

EVALUATION OF THE ANALYTICAL WORKFLOW AND LABORATORY ADVANTAGES BY THE SIMULTANEOUS EXECUTION OF SIX DIFFERENT MOLECULAR ASSAYS USING THE AUTOMATED VERSANT® KPCR MOLECULAR SYSTEM WITH THE MIPLX SOFTWARE

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Objectives:

Cost-effective processing, short time to result, and multi-specimen processing collectively represent a major challenge for the molecular routine laboratory. This is especially true for viral load monitoring in immunocompromised patients. Every day there are multiple and specific viral load requests for Cytomegalovirus (CMV), Epstein-Barr Virus (EBV), Polyomavirus BK (BKV), Polyomavirus JC (JCV), Parvovirus B19 (PARVO), and Human Herpes Virus 6 (HHV-6) from different specimens from patients that have to be processed and reported. The goal of this study was to evaluate the Analytical Workflow and Laboratory Advantages through the simultaneous execution of six different molecular assays Using the Automated VERSANT® kPCR Molecular System with the MiPLX Software Solution

Methods:

The VERSANT® kPCR Molecular System* equipped with the new VERSANT® MiPLX Software Solution and the VERSANT® Sample Preparation 1.2 Reagents kit (Siemens Healthcare Diagnostics, Tarrytown, NY, USA) were used to extract DNA from clinical specimens and automate PCR set-up for single or multiplex assays (Figure 1). Approximately 100 whole blood samples spiked with commercial viral standards were analyzed in about 10 automated runs for determination of the six quantitative viral load assays. kPCR PLX Assay Kits for CMV, EBV, BKV, JCV, Parvovirus B19, and HHV-6 A/B were used. Extracted assay specific internal control and quantitative standards were included for each kit. Viral target amplification, detection and quantification was performed automatically on the AD module of the VERSANT® kPCR Molecular System. MiPLX software comes with "dynamic protocols" which enables automated PCR assay set-up and assay multiplexing capability of up to six PCR assays by eluting splitting from one extracted sample (Figures 2, 3, 4).

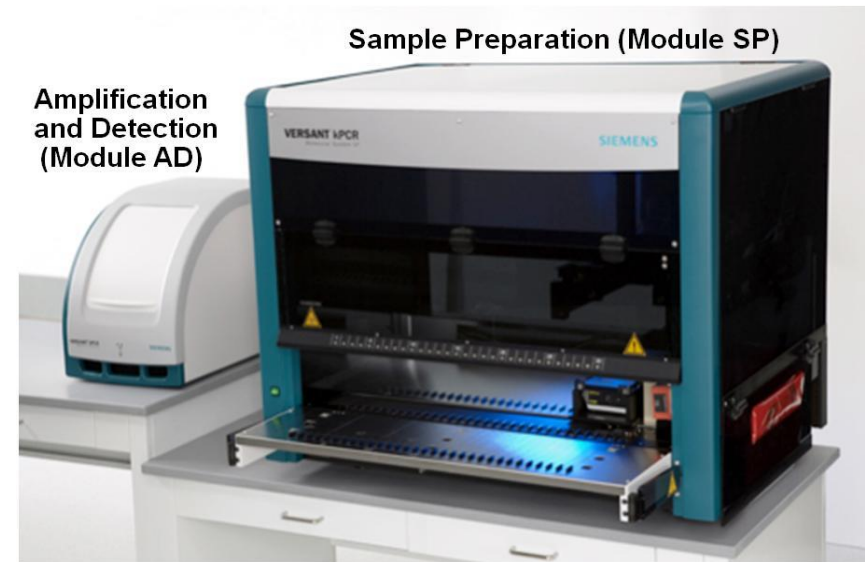


Figure 1: VERSANT® kPCR Molecular System* equipment

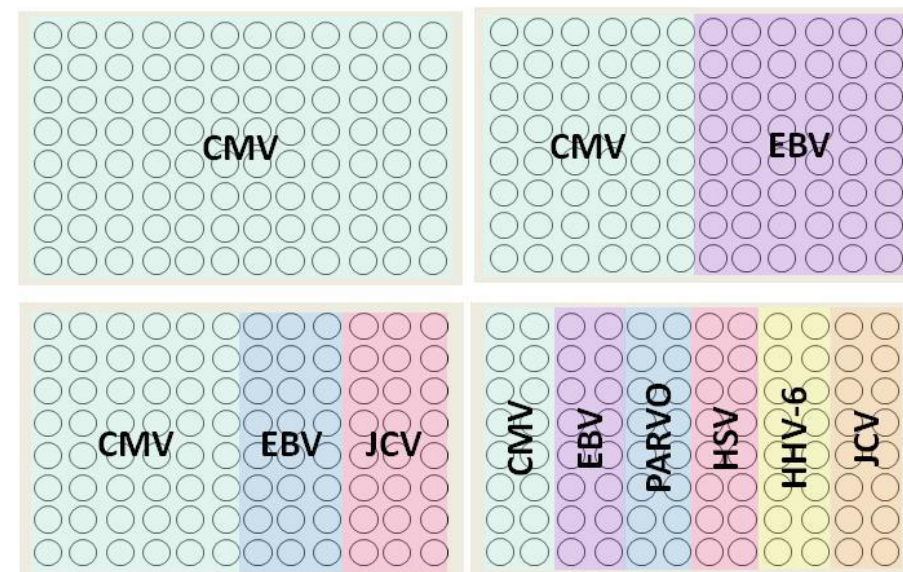


Figure 2:

Multiple Assays per Sample and PCR Plate. Dynamic protocols allow you to run 1, 2, 4, or 6 assays from one extracted sample on a single PCR plate

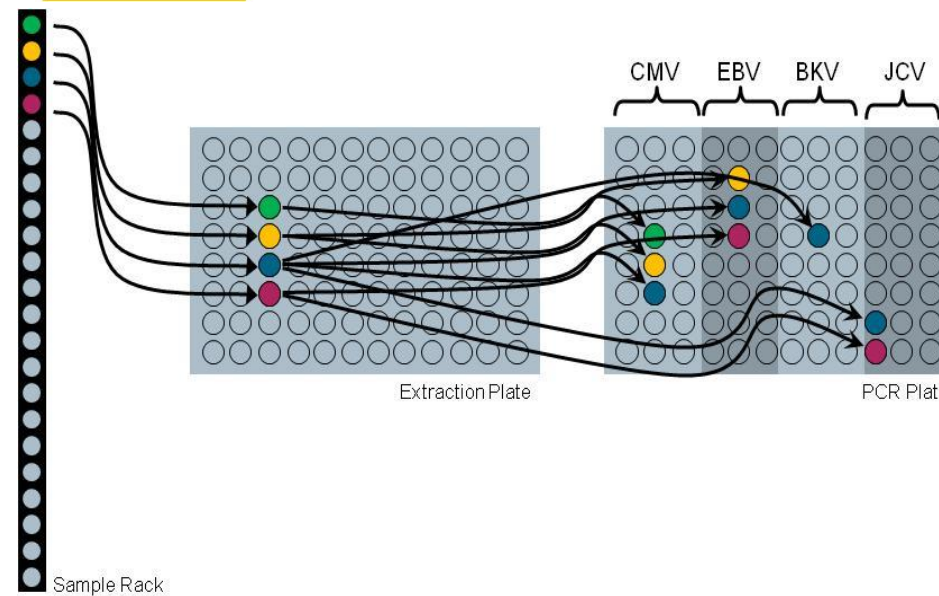


Figure 3: Eluate Splitting

Dynamic protocols provide the ability to get more information out of a single sample extraction by splitting the eluate instead of the sample

	CMV	EBV	PARVO	BKV	HHV6	JCV
A	CMV-A1 0004	EBV-A3 0005	PARVO-A6 0006	BKV-A7 0007	HHV6-A9 0008	JCV-A11 0009
B	CMV-B1 0005	EBV-B3 0006	PARVO-B6 0007	BKV-B7 0008	HHV6-B9 0009	JCV-B11 0010
C	CMV-C1 0006	EBV-C3 0007	PARVO-C6 0008	BKV-C7 0009	HHV6-C9 0010	JCV-C11 0011
D	CMV-D1 0007	EBV-D3 0008	PARVO-D6 0009	BKV-D7 0010	HHV6-D9 0011	JCV-D11 0012
E	Universal 0008	Universal 0010	Universal 0011	Universal 0012	Universal 0013	Universal 0014
F	0001	0009	0001	0001	0001	0001
G	0002	0010	0002	0002	0002	0002
H	0003	0003	0003	0003	0003	0003

Figure 4: MiPLX software

Results:

In this study we reported on the utility of the VERSANT® kPCR Molecular System* and the dedicated software to reduce the time necessary to perform the complete analysis of whole blood samples of subjects with viral infection and Figure 5 shows the results obtained for the 100 samples analyzed. In some samples multiple targets are found. The MiPLX software allows to analyze simultaneously six PCR assays for CMV, EBV, BKV, JCV, Parvovirus B19, and HHV-6 A/B. This procedure is quite fast (three and half hours for the workflow time), reduces the operator-dependent variability (primary tube, uracyl-N-glycosylase and LIS-interface) and, last but not the least, is carried out by using one automated platform (Figure 6).

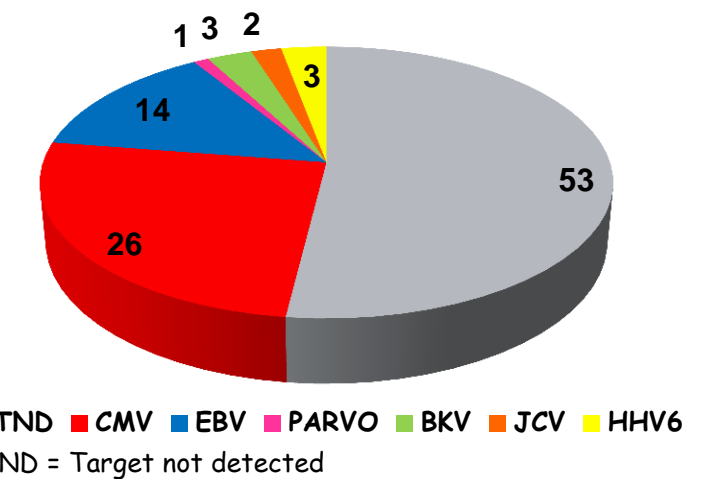


Figure 5: Results of the 100 analyzed samples

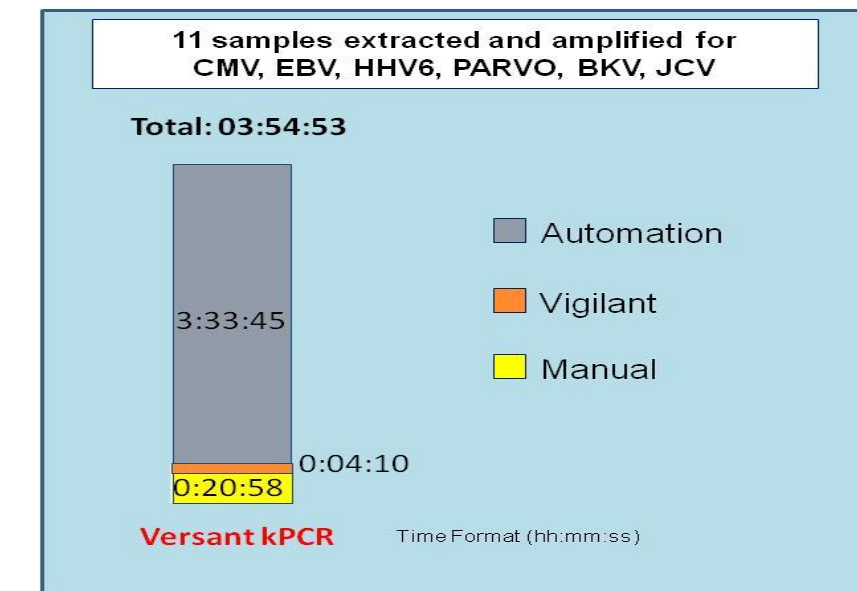


Figure 6: Workflow time

Conclusions:

The 6 kPCR PLX assays, when automated on the VERSANT® kPCR Molecular System with the new VERSANT® MiPLX Software Solution, showed good and robust assay performance. The VERSANT® MiPLX Software Solution, in combination with kPCR PLX assays, allows multiplexing of up to six assays from one sample in one run. This unique combination enables laboratories to manage complex viral panel orders for a more effective, consolidated, automated, and flexible workflow