# Evaluation of the new HSV1&2 VZV R-GENE® and the CELL control R-GENE® kits for the quantification of herpes simplex virus 1 genome in broncho-alveolar lavage from patients with bronchopneumonitis

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## INTRODUCTION

In immunocompetent patients undergoing prolonged mechanical ventilation, herpes simplex virus 1 (HSV-1) may reactivate in the oropharynx and contaminate gradually the lower respiratory tract, leading to bronchopneumonitis (BPn). We previously showed that HSV-1 load in bronchoalveolar lavage (BAL) above 80.000 copies/million of cells was predictive of the onset of BPn in patients from intensive care unit [1]. The objective of this study was to evaluate the new HSV1&2 VZV R-GENE® kit (reference 69-014B under development) and the CELL control R-GENE® kit (ARGENE®, BIOMERIEUX) in comparison to the routine real-time PCR laboratory-developed tests (LDTs) implemented in the National Reference Center for Herpesviruses for the quantification of HSV-1 genome and cells in BALs.

## PATIENTS AND METHODS

Fifty sequential BALs from 18 different patients were analyzed.

Nucleic acid extraction was performed using EMAG® (BIOMERIEUX), assay set-up using ESTREAM® (BIOMERIEUX), and DNA amplification using LightCycler®480 (ROCHE DIAGNOSTICS).

All assays for both HSV-1 genome and cell DNA quantification were performed on the same day with the same nucleic acid eluate, previously stored at -80° C.

Commercial assays were performed according to the manufacturer's recommendations, and the LDTs were performed as previously published [2,3]. Methods were compared using MedCalc® and Validation Manager™ softwares.

## RESULTS

No PCR inhibition was observed. The analysis of the 50 BALs with R-GENE® kits and LDTs led to a concordance of 100% for the quantification of cells (results expressed in cells/mL) and 94% for the quantification of HSV-1 genome (results expressed in number of copies/mL) (Table 1). The 3 discrepant results corresponded to low HSV-1 loads, below 2.5 log copies/mL. The comparison of the 33 HSV-1-positive BALs showed a good correlation between HSV-1 loads (expressed in copies/million of cells) measured by both techniques (Spearman's coefficient of rank correlation = 0.97; p<0.0001) with an average bias of -0.75 log copies/million of cells (Bland-Altman test) (Table 1 and Figure 1). Kinetics of HSV-1 loads in sequential BALs were similar with both techniques, as exemplified for one patient in Figure 2.

**Table 1. Comparison of HSV1&2 VZV R-GENE® and CELL control R-GENE® kits with LDTs for the quantification of HSV-1 genome in BALs.**

| Parameter | Kit[1] | Concordance | Correlation | |
|---|---|---|---|---|
| | | | Spearman test (Rho; p-value) | Bland-Altman test (average bias) |
| Cell quantification[2] | CELL control R-GENE® | 100% | Rho=0.96 p<0.0001 | +0.37 log |
| HSV-1 genome quantification[3] | HSV1&2 VZV R-GENE® | 94% | Rho=0.99 p<0.0001 | -0.37 log |
| HSV-1 genome quantification[4] | HSV1&2 VZV + CELL control R-GENE® | 94% | Rho=0.97 p<0.0001 | -0.75 log |

[1]Results obtained with R-GENE® kits were compared to LDTs.
Results were expressed in [2]cells/mL, [3]HSV-1 copies/mL, [4]HSV-1 copies/million of cells

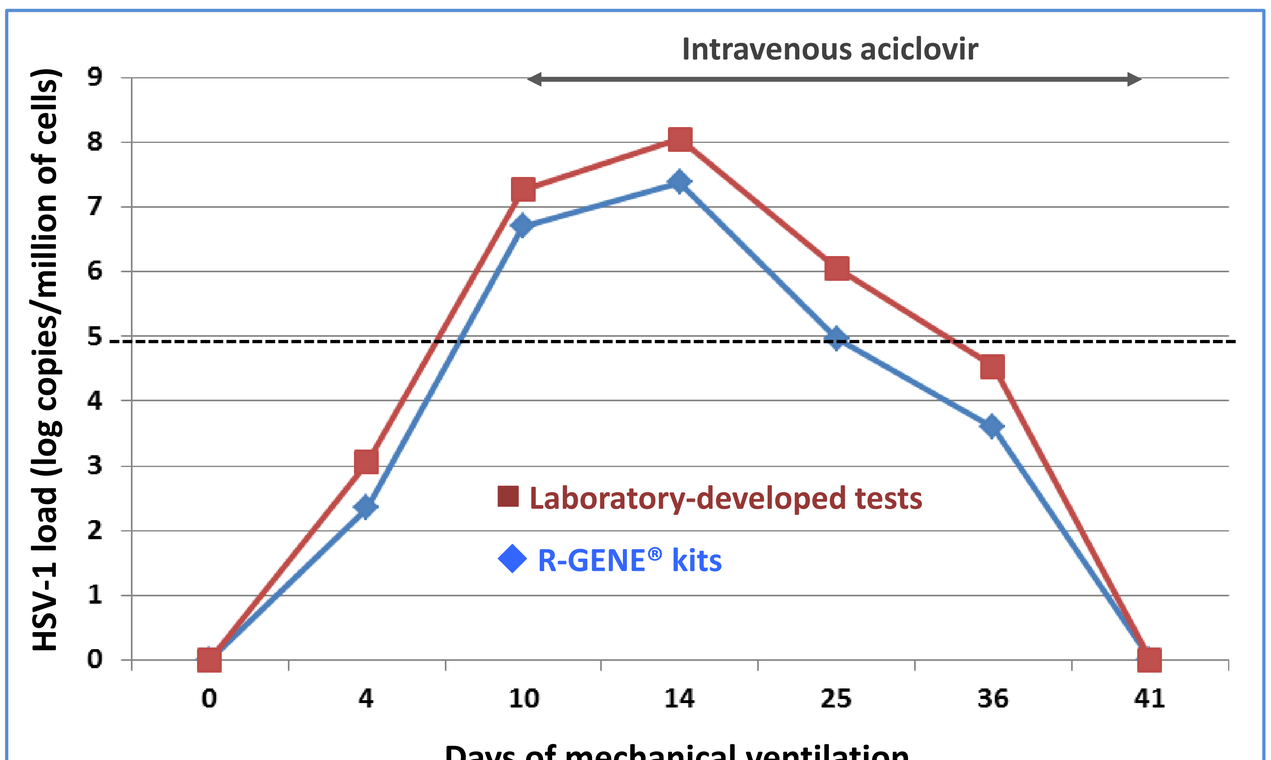


**Figure 2. Monitoring of HSV-1 load in BAL.** HSV-1 load in sequential BALs from a 57-year-old man undergoing prolonged mechanical ventilation was measured by both methods. HSV-1 bronchopneumonitis (BPn) was treated successfully with intravenous aciclovir. The predictive threshold of HSV-1 BPn previously reported [1] is indicated (black dotted line).

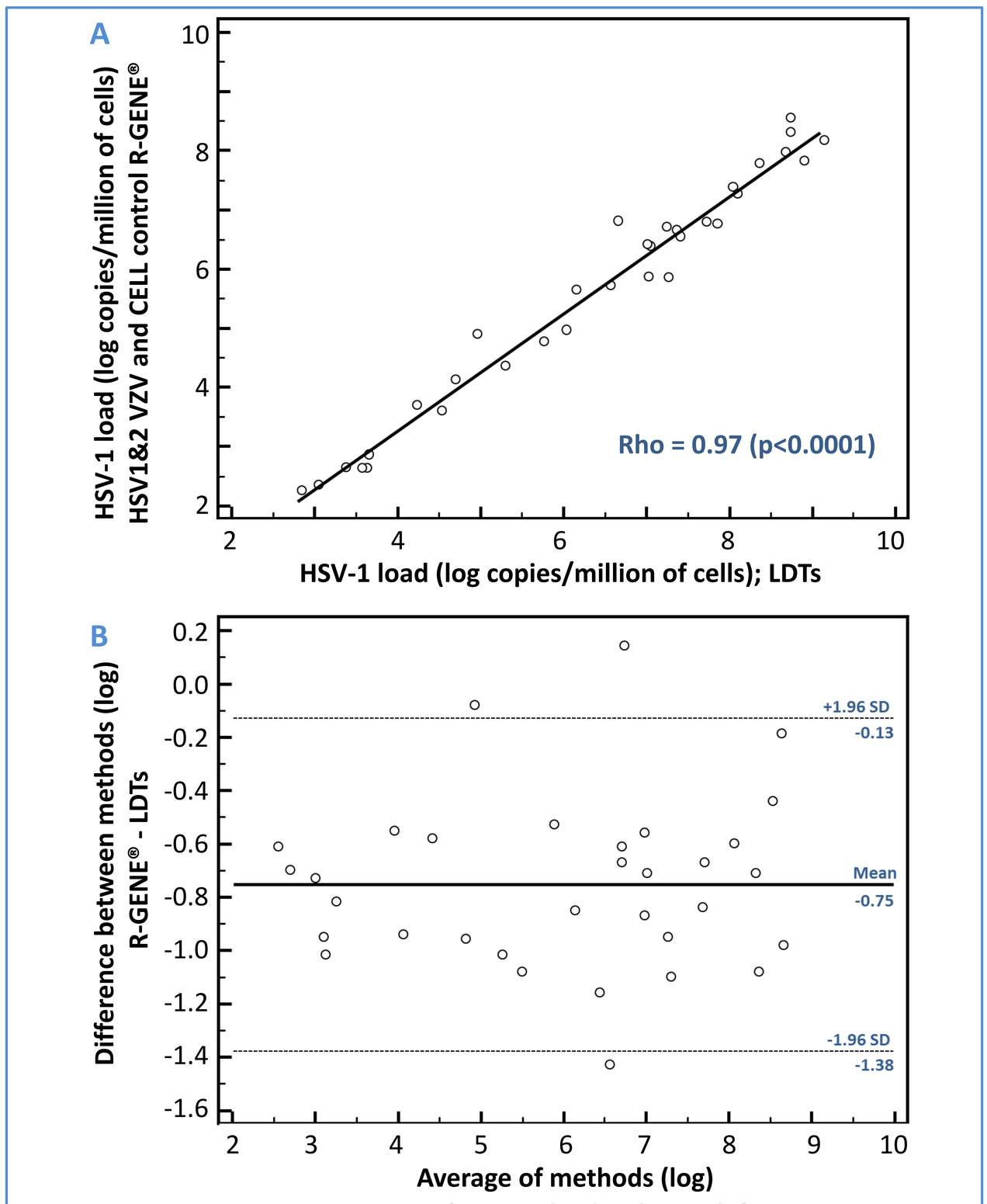


**Figure 1. Quantitative comparison of HSV-1 loads obtained from 33 LBAs using HSV1&2 VZV and CELL control R-GENE® kits and LDTs.** Spearman rank correlation test (A) and Bland-Altman test (B) were performed using Medcalc® software. SD: standard deviation.

## CONCLUSION

HSV1&2 VZV R-GENE® and CELL control R-GENE® kits allow the accurate quantification of HSV-1 load in BALs in a routine laboratory setting for the diagnosis and the monitoring of antiviral treatment of HSV-1 BPn among patients undergoing prolonged mechanical ventilation.

## REFERENCES

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