Cleveland Clinic

Clinical Evaluation of a Novel Multiplex Viral Load Assay for Transplant Patients

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Revised Abstract

Background: The availability of potent immunosuppressive drugs has significantly reduced the incidence of acute organ rejection in transplant recipients. The increased usage of these immunosuppressive drugs also coincides with increased incidence of post-transplant complications due to opportunistic infections. Cytomegalovirus, Epstein Barr virus, BK virus are among some of the more frequently diagnosed post-transplant viral infections.We evaluated a newly developed ViraQuant™ Multiplex Assay (Primera Biosystems, Inc. Boston, Mass,) that can quantitatively measure five different viruses (CMV, EBV, BKV, HHV6 & HHV7), and compared to our current CMV assay (Digene Hybrid Capture® System) (Digene/Qiagen, Gaithersburg, MD).

Materials & Methods: One hundred and thirty four whole bloods received in the Clinical Virology Laboratory for CMV viral load test were used to evaluate the ViraQuant™ Multiplex (STAR Technology) Assay. DNA was extracted from 200 µl of whole blood using Corbett Xtractor and eluted into 75 µl as per manufacturer's protocol. 10µl of extracted DNA was added to 40 µl of ViraQuant™ master mix and placed in the thermocycler of the STACE system for amplification and automated sample collection. Starting with cycle 20, aliquots are taken at the end of the extension phase and dispensed into a destination plate containing formamide and ROX standards. The plates are placed in an ABI 3730xl CE analyzer for detection and analysis by STACE software. Quantification of amplified products is performed by comparing the increasing fluorescent signal after alternate PCR cycles following the first 20 cycles with the fluorescent signal of the DNA calibration standards in each PCR reaction.

Results: Of the 134 samples tested 30 (22%) were positive for CMV by our method with a range of 450 copies/ml to 270,000 copies/ml. The ViraQuant assay detected 28 (21%) positive samples with a range of 134 copies/ml to 720,000 copies/ml. Of the 104 CMV-negative samples 48 (46%) were positive for one or more viruses. Thirty of 134 samples (22%) had 2 or more viruses detected. EBV was detected in 39 samples, BKV in 12, HHV-6 in 9 and HHV-7 in 30.

Conclusion: The ViraQuant assay compares favorably to our current method of CMV detection and quantification. In addition, the ViraQuant assay detected 46 % patients with other viral infections and 22% co-infections which could significantly improve the management of transplant patients.

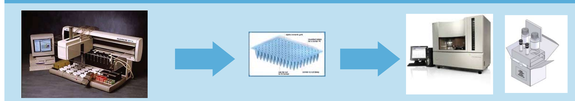
Background

- Cytomegalovirus, Epstein Barr virus, BK virus are among some of the more frequently diagnosed post-transplant viral infections.
- Coinfection with 2 or more of these viruses may play a significant part in organ rejection, graft dysfunction and other complications.
- We evaluated a newly developed Multiplex ViraQuant™ Assay (Primera Biosystems, Inc., Boston, Mass) that can quantitatively measure five different viruses simultaneously (CMV, EBV, BKV, HHV6 & HHV7) from a single sample, and compared to our monoplex CMV viral load assay (Hybrid Capture® System) (Digene/Qiagen, Gaithersburg, MD)

Methods and Materials

One hundred and thirty four whole bloods received in the Clinical Virology Laboratory for CMV viral load test were used to evaluate the Multiplex ViraQuant Assay. DNA was extracted from 200 µl of whole blood using Corbett Xtractor and eluted into 75 µl as per manufacturer's protocol. 10 µl of extracted DNA was added to 40 µl of ViraQuant master mix and placed in the thermocycler of the STACE system for amplification and automated sample collection. Aliquots are taken at the end of each extension phase and dispensed into a destination plate containing formamide and ROX standards. Detection and analysis of the destination plate were performed using ABI 3730xl CE.

Semi-Automated Modular Instrumentation System (STACE)



Perkin Elmer MultiProbe II Liquid Handler with Integrated PCR deck

Applied Biosystems 3730xl DNA Analyzer

Technology Principals

- ViraQuant is based on STAR (Scalable Target Analysis Routine) technology, a gene expression analysis platform that couples quantitative multiplex PCR and capillary electrophoresis (CE), allowing the simultaneous measurement of multiple targets in a single sample with high sensitivity.
- ViraQuant Reagents contain specific fluorescent primers to CMV, EBV, BKV, HHV6 and HHV7 viruses as well as quantification calibrators and sensitivity controls.
- A thermostable DNA polymerase is used to generate specific sized amplicons from any target virus present in samples as well as the co-amplification of controls.
- Quantification of amplified products is performed by comparing the increasing fluorescence signal after alternate PCR cycles following the first 20 PCR cycles with the fluorescence signal from the DNA calibration standards within each PCR reaction.

Results

- The Multiplex ViraQuant Assay detected CMV in 28/134 (21%) of samples (range 134 to 720,000 c/ml) which is comparable to our monoplex CMV test (30/ 134, 22%).
- Of the 104 CMV samples negative by our assay, 48 samples (46%) were positive for one or more viruses by the Multiplex assay. EBV was detected in 39 samples, BKV in 12, HHV-6 in 9 and HHV-7 in 30.
- Coinfection occurred in 30/134 (22%).

Table 1: Additional Positives Detected By ViraQuant

	CCF	ViraQuant Results (c/ml)				
Study #	CMV (C/ML)	CMV	EBV	BKV	HHV-6	HHV-7
CCF 25	905	510	387	**1998**	289	870
CCF 7	972	1124	**2207**			143
CCF 5	1558	2805				**3307**
CCF 14	2233	9014	**2368695**			
CCF 08	5635	24001	**1687**	**5139**		
CCF 02	29989	26673	1109	**6532**		
ccf032	N		426			**1681**
ccf040	N		916	**158213**		
CCF 12	N		1061			**5037**
ccf024	N		1417		**6450000**	
ccf023	N		**1613**		**4265**	
ccf002	N		**1737**			
ccf014	N		**2473**			
ccf035	N		**3406**			
CCF 10	N		**3798**			
ccf067	N		**4148**			
CCF 11	N		**4939**			**7056**
ccf012	N		**5094**			
ccf074	N	1094	**5349**			
CCF 15	N		**6788**			
ccf007	N		**8226**			
CCF 16	N		**17251**		937	**4844**
ccf051	N		**21527**			
CCF 38	N		**34003**	**29016**		
ccf054	N		**41925**		906	
CCF 7	N		**99842**			**7981**
CCF 2	N		**203237**			**5740**
ccf008	N			**5301**	753	
ccf062	N					**1590**
CCF 10	N					**6790**
ccf016	N					**39375**
ccf027	N			**2766**		
ccf039	N			**1017**		**1562**
ccf006	N					**2108**
CCF 07	N					

N=Not Detected

Discussion

Detection of CMV by the Multiplex assay was comparable to the Digene HC assay with a sensitivity of 87% and specificity of 98 %.

Of the 134 samples 93 were from solid organ transplant (SOT). A retrospective review of physician ordering patterns showed that in SOT patients quantitative viral load for EBV was also ordered 26% of the time and BKV 9% the time. (Table 2). Of particular interest is that EBV was concurrently ordered in 88% of lung transplants and BKV in 23% of kidney transplants.

Table 2: Ordering Pattern For Solid Organ Transplants

Transplant Type	# CMV Ordered	# EBV Ordered	# BKV Ordered	% Ordered Together EBV	% Ordered Together BKV
Heart	6	1	1	17	17
Kidney	31	2	7	6	23
Liver	31	1	0	3	0
Lung	25	22	0	88	0
Total Sot	**93**	**26**	**8**	**28**	**9**

Conclusion

- The Multiplex ViraQuant assay compares favorably to our current method of CMV detection and quantification.
- The ViraQuant assay detected 32.2% patients with other viral infections and 23% co-infections which could significantly improve the management of transplant patients.
- Analysis of the ordering pattern for transplant patients and the occurrences of significant viral levels of EBV, BKV, HHV6 and HHV7 that are not tested for indicate that the Multiplex Viraquant Assay would be of considerable utility in the management of transplant patients.