

Spartan DX-12™ Instrument Repeatability and Reproducibility

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Here we demonstrate reproducibility and repeatability across several Spartan DX-12™ instruments.

Introduction

Repeatability and reproducibility, or intra- and inter-assay variance, are important indicators of instrument performance. Repeatability is defined as short term precision of an assay run and analyzed multiple times in the same way, on the same instrument. Reproducibility refers to long-term precision, between runs on different instruments and is subject to inherent inter-run variations (1). These measurements help to validate the thermal performance and manufacturing consistency of the instrument.

The purpose of this study was to determine the repeatability and reproducibility of Real-Time PCR assays performed across several Spartan DX-12™ instruments.

Materials and Methods

DNA preparation

A synthetic plasmid construct was designed in-house and produced for Spartan Bioscience by GENEART Inc. (Toronto). Plasmid DNA received at a stock concentration of 1.5 mg/ml was reconstituted to 1.5 mM stock in sterile water, and stored in aliquotes at -20°C.

Input DNA

Plasmid DNA was diluted to a concentration of 0.75 ng/μl for the low Ct value (high copy number), and a 2¹⁸ dilution (15.75 fg/μl) of that was prepared in sterile water for the high Ct value (low copy number).

Real-time PCR

Oligonucleotide primers and probe designed against the Norovirus Open Reading Frame exon 1-2 junction (ORF 1-2) were used (2, 3). The primer and probe sequences are

Primer/Probe	Forward (5'-3')	Reverse (5'-3')	Amplicon size (bp)
COG1 primers	CgY Tgg ATg CgN TTY CAT gA	CTT AgA CgC CAT CAT CAT TYA C	85
COG1 probe	6-FAM-AgA TYg CgR TCY CCT gTC CA g-BHQ1™		
COG2 primers	CAR gAR BCN ATg TTY AgR Tgg ATg Ag	TCg ACg CCA TCT TCA TTC ACA	98
COG2 probe	CAL Fluor® Red 610 -Tgg gAg ggC gAT CgC AAT CT-BHQ2™		

6-FAM = 6-carboxy-fluorescein, BHQ™ = Black Hole Quencher

Table 1. Primer/probe sequences and amplicon sizes.

Component	Final amount
10X PCR Reaction Buffer (No MgCl ₂) (Invitrogen)	1 X
MgCl ₂ (Invitrogen)	2.5 mM
dNTP mix (Invitrogen)	0.125mM
Taq DNA polymerase (Invitrogen)	1 U
Probes (Biosearch Technologies)	0.2 μM (COG1), 0.05 μM (COG2)
PCR primers (IDT)	0.2 μM (each)
Template DNA	2 μl
Sterile water	up to 20 μl
Total reaction volume	20 μl

Table 2. Components of PCR amplification mixture.

Step	Temperature	Time	Cycles
Initial denaturation	95°C	30 s	1
Denaturation	95°C	25 s	40
Annealing/extension	55°C	25 s	40

Table 3. Cycling parameters.

shown in Table 1.

Components of the Real-Time amplification mixture are listed in Table 2. Samples were run as duplex reactions (COG1, COG2), across 12 wells and 5 units, at both low and high copy DNA concentrations. Samples run on the Spartan DX-12™ were run with Spartan Tubes (20μl PCR Tube Assembly, Tubes - Cat.No.01004153 and Caps - Cat.No.01004155), and samples run on the StepOne (Figure 1) were run with ABI tubes (Fast reaction tube with cap kit - Cat. No. 4358297). Table 3 shows the cycling parameters used (2-temperature

program).

DNA analysis

Fluorescence data was transferred from the Spartan DX-12™ and graphed using the Spartan Graphing & Analysis Software (ver. 3.10).

Results

Figure 2 shows representative plots of normalized fluorescence across 12 wells, for both the green and the red channels, at both high (1.5 ng) and low copy (31.5 fg) input DNA. Here we see highly repeatable results performed on the instrument in replicate assays.

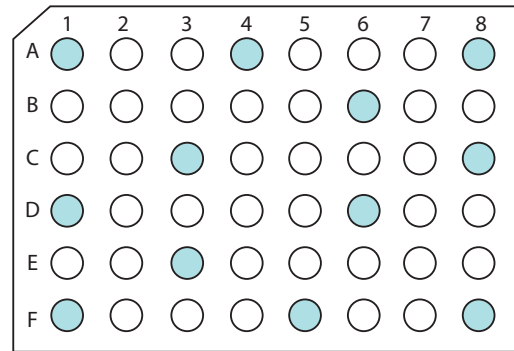


Figure 1. Distribution of tubes within the Step One, used for comparison (one run high copy, a second run low copy).

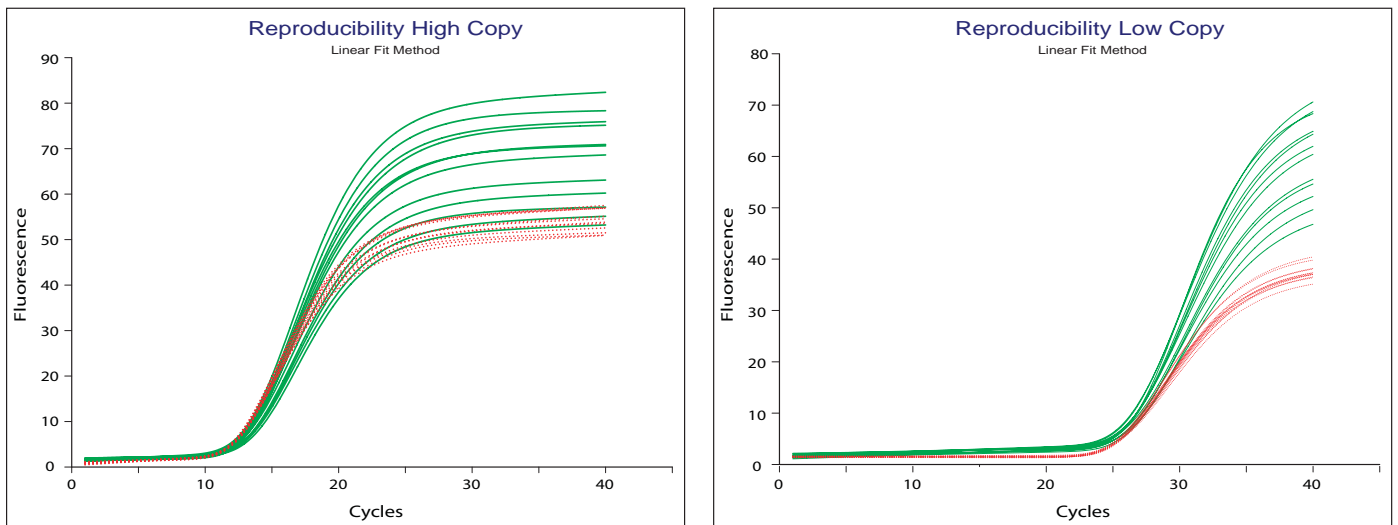


Figure 2. Real-time PCR results for 12 wells of high copy (1.5 ng) and low copy (31.5 fg) repeatability on the Spartan DX-12

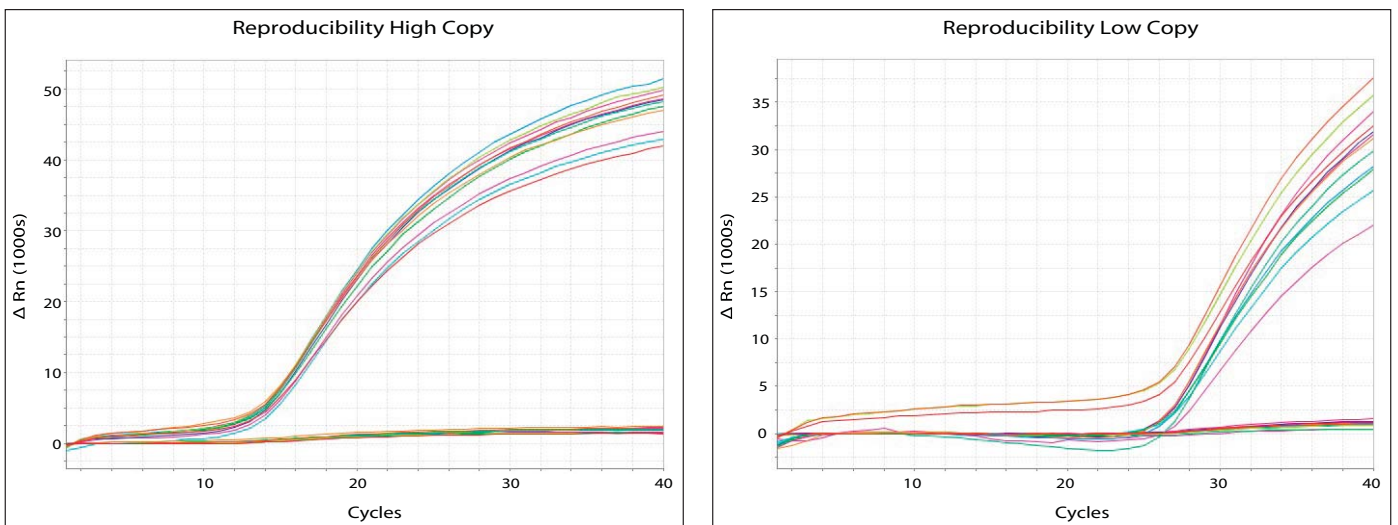


Figure 3. Real-time PCR results for 12 wells of high copy (1.5 ng) and low copy (31.5 fg) repeatability on the StepOne.

Intra-instrument repeatability on the Spartan DX-12™ shows consistent results across all 12 wells. High copy assays show standard deviations across 12 wells to be between ± 0.16 - 0.42 in the green channel, and ± 0.06 - 0.30 in the red channel (Table 4). Low copy intra-assay variations were between ± 0.15 - 0.22 in the green channel, and ± 0.13 - 0.21 in the red channel (Table 5). In a parallel experiment done on the StepOne, intra-instrument variability (across 12 wells, identified in Figure 1) showed an average Ct value of 14.26 ± 0.35 in the green channel and 12.31 ± 1.79 in the red channel,

during high copy testing, and 22.62 ± 7.64 in the green channel and 26.84 ± 1.67 in the red channel during low copy testing (Tables 4 and 5).

Inter-instrument testing on the Spartan DX-12™ also showed consistent results across instruments, demonstrating excellent reproducibility (Table 6). Average Ct values across all 12 wells of 5 different instruments were 13.33 ± 0.29 for the green channel and 12.41 ± 0.19 for the red channel when using high copy DNA; and 27.16 ± 0.53 for the green channel

Channel		Instrument 1	Instrument 2	Instrument 3	Instrument 4	Instrument 5	Step One
Green	Average Ct Value	13.51	13.37	13.11	13.34	13.31	14.26
	Standard Deviation	0.16	0.42	0.21	0.25	0.20	0.35
Red	Average Ct Value	12.47	12.48	12.32	12.47	12.30	12.31
	Standard Deviation	0.13	0.30	0.06	0.19	0.10	1.79

Table 4. High copy number real-time PCR intra-instrument repeatability (across 12 wells).

Channel		Instrument 1	Instrument 2	Instrument 3	Instrument 4	Instrument 5	Step One
Green	Average Ct Value	27.63	26.68	27.43	27.62	26.46	22.62
	Standard Deviation	0.22	0.20	0.20	0.15	0.12	7.64
Red	Average Ct Value	26.48	25.63	26.30	26.28	25.39	26.84
	Standard Deviation	0.13	0.21	0.13	0.12	0.14	1.67

Table 5. Low copy number real-time PCR intra-instrument repeatability (across 12 wells).

Channel		High Copy (1.5 ng)	Low Copy (31.5 fg)
Green	Average Ct Value	13.33	27.16
	Standard Deviation	0.29	0.53
Red	Average Ct Value	12.41	26.02
	Standard Deviation	0.19	0.45

Table 6. Inter-instrument real-time PCR reproducibility

and 26.02 ± 0.45 for the red channel when using low copy DNA (Table 6).

Discussion and Conclusions

The results demonstrate that the Spartan DX-12™ is capable of achieving excellent inter- and intra-instrument

repeatability and reproducibility. These results validate the thermal stability and functionality of the Spartan DX-12™, indicating that the instrument is a dependable and reliable real-time PCR instrument.

References

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3. Kageyama T *et al.* (2003). Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. *Journal of Clinical Microbiology*. 41(4): 1548-57.

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