EVALUATION OF THE 3M INTEGRATED CYCLER, AN INNOVATIVE RAPID THERMAL CYCLING SYSTEM

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Abstract/Introduction

The 3M Integrated Cycler is a compact rapid thermal cycling instrument with multiplex real-time fluorescence detection capability. The instrument uses a uniquely designed 96-well test plate in the form of a circular disposable disc that contains microfluidic channels connecting loading ports to reaction chambers at the periphery of the disc. Liquid movement from the loading well to the reaction chambers is aided by means of centrifugal force. We evaluated the instrument's performance characteristics, including detection sensitivity, reproducibility, compatibility with different enzyme systems and thermal cycling speed. The 3M Integrated Cycler demonstrated a linear dynamic range of at least 9 orders of magnitude and it provided consistent well to well signal reproducibility. The system displayed excellent sensitivity, with detection of target DNA observed at levels down to 2 copies per reaction. All enzymes tested, including GeneAmp (Applied Biosystems), FastStart (Roche), QuantiFast (QIAGEN), Platinum Taq (Invitrogen) and GoTaq (Promega) were found to be effective on the instrument. Because the instrument has the capability to perform rapid cycling (heating rates up to 5°C per second and cooling rates up to 3°C per second), experiments were conducted to determine minimum effective cycling times. The studies indicated that the instrument was capable of running 45 amplification cycles in as little as 33 minutes without affecting assay performance. These findings indicate that the 3M Integrated Cycler is an effective real-time PCR instrument, and its rapid cycling capability and flexibility with different chemistries demonstrates its potential as a high throughput clinical diagnostic tool.

Methods

Linear Dynamic Range – Serially diluted plasmid containing the CMV target sequence was used for linearity studies. 8 replicates were tested for each dilution. In addition, the Focus Diagnostics' Simplexa™ Extraction and Amplification Control (SEAC) was added to each reaction. Following amplification, slope and R^2 values were calculated to assess amplification efficiency and linearity, respectively.

Reproducibility (Well-to-Well) – Samples were added to all 96 wells of a disc and amplification/detection was performed with all 4 detectors (FAM, JOE, CFR 610 and Q670) using an assay for detection of Influenza A H1N1 (2009), Influenza B and an Armored RNA Internal Control. Assessment of reproducibility was based on standard deviations of Ct values and %CVs calculated for all 96 wells in each of the 4 detector channels.

Sensitivity – The ability of the instrument to detect low copy numbers of target was assessed by amplifying serial dilutions of quantitated CMV viral DNA (Advanced Biotechnologies Inc). Quantities of DNA used ranged from 50,000 down to 2 copies/reaction and replicates of each concentration were analyzed.

Compatibility with Different Enzymes – Primers designed to detect B. pertussis (Bp) and B. parapertussis (Bpp) were added to PCR mastermixes containing different enzymes including GeneAmp® Fast PCR Master Mix (Applied Biosystems™), 2x QuantiFast Multiplex PCR Master Mix (QIAGEN), Platinum® *Taq* DNA Polymerase (Invitrogen™), FastStart Taq DNA Polymerase (Roche) and GoTaq® (Promega). Ct values were compared, as was the ability of each system to detect low levels of target DNA.

Rapid Cycling Parameters – Annealing and extension times were varied in an attempt to decrease thermocycling times without decreasing assay sensitivity. Rapid cycling times were tested using CMV and HSV-1 targets, and the ability to detect targets using rapid cycling protocols was analyzed.

Methods (Cont.)

Table 1. Thermocycling parameters

Study	RT	HotStart	Denaturation/Extension			Primer Conc. (nM)	
	47°C	97°C	97°C	60°C	# Cycles	Target	IC
Linear Dynamic Range	N/A	10 min	15 sec	35 sec	50	200	100
Reproducibility	15 min	10 min	15 sec	30 sec	40	600	100
Sensitivity	N/A	5 min	15 sec	35 sec	45	600	100
Enzyme Comparison	N/A	10 min	15 sec	35 sec	45	200 (Bp) 150 (Bpp)	100
Reduced Cycling (HSV-1)	See Table 5					200	100
Reduced Cycling (CMV)	See Table 5					600	100

Results

Linear Dynamic Range: Results showed that the linear dynamic range of the 3M Integrated Cycler was at least 9 orders of magnitude (Table 2 and Figure 1). Linearity was achieved even by samples with Ct values as low as 5.46. In addition, the internal control reaction was unaffected even when high target concentrations were present.

Reproducibility (Well-to Well): Reproducibility across all 96 wells and across all four detection channels demonstrated low standard deviations ranging from 0.200 to 0.397 (Figure 2 and Table 3) and low %CVs ranging from 0.641 to 1.374.

Sensitivity: The instrument was consistently able to detect viral DNA at amounts as low as 2 copies/reaction (Figure 3).

Compatibility with Different Enzymes: A comparison of all enzyme systems shows that even without any extensive optimization of reaction conditions, all enzymes/systems amplify successfully on the 3M Integrated Cycler with a maximum of 2.1 Ct difference between enzyme systems for a given target concentration (Table 4).

Rapid Cycling Parameters: In comparison to longer cycling parameters, rapid cycling parameters for both CMV and HSV-1 assays demonstrated minimal affect on the average Ct values for all concentrations (Table 5).

Table 2. Linearity over 9 orders of magnitude. Note that the internal control is consistently detected even at very high concentrations of target, and that linearity is maintained to Ct values as low as 5.46.

Log Dilution	CMV Ave Ct	CMV Std Dev	IC Ave Ct	IC Std Dev
-1	5.46	0.03	26.54	0.06
-2	8.83	0.06	26.47	
-3	12.55	0.06	26.39	
-4	16.34	0.07	26.34	
-5	19.54	0.03	26.48	
-6	22.80	0.22	26.47	
-7	26.09	0.17	26.45	
-8	29.58	0.10	26.47	
-9	33.06	0.43	26.53	

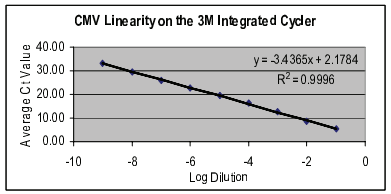

Figure 1. Linearity over 9 orders of magnitude

Results (Cont.)

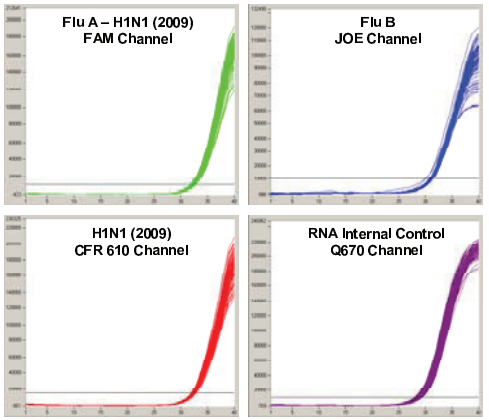

Figure 2. Well-to-Well reproducibility. Amplification curves for each channel/dye are shown for all 96 reaction wells on a 96-well disc.

Table 3. Average Ct value and standard deviation across all 96 wells in all 4 channels on a 96-well disc.

Channel	FAM	JOE	CFR610	Q670
Ave Ct.	32.41	31.22	32.55	28.86
Std. Dev	0.220	0.200	0.220	0.397
%CV	0.679	0.641	0.677	1.374

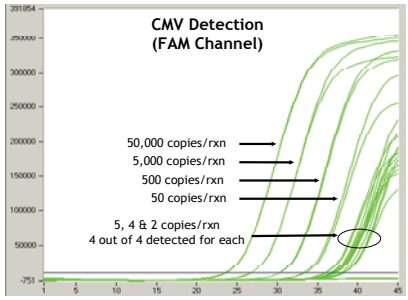

Figure 3. Sensitivity (limit of detection) studies for CMV assay showing that the 3M Integrated Cycler can routinely detect CMV viral DNA with concentrations as low as 2 copies/reaction.

Results (Cont.)

Table 4. Enzyme comparison *50 IC Copies/Rxn

Analyte	Enzyme System	Copies/Rxn		
		1000	100	10
Bp (FAM)	GeneAmp (Applied Biosystems) Run 1	27.70	31.03	34.92
	Quantifast (QIAGEN)	28.85	32.41	36.12
	Platinum Taq (Invitrogen)	30.36	33.91	35.55
Bpp (CFR 610)	GeneAmp (Applied Biosystems) Run 1	26.54	29.85	33.32
	Quantifast (QIAGEN)	26.91	30.44	34.35
	Platinum Taq (Invitrogen)	29.65	33.33	35.35
IC* (Q670)	GeneAmp (Applied Biosystems) Run 1	28.86		
	Quantifast (QIAGEN)	29.53		
	Platinum Taq (Invitrogen)	29.99		
Bp (FAM)	GeneAmp (Applied Biosystems) Run 2	27.50	30.66	33.44
	FastStart (Roche)	28.33	30.47	32.79
	GoTaq (Promega)	28.72	31.58	34.12
Bpp (CFR 610)	GeneAmp (Applied Biosystems) Run 2	27.30	30.67	34.12
	FastStart (Roche)	28.75	31.06	33.93
	GoTaq (Promega)	28.77	32.52	35.36
IC* (Q670)	GeneAmp (Applied Biosystems) Run 2	28.39		
	FastStart (Roche)	29.35		
	GoTaq (Promega)	28.73		

Table 5. Rapid cycling experiments showing sensitive detection of CMV and HSV-1 viruses.

Assay	Run	Stage 1	Stage 2		Run Time	Copies/Rxn		
		1 Cycle	45 Cycles					
		97°C	97°C	60°C		1000	100	10
CMV	Original Cycling	5 min	15 sec	35 sec	71 min	31.23	34.98	37.75
	Rapid Cycling	10 sec	2 sec	9 sec	35 min	33.38	36.57	38.50
HSV-1	Original Cycling	1 min	5 sec	35 sec	58 min	30.95	35.27	38.87
	Rapid Cycling	10 sec	2 sec	6 sec	33 min	31.23	34.16	36.71

Conclusions

- The 3M Integrated Cycler can perform efficiently across a very wide linear dynamic range and it demonstrates excellent reproducibility.
- The instrument is sensitive, with consistent detection in reactions containing as little as 2 copies per reaction.
- Given the similar performances observed, the enzyme comparison study demonstrated that the 3M Integrated Cycler has the capability of utilizing various enzyme systems. Note: Chemistry optimization for each enzyme system may produce even better results.
- Preliminary studies show that the 3M Integrated Cycler has the capability of running rapid cycling parameters with minimal effect on sensitivity.

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