Evaluation of the performance of the Alinity m MPXV Assay

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Robert Ehret, Malik Prentice, Martin Obermeier
Medical Center for Infectious Diseases, Berlin (MIB)
Contact: Ehret@mvz-mib.de

BACKGROUND

Timely and reliable laboratory diagnosis of monkeypox virus (MPXV) is of critical importance to patient care, contact tracing, and decreasing transmission.
The new Alinity m MPXV Research Use Only (RUO) assay developed for direct qualitative detection of MPXV DNA in clinical specimens on the Alinity m System was evaluated by comparison to a MPXV in-house multiplex real-time PCR (LDT) established with start of the global MPXV outbreak in summer 2022.

METHODS

The MPXV LDT utilizes the Nimbus system (Seegene, Korea) for nucleic acid extraction. The mastermix (TaqPath ProAmp Multiplex Master Mix, Applied BioSystems, USA) contains primer/probe pairs for MPXV, ortho-pox, internal process control [PhHV-1 IC], a cellularity control [ß-globin] (TIB Molbiol, Germany), and PCR was run on a Cfx96 system (Bio-Rad, Germany).
The Alinity m MPXV RUO assay (Abbott Molecular Inc., USA) utilizes real-time polymerase chain reaction (PCR) to amplify and detect monkeypox virus genomic DNA sequences, internal control sequences, and human genomic DNA sequence (ß-globin) extracted from clinical specimens.
Assay linearity and precision were assessed with a dilutional series prepared from a cell-culture supernatant (INSTAND e.V., Germany) pre-quantified by digital PCR, with concentrations ranging from 500,000 copies/mL to 160 copies/mL and tested at ten replicates each.
Detection limits of both tests (95% hit-rates) were determined by Probit analysis.
Residual archived patient swab samples (MPXV-negative/positive 100/300) were selected based on historical results generated with the MPXV LDT in 2022. The positive samples were diluted 1:10 in AVL buffer (QIAGEN, Germany) to obtain sufficient material for parallel testing of specimens and an option of repeating measurements with the Alinity m MPXV RUO and MPXV LDT assays to estimate the correlation between both tests.

RESULTS

The dilution series demonstrated linearity for both assays (Fig. 1). The precision of the individual samples measured repeatedly (10x) was higher with the Alinity m showing CVs (coefficient of variation) between 0.3% (for the high-titre dilution level) and 1.2% than with the LDT with CVs of 1.4 - 2.5%. Probit analyses showed 95% hit-rates of 293 copies/mL for the Alinity m assay and 446 copies/mL for the MPXV LDT (Fig. 2).
All patient samples with initial MPXV LDT-negative results tested negative with both assays. Dilutions of 286/300 samples (95.3%) with initial MPXV LDT-positive results were found positive again with both assays. Samples that were not positively confirmed by both tests after dilution had Ct values of >37 cycles upon initial testing of undiluted material. The correlation between results of both tests was very high (R^2 0.96) (Fig. 3). The mean difference in Ct values between both tests determined by Bland-Altman analysis was 0.54 cycles (Fig. 4), with lower Ct values for the Alinity m MPXV RUO assay.

RESULTS

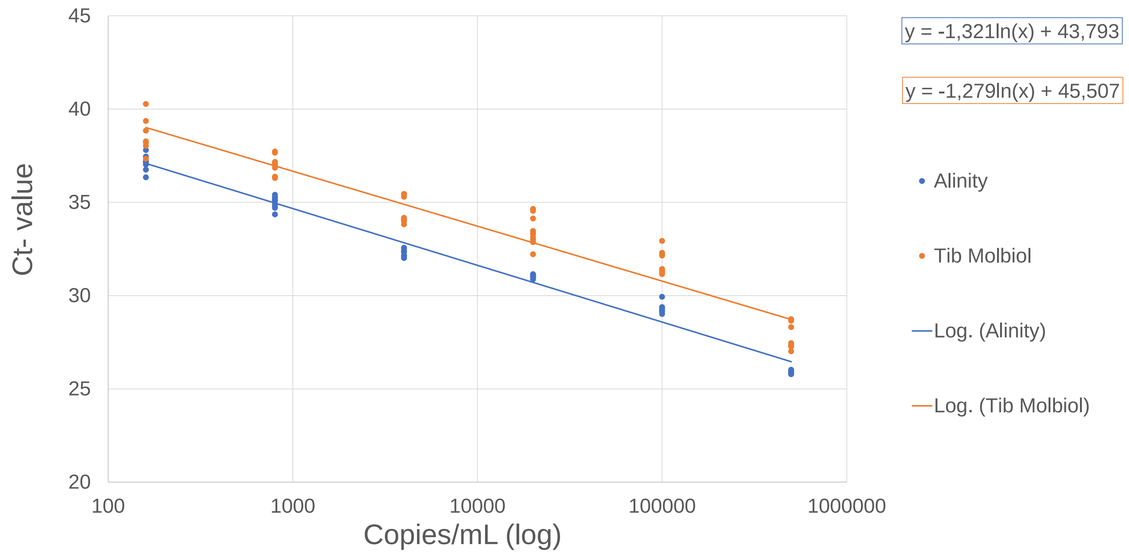

Fig. 1: Linear dilutional series MPXV Ct-values

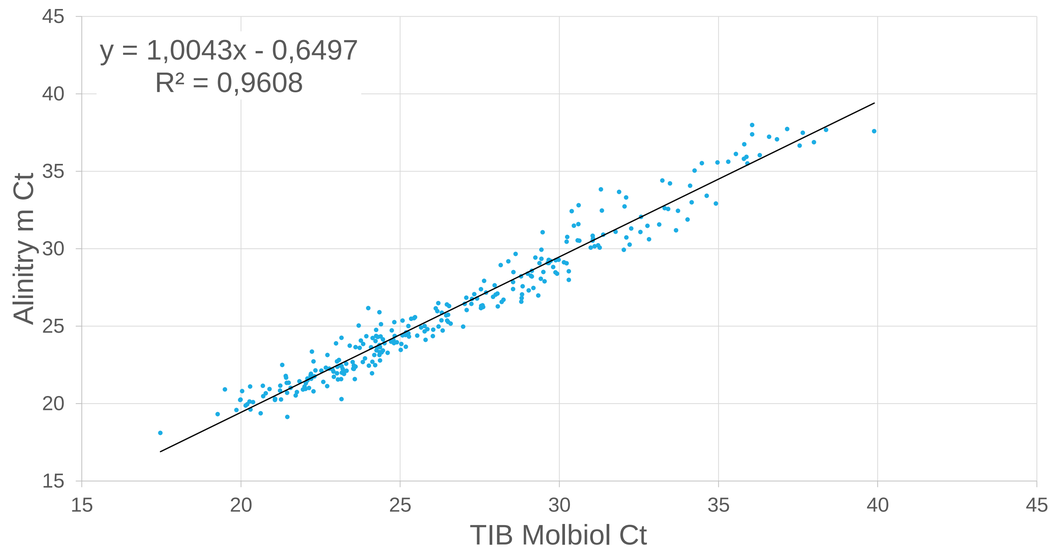

Fig. 3: Correlation MPXV Ct-values

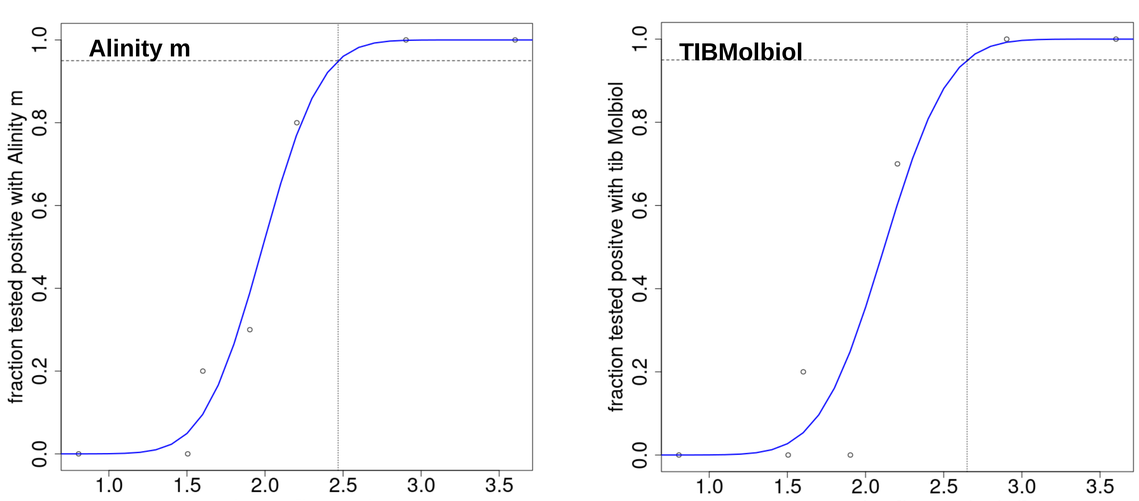

Fig. 2: Probit analysis MPXV (95% hit-rates)

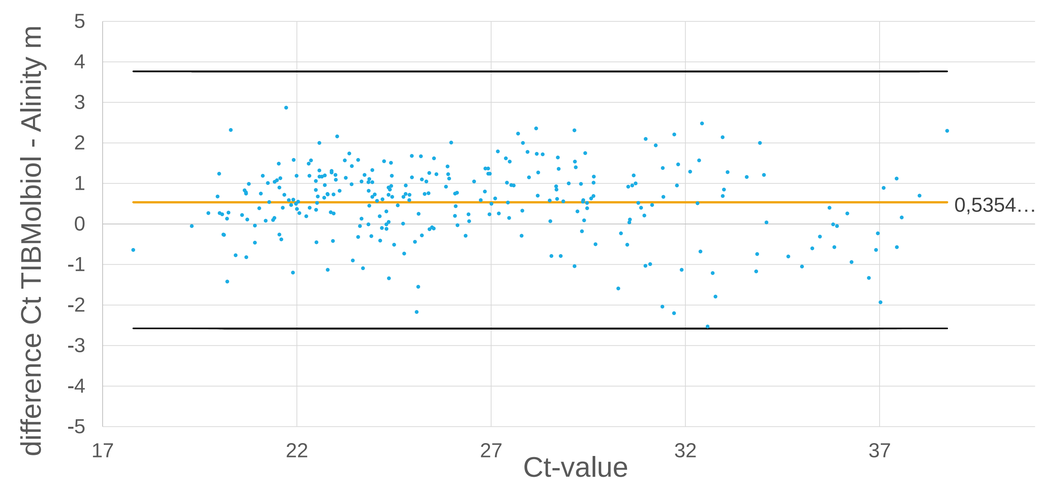

Fig. 4: Bland-Altman plot MPXV Ct-values

CONCLUSIONS

In our comparative analysis, the Alinity m MPXV RUO assay showed very high specificity and sensitivity with a lower detection limit of 293 copies/mL at a 95% hit rate. The correlation between the Alinity assay and the LDT was very high, with clearly lower CVs in repeated measurements and higher sensitivity for the Alinity m MPXV RUO assay. Continuous random access and stat capabilities of the Alinity m system allowed for improving turn-around-time of results in comparison to the batch-based LDT.

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Medical Center for Infectious Diseases, Berlin