Detection of HSV-1/2 and VZV Using Real-Time PCR and the altona RealStar® *alpha* Herpesvirus Assay

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ABSTRACT (Revised)

Background: Herpes Simplex Virus (HSV) and Varicella-Zoster Virus (VZV) can infect both skin and nerves and cause severe infections, including meningitis and encephalitis, particularly in the immunocompromised hosts. Laboratory testing is required to definitively determine the causative agent. The aim of this study was to evaluate the analytic performance characteristics of altona RealStar *alpha* Herpesvirus PCR Kit for the simultaneous detection of HSV-1/2 and VZV.

Method: A total of 136 clinical specimens were analyzed with altona *alpha* Herpesvirus kit using the 7500 Real-Time PCR System. Accuracy, precision, analytical sensitivity and specificity, linearity and stability of the assay were determined.

Results: For accuracy, 136 clinical specimens were tested using altona assay and results were compared to our lab-developed HSV assay and the Focus Diagnostics VZV ASR assay. The overall agreement was 100% for all targets. Intra- and inter-assay precisions were performed using high, medium, low positive and negative sample pools derived from HSV-1/2 and VZV positive controls. The samples were extracted, and tested in triplicate over three runs. The mean, standard deviation (SD) and coefficient of variation (% CV) of the Cycle Threshold (Ct) for each target were calculated. The CV and SD for intra- and inter-assay precision studies were less than 3% and 0.7, respectively. Linearity was determined by extracting and testing the 10-fold serial dilutions of the HSV-1/2 and VZV positive control ($2x10^6$ copies/mL) in triplicate. For analytical sensitivity, the 2-fold serial dilutions of the positive control were assayed 8 times. The limit of detection (LOD) were 100 copies/mL for HSV-1 and HSV-2 and 200 copies/mL for VZV. No cross-reactivity was detected with 10 other viruses. The detection of internal control in all PCR reactions confirmed the absence of interfering substances. Stability studies were performed by testing positive and negative patient samples stored at -80ºC over 7-day period and the samples were found to be stable.

Conclusion: altona RealStar *alpha* Herpesvirus PCR is an accurate and sensitive assay for clinical use.

INTRODUCTION

- HSV-1 and HSV-2 cause a variety of clinical symptoms in the central nervous system (CNS) and in immunocompromised patients. VZV can also cause severe systemic infections of the CNS and in immunocompromised patients. Rapid diagnosis is needed to distinguish HSV from VZV infections.
- Currently HSV and VZV tests are performed on two different platforms: a lab-developed test (LDT) for HSV 1/2 on ABI 7500 and a VZV ASR assay on 3M Integrated Cycler. The current HSV assay cannot differentiate between HSV-1 and HSV-2.
- The altona *alpha* Herpesvirus PCR Kit is a Research Use Only (RUO) kit that is based on multiplex real-time PCR, for the detection and differentiation of HSV-1, HSV-2, and VZV specific DNA.

Goal of this study:

- Evaluate the analytic performance characteristics of altona RealStar *alpha* Herpesvirus PCR Kit for the simultaneous detection of HSV-1/2 and VZV.

METHODS

- 136 clinical specimens including respiratory samples, blood, CSF, Fluids, swabs and tissues were tested with altona kit. Results were compared to the HSV LDT and Focus VZV ASR PCR assay.
- HSV-1/2 differentiation were compared to the results from Pathology Inc. using the altona assay on Rotor-Gene platform.
- Precision study was performed with high, medium, low positive and negative sample pools. Triplicates of each were assayed in a single run for intra-assay precision. Triplicates of each were assayed in 3 separate runs for inter-assay precision. The mean, SD and %CV of the Ct for each target were calculated.
- For linearity and limit of detection (LOD), 10-fold serial dilutions from $2x10^6$ to 20 copies/mL of the targets were run in triplicate to determine the linearity. 2-fold serial dilutions from 400 to 100 copies/mL of the targets were run in 8 replicates to determine the LOD (≥95% detection rate was used as LOD cutoff).
- Specificity was performed by testing 10 related and unrelated organisms with altona kit.
- altona internal control was included in all the PCR reactions to detect interfering substances.
- Stability at -80ºC was determined by testing the high, medium, and low positive samples in triplicate over 7-day period.

RESULTS

- Of the 136 clinical specimens, 53 were positive for HSV and 16 were positive for VZV.
- Sensitivity, specificity and overall concordance were 100% for all targets (Table 1).
- The detection of internal control in all the PCR reactions confirmed that no interfering substances were found in the tested sample types.

Table 1A. Accuracy Study - altona vs Reference

		Reference Method		
altona	HSV	Positive	Negative	Sensitivity = 100%
	Positive	53	0	Specificity = 100%
	Negative	0	32	Concordance = 100%
	VZV	Positive	Negative	Sensitivity = 100%
	Positive	16	0	Specificity = 100%
	Negative	0	35	Concordance = 100%

Table 1B. HSV-1/2 Typing

		Pathology Inc.		
CHLA		HSV-1	HSV-2	altona HSV Typing
	HSV-1	42	0	HSV-1 Concordance = 100%
	HSV-2	0	6	HSV-2 Concordance = 100%

RESULTS

- For intra-assay precision, the SD was ≤0.4 and the CV was ≤1.4%. For inter-assay precision, the SD was ≤0.7 and the CV was ≤2.6% (Table 2).

Table 2A. Intra-Assay Precision

Specimen	HSV-1 Mean Ct±SD(CV)	HSV-2 Mean Ct±SD(CV)	VZV Mean Ct±SD(CV)
High Positive	17.9 ± 0.15 (0.84%)	19.0 ± 0.25 (1.32%)	19.5 ± 0.12 (0.60%)
Med Positive	24.6 ± 0.13 (0.53%)	24.0 ± 0.15 (0.6%)	25.9 ± 0.05 (0.21%)
Low Positive	29.8 ± 0.05 (0.16%)	31.5 ± 0.35 (1.10%)	32.6 ± 0.40 (1.23%)
Negative	0 (0%)	0 (0%)	0 (0%)

Table 2B. Inter-Assay Precision

Specimen	HSV-1 Mean Ct±SD(CV)	HSV-2 Mean Ct±SD(CV)	VZV Mean Ct±SD(CV)
High Positive	18.2 ± 0.32 (1.78%)	19.4 0.35 (1.80%)	19.8 0.38 (1.91%)
Med Positive	24.8 0.23 (0.93%)	24.5 0.62 (2.51%)	26.2 0.52 (2.00%)
Low Positive	30.0 0.26 (0.85%)	31.9 0.70 (2.18%)	33.0 ± 0.61 (1.86%)
Negative	0 (0%)	0 (0%)	0 (0%)

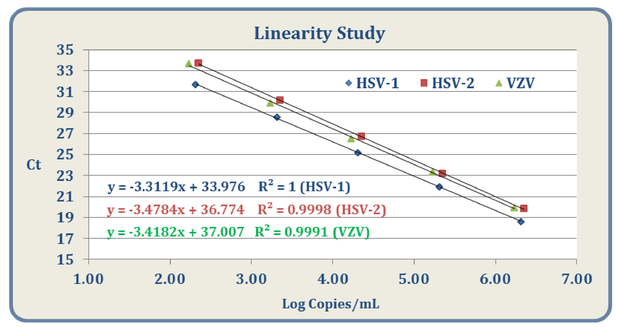
RESULTS

- The linear range for HSV-1/2 and VZV is from 20 to $2x10^6$ copies/mL (Figure 1).
- The LODs are 100 copies/mL for HSV-1 and HSV-2 and 200 copies/mL for VZV (Table 3).

Table 3. Limit of Detection

	Copies/mL	Ct Range	Mean	SD	CV	% Detection
HSV-1	400	31.00-31.56	31.27	0.22	0.70%	100%
	200	32.37-32.96	32.73	0.2	0.62%	100%
	100 (LOD)	31.95-33.72	32.97	0.49	1.49%	100%
HSV-2	400	34.19-43.54	36.42	3.12	8.57%	100%
	200	35.73-44.23	38.21	2.75	7.20%	100%
	100 (LOD)	34.51-40.10	37.19	1.86	5.00%	100%
VZV	400	35.18-37.19	36.18	0.82	2.28%	100%
	200 (LOD)	34.59-38.92	35.77	1.39	3.88%	100%
	100	36.24-Neg	37.51	1.06	2.82%	75%

Figure 1. Linearity



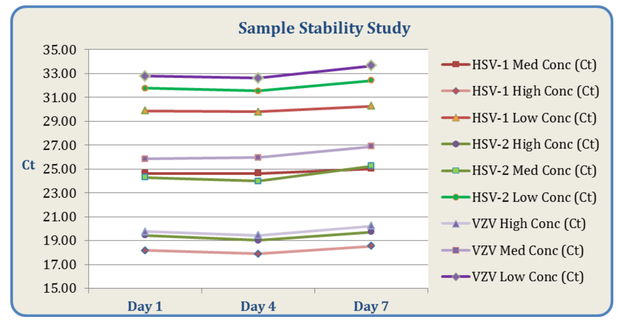
RESULTS

- For analytical specificity, no cross-reactivity was detected in clinical specimens positive for a variety of viruses (Table 4).
- Samples were found to be stable at -80ºC for at least 7 days (Figure 2).

Table 4. Cross-Reactivity

Pathogen	Specimen Type	altona HSV/VZV
Adenovirus	Blood	Not Detected
BKV	Blood	Not Detected
CMV	BAL	Not Detected
EBV	Blood	Not Detected
Enterovirus	CSF	Not Detected
HHV-6	Tissue	Not Detected
Parainfluenza 1	NP Swab	Not Detected
Parainfluenza 3	Tracheal Aspirate	Not Detected
Rhinovirus	NP Swab	Not Detected
RSV	NP Wash	Not Detected

Figure 2. Sample Stability



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CONCLUSION

- Compare to our current HSV and VZV assay, the altona multiplex PCR is simple, accurate, sensitive and can differentiate HSV-1 and HSV-2.
- Simultaneous detection of HSV-1/2 and VZV can reduce the reagent usage, labor time and improve the turn-around-time and patient care.