Enhancing Mpox Diagnostics: Insights from an External Quality Assessment Program

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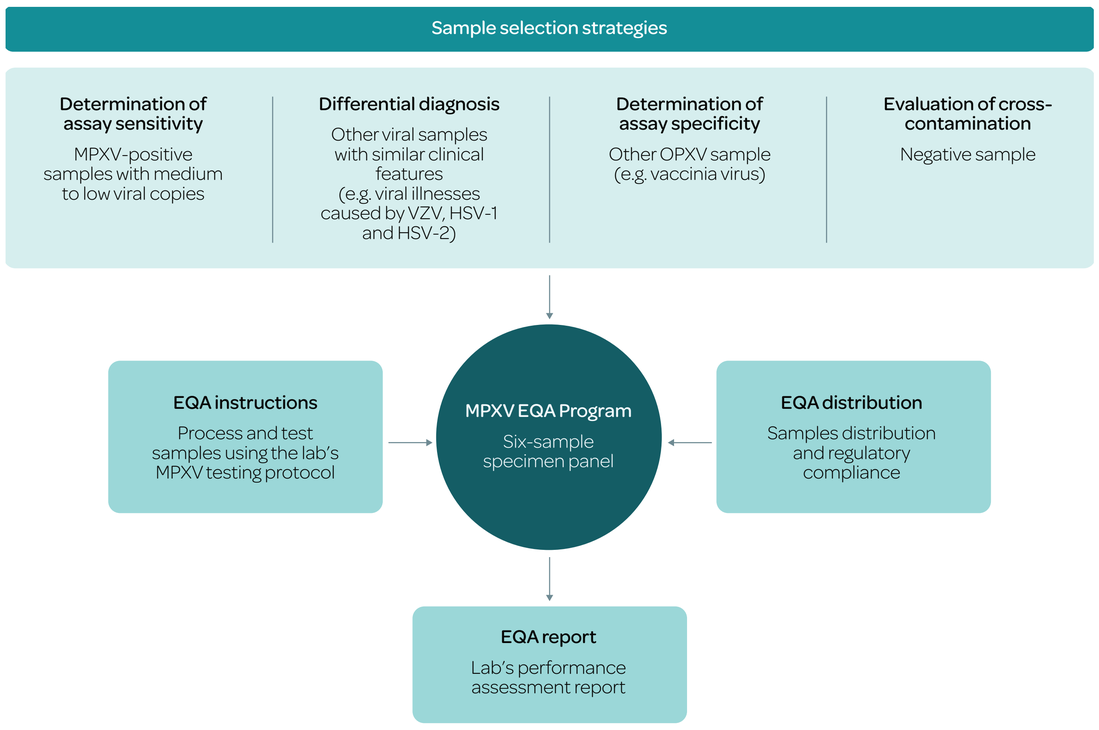
Introduction

Mpox is a viral illness caused by the monkeypox virus (MPXV), a species of the genus orthopoxvirus (OPXV), which includes vaccinia and other viruses. The preferred diagnostic test for mpox is the molecular detection of viral DNA by PCR. An initial positive detection using an OPXV PCR assay, followed by confirmation of MPXV via a viral-specific PCR assay, is essential to confirm mpox.

Method

To support public health and diagnostic testing capacity for MPXV throughout Australia, the Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP) implemented an annual external quality assessment (EQA) program for MPXV detection in 2022. The details of the program are as follows:



Results & Discussion

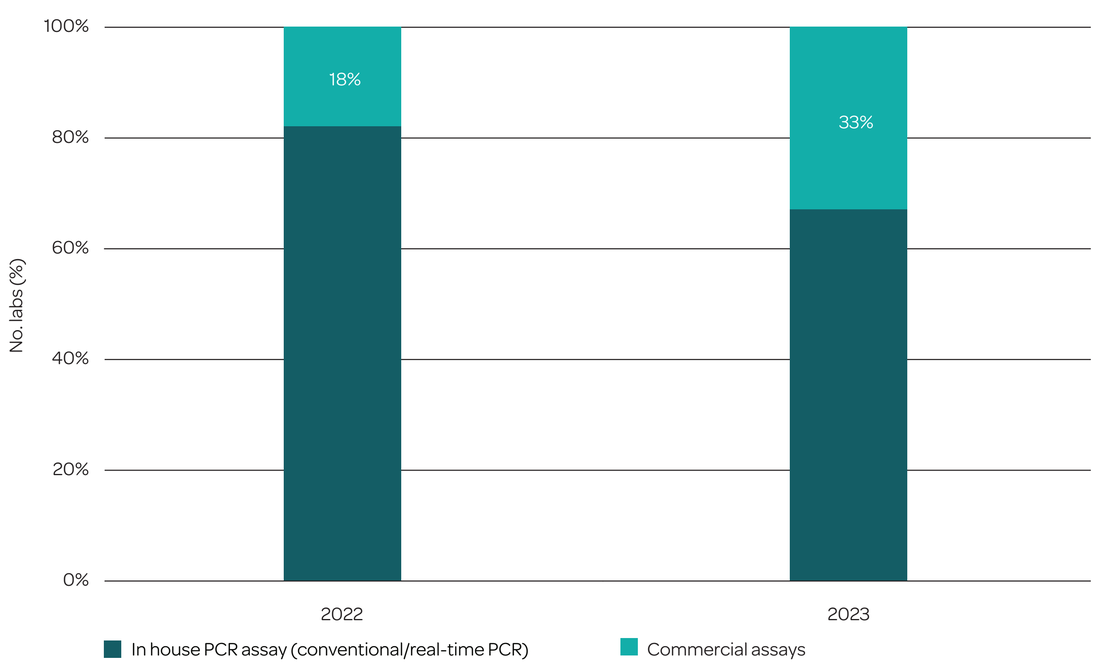


Figure 1 Comparison of assays used in the MPXV final identification

Most laboratories used in-house PCR assays to identify MPXV, with increased use of commercial testing kits, as reported by 18% and 33% of laboratories in 2022 and 2023, respectively. This was likely due to the increasing number of commercial testing kits that are more accessible to laboratories.

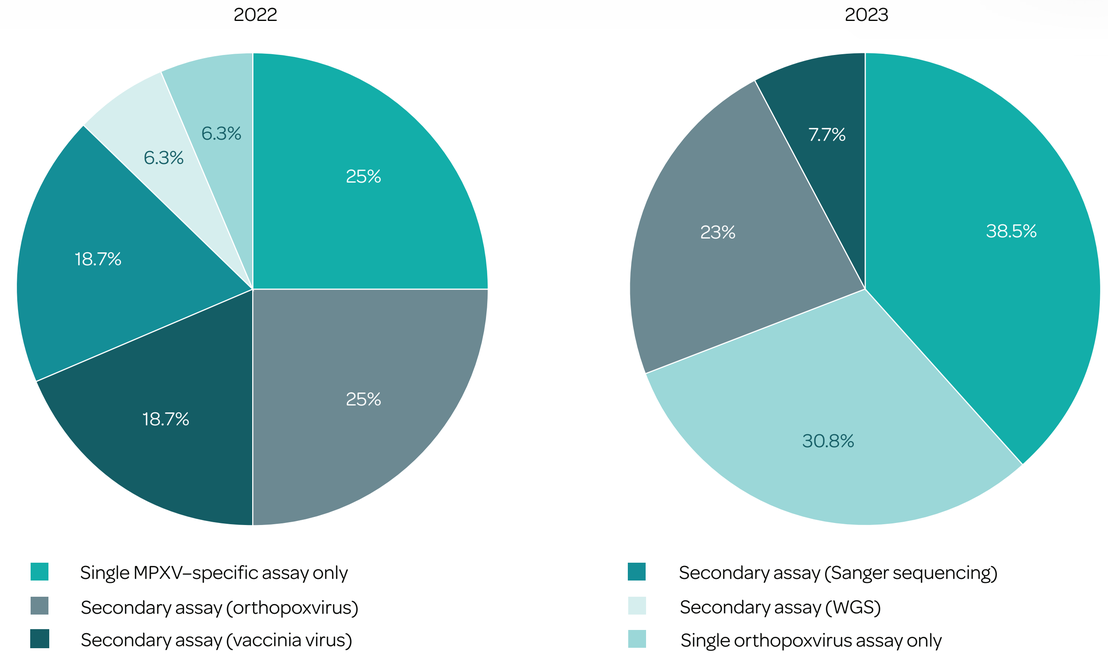


Figure 2 Laboratory testing algorithms and choice of assays (single versus secondary assay) included in the MPXV testing protocol

In 2022, 69% of participating laboratories performed the testing using a secondary assay, compared to 31% of participating laboratories in 2023. WHO recommends confirmation of MPXV infection based on a PCR assay (MPXV-specific or clade-specific PCR) for the detection of a unique viral DNA sequence, following a positive result from an initial PCR assay (a highly conserved assay targeting a generic OPXV or MPXV sequence). Alternate diagnoses, such as WGS and Sanger sequencing, can also be considered for subsequent clade identification.

The sensitivity of an MPXV-specific assay for correct identification of the MPXV-positive samples was also assessed. All laboratories that tested the samples using an MPXV-specific assay were able to correctly identify MPXV, even at low viral loads, confirming that their assays could detect MPXV accurately.

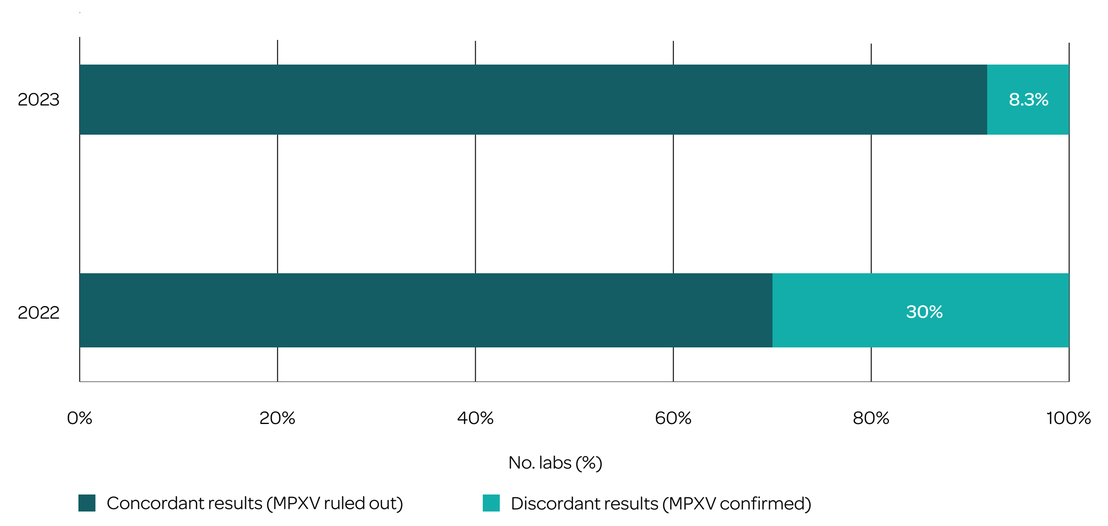


Figure 3 False positive results reported for vaccinia virus sample

Some laboratories reported false positive results for the vaccinia virus sample, indicating cross-reactivity in certain in-house MPXV-specific assays and the vaccinia virus. This diagnostic error highlighted the need for stringent assay validation processes within laboratories before deploying in-house assays for testing and the value of implementing a second confirmatory assay in the MPXV molecular testing algorithm.

Conclusion

The EQA program led to significant improvements, as the false-positive rate for the vaccinia virus sample decreased from 30% in 2022 to 8% in 2023, illustrating its positive impact on diagnostic practices in strengthening laboratory capacity.

Acknowledgment

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