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Validation of two QuantStudio™ 5 Real-Time PCR Systems

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Document Details

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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
- Repeatability and Reproducibility
- Y Intercept Thresholds

The results of this verification found that the second QIAsymphony® instrument is suitable to perform both DNA extractions and quantification assay preparation. Cross contamination was not detected in this verification and the QIAsymphony® SP/AS instrument gave repeatable and reproducible results.

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 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 have been purchased.

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

Validation and implementation of the two QS5s will be staggered. QS5-A was validated first, whilst maintaining one 7500 in operation for routine processing. Once QS5-A had been validated and implemented the remaining 7500 was removed from

use and QS5-B commenced validation. The validation experiments for both QS5s were be 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 Quant Studio Real Time PCR (June 2017). This document will be referred to as the Experimental Design. The following QIS documents are referenced throughout this report:

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

Methods

The methods for each experiment in this verification were as per the approved in-house document Project Proposal #185 – Validation of Quant Studio Real Time PCR (June 2017) [4] unless otherwise specified. This document will be referred to as the Experimental Design.

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].

Experiments and Results

Experiment 1: Sensitivity and Limit of Detection

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 instruments.

Results

Two plates of NIST standards A, B, and C serial dilution duplicates were prepared each for the 7500 and QS5A 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

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								

Plates were prepared as per (QIS 34050) Operation and Maintenance of the Microlab STARlet and LabElite Integrated I.D.Capper and (QIS 33407) Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit for the 7500 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 3 outlines the expected and the average quantification values and % inaccuracy for each serial dilution obtained from the 7500A and QS5A instruments. The SAT, LAT and Y-Target results for both instruments all gave quantification results down to 0.0001 ng/µL.

The % inaccuracy for SAT and LAT for the 7500A was markedly higher (>180%) at 0.0001 ng/µL than for QS5A (<70%), which supports the recommendation of previous studies [2] that the LOD for Quant Trio on the 7500s should be set at 0.001 ng/µL. The data indicates that the QS5A is more accurate than 7500A at the lowest dilution concentration tested (0.0001 ng/µL) for SAT and LAT, although it should be noted that the inaccuracy % for both instruments fluctuates across the range of dilutions tested (Figure 1-3).

Table 3: Average quantification results and % inaccuracy

	7500A						QS5A					
Concentration (ng/µL)	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
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
0.5	0.40486	-19.0	0.47043	-5.9	0.53297	6.6	0.40410	-19 2	0.55648	11.3	0.53550	7.1
0.1	0.08333	-16.7	0.10740	7.4	0.12445	24.5	0.09544	-4.6	0.12827	28.3	0.13520	35.2
0.09	0.07025	-21.9	0.09250	2.8	0.11651	29.5	0.07659	-14 9	0.11041	22.7	0.11979	33.1
0.07	0.05418	-22.6	0.07967	13.8	0.10983	56.9	0.07768	11 0	0.10107	44.4	0.13110	87.3
0.05	0.03357	-32.9	0.04646	-7.1	0.05238	4 8	0.04542	-9.2	0.05750	15.0	0.05022	0.4
0.03	0.01906	-36.5	0.02510	-16.3	0.02913	-2 9	0.02372	-20 9	0.03104	3.5	0.03598	19.9
0.01	0.00898	-10.2	0.01146	14.6	0.01457	45.7	0.01172	17 2	0.01321	32.1	0.01511	51.1
0 009	0.00815	-9.4	0.01009	12.1	0.01543	71.4	0.01008	12 0	0.01152	27.9	0.01234	37.1
0 008	0.00768	-4 0	0.00922	15.2	0.01249	56.2	0.01025	28.1	0.01051	31.3	0.01435	79.3
0 007	0.00684	-2 3	0.00769	9.9	0.01013	44.7	0.00958	36 9	0.00939	34.1	0.00703	0.5
0 006	0.00597	-0.6	0.00681	13.5	0.00658	9.6	0.00638	6.4	0.00730	21.6	0.00939	56.6
0 005	0.00582	16.4	0.00487	-2.5	0.00806	61.3	0.00735	47 0	0.00611	22.2	0.00964	92.8
0 004	0.00397	-0.7	0.00431	7.7	0.00313	-21.8	0.00421	5.2	0.00382	-4.6	0.00328	-18.0
0 003	0.00299	-0.4	0.00317	5.6	0.00339	13.0	0.00340	13.4	0.00265	-11.6	0.00518	72.7
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
0 001	0.00103	3.1	0.00096	-4.0	0.00166	65.6	0.00155	55 0	0.00095	-4.7	0.00197	96.9
0.0001	0.00028	181.6	0.00030	198 0	0.00019	92.9	0.00015	47 0	0.00017	67.6	0.00019	90.8
Average % Inacc.		1.1		16.4		36.1		12 9		20.5		44.1

Cells shaded in green indicate a higher accuracy comparing the two instruments

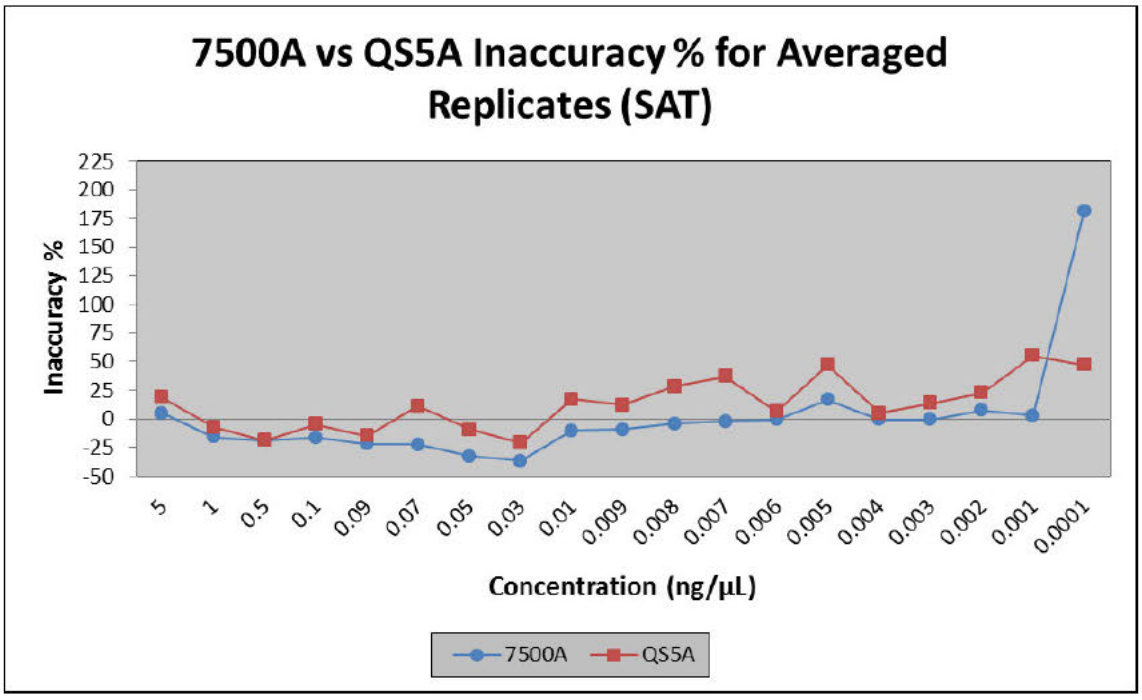

Figure 1: Percent inaccuracy for SAT

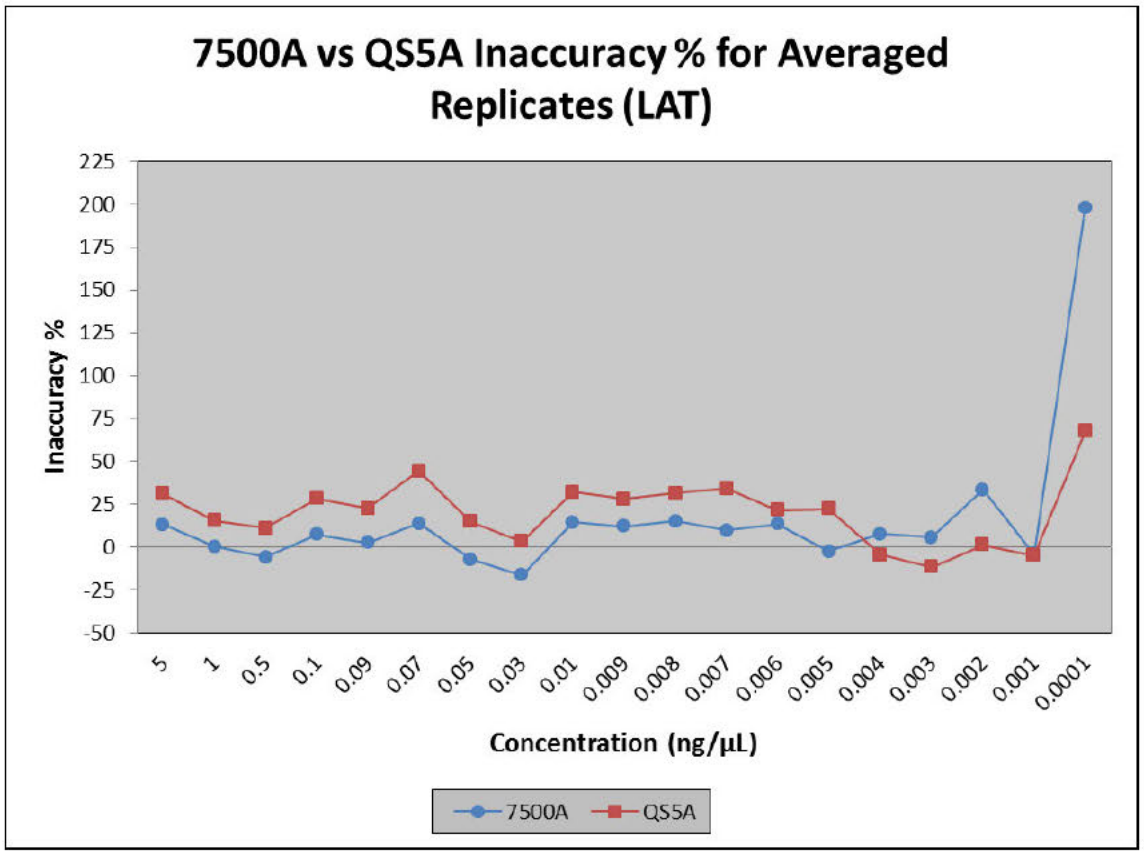

Figure 2: Percent inaccuracy for LAT

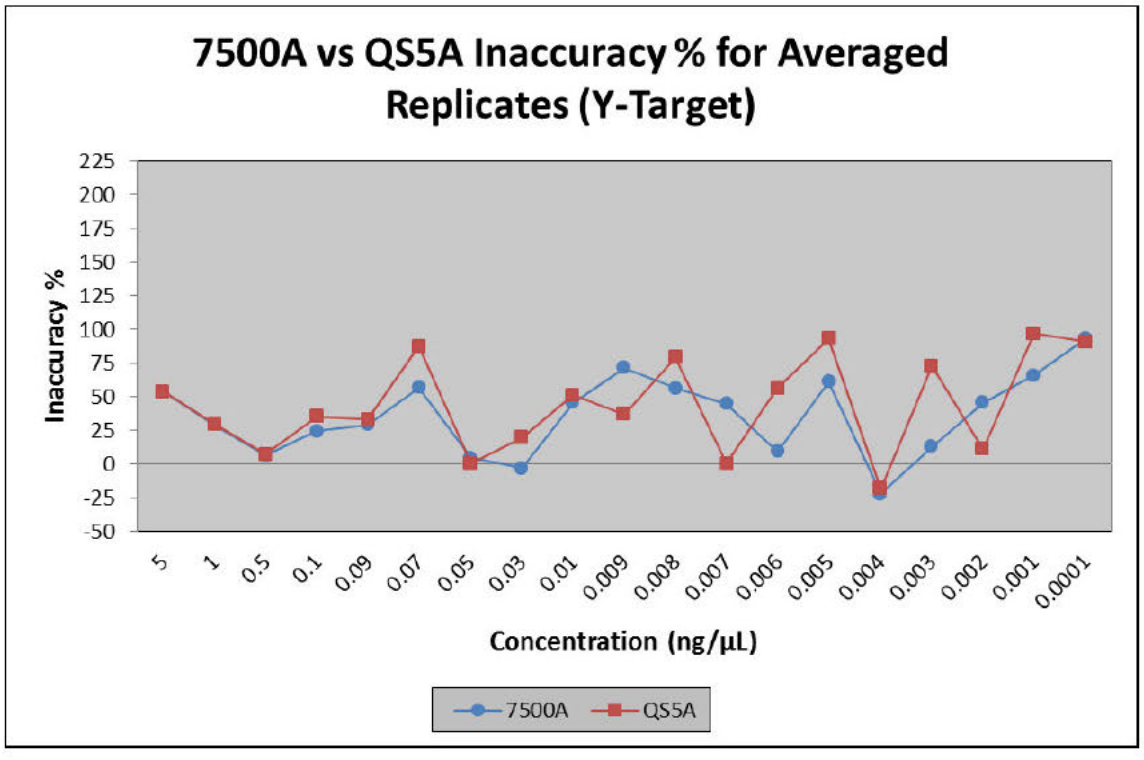

Figure 3: Percent inaccuracy for Y-Target

Discussion

The percent inaccuracy for 7500A and QS5A 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 LOD appears to be the same (0.001 ng/µL) for both the 7500A and QS5A as this is the lowest dilution for which all replicates for each instrument gave a quantification result. At the 0.0001 ng/µL dilution level, 8/16 replicates gave an undetermined result compared to 6/16 replicates for QS5A (data not shown) which suggests that the LOD for QS5A may be lower than that of 7500A (between 0.001 ng/µL and 0.0001 ng/µL) however dilutions between these values were not investigated in this study. The large disparity between instruments observed for SAT and LAT at 0.0001 ng/µL also supports the recommendations of previous studies [2] that the LOD for Quant Trio on the 7500s should be set at 0.001 ng/µL, and suggests that the QS5A is more accurate for these targets than the 7500A below 0.0001 ng/µL.

Acceptance Criteria

The results suggest that the LOD for Quant Trio on the QS5A is as good or better than the 7500A. Considering all the results, it is recommended that the QS5A LOD for SAT, LAT and Y-Target be set at 0.001 ng/µL.

Experiment 2: Comparison of QS5 and 7500

Purpose

To compare the performance of the two instruments, the Student *t*-test (two-tailed distribution, two-sample unequal variance) was performed to determine if there was a significant difference in combined quantification results using all the dilution series. Student *t*-tests were performed separately for SAT, LAT and Y-Targets. NIST A, B and C results (replicates 1 and 2) were combined for SAT and LAT, and replicates 1 and 2 for NIST A only were used for Y-Target results.

Results

The *t*-Test results indicate that there is no significant difference between QS5A and 7500A instruments at the three quantification targets SAT, LAT and Y-Target as shown in Table 4.

Table 4: Student's *t*-test P-values for comparison of QS5A and 7500A.

t-test data input	QS5A SAT P-Value	QS5A LAT P-Value	QS5A Y-Target P-Value
Average of values for all dilutions	0.46711	0.15677	0.87729
Sum of values for all dilutions	0.40012	0.19933	0.63876

For SAT and LAT, the quantification values produced for each standard and replicate (NIST A, B and C, replicates 1 and 2) were averaged/summed across all dilutions for each instrument (6 values used for each *t*-test array). For Y-Target, NIST A replicates 1 and 2 were used to produce 2 values for each *t*-test array.

Discussion

The results indicate the difference between quantification values for QS5A and 7500A is the least significant at the Y-Target, followed by SAT, with LAT having the highest level of difference. However the LAT P-Value is considerably higher than the 0.05 value, below which the test indicates a significant difference.

Acceptance Criteria

The comparison of QS5A and 7500A quantification results using student *t*-tests indicates there is no significant difference in the ability to quantify SAT, LAT and Y-Targets, therefore the instruments are comparable.

Experiment 3a: Repeatability

Purpose

To assess whether the QS5A produces the same results when one sample set is processed in duplicate by one user under the same conditions, the results from plates 1 and 2 (Tables 1 and 2 respectively) for the dilution series were compared using percentage change between the two replicates for SAT, LAT and Y-Target for each of the NIST standards.

A Student *t*-test (two-tailed distribution, two-sample unequal variance) was also performed separately for SAT and LAT to compare the repeatability results for the QS5A and 7500A.

Results

The percentage change between replicates for NIST A, B and C dilution series are shown in Figure 4, 5 and 6 respectively. The percentage change for NIST A (Figure 4) targets appear to be variable and show no specific trends. As one 0.0001 ng/µL replicate for Y-Target produced an undetermined result, this data point could not be included.

Similarly, the percentage changes for NIST B and C also do not exhibit continuous trends (i.e. one target having a consistently lower change than another, or one target fluctuating more than another). At the 0.0001 ng/µL dilution, no data is available for NIST A Y-Target, NIST B SAT and NIST C SAT and LAT as one or more replicates produced an undetermined quantification value.

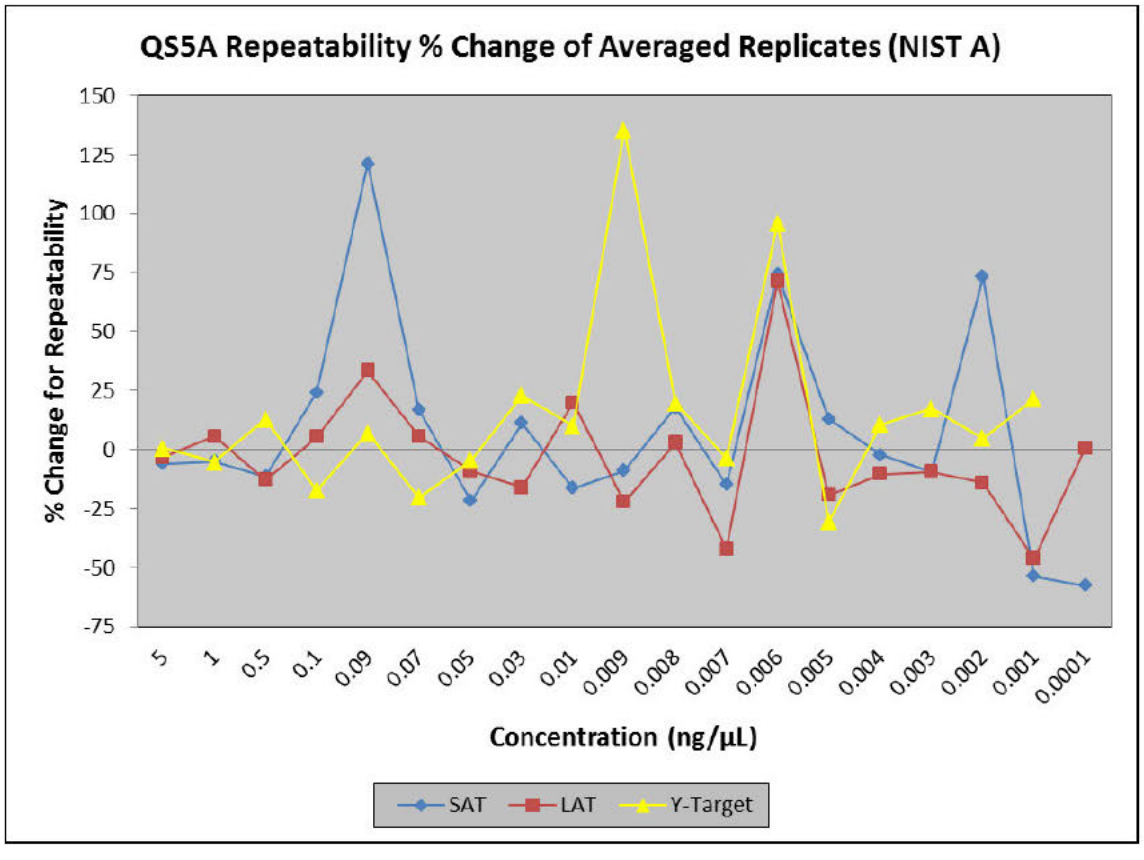

Figure 4: Percent change in repeatability for NIST A

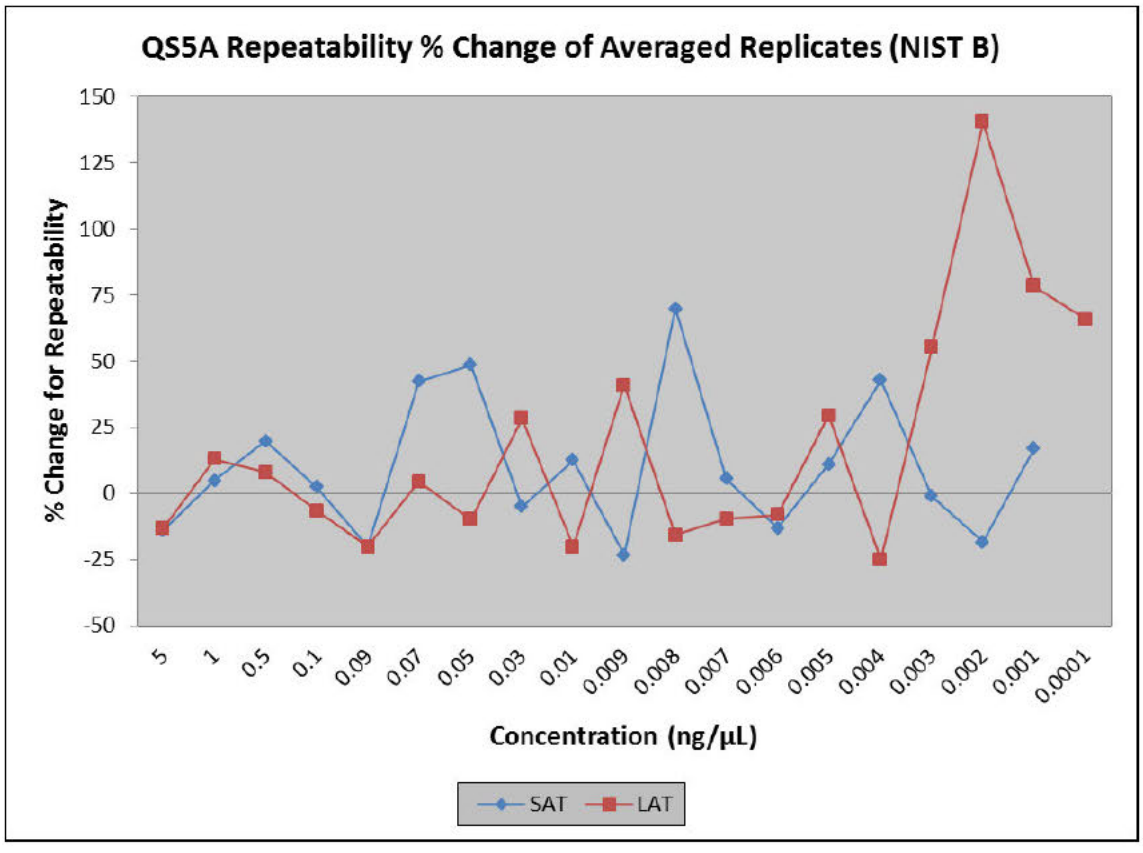

Figure 5: Percent change in repeatability for NIST B

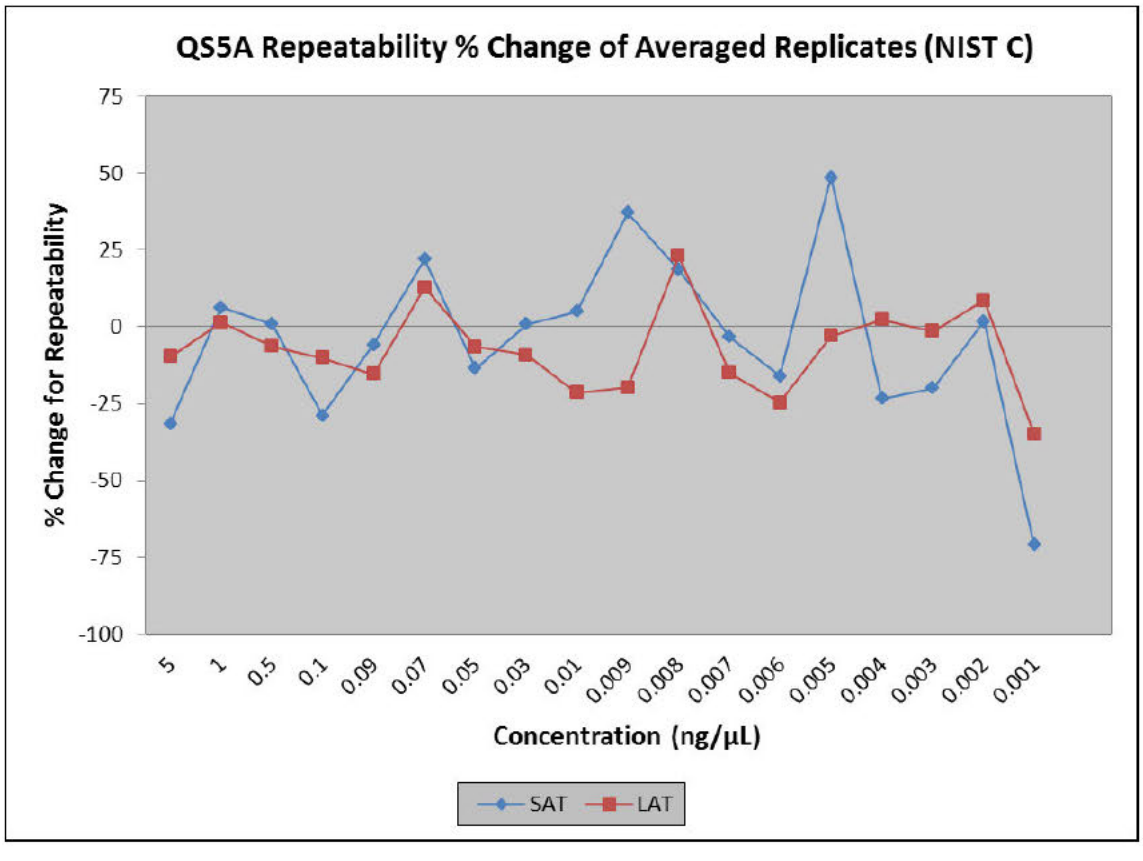

Figure 6: Percent change in repeatability for NIST C

The Student's *t*-test scores shows that no significant differences in repeatability results were observed for all of the 19 serial dilutions (Table 4) for the QS5A and 7500A. At the 0.001 ng/µL concentration it is evident that repeatability is demonstrable for both the QS5A and 7500A for the SAT and LAT targets. Analysis could not be performed for the SAT target for both instruments at the 0.0001 ng/µL dilution due to undetermined quantification values.

Table 4: Student's *t*-test score for repeatability comparison between QS5A and 7500A.

DNA Concentration (ng/µL)	QS5A SAT P-Value	7500A SAT P-Value	QS5A LAT P-Value	7500A LAT P-Value
5	0.43464	0.86890	0.63798	0.94500
1	0.92266	0.88435	0.75029	0.96900
0.5	0.95167	0.74397	0.62187	0.80791
0.1	0.75810	0.64242	0.86983	0.63072
0.09	0.68367	0.53703	0.94447	0.85757
0.07	0.06821	0.43891	0.68933	0.96881
0.05	0.99362	0.64511	0.37713	0.93425
0.03	0.89182	0.90010	0.90498	0.73559
0.01	0.92790	0.98261	0.82925	0.92494
0.009	0.94815	0.92194	0.83023	0.84420
0.008	0.27548	0.59479	0.97099	0.84258
0.007	0.85551	0.96689	0.25179	0.70795
0.006	0.82723	0.78577	0.74708	0.88341
0.005	0.27769	0.85790	0.95724	0.83584
0.004	0.93673	0.91370	0.44248	0.33716
0.003	0.38137	0.99742	0.77093	0.81520
0.002	0.57272	0.25556	0.42902	0.78322
0.001	0.20810	0.21849	0.74220	0.22692
0.0001	N/A	N/A	0.79942	0.46886

Cells shaded in orange indicate a P-Value <0.05. N/A indicates analysis was not performed due to undetermined quantification values. Cells shaded green indicate a higher accuracy of repeatability between QS5A and 7500A for each target.

Discussion

The variability observed between targets at specific dilutions, and across the entire dilution series for each NIST standard suggests that certain targets do not have a greater propensity to produce repeatable results. This is particularly evident for NIST A (Figure 4) where the % change for SAT and Y-Target at 0.09 ng/µL and 0.009 ng/µL

varies noticeably, whereas at 5 ng/µL and 0.006 ng/µL all targets are relatively similar despite the vast difference in concentration.

Comparing the repeatability *t*-test results of the QS5A to 7500A (Table 4) shows that repeatability results were not significantly different for both instruments for all compared dilutions. The lack of P-Value results for SAT at 0.0001 ng/µL for both instruments supports previous studies [2] which recommended the LOD be set at 0.001 ng/µL.

It is important to highlight that variability in quantification result repeatability using both instruments at specific dilutions is evident as can be seen from the P-Values in Table 4. It is expected that repeatability accuracy would decrease with decreasing concentration, however at 0.004 ng/µL both instruments resulted in higher accuracy than at 0.07 ng/µL for SAT, further highlighting the variability.

Furthermore, repeatability accuracy between instruments varies considerably at specific concentrations as can be seen for SAT at 0.003 ng/µL where the 7500A was more accurate, and at 0.05 ng/µL where the QS5A was more accurate.

Also, comparing repeatability P-Values between SAT and LAT at specific dilutions shows considerable variation for both instrument as can be seen at 0.004 ng/µL where the repeatability is more accurate for SAT than LAT for both instruments, but less accurate for SAT at 0.008 ng/µL.

Acceptance Criteria

Repeatability at all dilutions for QS5A and 7500A were shown to not differ significantly between replicates. The QS5A was shown to have a more accurate repeatability than the 7500A for 10 of the 18 dilution points compared for SAT, however the 7500A was shown to be more accurate for 11 of the 19 dilutions compared for LAT. These findings indicate that QS5A has produced results that are comparable to the 7500A.

Experiment 3b: Reproducibility

Purpose

To assess whether the QS5A produces the same results when one sample set is processed by different operators under different conditions, dilution series replicates plate 1 (Table 1) from experiment 1 were compared to a reproduced plate 1 using percentage change.

A Student *t*-test (two-tailed distribution, two-sample unequal variance) was also performed on the SAT and LAT results to determine if there was a significant difference between reproduced results for each dilution.

Results

The percentage change between reproduced replicates for NIST A, B and C dilution series are shown in Figure 7, 8 and 9 respectively. The percentage change for NIST A (Figure 7) appear to be similar between targets at higher concentrations (5-0.1 ng/µL), however similarities can also be seen at the 0.005 ng/µL dilution which indicates variability and no specific trends for the three targets across the dilution series.

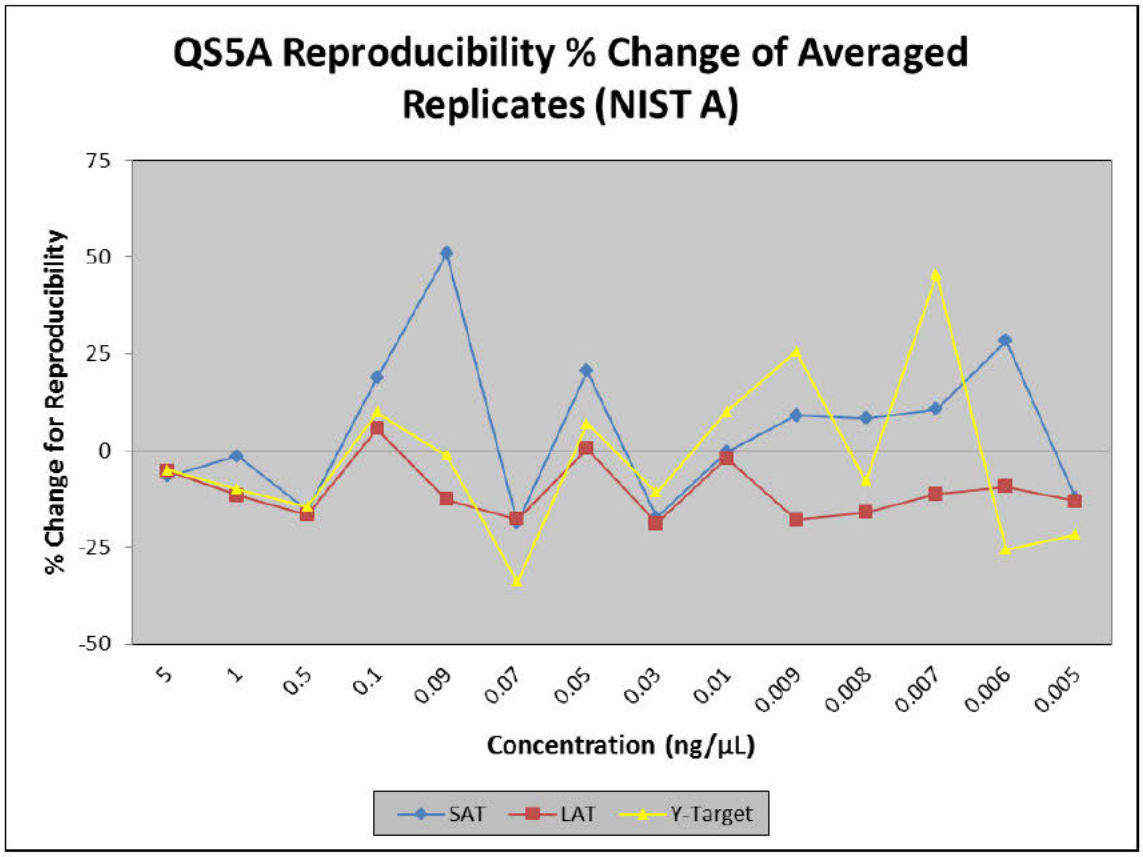

Figure 7: Percent change in reproducibility for NIST A

Similarly, the percentage changes for NIST B and C also do not exhibit continuous trends (i.e. one target having a consistently lower % change than another, or one target fluctuating more than another).

Both SAT and LAT targets for NIST B and C appear to not vary by more than +/- ~30%. One replicate of dilution 0.07 ng/µL for NIST C SAT on the reproduced plate produced a quantification value of ~6.5 ng/µL, this sample was quantified again in duplicate using identical consumables, reagents and instruments, and the new results used for Figure 9 and Table 5.

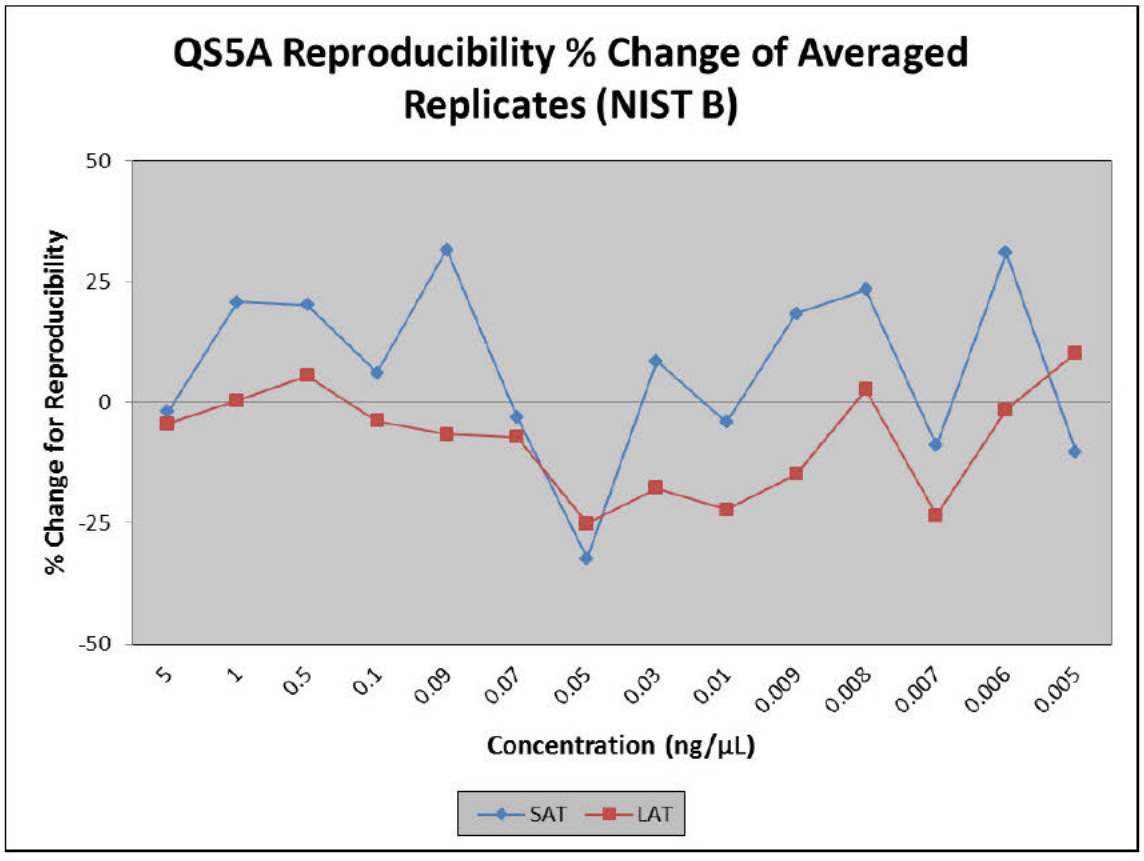

Figure 8: Percent change in reproducibility for NIST B

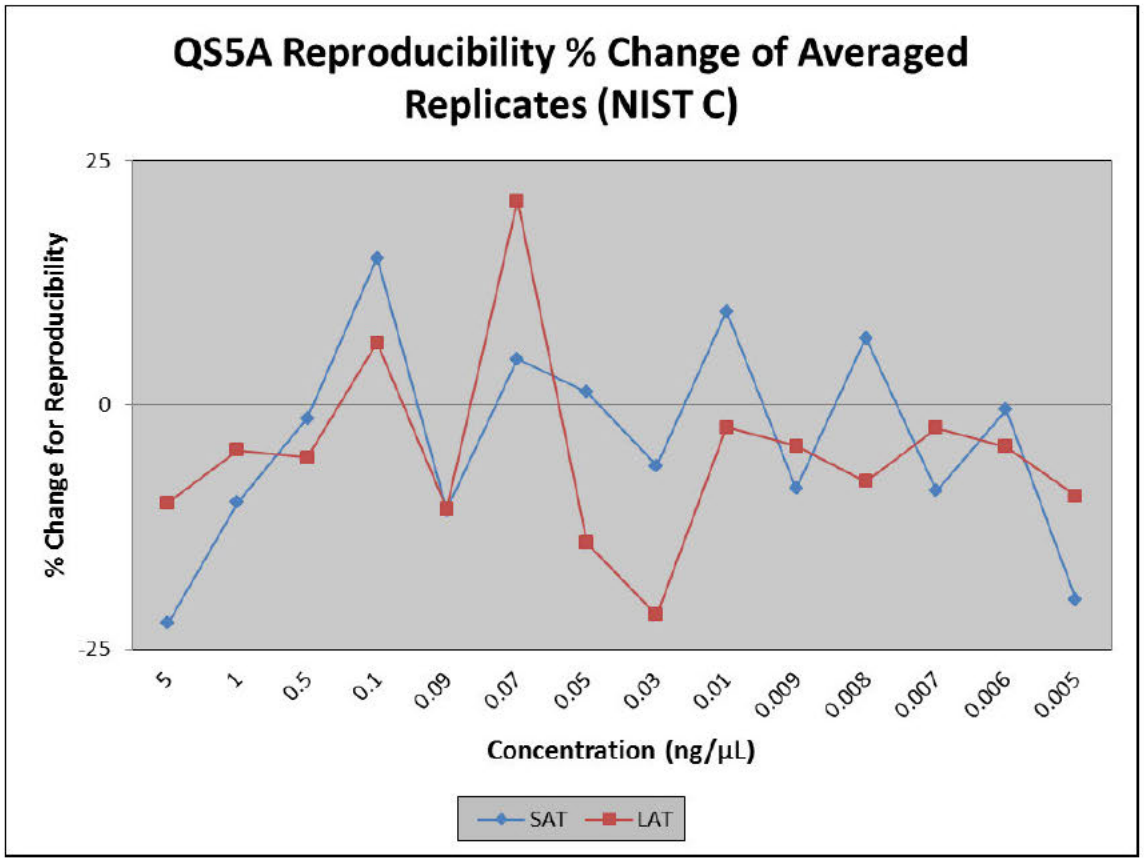

Figure 9: Percent change in reproducibility for NIST C

Table 5: Student's *t*-test score for reproducibility comparison between QS5A and 7500A.

DNA Concentration (ng/µL)	P-Value SAT	P-Value LAT
5	0.38085	0.57617
1	0.96049	0.58180
0.5	0.98437	0.33207
0.1	0.20177	0.82605
0.09	0.24754	0.53620
0.07	0.65340	0.69916
0.05	0.79259	0.27079
0.03	0.58615	0.17939
0.01	0.82323	0.60108
0.009	0.65022	0.39104
0.008	0.42330	0.63533
0.007	0.78140	0.30683
0.006	0.23013	0.74127
0.005	0.16601	0.83341

The Student's *t*-test scores shows that no significant differences were observed for all of the 14 serial dilutions (QS5A) for both the SAT and LAT targets.

Discussion

As for experiment 3A (repeatability), variability in repeatability between targets at specific dilutions and across the entire dilution series for each NIST standard do not indicate higher degrees of reproducibility for a particular target.

The greatest percentage change observed for the reproducibility data was approximately 50%, whereas the results from the repeatability experiment produced figures >125%. This is possibly due to the repeatability % being calculated from two replicates whereas the reproducibility is calculated using the averages of two replicates.

As for experiment 3A (repeatability), variability in quantification result reproducibility using QS5A at specific dilutions is apparent as can be seen from the P-Values in Table 5. Again there was no correlation observed between an increase in concentration and a higher P-Value indicating greater reproducibility accuracy. There were no significant differences observed for reproduced results observed at all dilutions tested.

Acceptance Criteria

(word this like repeatability acceptance criteria)

The *t*-test P-Values are concordant with the original Quantifiler® Trio validation study [2] which also did not report significant differences in reproducibility results for dilutions ranging from 0.09 ng/µL – 0.005 ng/µL. These findings indicate that QS5A reproducibility is as good as the original Quantifiler® Trio validation [2].

Experiment 4: Y-Intercept Thresholds

Purpose

To determine the Y-Intercept thresholds for the SAT, LAT and Y-Targets, the values from four plates run on the QS5 (Plate 1, Plate 2, reproduced Plate 1 and re-quant of dilution 0.07 ng/µL NIST C) 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 four plates ran on QS5A +/- 3 x standard deviations was calculated and compared to the current Y-Intercept thresholds [5] as shown in Table 6.

Table 6: Y-Intercept ranges calculated for QS5A compared to current ranges.

	QS5 Y-Int. Range	Current Y-Int. Range
LAT	24.63 – 25.31	24-28 – 26.30
SAT	26.66 – 27.51	26.36 – 28.63
Y-Target	25.74 – 25.94	25.51 – 28.11

The QS5A Y-Intercept ranges for SAT, LAT and Y-Target all fall into the current ranges outlined in the Quantification SOP [5].

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 run 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 but fall within the current ranges, the QS5 implementation will utilise the current ranges until more data is available to allow recalculation for QS5.

Conclusion

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.

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 #185 – Experimental Design - Validation of QuantStudio Real Time PCR

[5] Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit. QIS 33407.