

# DETECTION OF MULTIPLE VIRUSES AND INVESTIGATION OF CO-INFECTION RATE USING THE VIRAQUANT™ MULTIPLEX ASSAY ON THE ICEPLEX™ ANALYZER



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## Revised Abstract

### Background

Solid organ and bone marrow transplant recipients often develop clinical complications due to post-transplant viral infections. Cytomegalovirus, Epstein Barr virus, Human Herpesvirus 6 and BK virus are some of the more frequently diagnosed infections. These viruses along with Human Herpesvirus 7 are quantified by the ViraQuant™ Multiplex Assay on the new ICEPlex™ analyzer (PrimerDx, Inc. Mansfield, Mass.). As a large transplant center Cleveland Clinic physicians frequently request tests for these viruses. Until recently all except CMV were sent to outside reference labs for testing. Currently, both CMV and EBV viral load testing are done in our lab but BKV, HHV6 and 7 are sent to reference labs. We investigated results of a new multiplex assay to determine the presence of viruses not tested for in our lab and to understand the co-infection rate of samples that have been tested by routine methods (CMV and EBV viral load assays by Qiagen/Digene, Gaithersburg, MD).

### Materials & Methods

Eighty-one whole blood samples, 36 for CMV and 45 for EBV viral load testing were used for the co-infection study. DNA was extracted from 265 ul of whole blood using the Qiagen MDx Robot as per manufacturer's protocol. A PCR plate containing 10 ul of extracted sample DNA and 40 ul of ViraQuant™ master mix was placed in the ICEPlex analyzer for amplification and detection. The ICEPlex analyzer utilizes STAR (Scalable Target Analysis Routine) technology for simultaneous quantitative measurement of multiple target nucleic acids. Because of the high level of multiplex enabled by STAR, multiple standards and calibrators are included in each sample along with primers for the intended targets.

### Results

The ViraQuant assay detected 20% (16/81) samples positive for viruses other than those requested and 7% (6/81) co-infections. Of the 81 samples submitted for CMV (1+ and 35- by our routine method) and EBV (14+ and 31- by the routine method) testing ViraQuant detected 1 CMV, 4 EBV, 1 BKV, 4 HHV-6 and 9 HHV-7.

### Conclusion

The finding of the detection of 20% viruses other than those requested and the 7% incidence of co-infection are significant. The ability to detect other viruses and determine co-infection could improve the management of transplant patients by indicating antiviral therapy and reduction in the degree of immunosuppression.

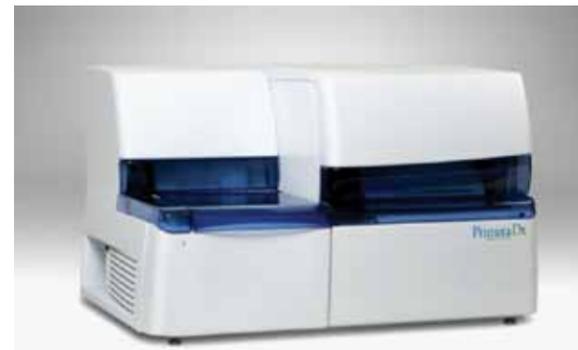
## Introduction

The beta herpesviruses, Cytomegalovirus, Epstein Barr virus, Human Herpesvirus 6 and 7 along with the BK polyoma virus can be frequently detected in solid organ and bone marrow transplant recipients, and other immunosuppressed patients. As a large transplant center Cleveland Clinic physicians frequently request tests for these viruses. Currently our lab determines CMV (1800/month) and EBV (700/month) viral load by 2 separate assays (*artus*® CMV™ and *artus*® EBV™ viral load assays by Qiagen, Gaithersburg, MD). Quantitative testing for BK (320/month), HHV-6 and 7 is sent to outside reference labs. The ability to multiplex testing for CMV and EBV along with bringing BK and HHV6 and 7 in-house would result in significant cost savings, reduction of tech time and decreased turn around time. We investigated a new multiplex assay to determine the presence of viruses not tested for in our lab and to understand the co-infection rate of samples that have been tested by routine methods.

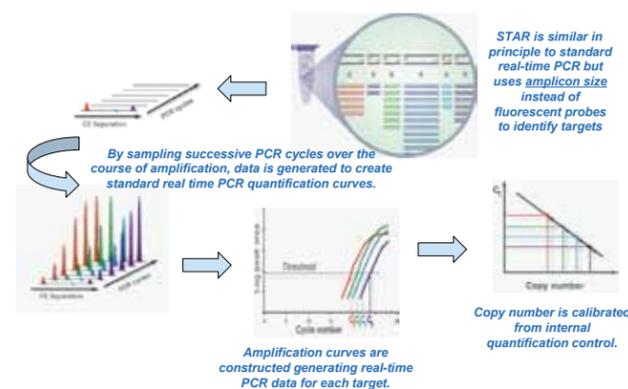
## Methods and Materials

- Eighty-one whole blood samples, 36 for CMV and 45 for EBV viral load testing were used.
- DNA was extracted from 265 ul of whole blood using the Qiagen MDx Robot as per manufacturer's protocol.
- 10 ul of extracted sample DNA and 40 ul of ViraQuant™ master mix was placed in the ICEPlex analyzer for amplification and detection.
- The ICEPlex analyzer utilizes STAR (Scalable Target Analysis Routine) technology for simultaneous quantitative measurement of multiple target nucleic acids.
- Multiple standards and calibrators are included in each sample along with primers for the intended targets

### ICEPlex™ System



### STAR Technology Description



## Results

Study #	CCF Results		Send Out Results			ViraQuant Results				
	CMV	EBV	BKV	HHV6	HHV7	CMV	EBV	BKV	HHV6	HHV7
IP018	-	-	-	-	-	-	-	-	-	-
IP045	579	-	-	-	-	-	-	-	-	-
IP024	7,942	-	-	-	-	19,499	1,240	-	-	-
IP025	-	-	-	-	-	-	-	-	2,573	-
IP027	-	4,732	-	-	-	-	2,699	-	-	-
IP031	-	-	-	-	-	-	-	-	3,069	-
IP037	-	-	-	-	-	-	1,023	-	-	-
IP039	-	571	-	-	-	-	620	-	-	-
IP040	-	-	28,500	-	-	-	-	53,692	-	-
IP041	-	1,063	-	-	-	-	341	-	-	-
IP043	-	-	-	-	-	-	19,933	-	-	-
IP046	-	-	-	-	-	-	-	-	-	1,116
IP048	-	-	-	-	-	-	372	-	-	-
IP054	-	579	-	-	-	-	800	248	-	248
IP055	-	16,711	-	-	-	-	7,894	-	-	403
IP058	-	40,704	-	-	-	-	29,194	-	-	-
IP059	-	2,980	-	-	-	-	3,348	-	-	-
IP061	-	633	-	-	-	-	403	-	-	-
IP062	-	544	-	-	-	-	-	-	-	-
IP063	-	1,145	-	-	-	-	699	-	-	-
IP073	-	-	-	-	-	-	-	-	-	-
IP074	-	-	-	-	-	2,125	-	-	-	682
IP076	-	645	-	-	-	-	341	-	-	-
IP077	-	1,529	-	-	-	-	521	-	-	403
IP079	-	-	-	-	-	-	-	-	-	403
IP081	-	527	-	-	-	-	124	-	-	-
IP084	-	-	-	-	-	-	-	-	-	372
IP085	-	9,982	-	-	-	-	2,220	-	-	-
IP086	-	-	-	-	-	-	369	-	-	-
IP089	-	-	-	-	-	-	1,596	-	-	-
IP090	-	1,537	-	-	-	-	615	-	-	615
IP091	-	162,800	-	-	-	-	46,500	-	589	372
IP093	-	43,928	-	-	-	-	9,137	-	1,921	-
48 negative samples	45	28	7	1	1	-	-	-	-	-

Presence of co-infection

## Conclusions

- The finding of the detection of 20% viruses other than those requested and the 7% incidence of co-infection are significant
- The ability to detect other viruses and determine co-infection could improve the management of transplant patients by indicating antiviral therapy and reduction in the degree of immunosuppression
- Ability to multiplex testing could result in cost saving and decreased turn around time
- Validation of the Viraquant assay compared to our routine method and studies to determine LOD and LOQ need to be done

\*ICEPlex and ViraQuant are for Research Use Only.  
Not for clinical diagnostic use.