Clinical Utility of A Noval Multiplex Quantitative Viral Loads Assay for the Management of 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. Increased use of these immunosuppressive drugs coincides with increased incidence of post-transplant complications due to opportunistic infections. Cytomegalovirus, Epstein Barr virus, BK virus are some of the more frequently diagnosed post-transplant viral infections. We evaluated a newly developed ViraQuant™ Multiplex Assay (Primera Biosystems, Inc. Boston, Mass,) that quantitatively measures five different viruses (CMV, EBV, BKV, HHV6 & HHV7) from one sample. We compared results of the new multiplex assay with our routinely used monoplex CMV viral load assay (Qiagen/Digene, Gaitherberg, MD).

Materials & Methods: One hundred and thirty-eight whole bloods from solid organ and bone marrow transplant recipients received in the Clinical Virology Laboratory for CMV viral load testing were used for the comparison. DNA was extracted from 200 ul of whole blood using the Corbett Xtractor or the Qiagen QiaCube as per manufacturer's protocol. A reaction mixture containing 10 ul extracted DNA and 40 ul of ViraQuant master mix was placed in the thermocycler of the ViraQuant STACE (Semiautomated Thermocycler and Capillary Electrophoresis) System for amplification and automated amplicon collection. Starting at cycle 20, aliquots of amplicon are collected after alternate PCR cycles and dispensed into a destination plate containing formamide and ROX standards. These plates are placed in an ABI 3730xl CE analyzer for detection and analysis by STACE software. Quantification is accomplished 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 138 samples tested, 15 (12%) were positive for CMV by our method with a range of 1,016 to 270,000 copies/ml. The ViraQuant Assay detected 17 (12%) CMV positive samples with a range of 1,042 to 720,000 copies/ml. The ViraQuant Assay also detected the following viruses: EBV 19/138 (14%), BKV 6/138 (4%), HHV-6 2/138 (1%) and HHV-7 12/138 (9%). Of the 123 samples CMV negative by the routine method, 31/123 (24%) were positive for an additional one or more viruses by the ViraQuant Assay. The ViraQuant Assay detected two or more viruses in 11/138 (8%) of the samples.

Conclusion: The ViraQuant Assay compares favorably to our current CMV viral load test. In addition, the ViraQuant assay detected 24% samples positive for other viral infections and 8% co-infections which could significantly improve the management of transplant patients.

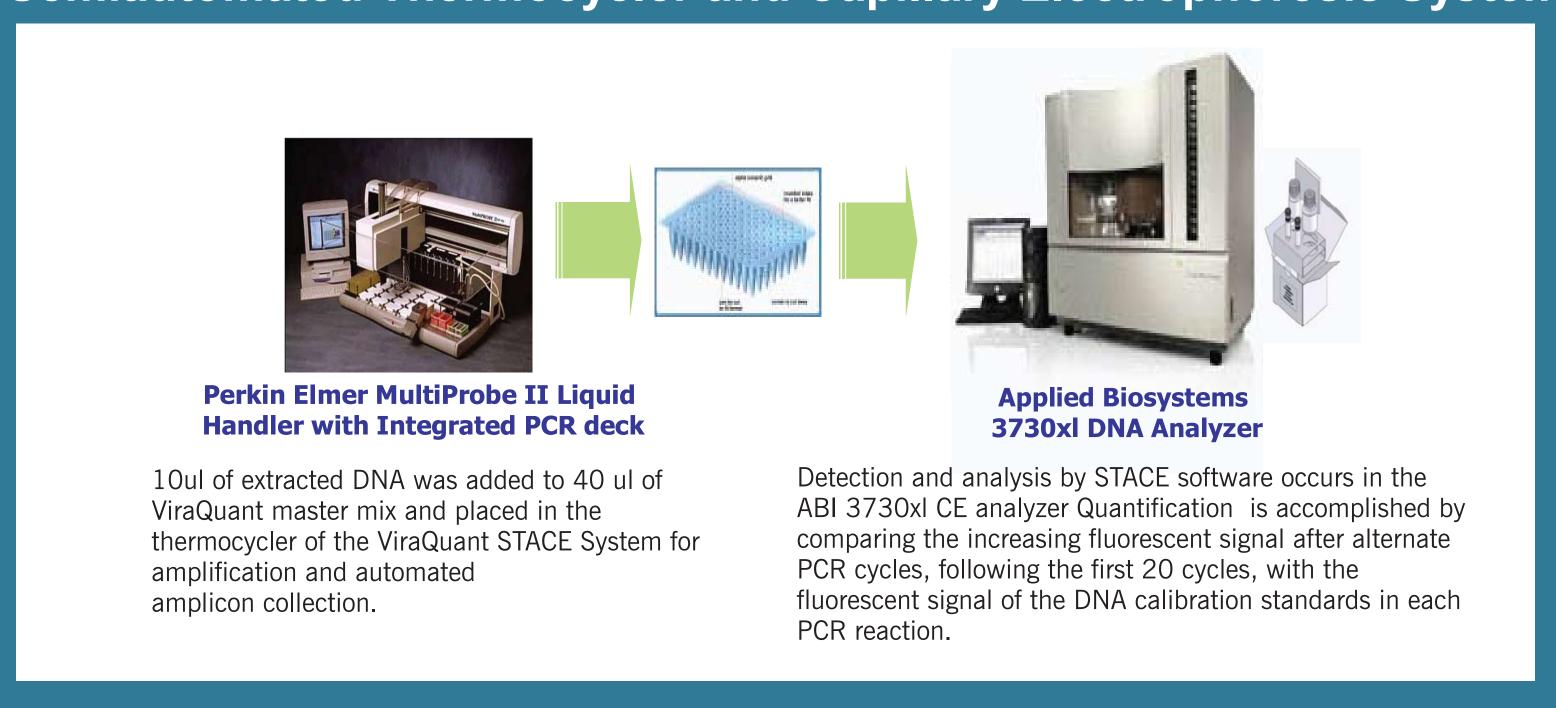
Background

- 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 that quantitatively measures CMV, EBV, BKV, HHV6 & HHV7 from a single sample, and compared to our routine monoplex CMV viral load assay.

Material & Methods

- 138 whole bloods from transplant recipients received in the Clinical Virology Laboratory for CMV viral load testing were used for the comparison.
- DNA was extracted from 200 ul of whole blood using the Corbett Xtractor or the Qiagen QiaCube.

Semiautomated Thermocycler and Capillary Electrophoresis System



Results

- 138 samples tested.
- 15 (12%) were positive for CMV by our method with a range of 1,016 to 270,000 copies/ml.
- 17 (12%) CMV by ViraQuant with a range of 1,042 to 720,000 copies/ml.
- The ViraQuant Assay also detected the following viruses: EBV 19/138 (14%), BKV 6/138 (4%), HHV-6 2/138 (1%) and HHV-7 12/138 (9%).
- Of the 123 samples CMV negative by the our method, 31/123 (24%) were positive for an additional one or more viruses by the ViraQuant Assay. The ViraQuant Assay detected two or more viruses in 11/138 (8%) of the samples.

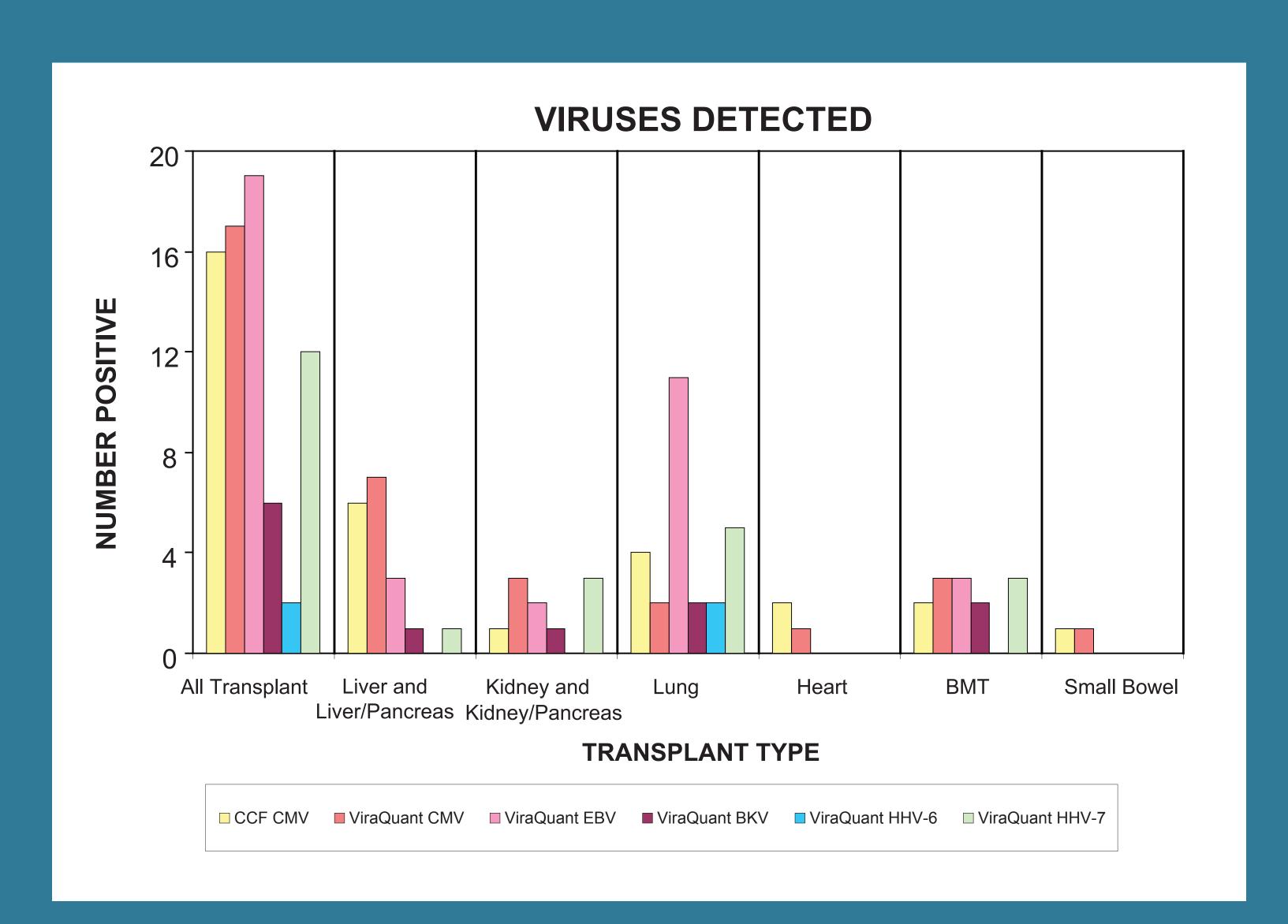


Table 1: Results by Transplant Type

	CCF	,	Transplant Type				
	Results						
Study #	CMV	CMV	EBV	BKV	HHV-6	HHV-7	71
CCF Sample12	3648	N	N	N	N	N	
CCF Sample7	N	1124	2207	N	N	N	
ccf036 TX-10	N N	1865 2000	1382 N	N N	N N	N N	
ccf027	N N	N	N N	2766	N	N N	Kidney and
CCF 10	N	N N	N N	N	N	6790	Kidney/Pancreas
CCF Sample36	N	N	N	N	N	1106	
ccf006	N	N	N	N	N	2108	
37 Negative For All	N	N	N	N	N	N	
CCF Sample1	1210	N	N	N	N	N	
ccf049	1234	4967	N N	N	N	N	
CCF Sample 27	1558	2805	N	N	N	3307	
CCF Sample27	1969	1042	N N	N	N	N N	
CCFVQET_05 TX-8	2759 4402	4526 3300	N N	N N	N N	N N	Liver and
ccf074	N	1094	5349	N N	N	N N	Liver/Pancreas
ccf019	N	1426	N	N	N	N	
CCF Sample10	N	N	3798	N	N	N	
ccf002	N	N	1737	N	N	N	
CCF Sample25	N	N	N	1998	N	N	
25 Negative For All	N	N	N	N	N	N	
CCFVQET_03	1445	5159	N	N	N	N	
TX-13	2026	N	N	N	N	N	
ccf044	2338	N	N	N	N	N	
CCF_Sample6	9406	1154	N	N	N	N	
CCF_7	N	N	99842	N	N	7981	
CCF_16	N	N	17251	N	N	4844	
ccf007	N	N	8226	N	N	N	
ccf012	N	N	5094	N	N	N	
CCF 11	N	N	4939	N	N	7056	
ccf014	N	N	2473	N	N	N	Lung
ccf023	N	N	1613	N	4265	N	
ccf024	N	N	1417	N	6450000	N	
CCF 8	N	N	1113	N	N	N	
CCF 12	N	N	1061	N	N	5037	
ccf013	N	N	1052	N	N	N	
ccf040	N	N	N	158213	N	N	
ccf008	N	N	N	5301	N	N	
ccf016	N	N	N	N	N	39375	
14 Negative For All	N	N	N	N	N	N	
ccf043	265,238	720,000	N	N	N	N	
TX-11	1,097	N	N	N	N	N	Heart
6 Negative For All	N	N	N	N	N	N	110411
TX-24	1080	2100	N	N	N	N	Small Bowel
CCF_4	N	N	N	N	N	N	Pancreas
TX-23	1016	N	N	N	N	N	. C. TOT GGG
CCF_Sample30	2635	1929	N	N	N	N	
ccf020	N	1741	N	N	N	N	
CCF 13	N	1321	N	N	N	N	
ccf054	N	N	41925	N	N	N	
CCF Sample38	N	N	34003	29016	N	N	
CCF 15	N	N	6788	N	N	N	Bone Marrow
ccf063	N	N	N	1427	N	N	
ccf068	N N	N N	N N	N 1427	N N	1465	
CCFVQET 07	N	N	N	N	N N	2373	
_					IV		
ccf062	N N	N N	N N	N N	NI	1590	
11 Negative For All	N	N	N	N	l N	N	

Discussion

A retrospective review of physician ordering patterns showed that the quantitative viral load ordered concurrently with CMV was 28% for EBV, 8% for BKV and about 1% for HHV-6. Of particular interest is that EBV was concurrently ordered in 96% of lung transplants and BKV in 26% of kidney transplants.

Table 2: Physician Ordering Pattern

TRANSPLANT TYPE	# CMV ORDERED	# EBV ORDERED	# BKV ORDERED	# HHV-6 ORDERED
HEART	8	0	0	0
KIDNEY	38	2 (5%)	10 (26%)	0
LIVER	36	1 (3%)	1 (3%)	0
LUNG	32	31 (97%)	0	1 (3%)
PANCREAS	1	0	0	0
SMALL BOWEL	1	1 (100%)	0	0
TOTAL SOT	116	37 (32%)	11 (9%)	1 (.8%)
ВМТ	22	2 (9%)	0	0
TOTAL	138	39 (28%)	11 (8%)	1 (.7%)

Conclusion

- Currently, quantitative CMV viral load testing is offered in-house while EBV, BK, HHV-6 and HHV-7 are sent out due to test volumes.
- Our preliminary study shows that the ViraQuant assay compares favorably to our current CMV assay. However the ViraQuant assay detected 24% more positive samples for viruses other than CMV and 8% co-infections were found.
- The number of positive results for EBV, BKV, HHV-6 and HHV-7, the incidence of co-infection, the decrease in turnaround time and hands on time strongly support that the ViraQuant Assay would be useful for our busy laboratory.
- Analysis of the ordering pattern for transplant patients and the occurrences of significant viral levels of EBV, BKV, HHV6 and HHV7 that are not currently tested for, indicate that the Multiplex Viraquant Assay would be of considerable utility in the management of transplant patients.