

# Diagnostic evaluation of Four Real-Time Qualitative PCR Kits for Human Monkeypox Virus

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## PURPOSE/OBJECTIVES

The emerging outbreak of Monkeypox (MPX), a rare zoonotic disease caused by human monkeypox virus which spreads strongly with close/intimate contact, is a new challenge for global public health. Since early May 2022, several human MPX cases were identified in non-endemic countries.

The aim of this study is to compare the diagnostic performances of four real-time PCR assays with a home-made PCR test, for MPX laboratory diagnosis, during the 2022 outbreak.

## MATERIALS & METHODS

In the Hygiene Unit laboratory at San Martino Hospital (Genoa), 27 positive and 10 negative specimens (swabs from lesions, crusts and exudates), harvested from 25 patients with a clinical picture suggestive for MPX disease between July to August 2022, were retrospectively tested with different multiplex real-time qualitative PCR assays, according to the manufacturer's instructions: RealCycler MONK-UX/-GX (Progenie Molecular), STANDARD M10 MPX/OPX (SD Biosensor), Novaplex - MPXV Assay (Seegene Inc.) and RealStar® Orthopoxvirus PCR Kit 1.0 (Altona Diagnostics) recognized as "Research use only (RUO)". All the specimens had been previously tested with a home-made real-time PCR for generic MPX virus DNA detection (MPXV generic G2R\_G). Like the home-made test, turn-around-time (TAT) of these assays was 3 hours, excluding STANDARD M10 MPX/OPX that is categorized as a one-hour TAT point-of-care test. Furthermore, STANDARD M10 MPX/OPX MPX differentiated West-African and Congo-Basin strains. The diagnostic characteristics of these different commercial tests were evaluated.

## RESULTS

The accuracy and sensitivity of these molecular RUO MPX assays ranged from 97.3% (86.2-99.5) to 100% (90.6-100) and 96.3% (81.72-99.34) to 100% (72.25-100), respectively. RealCycler® MONK-UX and STANDARD M10 MPX/OPX did not detect a positive sample with low viral load [cycle threshold (ct) of 36]. The overall specificity was 100% (72.25-100). Furthermore, these tests had Cohen's k values ranging from 1 (0.67-1) to 0.93 (0.61-1). The diagnostic performances data were reported in table 1. All MPX patients were affected by West African strain.

MPX real-time PCR assays	Accuracy	Sensitivity	Specificity	Cohen's k
RealStar® Orthopoxvirus PCR Kit 1.0	100% (90.6-100)	100 (87.54-100)	100 (72.25-100)	1 (0.67-1)
RealCycler® MONK-UX/-GX	97.3% (86.2-99.5)	96.3 (81.72-99.34)	100 (72.25-100)	0.93 (0.61-1)
Novaplex™ - MPXV Assay	100% (90.6-100)	100 (87.54-100)	100 (72.25-100)	1 (0.67-1)
STANDARD M10 MPX/OPX	97.3% (86.2-99.5)	96.3 (81.72-99.34)	100 (72.25-100)	0.93 (0.61-1)

Table 1 - Diagnostic performances of novel molecular MPX assays compared to the home-made PCR procedure in use. Data were analyzed computing at 95% confidence interval.

In-house or RUO molecular tests showed a different Ct distribution for high viral load ( $Ct \leq 30$ ) specimens (Wilcoxon's test:  $p < 0.05$ ). However, the interpretation of these results as positive unchanged for all molecular assays in use and evaluation. Instead, skin swabs with low viral load ( $Ct$  of 31-35) had  $Ct$  values which they were similarly distributed, including the RUO tests and the method of reference, even if the sample size of this group were low ( $N = 7$ ) (Wilcoxon's test:  $p > 0.05$ ).

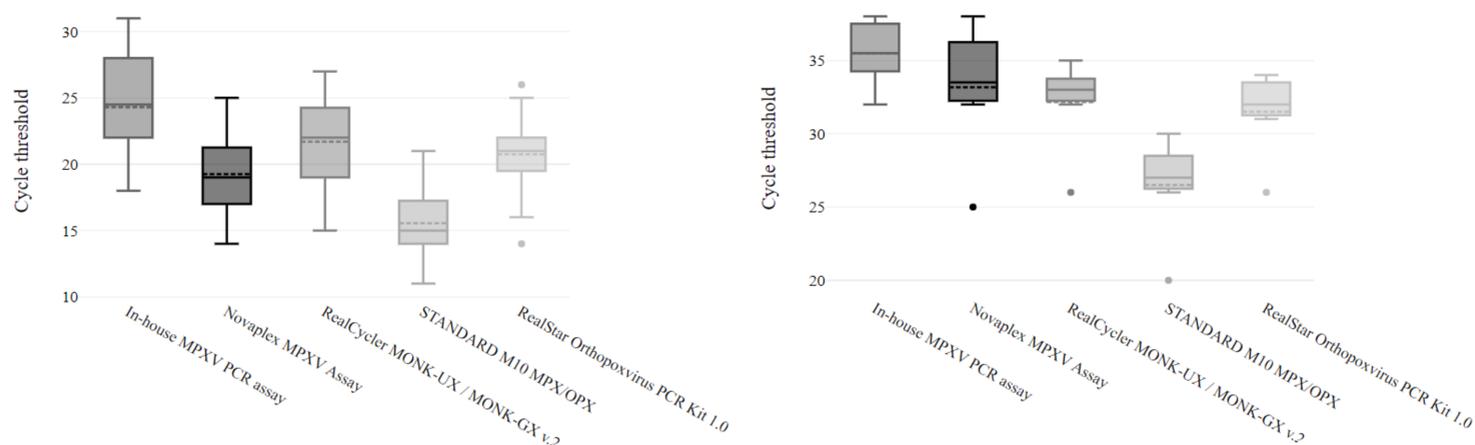


Figure 1 - MPXV molecular diagnosis: graphical results of cycle threshold using in-house and Research Use Only PCR assays.

Furthermore, RUO MPX assays showed no cross-reactivity testing a HSV1, HSV2 and VZV pool.

## SUMMARY/CONCLUSION

As they are very accurate, reliable and user-friendly, these tests are highly recommended for daily or rapid laboratory discrimination of MPX infections from other rash illness.