

Quantification of HCV Control cRNA using two different *Tth* DNA Polymerases

Experimental Protocol:

Material:

- HCV Control cRNA (10,000,000; 1,000,000; 100,000; 10,000; 1,000; 100; 50; and 10 molecules per 8-well Control strip [8 tubes/strip or 0.1 ml tubes, respectively]), Lot 008
- *rTth* DNA Polymerase, 2.5 U/ μ l (Applied Biosystems; supplier ABI)
 - plus:
 - 5x EZ-buffer (Roboscreen)
 - Mn-acetate-solution, 25 mM (Roboscreen)
- *Tth* DNA Polymerase, 5 U/ μ l (supplier GC)
 - plus:
 - 10x PCR-buffer (supplier GC)
 - Mn-acetate-solution, 50 mM (supplier GC)

Instruments:

- ABI PRISM 7000 Sequence Detection System (Applied Biosystems)
- Rotor-Gene 2000 (Corbett Research)

Reaction conditions (both instruments):

Reaction component	Final concentration (25 μ l-Assay)
10x/5x buffer	1x
Mn-acetate solution	3.5 mM
Nucleotide mix	0.3 mM dATP, dCTP and dGTP, 0.6 mM dUTP
Forward and reverse primer	7.5 pmol each
TaqMan [®] probe (FAM/TAMRA)	3.4 pmol
<i>Tth</i> DNA Polymerase	1.5 U

Cycler Program

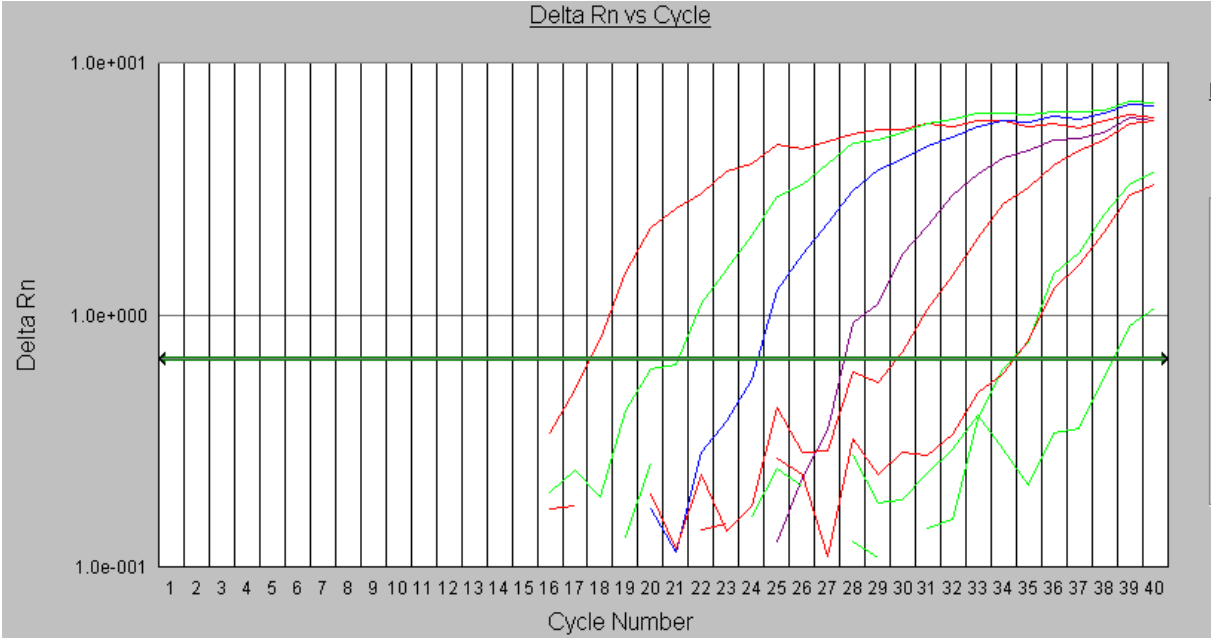
Rotor-Gene					
	RT	Hold	Cycle		Hold
Temperature (°C)	59	95	95	59	25
Time (min:s)	60	10:00	00:15	01:00	∞
Cycles			45		

7000 SDS					
	RT	Hold	Cycle		Hold
shut off "9600 emulation" (window "instrument")					
Temperature (°C)	59	95	95	59	25
Time (min:s)	60	10:00	00:30	01:30	∞
Cycles			40		

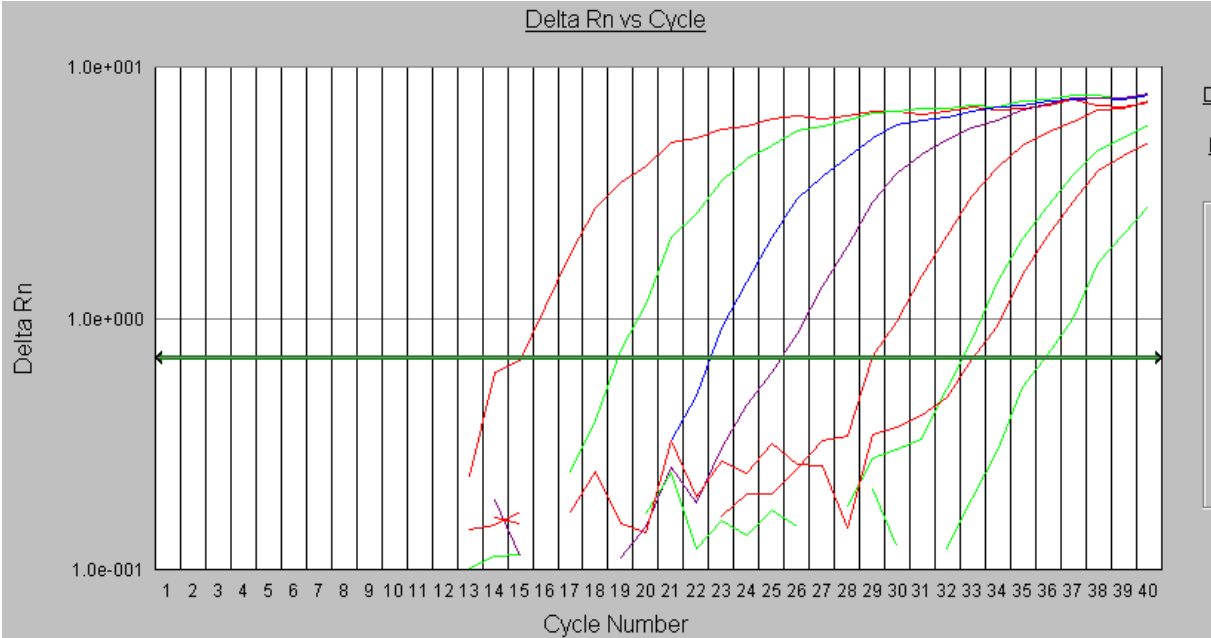
Results:

Saturation curves

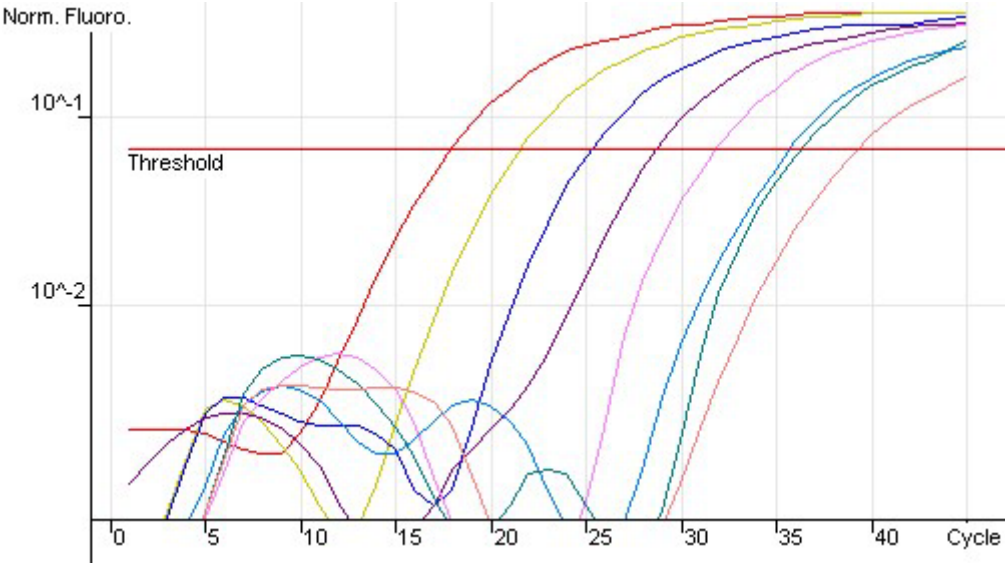
Enzyme: *rTth* DNA Polymerase (Applied Biosystems, supplier ABI); **instrument:** 7000SDS



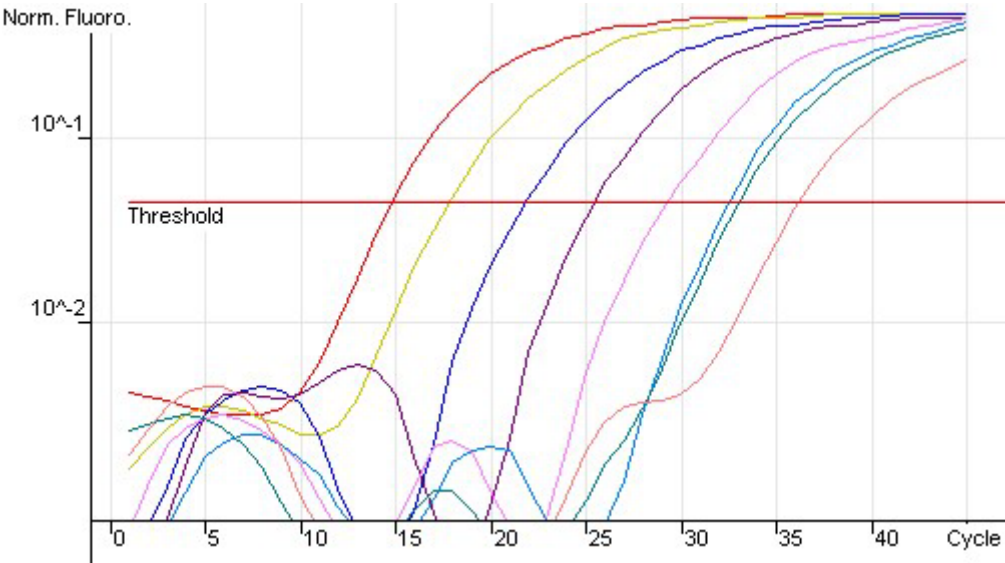
Enzyme: *Tth* DNA Polymerase (supplier GC); **instrument:** 7000SDS



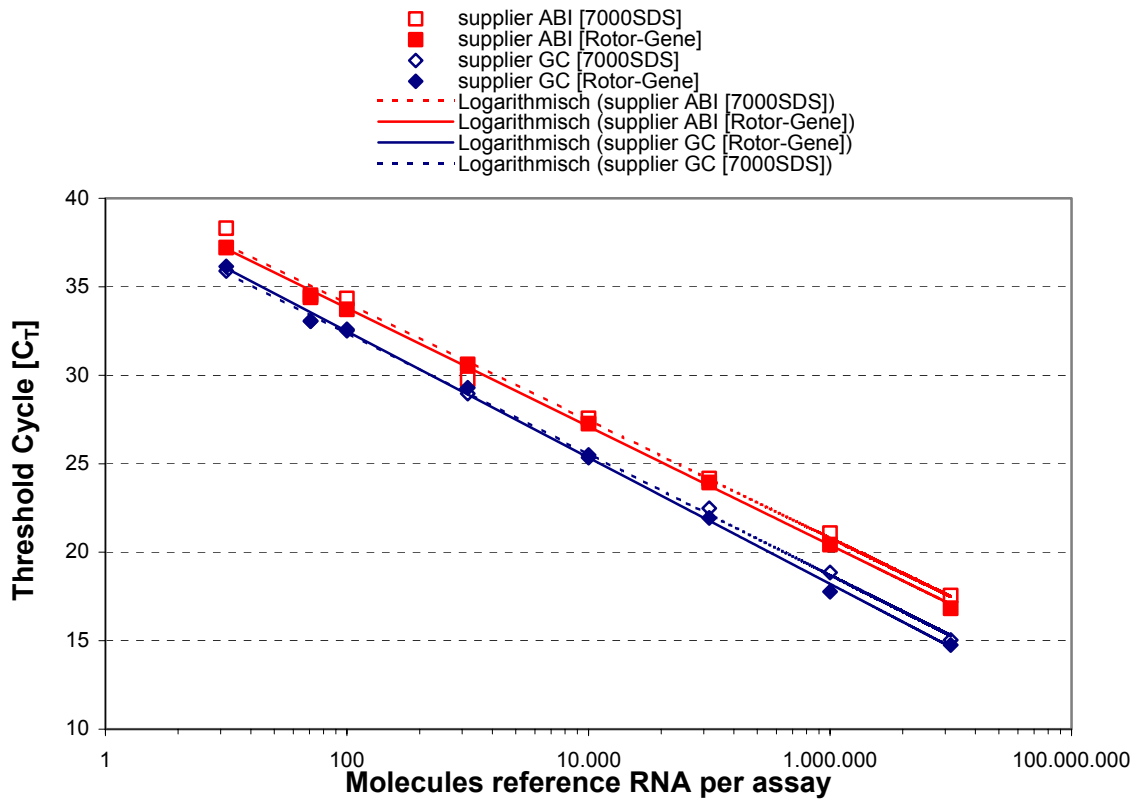
Enzyme: *rTth* DNA Polymerase (Applied Biosystems, supplier ABI); **instrument:** Rotor-Gene



Enzyme: *Tth* DNA Polymerase (supplier GC); **instrument:** Rotor-Gene



Reference curves



Instrument		7000SDS		RG, 0,1 ml	
Enzyme		supplier ABI	supplier GC	supplier ABI	supplier GC
		16.10.2002	17.10.2002	16.10.2002	17.10.2002
Tube No.	Molecules per tube	161002_HCV_1	171002_HCV_1	161002_HCV_2RG	171002_HCV_2RG
1	10.000.000	17,55	15,04	16,83	14,76
2	1.000.000	21,08	18,85	20,43	17,77
3	100.000	24,16	22,48	23,94	21,94
4	10.000	27,56	25,35	27,27	25,49
5	1.000	29,77	28,97	30,61	29,29
6	100	34,34	32,59	33,72	32,52
7	50	34,4	33,05	34,52	33,07
8	10	38,31	35,89	37,21	36,15

Summary

The use of the *Tth* DNA Polymerase (supplier GC) improved the sensitivity of the of HCV RNA quantification significantly.