Altona Diagnostics RealStar™ *Varicella Zoster Virus* Real Time Quantitative and In House Qualitative Polymerase Chain Reaction Assays Comparative Analysis

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BACKGROUND

- *Varicella Zoster Virus* (VZV) can be responsible for cerebral, cutaneous and congenital infections.
- Since effective antiviral treatment and prophylaxis are available, rapid and accurate laboratory diagnosis is mandatory.
- No commercial VZV polymerase chain reaction (PCR) assay has yet been approved by the FDA.

OBJECTIVES

- Compare Altona Diagnostics RealStar™ VZV Kit 1.0 commercial real time quantitative PCR assay with in house conventional qualitative PCR assay.

METHODS

- This study was performed on clinical specimens submitted for routine VZV PCR testing in our clinical diagnostic microbiology laboratory at Centre Hospitalier Sainte-Justine, a tertiary care pediatric center.
- 145 specimens including cerebrospinal fluid (CSF) (43%), cutaneous (43%) and other specimen types (14%) such as serum, ocular and respiratory secretions were included.
- Paramagnetic particles based DNA extraction was performed using Promega™ extraction kit on a Maxwell™ instrument.
- In house qualitative PCR assay targeting the UL21 gene and producing a 647-bp amplicons for detection by agarose electrophoresis was performed on the ABI 9700 thermal cycler.
- Altona Diagnostics RealStar™ VZV Kit 1.0 commercial real time quantitative PCR assay was performed according to manufacturer's recommendations on the ABI 7500 thermal cycler.
- Specimens were selected on the basis of their result on the in house assay. To account for this potential selection bias, a variety of positive and negative specimens for each specimen types were included.

RESULTS

Table 1. VZV PCR assays results

Specimen	In house + RealStar™ +	In house + RealStar™ -	In house - RealStar™ +	In house - RealStar™ -
CSF	24	0	1	37
Cutaneous	24	0	0	37
Other	14	1	0	7
Total	62	1	1	81

- One corneal scrapping specimen was only positive on the in house PCR assay. This specimen produced a weak signal at a Ct of 35.26 on RealStar™ PCR.
- One CSF specimen was only positive on RealStar™ PCR at a Ct of 30 which corresponded to a quantification of 392 copies/mL.

- Positive specimens on RealStar™ PCR were so at an average Ct of 23.98 (SD 6.18) and quantitative viral loads varied from $1.85x10^2$ and $6.67x10^7$ copies/mL.
- Average quantitative viral loads in CSF and cutaneous specimens were respectively $4.4x10^5$ and $1.1x10^7$ copies/mL, a difference found to be statistically significant (p=0.004).

Table 2. VZV PCR assays concordance analysis

Total concordance % (95% CI)	Positive concordance % (95% CI)	Negative concordance % (95% CI)	Kappa statistic value k (95% CI)
98.6 (94.8–99.9)	98.4 (90.7–99.9)	98.8 (92.8–99.9)	0.97 (0.93–1.00)

- Total, positive and negative concordance percentages between both assays were respectively 98.6% (95% CI 94.8–99.9), 98.4% (95% CI 90.7–99.9) and 98.8% (95% CI 92.8–99.9).
- Kappa statistic value was 0.97 (95% CI 0.93–1.00) indicating excellent agreement beyond chance.

Figure 1. Conventional in house VZV PCR

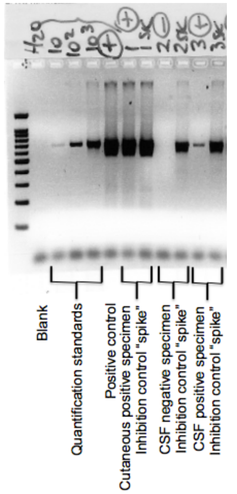
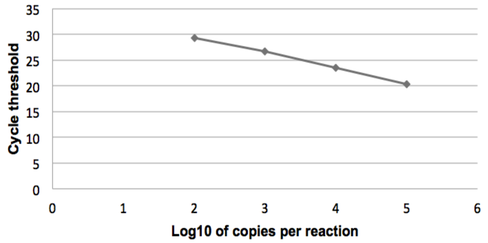


Figure 2. RealStar™ PCR standard curve



RealStar™ VZV Kit 1.0 quantification standards analysis produced standard curves with the following average parameters;

- Slope -3.15
- R^2 0.999
- Crossing point of 36.03
- Efficiency of 107.94.

DISCUSSION

- Performance characteristics such as sensitivity and specificity could not be evaluated since PCR assays are more sensitive than viral culture based reference methods and since no clinical correlations were obtained.

CONCLUSIONS

- RealStar™ VZV Kit 1.0 real time quantitative PCR found quantitative viral loads to be significantly higher in cutaneous than in CSF positive specimens.
- RealStar™ VZV Kit 1.0 real time quantitative and in house conventional qualitative VZV PCR assays have excellent concordance in CSF, cutaneous and other specimen types.

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