



Project Report #185

Validation of two QuantStudio™ 5 Real-Time PCR Systems

*Adam Kaity, Luke Ryan, Megan Mathieson and
Cathie Allen
February 2019*



Document Details

Contact for enquiries and proposed changes

If you have any questions regarding this document or if you have a suggestion for improvements, please contact:

Contact officer: Luke Ryan

Title: Senior Scientist – Analytical

Phone:

Email:

Version history

Version	Date	Changed by	Description
1.0	March 2018	Adam Kaity	Creation of Document
2.0	April 2018	Adam Kaity	Feedback from LBR/MLM
3.0 and 3.1	May 2018	Adam Kaity	Tech Review feedback and LBR/MLM feedback
3.2	June 2018	Adam Kaity	Revise Exp 2
3.3	June 2018	Adam Kaity	Final report including feedback amendments
4.0	November 2018	Adam Kaity	Report incorporating revised Experiment 3
5.0	December 2018	Adam Kaity	Report incorporating revised Experiment 3
6.0	February	Adam Kaity	New figures incorporated for experiment 1. Additional feedback added.

Document sign off

This document has been approved by:

Name	Position	Signature	Date
Cathie Allen	Managing Scientist		

The following officers have endorsed this document

Name	Position	Signature	Date
Paula Brisotto	Team Leader ER&Q		
Justin Howes	Team Leader FRIT		
Luke Ryan	Senior Scientist Analytical		
Kirsten Scott	Senior Scientist Q&P		
Alan McNevin	Senior Scientist ER		
Kylie Rika	Senior Scientist Reporting 2		
Sharon Johnstone	Senior Scientist Intel		

Contents

Contents	2
Abstract.....	3
Introduction.....	3
Resources	4
Methods	4
Sample Selection	4
Experiments and Results	4
Experiment 1: Sensitivity, Limit of Detection and Inaccuracy	4
Experiment 2: Comparison of QS5s and 7500	14
Experiment 3a: Repeatability	15
Experiment 3b: Reproducibility	18
Experiment 4: Y-Intercept Thresholds.....	21
Conclusion.....	22
Recommendations	23
References	23

Abstract

The purpose of this project was to validate both QuantStudio™ 5 (QS5) instruments for the analysis of Quantifiler® Trio (Quant Trio) DNA quantification reactions. Both QS5-A and QS5-B were validated separately using the experiments outlined below.

The following experiments were performed on both QS5-A and QS5-B:

- Sensitivity and Limit of Detection
- Comparison of QS5 and 7500
- Y-Intercept Thresholds

Repeatability and reproducibility was performed on QS5-A only.

The results of this verification found that both QS5-A and QS5-B instruments are suitable to perform DNA quantification using the Quantifiler® Trio quantification kit, and can replace the two 7500 instruments that are currently in use.

Introduction

Forensic DNA Analysis has two 7500 Real-Time PCR instruments (7500s) which are used to analyse Quantifiler® Trio DNA quantification reactions. Both 7500s are at the end of life and are being replaced under the Health Technology Equipment Replacement Program (HTER). The HTER process identified the QuantStudio™ 5 Real-Time PCR System (QS5) as the most suitable replacement for the 7500s. Two QS5s were purchased.

Both QS5s were validated for the analysis of Quantifiler® Trio DNA quantification reactions by the manufacturer. The QS5s were delivered with pre-installed protocols for the Quantifiler® Trio kit.

Validation of the two QS5s were performed separately (except for repeatability and reproducibility), QS5-A followed by QS5-B. Both QS5s will be implemented concurrently and replace the two 7500s. The validation experiments for both QS5s were the same.

Resources

All reagents, materials and equipment used in this project were as specified in the approved in-house document Project Proposal #185 – Validation of QuantStudio™ Real-Time PCR Systems (June 2017) ^[4]. This document will be referred to as the Experimental Design. The following QIS documents are referenced throughout this report:

- Operation and Maintenance of the Microlab STARlet and LabElite Integrated I.D.Capper. QIS 34050. ^[5]
- Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit. QIS 33407. ^[6]

Methods

The methods for each experiment in this verification were as per the Experimental Design unless otherwise specified.

Sample Selection

NIST standards were used for this validation. NIST Standard sets A, B and C were used to create serial dilutions using TE-4 buffer with final concentrations as per the Experimental Design. NIST Standards A, B, and C, are derived from a single male donor, multiple female donors, and multiple male and female donors, respectively ^[3].

Experiment 3 will utilise twelve previously extracted Collaborative Testing Service (CTS) samples with volumes greater than 70 µL.

Experiments and Results

Experiment 1: Sensitivity, Limit of Detection and Inaccuracy

Purpose

Quantifiler® Trio has been shown to have a single source sensitivity down to concentrations of 0.005 ng/µL^[1]. The validation of Quantifiler® Trio on the 7500s determined the Limit of Detection (LOD) to be 0.001 ng/µL^[2]. Serial dilutions of NIST standards were used to determine the LOD for Quantifiler® Trio on the QS5 instruments. Percent change (inaccuracy) was calculated from the expected and

observed result. This was performed for each of the quantification targets: SAT, LAT and Y-Target for both QS5 instruments and 7500-A.

Results

Two plates of NIST standards A, B, and C serial dilution duplicates were prepared each for the 7500-A and both QS5s as outlined in Tables 1 and 2 below. Dilutions ranged from 5 – 0.0001 ng/μL.

Table 1: NIST Standards Serial Dilutions – Platemap 1 of 2

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD 1 50 ng/μL	STD 5 0.005 ng/μL	NIST C 5.0 ng/μL	NIST B 0.5 ng/μL	NIST A 0.1 ng/μL	NIST C 0.09 ng/μL	NIST B 0.05 ng/μL	NIST A 0.03 ng/μL	NIST C 0.01 ng/μL	NIST B 0.008 ng/μL	NIST A 0.007 ng/μL	NIST C 0.006 ng/μL
B	STD 1 50 ng/μL	STD 5 0.005 ng/μL	NIST A 1.0 ng/μL	NIST C 0.5 ng/μL	NIST B 0.1 ng/μL	NIST A 0.07 ng/μL	NIST C 0.05 ng/μL	NIST B 0.03 ng/μL	NIST A 0.009 ng/μL	NIST C 0.008 ng/μL	NIST B 0.007 ng/μL	NIST A 0.005 ng/μL
C	STD 2 5.000 ng/μL	Reagent Blank	NIST B 1.0 ng/μL	NIST A 0.5 ng/μL	NIST C 0.1 ng/μL	NIST B 0.07 ng/μL	NIST A 0.05 ng/μL	NIST C 0.03 ng/μL	NIST B 0.009 ng/μL	NIST A 0.008 ng/μL	NIST C 0.007 ng/μL	NIST B 0.005 ng/μL
D	STD 2 5.000 ng/μL	NIST A 5.0 ng/μL	NIST C 1.0 ng/μL	NIST B 0.5 ng/μL	NIST A 0.09 ng/μL	NIST C 0.07 ng/μL	NIST B 0.05 ng/μL	NIST A 0.01 ng/μL	NIST C 0.009 ng/μL	NIST B 0.008 ng/μL	NIST A 0.006 ng/μL	NIST C 0.005 ng/μL
E	STD 3 0.500 ng/μL	NIST B 5.0 ng/μL	NIST A 1.0 ng/μL	NIST C 0.5 ng/μL	NIST B 0.09 ng/μL	NIST A 0.07 ng/μL	NIST C 0.05 ng/μL	NIST B 0.01 ng/μL	NIST A 0.009 ng/μL	NIST C 0.008 ng/μL	NIST B 0.006 ng/μL	NIST A 0.005 ng/μL
F	STD 3 0.500 ng/μL	NIST C 5.0 ng/μL	NIST B 1.0 ng/μL	NIST A 0.1 ng/μL	NIST C 0.09 ng/μL	NIST B 0.07 ng/μL	NIST A 0.03 ng/μL	NIST C 0.01 ng/μL	NIST B 0.009 ng/μL	NIST A 0.007 ng/μL	NIST C 0.006 ng/μL	NIST B 0.005 ng/μL
G	STD 4 0.050 ng/μL	NIST A 5.0 ng/μL	NIST C 1.0 ng/μL	NIST B 0.1 ng/μL	NIST A 0.09 ng/μL	NIST C 0.07 ng/μL	NIST B 0.03 ng/μL	NIST A 0.01 ng/μL	NIST C 0.009 ng/μL	NIST B 0.007 ng/μL	NIST A 0.006 ng/μL	NIST C 0.005 ng/μL
H	STD 4 0.050 ng/μL	NIST B 5.0 ng/μL	NIST A 0.5 ng/μL	NIST C 0.1 ng/μL	NIST B 0.09 ng/μL	NIST A 0.05 ng/μL	NIST C 0.03 ng/μL	NIST B 0.01 ng/μL	NIST A 0.008 ng/μL	NIST C 0.007 ng/μL	NIST B 0.006 ng/μL	Reagent Blank

Plates were prepared as per Operation and Maintenance of the Microlab STARlet and LabElite Integrated I.D.Capper (QIS 34050) ^[5] and Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit (QIS 33407) ^[6] for 7500-A and both QS5s.

Combined results for NIST A, B and C were used to determine the LOD for the SAT and LAT. Results from only NIST A were used to determine the LOD for the Y-Target.

Table 2: NIST Standards Serial Dilutions – Platemap 2 of 2

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD 1 50 ng/μL	STD 5 0.005 ng/μL	NIST C 0.004 ng/μL	NIST B 0.002 ng/μL	NIST A 0.001 ng/μL	NIST C 0.0001 ng/μL						
B	STD 1 50 ng/μL	STD 5 0.005 ng/μL	NIST A 0.003 ng/μL	NIST C 0.002 ng/μL	NIST B 0.001 ng/μL							
C	STD 2 5.000 ng/μL	Reagent Blank	NIST B 0.003 ng/μL	NIST A 0.002 ng/μL	NIST C 0.001 ng/μL							
D	STD 2 5.000 ng/μL	NIST A 0.004 ng/μL	NIST C 0.003 ng/μL	NIST B 0.002 ng/μL	NIST A 0.0001 ng/μL							
E	STD 3 0.500 ng/μL	NIST B 0.004 ng/μL	NIST A 0.003 ng/μL	NIST C 0.002 ng/μL	NIST B 0.0001 ng/μL							
F	STD 3 0.500 ng/μL	NIST C 0.004 ng/μL	NIST B 0.003 ng/μL	NIST A 0.001 ng/μL	NIST C 0.0001 ng/μL							
G	STD 4 0.050 ng/μL	NIST A 0.004 ng/μL	NIST C 0.003 ng/μL	NIST B 0.001 ng/μL	NIST A 0.0001 ng/μL							
H	STD 4 0.050 ng/μL	NIST B 0.004 ng/μL	NIST A 0.002 ng/μL	NIST C 0.001 ng/μL	NIST B 0.0001 ng/μL							

Table 3 outlines the expected and the average quantification values and % inaccuracy for each serial dilution obtained from the 7500-A and QS5 instruments. The SAT, LAT and Y-Target results for both instrument types all gave quantification results down to 0.0001 ng/μL. Figure 1 displays the % inaccuracy for both SAT replicate values obtained for the 7500, QS5A and QS5B (Figures 2-4 shows % inaccuracy for NIST A, B and C respectively).

The % inaccuracy for SAT and LAT for the 7500-A was markedly higher (>180%) at 0.0001 ng/μL than for QS5-A (<70%) and QS5-B (<117%), which supports the recommendation of previous studies [2] that the LOD for Quantifiler® Trio on the 7500s should be set at 0.001 ng/μL. The data indicates that both QS5s are more accurate than 7500-A at the lowest dilution concentration tested (0.0001 ng/μL) for SAT and LAT, although it should be noted that the inaccuracy % for all instruments fluctuates across the range of dilutions tested (Figures 1-7).

Y-Target % inaccuracy appeared to increase with decreasing concentration for all instruments with QS5-B registering the greatest inaccuracy reading for the data set at 0.0001 ng/μL which was produced by a single outlying quantification value (0.00056 ng/μL) as the replicate failed to produce a value from which an average could be calculated.

Table 3: Average quantification results and % inaccuracy

Concentration (ng/μL)	7500-A						Q55-A						Q55-B					
	SAT Average (ng/μL)	SAT % Inacc.	LAT Average (ng/μL)	LAT % Inacc.	Y-Target Average (ng/μL)	Y-Target % Inacc.	SAT Average (ng/μL)	SAT % Inacc.	LAT Average (ng/μL)	LAT % Inacc.	Y-Target Average (ng/μL)	Y-Target % Inacc.	SAT Average (ng/μL)	SAT % Inacc.	LAT Average (ng/μL)	LAT % Inacc.	Y-Target Average (ng/μL)	Y-Target % Inacc.
5	5.23438	4.7	5.65350	13.1	7.69158	53.8	5.93264	18.7	6.55684	31.1	7.69477	53.9	5.64724	12.9	6.12456	22.5	7.92748	58.5
1	0.83839	-16.2	1.00262	0.3	1.29179	29.2	0.92602	-7.4	1.15516	15.5	1.29869	29.9	0.68532	-31.5	0.81726	-18.3	0.84837	-15.2
0.5	0.40486	-19.0	0.47043	-5.9	0.53297	6.6	0.40410	-19.2	0.55648	11.3	0.53550	7.1	0.36752	-26.5	0.45626	-8.7	0.37763	-24.5
0.1	0.08333	-16.7	0.10740	7.4	0.12445	24.5	0.09544	-4.6	0.12827	28.3	0.13520	35.2	0.08792	-12.1	0.10440	4.4	0.10567	5.7
0.09	0.07025	-21.9	0.09250	2.8	0.11651	29.5	0.07659	-14.9	0.11041	22.7	0.11979	33.1	0.06873	-23.6	0.08394	-6.7	0.09569	6.3
0.07	0.05418	-22.6	0.07967	13.8	0.10983	56.9	0.07768	11.0	0.10107	44.4	0.13110	87.3	0.05848	-16.5	0.07519	7.4	0.06782	-3.1
0.05	0.03357	-32.9	0.04646	-7.1	0.05238	4.8	0.04542	-9.2	0.05750	15.0	0.05022	0.4	0.03041	-39.2	0.03950	-21.0	0.03315	-33.7
0.03	0.01906	-36.5	0.02510	-16.3	0.02913	-2.9	0.02372	-20.9	0.03104	3.5	0.03598	19.9	0.01678	-44.1	0.02045	-31.8	0.02557	-14.8
0.01	0.00898	-10.2	0.01146	14.6	0.01457	45.7	0.01172	17.2	0.01321	32.1	0.01511	51.1	0.00942	-5.8	0.00957	-4.3	0.01337	33.7
0.009	0.00815	-9.4	0.01009	12.1	0.01543	71.4	0.01008	12.0	0.01152	27.9	0.01234	37.1	0.00724	-19.5	0.00791	-12.1	0.00974	8.3
0.008	0.00768	-4.0	0.00922	15.2	0.01249	56.2	0.01025	28.1	0.01051	31.3	0.01435	79.3	0.00744	-7.0	0.00897	12.1	0.01147	43.3
0.007	0.00684	-2.3	0.00769	9.9	0.01013	44.7	0.00958	36.9	0.00939	34.1	0.00703	0.5	0.00563	-19.6	0.00602	-13.9	0.00863	23.3
0.006	0.00597	-0.6	0.00681	13.5	0.00658	9.6	0.00638	6.4	0.00730	21.6	0.00939	56.6	0.00390	-35.1	0.00417	-30.5	0.00534	-11.0
0.005	0.00582	16.4	0.00487	-2.5	0.00806	61.3	0.00735	47.0	0.00611	22.2	0.00964	92.8	0.00444	-11.2	0.00445	-11.1	0.00507	1.5
0.004	0.00397	-0.7	0.00431	7.7	0.00313	-21.8	0.00421	5.2	0.00382	-4.6	0.00328	-18.0	0.00315	-21.1	0.00281	-29.7	0.00146	-63.5
0.003	0.00299	-0.4	0.00317	5.6	0.00339	13.0	0.00340	13.4	0.00286	-4.7	0.00518	72.7	0.00177	-41.0	0.00155	-48.2	0.00185	-38.4
0.002	0.00215	7.6	0.00267	33.6	0.00291	45.5	0.00246	22.8	0.00202	1.2	0.00223	11.4	0.00108	-46.1	0.00063	-68.4	0.00093	-53.5
0.001	0.00103	3.1	0.00096	-4.0	0.00166	65.6	0.00155	55.0	0.00095	-4.7	0.00197	96.9	0.00081	-19.3	0.00057	-43.1	0.00084	-16.5
0.0001	0.00028	181.6	0.00030	198.0	0.00019	92.9	0.00015	47.0	0.00017	67.6	0.00019	90.8	0.00015	52.3	0.00022	116.2	0.00056	461.2

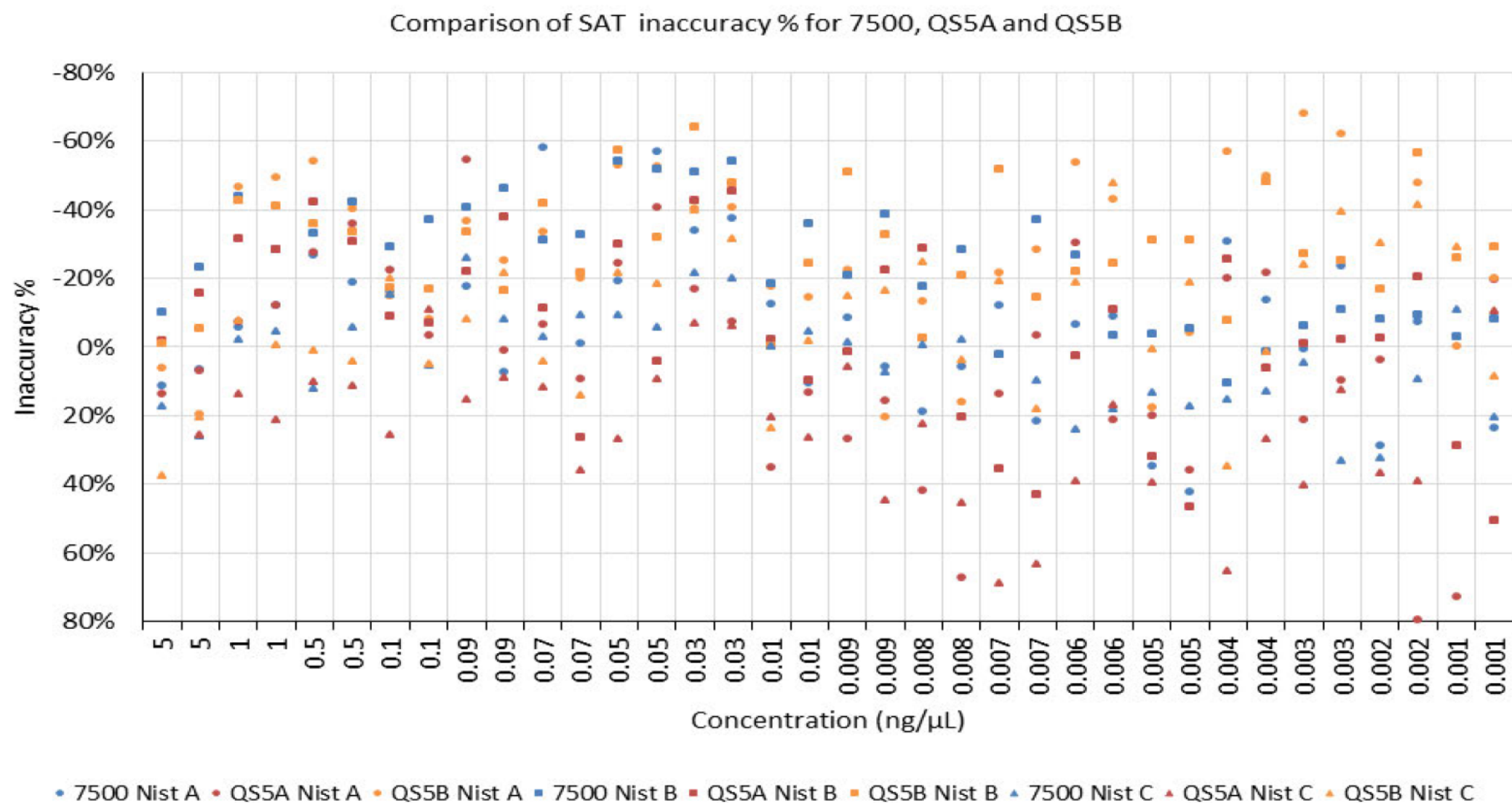


Figure 1: Comparison of SAT inaccuracy percentage for 7500, QS5A and QS5B

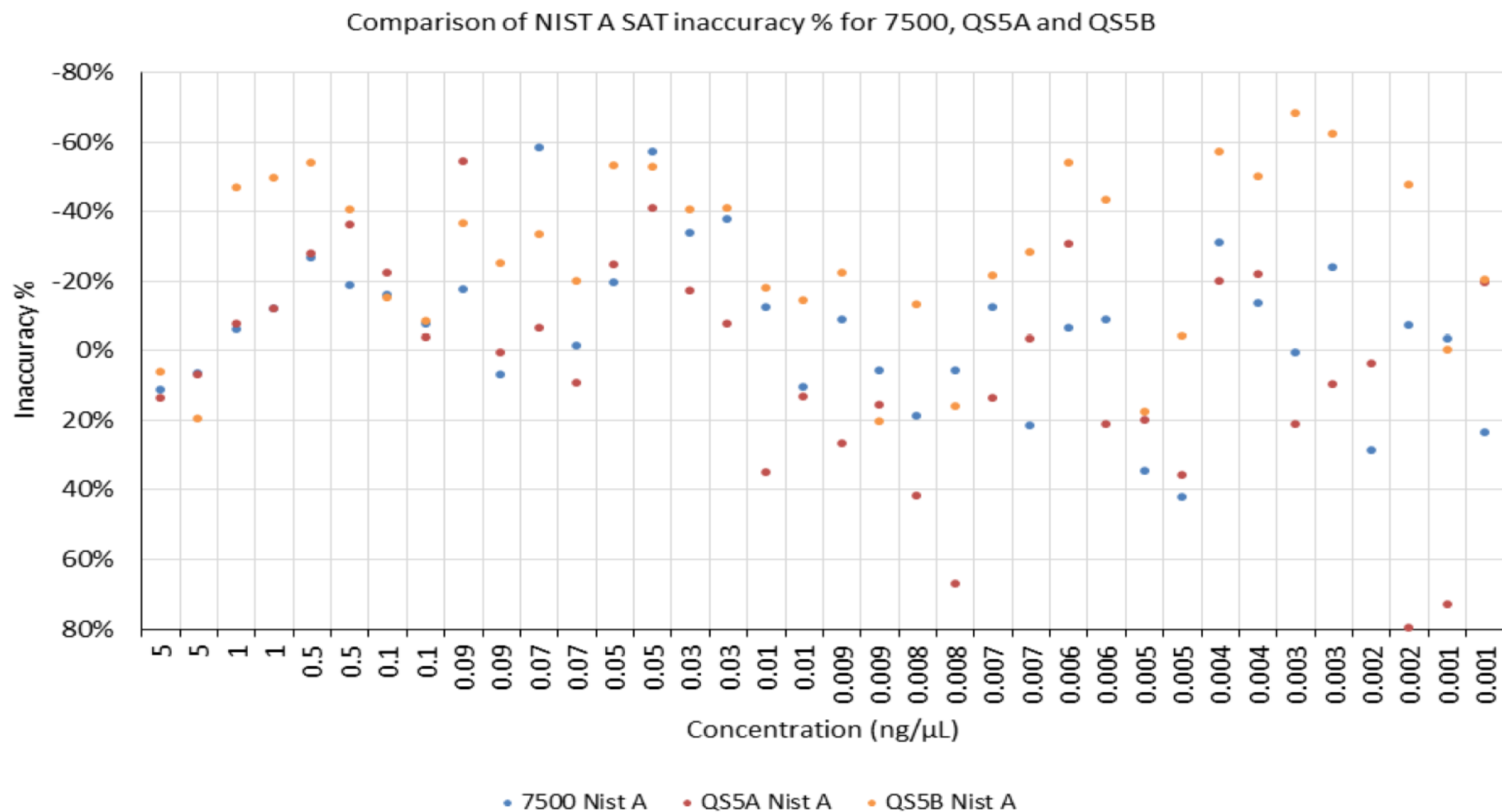


Figure 2: Comparison of NIST A SAT inaccuracy percentage for 7500, QS5A and QS5B

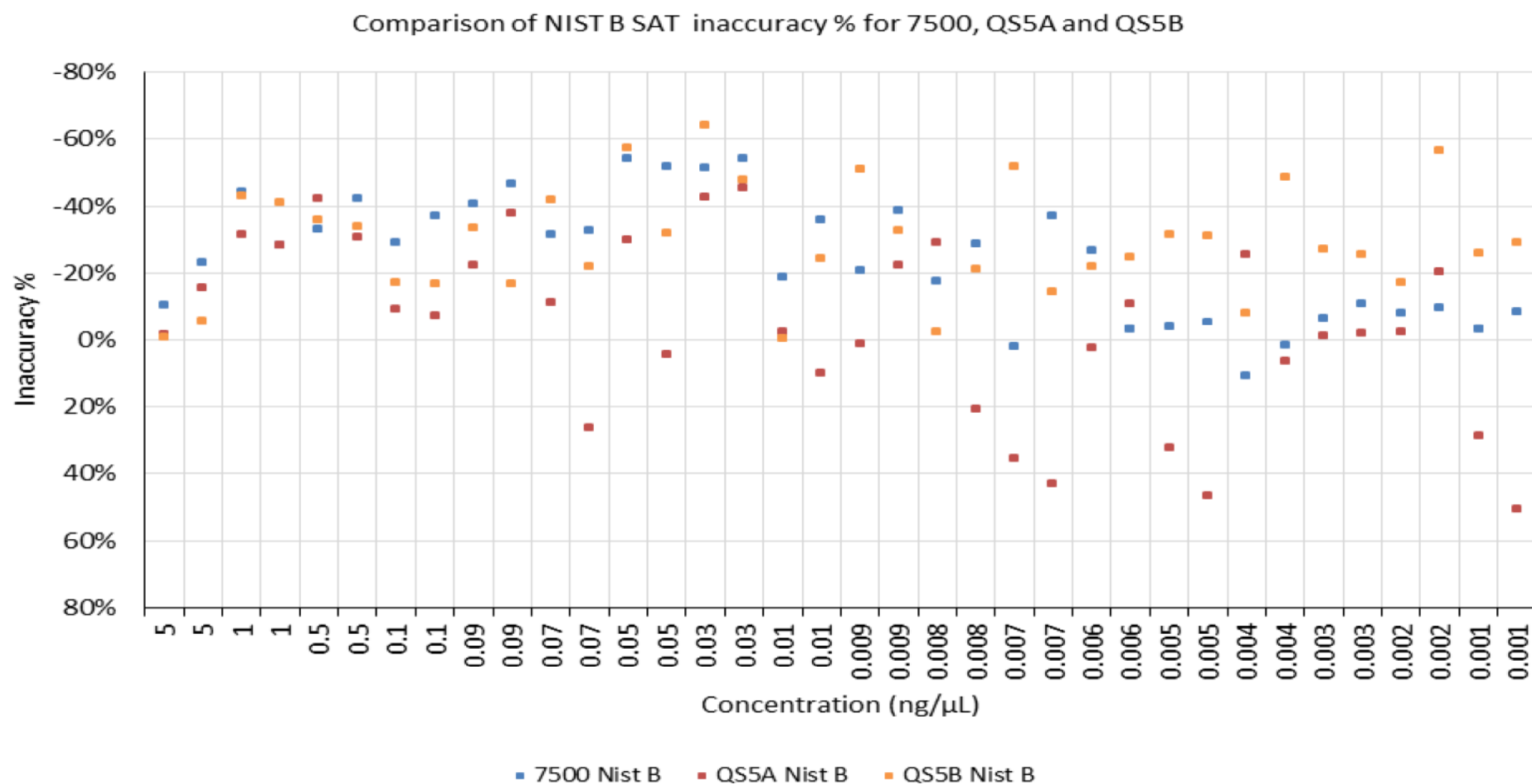


Figure 3: Comparison of NIST B SAT inaccuracy percentage for 7500, QS5A and QS5B

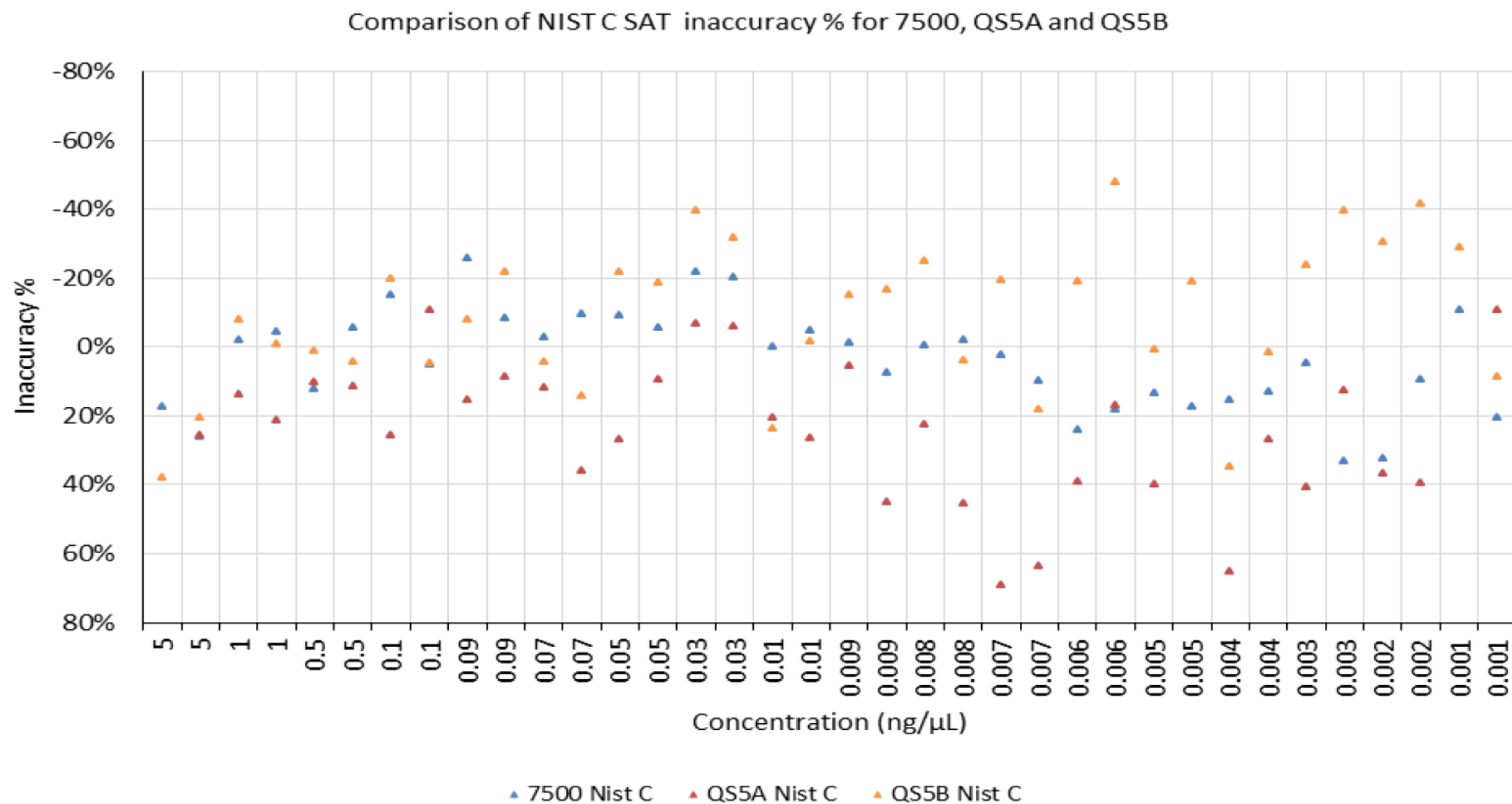


Figure 4: Comparison of NIST C SAT inaccuracy percentage for 7500, QS5A and QS5B

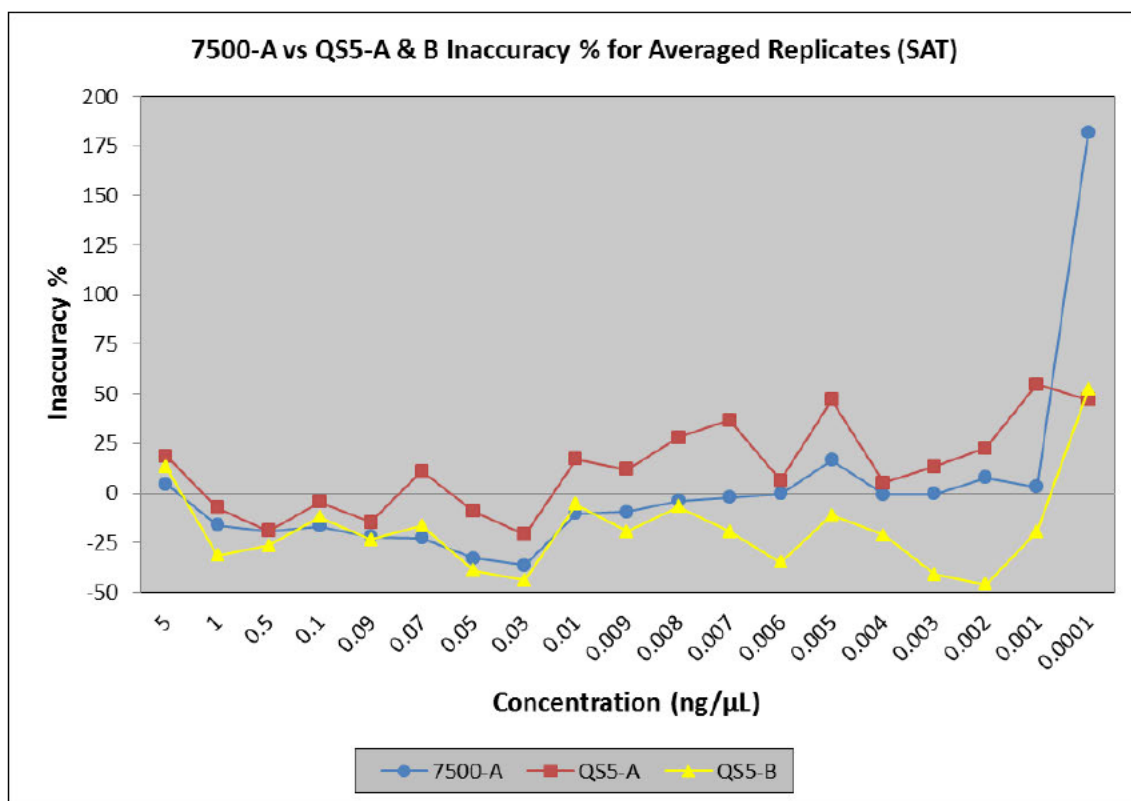


Figure 5: Percent inaccuracy for SAT

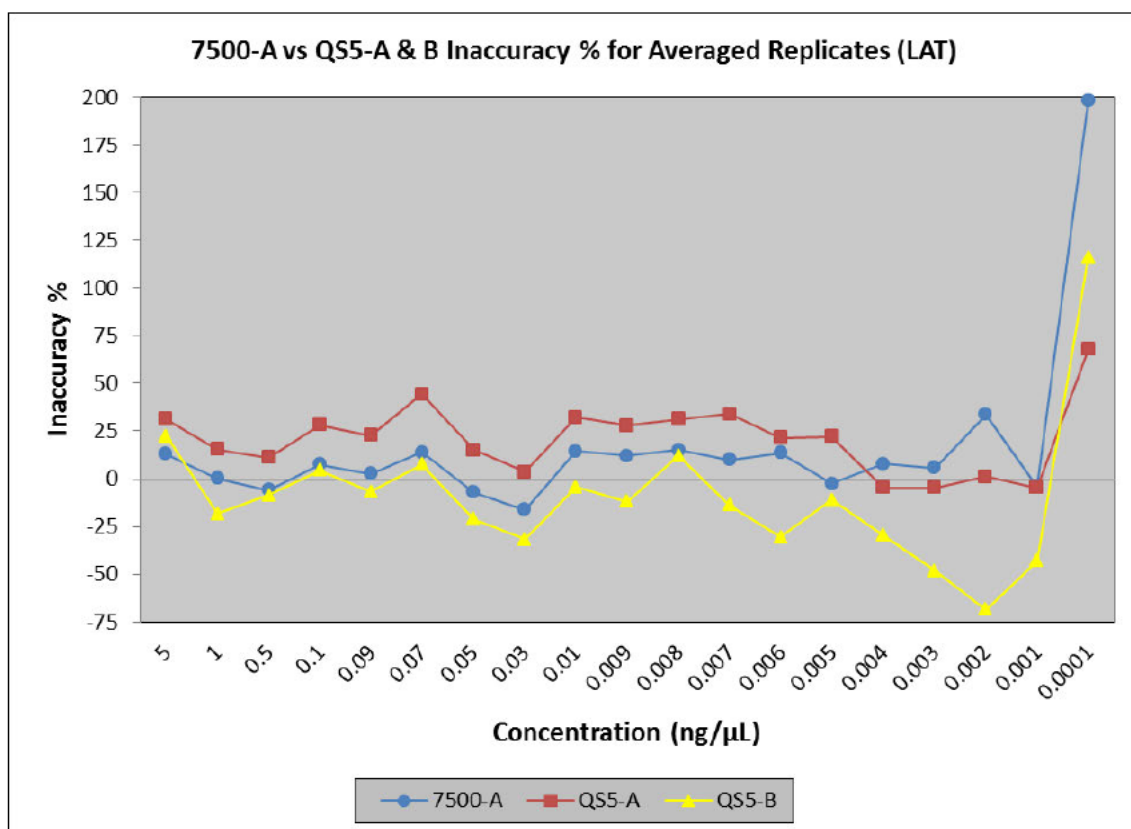


Figure 6: Percent inaccuracy for LAT

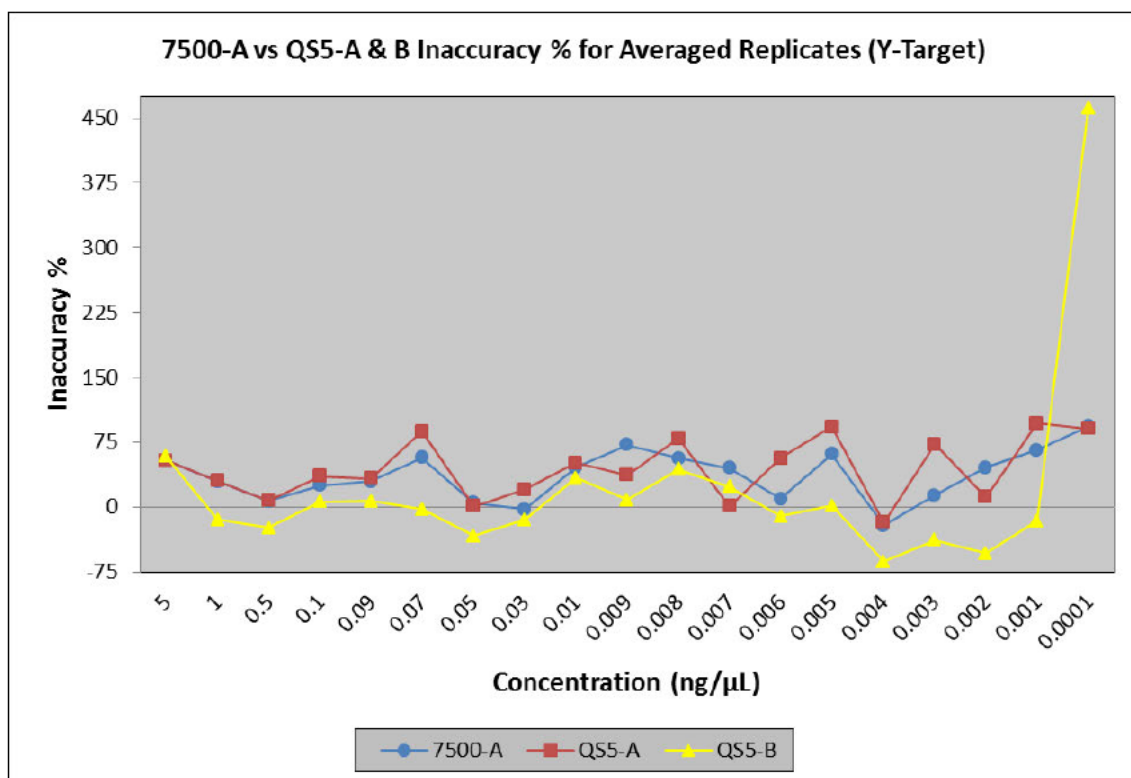


Figure 7: Percent inaccuracy for Y-Target

Discussion

The percent inaccuracy for 7500-A and the QS5s for all quantification targets (SAT, LAT and Y-Target) were similar for most dilutions, although the difference in inaccuracy was greater for some dilutions which is to be expected considering the observations of previous studies [2], and the inherent variation that is routinely observed between replicates using the Quant Trio kit.

The lowest dilution for which all replicates gave a quantification result for all targets on the 7500-A and QS5-A was 0.001 ng/μL. On QS5-B one replicate each of NIST A SAT, NIST B LAT and NIST C Y-target showed quantification values of undetermined at the 0.001 ng/μL dilution.

At the 0.0001 ng/μL dilution, 8/16 replicates gave an undetermined result for the 7500-A compared to 6/16 replicates for QS5-A and 11/16 for QS5-B (data not shown). This suggests the LOD for the QS5s are comparable to the 7500-A.

The large disparity between the 7500-A and the QS5s observed for SAT and LAT at 0.0001 ng/μL (Figures 5 & 6) supports the recommendations of previous studies ^[2] that the LOD for Quant Trio on the 7500s should be set at 0.001 ng/μL. This suggests the QS5s may be more accurate than the 7500-A at concentrations between 0.001 ng/μL and 0.0001 ng/μL.

Acceptance Criteria

The results indicate the LOD for Quant Trio on the QS5s is as good or better than the 7500A. Considering all the results, it is recommended the LOD for Quant Trio on the QS5 for SAT, LAT and Y-Target be set at 0.001 ng/μL.

Experiment 2: Comparison of QS5s and 7500

Purpose

To compare the performance of the two instrument types, the Student *t*-test (two-tailed distribution, paired) was performed to determine if there was a significant difference in quantification results across the entire dilution series. Student *t*-tests were performed separately for SAT, LAT and Y-Targets specific to each of the NIST standards using both replicates for each instrument. Only NIST A and C were used for Y-Target results. The two QS5s were compared to 7500-A using separate *t*-tests.

Results

The *t*-test results indicate that there is no significant difference between the quantification values between 7500-A and the QS5 instruments at quantification targets SAT, LAT and Y-Target as shown in Table 4.

Table 4: Student's *t*-test P-values for comparison of QS5-A and QS5-B with 7500-A

Standard	Instruments compared	SAT	LAT	Y-Target
NIST A	QS5-A & 7500-A	0.70050	0.06813	0.42519
	QS5-B & 7500-A	0.44247	0.77529	0.19765
NIST B	QS5-A & 7500-A	0.05212	0.06054	N/A
	QS5-B & 7500-A	0.19258	0.15191	
NIST C	QS5-A & 7500-A	0.23834	0.09180	0.39582
	QS5-B & 7500-A	0.52538	0.45386	0.32165

Note: P-values < 0.05 indicate a significant difference between results produced by the two instruments.

Discussion

The results indicate the difference between quantification values for 7500-A and the QS5s are not significant for the SAT, LAT and Y-Targets for both the QS5s. The difference in LAT values for the QS5-A comparison was observed to be higher than for QS5-B, however the opposite trend was evident for the Y-Target comparison, where the QS5-B comparison showed a greater difference. The difference between SAT values showed no specific trend with QS5-A showing a greater difference than QS5-B for NIST B and C, but not for A.

As the LAT region component of the Quant Trio kit is designed to provide only an approximate estimation of the level of degradation for samples, it is expected quantification values for this target would vary over time and with freeze/thaw cycles since the target is more than twice the size of the SAT and Y-Targets ^[1]. The LAT and degradation index is currently not used by Forensic DNA Analysis.

Acceptance Criteria

The comparison of the QS5s and 7500-A quantification results using student *t*-tests indicates there is no significant difference in the ability to quantify SAT, LAT and Y-Targets, therefore both the QS5 instruments are comparable to 7500-A for these parameters and should be accepted.

Experiment 3a: Repeatability

Purpose

To assess whether the QS5-A produced the same results when a set of twelve CTS samples was processed in replicates of seven by one operator under the same conditions. The SAT results from the repeatability plate (Table 5) were compiled using a scatter plot, and the comparability of results was assessed qualitatively.

Table 5: CTS Sample Platemap for Repeatability

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD 1 50 ng/μL	STD 5 0.005 ng/μL	CTS 1	CTS 2	CTS 4	CTS 5	CTS 6	CTS 7	CTS 8	CTS 9	CTS 10	CTS 12
B	STD 1 50 ng/μL	STD 5 0.005 ng/μL	CTS 1	CTS 3	CTS 4	CTS 5	CTS 6	CTS 7	CTS 8	CTS 9	CTS 11	CTS 12
C	STD 2 5.000 ng/μL	Reagent Blank	CTS 2	CTS 3	CTS 4	CTS 5	CTS 6	CTS 7	CTS 8	CTS 10	CTS 11	CTS 12
D	STD 2 5.000 ng/μL	CTS 1	CTS 2	CTS 3	CTS 4	CTS 5	CTS 6	CTS 7	CTS 9	CTS 10	CTS 11	CTS 12
E	STD 3 0.500 ng/μL	CTS 1	CTS 2	CTS 3	CTS 4	CTS 5	CTS 6	CTS 8	CTS 9	CTS 10	CTS 11	CTS 12
F	STD 3 0.500 ng/μL	CTS 1	CTS 2	CTS 3	CTS 4	CTS 5	CTS 7	CTS 8	CTS 9	CTS 10	CTS 11	CTS 12
G	STD 4 0.050 ng/μL	CTS 1	CTS 2	CTS 3	CTS 4	CTS 6	CTS 7	CTS 8	CTS 9	CTS 10	CTS 11	CTS 12
H	STD 4 0.050 ng/μL	CTS 1	CTS 2	CTS 3	CTS 5	CTS 6	CTS 7	CTS 8	CTS 9	CTS 10	CTS 11	Reagent Blank

Results

The quantification results for the twelve CTS sample replicates are displayed in a scatter plot (Figure 8). It is evident from these results the repeatability of measured quantification values between each of the seven replicates is qualitatively comparable for all twelve samples.

A clear trend is apparent in the variability between replicates for each sample, with extracts of a higher concentration displaying a greater spread of quantification values than extracts with concentrations below 2 ng/μL.

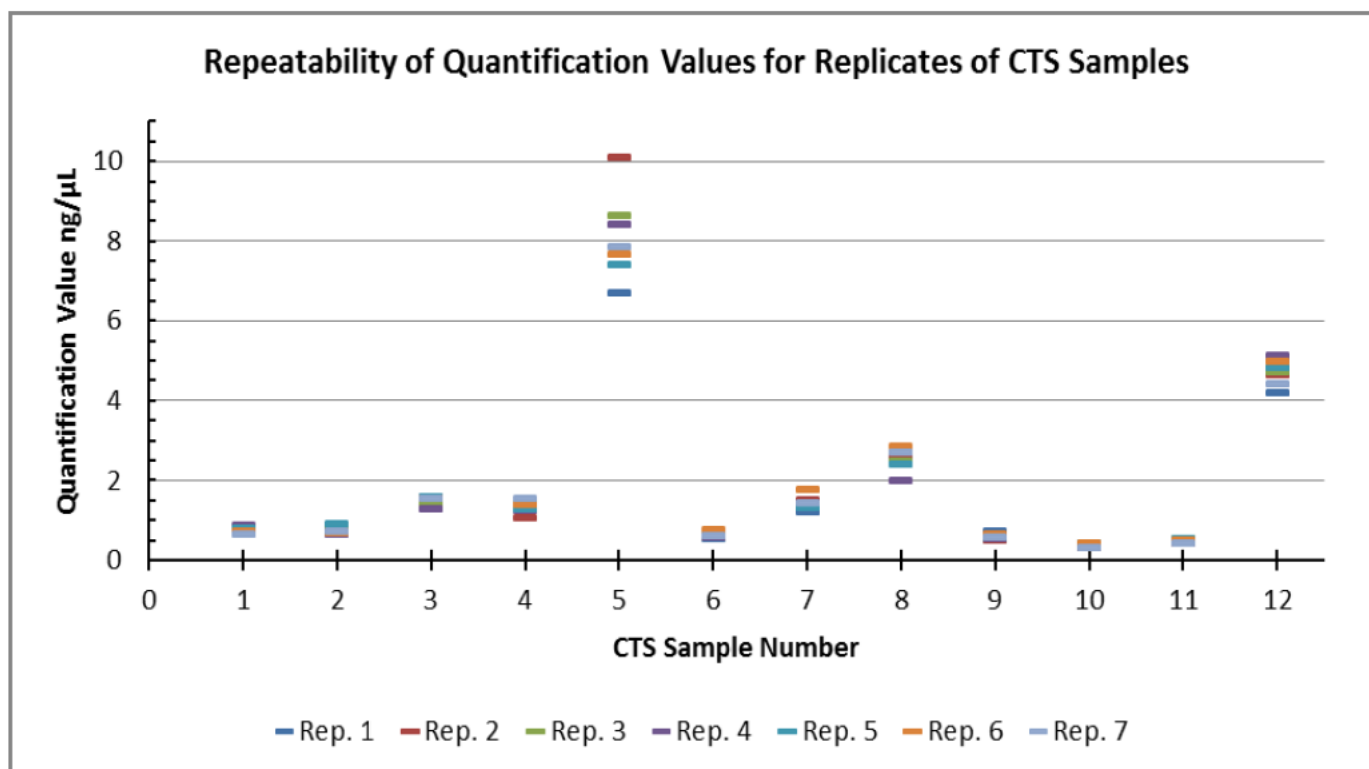


Figure 8: Repeatability of Quantification Values for Replicates of CTS Samples

Discussion

The variability observed between replicates for each CTS sample provides evidence that a degree of variation is present in the Quantifiler Trio system's ability to produce repeatable results. This is particularly evident for CTS sample 5, which had the highest measured concentrations for each of the replicates.

Variation among replicates is seen to reduce dramatically as the quantification values decrease below 2 ng/μL, with CTS sample 10 displaying the lowest variation. This observation is to be expected as variations in amplification efficiency are more likely to occur in samples with higher concentrations, which is further exacerbated by the exponential increase of amplicons during PCR ultimately leading to a wider range of replicate variability.

Variability in quantification result repeatability using Quantifiler Trio has also been documented in previous studies ^[2], and as in the current study the values for each replicate of a specific samples were comparable.

Acceptance Criteria

Repeatability across all CTS samples were shown to be comparable between the seven replicates. These findings indicate the QS5 has produced results that are comparable to the original Quantifiler® Trio validation using the 7500 instrument [2], which also showed comparably similar results between replicates. Therefore the QS5 should be accepted.

Experiment 3b: Reproducibility

Purpose

To assess whether the QS5 reproduces the same quantification results by different operators on 5 different days, the results from the twelve CTS samples were compiled on a scatter plot together with the minimum and maximum values recorded for each sample in experiment 3a (Repeatability).

Table 6: CTS Sample Platemap for Reproducibility

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD 1 50 ng/μL	STD 5 0.005 ng/μL	CTS 6									
B	STD 1 50 ng/μL	STD 5 0.005 ng/μL	CTS 7									
C	STD 2 5.000 ng/μL	Reagent Blank	CTS 8									
D	STD 2 5.000 ng/μL	CTS 1	CTS 9									
E	STD 3 0.500 ng/μL	CTS 2	CTS 10									
F	STD 3 0.500 ng/μL	CTS 3	CTS 11									
G	STD 4 0.050 ng/μL	CTS 4	CTS 12									
H	STD 4 0.050 ng/μL	CTS 5	Reagent Blank									

Results

The quantification results for the twelve CTS samples reproduced over five days are displayed in a scatter plot (Figure 9). The majority of values are close to or within the maximum/minimum values recorded in the repeatability experiment, however as expected, some of the reproducibility results are above or below these earlier observed upper and lower values.

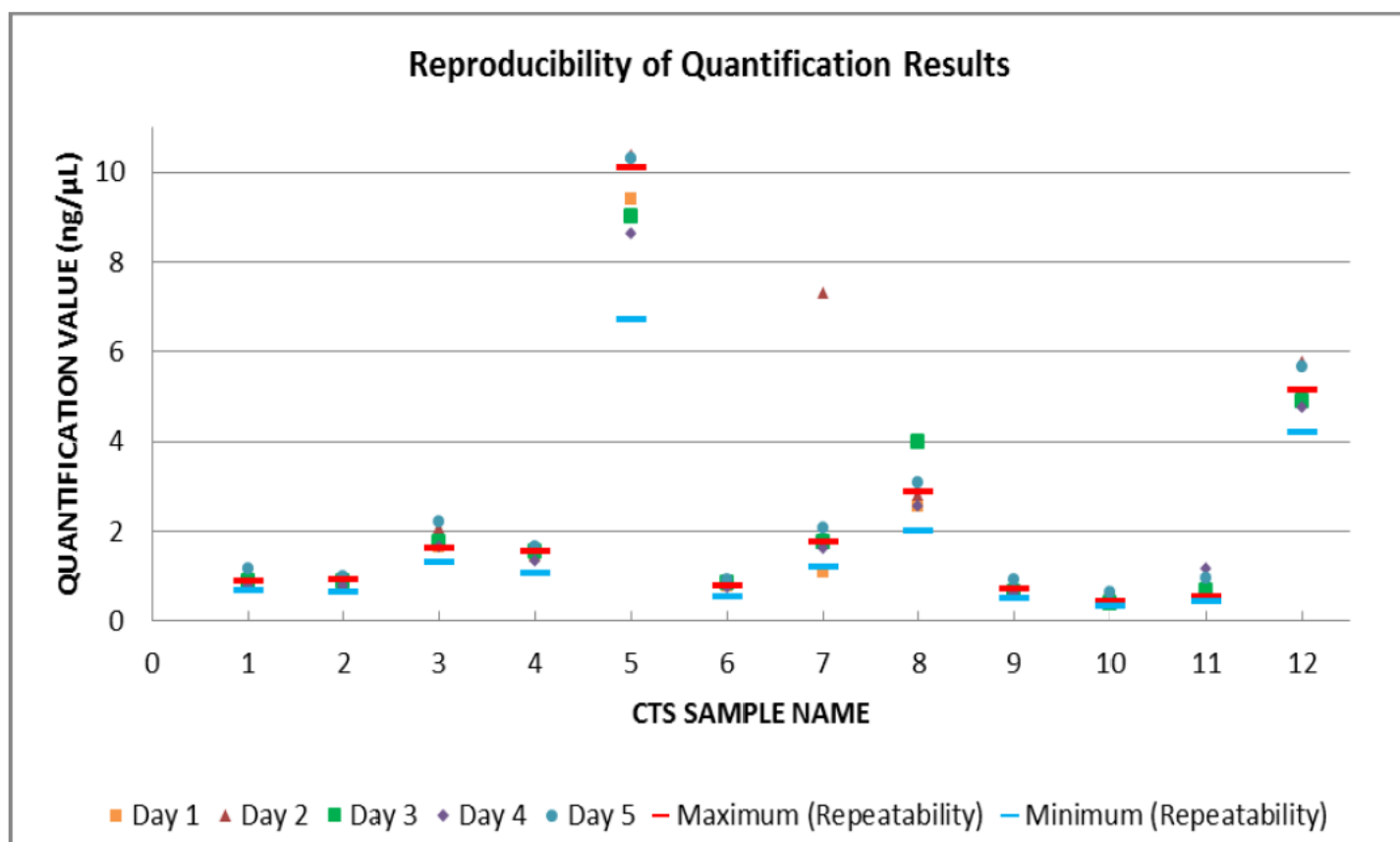


Figure 9: Reproducibility of Quantification Values for CTS Samples over 5 days

The quantification result for sample seven reproduced on day two resulted in a value that was dramatically higher than all other recorded quantification values for this sample. It is relevant to note here that a singular, aberrant, result was observed in experiments that were conducted as part of an earlier version of this report (data not shown).

Discussion

As for experiment 3a (repeatability), the variability between reproduced results for each of the twelve CTS samples supports the premise that a degree of variation is present in the Quantifiler Trio system's ability to generate reproducible results. As previously

discussed in Experiment 3a, the degree of this variation appears to correlate with concentration as observed for samples 5, 8 & 12, which had the three highest concentrations. Furthermore, samples that provide overestimated quantification results would likely produce partial or No Signal Detected Profiles during the Profile Data Analysis stage which would either be re-quantified or re-amplified at a higher template input to provide an uncompromised result.

Overall, the data shows that for a range of samples, the QS5 reproduces comparable results regardless of which personnel are operating the PCR setup and QS5 instruments, or on which day the assay and analysis was performed.

The unexpected high quantification value for sample seven on day two could not be attributed to any one source, and may be the result of a range of factors including the Quantification PCR variations, pipetting variation and QS5 detection anomalies. Standard operating procedures within Forensic DNA Analysis requires samples that produce quantification values >5 ng/ μ L to be diluted which effectively eliminates the chance of overloading subsequent amplification reactions.

Acceptance Criteria

Reproducibility of quantification values across the twelve CTS samples were shown to be qualitatively comparable despite the presence of some outlying data points. It is expected to observe some of the five reproducibility values that fall outside the minimum/maximum value range (obtained in experiment 3a) since only seven replicates were performed in experiment 3a to establish this range. Replicate numbers were limited to seven by the number of sample wells in the 96 well plate.

These findings indicate that the QS5 has produced results that are comparable to the original Quantifiler® Trio validation using the 7500 instrument [2], which showed that the same results can be produced for one sample set by different operators under the same conditions – i.e the results are reproducible and the QS5 should be accepted.

Experiment 4: Y-Intercept Thresholds

Purpose

To determine the Y-Intercept thresholds for the SAT, LAT and Y-Targets, the values from eleven plates run on the QS5s (Plate 1 (QS5-A & B), Plate 2 (QS5-A & B), QS5-B standards only, repeatability QS5-A, and five reproducibility plates QS5-A) were used. The current ranges ^[5] will be used for the implementation of the two QS5 instruments with Quantifiler® Trio if the calculated Y-intercept values fall within these ranges.

Results

The average Y-intercept values taken from the thirteen plates ran on the QS5s $\pm 3 \times$ standard deviations was calculated and compared to the current Y-Intercept thresholds ^[5] as shown in Table 7.

Table 7: Y-Intercept ranges calculated for QS5 compared to current ranges.

	QS5 Y-Int. Range	Current Y-Int. Range
LAT	23.85 – 25.63	24.28 – 26.30
SAT	25.83 – 27.73	26.36 – 28.63
Y-Target	24.68 – 26.81	25.51 – 28.11

The QS5 Y-Intercept upper ranges for SAT, LAT and Y-Target are all within the current ranges, and below the current upper ranges. However the calculated QS5 lower ranges all fall outside (below) the current ranges outlined in the Quantification SOP ^[5]. It is important to note that only one of the eleven QS5 plates had Y-Intercept ranges that fell outside of the current ranges, therefore this one plate contributed greatly to shift the newly calculated ranges out of the current ranges.

Discussion

The newly calculated Y-Intercept ranges for QS5 are considerably narrower than the current ranges, which is in part due to the relatively small number of plates used to calculate them. It is important to consider that calculated thresholds are instrument and kit specific so variation is to be expected. As more plates are processed after implementation, the cumulative data will be used to recalculate these ranges over time.

Acceptance Criteria

Since the newly calculated QS5 Y-Intercept ranges are relatively narrow and fall under the current upper ranges but below the lower ranges, the QS5 implementation will utilise the current ranges until more data is available to allow recalculation for QS5.

Conclusion

The results of experiment 1 showed the LOD for QS5 is similar to that of 7500 and possibly even more sensitive although more studies are required to confirm this. These findings support the recommendations of the original Quantifiler® Trio validation that the LOD be set to 0.001 ng/μL.

Independently comparing the results of both QS5 instruments to those produced by 7500-A showed no significant differences in SAT, LAT and Y-Target quantification results demonstrating comparability between 7500 and QS5.

The QS5 instrument showed qualitative comparability in repeatability results across all CTS samples demonstrating comparability to the 7500 instrument which also produced repeatable results in the original Quantifiler® Trio validation.

The QS5 instrument was also able to demonstrate comparable results reproduced by different operators on different days for the selected CTS samples. These results are comparable to the findings for the original Quantifiler® Trio validation using the 7500.

The Y-intercept ranges calculated from the values obtained from all eleven QS5 plates produced in this study all fall below the upper ranges that are currently in use, however these new ranges also fall below the lower current ranges. Given the ranges calculated for QS5 are considerably narrower than current ranges, it is recommended that the current ranges be used for QS5 implementation, and the thresholds revised every 2 weeks for the first 3 months once the data set is expanded.

Recommendations

1. QuantStudio™ 5 Real-Time PCR systems A and B be implemented for DNA quantification using the Quantifiler® Trio DNA quantification kit, and thus replacing the two 7500 Real-Time PCR systems.
2. Y-Intercept data for SAT, LAT and Y-Targets are to be collated and used to recalculate/monitor ranges over time after implementation of the QS5s (not greater than 6 months).

References

- [1] Thermo Fisher Scientific, Quantifiler® HP and Trio DNA Quantification Kits UserGuide, Publication Number 4485354, Revision A. Publication Number 4485354, Revision A ed2014.
- [2] Validation of Quantifiler® Trio. P. Acedo, M. Mathieson, L. Ryan, C. Allen. September 2015. Forensic DNA Analysis.
- [3] Certificate of Analysis – Standard Reference Material® 2372 Human DNA Quantitation Standard. National Institute of Standards & Technology.
- [4] Project Proposal #185 – Validation of the QuantStudio™ Real-Time PCR Systems (June 2017).
- [5] Operation and Maintenance of the Microlab STARlet and LabElite Integrated I.D.Capper. QIS 34050.
- [6] Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit. QIS 33407.