

Supplementary Materials

Implementation and Validation of the Roche Light Cyclar 480 96-Well Plate Platform as a Real-Time PCR Assay for the Quantitative Detection of Cytomegalovirus (CMV) in Clinical Specimens Using the Luminex MultiCode ASRs System

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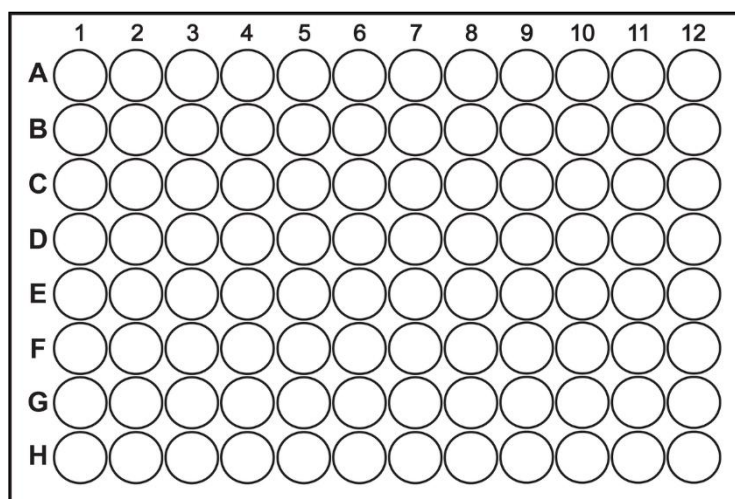
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Notes for All the supplemental Figures and Tables: PCR plate grid template shows the location (as known as “Well” in the plate) is labeled as: A1, A2, ..., A12; B1 – B12, which is shown on all the supplemental Tables (as known as Location in Tables) or the original PCR profiles (as known as Wells) below.

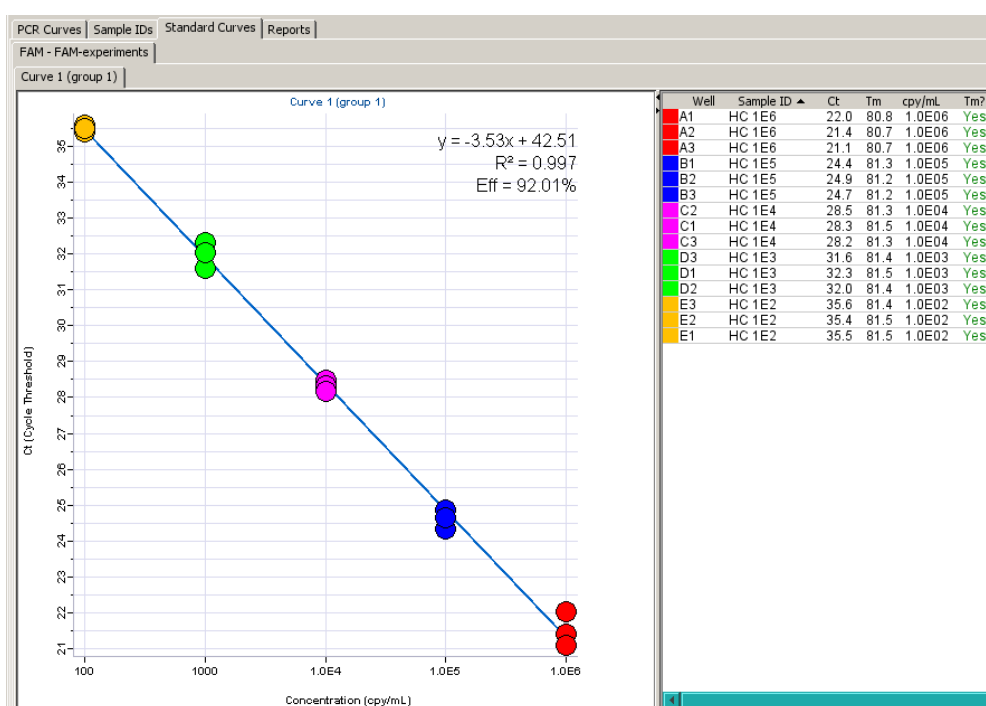
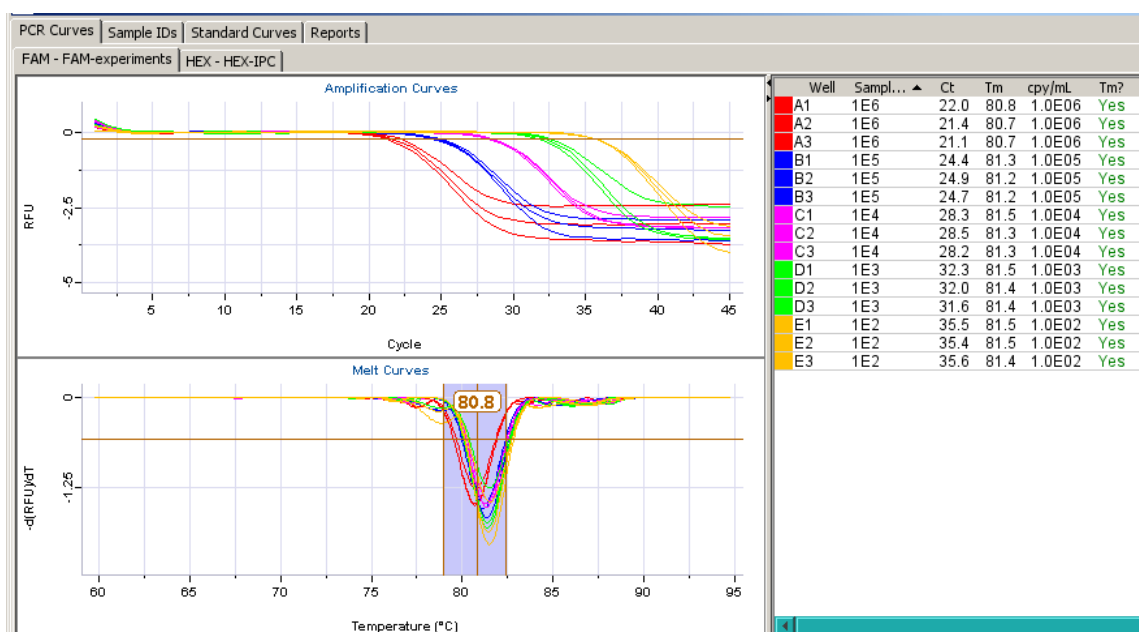


Figure S1. LC480's original PCR profiles show the Reportable range. We performed the linearity study by determining a series of dilutions from a standard stock across anticipated measuring range with triplicates in each data point (performed on March 21, 2011). Upper panel: qPCR reaction curve; lower panel: measuring range. Note: Plot of results from a linearity experiment to determine reportable range. Seven concentrations of analyte prepared by dilution of a high-concentration standard were tested in triplicate. Assigned values, (converted to $\log_{10}$) were plotted on the x axis versus measured values (converted to $\log_{10}$) on the y axis using Microsoft Excel. Linear regression analysis gave the equation $y = -3.53x + 42.51$ ($R^2 = 0.997$) (Reference: Burd EM, 2010). (abbreviations: LC480, Roche Light Cycler 480 96-well Plate Platform; LC2, Roche Light Cycler 2 microcapillary Platform).

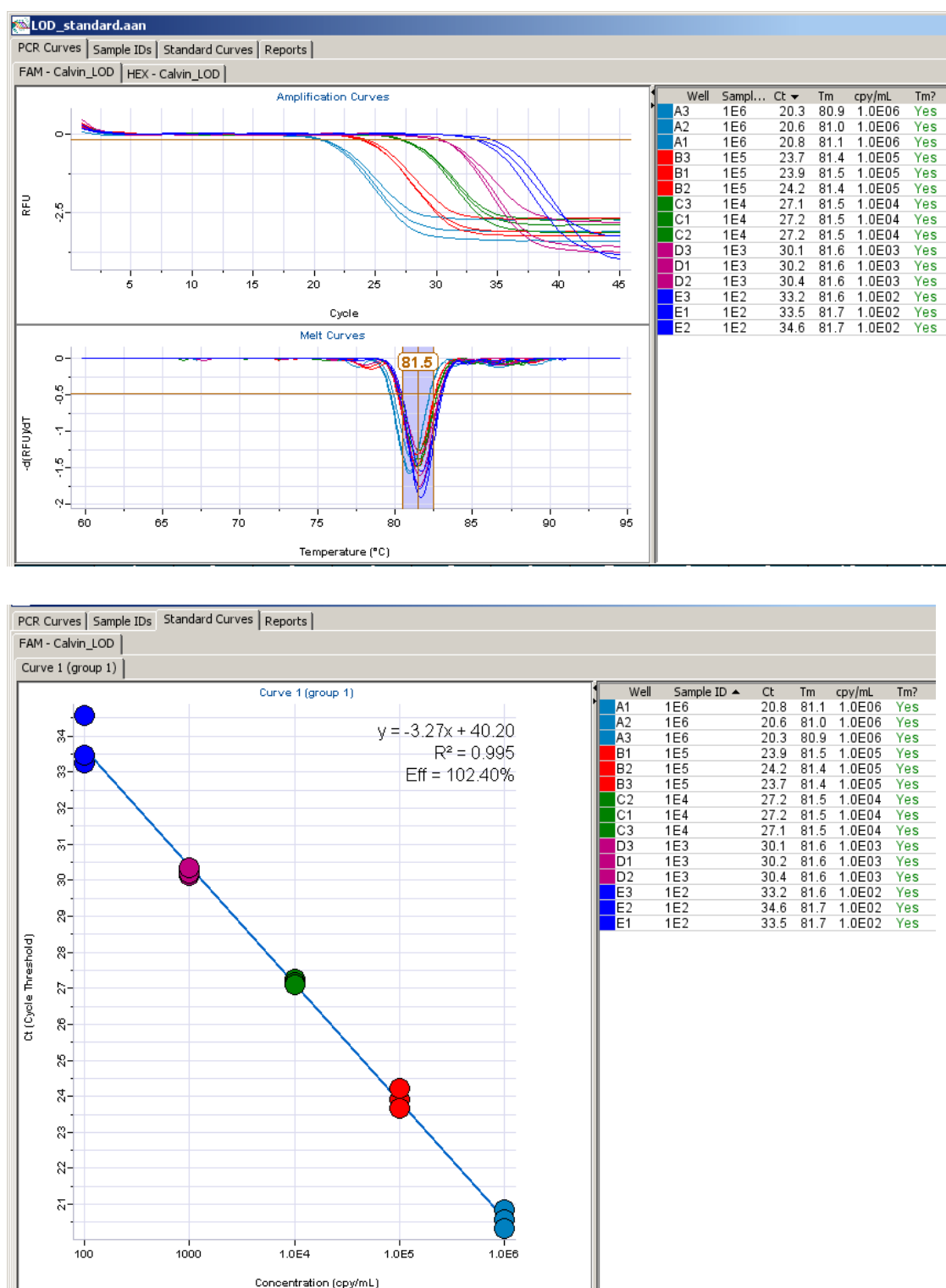


Figure 2. LC480's original PCR profiles show the Reportable range. We performed the linearity study by determining a series of dilutions from a standard stock across anticipated measuring range with triplicates in each data point (performed on April 23, 2011). Upper panel: qPCR reaction curve; lower panel: measuring range. Note: Plot of results from a linearity experiment to determine reportable range. Seven concentrations of analyte prepared by dilution of a high-concentration standard were tested in triplicate. Assigned values, (converted to $\log_{10}$) were plotted on the x axis versus measured values (converted to $\log_{10}$) on the y axis using Microsoft Excel. A second-order polynomial trendline of the linear regression analysis gave the equation " $y = -3.27x + 40.20$ ($R^2 = 0.995$)" (Reference: Burd EM, 2010).

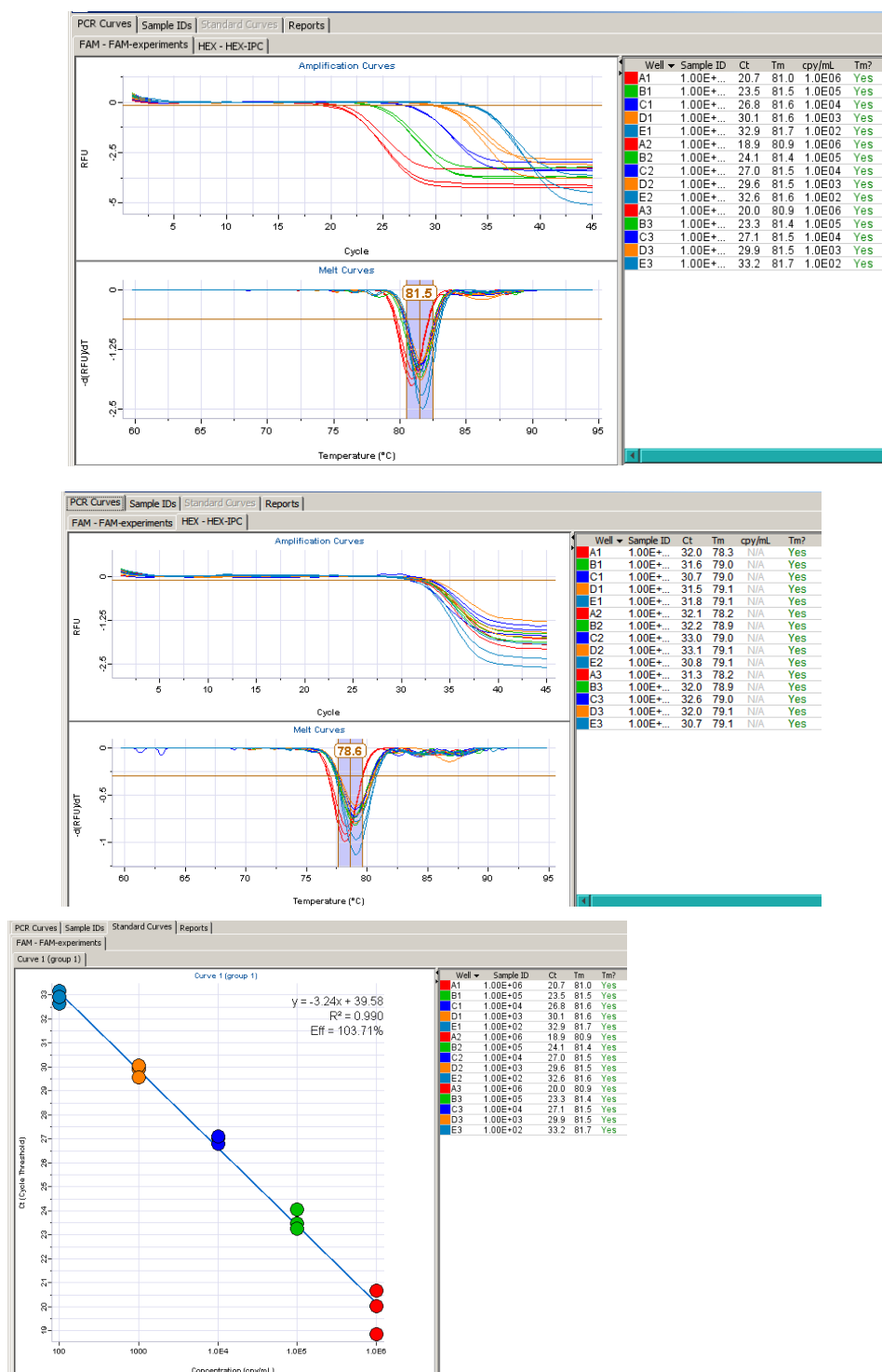


Figure S3. LC480's original PCR profiles show the Reportable range. We performed the linearity study by determining a series of dilutions from a standard stock across anticipated measuring range with triplicates in each data point (performed on May 24, 2011). Upper panel: qPCR reaction curve; Middle panel: qPCR reaction internal positive control (IPC-HEX); lower panel: measuring range. Note: Plot of results from a linearity experiment to determine reportable range. Seven concentrations of analyte prepared by dilution of a high-concentration standard were tested in triplicate. Assigned values, (converted to $\log_{10}$) were plotted on the x axis versus measured values (converted to $\log_{10}$) on the y axis using Microsoft Excel. A third-order polynomial trendline of the linear regression analysis gave the equation " $y = -3.24x + 40.20$ ($R^2 = 0.990$)" (Reference: Burd EM, 2010).

Table S4.0.1. Overall Log difference in the limit of detection (LOD) variations between standard curves

Std1 LOD (100)			std2(6/7/12) LOD (100)			Std3 LOD (100)		
Date	CN	Log	Date	CN	Log	Date	CN	Log
4/23/2012	1.60E+02	2.20412	4/23/2012	5.50E+01	1.740363	4/23/2012	3.40E+01	1.531479
	2.00E+02	2.30103		6.60E+01	1.819544		4.10E+01	1.612784
	1.10E+02	2.041393		3.60E+01	1.556303		2.20E+01	1.342423
4/25/2012	1.60E+02	2.20412	4/25/2012	6.70E+01	1.826075	4/25/2012	3.40E+01	1.531479
	1.10E+02	2.041393		6.50E+01	1.812913		3.20E+01	1.50515
	2.10E+02	2.322219		5.50E+01	1.740363		2.00E+01	1.30103
	1.70E+02	2.230449		5.10E+01	1.70757		4.20E+01	1.623249
	2.40E+02	2.380211		3.30E+01	1.518514		4.80E+01	1.681241
	2.80E+02	2.447158		6.80E+01	1.832509		5.90E+01	1.770852
	1.60E+02	2.20412		7.80E+01	1.892095		3.20E+01	1.50515
	2.20E+02	2.342423		9.40E+01	1.973128		4.60E+01	1.662758
	2.20E+02	2.342423		5.20E+01	1.716003		4.40E+01	1.643453
	2.30E+02	2.361728		7.40E+01	1.869232		4.70E+01	1.672098
	2.90E+02	2.462398		7.00E+01	1.845098		6.10E+01	1.78533
	3.10E+02	2.491362		7.60E+01	1.880814		6.50E+01	1.812913
	1.40E+02	2.146128		9.80E+01	1.991226		2.60E+01	1.414973
	1.70E+02	2.230449		1.00E+02	2		3.50E+01	1.544068
	2.00E+02	2.30103		4.30E+01	1.633468		4.00E+01	1.60206
	3.30E+02	2.518514		5.60E+01	1.748188		7.00E+01	1.845098
	1.90E+02	2.278754		6.50E+01	1.812913		3.90E+01	1.591065
	2.60E+02	2.414973		1.10E+02	2.041393		5.40E+01	1.732394
	2.10E+02	2.322219		6.30E+01	1.799341		4.20E+01	1.623249
	2.00E+02	2.30103		8.80E+01	1.944483		4.10E+01	1.612784
	2.10E+02	2.322219		6.90E+01	1.838849		4.30E+01	1.633468
	1.90E+02	2.278754		6.00E+01	1.778151		3.70E+01	1.568202
	1.10E+02	2.041393		3.30E+01	1.518514		2.10E+01	1.322219
5/24/2012 100cp14Rep Std1	2.00E+02	2.30103	5/24/2012 std2(6/7/12) 100cp14Rep	7.20E+01	1.857332	5/24/2012 100cp14Rep Std3	4.10E+01	1.612784
	2.30E+02	2.361728		7.80E+01	1.892095		4.80E+01	1.681241
	2.20E+02	2.342423		8.00E+01	1.90309		4.50E+01	1.653213
	1.80E+02	2.255273		1.30E+02	2.113943		3.70E+01	1.568202
	2.40E+02	2.380211		6.30E+01	1.799341		4.90E+01	1.690196
	2.30E+02	2.361728		6.90E+01	1.838849		4.80E+01	1.681241
	2.40E+02	2.380211		6.60E+01	1.819544		5.00E+01	1.69897
	2.30E+02	2.361728		7.70E+01	1.886491		4.60E+01	1.662758
	3.80E+02	2.579784		5.90E+01	1.770852		8.10E+01	1.908485
	2.40E+02	2.380211		7.70E+01	1.886491		5.00E+01	1.69897
	1.90E+02	2.278754		7.50E+01	1.875061		3.90E+01	1.591065
	5.40E+02	2.732394		8.00E+01	1.90309		1.20E+02	2.079181
mean	2.25E+02	2.329019	mean	7.43E+01	1.844398	mean	4.64E+01	1.638876
	SD	80.54303		SD	28.61324		SD	18.32163
Overall Log SD (across all three standard curves):			0.006782					

Table S4.0.2. Overall Log difference in the high copy (HC) variations between standard curves

std1 HC (100000)			Std2(6/7/12) HC (100000)			std3 HC (100000)		
Date	CN	Log	Date	CN	Log	Date	CN	Log
4/23/2012	1.00E+05	5.00E+00	4/23/2012	5.70E+04	4.76E+00	4/23/2012	3.70E+04	4.57E+00
	1.70E+05	5.23E+00		9.70E+04	4.99E+00		6.40E+04	4.81E+00
	1.40E+05	5.15E+00		7.90E+04	4.90E+00		5.20E+04	4.72E+00
	2.00E+05	5.30E+00		1.10E+05	5.04E+00		7.40E+04	4.87E+00
	8.00E+04	4.90E+00		4.20E+04	4.62E+00		2.80E+04	4.45E+00
	1.10E+05	5.04E+00		6.30E+04	4.80E+00		4.10E+04	4.61E+00
	8.40E+04	4.92E+00		4.50E+04	4.65E+00		2.90E+04	4.46E+00
	8.10E+04	4.91E+00		4.30E+04	4.63E+00		2.80E+04	4.45E+00
	8.90E+04	4.95E+00		4.80E+04	4.68E+00		3.10E+04	4.49E+00
	1.00E+05	5.00E+00		5.60E+04	4.75E+00		3.60E+04	4.56E+00
	6.10E+04	4.79E+00		3.20E+04	4.51E+00		2.10E+04	4.32E+00
	8.90E+04	4.95E+00		4.80E+04	4.68E+00		3.10E+04	4.49E+00
	8.70E+04	4.94E+00		4.70E+04	4.67E+00		3.00E+04	4.48E+00
5/18/2012	1.40E+05	5.15E+00	5/18/2012	7.90E+04	4.90E+00	5/18/2012	5.00E+04	4.70E+00
	1.50E+05	5.18E+00		8.50E+04	4.93E+00		5.20E+04	4.72E+00
	9.30E+04	4.97E+00		5.00E+04	4.70E+00		5.60E+04	4.75E+00
	1.60E+05	5.20E+00		8.90E+04	4.95E+00		3.30E+04	4.52E+00
	1.50E+05	5.18E+00		8.40E+04	4.92E+00		5.80E+04	4.76E+00
	2.00E+05	5.30E+00		1.20E+05	5.08E+00		5.50E+04	4.74E+00
	1.10E+05	5.04E+00		6.20E+04	4.79E+00		7.70E+04	4.89E+00
	1.50E+05	5.18E+00		8.70E+04	4.94E+00		4.00E+04	4.60E+00
	1.30E+05	5.11E+00		7.30E+04	4.86E+00		5.70E+04	4.76E+00
	1.30E+05	5.11E+00		7.50E+04	4.88E+00		4.80E+04	4.68E+00
	2.00E+05	5.30E+00		1.10E+05	5.04E+00		4.90E+04	4.69E+00
	1.40E+05	5.15E+00		7.80E+04	4.89E+00		7.30E+04	4.86E+00
	2.10E+05	5.32E+00		1.20E+05	5.08E+00		5.10E+04	4.71E+00
	1.40E+05	5.15E+00		8.00E+04	4.90E+00		7.80E+04	4.89E+00
	1.70E+05	5.23E+00		9.60E+04	4.98E+00		5.20E+04	4.72E+00
	1.40E+05	5.15E+00		8.10E+04	4.91E+00		6.30E+04	4.80E+00
	2.20E+05	5.34E+00		1.30E+05	5.11E+00		5.30E+04	4.72E+00
	1.30E+05	5.11E+00		7.30E+04	4.86E+00		8.20E+04	4.91E+00
	1.70E+05	5.23E+00		9.80E+04	4.99E+00		4.80E+04	4.68E+00
	2.70E+05	5.43E+00		1.60E+05	5.20E+00		6.40E+04	4.81E+00
	1.90E+05	5.28E+00		1.10E+05	5.04E+00		1.00E+05	5.00E+00
	2.50E+05	5.40E+00		1.50E+05	5.18E+00		7.00E+04	4.85E+00
	1.60E+05	5.20E+00		8.80E+04	4.94E+00		9.60E+04	4.98E+00
	1.50E+05	5.18E+00		8.30E+04	4.92E+00		5.70E+04	4.76E+00
	2.70E+05	5.43E+00		1.60E+05	5.20E+00		5.40E+04	4.73E+00
	2.30E+05	5.36E+00		1.40E+05	5.15E+00		1.00E+05	5.00E+00
	1.90E+05	5.28E+00		1.10E+05	5.04E+00		9.00E+04	4.95E+00
	3.10E+05	5.49E+00		1.90E+05	5.28E+00		7.00E+04	4.85E+00
	1.50E+05	5.18E+00		8.40E+04	4.92E+00		1.20E+05	5.08E+00
	3.70E+04	4.57E+00		1.40E+05	5.15E+00		5.50E+04	4.74E+00
mean	1.52E+05	5.15E+00	mean	8.96E+04	4.92E+00	mean	5.70E+04	4.72E+00
SD	59863.8174	1.86E-01	SD	36419.6654	1.80E-01	SD	22353.1754	1.73E-01

Overall Log SD across all three standard curves:

0.006186

Table S4.0.3. Overall Log difference in the low copy (LC) variations between standard curve

stdn1 LC (400)			stdn2(6/7/12) LC (400)			stdn3 LC (400)		
Date	CN	Log	Date	CN	Log	Date	CN	Log
4/23/2012	7.80E+02	2.892095	4/23/2012	2.20E+02	2.342423	4/23/2012	1.80E+02	2.255273
	3.30E+02	2.518514		2.40E+02	2.380211		7.00E+01	1.845098
	4.00E+02	2.60206		2.10E+02	2.322219		8.50E+01	1.929419
	4.20E+02	2.623249		2.80E+02	2.447158		8.90E+01	1.94939
	4.70E+02	2.672098		1.10E+02	2.041393		1.00E+02	2
	3.20E+02	2.50515		1.40E+02	2.146128		6.70E+01	1.826075
	4.00E+02	2.60206		1.40E+02	2.146128		8.50E+01	1.929419
	3.30E+02	2.518514		1.60E+02	2.20412		7.10E+01	1.851258
	3.90E+02	2.591065		1.10E+02	2.041393		8.40E+01	1.924279
	7.10E+02	2.851258		1.40E+02	2.146128		1.60E+02	2.20412
	6.20E+02	2.792392		1.10E+02	2.041393		1.40E+02	2.146128
	6.70E+02	2.826075		1.30E+02	2.113943		1.50E+02	2.176091
	5.90E+02	2.770852		2.60E+02	2.414973		1.30E+02	2.113943
4/25/2012	7.70E+02	2.886491	4/25/2012	2.80E+02	2.447158	4/25/2012	1.80E+02	2.255273
	4.20E+02	2.623249		1.50E+02	2.176091		9.10E+01	1.959041
	4.50E+02	2.653213		1.50E+02	2.176091		9.60E+01	1.982271
5/18/2012 stdn1	9.20E+02	2.963788	5/18/2012	3.10E+02	2.491362	5/18/2012	2.10E+02	2.322219
	7.30E+02	2.863323		2.40E+02	2.380211		1.60E+02	2.20412
	5.00E+02	2.69897		1.60E+02	2.20412		1.10E+02	2.041393
	1.00E+03	3		3.60E+02	2.556303		2.40E+02	2.380211
	6.60E+02	2.819544		2.20E+02	2.342423		1.50E+02	2.176091
5/24/2012	9.10E+02	2.959041	5/24/2012	3.10E+02	2.491362	5/24/2012	2.10E+02	2.322219
	7.60E+02	2.880814		3.00E+02	2.477121		1.70E+02	2.230449
	1.10E+03	3.041393		2.30E+02	2.361728		2.50E+02	2.39794
	7.00E+02	2.845098		3.60E+02	2.556303		1.60E+02	2.20412
	9.40E+02	2.973128		2.20E+02	2.342423		2.20E+02	2.342423
	5.90E+02	2.770852		2.90E+02	2.462398		1.30E+02	2.113943
	1.60E+03	3.20412		4.00E+02	2.60206		3.90E+02	2.591065
	8.50E+02	2.929419		3.70E+02	2.568202		1.90E+02	2.278754
	9.90E+02	2.995635		2.70E+02	2.431364		2.30E+02	2.361728
	1.10E+03	3.041393		3.40E+02	2.531479		2.50E+02	2.39794
	8.50E+02	2.929419		3.90E+02	2.591065		2.00E+02	2.30103
	8.20E+02	2.913814		3.50E+02	2.544068		1.90E+02	2.278754
	6.20E+02	2.792392		2.10E+02	2.322219		1.40E+02	2.146128
	1.00E+03	3		6.30E+02	2.799341		2.30E+02	2.361728
	5.90E+02	2.770852		3.10E+02	2.491362		1.30E+02	2.113943
	6.30E+02	2.799341		2.10E+02	2.322219		1.40E+02	2.146128
	8.00E+02	2.90309		5.00E+02	2.69897		1.80E+02	2.255273
	7.60E+02	2.880814		2.70E+02	2.431364		1.70E+02	2.230449
	1.30E+03	3.113943		2.50E+02	2.39794		3.10E+02	2.491362
mean	7.20E+02	2.825463	mean	2.58E+02	2.374609	mean	1.63E+02	2.175912
SD	279.21582	0.170171	SD	1.10E+02	0.184353	SD	69.155696	0.18515

Overall Log SD across all the three standard curves:

0.0084

Table S4.1.1 LOD was determined by replication experiments performed by different dates (CN – Copy Number per mL; LOD – Limit of Detection; SD – standard deviation).

Standard Curve 1

std1 LC (400)			std2(6/7/12) LC (400)			std3 LC (400)		
Date	CN	Log	Date	CN	Log	Date	CN	Log
4/23/2012	7.80E+02	2.892095	4/23/2012	2.20E+02	2.342423	4/23/2012	1.80E+02	2.255273
	3.30E+02	2.518514		2.40E+02	2.380211		7.00E+01	1.845098
	4.00E+02	2.60206		2.10E+02	2.322219		8.50E+01	1.929419
	4.20E+02	2.623249		2.80E+02	2.447158		8.90E+01	1.94939
	4.70E+02	2.672098		1.10E+02	2.041393		1.00E+02	2
	3.20E+02	2.50515		1.40E+02	2.146128		6.70E+01	1.826075
	4.00E+02	2.60206		1.40E+02	2.146128		8.50E+01	1.929419
	3.30E+02	2.518514		1.60E+02	2.20412		7.10E+01	1.851258
	3.90E+02	2.591065		1.10E+02	2.041393		8.40E+01	1.924279
	7.10E+02	2.851258		1.40E+02	2.146128		1.60E+02	2.20412
	6.20E+02	2.792392		1.10E+02	2.041393		1.40E+02	2.146128
	6.70E+02	2.826075		1.30E+02	2.113943		1.50E+02	2.176091
	5.90E+02	2.770852		2.60E+02	2.414973		1.30E+02	2.113943
4/25/2012	7.70E+02	2.886491	4/25/2012	2.80E+02	2.447158	4/25/2012	1.80E+02	2.255273
	4.20E+02	2.623249		1.50E+02	2.176091		9.10E+01	1.959041
	4.50E+02	2.653213		1.50E+02	2.176091		9.60E+01	1.982271
5/18/2012 std1	9.20E+02	2.963788	5/18/2012	3.10E+02	2.491362	5/18/2012	2.10E+02	2.322219
	7.30E+02	2.863323		2.40E+02	2.380211		1.60E+02	2.20412
	5.00E+02	2.69897		1.60E+02	2.20412		1.10E+02	2.041393
	1.00E+03	3		3.60E+02	2.556303		2.40E+02	2.380211
	6.60E+02	2.819544		2.20E+02	2.342423		1.50E+02	2.176091
5/24/2012	9.10E+02	2.959041	5/24/2012	3.10E+02	2.491362	5/24/2012	2.10E+02	2.322219
	7.60E+02	2.880814		3.00E+02	2.477121		1.70E+02	2.230449
	1.10E+03	3.041393		2.30E+02	2.361728		2.50E+02	2.39794
	7.00E+02	2.845098		3.60E+02	2.556303		1.60E+02	2.20412
	9.40E+02	2.973128		2.20E+02	2.342423		2.20E+02	2.342423
	5.90E+02	2.770852		2.90E+02	2.462398		1.30E+02	2.113943
	1.60E+03	3.20412		4.00E+02	2.60206		3.90E+02	2.591065
	8.50E+02	2.929419		3.70E+02	2.568202		1.90E+02	2.278754
	9.90E+02	2.995635		2.70E+02	2.431364		2.30E+02	2.361728
	1.10E+03	3.041393		3.40E+02	2.531479		2.50E+02	2.39794
	8.50E+02	2.929419		3.90E+02	2.591065		2.00E+02	2.30103
	8.20E+02	2.913814		3.50E+02	2.544068		1.90E+02	2.278754
	6.20E+02	2.792392		2.10E+02	2.322219		1.40E+02	2.146128
	1.00E+03	3		6.30E+02	2.799341		2.30E+02	2.361728
	5.90E+02	2.770852		3.10E+02	2.491362		1.30E+02	2.113943
	6.30E+02	2.799341		2.10E+02	2.322219		1.40E+02	2.146128
	8.00E+02	2.90309		5.00E+02	2.69897		1.80E+02	2.255273
	7.60E+02	2.880814		2.70E+02	2.431364		1.70E+02	2.230449
	1.30E+03	3.113943		2.50E+02	2.39794		3.10E+02	2.491362
mean	7.20E+02	2.825463	mean	2.58E+02	2.374609	mean	1.63E+02	2.175912
SD	279.21582	0.170171	SD	1.10E+02	0.184353	SD	69.155696	0.18515

Overall Log SD across all the three standard curves:

0.0084

LOD (100)			LOD (100)			LOD (100)		
Date	CN	Log	Date	CN	Log	Date	CN	Log
4/23/2012	1.60E+02	2.20412	4/25/2012	1.60E+02	2.20412	5/24/2012	2.00E+02	2.30103
	2.00E+02	2.30103	stnd1	1.10E+02	2.041393	100cp14Rep	2.30E+02	2.361728
stnd1	1.10E+02	2.041393	100cp23Rep	2.10E+02	2.322219	Stnd1	2.20E+02	2.342423
mean	1.57E+02	2.182181		1.70E+02	2.230449		1.80E+02	2.255273
SD	45.0925	0.131202		2.40E+02	2.380211		2.40E+02	2.380211
				2.80E+02	2.447158		2.30E+02	2.361728
				1.60E+02	2.20412		2.40E+02	2.380211
				2.20E+02	2.342423		2.30E+02	2.361728
				2.20E+02	2.342423		3.80E+02	2.579784
				2.30E+02	2.361728		2.40E+02	2.380211
				2.90E+02	2.462398		1.90E+02	2.278754
				3.10E+02	2.491362		5.40E+02	2.732394
				1.40E+02	2.146128		2.10E+02	2.322219
				1.70E+02	2.230449		3.90E+02	2.591065
				2.00E+02	2.30103	mean	2.66E+02	2.402054
				3.30E+02	2.518514	SD	100.8208	0.135769
				1.90E+02	2.278754			
				2.60E+02	2.414973			
				2.10E+02	2.322219			
				2.00E+02	2.30103			
				2.10E+02	2.322219			
				1.90E+02	2.278754			
				1.10E+02	2.041393			
			mean	2.09E+02	2.303716			
			SD	57.91161	0.125593			

LC			LC			LC		
Date	CN	Log	Date	CN	L o g	Date	CN	Log
4/23/2012	1.80E+02	2.255273	5/18/2012	2.10E+02	2	5/24/2012	2.10E+02	2.322219
std3	7.00E+01	1.845098	std3	1.60E+02	.3	std3	1.70E+02	2.230449
400cp13Rep	8.50E+01	1.929419		1.10E+02	2	400cp19Rep	2.50E+02	2.39794
	8.90E+01	1.94939		2.40E+02	2		1.60E+02	2.20412
	1.00E+02	2		1.50E+02	2		2.20E+02	2.342423
					.1			
					7			
					6			
					0			

	6.70E+01	1.826075	mean	1.74E+02	9	1.30E+02	2.113943										
					1												
	8.50E+01	1.929419			SD			51.28353	2								
									.								
									2								
									2								
									4								
									8								
			0														
			7														
	7.10E+01	1.851258			0												
					.												
					1												
					3												
					2												
					3												
					6												
					5												
mean	1.09E+02	2.011576		1.90E+02	2.278754												
SD	38.41574	0.148621															
4/25/2012	1.80E+02	2.255273				2.30E+02	2.361728										
	9.10E+01	1.959041						2.50E+02	2.39794								
	9.60E+01	1.982271								2.00E+02	2.30103						
	mean	1.22E+02										2.065528	1.90E+02	2.278754			
	SD	50.00333										0.164733			1.40E+02	2.146128	
	mean	1.09E+02										2.011576					2.30E+02
SD	38.41574	0.148621				1.30E+02	2.113943										
400cp3Rep	1.80E+02	2.255273						1.40E+02	2.146128								
	9.10E+01	1.959041								1.80E+02	2.255273						
	9.60E+01	1.982271										1.70E+02	2.230449				
	mean	1.22E+02												2.065528	3.10E+02	2.491362	
	SD	50.00333												0.164733			mean
	mean	1.09E+02				2.011576	SD							65.01012			
SD	38.41574	0.148621															
mean	1.09E+02	2.011576															
SD	38.41574	0.148621															
mean	1.09E+02	2.011576															
SD	38.41574	0.148621															

Table S4.1.2 High-copy control (HC) was evaluated by replication experiments performed by different dates (The measurement of Day-to-day variations) (CN – Copy Number per mL; HC – High Copy number control; SD – standard deviation).

Standard Curve 1 (file name: Stnd1-HC_CN-Log_day-to-day-variation.xlsx)

HC			HC		
Date	CN	Log	Date	CN	Log
4/23/2012 (stdn1) 13 Rep	1.00E+05	5.00E+00	5/18/2012 stdn1	1.40E+05	5.15E+00
	1.70E+05	5.23E+00		1.50E+05	5.18E+00
	1.40E+05	5.15E+00		9.30E+04	4.97E+00
	2.00E+05	5.30E+00		1.60E+05	5.20E+00
	8.00E+04	4.90E+00		1.50E+05	5.18E+00
	1.10E+05	5.04E+00		2.00E+05	5.30E+00
	8.40E+04	4.92E+00		1.10E+05	5.04E+00
	8.10E+04	4.91E+00		1.50E+05	5.18E+00
	8.90E+04	4.95E+00		1.30E+05	5.11E+00
	1.00E+05	5.00E+00		1.30E+05	5.11E+00
	6.10E+04	4.79E+00		2.00E+05	5.30E+00
	8.90E+04	4.95E+00		1.40E+05	5.15E+00
	8.70E+04	4.94E+00		2.10E+05	5.32E+00
mean	1.07E+05	5.01E+00		1.40E+05	5.15E+00
SD	39698.87	1.43E-01		1.70E+05	5.23E+00
				1.40E+05	5.15E+00
				2.20E+05	5.34E+00
				1.30E+05	5.11E+00
				1.70E+05	5.23E+00
				2.70E+05	5.43E+00
				1.90E+05	5.28E+00
				2.50E+05	5.40E+00
				1.60E+05	5.20E+00
				1.50E+05	5.18E+00
				2.70E+05	5.43E+00
				2.30E+05	5.36E+00
				1.90E+05	5.28E+00
				3.10E+05	5.49E+00
				1.50E+05	5.18E+00
				3.70E+04	4.57E+00
			mean	1.71E+05	5.21E+00
			SD	56952.69	1.70E-01

Standard Curve 2 (File: Stnd2-HC_CN-Log_day-to-day-variation.xlsx)

HC			HC		
Date	CN	Log	Date	CN	Log
4/23/2012	5.70E+04	4.76E+00	5/18/2012	7.90E+04	4.90E+00
1E5 13Rep	9.70E+04	4.99E+00	stnd2(6/7/12)	8.50E+04	4.93E+00
Stnd2(6/7/12)	7.90E+04	4.90E+00		5.00E+04	4.70E+00
	1.10E+05	5.04E+00		8.90E+04	4.95E+00
	4.20E+04	4.62E+00		8.40E+04	4.92E+00
	6.30E+04	4.80E+00		1.20E+05	5.08E+00
	4.50E+04	4.65E+00		6.20E+04	4.79E+00
	4.30E+04	4.63E+00		8.70E+04	4.94E+00
	4.80E+04	4.68E+00		7.30E+04	4.86E+00
	5.60E+04	4.75E+00		7.50E+04	4.88E+00
	3.20E+04	4.51E+00		1.10E+05	5.04E+00
	4.80E+04	4.68E+00		7.80E+04	4.89E+00
	4.70E+04	4.67E+00		1.20E+05	5.08E+00
mean	5.90E+04	4.74E+00		8.00E+04	4.90E+00
SD	22967.37	1.53E-01		9.60E+04	4.98E+00
				8.10E+04	4.91E+00
				1.30E+05	5.11E+00
				7.30E+04	4.86E+00
				9.80E+04	4.99E+00
				1.60E+05	5.20E+00
				1.10E+05	5.04E+00
				1.50E+05	5.18E+00
				8.80E+04	4.94E+00
				8.30E+04	4.92E+00
				1.60E+05	5.20E+00
				1.40E+05	5.15E+00
				1.10E+05	5.04E+00
				1.90E+05	5.28E+00
				8.40E+04	4.92E+00
				1.40E+05	5.15E+00
			mean	1.03E+05	4.99E+00
			SD	33193.36	1.34E-01

Standard Curve 3 (File: Stnd3-HC_CN-Log_day-to-day-variation.xlsx)

HC			HC		
Date	CN	Log	Date	CN	Log
4/23/2012	3.70E+04	4.57E+00	5/18/2012	5.00E+04	4.70E+00
(stnd3)	6.40E+04	4.81E+00	(stnd3)	5.20E+04	4.72E+00
1E5_13Rep	5.20E+04	4.72E+00		5.60E+04	4.75E+00
	7.40E+04	4.87E+00		3.30E+04	4.52E+00
	2.80E+04	4.45E+00		5.80E+04	4.76E+00
	4.10E+04	4.61E+00		5.50E+04	4.74E+00
	2.90E+04	4.46E+00		7.70E+04	4.89E+00
	2.80E+04	4.45E+00		4.00E+04	4.60E+00
	3.10E+04	4.49E+00		5.70E+04	4.76E+00
	3.60E+04	4.56E+00		4.80E+04	4.68E+00
	2.10E+04	4.32E+00		4.90E+04	4.69E+00
	3.10E+04	4.49E+00		7.30E+04	4.86E+00
	3.00E+04	4.48E+00		5.10E+04	4.71E+00
mean	3.86E+04	4.56E+00		7.80E+04	4.89E+00
SD	15569.94	1.56E-01		5.20E+04	4.72E+00
				6.30E+04	4.80E+00
				5.30E+04	4.72E+00
				8.20E+04	4.91E+00
				4.80E+04	4.68E+00
				6.40E+04	4.81E+00
				1.00E+05	5.00E+00
				7.00E+04	4.85E+00
				9.60E+04	4.98E+00
				5.70E+04	4.76E+00
				5.40E+04	4.73E+00
				1.00E+05	5.00E+00
				9.00E+04	4.95E+00
				7.00E+04	4.85E+00
				1.20E+05	5.08E+00
				5.50E+04	4.74E+00
			mean	6.50E+04	4.79E+00
			SD	20126.3	1.27E-01

Table S4.1.3 Low-copy control (LC) was evaluated with replication experiments performed by different dates (The measurement of Day-to-day variations) (CN – Copy Number per mL; LC – Low Copy number control; SD – standard deviation).

Standard Curve 1 (file name: Stnd1-LC_CN-Log_day-to-day-variation.xlsx)

LC			LC			LC			
Date	CN	Log	Date	CN	Log	Date	CN	Log	
4/23/2012 (stnd1) 13 Rep	7.80E+02	2.892095	5/18/2012 stnd1	9.20E+02	2.963788	5/24/2012 stnd1 400cp19Re P	9.10E+02	2.959041	
	3.30E+02	2.518514		7.30E+02	2.863323		7.60E+02	2.880814	
	4.00E+02	2.60206		5.00E+02	2.69897		1.10E+03	3.041393	
	4.20E+02	2.623249		1.00E+03	3		7.00E+02	2.845098	
	4.70E+02	2.672098		6.60E+02	2.819544		9.40E+02	2.973128	
	3.20E+02	2.50515	mean	7.62E+02	2.869125		5.90E+02	2.770852	
	4.00E+02	2.60206	SD	201.0473	0.119931		1.60E+03	3.20412	
	3.30E+02	2.518514					8.50E+02	2.929419	
	3.90E+02	2.591065					9.90E+02	2.995635	
	7.10E+02	2.851258					1.10E+03	3.041393	
	6.20E+02	2.792392					8.50E+02	2.929419	
	6.70E+02	2.826075					8.20E+02	2.913814	
	5.90E+02	2.770852					6.20E+02	2.792392	
	mean	4.95E+02	2.67426				1.00E+03	3	
	SD	158.9348	0.13607				5.90E+02	2.770852	
						6.30E+02	2.799341		
						8.00E+02	2.90309		
						7.60E+02	2.880814		
						1.30E+03	3.113943		
						mean	8.90E+02	2.933924	
						SD	257.3368	0.116693	

4/25/2012 (stnd1)	7.70E+02	2.886491
	4.20E+02	2.623249
	4.50E+02	2.653213
mean	5.47E+02	2.720984
SD	193.9931	0.144114

Standard Curve 2 (file name: Stnd2-LC_CN-Log_day-to-day-variation.xlsx)

HC			HC		
Date	CN	Log	Date	CN	Log
4/23/2012	5.70E+04	4.76E+00	5/18/2012	7.90E+04	4.90E+00
1E5 13Rep	9.70E+04	4.99E+00	stnd2(6/7/12)	8.50E+04	4.93E+00
Stnd2(6/7/12)	7.90E+04	4.90E+00		5.00E+04	4.70E+00
	1.10E+05	5.04E+00		8.90E+04	4.95E+00
	4.20E+04	4.62E+00		8.40E+04	4.92E+00
	6.30E+04	4.80E+00		1.20E+05	5.08E+00
	4.50E+04	4.65E+00		6.20E+04	4.79E+00
	4.30E+04	4.63E+00		8.70E+04	4.94E+00
	4.80E+04	4.68E+00		7.30E+04	4.86E+00
	5.60E+04	4.75E+00		7.50E+04	4.88E+00
	3.20E+04	4.51E+00		1.10E+05	5.04E+00
	4.80E+04	4.68E+00		7.80E+04	4.89E+00
	4.70E+04	4.67E+00		1.20E+05	5.08E+00
mean	5.90E+04	4.74E+00		8.00E+04	4.90E+00
SD	22967.37	1.53E-01		9.60E+04	4.98E+00
				8.10E+04	4.91E+00
				1.30E+05	5.11E+00
				7.30E+04	4.86E+00
				9.80E+04	4.99E+00
				1.60E+05	5.20E+00
				1.10E+05	5.04E+00
				1.50E+05	5.18E+00
				8.80E+04	4.94E+00
				8.30E+04	4.92E+00
				1.60E+05	5.20E+00
				1.40E+05	5.15E+00
				1.10E+05	5.04E+00
				1.90E+05	5.28E+00
				8.40E+04	4.92E+00
				1.40E+05	5.15E+00
			mean	1.03E+05	4.99E+00
			SD	33193.36	1.34E-01

Standard Curve 3 (file name: Stnd3-LC_CN-Log_day-to-day-variation.xlsx)

LC			LC			LC		
Date	CN	Log	Date	CN	Log	Date	CN	Log
4/23/2012 stnd3 400cp13Rep	1.80E+02	2.255273	5/18/2012 stnd3	2.10E+02	2.322219	5/24/2012 stnd3 400cp19Rep	2.10E+02	2.322219
	7.00E+01	1.845098		1.60E+02	2.20412		1.70E+02	2.230449
	8.50E+01	1.929419		1.10E+02	2.041393		2.50E+02	2.39794
	8.90E+01	1.94939		2.40E+02	2.380211		1.60E+02	2.20412
	1.00E+02	2		1.50E+02	2.176091		2.20E+02	2.342423
	6.70E+01	1.826075	mean	1.74E+02	2.224807		1.30E+02	2.113943
	8.50E+01	1.929419	SD	51.28353	0.132365		3.90E+02	2.591065
	7.10E+01	1.851258					1.90E+02	2.278754
	8.40E+01	1.924279					2.30E+02	2.361728
	1.60E+02	2.20412					2.50E+02	2.39794
	1.40E+02	2.146128					2.00E+02	2.30103
	1.50E+02	2.176091					1.90E+02	2.278754
	1.30E+02	2.113943	1.40E+02	2.146128				
mean	1.09E+02	2.011576	2.30E+02	2.361728				
SD	38.41574	0.148621	1.30E+02	2.113943				
							1.40E+02	2.146128
							1.80E+02	2.255273
							1.70E+02	2.230449
							3.10E+02	2.491362
						mean	2.05E+02	2.292914
						SD	65.01012	0.126456

4/25/2012	1.80E+02	2.255273
400cp3Rep	9.10E+01	1.959041
stnd3	9.60E+01	1.982271
mean	1.22E+02	2.065528
SD	50.00333	0.164733

Table S4.2.1 Replication experiments performed by different dates and different concentrations with the standards 1, 2, 3 (CN – Copy Number per mL; HC- high copy; LC – Low Copy number control; SD – standard deviation).

Standard Curve 1 (File name: CN_Log_HC-LC-LOD_40_Std1_vf.xlsx)

HC (100000)			LC (400)			LOD (100)		
Date	CN	Log	Date	CN	Log	Date	CN	Log
4/23/2012 (stdn1) 13 Rep	1.00E+05	5.00E+00	4/23/2012 (stdn1) 13 Rep	7.80E+02	2.892095	4/23/2012 400cp3Rep dil stdn1	1.60E+02	2.20412
	1.70E+05	5.23E+00		3.30E+02	2.518514		2.00E+02	2.30103
							1.10E+02	2.041393
	1.40E+05	5.15E+00		4.00E+02	2.60206	4/25/2012 stdn1 100cp23Rep	1.60E+02	2.20412
	2.00E+05	5.30E+00		4.20E+02	2.623249		1.10E+02	2.041393
	8.00E+04	4.90E+00		4.70E+02	2.672098		2.10E+02	2.322219
	1.10E+05	5.04E+00		3.20E+02	2.50515		1.70E+02	2.230449
	8.40E+04	4.92E+00		4.00E+02	2.60206		2.40E+02	2.380211
	8.10E+04	4.91E+00		3.30E+02	2.518514		2.80E+02	2.447158
	8.90E+04	4.95E+00		3.90E+02	2.591065		1.60E+02	2.20412
	1.00E+05	5.00E+00		7.10E+02	2.851258		2.20E+02	2.342423
	6.10E+04	4.79E+00		6.20E+02	2.792392		2.20E+02	2.342423
	8.90E+04	4.95E+00		6.70E+02	2.826075		2.30E+02	2.361728
	8.70E+04	4.94E+00		5.90E+02	2.770852		2.90E+02	2.462398
							3.10E+02	2.491362
							1.40E+02	2.146128
5/18/2012 stdn1	1.40E+05	5.15E+00	4/25/2012 (stdn1)	7.70E+02	2.886491		1.70E+02	2.230449
	1.50E+05	5.18E+00		4.20E+02	2.623249		2.00E+02	2.30103
	9.30E+04	4.97E+00		4.50E+02	2.653213		3.30E+02	2.518514
	1.60E+05	5.20E+00	5/18/2012 stdn1	9.20E+02	2.963788		1.90E+02	2.278754
	1.50E+05	5.18E+00		7.30E+02	2.863323		2.60E+02	2.414973
	2.00E+05	5.30E+00		5.00E+02	2.69897		2.10E+02	2.322219
	1.10E+05	5.04E+00		1.00E+03	3		2.00E+02	2.30103
	1.50E+05	5.18E+00		6.60E+02	2.819544		2.10E+02	2.322219
	1.30E+05	5.11E+00	5/24/2012 stdn1 400cp19Rep	9.10E+02	2.959041	5/24/2012 100cp14Rep Std1	1.90E+02	2.278754
	1.30E+05	5.11E+00		7.60E+02	2.880814		1.10E+02	2.041393
	2.00E+05	5.30E+00		1.10E+03	3.041393		2.00E+02	2.30103
							2.30E+02	2.361728
	1.40E+05	5.15E+00		7.00E+02	2.845098		2.20E+02	2.342423
	2.10E+05	5.32E+00		9.40E+02	2.973128		1.80E+02	2.255273
	1.40E+05	5.15E+00		5.90E+02	2.770852		2.40E+02	2.380211
	1.70E+05	5.23E+00		1.60E+03	3.20412		2.30E+02	2.361728
	1.40E+05	5.15E+00		8.50E+02	2.929419		2.40E+02	2.380211
	2.20E+05	5.34E+00		9.90E+02	2.995635		2.30E+02	2.361728
	1.30E+05	5.11E+00		1.10E+03	3.041393		3.80E+02	2.579784
	1.70E+05	5.23E+00		8.50E+02	2.929419		2.40E+02	2.380211
	2.70E+05	5.43E+00		8.20E+02	2.913814		1.90E+02	2.278754
	1.90E+05	5.28E+00		6.20E+02	2.792392		5.40E+02	2.732394
	2.50E+05	5.40E+00		1.00E+03	3			
	1.60E+05	5.20E+00		5.90E+02	2.770852			
	1.50E+05	5.18E+00		6.30E+02	2.799341			
	2.70E+05	5.43E+00		8.00E+02	2.90309			

	2.30E+05	5.36E+00		7.60E+02	2.880814		2.10E+02	2.322219
	1.90E+05	5.28E+00		1.30E+03	3.113943		3.90E+02	2.591065
	3.10E+05	5.49E+00	mean	7.20E+02	2.825463	mean	2.25E+02	2.329019
	1.50E+05	5.18E+00	SD	279.2158	0.170171	SD	80.54303	0.140982
	3.70E+04	4.57E+00						
mean	1.52E+05	5.15E+00						
SD	59863.82	1.86E-01						

Standard Curve 2 (File name: CN_Log_HC-LC-LOD_40_Std2_vf.xlsx)

HC (100000)			LC (400)			LOD (100)			
Date	CN	Log	Date	CN	Log	Date	CN	Log	
4/23/2012 1E5 13Rep Std2(6/7/12)	5.70E+04	4.76E+00	4/23/2012 400cp13Rep std2(6/7/12)	2.20E+02	2.342423	4/23/2012 400cp3Rep dil std2(6/7/12)	5.50E+01	1.740363	
	9.70E+04	4.99E+00		2.40E+02	2.380211		6.60E+01	1.819544	
	7.90E+04	4.90E+00		2.10E+02	2.322219		3.60E+01	1.556303	
	1.10E+05	5.04E+00	2.80E+02	2.447158	4/25/2012 100cp23Rep std2(6/7/12)	6.70E+01	1.826075		
	4.20E+04	4.62E+00	1.10E+02	2.041393		6.50E+01	1.812913		
	6.30E+04	4.80E+00	1.40E+02	2.146128		5.50E+01	1.740363		
	4.50E+04	4.65E+00	1.40E+02	2.146128		5.10E+01	1.70757		
	4.30E+04	4.63E+00	1.60E+02	2.20412		3.30E+01	1.518514		
	4.80E+04	4.68E+00	1.10E+02	2.041393		6.80E+01	1.832509		
	5.60E+04	4.75E+00	1.40E+02	2.146128		7.80E+01	1.892095		
	3.20E+04	4.51E+00	1.10E+02	2.041393		9.40E+01	1.973128		
	4.80E+04	4.68E+00	1.30E+02	2.113943		5.20E+01	1.716003		
	4.70E+04	4.67E+00	2.60E+02	2.414973		7.40E+01	1.869232		
5/18/2012 std2(6/7/12)	7.90E+04	4.90E+00	4/25/2012 std2(6/7/12)	2.80E+02	2.447158	7.00E+01 1.845098 7.60E+01 1.880814 9.80E+01 1.991226 1.00E+02 2 4.30E+01 1.633468 5.60E+01 1.748188 6.50E+01 1.812913 1.10E+02 2.041393 6.30E+01 1.799341 8.80E+01 1.944483 6.90E+01 1.838849 6.00E+01 1.778151 3.30E+01 1.518514 5/24/2012 std2(6/7/12) 100cp14Rep 7.20E+01 1.857332 7.80E+01 1.892095 8.00E+01 1.90309 1.30E+02 2.113943 6.30E+01 1.799341 6.90E+01 1.838849 6.60E+01 1.819544 7.70E+01 1.886491 5.90E+01 1.770852 7.70E+01 1.886491 7.50E+01 1.875061 8.00E+01 1.90309 1.90E+02 2.278754 1.30E+02 2.113943 mean 7.43E+01 1.844398 SD 28.61324 0.150792	7.00E+01	1.845098	
	8.50E+04	4.93E+00		1.50E+02	2.176091		7.60E+01	1.880814	
	5.00E+04	4.70E+00		1.50E+02	2.176091		9.80E+01	1.991226	
	8.90E+04	4.95E+00	5/18/2012 std2(6/7/12)	3.10E+02	2.491362		1.00E+02	2	
	8.40E+04	4.92E+00		2.40E+02	2.380211		4.30E+01	1.633468	
	1.20E+05	5.08E+00		1.60E+02	2.20412		5.60E+01	1.748188	
	6.20E+04	4.79E+00		3.60E+02	2.556303		6.50E+01	1.812913	
	8.70E+04	4.94E+00		2.20E+02	2.342423		1.10E+02	2.041393	
	7.30E+04	4.86E+00	5/24/2012 std2(6/7/12) 400cp19Rep	3.10E+02	2.491362		6.30E+01	1.799341	
	7.50E+04	4.88E+00		3.00E+02	2.477121		8.80E+01	1.944483	
	1.10E+05	5.04E+00		2.30E+02	2.361728		6.90E+01	1.838849	
	7.80E+04	4.89E+00		3.60E+02	2.556303		6.00E+01	1.778151	
	1.20E+05	5.08E+00		2.20E+02	2.342423		3.30E+01	1.518514	
	8.00E+04	4.90E+00		2.90E+02	2.462398		7.20E+01	1.857332	
	9.60E+04	4.98E+00		4.00E+02	2.60206		7.80E+01	1.892095	
	8.10E+04	4.91E+00		3.70E+02	2.568202		8.00E+01	1.90309	
	1.30E+05	5.11E+00		2.70E+02	2.431364		1.30E+02	2.113943	
	7.30E+04	4.86E+00		3.40E+02	2.531479		6.30E+01	1.799341	
	9.80E+04	4.99E+00		3.90E+02	2.591065		6.90E+01	1.838849	
	1.60E+05	5.20E+00		3.50E+02	2.544068		6.60E+01	1.819544	
	1.10E+05	5.04E+00		2.10E+02	2.322219		7.70E+01	1.886491	
	1.50E+05	5.18E+00		6.30E+02	2.799341		5.90E+01	1.770852	
	8.80E+04	4.94E+00	3.10E+02	2.491362	7.70E+01		1.886491		
	8.30E+04	4.92E+00	2.10E+02	2.322219	7.50E+01		1.875061		
	1.60E+05	5.20E+00	5.00E+02	2.69897	8.00E+01		1.90309		
	1.40E+05	5.15E+00	2.70E+02	2.431364	1.90E+02		2.278754		
	1.10E+05	5.04E+00	2.50E+02	2.39794	1.30E+02		2.113943		
	1.90E+05	5.28E+00	mean	2.58E+02	2.374609		mean	7.43E+01	1.844398
	8.40E+04	4.92E+00	SD	1.10E+02	0.184353		SD	28.61324	0.150792
	1.40E+05	5.15E+00							
	mean	8.96E+04	4.92E+00						
	SD	36419.67	1.80E-01						

Standard Curve 3 (File name: CN_Log_HC-LC-LOD_40_Std3_vf.xlsx)

HC (100000)			LC (400)			LOD (100)		
Date	CN	Log	Date	CN	Log	Date	CN	Log
4/23/2012 (stdn3) 1E5_13Rep	3.70E+04	4.57E+00	4/23/2012 stdn3 400cp13Rep	1.80E+02	2.255273	4/23/2012 stdn3 400cp3Rep dil	3.40E+01	1.531479
	6.40E+04	4.81E+00		7.00E+01	1.845098		4.10E+01	1.612784
	5.20E+04	4.72E+00		8.50E+01	1.929419		2.20E+01	1.342423
	7.40E+04	4.87E+00		8.90E+01	1.94939	4/25/2012 100cp23Rep stdn3	3.40E+01	1.531479
	2.80E+04	4.45E+00		1.00E+02	2		3.20E+01	1.50515
	4.10E+04	4.61E+00		6.70E+01	1.826075		2.00E+01	1.30103
	2.90E+04	4.46E+00		8.50E+01	1.929419		4.20E+01	1.623249
	2.80E+04	4.45E+00		7.10E+01	1.851258		4.80E+01	1.681241
	3.10E+04	4.49E+00		8.40E+01	1.924279		5.90E+01	1.770852
	3.60E+04	4.56E+00		1.60E+02	2.20412		3.20E+01	1.50515
	2.10E+04	4.32E+00		1.40E+02	2.146128		4.60E+01	1.662758
	3.10E+04	4.49E+00		1.50E+02	2.176091		4.40E+01	1.643453
	3.00E+04	4.48E+00		1.30E+02	2.113943		4.70E+01	1.672098
							6.10E+01	1.78533
							6.50E+01	1.812913
							2.60E+01	1.414973
5/18/2012 (stdn3)	5.00E+04	4.70E+00	4/25/2012 400cp3Rep stdn3	1.80E+02	2.255273		3.50E+01	1.544068
	5.20E+04	4.72E+00		9.10E+01	1.959041		4.00E+01	1.60206
	5.60E+04	4.75E+00		9.60E+01	1.982271		7.00E+01	1.845098
	3.30E+04	4.52E+00	5/18/2012 stdn3	2.10E+02	2.322219		3.90E+01	1.591065
	5.80E+04	4.76E+00		1.60E+02	2.20412		5.40E+01	1.732394
	5.50E+04	4.74E+00		1.10E+02	2.041393		4.20E+01	1.623249
	7.70E+04	4.89E+00		2.40E+02	2.380211		4.10E+01	1.612784
	4.00E+04	4.60E+00	5/24/2012 stdn3 400cp19Rep	1.50E+02	2.176091		4.30E+01	1.633468
	5.70E+04	4.76E+00		2.10E+02	2.322219		3.70E+01	1.568202
	4.80E+04	4.68E+00		1.70E+02	2.230449		2.10E+01	1.322219
	4.90E+04	4.69E+00		2.50E+02	2.39794	5/24/2012 100cp14Rep Std3	4.10E+01	1.612784
	7.30E+04	4.86E+00		1.60E+02	2.20412		4.80E+01	1.681241
	5.10E+04	4.71E+00		2.20E+02	2.342423		4.50E+01	1.653213
	7.80E+04	4.89E+00		1.30E+02	2.113943		3.70E+01	1.568202
	5.20E+04	4.72E+00		3.90E+02	2.591065		4.90E+01	1.690196
	6.30E+04	4.80E+00		1.90E+02	2.278754		4.80E+01	1.681241
	5.30E+04	4.72E+00		2.30E+02	2.361728		5.00E+01	1.69897
	8.20E+04	4.91E+00		2.50E+02	2.39794		4.60E+01	1.662758
	4.80E+04	4.68E+00		2.00E+02	2.30103		8.10E+01	1.908485
	6.40E+04	4.81E+00		1.90E+02	2.278754		5.00E+01	1.69897
	1.00E+05	5.00E+00		1.40E+02	2.146128		3.90E+01	1.591065
	7.00E+04	4.85E+00		2.30E+02	2.361728		1.20E+02	2.079181
	9.60E+04	4.98E+00		1.30E+02	2.113943		4.30E+01	1.633468
	5.70E+04	4.76E+00		1.40E+02	2.146128		8.40E+01	1.924279
	5.40E+04	4.73E+00		1.80E+02	2.255273			
	1.00E+05	5.00E+00		1.70E+02	2.230449			
	9.00E+04	4.95E+00		3.10E+02	2.491362			
	7.00E+04	4.85E+00	mean	1.63E+02	2.175912	mean	4.64E+01	1.638876
	1.20E+05	5.08E+00	SD	69.1557	0.18515	SD	18.32163	0.153999
	5.50E+04	4.74E+00						
mean	5.70E+04	4.72E+00						
SD	22353.18	1.73E-01						

Table S4.2.4 (Figure S4.2.4) Quantitative real-time PCR of 50 patients' viral DNA was run side-by-side with calibrated DNA standard reagents for creating standard curve 1 (1E6, 1E5, 1E4, 1E3, 1E2) with controls of LC (low copy: LCA, LCA-B)), CAP (VLS9, V3, V4), NTC (water), IPC (HEX) within the same 96-well plate for intra-assay comparison.
(File name: 03-21-2012_std1-patients_CN_vf.xlsx)

Sample ID	Copy/mL
1.00E+05	1.00E+05
1.00E+05	1.00E+05
1.00E+05	1.00E+05
1129:Z0	6.70E+03
1201:Z00	2.20E+02
0108:Z000	8.40E+00
1124:Z0	3.50E+01
1125:Z0	1.40E+02
0131:Z00	2.70E+02
0206:Z000	3.50E+02
0109:Z00	3.00E+04
2-B	9.00E+01
1124:Z0	1.40E+02
0112:Z00	5.60E+04
0116:Z00	2.40E+03
1128:ZR	7.70E+01
0119:Z0R	6.90E+01
LCA-B	3.60E+02
1121:Z00023R	4.40E+01
VL510	1.90E+02
1201:Z11R	1.50E+02
1128:16R	1.90E+02
VLS9	1.50E+04
1127:05R	1.10E+02
1129:Z3R	9.10E+00
LCA	3.10E+02
LCA	4.70E+02
LCA	5.50E+02
0103:Z5R	1.00E+01
V3	1.90E+03
1128:41R	1.80E+02
0110:Z1S	2.20E+01
1201:ZR	N/A
1205:Z0R	3.90E+02
Z005R	9.00E+04
Z0015R	N/A
1201:Z7R	7.40E+01
0105:Z18R	5.10E+04
0106:Z35R	N/A
1205:Z5R	3.30E+02

1205:Z8R	9.40E+02
Water	N/A
1.00E+03	1.00E+03
1.00E+03	1.00E+03
1.00E+03	1.00E+03
1.00E+06	1.00E+06
1.00E+06	1.00E+06
1.00E+06	1.00E+06
1.00E+04	1.00E+04
1.00E+04	1.00E+04
1.00E+04	1.00E+04
1.00E+02	1.00E+02
1.00E+02	1.00E+02
1.00E+02	1.00E+02

Table S5.1 Replication experiments performed for determining the day-to-day variation and standard-to-standard variation across three different standard curves (standard curve 1, standard curve 2, and standard curve 3) (CN – Copy Number per mL; LOD – Limit of Detection; SD – standard deviation). (File name: Overall-Stnds-1-2-3_100cp_LOD_variation.xlsx)

Std1			std2(6/7/12)			Std3		
LOD (100)			LOD (100)			LOD (100)		
Date	CN	Log	Date	CN	Log	Date	CN	Log
4/23/2012	1.60E+02	2.20412	4/23/2012	5.50E+01	1.740363	4/23/2012	3.40E+01	1.531479
	2.00E+02	2.30103		6.60E+01	1.819544		4.10E+01	1.612784
	1.10E+02	2.041393		3.60E+01	1.556303		2.20E+01	1.342423
4/25/2012	1.60E+02	2.20412	4/25/2012	6.70E+01	1.826075	4/25/2012	3.40E+01	1.531479
	1.10E+02	2.041393		6.50E+01	1.812913		3.20E+01	1.50515
	2.10E+02	2.322219		5.50E+01	1.740363		2.00E+01	1.30103
	1.70E+02	2.230449		5.10E+01	1.70757		4.20E+01	1.623249
	2.40E+02	2.380211		3.30E+01	1.518514		4.80E+01	1.681241
	2.80E+02	2.447158		6.80E+01	1.832509		5.90E+01	1.770852
	1.60E+02	2.20412		7.80E+01	1.892095		3.20E+01	1.50515
	2.20E+02	2.342423		9.40E+01	1.973128		4.60E+01	1.662758
	2.20E+02	2.342423		5.20E+01	1.716003		4.40E+01	1.643453
	2.30E+02	2.361728		7.40E+01	1.869232		4.70E+01	1.672098
	2.90E+02	2.462398		7.00E+01	1.845098		6.10E+01	1.78533

	3.10E+02	2.49136 2		7.60E+01	1.880814		6.50E+01	1.812913
	1.40E+02	2.14612 8		9.80E+01	1.991226		2.60E+01	1.414973
	1.70E+02	2.23044 9		1.00E+02	2		3.50E+01	1.544068
	2.00E+02	2.30103		4.30E+01	1.633468		4.00E+01	1.60206
	3.30E+02	2.51851 4		5.60E+01	1.748188		7.00E+01	1.845098
	1.90E+02	2.27875 4		6.50E+01	1.812913		3.90E+01	1.591065
	2.60E+02	2.41497 3		1.10E+02	2.041393		5.40E+01	1.732394
	2.10E+02	2.32221 9		6.30E+01	1.799341		4.20E+01	1.623249
	2.00E+02	2.30103		8.80E+01	1.944483		4.10E+01	1.612784
	2.10E+02	2.32221 9		6.90E+01	1.838849		4.30E+01	1.633468
	1.90E+02	2.27875 4		6.00E+01	1.778151		3.70E+01	1.568202
	1.10E+02	2.04139 3		3.30E+01	1.518514		2.10E+01	1.322219
5/24/2012 100cp14Rep Std1	2.00E+02	2.30103	5/24/2012 std2(6/7/1 2) 100cp14Rep	7.20E+01	1.857332	5/24/2012 100cp14Rep Std3	4.10E+01	1.612784
	2.30E+02	2.36172 8		7.80E+01	1.892095		4.80E+01	1.681241
	2.20E+02	2.34242 3		8.00E+01	1.90309		4.50E+01	1.653213
	1.80E+02	2.25527 3		1.30E+02	2.113943		3.70E+01	1.568202
	2.40E+02	2.38021 1		6.30E+01	1.799341		4.90E+01	1.690196
	2.30E+02	2.36172 8		6.90E+01	1.838849		4.80E+01	1.681241
	2.40E+02	2.38021 1		6.60E+01	1.819544		5.00E+01	1.69897
	2.30E+02	2.36172 8		7.70E+01	1.886491		4.60E+01	1.662758
	3.80E+02	2.57978 4		5.90E+01	1.770852		8.10E+01	1.908485
	2.40E+02	2.38021 1		7.70E+01	1.886491		5.00E+01	1.69897
	1.90E+02	2.27875 4		7.50E+01	1.875061		3.90E+01	1.591065
	5.40E+02	2.73239 4		8.00E+01	1.90309		1.20E+02	2.079181
	2.10E+02	2.32221 9		1.90E+02	2.278754		4.30E+01	1.633468
	3.90E+02	2.59106 5		1.30E+02	2.113943		8.40E+01	1.924279
mean	2.25E+02	2.32901 9	mean	7.43E+01	1.844398	mean	4.64E+01	1.638876

SD	80.54303	0.14098 2	SD	28.61324	0.150792	SD	18.32163	0.153999
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Overall Log SD (across all three standard curves): 0.006782

Table S5.2 Replication experiments performed for determining the day-to-day variation and standard-to-standard variation across three different standard curves (standard curve 1, standard curve 2, and standard curve 3) (CN – Copy Number per mL; HC – High Copy number; SD – standard deviation). (File name: Overall-Stnds-1-2-3_HC_variation_1Column.xlsx)

stnd1 HC (100000)			Stnd2(6/7/12) HC (100000)			stnd3 HC (100000)		
Date	CN	Log	Date	CN	Log	Date	CN	Log
4/23/2012	1.00E+05	5.00E+00	4/23/2012	5.70E+04	4.76E+00	4/23/2012	3.70E+04	4.57E+00
	1.70E+05	5.23E+00		9.70E+04	4.99E+00		6.40E+04	4.81E+00
	1.40E+05	5.15E+00		7.90E+04	4.90E+00		5.20E+04	4.72E+00
	2.00E+05	5.30E+00		1.10E+05	5.04E+00		7.40E+04	4.87E+00
	8.00E+04	4.90E+00		4.20E+04	4.62E+00		2.80E+04	4.45E+00
	1.10E+05	5.04E+00		6.30E+04	4.80E+00		4.10E+04	4.61E+00
	8.40E+04	4.92E+00		4.50E+04	4.65E+00		2.90E+04	4.46E+00
	8.10E+04	4.91E+00		4.30E+04	4.63E+00		2.80E+04	4.45E+00
	8.90E+04	4.95E+00		4.80E+04	4.68E+00		3.10E+04	4.49E+00
	1.00E+05	5.00E+00		5.60E+04	4.75E+00		3.60E+04	4.56E+00
	6.10E+04	4.79E+00		3.20E+04	4.51E+00		2.10E+04	4.32E+00
	8.90E+04	4.95E+00		4.80E+04	4.68E+00		3.10E+04	4.49E+00
	8.70E+04	4.94E+00		4.70E+04	4.67E+00		3.00E+04	4.48E+00
5/18/2012	1.40E+05	5.15E+00	5/18/2012	7.90E+04	4.90E+00	5/18/2012	5.00E+04	4.70E+00
	1.50E+05	5.18E+00		8.50E+04	4.93E+00		5.20E+04	4.72E+00
	9.30E+04	4.97E+00		5.00E+04	4.70E+00		5.60E+04	4.75E+00
	1.60E+05	5.20E+00		8.90E+04	4.95E+00		3.30E+04	4.52E+00
	1.50E+05	5.18E+00		8.40E+04	4.92E+00		5.80E+04	4.76E+00
	2.00E+05	5.30E+00		1.20E+05	5.08E+00		5.50E+04	4.74E+00
	1.10E+05	5.04E+00		6.20E+04	4.79E+00		7.70E+04	4.89E+00
	1.50E+05	5.18E+00		8.70E+04	4.94E+00		4.00E+04	4.60E+00
	1.30E+05	5.11E+00		7.30E+04	4.86E+00		5.70E+04	4.76E+00
	1.30E+05	5.11E+00		7.50E+04	4.88E+00		4.80E+04	4.68E+00
	2.00E+05	5.30E+00		1.10E+05	5.04E+00		4.90E+04	4.69E+00
	1.40E+05	5.15E+00		7.80E+04	4.89E+00		7.30E+04	4.86E+00
	2.10E+05	5.32E+00		1.20E+05	5.08E+00		5.10E+04	4.71E+00
	1.40E+05	5.15E+00		8.00E+04	4.90E+00		7.80E+04	4.89E+00
	1.70E+05	5.23E+00		9.60E+04	4.98E+00		5.20E+04	4.72E+00
	1.40E+05	5.15E+00		8.10E+04	4.91E+00		6.30E+04	4.80E+00
	2.20E+05	5.34E+00		1.30E+05	5.11E+00		5.30E+04	4.72E+00
	1.30E+05	5.11E+00		7.30E+04	4.86E+00		8.20E+04	4.91E+00
	1.70E+05	5.23E+00		9.80E+04	4.99E+00		4.80E+04	4.68E+00
	2.70E+05	5.43E+00		1.60E+05	5.20E+00		6.40E+04	4.81E+00
	1.90E+05	5.28E+00		1.10E+05	5.04E+00		1.00E+05	5.00E+00
	2.50E+05	5.40E+00		1.50E+05	5.18E+00		7.00E+04	4.85E+00

	1.60E+05	5.20E+00		8.80E+04	4.94E+00		9.60E+04	4.98E+00
	1.50E+05	5.18E+00		8.30E+04	4.92E+00		5.70E+04	4.76E+00
	2.70E+05	5.43E+00		1.60E+05	5.20E+00		5.40E+04	4.73E+00
	2.30E+05	5.36E+00		1.40E+05	5.15E+00		1.00E+05	5.00E+00
	1.90E+05	5.28E+00		1.10E+05	5.04E+00		9.00E+04	4.95E+00
	3.10E+05	5.49E+00		1.90E+05	5.28E+00		7.00E+04	4.85E+00
	1.50E+05	5.18E+00		8.40E+04	4.92E+00		1.20E+05	5.08E+00
	3.70E+04	4.57E+00		1.40E+05	5.15E+00		5.50E+04	4.74E+00
mean	1.52E+05	5.15E+00	mean	8.96E+04	4.92E+00	mean	5.70E+04	4.72E+00
SD	59863.82	1.86E-01	SD	36419.67	1.80E-01	SD	22353.18	1.73E-01

Overall Log SD across all three standard curves:

0.006186

Table S5.3 Replication experiments performed for determining the day-to-day variation and standard-to-standard variation across three different standard curves (standard curve 1, standard curve 2, and standard curve 3) (CN – Copy Number per mL; **LC – Low Copy number**; SD – standard deviation) (File name: Overall-Stnds-1-2-3_LC_variation-1column.xlsx)

stnd1 LC (400)			stnd2(6/7/12) LC (400)			stnd3 LC (400)		
Date	CN	Log	Date	CN	Log	Date	CN	Log
4/23/2012	7.80E+02	2.8920946	4/23/2012	2.20E+02	2.34242268	4/23/2012	1.80E+02	2.25527251
	3.30E+02	2.51851394		2.40E+02	2.38021124		7.00E+01	1.84509804
	4.00E+02	2.60205999		2.10E+02	2.32221929		8.50E+01	1.92941893
	4.20E+02	2.62324929		2.80E+02	2.44715803		8.90E+01	1.94939001
	4.70E+02	2.67209786		1.10E+02	2.04139269		1.00E+02	2
	3.20E+02	2.50514998		1.40E+02	2.14612804		6.70E+01	1.8260748
	4.00E+02	2.60205999		1.40E+02	2.14612804		8.50E+01	1.92941893
	3.30E+02	2.51851394		1.60E+02	2.20411998		7.10E+01	1.85125835
	3.90E+02	2.59106461		1.10E+02	2.04139269		8.40E+01	1.92427929
	7.10E+02	2.85125835		1.40E+02	2.14612804		1.60E+02	2.20411998
	6.20E+02	2.79239169		1.10E+02	2.04139269		1.40E+02	2.14612804
	6.70E+02	2.8260748		1.30E+02	2.11394335		1.50E+02	2.17609126
	5.90E+02	2.77085201		2.60E+02	2.41497335		1.30E+02	2.11394335
4/25/2012	7.70E+02	2.88649073	4/25/2012	2.80E+02	2.44715803	4/25/2012	1.80E+02	2.25527251
	4.20E+02	2.62324929		1.50E+02	2.17609126		9.10E+01	1.95904139
	4.50E+02	2.65321251		1.50E+02	2.17609126		9.60E+01	1.98227123
5/18/2012 stnd1	9.20E+02	2.96378783	5/18/2012	3.10E+02	2.49136169	5/18/2012	2.10E+02	2.32221929
	7.30E+02	2.86332286		2.40E+02	2.38021124		1.60E+02	2.20411998
	5.00E+02	2.69897		1.60E+02	2.20411998		1.10E+02	2.04139269
	1.00E+03	3		3.60E+02	2.5563025		2.40E+02	2.38021124
	6.60E+02	2.81954394		2.20E+02	2.34242268		1.50E+02	2.17609126
5/24/2012	9.10E+02	2.95904139	5/24/2012	3.10E+02	2.49136169	5/24/2012	2.10E+02	2.32221929
	7.60E+02	2.88081359		3.00E+02	2.47712125		1.70E+02	2.23044892
	1.10E+03	3.04139269		2.30E+02	2.36172784		2.50E+02	2.39794001
	7.00E+02	2.84509804		3.60E+02	2.5563025		1.60E+02	2.20411998
	9.40E+02	2.97312785		2.20E+02	2.34242268		2.20E+02	2.34242268
	5.90E+02	2.77085201		2.90E+02	2.462398		1.30E+02	2.11394335
	1.60E+03	3.20411998		4.00E+02	2.60205999		3.90E+02	2.59106461

	8.50E+02	2.92941893		3.70E+02	2.56820172		1.90E+02	2.2787536
	9.90E+02	2.99563519		2.70E+02	2.43136376		2.30E+02	2.36172784
	1.10E+03	3.04139269		3.40E+02	2.53147892		2.50E+02	2.39794001
	8.50E+02	2.92941893		3.90E+02	2.59106461		2.00E+02	2.30103
	8.20E+02	2.91381385		3.50E+02	2.54406804		1.90E+02	2.2787536
	6.20E+02	2.79239169		2.10E+02	2.32221929		1.40E+02	2.14612804
	1.00E+03	3		6.30E+02	2.79934055		2.30E+02	2.36172784
	5.90E+02	2.77085201		3.10E+02	2.49136169		1.30E+02	2.11394335
	6.30E+02	2.79934055		2.10E+02	2.32221929		1.40E+02	2.14612804
	8.00E+02	2.90308999		5.00E+02	2.69897		1.80E+02	2.25527251
	7.60E+02	2.88081359		2.70E+02	2.43136376		1.70E+02	2.23044892
	1.30E+03	3.11394335		2.50E+02	2.39794001		3.10E+02	2.49136169
mean	7.20E+02	2.82546286	mean	2.58E+02	2.37460886	mean	1.63E+02	2.17591218
SD	279.21582	0.17017053	SD	1.10E+02	0.1843528	SD	69.1556958	0.18515036

Overall Log SD across all the
three standard curves:

0.00843

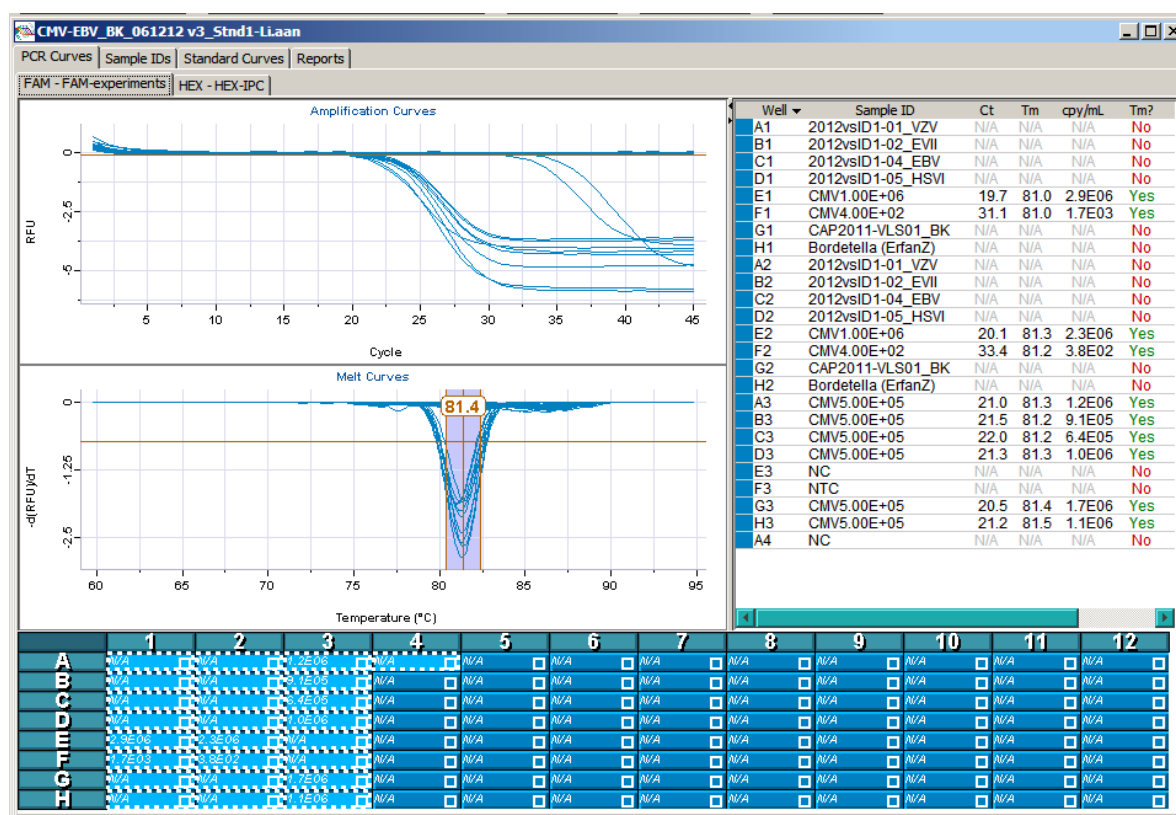


Figure S5. Analytical specificity was established first by different control virus sample studies of CMV, BK, EBV, EVII, HSVI, bordetella bacterial DNA (**quantitation with the standard curve 1**). "2012 viral survey ID1-01_VZV" was for Varicella-zoster virus. "2012_ID1-02_EVII" was positive for echovirus type II. "2012 viral survey ID1-04_EBV" was positive for Epstein-Barr virus. "2012vsID1-05_HSVI" was positive for Herpes simplex type I virus. "CAP2011-VLS01_BK" was positive for BK viral load (performed on June 12, 2012).

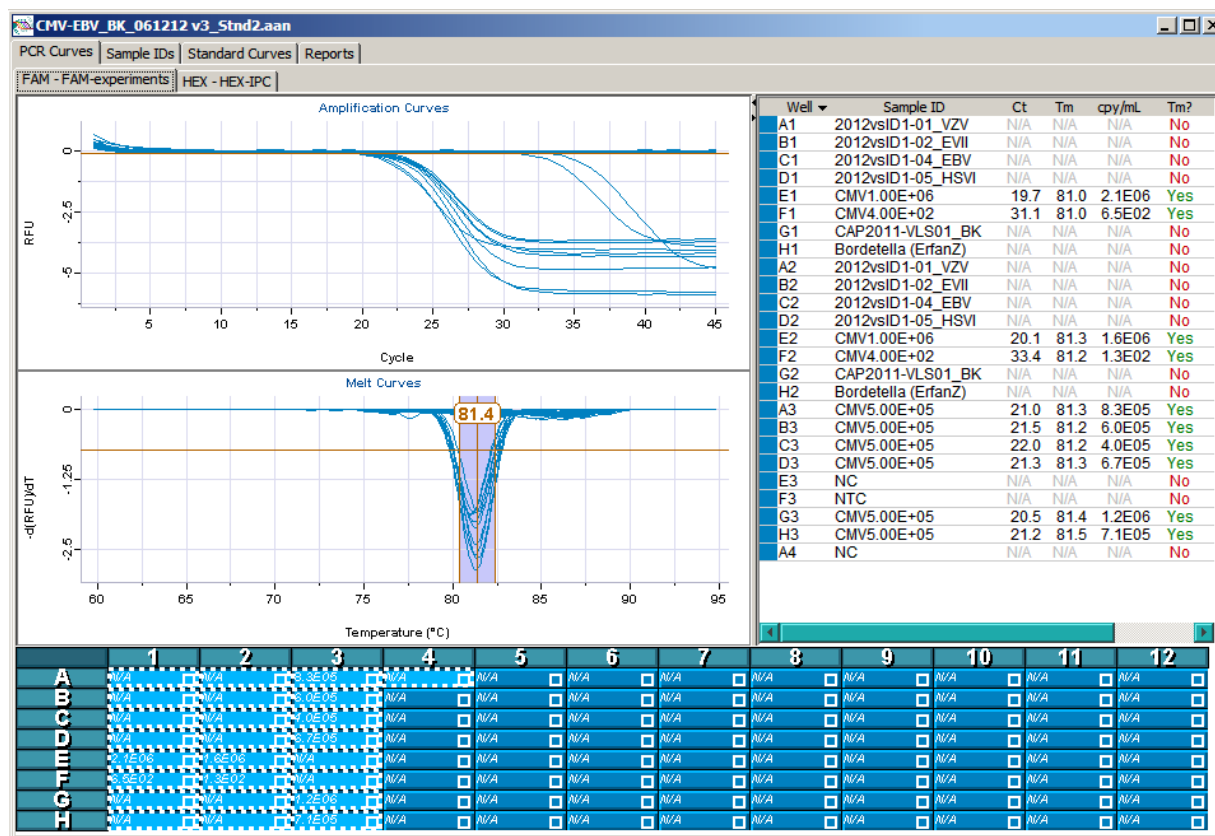


Figure S6. Analytical specificity was established first by different control virus sample studies of CMV, BK, EBV, EVII, HSVI, bordetella bacterial DNA (**quantitation with the standard curve 2**). "2012 viral survey ID1-01_VZV" was for Varicella-zoster virus. "2012_ID1-02_EVII" was positive for echovirus type II. "2012 viral survey ID1-04_EBV" was positive for Epstein-Barr virus. "2012vsID1-05_HSVI" was positive for Herpes simplex type I virus. "CAP2011-VLS01_BK" was positive for BK viral load (performed on June 12, 2012).

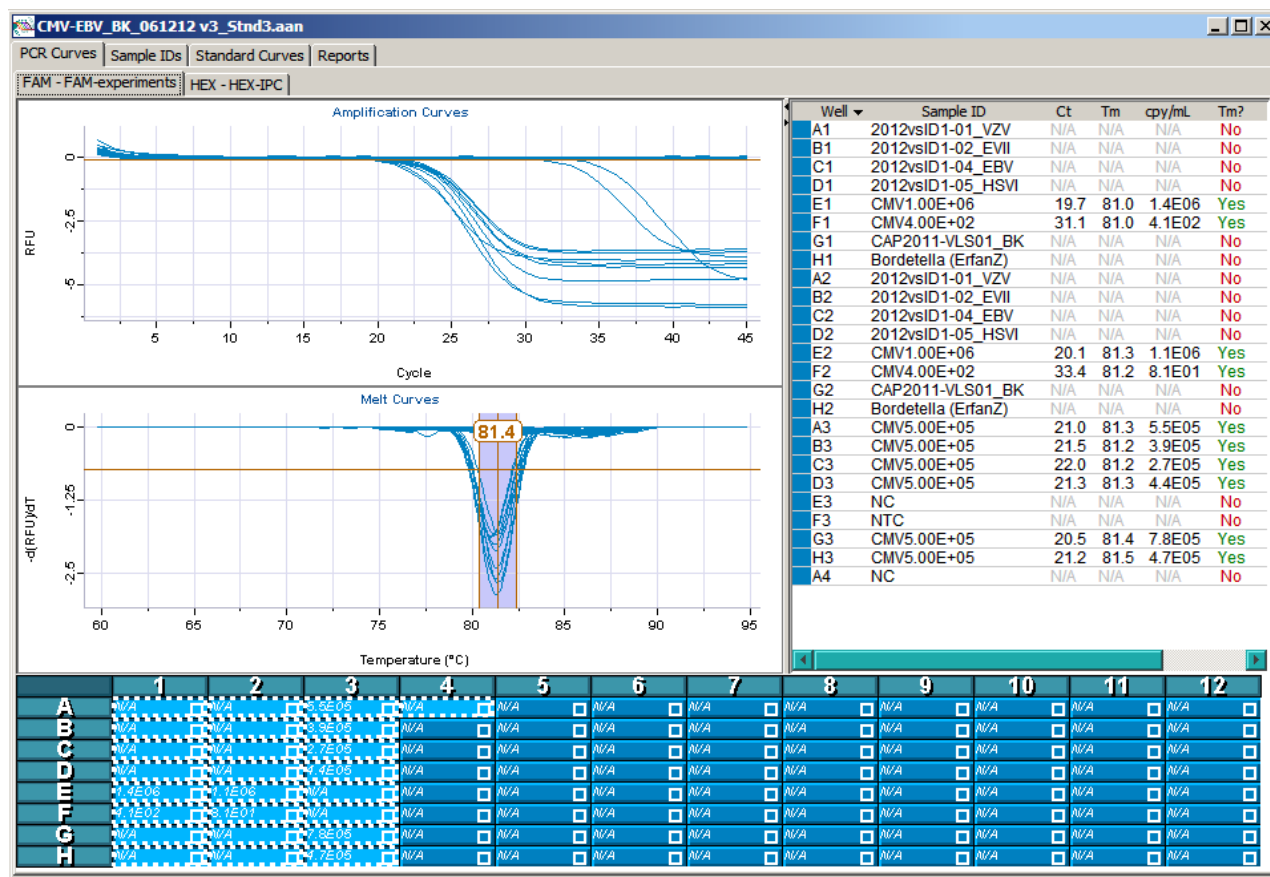


Figure S7. Analytical specificity was established first by different control virus sample studies of CMV, BK, EBV, EVII, HSVI, bordetella bacterial DNA (**quantitation with the standard curve 3**). "2012 viral survey ID1-01_VZV" was for Varicella-zoster virus. "2012_ID1-02_EVII" was positive for echovirus type II. "2012 viral survey ID1-04_EBV" was positive for Epstein-Barr virus. "2012vsID1-05_HSVI" was positive for Herpes simplex type I virus. "CAP2011-VLS01_BK" was positive for BK viral load (performed on June 12, 2012).

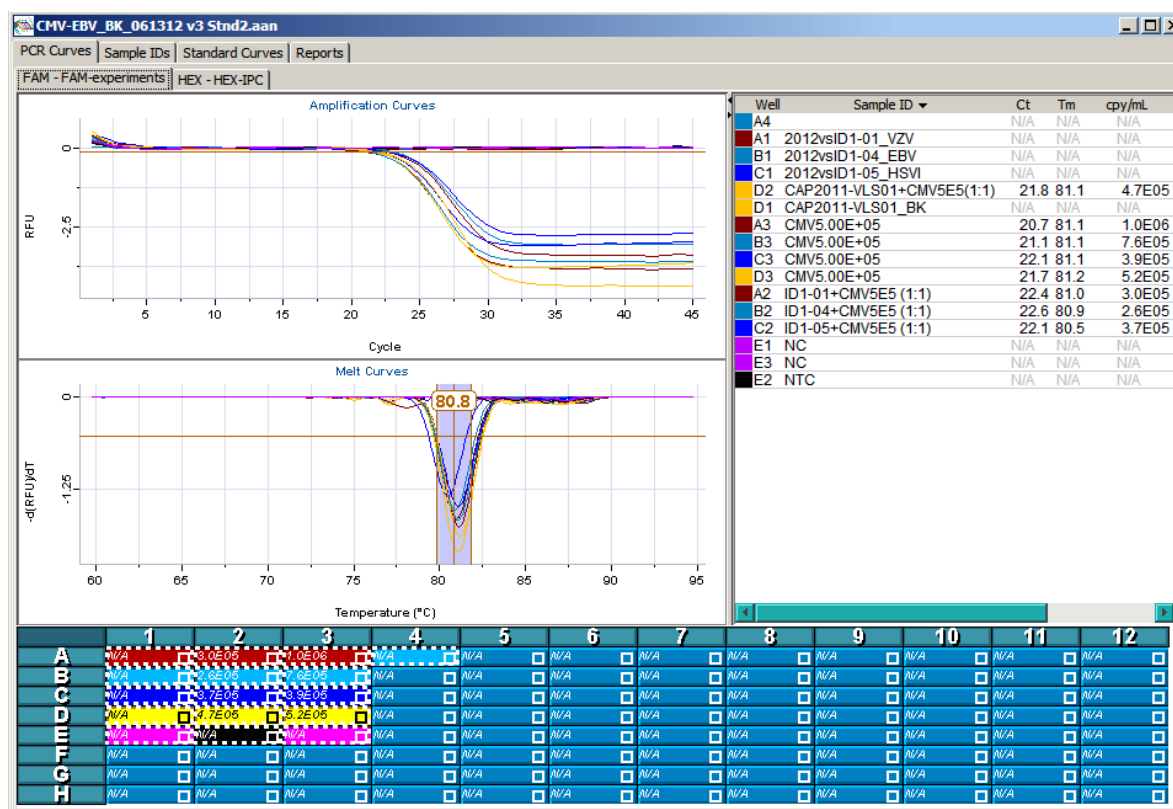


Figure S8. Analytical specificity was established secondly by interference study of CMV with BK, EBV, HSVI (quantitation with the standard curve 2). "2012 viral survey ID1-01_VZV" was for Varicella-zoster virus. "2012 viral survey ID1-04_EBV" was positive for Epstein-Barr virus. "2012vsID1-05_HSVI" was positive for Herpes simplex type I virus. "CAP2011-VLS01_BK" was positive for BK viral load.

Note: In all the mixtures, 2.5 micro-liter of CAP were mixed with 2.5 micro-liter of CMV 5E5 ($5.00E+05$) while in the controls either 5.0 micro-liter of CMV 5E5 ($5.00E+05$) or 5.0 micro-liter of CAP samples were used (performed on June 13, 2012).

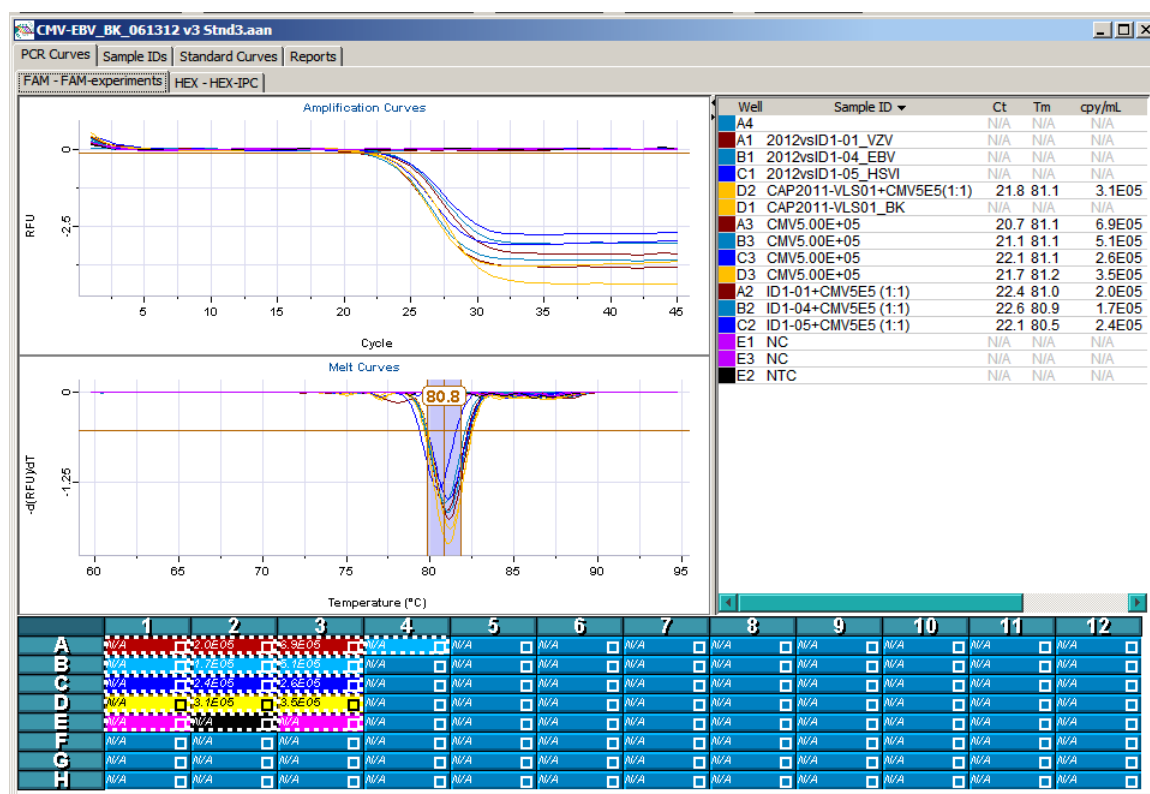


Figure S9. Analytical specificity was established second by interference study of CMV with BK, EBV, HSVI (quantitation with the standard curve 3). "2012 viral survey ID1-01_VZV" was for Varicella-zoster virus. "2012 viral survey ID1-04_EBV" was positive for Epstein-Barr virus. "2012vsID1-05_HSVI" was positive for Herpes simplex type I virus. "CAP2011-VLS01_BK" was positive for BK viral load. Note: In all the mixtures, 2.5 micro-liter of CAP were mixed with 2.5 micro-liter of CMV 5E5 ($5.00E+05$) while in the controls either 5.0 micro-liter of CMV 5E5 ($5.00E+05$) or 5.0 micro-liter of CAP samples were used (performed on June 12, 2012).