Evaluation of the analytical performances of the HHV6 R-GENE® assay (ARGENE®, bioMérieux)

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INTRODUCTION

Human herpesvirus 6 (HHV6) causes infection in early childhood (roseola) with lifelong latency. Reactivations are most of the time asymptomatic in immunocompetent people. However, the HHV6 infection can induce serious diseases in immunocompromised patients: encephalitis, bone marrow suppression, colitis and pneumopathy. The severity of HHV6 infection-induced pathologies is primarily determined by the patient's immune status. At-risk patients are mainly: hematopoietic stem cell transplantation (HSCT) patients, solid organ transplant (SOT) recipients and AIDS patients.

The new **HHV6 R-GENE® kit* (ref. 69-006B, ARGENE®, bioMérieux)** enables to detect and quantify HHV6 (HHV-6A and HHV-6B) viral load in duplex amplification with an internal control (generic to the whole ARGENE® transplant range) in whole blood, plasma, CSF and BAL by real-time PCR after extraction of viral DNA. Results obtained are expressed as number of copies/mL of sample. Viral load measurement and monitoring allow clinicians to initiate or adjust antiviral or immunosuppressive treatments.

As part of requirements for CE IVD marking, analytical performances were determined on this new HHV6 R-GENE® assay*:
- Determination of **analytical sensitivity (LoD)**,
- Determination of **linearity range**,
- Testing of different **commercialized panels** including HHV-6A and HHV-6B
  - 2016 past-panel from QCMD,
  - HHV-6A and HHV-6B verification panels from Exact Diagnostics,
  - HHV-6A analytical panel samples from Qnostics.

MATERIAL AND METHODS

Analytical performances of the **HHV6 R-GENE® kit*** were determined with EMAG® or NUCLISENS® easyMAG® and ABI 7500 Fast or ABI 7500 Fast Dx instruments.

Determination of analytical sensitivity (LoD)

The analytical sensitivity of the HHV6 R-GENE® kit* was determined using a range of dilutions of HHV-6B. The serial dilutions were performed in whole blood, plasma, CSF and BAL matrices, previously characterized negative for HHV-6. Each dilution was extracted 20 times.

Determination of linearity range

Series of dilutions of highly-positive HHV-6A and HHV-6B were performed for whole blood, plasma, CSF and BAL matrices with 3 replicates per concentration. An analysis was performed on calculated concentrations versus theoretical concentrations.

Testing of different commercialized panels

The **2016 past panel from QCMD** consists of 10 vials containing frozen plasma samples with various concentrations of HHV-6A and HHV-6B. Only the positive core samples were tested which corresponds to 7 samples. An analysis was performed on calculated concentrations versus theoretical concentrations.

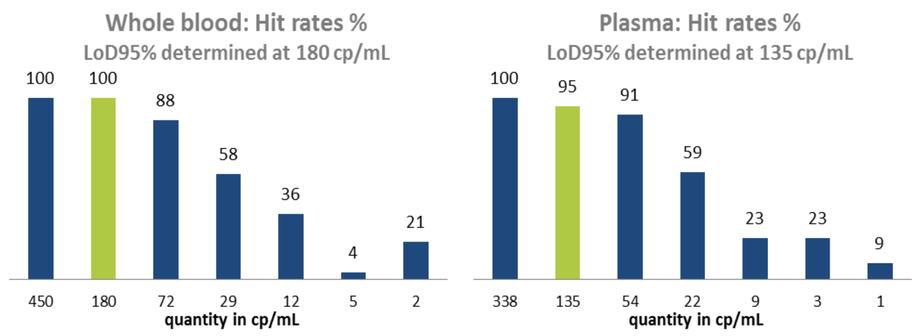
HHV-6A verification panel and HHV-6B verification panel from Exact Diagnostics consist of 5 vials containing frozen plasma samples with various concentrations of HHV6 (ranging from 500 cp/mL to 5 000 000 cp/mL). For each panel, an analysis was performed on calculated concentrations versus theoretical concentrations.

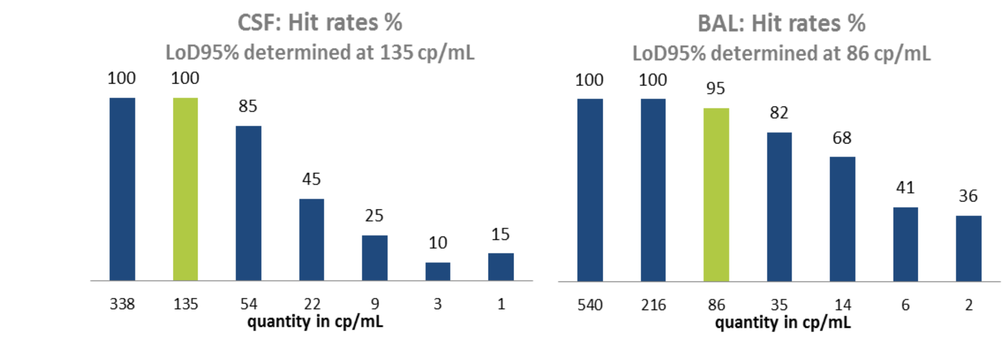
HHV-6A analytical panel from Qnostics was tested with HHV6 R-GENE® kit* and with RealStar® HHV-6 PCR Kit 1.0 (altona Diagnostics). This Qnostics panel consists of 1 negative and 9 positive samples with different concentrations of HHV-6A into human plasma. An analysis was performed on calculated concentrations with **HHV6 R-GENE® kit* (ARGENE®) versus RealStar® HHV-6 PCR Kit (altona Diagnostics)**.

RESULTS

Determination of analytical sensitivity (LoD)

The LoD was the lowest concentration giving at least 95% hit rate. The following graphs show the different hit rates obtained for each matrix: whole blood, plasma, CSF and BAL.

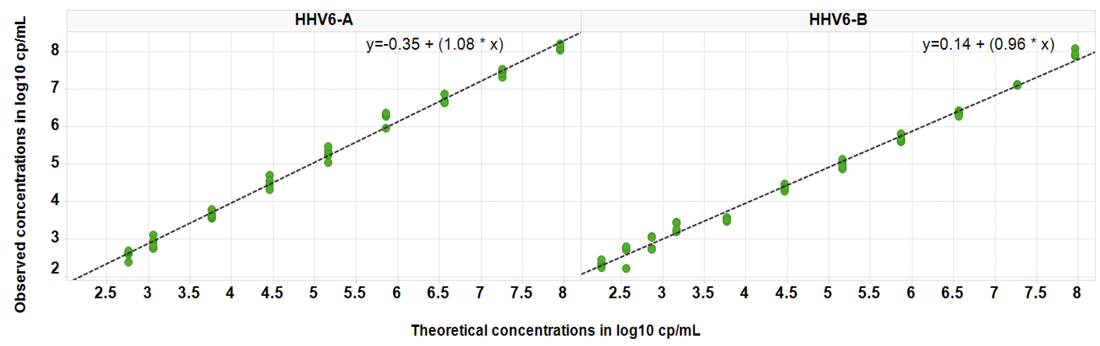




As a consensus value, the claimed limit of detection (LoD 95%) for the HHV6 R-GENE® kit* is **200 copies/mL (2.3 log_{10} copies/mL)** for whole blood, plasma, CSF and BAL.

Determination of linearity range

The following figures present only the results for whole blood.



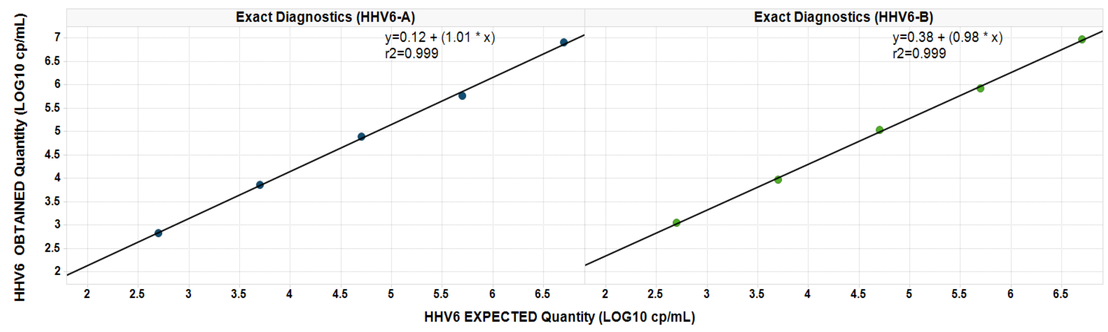
Linearity ranges are:
- For whole blood: 180 → 9.2E+07 cp/mL (2.3 → 8.0 log_{10} cp/mL)
- For plasma: 360 → 9.0E+07 cp/mL (2.6 → 8.0 log_{10} cp/mL)
- For CSF: 420 → 2.1E+08 cp/mL (2.6 → 8.3 log_{10} cp/mL)
- For BAL: 200 → 1.3E+08 cp/mL (2.3 → 8.1 log_{10} cp/mL)

Combining those data to precision and accuracy results (data not shown), the claimed range of quantification for the HHV6 R-GENE® test is from **500 to 9.0E+07 copies/mL (2.7 to 8.0 log_{10} copies/mL)** in whole blood, plasma, CSF and BAL.

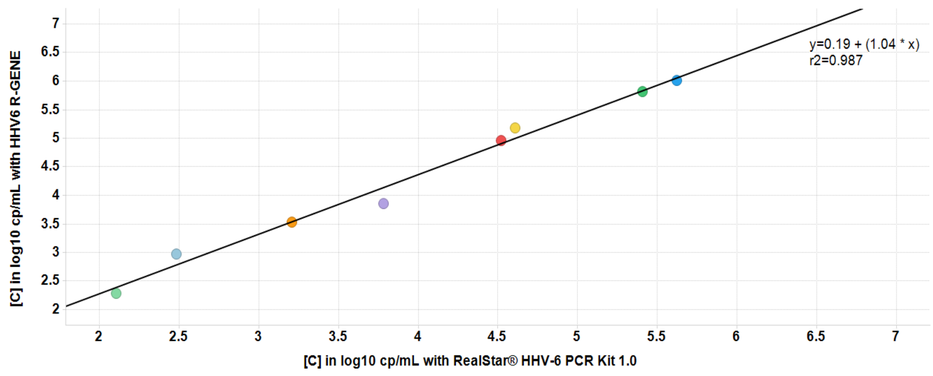
Testing of different commercialized panels

The 2016 past panel from QCMD was assessed and **compared to the expected values**. A maximum absolute difference of 0.2 log_{10} cp/mL versus the expected values was observed.

The **verification panels from Exact Diagnostics** were also assessed and compared to the expected values. The following graphs show a good correlation between the values obtained with HHV6 R-GENE® and the expected values.



The HHV-6A analytical panel from Qnostics:
A comparison was done between **HHV6 R-GENE® kit*** and **RealStar® HHV-6 PCR Kit 1.0.** Only the samples in the range 500 to 9.0E+07 copies/mL are shown on the following graph (8 from the 9 positive samples). There is a good correlation between the HHV6 R-GENE® assay* and the RealStar® HHV-6 PCR Kit.



CONCLUSION

In the 4 matrices validated (whole blood, plasma, CSF and BAL), the LoD is claimed at 200 cp/mL and the quantification range is demonstrated from 2.7 to 8.0 log_{10} copies/mL.
The results with commercialized panels (QCMD, Exact Diagnostics and Qnostics) show a good correlation with the expected values and comparable results between the HHV6 R-GENE® kit* and the RealStar® HHV-6 PCR Kit 1.0.

Overall, these results show the good analytical performances of the new HHV6 R-GENE® assay* on HHV-6A and HHV-6B.

* Product under development intended to be CE marked in November 2018