

IVD_15 - Development of a new multiplex platform for detection and screening for virus-caused exanthematic diseases

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Introduction: Skin exanthems are associated with self-limited diseases. Monkeypox (Mpox) is a viral zoonotic disease caused by the Monkeypox virus – *Orthopoxvirus* genus – characterized by rash or skin lesions, usually concentrated in the face, hand, or genital area. In 2022, several cases were reported in non-endemic countries. Bio-Manguinhos registered two real time PCR IVD kits, supporting the Brazilian Ministry of Health. One of these kits allows differentiation in cases previously classified as negative, providing greater capacity for diagnostic clarification of related viruses. However, there are limitations for multiplexing which allows no more than 5 targets to be detected among commercial real time PCR equipment.

Objectives: The aim of this work is the development of a new 7-plex reaction that allows the detection and differentiation of Mpox and other exanthematic viruses.

Methodology: The real time PCR reaction was made using a microchip, designed with 9 separated wells – Genoplexor™ (OPTOLANE technologies). Each well will be amplified for two different targets (FAM and CalRED610 dyes) probes. With this, one sample loaded to the chip can detect Mpox, pan-*Orthopoxvirus* (OPV), *Varicela zoster Virus* (VZV), *Molluscum contagiosum virus* (MOCV), *Herpes Simplex Virus* (HSV) serotypes 1 and 2 and human *Beta-globulin* gene as internal control (IC) using DNA extracted truly positive and negative samples.

Results: The reaction was validated for fast protocol and cycling conditions. An equipment that could be used in fields for faster diagnostic as a point of care device (POC) has been evaluated. Two DNA samples of each target were tested using Genoplexor™ and compared with IVD Molecular OPXV/MPXV/VZV/RP Bio-Manguinhos Kit. Regarding the specificity, 10 negative skin swab samples have shown no false-positive for any targets. Considering positive samples, only targets matching the input viral tested showed positive results – 100% concordance. Related to sensitivity, Genoplexor™ had equivalent results compared to IVD kit.

Conclusion: The goal of this kit is the usedness flexibility, detecting 7 different targets at the same time. Furthermore, we are developing an innovative way to prepare samples without DNA isolation and the possibility of reaction prepare on fields.

Keywords: Molecular point of care; Monkeypox; R&D