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## BACKGROUND & OBJECTIVES

Nucleic acid amplification testing has greatly facilitated the monitoring of transplant patients for infections due to CMV, EBV and HHV6 in blood specimens. Real-time PCR assays are replacing conventional PCRs as they are less prone to contamination and do not require gel electrophoresis for detection. Our screening method for detection of cytomegalovirus (CMV), Epstein-Barr virus (EBV) and human herpesvirus 6 were in-house conventional qualitative PCR assays followed by detection of amplicons by agarose gel electrophoresis and positive PCR products were further characterized by restriction digestion using *Bam*HI and *Bst*UI enzymes.

In-house PCR assays were CMVPC: specifically designed for CMV detection, HERPC: designed for CMV and EBV detection, VZVPC: designed for HHV6A, HHV6B, HHV7 and varicella-zoster virus (VZV) detection.

The *ReaStar*® HHV4/5/6 PCR kit 1.0 from Altona Diagnostics (Hamburg, Germany) is a multiplex qualitative method capable of simultaneous detection of CMV, EBV and HHV6 (A and B). The assay includes an internal control to monitor the presence of amplification inhibition.

The objectives of this study were to:

- 1) Assess the sensitivity of detection of the Altona *ReaStar*® HHV4/5/6 PCR kit 1.0 compared to in-house PCR assays
- 2) To determine the limit of detection (LoD) of the Altona *ReaStar*® HHV4/5/6 PCR kit 1.0 for each one of CMV, EBV and HHV6 targets and compare it to the in-house assays.

## METHODS

**Detection of CMV, EBV and HHV6 in Whole blood:** One hundred and thirty four blood samples from pediatric and adult patients were extracted using Chemagic DNA blood kits (Chemagen, Perkin Elmer)/KingFisher Flex (Thermo Scientific) and tested with three conventional PCR assays; 1. HERPC PCR detects CMV and EBV, 2. VZVPC PCR detects VZV and HHV6a/6b and 3. CMV PCR detects CMV (modified from Johnson et al., 2000).

**Conventional PCRs:** 10 µL of extracted nucleic acid was mixed with 40 µL of master mix containing the following primers:

**HERPC Master mix:**

Forward primer (26 mer): 5'-GTG GTG GAC TTT GCC AGC CTG TAC CC-3'

Reverse primer (30 mer): 5'-TAA ACA TGG AGT CCG TGT CGC CGT AGA TGA-3'

The amplification parameters were as follows:

95°C 10 min  
54°C 45 sec 1 cycle  
72°C 1 min  
95°C 45 sec  
54°C 45 sec 40 cycles  
72°C 1 min

**VZV Master Mix:**

Forward primer (29 mer): 5'-GTC GTC TTT GAT TTT CAA AGT TTA TAT CC-3'

Reverse primer (34 mer): 5'-ATA AAC ACA CAA TCC GTA TCA CCA TAA ATA ACC T-3'

The amplification parameters were as follows:

95° 10 min  
95°C 1 min  
47°C 1 min 40 cycles  
72°C 1 min  
72°C 3 min

PCR products were detected and visualized by ethidium bromide containing agarose gel and positive PCR products were further characterized by restriction digestion using *Bam*HI and *Bst*UI enzymes.

***ReaStar*® HHV4/5/6 1.0 PCR kit:** a real-time based multiplex assay for the simultaneous detection of CMV, EBV and HHV6 (Altona Diagnostics, Hamburg Germany) and an Internal Control provided with the assay.

10 µL of extracted nucleic acid was mixed with 20 µL of master mix (Master A + Master B) and the amplification was performed on a Rotor-Gene 6000 thermocycler (Qiagen Canada, Toronto, ON) according to the manufacturer's instructions.

## RESULTS

The sensitivity of the new multiplex assay was 100% for both EBV (41/41) and HHV6 (34/34) compared to the conventional PCR assays. The sensitivity of the new multiplex for CMV detection was 91.8% (45/49), however, 4 discordant samples could not be re-extracted due to inadequate specimen volume. For two of these specimens the initial quantitation of CMV in these specimens was <600 copies per mL. Specificity of the assay for CMV, EBV and HHV6 was 98.7% (74/75), 98.8% (82/83), and 89.0% (89/100), respectively (Table 1). Limit of detection of the *ReaStar*® HHV4/5/6 PCR for all three targets was <1,000 copies per mL blood (Table 2).

**Table 1.** Sensitivity and specificity of the Altona *ReaStar*® HHV4/5/6 PCR for CMV, EBV and HHV6

TARGET	SENSITIVITY	SPECIFICITY
CMV	91.8% (45/49)	98.7% (74/75)
EBV	100% (41/41)	98.8% (82/83)
HHV6	100% (34/34)	89.0% (89/100)

**Table 2.** LODs determined by testing viral concentrations at or near the detection limit in triplicate

Copies/mL	CMV		EBV		HHV6	
	HERPC	altona	HERPC	altona	VZVPC	altona
10,000	3/3	3/3	3/3	3/3	3/3	3/3
5,000	3/3	3/3	3/3	3/3	3/3	3/3
1,000	3/3	3/3	3/3	3/3	3/3	3/3
500	3/3	1/3	2/3	2/3	3/3	3/3

## DISCUSSION & CONCLUSIONS

The new *ReaStar*® HHV4/5/6 PCR kit is a multiplex assay capable of detecting CMV, EBV and HHV6 in blood specimens with high sensitivity and specificity. The performance of the commercial assay for detection of CMV, EBV and HHV6 in blood specimens was comparable if not better than the conventional in-house PCR assays and could markedly improve workflow and turnaround time. The assay could also be suitable for other specimens types including respiratory, tissue and sterile body fluids.

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