Evaluation of the new AltoStar® HHV-7/-8 PCR Kit 1.5 RUO for the quantitative detection of HHV-8 DNA in Plasma, whole blood and PBMCs

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BACKGROUND

In persons whose immune system is compromised, HHV-8 is the causative agent for Kaposi's sarcoma, multicentric cattleman's disease and primary effusion lymphoma. Most of the assays used to detect HHV-8 DNA are qualitative laboratory developed assay. We evaluated the research use only quantitative AltoStar® HHV-7/-8 PCR Kit 1.5 RUO (Altostar).

METHODS

34 Anonymized residual sample material from persons with known HHV-8 infection was either tested as whole blood sample, plasma or separated PBMCs and viral load was compared between those compartments. Dilution series of three different cell lines infected with HHV-8 were generated and quantity of HHV-8 DNA in these samples was determined by digital PCR before testing with the Altostar. Limit of detection was calculated using probit 95% hit rate.

RESULTS

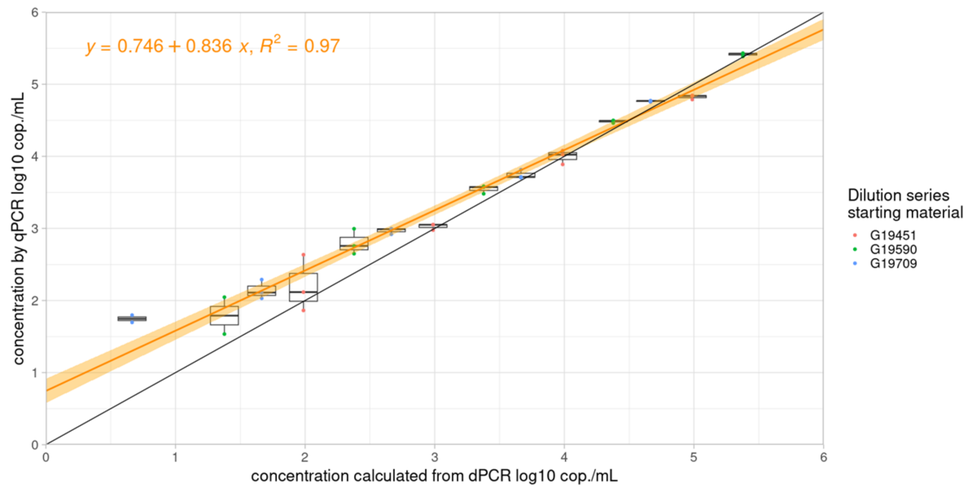

Figure 1: Comparison of dilutions from cell culture material

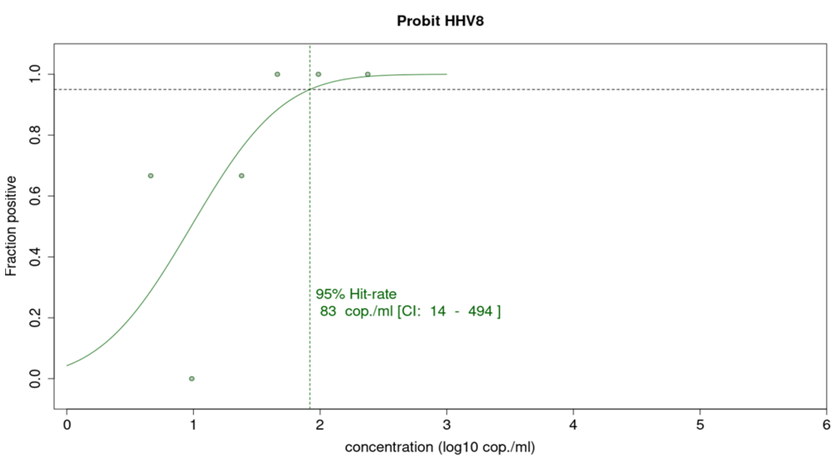

Figure 2: Orientation calculation of sensitivity

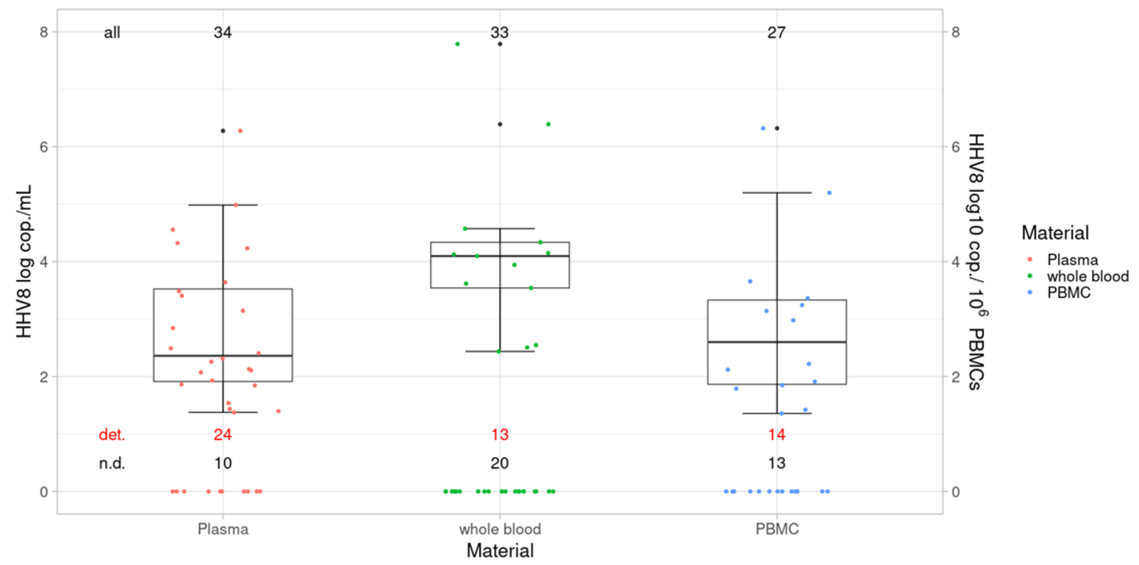

Figure 3: Distribution of HHV-8 DNA in different compartments

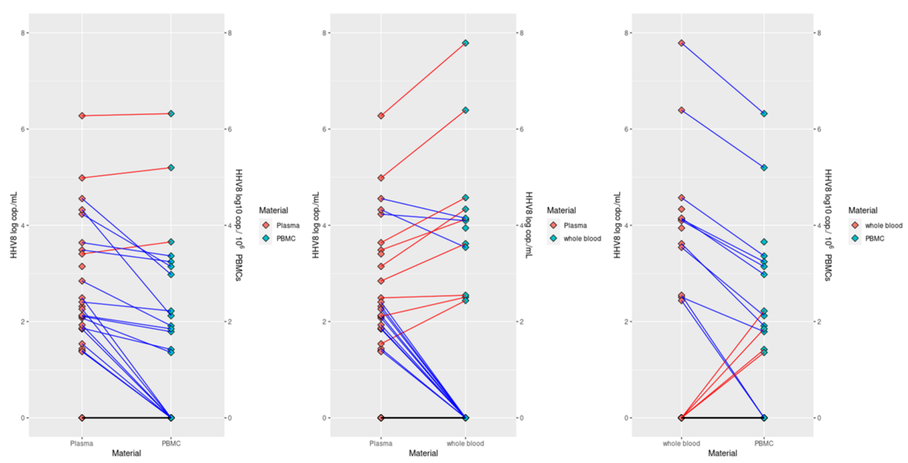

Figure 4: Relation between different compartments

RESULTS

Limit of detection of the assay was calculated at 83 cop./ml (95% probit hit rate). The assay showed good linearity between 100 and 1.000.000 cop./ml. In 24 (70%) of the plasma samples HHV-8 DNA could be detected between 83 copies/ml and 1.900.000 cop./ml. Detection rates were lower in whole blood (40%) and PBMCs (51%). If HHV-8 DNA could be detected in whole blood, the viral load was in 75% of the cases higher than in plasma.

CONCLUSIONS

The new Altostar assay is a substantial addition to the repertoire of HHV-8 testing and allows with a high flexibility to detect HHV-8 DNA in different blood compartments. Although the limit of detection in whole blood is decreased due to the lower possible input volume in the used nucleic acid extraction system, additional information can be gained, especially from the perspective of clonal expansion of infected cells due to lymphoproliferation by testing this compartment.

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