

Performance Evaluation of Real-Time PCR Instruments

B. Jaitner, B. Phelps, D. Chien and Y-L. Fong

Blood Testing Division, Chiron Corporation, Emeryville, California 94608

OBJECTIVE

Real-time PCR instruments from Applied Biosystems (7900HT), Cepheid (Smartcycler), Corbett Research (Rotor-Gene), MJ Research (Opticon) and Stratagene (Mx4000) were compared with regard to detection sensitivity, linearity, uniformity and precision.

METHODS

- Amplification and SybrGreen detection of a 170bp fragment of the six 23S rRNA genes in genomic DNA from Staphylococcus aureus were used to evaluate the performance of each instrument.
- The Staphylococcus aureus genome encompasses 2.8 Mb, which correspond to approx. 3 fg genomic DNA. The genomic DNA from this microorganism was tested in twofold serial dilutions, from a single copy to approx. 2100 genomic copies

Default cycling protocol:

95°C 10 min
 95°C 15 sec
 55°C 45 sec
 72°C 15 sec } 40 Cycles

Table 1: Instrument settings were defined for each instrument individually.

Instrument	Baseline	Threshold
7900	Subtract baseline for cycle range 3-15.	Set manually: 0.2
Mx4000	Adaptive baseline.	Set manually: 0.01
Opticon	Drift in fluorescence over initial cycles, therefore choose cycle range 10-18.	Set manually: 0.008
Rotor-Gene	Set automatically.	Set manually: 0.03
Smartcycler	Automatically set baseline for 5 cycles between cycles 5-40.	Set manually: 30 RFU

RESULTS

Detection sensitivity of the different real-time PCR instruments

Table 2. Genomic DNA from Staph. aureus was twofold serially diluted between 1 and 27 copies/reaction. The detection sensitivity of the 23S rRNA genes was determined in two independent experiments.

genomic copies	7900HT		Mx4000		Opticon		Smartcycler	
	ave Ct	%CV	ave Ct	%CV	ave Ct	%CV	ave Ct	%CV
27	26.7	0.3	30.1	0.4	28.9	0.5	31.6	0.2
13	27.6	0.4	30.9	1.5	29.8	1.4	33.1	1.8
7	28.6	0.9	31.7	1.2	31.8	1.7	34.1	0.4
3	29.4	0.3	32.9	1.2	32.1	2.2	35.1	1.3
2	30.4	0.1			33.7	6.0		
1	31.5	1.3			34.0	2.3	37.1	

genomic copies	7900HT		Mx4000		Opticon		Smartcycler	
	ave Ct	%CV	ave Ct	%CV	ave Ct	%CV	ave Ct	%CV
27	26.9	0.3	29.8	0.5	28.7	0.4	31.6	0.8
13	27.8	0.4	30.8	0.7	29.7	1.0	33.1	0.5
7	28.6	0.9	31.9	1.5	31.3	1.1	34.7	1.4
3	29.8	0.7	33.2	1.3	32.0	1.0	34.7	
2	30.4	1.1	33.7	3.0	32.6	2.2		
1	31.6	0.5	35.7	4.5	34.7	2.0	36.4	2.6

Instrument precision for the detection of 1 to 214 copies of genomic DNA

Table 3. Genomic DNA from Staph aureus was twofold serially diluted between 1 and 214 copies/reaction. The instrument precision for the detection of the 23S rRNA genes was determined in two independent experiments.

genomic copies	7900HT		Rotor-Gene		Opticon		Smartcycler	
	ave Ct	%CV	ave Ct	%CV	ave Ct	%CV	ave Ct	%CV
214	23.2	0.4	25.1	1.2	25.0	1.2	28.1	0.9
107	24.4	0.3	26.2	0.4	26.0	1.0	N/A	N/A
54	25.6	0.1	N/A	N/A	27.0	1.0	N/A	N/A
27	26.7	0.3	28.2	0.6	28.2	0.5	31.2	0.4
13	27.6	0.4	29.7	0.3	29.4	0.3	32.1	0.4
7	28.6	0.9	30.2	0.3	30.7	1.5	33.6	2.1
3.5	29.4	0.3	31.9	1.1	31.4	1.8	34.9	2.3
2	30.4	0.1	33.0	1.6	32.4	1.2	35.0	0.3
1	31.5	1.3	33.2	2.4	33.7	2.2		

genomic copies	7900HT		Rotor-Gene		Opticon		Smartcycler	
	ave Ct	%CV	ave Ct	%CV	ave Ct	%CV	ave Ct	%CV
214	23.3	0.3	24.7	1.0	24.9	0.6	28.3	1.6
107	24.3	0.3	25.6	0.5	25.9	0.4	N/A	N/A
54	25.7	0.7	26.5	1.0	27.1	1.0	N/A	N/A
27	26.9	0.3	27.8	1.4	28.0	0.3	31.6	0.6
13	27.8	0.4	29.0	0.9	29.2	0.5	32.4	0.7
7	28.6	0.9	29.9	1.0	30.5	0.6	33.5	1.2
3.5	29.8	0.7	31.3	0.6	30.9	1.2	35.4	2.2
2	30.4	1.1	32.4	1.1	32.2	4.8	36.5	2.9
1	31.6	0.5	32.6	0.8	32.7	2.2	37.7	2.8

Table 4: The average Tm value and %CV for four representative runs on each instrument is shown.

Instrument	ave Tm	%CV
7900HT	84.5	0.3
Mx4000	84.8	0.3
Opticon	83.9	0.2
Smartcycler	85.0	0.2

Well-to-Well Uniformity

Table 5. Uniformity of melt peak heights and endpoint fluorescence. The melt peak heights and endpoint fluorescence of the 170bp 23S rRNA amplicon from Staph. aureus was determined for 7 genomic copies. The average values for 32 (Smartcycler) or 96 individual reactions (all other instruments) and %CV are shown.

Instrument	Melt peak heights ave	%CV	Endpoint RFU ave	%CV
7900HT	0.12	4.66	1.56	3.93
Smartcycler	33.10	16.76	105.50	13.84
Opticon	1.12	4.31	N/A	
Mx4000	N/A		0.05	7.20

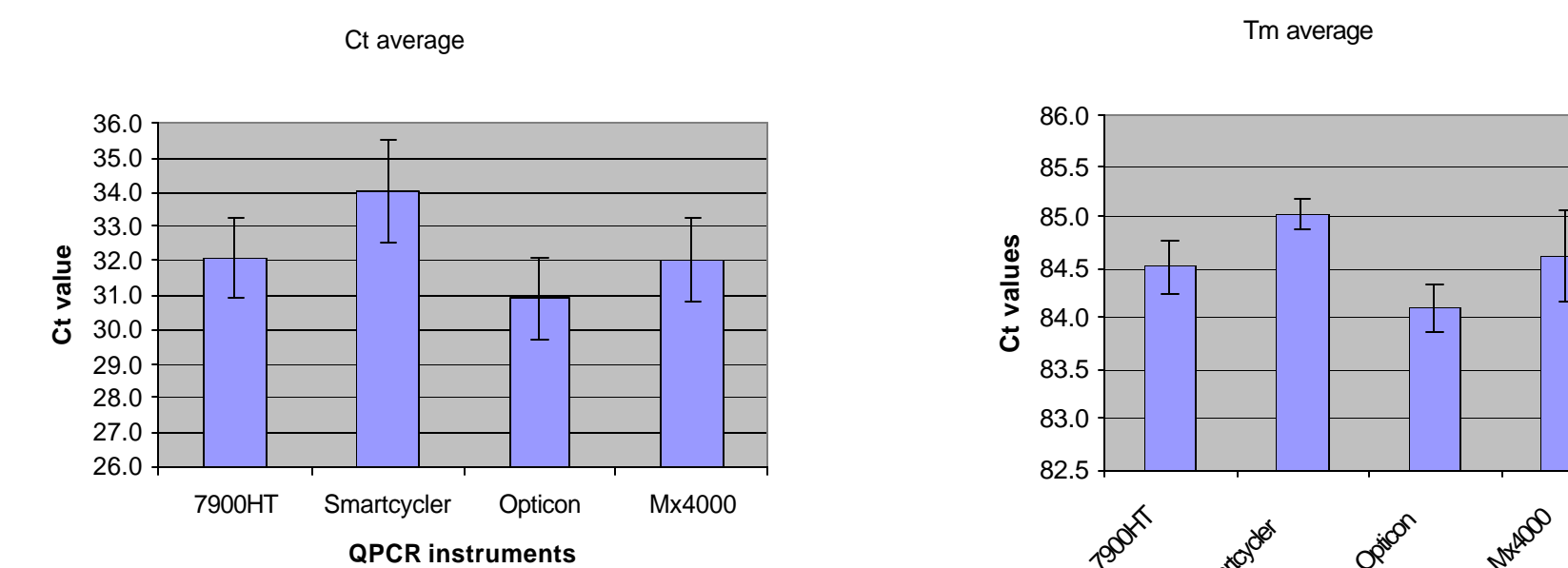


Fig. 1: Uniformity of Ct and Tm values. The amplification and detection of the 23S rRNA genes from 7 copies of Staph. aureus genomic DNA was determined for 32 (Smartcycler) or 96 individual reactions (all other instruments). The average Ct and Tm values and the %CV for a representative run are shown.

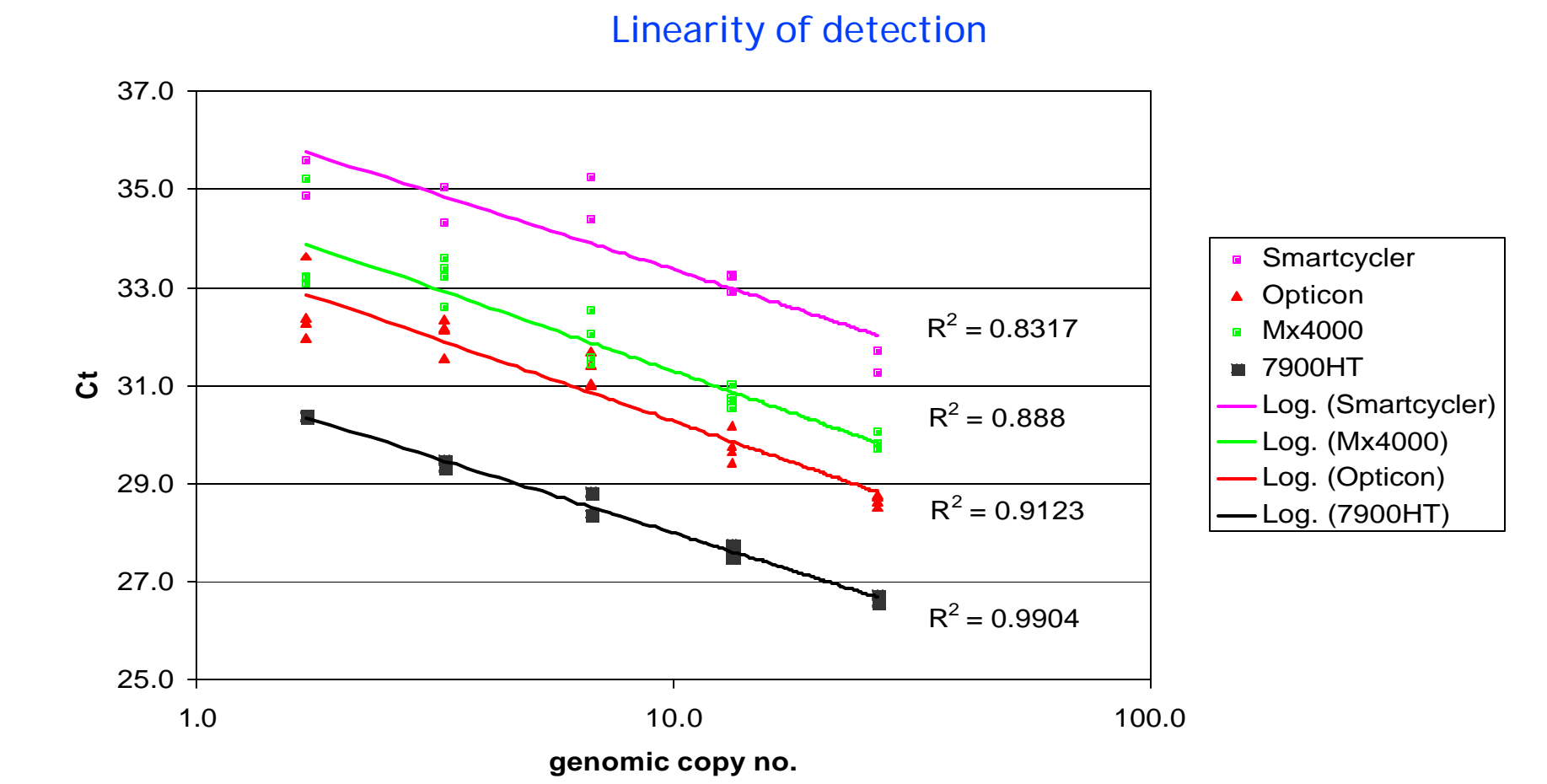


Fig. 2 Genomic DNA from Staph aureus was two-fold serially diluted between 2 to 27 copies/reaction and the amplification and detection analyzed for the Smartcycler, Opticon, Mx4000 and the 7900HT.

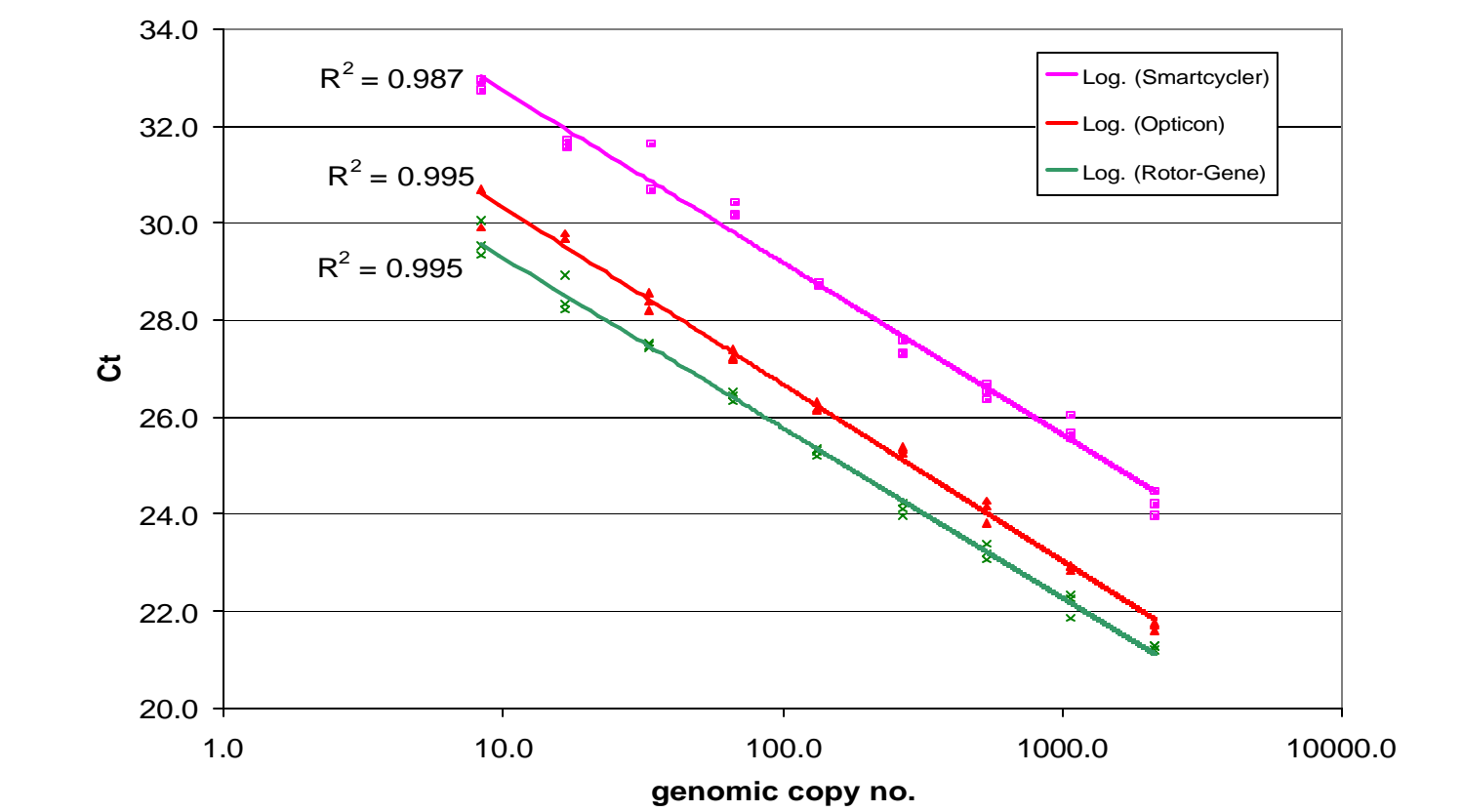


Fig. 3 Genomic DNA from Staph aureus was two-fold serially diluted between 8 to 2133 copies/reaction and the amplification and detection analyzed for the Opticon, Rotor-Gene and Smartcycler.

CONCLUSIONS

- One genomic copy is reproducibly detected by the 7900HT, the Rotor-Gene and the Opticon
- The earliest detection of one genomic copy of Staphylococcus aureus DNA is observed in the 7900HT with a Ct value of 31.5 +/- 1.3 (% CV)
- The instrument precision for the Tm value is best for the Smartcycler with a Tm of 85°C +/- 0.2 (% CV) and the Opticon with a Tm of 83.9°C +/- 0.2

- The amplification and detection is linear over a range of 10 to 2100 genomic copies with a R2 value of 0.99 for the Opticon, and Rotor-Gene and the 7900HT, and a R2 value of 0.98 for the Smartcycler and the Mx4000 (data not shown)
- In respect of well-to-well uniformity, the %CV of the Ct and the Tm value is below 1.5 for all studied instruments. In contrast, the %CV for the endpoint fluorescence and the melt peak heights is < 5 for the 7900HT and higher for all other instruments