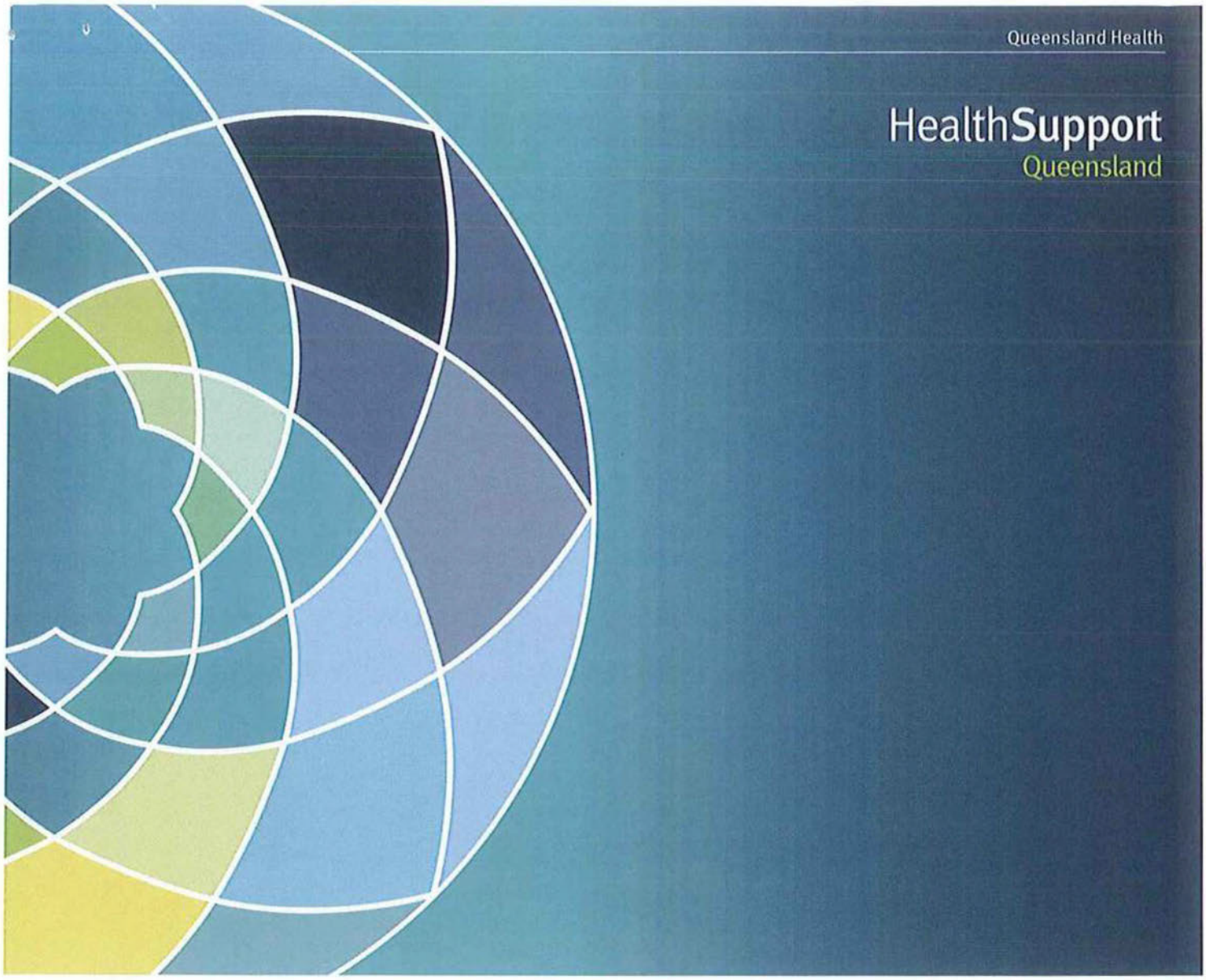

Project Proposal #173

Verification of Hamilton STARlet B for Quantification and Amplification Assay Setup

July 2017

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Queensland Government

Document Details

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Version history

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1.0	July 2017	Tegan Dwyer, Luke Ryan	Added results for new STARlet B PowerPlex21 protocol

Document sign off

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Contents

Abstract

Following a tender process, Forensic DNA Analysis has purchased two Hamilton Microlab® STARlet and LabElite® Integrated I.D. Capper™ instruments (STARlet A and B) to replace the Multiprobe® II PLUS HT EX (Multiprobe® II) liquid handlers.

The purpose of this project was to validate STARlet B for:

- Preparation of DNA quantification standards for Quantifiler® Trio
- Quantification assay setup using Quantifiler® Trio
- Amplification assay setup for Profiler® Plus and Powerplex®21

A total of five experiments were performed in the validation of the STARlet B:

1. Verification with ARTEL MVS
2. Preparation of DNA quantification standards
3. Quantifiler® Trio contamination checks
4. Profiler® Plus contamination checks
5. Powerplex®21 contamination checks

These core validation experiments were used to assess the STARlet in terms of the core functions of the instrument:

- Verification of the pipetting accuracy using the ARTEL MVS in Experiment 1.
- Verification that the STARlet protocols use of plate maps to pipette the correct sample into the correct well on the assay plate, without contaminating other assay plate wells. This was assessed in Experiments 2, 3, 4 and 5.

The STARlet B was found to be suitable for the above methods and performed as expected in all cases with no signs of cross-contamination.

Introduction

Forensic DNA Analysis has two Multiprobe® II liquid handlers which have been used to setup quantification and amplification assays in 96 well plate format. The two Multiprobe® II liquid handlers were used to setup Quantifiler® Trio quantification assays and Profiler® Plus and PowerPlex®21 amplification assays.

Both Multiprobe® II liquid handlers are at end of life and have been replaced following a tender process. The tender process identified the Hamilton STARlet as the most

suitable replacement for the Multiprobe® II liquid handlers for quantification and amplification setup.

The two STARlets are to be validated for setup of Quantifiler® Trio assays for quantification and Profiler® Plus and PowerPlex®21 assays for amplification. The STARlets were to be delivered with pre-installed scripts for the setup of Quantifiler® Trio, Profiler® Plus and PowerPlex®21 however due to time constraints new methods were developed in house with the support of the vendor's programming expert.

Validation and implementation of the two STARlets has been staggered. STARlet A has been validated first, whilst maintaining one Multiprobe® II for routine processing. Once STARlet A was validated and implemented, verification of STARlet B commenced. Once STARlet B is implemented the second Multiprobe® II instrument will be retired.

Resources

All reagents, materials and equipment used in this project were as specified in the approved in-house document Project Proposal #173: Validation of Hamilton STARlet B for Quantification and Amplification Assay Setup (March 2016). This document will be referred to as the Experimental Design. The following QIS documents are referenced throughout this report:

- QIS 17130 Capillary Electrophoresis Quality (CEQ) Check
- QIS 17137 Procedure for STR fragment analysis using GeneMapper ID-X software
- QIS 19976 Amplification of Extracted DNA using the AmpFISTR Profiler® Plus kit
- QIS 19994 Procedure for testing DNA Quantification Standards, DNA Quantification and Amplification kits & Reagents, and Quality Control Samples
- QIS 26628 Verifications using the Artel MVS
- QIS 31389 STR fragment analysis of PowerPlex® 21 profiles using GeneMapper® ID-X software
- QIS 31511 Amplification of Extracted DNA using the PowerPlex®21 System
- QIS 33407 Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit

Where Allelic Imbalance (AI) is mentioned throughout this document, this is in accordance with validated values from Project# 33 Peak Height and Allelic Imbalance

Thresholds and Project# 171 Verification of PowerPlex®21 New Internal Lane Standard and Matrix.

Sample Selection

43 Reference FTA™ buccal samples which have been submitted by the Queensland Police Service for routine testing, and have given full DNA profiles using Powerplex®21, were used to generate the required data sets.

Experiments and Results

The STARlet B was intended to come with pre-loaded scripts for the Quantifiler® Trio, Profiler® Plus and Powerplex®21 assays however during consultations with the vendor and third parties involved in the procurement of this script, it became apparent it would be more efficient and flexible to develop a protocol in house with the collaboration of the vendor's programming expert. As such, all protocols were written with a programming expert, then tested and optimised by in-house staff. These protocols were validated and optimised during the validation of STARlet A and the final versions were transferred to STARlet B and assessed. All protocols were deemed suitable without change except for the PowerPlex®21 protocol. As the results produced by this protocol were sub-optimal on STARlet B, but ideal for STARlet A, a new protocol was developed and tested specifically for STARlet B.

Experiment 1: Verification of STARlet B with ARTEL MVS

Purpose

To verify the Low and High Volumes of the Tips required for all protocols on STARlet B using the Artel MVS.

Method

A protocol was developed by vendor experts and in-house staff. The Artel instrument and software, which is used to test pipetting accuracy, was used in this experiment according to QIS 26628. Forensic DNA Analysis uses the Artel instrument for three monthly verifications of handheld pipettes (POVAs) as well as for the Multiprobe® II. Specifically, the verification using the Artel assesses the precision and accuracy of the volume(s) delivered by each channel of the liquid handler being assessed.

Results

STARlet B passed internal verification criteria at all volumes tested (see below). Each verification plate was analysed using the Artel Software. The Data Manager software generated and displayed an Output Report, with a 'PASSED' or a 'FAILED' result. Yellow or orange coloured data points represented dispensed volumes that exceeded the limits for Relative Inaccuracy and/or Coefficient of Variation. For the STARlet B to pass overall, each individual channel was required to pass.

50 µL Tips at 1 µL

The STARlet B was verified with the 50 µL tips at 1 µL. Acceptance criteria for Forensic DNA Analysis POVAs (Piston Operated Volumetric Apparatus) is a %CV and %inaccuracy of +/- 5 % (10 % for volumes <10 µL).

Results for the 1 µL verification with 50 µL tips for the STARlet A were the following:

- %CV = 2.41 %
- %inaccuracy = 2.61 % (see Table 1)

Although the results are summarised for all channels in Table 1, each channel is analysed individually, and each channel must pass for the overall verification to pass. See Table 2 for individual channel results.

Table 1 Summary of Verification Statistics of 50 µL Tips at 1 µL.

Target volume (µL)	1
Target solution	Range D
Number of data points per channel	12
Mean volume for all channels (µL)	**1.02612**
Relative inaccuracy for all channels	**2.61%**
Standard deviation for all channels (µL)	0.02472
Coefficient of variation (CV) for all channels	**2.41%**
Relative inaccuracy pass/fail limit	10%
Coefficient of variation pass/fail limit	10%
Status based on channel results	**Passed**
Status based on run statistics	**Passed**

All channels must pass to accept the verification. The STARlet B prepared a full plate of 1 µL volumes with 50 µL tips – this generates 12 repeats of each channel. All channels passed at 1 µL (see Tables 2 and 3).

Table 2 Channel Statistics of 50 µL Tips at 1 µL.

Channel	Mean Volume	Inaccuracy	Standard Deviation	CV	Status
1	1.01276	1.28%	0.01166	1.15%	Passed
2	1.04908	4.91%	0.01080	1.03%	Passed
3	1.02851	2.85%	0.00832	0.81%	Passed
4	0.99916	-0.08%	0.01345	1.35%	Passed
5	1.03925	3.93%	0.01544	1.49%	Passed
6	1.02668	2.67%	0.01512	1.47%	Passed
7	0.99585	-0.41%	0.01461	1.47%	Passed
8	1.05768	5.77%	0.01580	1.49%	Passed

Table 3 Verification Results of 50 µL Tips at 1 µL.

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.006	0.999	0.990	1.017	1.019	1.025	1.007	1.017	1.024	1.016	1.030	1.004
B	1.053	1.037	1.027	1.044	1.052	1.060	1.066	1.057	1.049	1.057	1.045	1.042
C	1.028	1.025	1.022	1.025	1.021	1.043	1.017	1.021	1.028	1.036	1.035	1.041
D	1.006	0.976	0.989	0.982	0.991	1.019	0.990	1.012	1.007	1.009	1.012	0.997
E	1.059	1.037	1.025	1.020	1.022	1.046	1.039	1.039	1.026	1.038	1.070	1.051
F	1.053	1.033	1.027	1.016	1.023	1.026	1.047	1.004	1.017	1.013	1.045	1.018
G	0.994	0.975	0.996	0.983	0.982	1.010	1.002	1.003	0.998	1.004	1.026	0.978
H	1.059	1.044	1.045	1.036	1.034	1.066	1.065	1.058	1.061	1.073	1.089	1.064

50 µL Tips at 50 µL

The STARlet B was verified with the 50 µL tips at 50 µL. Acceptance criteria for Forensic DNA Analysis POVAs is a %CV and %inaccuracy of +/- 5 % (10 % for volumes <10 µL).

Results for the 50 µL verification with 50 µL tips for the STARlet B were the following:

- %CV = 0.17 %
- %inaccuracy = -0.38 % (see Table 4)

Although the results are summarised for all channels in Table 4, each channel is analysed individually, and each channel must pass for the overall verification to pass. See Table 5 for individual channel results.

Table 4 Verification Statistics of 50 µL Tips at 50 µL.

Target volume (µL)	50
Target solution	Range A
Number of data points per channel	12
Mean volume for all channels (µL)	**49.812**
Relative inaccuracy for all channels	**-0.38%**
Standard deviation for all channels (µL)	0.084
Coefficient of variation (CV) for all channels	**0.17%**
Relative inaccuracy pass/fail limit	5%
Coefficient of variation pass/fail limit	5%
Status based on channel results	**Passed**
Status based on run statistics	**Passed**

All channels must pass to accept the verification. The STARlet B prepared a full plate of 50 µL volumes with 50 µL tips – this generates 12 repeats of each channel. All channels passed at 50 µL (see Tables 5 and 6).

Table 5 Channel Statistics of 50 µL Tips at 50 µL.

Channel	Mean Volume	Inaccuracy	Standard Deviation	CV	Status
1	49.788	-0.42%	0.077	0.15%	Passed
2	49.823	-0.35%	0.092	0.18%	Passed
3	49.813	-0.37%	0.091	0.18%	Passed
4	49.803	-0.39%	0.056	0.11%	Passed
5	49.815	-0.37%	0.066	0.13%	Passed
6	49.891	-0.22%	0.110	0.22%	Passed
7	49.790	-0.42%	0.085	0.17%	Passed
8	49.774	-0.45%	0.049	0.10%	Passed

Table 6 Verification Results of 50 µL Tips at 50 µL.

	1	2	3	4	5	6	7	8	9	10	11	12
A	49.72	49.84	49.94	49.74	49.75	49.76	49.69	49.80	49.71	49.85	49.77	49.89
B	49.83	49.77	49.86	49.75	50.08	49.80	49.84	49.86	49.79	49.77	49.80	49.72
C	49.73	49.80	50.07	49.80	49.77	49.85	49.76	49.83	49.77	49.87	49.74	49.77
D	49.74	49.77	49.86	49.79	49.86	49.81	49.74	49.87	49.79	49.87	49.83	49.71
E	49.69	49.74	49.75	49.87	49.83	49.85	49.85	49.91	49.80	49.90	49.80	49.79
F	49.82	49.79	49.97	49.86	49.96	49.80	50.18	49.88	49.90	49.87	49.88	49.78
G	49.65	49.71	49.77	49.85	49.85	49.82	49.72	49.94	49.77	49.90	49.72	49.78
H	49.83	49.83	49.80	49.78	49.77	49.65	49.77	49.78	49.79	49.73	49.81	49.75

300 µL Tips at 15 µL

The STARlet B was verified with the 300 µL tips at 15 µL. Acceptance criteria for Forensic DNA Analysis POVAs is a %CV and %inaccuracy of +/- 5 % (10 % for volumes <10 µL).

Results for the 15 µL verification with 300 µL tips for the STARlet B were the following:

- %CV = 1.29 %
- %inaccuracy = 0.58 % (see Table 7)

Although the results are summarised for all channels in Table 7, each channel is analysed individually, and each channel must pass for the overall verification to pass. See Table 8 for individual channel results.

Table 7 Verification Statistics of 300 µL Tips at 15 µL.

Target volume (µL)	15
Target solution	Range B
Number of data points per channel	12
Mean volume for all channels (µL)	**15.087**
Relative inaccuracy for all channels	**0.58%**
Standard deviation for all channels (µL)	0.194
Coefficient of variation (CV) for all channels	**1.29%**
Relative inaccuracy pass/fail limit	5%
Coefficient of variation pass/fail limit	5%
Status based on channel results	**Passed**
Status based on run statistics	**Passed**

All channels must pass to accept the verification. The STARlet B prepared a full plate of 15 µL volumes with 300 µL tips – this generates 12 repeats of each channel. All channels passed at 15 µL (see Tables 8 and 9).

Table 8 Channel Statistics of 300 µL Tips at 15 µL.

Channel	Mean Volume	Inaccuracy	Standard Deviation	CV	Status
1	15.150	1.00%	0.101	0.67%	Passed
2	15.127	0.85%	0.146	0.97%	Passed
3	15.169	1.13%	0.118	0.78%	Passed
4	15.017	0.11%	0.222	1.48%	Passed
5	14.998	-0.01%	0.235	1.57%	Passed
6	15.038	0.25%	0.249	1.66%	Passed
7	14.998	-0.01%	0.191	1.27%	Passed
8	15.200	1.33%	0.154	1.01%	Passed

Table 9 300 µL Verification Results at 15 µL

	1	2	3	4	5	6	7	8	9	10	11	12
A	15.25	15.20	15.16	15.17	15.19	15.18	15.24	15.15	15.19	15.18	14.92	14.97
B	15.27	15.18	15.16	15.20	15.25	15.21	15.24	15.16	15.16	14.82	14.92	14.95
C	15.29	15.28	15.23	15.28	15.29	15.14	15.18	15.10	15.11	15.19	14.91	15.03
D	15.31	15.27	15.22	15.28	14.87	14.99	15.06	15.03	14.98	14.66	14.72	14.81
E	15.29	15.22	15.18	15.19	14.86	15.00	15.00	15.01	14.92	14.51	15.14	14.65
F	15.33	15.30	15.28	15.31	15.00	14.96	14.98	15.01	14.94	14.57	15.12	14.65
G	15.25	15.21	15.16	15.17	14.86	14.97	15.01	15.03	14.97	14.60	14.99	14.76
H	15.34	15.32	15.26	15.21	15.37	15.20	15.23	15.21	15.12	15.15	14.77	15.22

300 µL Tips at 200 µL

The STARlet B was verified with the 300 µL tips at 200 µL. Acceptance criteria for Forensic DNA Analysis POVAs is a %CV and %inaccuracy of +/- 5 % (10 % for volumes <10 µL).

Results for the 200 µL verification with 300 µL tips for the STARlet B were the following:

- %CV = 0.22 %
- %inaccuracy = 1.11 % (See Table 10)

Although the results are summarised for all channels in Table 1, each channel is analysed individually, and each channel must pass for the overall verification to pass. See Table 11 for individual channel results.

Table 10 Verification Statistics of 300 µL Tips at 200 µL.

Target volume (µL)	200
Target solution	Range A
Number of data points per channel	12
Mean volume for all channels (µL)	**202.23**
Relative inaccuracy for all channels	**1.11%**
Standard deviation for all channels (µL)	0.45
Coefficient of variation (CV) for all channels	**0.22%**
Relative inaccuracy pass/fail limit	5%
Coefficient of variation pass/fail limit	5%
Status based on channel results	**Passed**
Status based on run statistics	**Passed**

All channels must pass to accept the verification. The STARlet B prepared a full plate of 200 µL volumes with 300 µL tips – this generates 12 repeats of each channel. All channels passed at 200 µL (see Tables 11 and 12).

Table 11 Channel Statistics of 300 µL Tips at 200 µL.

Channel	Mean Volume	Inaccuracy	Standard Deviation	CV	Status
1	202.53	1.27%	0.45	0.22%	Passed
2	202.17	1.08%	0.41	0.20%	Passed
3	201.78	0.89%	0.31	0.15%	Passed
4	202.34	1.17%	0.32	0.16%	Passed
5	202.28	1.14%	0.31	0.15%	Passed
6	201.94	0.97%	0.47	0.23%	Passed
7	202.28	1.14%	0.39	0.19%	Passed
8	202.53	1.27%	0.46	0.23%	Passed

Table 12 Verification Results of 300 µL Tips at 200 µL.

	1	2	3	4	5	6	7	8	9	10	11	12
A	202.5	202.6	202.1	202.2	202.7	202.1	201.9	202.2	202.6	202.9	203.2	203.3
B	202.5	202.1	202.1	201.9	201.8	201.6	201.7	202.1	202.2	202.4	202.6	203.0
C	202.2	202.1	201.9	201.8	201.7	201.4	201.2	201.6	201.6	201.8	201.9	202.2
D	202.1	202.2	202.0	202.1	202.3	203.1	202.1	202.4	202.2	202.3	202.5	202.8
E	202.5	202.5	202.0	201.9	202.0	202.0	202.0	202.3	202.4	202.3	202.6	202.9
F	202.3	202.1	201.4	201.7	201.9	201.4	201.1	202.1	201.9	202.3	202.7	202.4
G	202.3	202.2	202.2	202.0	201.9	201.8	201.8	202.3	202.5	202.6	202.7	203.1
H	202.7	201.8	202.2	202.3	202.4	202.1	202.1	202.5	202.8	203.1	203.1	203.2

Acceptance Criteria

As per NATA requirements, all instruments are to be verified 3-monthly using this protocol (including analysis of individual channels) and the following acceptance criteria for DNA Analysis POVAs with a %CV and %inaccuracy of +/- 5 % (10 % for volumes <10 µL).

As the results for all tested volumes (1 µL, 50 µL, 15 µL and 200 µL) passed these acceptance criteria, this protocol can be accepted and pipetting accuracy of the STARlet B confirmed.

Experiment 2: Preparation of DNA quantification standards

Purpose

To develop a protocol for the preparation of DNA Standards for Quantifiler® Trio and use this protocol to successfully prepare DNA quantification standards.

Method

A protocol was developed by in-house staff. This protocol was used to prepare standard sets N5_20170208 and N6_20170208. This protocol was also used to prepare 2 standard test plates which were was analysed on 7500B according to QIS 19994.

Results

The results from both sets of standards diluted and tested on plates prepped by the STARlet B fell within the acceptable ranges as outlined in QIS 19994. STARlet B successfully prepared two sets of Quantifiler® Trio standards which passed internal criteria for Small Autosomal (SAT), Large Autosomal (LAT) and Y-Target standard curves as documented below (see Table 13):

Table 13 Quantifiler® Trio Standards Setup Results.

Standard Set	1	2
Batch and Instrument	**N5**	**N6**
SAT Slope (-3.6 to -3.0)	-3.165	-3.143
SAT Y-Intercept (27.4617 – 28.2614 7500A) (27.1275 – 27.9105 7500B)	27.344	27.165
SAT R^2 value (≥0.980000)	0.998	0.998
LAT Slope (-3.7 to -3.1)	-3.352	-3.34
LAT Y-Intercept (25.2902 – 26.1106 7500A) (25.1181 – 25.6552 7500B)	25.412	25.235
LAT R^2 value (≥0.980000)	1.0	1.0
Y-Target Slope (-3.6 to -3.0)	-3.169	-3.457
Y-Target Y-Intercept (26.9899 – 27.7212 7500A) (26.3689 – 26.8339 7500B)	26.471	26.612
Y-Target R^2 value (≥0.980000)	0.998	0.999
Reagent blank (Undet. to 0.00241 ng/µL)	Undetermined	Undetermined

Discussion

As of the 08/02/2017 new Y-Intercept thresholds were added to QIS 19994 to account for the variability between the two 7500 instruments observed in routine processing. These new standard curve thresholds were used in this validation with standards made on the 08/02/2017.

Acceptance Criteria

The results from both sets of standards diluted and tested on plates prepared by the STARlet B fell within the acceptable ranges as outlined in QIS 19994, therefore this protocol can be accepted.

Experiment 3: Quantifiler® Trio Contamination Checks

Purpose

To assess performance and contamination of the Quantifiler® Trio protocol.

Method

The final Quantifiler® Trio Protocol v2.0 was used in this project. Checkerboard test plates was prepared according to section 4.2.1 of the Experimental Design. Results were analysed according to QIS 33407.

Results

The STARlet B prepared a Checkerboard Quantifiler® Trio assay plate as per the Experimental Design. All samples containing DNA gave quantification results, and blank samples (including reagent blanks) gave undetermined results (with the exception of one reagent blank which gave a quantification result of 0.0001 ng/µL which is below the laboratory's LOD) see Table 14.

Table 14 Quantifiler® Trio Contamination Check Results.

Experiment #	Batch ID	Results	Batch in AUSLAB
EXP 3 Checkerboard	[redacted]	Standards Passed. E12 - Reagent Blank had a SAT quant value 0.0001 which is below the LOD for Quant Trio. No Contamination.	YES

Table 15 Quantifiler® Trio Contamination Check Standard Curve Results.

Standard Set	1
Batch	EXP 3 Checkerboard
Instrument	7500 B
SAT Slope (-3.6 to -3.0)	-3.317
SAT Y-Intercept (27.4617 – 28.2614 7500A) (27.1275 – 27.9105 7500B)	27.406
SAT R^2 value (≥0.980000)	0.999
LAT Slope (-3.7 to -3.1)	-3.375
LAT Y-Intercept (25.2902 – 26.1106 7500A) (25.1181 – 25.6552 7500B)	25.392
LAT R^2 value (≥0.980000)	1.0
Y-Target Slope (-3.6 to -3.0)	-3.213
Y-Target Y-Intercept (26.9899 – 27.7212 7500A) (26.3689 – 26.8339 7500B)	26.51
Y-Target R^2 value (≥0.980000)	0.997
Reagent blank (Undet. to 0.00241 ng/µL)	Undetermined

Discussion

All samples gave quantification results – ranging from a quantification value of 0.1369 ng/µL to 8.1154 ng/µL - which is typical for reference samples such as those used in this data set. All blanks were expected to and produced undetermined results (with the exception of one reagent blank which gave a quantification result of 0.0001 ng/µL) which indicates that during plate preparation on the STARlet B no detectable cross-contamination occurred.

Acceptance criteria

All samples containing DNA gave a quantification result and all blanks (including reagent blanks) were undetermined or below LOD - which indicates that there was no

detectable cross-contamination during assay preparation on the STARlet B. Therefore this protocol can be accepted.

Experiment 4: Profiler® Plus Contamination Checks

Purpose

To assess contamination and performance of the Profiler® Plus protocol.

Method

The final Profiler Plus Protocol v2.0 was used in this project. Checkerboard test plates was prepared according to section 4.2.1 of the Experimental Design. Results were analysed according to QIS 17137.

Results

The STARlet B prepared a Checkerboard plate as per the Experimental Design. On this plate all samples with DNA gave results which had 100% allele concordance with the original run prepared on the Multiprobe® II. All ladders, positive and negative controls passed normal quality criteria as per QIS 17130. All blanks gave NSD profiles (see Table 16).

Table 16 Profiler® Plus Contamination Check Results

Experiment 4 Profiler® Plus Amplification Contamination Checks			
Experiment #	**Batch ID**	**GM Batch ID**	**Results**
EXP 4 Checkerboard	[redacted]	[redacted]	100% Allele Concordance – 100% full profiles. No contamination.

Discussion

All samples containing DNA used in this data set returned profiles consistent with the original results. All blanks produced NSD profiles demonstrating there was no detectable cross-contamination during plate preparation.

Acceptance Criteria

As the plate passed the quality acceptance criteria as per QIS 17130, no blanks gave DNA profile results, and allele designations for all samples containing DNA were concordant with results obtained previously on assays prepared using the Multiprobe® II, this method can be accepted.

Experiment 5: PowerPlex® 21 Contamination Checks

Purpose

To assess contamination and performance of the PowerPlex® 21 protocol.

Method

The PowerPlex21 Protocol v2.0 from STARlet A was initially used in this project and was found to be unsuitable for STARlet B, i.e. multiple samples which gave full DNA profiles on assays prepared with the Multiprobe® II gave partial DNA profiles on the STARlet B assays (full results available in the project folder). New liquid classes, specific to the PP21 protocol and STARlet B, were developed and tested extensively to produce PowerPlex 21 STARlet B Protocol v1.0. A Checkerboard test plate was prepped according to section 5 of the Experimental Design. Results were analysed according to QIS 31389.

Results

The STARlet B prepared a Checkerboard plate as per the Experimental Design. On this plate all samples with DNA gave results which had 100% allele concordance with the original run on the Multiprobe® II. All ladders, positive and negative controls passed normal quality criteria as per QIS 17130. All blanks gave NSD profiles (see Table 17).

Table 17 PowerPlex® 21 Contamination Check Results

Experiment 5 PowerPlex® 21 Amplification Contamination & Performance Checks			
Experiment #	**Batch ID**	**GM Batch ID**	**Results**
EXP 5 Checkerboard	[redacted]	[redacted]	100 % Allele Concordance. No Contamination.

Discussion

All samples containing DNA used in this data set returned profiles consistent with the original runs prepared using the Multiprobe® II. All blanks produced NSD profiles demonstrating there was no detectable cross-contamination during plate preparation.

Acceptance Criteria

As the plate passed the quality acceptance criteria as per QIS 17130, no blanks gave DNA profile results, and allele designations for all samples containing DNA were concordant with results obtained previously on assays prepared using the Multiprobe® II, this method can be accepted.

Conclusion

The STARlet B was verified with the ARTEL at all critical volumes in Experiment 1 (i.e. 50 µL tips at 1 µL and 50 µL volumes; and 300 µL tips at 15 µL and 200 µL volumes) and is therefore pipetting accurately and within verification thresholds.

Two sets of Quantifiler® Trio Standards were prepared by the STARlet B in Experiment 2. These standards passed manufacturer and internal thresholds on a 7500 instrument. Therefore the STARlet B has been validated for the preparation of Quantifiler® Trio Standards.

All contamination check plates (Quantifiler® Trio, PowerPlex® 21 and Profiler® Plus) results showed complete allele concordance with no detectable signs of contamination.

This validation study has determined that the STARlet B using the methods developed in-house is suitable for routine preparation of Quantifiler® Trio Standards, Quantifiler® Trio assays, PowerPlex® 21 assays and Profiler® Plus assays in the Forensic DNA Analysis laboratory. No evidence of cross contamination between samples (between runs or between samples within a run) was identified in this study.

Recommendations

1. The STARlet B be implemented for the preparation and testing of Quantifiler® Trio Standards;
2. The STARlet B be implemented for the preparation of Quantifiler® Trio assays;
3. The STARlet B be implemented for the preparation of PowerPlex® 21 assays;
4. The STARlet B be implemented for the preparation of Profiler® Plus assays;
5. The STARlet B be implemented for the testing of Quantifiler® Trio, PowerPlex® 21 and Profiler® Plus kits;
6. The remaining Multiprobe® II PLUS HT EX be decommissioned and removed from the laboratory.

Appendix 1 Final Versions of Protocols for Implementation

- AmpTest_PowerPlex_21_Setup_v1.0
- AmpTest_ProfilerPlus_Setup_v2.0
- ARTEL_All_Tips_v1.0
- PowerPlex_21_Setup_v2.0
- ProfilerPlus_Setup_v2.0
- Quant_Trio_STD_Dilution_and_Test_Setup_v2.0
- Quant_Trio_STD_Test_Setup_v1.0
- Quant_Trio_Kit_Test_Setup_v1.0
- Quantifiler_Trio_Setup_v2.0

References

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