Comparative evaluation of the new HSV1&2 VZV R-GENE® kit and a real-time PCR laboratory-developed test for the detection and quantification of varicella-zoster virus (VZV) genome in clinical samples

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INTRODUCTION

Varicella-zoster virus (VZV) is a common pathogen responsible for mucocutaneous, neurological, ocular and disseminated infections. Rapid and accurate laboratory diagnosis of VZV infections in a large variety of specimens is essential for optimal clinical and therapeutic management. The objective of this study was to evaluate the new HSV1&2 VZV R-GENE® kit (reference 69-014B under development, ARGENE®, BIOMERIEUX) in comparison to the routine real-time PCR laboratory-developed test (LDT) implemented in the National Reference Center for Herpesviruses for the detection and quantification of VZV genome.

Table 1. Samples tested for the comparison of HSV1&2 VZV R-GENE® kit and LDT for VZV genome detection and quantification.

Sample	VZV negative (n)	VZV positive (n)
Whole bloods	1	15
Mucocutaneous swabs	2	22
CSFs from patients	2	8
Spiked CSFs	0	10
QCMD VZV DNA EQA Programme samples	1	9
Total	**6**	**64**

CSF: cerebrospinal fluid; EQA: external quality assessment.

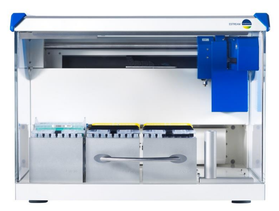
PATIENTS AND METHODS

A total of 70 VZV positive and negative samples were included **(Table 1)**: whole bloods (16), mucocutaneous swabs (24), cerebrospinal fluids (CSFs) from patients (10), VZV-negative CSFs spiked with different concentrations of VZV ATCC strains Webster and Ellen (10), samples from QCMD 2018 VZV DNA EQA Programme (10). Nucleic acid extraction was performed using EMAG® (BIOMERIEUX), assay set-up using ESTREAM® (BIOMERIEUX), and DNA amplification using LightCycler®480 (ROCHE DIAGNOSTICS). Both assays were performed on the same day with the same nucleic acid eluate, previously stored at -80° C. R-GENE® assay was performed according to the manufacturer's recommendations, and LDT was performed as previously published **[1]**. Methods were compared using MedCalc® and Validation Manager™ softwares.

EMAG® (BIOMERIEUX)

Nucleic acid extraction (200 µL of sample -> 50 µL of eluate)

ESTREAM® (BIOMERIEUX)



Assay set-up

LightCycler®480 (ROCHE DIAGNOSTICS)

DNA amplification

Figure 1. PCR workflow used for the comparison of HSV1&2 VZV R-GENE® kit and LDT for VZV genome detection and quantification.

RESULTS

No PCR inhibition was observed. The concordance between HSV1&2 VZV R-GENE® kit and LDT was 100%. The comparison of the 64 positive samples showed an excellent correlation between the VZV loads measured by both techniques (Spearman's coefficient of rank correlation = 0.98; p<0.0001) with an average bias of -0.28 log copies/mL (Bland-Altman test) **(Figure 2)**. Mucocutaneous swabs were included in the quantitative comparison despite only qualitative results are claimed by the manufacturer for this type of clinical sample. Of note, the average bias obtained with the 8 VZV positive CSFs from 5 distinct patients was higher (-0.79) than the average bias obtained with all other types of tested samples, including spiked CSFs (average bias ranging from -0.10 to -0.30). Further investigations are under progress to explain this phenomenon.

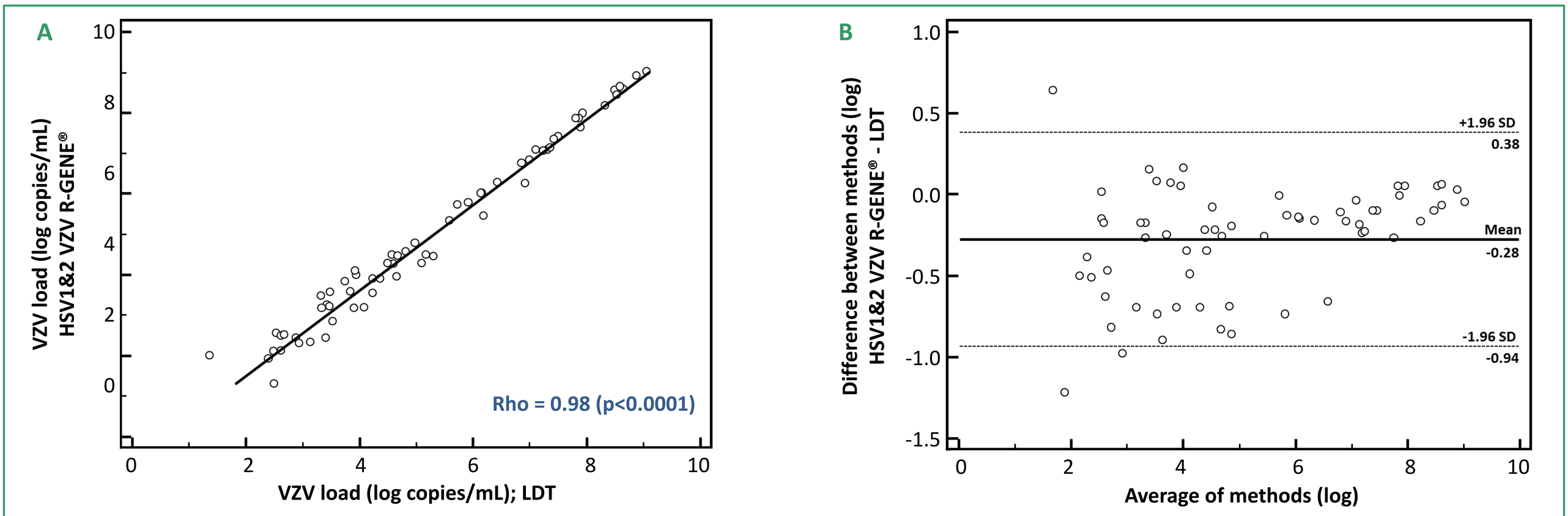


Figure 2. Quantitative comparison of VZV loads obtained from 64 samples using HSV1&2 VZV R-GENE® kit and laboratory-developed test (LDT).
Spearman rank correlation test (A) and Bland-Altman test (B) were performed using Medcalc® software. SD: standard deviation.

CONCLUSION

HSV1&2 VZV R-GENE® kit constitutes a suitable method for the detection and quantification of VZV genome in clinical samples in a routine laboratory setting.

REFERENCES

1- Burrel S, Fovet C, Brunet C, Ovaguimian L, Conan F, Kalkias L, Agut H, Boutolleau D. **Routine use of duplex real-time PCR assays including a commercial internal control for molecular diagnosis of opportunistic DNA virus infections**. J Virol Methods 2012; 185: 136-141.