1): 223-227, 2010.
- Maksyutov RA, et al. J Virol Methods 236: 215-220, 2016.
- Negrón DA, et al. bioRxiv 2023.04.24.538151, 2023.
- Neubauer H, et al. J Virol Methods 74(2): 201-207, 1998.
- Saijo M, et al. Jpn J Infect Dis 61(2): 140-142, 2008.
- Shcheklkunov SN, et al. J Virol Methods 175(2): 163-169, 2011.

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