

EVALUATION OF ViraQuant™: 5 VIRUSES MULTIPLEX ASSAY RUN ON ICEPlex™, AN AUTOMATED QUANTITATIVE MULTIPLEX PCR PLATFORM.

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Multiply Your Results

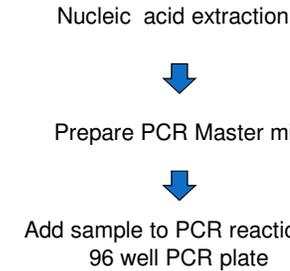
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Introduction

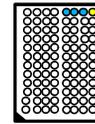
Measurement of the load of herpes viruses, such as CMV, EBV, HHV-6 and 7 as well as measurement of BKV polyomavirus today is mostly done by Real-Time PCR. Existing Real-Time PCR based assays have very limited multiplexing capabilities, while external calibration and controls are an additional burden for each run. ViraQuant™, a multiplex assay designed for viral load monitoring, was evaluated for its performance on ICEPlex™, an integrated PCR and capillary electrophoresis platform with quantitative multiplexing capability. ICEPlex utilizes a novel approach for PCR amplicon detection, which greatly exceeds the multiplex capacity of traditional Real-Time PCR. ViraQuant can detect and quantify 5 individual viruses (CMV, EBV, BKV, HHV-6, and HHV-7) simultaneously. Each reaction also includes amplification controls for each of the 5 viruses, an extraction control and quantification calibrators.

ICEPlex Work Flow



→ Load PCR plate in ICEPlex instrument

Run automated PCR and CE with injection from PCR reactions at alternate cycles.

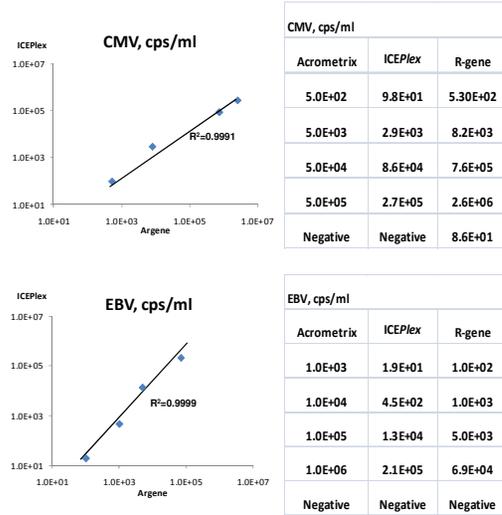


ICEPlex system automatically processes collected data and provides user with the result.

ViraQuant™ features:

- Multiplex assay for simultaneous quantification of 5 viruses: CMV, EBV, HHV-6, HHV-7 and BKV
- In-reaction sensitivity control for each of the targets
- In-reaction extraction control
- In-reaction quantification calibrators

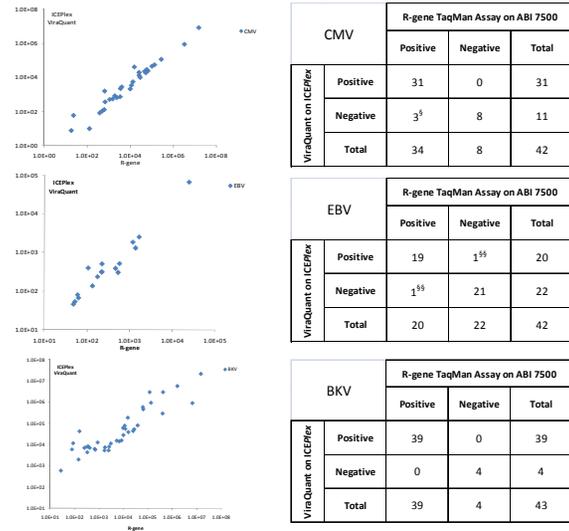
ICEPlex ViraQuant and TaqMan assay show excellent correlation measuring Acrometrix OptiQuant standards



Copy numbers determined for the OptiQuant CMV and EBV viral particles by both ICEPlex ViraQuant assay and R-gene assays showed excellent correlation for the measurement. For CMV, the reported copy numbers were in good agreement with the data provided by Acrometrix. For EBV, both ICEPlex ViraQuant and R-gene assays reported lower copy numbers than the figure provided by the supplier. A potential reason for this is that both ICEPlex ViraQuant and R-gene assays target a gene present as a single copy in the viral genome, while the other assay targets a multi-copy gene. Also, the R-gene CMV assay was found to report a false positive (low copy number) for the OptiQuant CMV negative control standard.

ICEPlex ViraQuant and TaqMan assay show good correlation measuring viral load in blood plasma samples

Leftover de-identified blood plasma samples positive or negative for studied viruses were obtained and tested with ICEPlex ViraQuant and R-gene CMV, EBV and BKV TaqMan assays on ABI 7500.



Overall, both assays exhibited very good correlation in reported numbers and concordance.

[§]Out of 3 discordant samples for CMV all were reported negative by ICEPlex ViraQuant and low positive by the R-gene assay. Out of these 3 samples one was reported to be negative by a reference lab, while the other two were reported as very low positives. This kind of discordance most likely reflects assay-specific reporting criteria for the samples falling close to the limit of detection.

^{§§}Both discordant EBV samples were reported as low positive by a reference lab.

Virus co-presence: type and occurrence

Using ICEPlex ViraQuant assay we identified following cases of virus co-presence:

Samples from CMV study discovered positive for other targets					
Sample ID	CMV	EBV	BKV	HHV-6	HHV-7
CMV2957	30000	-	3200	-	-
CMV3189	19000	-	70000	-	-
CMV4930	2300	-	150	-	-
CMV4817	32000	-	13000	-	-
CMV5408	850	-	8400	-	-
CMV6428	2300	-	530	-	-
CMV0019	24000	-	9000	8900	-
CMV0022	10	-	1300	-	-
CMV0037	510	-	160000	-	-
CMV0033	-	-	2900	-	-
CMV0007	-	-	5800	-	-

Samples from EBV study discovered positive for other targets					
Sample ID	CMV	EBV	BKV	HHV-6	HHV-7
EBV0003	-	70	-	-	230
EBV0015	180	10	940	-	-
EBV0023	-	340	127000	-	-
EBV0027	-	340	280	-	-

Samples from BKV study discovered positive for other targets					
Sample ID	CMV	EBV	BKV	HHV-6	HHV-7
BB0011	-	-	455000	1300	-
BB1313	-	-	25000000	6500	-

In 42 samples from a CMV study, 11 were subsequently found to be positive for other viruses: 9 for BKV and 1 for HHV-6. One sample was found to be positive for two other viruses, BKV and HHV-6.

In 42 samples from an EBV study, 4 were subsequently found to be positive for other viruses: 2 for BKV, one for HHV-7 and one for CMV and BKV.

In 43 samples from a BKV study, 2 were subsequently found to be positive for HHV-6.

In several of the samples, the viral load of the originally untested viruses (most commonly BKV) was quite significant.

Conclusions

The multiplex ViraQuant assay has demonstrated excellent quantitative performance on ICEPlex compared to the single viral load monitoring assays.

ICEPlex offers the convenience of detecting and measuring 5 viruses with controls and quantitative calibration in a single PCR reaction.

The frequency of co-presence of different viruses in plasma samples demonstrates the benefit of using multiplex assay.

*ICEPlex™ and ViraQuant™ are for Research Use Only (RUO). Not for clinical diagnostic use.