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# Clinical implementation of real-time PCRs for the detection of Mpox

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## Introduction

Mpox is a zoonotic disease caused by monkeypox virus (MPXV), member of the Orthopoxvirus (OPXV) genus in the Poxviridae family. It is a dsDNA virus with two distinct clades identified: Clade I (former Congo Basin clade) and Clade II (former West African clade), which caused the current outbreak. Clinical mpox disease presents with a localised or disseminated rash, represented via various lesions depending on the stage of the disease. Laboratory diagnosis is determined through PCR testing.

The UK was an epicentre of the mpox outbreak detected in early May 2022, when the first cases were reported outside of Africa. In the absence of clear understanding of the extent of local transmission and the possibility of further spread to the population, development of local diagnostic testing was warranted.

## Methods

The laboratory tested 113 specimens from 40 patients received for MPXV investigation, which were tested using the RealStar® Orthopoxvirus PCR Kit 1.0 (Altona Diagnostics, Germany) and an in-house developed real-time PCR assay detecting the presence of MPXV and non-variola OPXV, as well as internal process control. All samples were referred to the Rare and Imported Pathogens Laboratory (RIPL), UKHSA Porton Down, UK, and the diagnostic performance was compared. The limit of detection (LoD) of the MPXV/OPXV PCR using serial dilutions of a MPXV synthetic DNA fragment provided by the National Measurement Laboratory, LGC, Teddington, UK.

## Results

### Reactivity

Skin and lesion swabs were the most frequent specimen types tested, with positive Ct values ranging from 16.25 to 32.48 by RealStar® Orthopoxvirus PCR and 18.83 to 36.4 by the in-house MPXV/OPXV PCR . Viraemia was indicated in 4/18 (22.2%) EDTA blood samples tested by RealStar® Orthopoxvirus PCR compared to 3/18 (16.6%) by the in-house MPXV/OPXV PCR. The EDTA specimens were collected from known positive patients, but not confirmed by RIPL. The positivity rate of the samples tested was 38.94%.

**Table 1. Specimen types used for the validation of the RealStar® Orthopoxvirus PCR Kit 1.0 (Altona Diagnostics, Germany) and an in-house developed MPXV/OPXV real-time PCR.**

| Specimen Type | No. Specimens | Positive Specimens | Positivity Rate |
|---|---|---|---|
| Anal swab | 2 | 1 | 50% |
| Genital swab | 4 | 3 | 75% |
| Mouth swab | 4 | 1 | 25% |
| Skin swab | 45 | 16 | 35.56% |
| Swab of unknown site | 7 | 3 | 42.86% |
| Lesion swab | 33 | 16 | 48.48% |
| EDTA blood | 18 | 4 | 22.22% |
| TOTAL | 113 | 44 | 38.94% |

### Statistical Analysis

Both the RealStar® Orthopoxvirus PCR and the in-house MPXV/OPXV PCR successfully detected 39/40 swabs as positive. Only one swab was detected as positive by RIPL but negative on both assays. The uncertainty of measurement (UOM) and coefficient of variation (CV) were calculated by the MPXV internal control used.

**Table 2. Statistical analysis of performance for the RealStar® Orthopoxvirus PCR and the in-house MPXV/OPXV PCR assays compared with RIPL.**

| Statistical Analysis | RealStar® Orthopoxvirus PCR | In-house MPXV PCR | In-house OPXV PCR |
|---|---|---|---|
| Analytical Sensitivity | 97.5% | 97.5% | 97.5% |
| Analytical Specificity | 100% | 100% | 100% |
| Positive Predictive Value | 100% | 100% | 100% |
| Negative Predictive Value | 98.21% | 98.21% | 98.21% |
| Uncertainty of Measurement | 1.22 | 0.76 | 0.75 |
| Coefficient of variation | 4.35% | 2.56% | 2.65% |

### Limit of Detection and Standard Curves

The monkeypox synthetic DNA was tested on a ten-fold serial dilution to produce a standard curve and determine the limit of detection (LoD). The LoD for the MPXV/OPXV PCR was equivalent to 1 dcp/µL (R2 = 0.9941 for MPXV and 0.9998 for OPXV, M = -3.626 for MPXV and -3.4 for OPXV). Unfortunately, the LoD calculated only for the in-house MPXV/OPXV PCR, as the monkeypox DNA sequence contained on the synthetic DNA was not compatible with the RealStar® Orthopoxvirus PCR.

**Table 3. Serial dilutions of the monkeypox synthetic DNA fragment for the determination of the limit of detection for the in-house MPXV/OPXV PCR.**

| DNA dcp/µL | DNA per reaction (dcp/µL) | Log10 DNA (dcp/µL) | MPXV Ct value | OPXV Ct value |
|---|---|---|---|---|
| 0.1 | 1 | 0.01 | 0 | 0 |
| 1 | 10 | 0.1 | 36 | 36.46 |
| 10 | 100 | 1 | 32.78 | 33.04 |
| 100 | 1000 | 2 | 28.67 | 29.62 |
| 1000 | 10000 | 3 | 26.22 | 26.39 |
| 10000 | 100000 | 4 | 21.65 | 22.84 |
| 100000 | 1000000 | 5 | 18.16 | 19.43 |

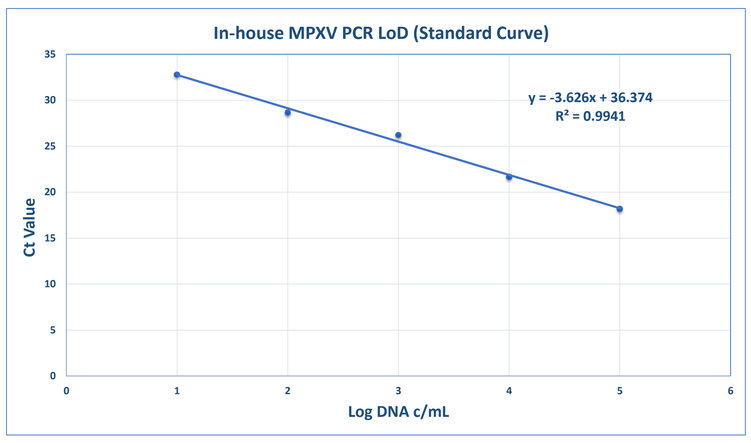


**Figure 1. Standard curve for the quantification of the in-house MPXV PCR using the monkeypox synthetic DNA fragment (NML,UK)**

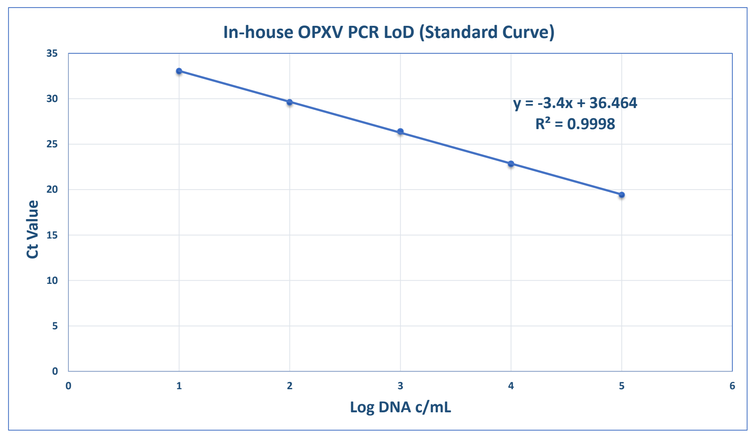


**Figure 2. Standard curve for the quantification of the in-house OPXV PCR using the monkeypox synthetic DNA fragment (NML,UK)**

## Conclusion

In response to urgent clinical need, the Department of Virology at Barts Health NHS Trust in the UK, evaluated and implemented the RealStar® Orthopoxvirus PCR Kit 1.0 (Altona Diagnostics, Germany). Localised laboratory testing improved result reporting times with added potential to help break onward transmission chains. The assay was subsequently replaced by the in-house developed MPXV/OPXV PCR assay. Both commercial and in-house assay demonstrated excellent clinical performance. Between May and December 2022, the department performed 1106 MPXV PCR tests for the population of East and South East London, of which 302 (27.31%) were detected as positive. This was 2.01% of the cases detected in the UK (75/3732). Between January and August 2023, the department performed 227 tests, of which 15 (6.61%) were detected as positive, comprising 20% of the cases detected in the UK (10/50).

## References

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