SCREENING OF MPXV, VZV, HSV-1, AND HSV-2 CAUSING MUCOCUTANEOUS VESICULAR RASHES BY MULTIPLEX HRM-PCR

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OBJECTIVES

Since May 2022, over 27,000 cases of monkeypox (MPOX) have been reported in Europe where the disease is not endemic. In the Democratic Republic of Congo (DRC), this disease has become a public health emergency, with 19,667 suspected cases and 575 deaths reported in 2024. Monkeypox virus (MPXV) represents a diagnostic challenge due to the diversity of clinical presentations. These symptoms often share similarities with infection caused by herpes simplex virus types 1 and 2 (HSV-1, HSV-2) and varicella-zoster virus (VZV). AFYIA Diagnostics has developed a qualitative screening test that allows the simultaneous identification of these viruses.

MATERIELS & METHODS

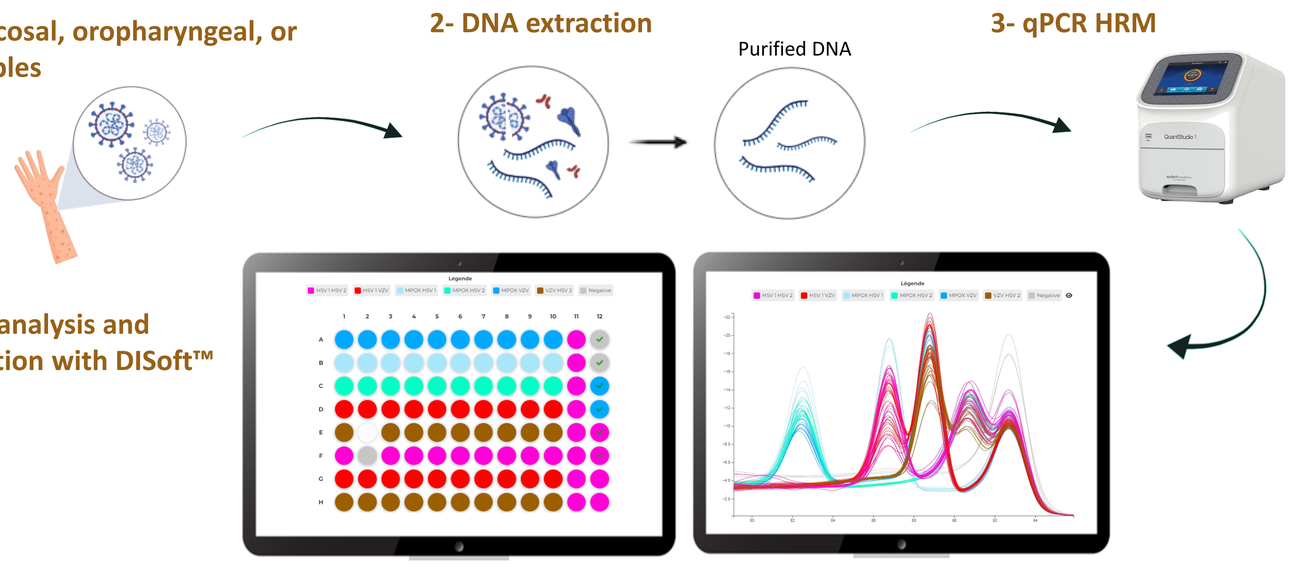
The DIRASH kit is a multiplex HRM assay, based on the simultaneous detection of 4 viruses. The associated DISoft™ software is used to automatically analyze and interpret the HRM profiles obtained. Three studies were carried out to monitor cases of cutaneous-mucosal febrile vesicular eruptions: 1 conducted by CHU Montpellier from January 2021 to December 2022 during the clade II epidemic, and 2 conducted by INRB in Kinshasa from December 2022 to June 2024 with clade Ia and Ib . Each patient was screened by commercial or in-house PCRs. These samples were then retrospectively tested with the DIRASH.

Syndromic diagnostics
Multiplex qPCR HRM test for screening viral DNA of MPOX, VZV, HSV-1, and HSV-2.

Automated interpretation of melt curves
The results obtained are analyzed and interpreted by software based on artificial intelligence: DISoft™.

Performance
LDPCR < 8 copies/µL for each pathogen
Exclusivity = 100% (tested on 84 pathogens)

Stable at room temperature
Lyophilized



RESULTS

A total of 850 samples, collected between January 2021 and January 2024, were retrospectively tested using the DIRASH kit to compare its clinical sensitivity with that of the kits used in the two partner laboratories: one at the CHU of Montpellier in France and the other at the INRB in Kinshasa, DRC.

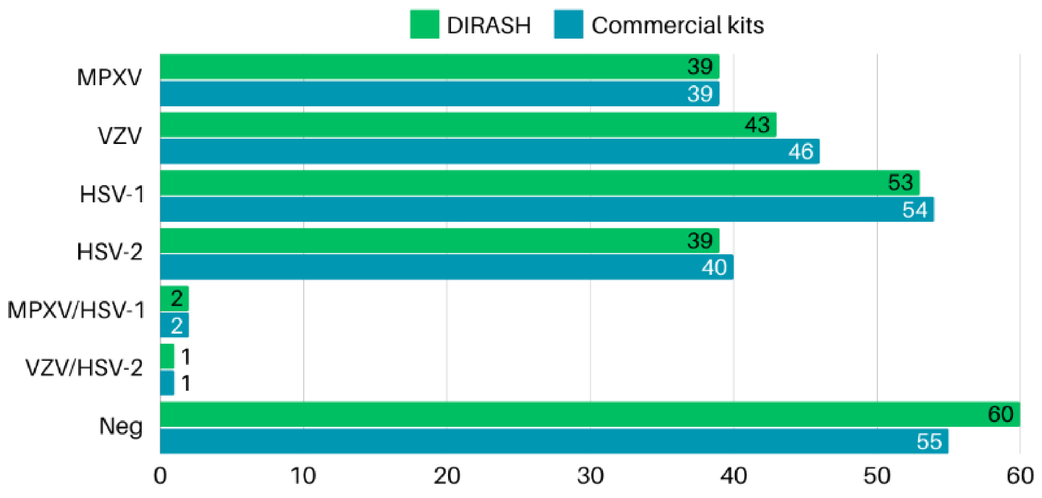


Figure 1: Comparison of the clinical sensitivity and specificity of the DIRASH kit versus the routine PCR tests of the CHU of Montpellier.

- **Sensitivity: MPXV = 100%; VZV = 94%; HSV-1 and HSV-2 = 98%**
- **Specificity: 100%**

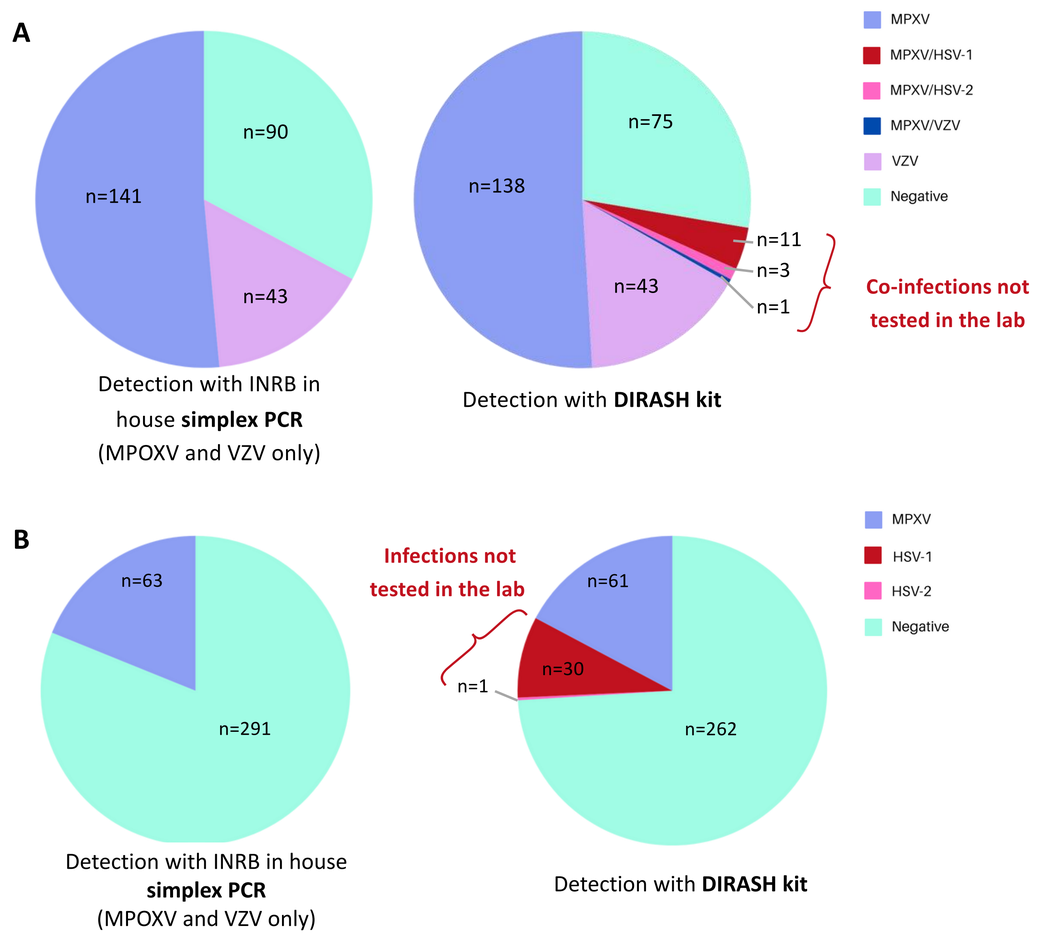


Figure 2: Comparison of the clinical sensitivity and specificity of the DIRASH kit versus the routine in house simplex PCR technology of the INRB with 259 samples collected between 2022 and 2024 (A), as well as 354 samples collected in 2024 (B).

- **Sensitivity: MPXV = 98%; VZV = 100%**
- **Specificity: 100%**

CONCLUSION

The DIRASH kit is rapid, reliable, transportable at room temperature, low-cost-multiplex test with automated result interpretation. Its ability to simultaneously detect viruses causing mucocutaneous vesicular eruptions meets the needs for differential diagnosis of MPXV, particularly in the DRC, where the epidemic is the largest in Africa, but also non-endemic countries. The kit is well-suited to strengthening diagnostic capabilities in the DRC, supporting patient management and epidemic control.

