M84

QUANTITATIVE REAL TIME PCR ON THE 3M INTEGRATED CYCLER REAL TIME PCR SYSTEM

Heather Gregson*, Mark Dobbs, Peter Lee, Regina Martin, Albert Castro, Robert Hazelo, Benedict Archer, Vishnu Mishra, and Maurice Exner

Focus Diagnostics Inc., Cypress, CA

Revised Abstract/Introduction

Background: The 3M Integrated Cycler is a rapid thermal cycling device that amplifies nucleic acid using a microfluidic disk. Instrument software has been developed that utilizes an innovative quantitative algorithm that provides amplification efficiencies in each reaction. We evaluated this system using 3 quantitative assays to detect cytomegalovirus (CMV), Epstein-Barr virus (EBV), and BK virus (BKV), all of which included a novel internal extraction and amplification control.

Methods: Dilutions of purified nucleic acids (ranging in concentration from 0-10^9 copies/reaction) were tested on the 3M Integrated Cycler using common cycling conditions, and quantitative results were analyzed using the Integrated Cycler Studio Software. The linear ranges and reproducibility were assessed.

Results: The instrument and software were able to provide accurate quantitation across a linear range of up to 9 orders of magnitude. Results obtained from quantitative methods using fluorescence thresholds were comparable to results from Chemical Model based PCR Analysis (CMPA) methods.

Conclusions: The 3M Integrated Cycler System is an effective alternative for quantitative assays. The System can quantitate over a broad range using two methods: a unique software algorithm, which analyzes efficiency of each individual reaction, and the traditional standard curve method.

Methods

Virus and Nucleic Acid Stocks: Viral stock material was obtained from Advanced Biotechnologies, Inc. (Columbia, MD) or American Type Tissue Collection (ATCC; Manassas, VA). Amplicons for each of the targets were generated and quantitated by NanoDrop 1000 (Wilmington, DE).

Nucleic Acid Extraction: Extraction/purification of nucleic acid from specimens, purified virus, and standards was performed on the Roche MagNA Pure LC instrument (Indianapolis, IN). A sample volume of 200µL was extracted and eluted into a 50µL volume. Extraction and Amplification Control (EAC) template DNA (200µL) was added into the MagNA Pure lysis buffer (12 mL) in a conical tube, mixed, then added into the appropriate tray on the MagNA Pure instrument.

Algorithm Comparison and Linear Range: Serially diluted DNA template (either purified virus or DNA amplicons containing the appropriate target sequence) were prepared and were run. Results were analyzed using an algorithm that involves the use of a fluorescence threshold, as well as by a novel algorithm based on a chemical model that quantifies based on reaction chemistry, and that is independent of a fluorescence threshold setting (unique to the 3M integrated cycler instrument).

Results

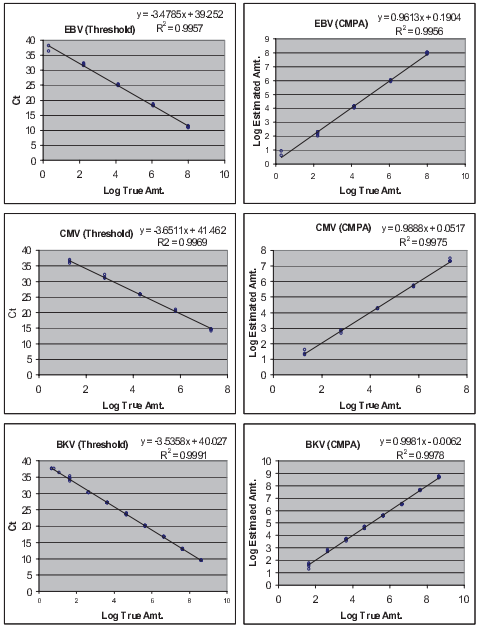

Figure 1. Linearity comparisons between Threshold and Chemical Model based PCR Analysis (CMPA) methods of quantitation, and comparison of expected and observed values in copies/reaction.

Algorithm Comparison and Linearity: The 3M Integrated Cycler can quantitate over a broad range using two methods: a unique software algorithm, which incorporates efficiency of each individual reaction, and the traditional standard curve method.. The results indicate that the chemistry based analysis method performed as well as the threshold-based method based on % error (Tables 1-3), and provided linearity across 9 orders of magnitude (Figure 1 and 2).

Results (Cont.)

Table 1. Comparison of expected and observed values for EBV assay using two algorithms for quantitative analysis.

	Chemistry Model Method		Threshold Method		
Expected Value copies/reaction	Observed Value copies/reaction	% Error	Observed Value copies/reaction	% Error	EAC Ct Value
11,553,750	11,499,218	-0.8%	11,748,748	1.7%	32.1
115,538	110,620	-4.3%	106,701	-7.6%	32.3
1,155	1,168	1.0%	1,141	-1.3%	31.9
116	131	13.6%	123	6.3%	31.3
29	51	75.0%	55	89.3%	30.9

Table 2. Comparison of expected and observed values for CMV assay using two algorithms for quantitative analysis.

	Chemistry Model Method		Threshold Method		
Expected Value copies/reaction	Observed Value copies/reaction	% Error	Observed Value copies/reaction	% Error	EAC Ct Value
20,000,000	23,230,397	16.2%	24,613,587	23.1%	31.9
631,000	513,657	-18.6%	519,480	-17.7%	31.3
20,000	18,395	-8.0%	18,682	-6.6%	30.2
631	618	-2.0%	566	-10.3%	31.4
20	27	33.6%	25	27.4%	30.7

Table 3. Comparison of expected and observed values for BKV assay using two algorithms for quantitative analysis.

	Chemistry Model Method		Threshold Method		
Expected Value copies/reaction	Observed Value copies/reaction	% Error	Observed Value copies/reaction	% Error	EAC Ct Value
438,000,000	482,919,600	10.3%	470,001,172	7.3%	39.9
43,800,000	44,433,038	1.4%	46,741,024	6.7%	39.8
4,380,000	3,264,491	-25.5%	3,937,416	-10.1%	35.3
438,000	373,827	-14.7%	446,326	1.9%	30.7
43,800	42,872	-2.1%	42,595	-2.8%	29.5
4,380	4,552	3.9%	4,124	-5.9%	29.1
438	571	30.4%	507	15.8%	29.4
44	40	-10.1%	33	-23.9%	31.7

Conclusion

- The novel algorithm, based on chemistry modeling, provided accurate quantitative results without the need to set a fluorescence threshold. This could provide advantages over a threshold-based method, because the chemical method provides an independent quantitative analysis for each sample and does not assume similar reaction efficiencies between samples.

The integrated cycler's companion Studio Software with quantitation is in development, it is not currently available for sale, and is not FDA cleared.

Results (Cont.)

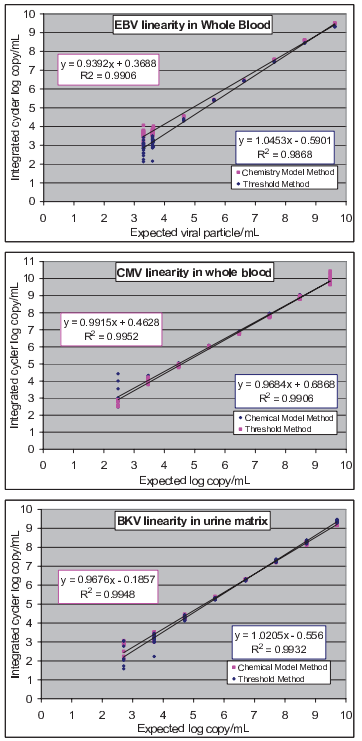

Figure 2. Dilution of purified viral particle into negative sample matrix. Quantitation was analyzed by chemistry model method and threshold method.

FOCUS Diagnostics

*Corresponding author: heatherg@focusdx.com