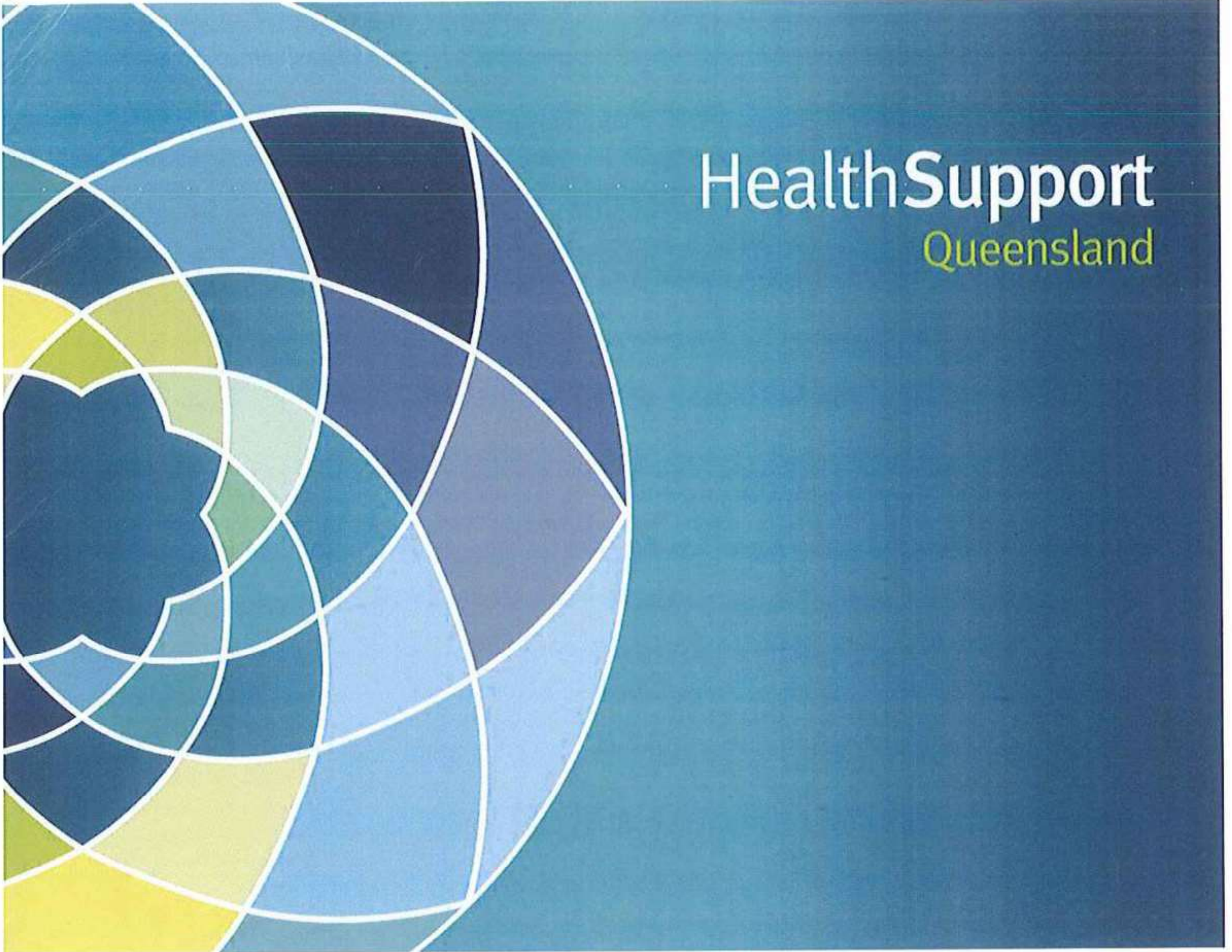

Validation of the QIAsymphony® SP/AS Modules

November, 2016

Maria Aguilera, Biljana Micic, Pierre Acedo, Luke Ryan and Cathie Allen

Validation of the QIAsymphony® SP/AS

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1 ABSTRACT

This project successfully validated the QIAsymphony® SP/AS instrument (using the QIAsymphony® DNA Investigator Kit) for the extraction of casework and reference forensic samples as a replacement for the existing automated extraction method using the Multiprobe® II PLUS HT EX with Gripper™ Integration. This project also successfully validated the QIAsymphony® SP/AS instrument for the extraction of a range of cell and substrate types currently extracted using the Maxwell®16 instrument.

The QIAsymphony® SP/AS instrument was also validated for the setup of Quantifiler® Trio quantification assays using the "integrated run" function, whereby samples which have been extracted on the SP module, are transferred to the AS module for quantification assay setup.

2 INTRODUCTION

Forensic DNA Analysis has performed automated DNA extraction using the Multiprobe® II PLUS HT EX with Gripper™ Integration (Multiprobe® II) up until recently when the Multiprobe® II instruments were assessed as being at the end of their life as the supplier, Perkin Elmer, advised that they would no longer be supporting the instruments after the end of 2016. Through the HTER evaluation process, the QIAsymphony® instrument in conjunction with the QIAsymphony® DNA Investigator Kit, was found to be the most suitable for the automated purification of total DNA from forensic casework and reference forensic samples. A preliminary trial completed in 2015 found that the QIAsymphony® instrument obtained considerably higher DNA yields when compared with automated DNA extractions using the Multiprobe® II PLUS HT EX with Gripper™[1].

During this validation study, following some modifications to the pre-lysis and extraction protocols, the QIAsymphony® instrument and QIAsymphony® DNA Investigator Kit[2] were found to give comparable DNA yields to the Maxwell®16 instrument using the Casework DNA IQ™ Pro Kit for Maxwell®16.

The QIAsymphony® instrument is a 4 channel modular automated system which enables the processing of up to 96 samples on a single run. This instrument can be used for the extraction and purification of DNA from forensic casework and reference samples. It uses pre-programmed optimised protocols and the

QIAGEN® cartridge-based magnetic-particle chemistry kit, the QIAsymphony® DNA Investigator Kit.

Preliminary testing during our trial study of the QIAsymphony® instrument found that better DNA yields were obtained to that of the Multiprobe® II with a modified version of the QIAsymphony® lysis protocol – pre-treatment of surface and buccal swabs[2,3] protocol to include the addition of 25µL DTT (prepared in-house) to each sample [1].

The QIAsymphony® AS module, which is also a four channel liquid handler, extends the capabilities of the QIAsymphony® SP by integrating automated PCR assay setup. At the end of the extraction protocol, extracts are automatically transferred from SP module to the AS module where the quantification assay is setup up. This is referred to as an "integrated run".

The primary purpose of this validation was to validate the QIAsymphony® SP/AS for the DNA extraction of casework and reference samples as a replacement for the Multiprobe® II automated extraction instrument. Two secondary validation goals were also included:

- Modifying the QIAsymphony® pre-lysis and/or extraction protocols to achieve DNA yields which were comparable to the Maxwell®16 instrument.
- Validating the AS module for quantification assay setup.

This validation used qualitative and quantitative acceptance and assessment criteria to assess the QIAsymphony® and to compare it to the Maxwell®16. Acceptance criteria are used to validate or fail the QIAsymphony®. Assessment criteria are used to form conclusions about performance, workflow, rework and other operational factors impacting on the QIAsymphony®.

3 GOVERNANCE

Project Personnel

- Project Manager: Luke Ryan, Senior Scientist, Analytical Team
- Primary Project Officer – Maria Aguilera, Scientist, Analytical Team
- Project Officers – Biljana Micic and Pierre Acedo, Scientists, Analytical Team

Decision Making Group

- The Management Team and the Senior Project Officer were the decision making group for this project.
- The Primary Project Officer is included in the Decision Making Group in their capacity as an expert user.

4 RESOURCES

The following resources were required for this validation:

4.1 Reagents

- QIAsymphony® DNA Investigator® Kit (QIAGEN Group, 40724 Hilden, DE)
- Casework DNA IQ™ Pro Kit for Maxwell®16 (Promega Corp., Madison, WI, USA)
- QIAamp® DNA Investigator® Kit (QIAGEN Group, 40724 Hilden, DE)
- DNA IQ™ Kit (Promega Corp., Madison, WI, USA)Microcon® Centrifugal Filters, Merck Millipore, Darmstadt, Germany.
- TNE (Forensic DNA Analysis, Brisbane, QLD, AU)
- Sarcosyl (Sigma-Aldrich® Corporation, St Louis, MO, US)
- Proteinase K (Pro K) – Affymetrix USB (USB® Products Affymetrix Inc., Cleveland, Ohio, USA)
- 1,4 Dithiothreitol (DTT) – Affymetrix USB (USB® Products Affymetrix Inc., Cleveland, Ohio, USA)
- Nanopure water (Forensic DNA Analysis Unit, Brisbane, QLD, AU)
- Quantifiler® Trio DNA Quantification Kit Life Technologies Applied Biosystems®, Foster City, CA, USA)
- Promega PowerPlex®21 system (Promega Corp., Madison, WI, US)
- 2800M Control DNA, 10ng/µl (Promega Corp., Madison, WI, US)
- Water amplification grade (Promega Corp., Madison, WI, US)
- Amphyl (0.2 % and 1 % v/v) (Rickitt Benckiser Inc. Parsippany, NJ, US)
- Promega PowerPlex® 5 Dye Matrix Standard (Promega Corp., Madison, WI, US)
- TE Running Buffer (Life Technologies Applied Biosystems, Foster City, CA, US)
- 3130 POP-4™ Polymer (Life Technologies Applied Biosystems, Foster City, CA, US)
- HID 5-DYE Installation Standard (Life Technologies Applied Biosystems®, Foster City, CA, USA)
- Hi-Di™ Formamide (Life Technologies Applied Biosystems®, Foster City, CA, US)
- Promega CC5 Internal Lane Standard 500 (Promega Corp., Madison, WI, US)
- Promega PowerPlex®21 Allelic Ladder Mix (Promega Corp., Madison, WI, US)
- Positive controls (Forensic DNA Analysis, Brisbane, QLD, AU)
- 5 % v/v Brite N White liquid bleach (Cleantec, Darra, QLD, AU)
- 5 % v/v Trigene Advance (In Vitro Pty. Ltd., Noble Park North, VIC, AU)
- Sodium Chloride 0.9% for Irrigation (Baxter Healthcare Pty Ltd, Old Toongabbie, NSW, AU)
- Indigo Carmine (Sigma-Aldrich, St Louis, MO 63103, US)
- Urea (Sigma-Aldrich, St Louis, MO 63103, US)
- Tannic Acid (Sigma-Aldrich, St Louis, MO 63103, US)

- Motor oil
- Ethanol (Recochem Incorporated, Wynnum, QLD,AU)

4.2 Consumables

- QIAsymphony® Sample prep cartridges, 8-well (QIAGEN Group, 40724 Hilden, DE)
- QIAsymphony® 8-Rod Covers (QIAGEN Group, 40724 Hilden, DE)
- QIAsymphony® 2mL and 5mL conical tubes (QIAGEN Group, 40724 Hilden, GERMANY)
- QIAsymphony® Filter-tips 1500, 200 & 50µL (QIAGEN Group, 40724 Hilden, GERMANY)
- Roborack 25µl and 175µl Conductive Filter Tips, Pre-Sterilized (PerkinElmer,l Downers Grove, IL, USA)
- Axygen 96-well half skirt PCR Microplate, clear (Axygen Scientific Inc., Union City, CA, US)
- MicroAmp® Optical 96-Well Reaction Plate with Barcode (Applied Biosystems by Life Technologies, Foster City, CA, USA)
- MicroAmp® Optical Adhesive Film (Life Technologies Applied Biosystems, Foster City, CA, US)
- QIAGEN Tape Pads (QIAGEN Group, 40724 Hilden, GERMANY)
- Axymat 96-Silicone septa mat (Axygen Inc. Union City, CA, US)
- 3130xL 16 capillary arrays (Life Technologies Applied Biosystems, Foster City, CA, US)
- Tape pad adhesive film (QIAGEN Pty. Ltd., 40724 Hilden, Germany)
- Sterile 1.5 and 2 mL screw-cap tubes (Axygen Scientific Inc., Union City, CA, US)
- Sterile 5 mL screw-cap tubes (Axygen Scientific Inc., Union City, CA, US)
- Reservoir septas (Life Technologies Applied Biosystems, Foster City, CA, US)
- Whatman FTA® Classic Card (Sanford, Maine 04073, US)
- Whatman Sterile Foam-Tipped Applicator (Sanford, Maine 04073, US)
- ART Filtered 1000 µL, 300 µL & 20p pipette tips (Molecular BioProducts Inc., San Diego, CA, US)
- F1-ClipTip pipette tips - 20µL, 50µL, 200µL & 1000 µL (Thermo Fisher Scientific Inc, 01621 Vantaa, FIN)
- Combitips advanced® 0.5mL (Eppendorf Biopur, Hamburg, DE)
- Cytobrush® Plus Cell collector (Medscand® Medical – a CooperSurgical company, 10625 Berlin, DE)
- 4mL Vacuette EDTA tubes (Greiner bio-one, Monroe, North Carolina 28110, US)
- Nunc™ Bank-It™ tubes (Nunc A/S DK-4000 Roskilde, DK)
- Rediwipes (Cello Paper Pty. Ltd., Fairfield, NSW, AU)
- Sterile rayon swabs (Copan Diagnostics Inc., Murrieta, CA, US)
- Kova® Glasstic® Slide 10 with grids (HYCOR Biomedical Inc., IN 46240, US)
- Olympus Lens Cleaning Tissues (Olympus America Corporation, Tokyo, Japan)

4.3 Equipment

- QIAsymphony® SP and AS modules (QIAGEN Group, 40724 Hilden, DE)
- Multiprobe® II PLUS ht ex with Gripper™ Integration Platforms (PerkinElmer, Downers Grove, IL, USA)
- Maxwell®16 MDx instrument (Promega Corp., Madison, WI, USA)
- Airstream® Biological safety cabinets class II (ESCO Pty. Ltd., Hornsham, PA, US)
- TOPSAFE Biological safety cabinets class II (LAF Technologies Pty. Ltd, Bayswater North, VIC 3153, AU)
- AB 7500 Real Time PCR System (Life Technologies by Applied Biosystems, Foster City, CA, US)
- GeneAmp PCR system 9700 (Applied Biosystems by Life Technologies, Foster City, CA, USA)
- AB 3130xL B Genetic Analyser (Life Technologies Applied Biosystems, Foster City, CA, USA)
- GeneMapper® ID-X ver. 1.4 (Life Technologies Applied Biosystems, Foster City, CA, USA)
- The BSD Duet 600 Series II (BSD Robotics, Australia)
- STORstar instrument (Process Analysis & Automation, Hampshire, UK)
- Eppendorf Thermomixer Comfort 5355 (Eppendorf AG, 22339 Hamburg, DE)
- LaboGene Scanspeed 1248 Centrifuge (Labgear, Lynge, Denmark)
- Vortex Mixer VM1 (Ratek instruments Pty Ltd, Melbourne, VIC, AU)
- MixMate (Eppendorf AG, Hamburg, DE)
- Micro centrifuge (Tomy, Tokyo, JP)
- Eppendorf 5424 centrifuge and Eppendorf 5804 centrifuge (Eppendorf, North Ryde, NSW, Australia)
- Dry Block Heater (Ratek, Boronia, NSW, Australia)
- Milli-Q® Integral 3 (A10) System with Q-POD™ (Millipore™, Billerica, MA, USA)
- Pipettes (Eppendorf, Hamburg, DE and Thermo Fisher Scientific (Finnpipette), Waltham, MA, US)
- ClipTip Pipettes (Thermo Fisher Scientific, 01621 Vantaa, Finland)
- BX41 Microscope (Olympus America Corporation, Tokyo, Japan)
- Labnet Shaker 20 (National Labnet Co., Woodbridge, NJ, USA)

Forensic DNA Analysis Analytical staff, computer and instrument time, as well as bench space in Forensic DNA Analysis Analytical Laboratory were also used in the duration of this project.

5 METHODS

5.1 Sample Selection and Collection

Samples used in this project were sourced from:

- Staff and non-staff voluntary donors. Appropriate consent was sought using QIS 33333 *Participant Information and Consent Form (PICF) – Common Biological Samples.*
- Coronial tissue samples submitted to Forensic DNA Analysis for routine testing were used.

Sample collection of these various substrate types sourced from various donors was conducted by the donors themselves with the assistance of the Analytical, Evidence Recovery and Project and Quality teams, and a phlebotomist. After preparation, all samples were given a unique barcode and stored appropriately. Sample and substrate types that were included in this project are shown in Table 1.

Table 1 - Description of sample types and donors which were used in the experiments for the validation of the QIAsymphony® instrument

Sample type	Designated Donor number	Sample Submitted
BLOOD		
Swabs	Donor 1 Donor 2 Donor 3 Donor 4 Donor 5 Donor 6 Donor 7 Donor 8 Donor 9 Donor 10 Donor 11 Donor 12	Substrate prepared with donor blood
Fabric	Donor 13	Substrate prepared with donor blood
CELLS		
Swabs	Donor 14	Substrate prepared with donor buccal cells
Tapelifts	Donor 14 & 15	Substrate prepared with donor buccal cells
FTA Paper	Donor 16	Substrate prepared with donor buccal cells
Cigarette Butts	Donor 17	Direct donor submission
Chewing Gum	Donor 18	Direct donor submission
OTHER SAMPLE TYPES		
Tissue	Donor 19	Direct donor submission
Hair	Male Hair – Donor 20 Female Hair – Donor 21	Direct donor submission Direct donor submission
Fingernails	Donor 22	Direct donor submission

5.1.1 Collection of blood

Blood donors (different to all other donors) were selected and blood was collected in EDTA tubes by a qualified phlebotomist and stored at 4°C until the blood was required for use. A total of 13 blood donors (Donors 1-13) were used for this validation.

5.1.2 Collection of buccal cells for cells suspension

Buccal cells were collected from a donor using the cytobrush™ method. For each collection, two cytobrush™ Plus Cell Collector devices were used to collect buccal cells from each cheek for 1 minute then collected into 500µL of 0.9% saline solution. The cell solutions were stored at 4°C until they were required for use. A total of two buccal cell donors (Donors 14 and 15) were used for this validation.

5.1.3 Collection of buccal cells for FTA paper

Buccal cells were collected from a donor using a sterile foam-tipped rubber applicator swab supplied in Whatman® FTA card collection kits. The swab was used to collect buccal cells from inside each cheek with a rubbing motion for 1 minute. The swab was then applied to the FTA card, and the FTA card firmly held over the swab for ten seconds. The FTA cards were stored at room temperature until they were required for use. A total of five buccal cell collections from Donor 16 were collected.

5.1.4 Collection of cigarette butts samples

The cigarette butts from smoked cigarettes were submitted by Donor 17 in 2mL tubes that were supplied. One cigarette smoker was used for this validation, with each tube containing one cigarette butt. These cigarette butts were then stored at 4°C until they were required for sampling.

Cigarette substrates were sampled in accordance to QIS 17142 *Examination of items,* section 6.9.4, and stored at 4°C until required for use.

5.1.5 Collection of chewing gum samples

Chewing gum was chewed by Donor 18 for five minutes before being collected into a 2mL tube.

5.1.6 Collection of tissue samples

Coronial tissue samples were originally sampled by the Evidence Recovery Team and further sub-sampled in Analytical in accordance to QIS 22903 *Procedure for Bone and Tissue Sample Examination and Preparation,* section 4.3.

5.1.7 Collection of hair samples

Hair was collected from two donors (Donor 20 – male, and Donor 21 – female). Each donor used their personal pre-sterilized tweezers to pluck 30 short hairs from their body and two hairs were placed into 15 sterile 2mL tubes (i.e. so that

each tube contains two hairs from the same individual) as per procedure in QIS 17140 *Procedure for the Identification and Examination of Hairs*. The hair samples were stored at 4°C until required.

5.1.8 Collection of fingernail samples

The donor (Donor 22) used their fingernails to scratch their scalp prior to trimming all five fingernails from one hand into a 2mL tube and then trimming all five fingernails of the opposite hand into a second 2mL tube as per procedures in QIS 17142 *Examination of items,* section 6.9.5. The donor used their personal pre-sterilised nail clippers to trim their nails. Fingernail samples were stored at 4°C until required. A total of five fingernail collections were taken from the donor, with at least two weeks between each collection to allow the fingernails to regrow.

5.2 Sample and Substrate Preparation

Sample preparation of the various substrate types sourced from various donors (as per section 5.1) was conducted by the Analytical and Evidence Recovery teams and used throughout this validation.

5.2.1 Negative extraction and blank controls

Negative extraction controls (blank reagent controls) were included throughout all batches for all experiments for the purpose of detecting any reagent contamination that may occur. All negative extraction controls were registered in AUSLAB with a unique barcode at the time of creating the extraction batches.

Additional blank controls were included in Experiment 8 which was designed to detect any cross-contamination during extraction and quantification batches using the QIAsymphony®. These were also registered in AUSLAB with a unique barcode at the time of creating the extraction batches.

5.2.2 Positive blood control samples

Positive blood extraction control swabs used in routine extraction batches in the Analytical laboratory were used throughout this validation. These were prepared as per QIS 25874 *Preparation of DNA Quantification Standards and In-house Quality Controls*. Positive blood swab controls used for the validation were registered in AUSLAB with a unique barcode at the time of creating the extraction batches.

5.2.3 Blood swab sample sets

Blood swab sample sets used in this validation were prepared in accordance to QIS 25874 *Preparation of DNA Quantification Standards and In-house Quality Controls,* with 10µL of blood inoculated onto each swab for use throughout this validation.

Blood swab sample sets were stored at 4°C, and registered in AUSLAB with a unique barcode when they were required at the time of creating the extraction batches.

5.2.4 Blood fabric sample sets

Cotton fabric was sampled in accordance to QIS 17142 *Examination of items*, section 6.8.4. The pieces of cotton fabric (approximately 5mm x 5mm in size) were inoculated with blood. Some experiments required varying amounts of blood to be added to each piece of fabric. These pieces of fabric were created as per the details in the relevant experimental design. 3.5µL of blood was inoculated onto pieces of fabric for Experiment 6 and 10µL for Experiment 5. The inoculated fabric was dried in the biohazard hood on a petri dish placed on a 56°C heater block. Once dry, each piece of fabric was transferred into an individual 2mL tube for storage.

Blood fabric sample sets were stored at 4°C, and registered in AUSLAB with a unique barcode at the time of creating the extraction batches.

5.2.5 Cell suspension and cell count

A single source buccal cell suspension was prepared and a cell count performed using the two buccal cell donors obtained using cytobrush method in 0.9% saline solution (section 5.1.2). Cell counts were performed according to Appendix B (section 9.2) of QIS 25874 *Preparation of DNA Quantification Standards and In-house Quality Controls*. Cell suspensions were stored at 4°C.

5.2.6 Cell suspension dilutions

Dilutions of a cell suspension were prepared using the stock cell suspension (Donor 14) and 0.9% saline solution according to calculations performed to achieve the desired concentration of cells per µL. QIS 24012 *Miscellaneous Analytical Section Tasks*, section 4.2 was used to prepare these dilutions. Cell suspension dilutions were stored at 4°C.

5.2.7 Cell swab samples

Cell swab samples used in this validation were prepared using a similar procedure to that of blood swabs (section 5.2.3). 10µL of stock suspension from Donor 14 was inoculated onto each swab for use throughout this validation.

For Experiment 4, a different amount of cells was required to be added to each swab. The cell suspension dilution was pipetted directly onto the swabs (see Table 6 for volumes) and allowed to dry on a heater block for 2 hours.

The cell swab samples were stored at 4°C, and registered in AUSLAB with a unique barcode at the time of creating the extraction batches.

5.2.8 Cell inoculated fabric

Cotton fabric was sampled in accordance with QIS 17142 *Examination of items*, section 6.8.4. The pieces of cotton fabric (approximately 5mm x 5mm in size) were inoculated with cell suspension from Donor 14.

25µL of this cell suspension was inoculated onto pieces of fabric for Experiment 7, and allowed to dry on a petri dish in the biohazard hood overnight. Once dry, each piece of fabric was transferred into an individual 2mL tube for storage.

Fabric cell sets were stored at 4°C, and registered in AUSLAB with a unique barcode when they were required at the time of creating the extraction batches.

5.2.9 Tapelift cells from fabric samples

One large piece of cotton fabric (approximately 100 mm x 500 mm) was sectioned and each section was spotted with 15µL of the stock cell suspension of Donor 15 (section 5.1.2). The fabric was allowed to dry in the biohazard hood overnight. Each section with spotted cell suspension was sampled individually using the tapelift method as per QIS 17142 *Examination of items*, section 6.8.2.

These cell fabric sample sets were stored at 4°C, and registered in AUSLAB with a unique barcode when they were required at the time of creating the extraction batches.

5.2.10 Tapelift directly inoculated with cells

For Experiment 5 (Batches 7-11), tapelift samples were prepared with 5µL of Donor 15 stock cell suspension (section 5.1.2) inoculated directly onto the tape. For Experiment 5 (Batches 12-22), tapelift samples were prepared with 15µL of Donor 14 stock cell suspension (section 5.1.2) inoculated directly onto the tape.

For Experiment 12 tapelift samples were prepared with 15µL of Donor 15 stock cell suspension (section 5.1.2) inoculated directly onto the tape.

All tapelifts were air dried in the biohazard hood overnight and then sampled into 2.0mL tubes as per QIS 17142 *Examination of items,* section 6.8.2.

These cell tapelift samples were stored at 4°C, and registered in AUSLAB with a unique barcode when they were required at the time of creating the extraction batches.

5.2.11 FTA paper Samples (buccal cells)

Cell FTA card samples collected as per section 5.1.3 were sampled using the BSD Duet 600 Series II as per QIS 24823 *FTA Processing and Work Instructions*. The five x 1.2mm punches (standard punch size for normal FTA sample processing) were sampled into each 2mL tube. FTA paper cards were stored at 4°C.

5.2.12 Inhibitor Samples

All the inhibitors except for the motor oil were obtained in powder form and were required to be made into solution. Each inhibitor was prepared at the highest and lowest concentrations as per the *Validation of the Manual Method for Extracting DNA using the DNA IQ™ System* [3] as well as additional concentrations between these.

Below outlines the concentrations prepared for each inhibitor which were peer reviewed by the Project Manager.

Hematin: MW: 633.49g/mol prepared in 1M NaOH.

The following formula was used:

mass (g) = conc.(mol/L) x volume (L) x molecular weight (g/mol)

For a 1000µM stock solution of Hematin:
= 0.633g/mM
= 0.00063g/µM
= 1000µL NaOH + 0.63mg Hematin

Using the stock solution, the following concentrations were prepared:
50µM = 1µL of stock Hematin + 19mL of 1M NaOH
75µM = 1µL of stock Hematin + 12.33mL of 1M NaOH
100µM = 1µL of stock Hematin + 9mL of 1M NaOH
125µM = 1µL of stock Hematin + 7mL of 1M NaOH
150µM = 1µL of stock Hematin + 5.7mL of 1M NaOH

Humic Acid: MW: 60.6g/mol

The following formula was used:

w/v = [mass of solute (g) / volume of solute (mL)] x 100

1% (w/v) = 1000µL H2O + 0.01g Humic Acid
2% (w/v) = 1000µL H2O + 0.02g Humic Acid
5% (w/v) = 1000µL H2O + 0.05g Humic Acid
10% (w/v) = 1000µL H2O + 0.10g Humic Acid
15% (w/v) = 1000µL H2O + 0.15g Humic Acid
20% (w/v) = 1000µL H2O + 0.20g Humic Acid

Tannic Acid: MW: 1701.2g/mol

The following formula was used:

mass (g) = conc.(mol/L) x volume (L) x molecular weight (g/mol)

0.2M = 5mL of H2O + 1.7g of Tannic Acid
0.8M = 5mL of H2O + 3.4g of Tannic Acid
1M = 2.5mL of H2O + 4.25g of Tannic Acid
1.2M = 1mL of H2O + 2g of Tannic Acid
1.6M = 1.25mL of H2O + 3.25g of Tannic Acid
2M = 1.25mL of H2O + 4.25g of Tannic Acid

Indigo Carmine Dye: MW: 466.35g/mol

The following formula was used:

mass (g) = conc.(mol/L) x volume (L) x molecular weight (g/mol)

100mM = 5mL of H_2O + 0.002g of Indigo Carmine
80mM = 5mL of H_2O + 0.0017g of Indigo Carmine
60mM = 5mL of H_2O + 0.0014g of Indigo Carmine
40mM = 5mL of H_2O + 0.0009g of Indigo Carmine
20mM = 5mL of H_2O + 0.00046g of Indigo Carmine
10mM = 5mL of H_2O + 0.0002g of Indigo Carmine

Urea: MW: 60g/mol

The following formula was used:

mass (g) = conc.(mol/L) x volume (L) x molecular weight (g/mol)

0.2M = 100mL of H_2O + 1.2g of Urea
0.4M = 100mL of H_2O + 2.4g of Urea
0.6M = 100mL of H_2O + 3.6g of Urea
0.8M = 100mL of H_2O + 4.8g of Urea
1M = 100mL of H_2O + 6g of Urea

Motor Oil: Neat motor oil in a variety of quantities was used.

Sanitary pads: Sanitary pads were excised and used.

Disposable Nappies: Disposable nappies were excised and used.

Powdered gloves: Powdered gloves were excised and used.

Non-powdered gloves: Non-powdered gloves were excised and used.

Nitrile gloves: Nitrile gloves were excised and used.

5.3 DNA Extractions

Extractions were performed using the QIAsymphony® and Maxwell®16 instruments. Both extraction methods included a pre-lysis procedure, followed by the completion of the extraction using the relevant instrument.

Samples extracted using the QIAsymphony® instrument were pre-lysed using varying modified versions of the QIAsymphony® lysis protocol: Pre-treatment of Surface and Buccal Swabs [2, 3] outlined in section 6.1 of this report. These lysates were then extracted using the QIAsymphony® instrument with the QIAsymphony® DNA Investigator Kit.

Samples extracted using the Maxwell®16 instrument were pre-lysed and then extracted using the Maxwell®16 instrument with the Casework DNA IQ™ Pro Kit for Maxwell®16 as per QIS 29344 *DNA IQ™ Extraction using the Maxwell® 16.*

Experiment 12 also used the current manual DNA IQ™ method of extraction (QIS 24897 *DNA IQ Method of Extracting DNA from Reference and Casework sample*, section 7.1) and the manual QIAamp® DNA Investigator® Kit [6] for comparison.

5.4 DNA Quantification

All samples were quantified using the AB 7500 Real Time PCR System instrument and Thermo Fisher Scientific Quantifiler® Trio DNA Quantification Kit (Quantifiler® Trio).

Where testing of the QIAsymphony® AS module was required, quantification assay setup was performed using the QIAsymphony® AS module using a custom protocol created by QIAGEN.

All other samples required to be quantified, were prepared using the Pre-PCR Multiprobe® II platform or manually in accordance to QIS 33407 *Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit.*

5.5 DNA Amplification

All samples requiring amplification were amplified with the Promega PowerPlex®21 PCR Amplification Kit using GeneAmp PCR system 9700 thermalcycler. Amplification volumes were calculated from the quantification results. A total of approximately 0.5ng of DNA template was added for each 25μL volume amplification reaction. PCR reactions were prepared using the Pre-PCR Multiprobe® II platform as per QIS 31511 *Amplification of Extracted DNA using the PowerPlex® 21 System.* The PCR cycling conditions utilised are in Table 2.

Table 2 - PCR cycling conditions for PowerPlex®21 System

PowerPlex® 21 Kit	Standard
GeneAmp 9700 mode	Max
	30 cycles
Taq Activation	96°C for 1 minute
Denaturation	94°C for 10 seconds
Annealing	59°C for 1 minute
Extension	72°C for 30 seconds
Final Extension	60°C for 10 minutes
Store	4°C Soak for up to 24 hours

5.6 DNA Fragment Analysis

Plates for DNA fragment analysis (capillary electrophoresis) were processed using the 3130*xl* B instrument as per QIS 19978 *Capillary Electrophoresis Setup* and QIS 15998 *Procedure for the Operation and Maintenance of the 3130xl Genetic Analysers*. Table 3 outlines the 3130*xl* Genetic Analyser running conditions. All samples had DNA fragment analysis performed as per QIS 17130 *Capillary Electrophoresis Quality (CEQ) Check*.

Table 3 - 3130xl CE protocol conditions

Injection time	Injection voltage	Run time
5sec	3kV	1500sec

5.7 Profile Interpretation

All sample results were interpreted using the GeneMapper® ID-X v1.4 software as per QIS 31389 *STR fragment analysis of PowerPlex® 21 profiles using GeneMapper® ID-X software.*

As per Section 4 of QIS 31389, the GMIDX sample/display plot screen was inspected for each sample to assess the quality of the profile result in terms of allele designation, OL or OMR peaks, unlabelled peaks, off ladder alleles, variants, cross over alleles, peak heights (including homozygote and heterozygote thresholds), excess samples, allelic imbalance, stutter peaks, pull up, incomplete adenlylation, reproducible artefacts, non-reproducible artefacts (including spikes, bad baseline and peak shadows), partial profiles, inhibition, degradation, preferential amplification, extra peaks, tri-alleles and mixed DNA profiles.

6 EXPERIMENTAL DESIGN

6.1 Experiment 1: Modification of Pre-lysis Protocol

Background
Preliminary testing during the trial study of the QIAsymphony® instrument found that better DNA yields were obtained to that of the Multiprobe® II with a modified version of the QIAsymphony® lysis protocol – pre-treatment of surface and buccal swabs[2,3] protocol. During the trial the protocol was modified to include the addition of 25µL DTT (prepared in-house) to each sample[1].

Each sample had the following added:

- 450µL ATL Buffer
- 25µL of Proteinase K solution
- 25µL of DTT

Samples were then incubated using the ThermoMixer at 56°C for 45 minutes whilst shaking.

Intent

There are a limited number of heating shakers (thermomixers) available in the Analytical laboratory. This experiment was performed to test alternative shaking/vortexing incubation protocols and equipment to determine whether an alternative could be used in place of the thermomixer whilst still obtaining optimal results.

Experimental Design

Three pre-lysis protocols were tested using the following sample set:

- 1 positive extraction control
- 1 negative extraction control
- 10 blood swab samples
- 10 tapelift samples (prepared as per 5.2.9)

DNA yields for samples processed with Protocols 2 and 3 were compared to Protocol 1 (deemed suitable during QIAsymphony® instrument trial). The following three pre-lysis treatments were initially compared:

Protocol 1 (deemed suitable during QIAsymphony® instrument trial)

- 450µL ATL Buffer added to the sample
- 25µL of the Proteinase K solution added to the sample
- 25µL DTT added to the sample
- Samples incubated using the thermomixer at 56°C, shaking at 900rpm for 45 minutes
- Samples pulse spun to remove condensation from lids
- Swabs transferred to spin baskets
- Centrifuged for 2 minutes at 15800g
- Swabs transferred into a separate 2mL/1.5mL tube for storage, and the flow through back into the original sample tube ready for the QIAsymphony®

Protocol 2 (modified protocol using the Labnet shaker 20)

- 450µL ATL Buffer added to the sample
- 25µL of the Proteinase K solution added to the sample
- 25µL DTT added to the sample
- Samples incubated on hotblock at 56°C (without shaking) for 45 minutes
- Mixed samples on shaker for 10 minutes at 1200rpm
- Samples pulse spun to remove condensation from lids
- Swabs transferred to spin baskets
- Centrifuged for 2 minutes at 15800g
- Swabs transferred into a separate 2mL/1.5mL tube for storage, and the flow through back into the original sample tube ready for the QIAsymphony®

Protocol 3 (modified protocol using manual vortex mixing)

- 450µL ATL Buffer added to the sample
- 25µL of the Proteinase K solution added to the sample
- 25µL DTT added to the sample
- Vortex mixed each sample for 10 seconds before incubation
- Incubated samples on hotblock at 56°C for 45 minutes
- Vortex mixed samples for 10 seconds after incubation

- Samples pulse spun to remove condensation from lids
- Swabs transferred to spin baskets
- Centrifuged for 2 minutes at 15800g
- Swabs transferred into a separate 2mL/1.5mL tube for storage, and the flow through back into the original sample tube ready for the QIAsymphony®

The results from the tapelift samples in Experiment 5 showed that the QIAsymphony® was giving lower DNA yields than the Maxwell®16. As a result of this, a modified pre-lysis protocol was tested to determine if further increases to DNA yields could be achieved.

Protocol 4 (modified protocol with increased temperature and using thermomixer at 1400rpm)

- 450µL ATL Buffer added to the sample
- 25µL of the Proteinase K solution added to the sample
- 25µL DTT added to the sample
- Vortex mixed each sample for 10 seconds before incubation
- Incubated samples on ThermoMixer 70°C for 45 minutes at 1400rpm
- Vortex mixed samples for 10 seconds after incubation
- Samples pulse spun to remove condensation from lids
- Swabs transferred to spin baskets
- Centrifuged for 2 minutes at 15800g
- Swabs transferred into a separate 2mL/1.5mL tube for storage, and the flow through back into the original sample tube ready for the QIAsymphony®

All samples were then extracted using the QIAsymphony® with the standard protocol 'Extraction CW500 Trial' used during the trial (originally called CW500 ADV CR21066 ID1358).

All samples were sent for quantification and these quantification assay plates were prepared manually as per method 5.4. All samples were quantified twice to obtain an average DNA yield.

Data Analysis
The DNA yields (quantification results) from each of the pre-lysis protocols were compared.

Acceptance Criteria
The protocol with the highest DNA yields was accepted as the most effective protocol and implemented.

6.2 Experiment 2: Modification of Extraction Protocol

Intent
The purpose of this experiment was to determine if modifying the QIAsymphony® instrument extraction protocol, used in the preliminary trial, would achieve higher DNA yields. The following protocol modifications were added into the existing protocol and tested:

- Increased number of resin mixing steps before the addition of the resin solution to the sample preparation cartridge.
- Addition of heating during the elution step (similar to that of the Maxwell®16 instrument).

Experimental Design
Separate protocols created by QIAGEN were used to test and assess the increased resin mixing and heated elution separately. A third protocol was also used to test increased resin mixing and heated elution in combination. These modified protocols were compared to the original un-modified protocol - Extraction CW500 Trial.

Four batches of samples were used in this experiment as per Table 4. Each sample batch consisted of:

- one positive extraction control
- one negative extraction control
- 10 blood swab samples
- 10 tapelift samples (prepared as per 5.2.9)

All batches underwent pre-lysis using Protocol 3, accepted initially in Experiment 1. Extraction using the QIAsymphony® was then performed using the protocols as per Table 4 below.

All samples underwent quantification in duplicate as per method 5.4.

Table 4 - Modified Extraction Protocols

Test Batch	Protocol	Modification made
1	Extraction CW500 Trial	Custom protocol with no modifications
2	Extraction CW500 Trial IM	Custom protocol with the addition of extra mixing of resin
3	Extraction CW500 Trial HE	Custom protocol with the addition of heating at the elution step
4	Extraction CW500 Trial HEIM	Custom protocol with the addition of both modifications from test batch 2 & 3

Data Analysis
DNA quantification yields for each protocol were compared.

Acceptance Criteria
The protocol with the highest average DNA yield, and which did not show inhibition via IPCCT results, was accepted as the most effective protocol and implemented.

6.3 Experiment 3: Lysate Storage

Intent

The purpose of this experiment was to determine if the lysate storage temperature and time of storage (between completion of pre-lysis and extraction using the QIAsymphony®), had any negative impacts on DNA yields and profile quality. Samples were stored for intervals from 0 to 96 hours at:

- Fridge storage (2˚C – 8˚C)
- Freezer storage (-10˚C – -20˚C)

The results of this experiment were used to determine acceptable storage times and conditions for lysates. The lysate storage refers to the time between the completion of the pre-lysis procedure to the start of the extraction using the QIAsymphony® SP module.

Experimental Design

A single sample set for this experiment consisted of:

- one positive extraction control
- one negative extraction control
- 5 blood swab samples

Table 5 describes the timeframes and conditions that each batch was stored at.

Table 5 - Lysate storage conditions and timeframes

Test Batch	Start Day	Storage conditions to be tested
1	Monday	Batch 1a - 96 hours (4 days) at fridge temperature
		Batch 1b - 96 hours (4 days) at freezer temperature
2	Tuesday	Batch 2a - 72 hours (3 days) at fridge temperature
		Batch 2b - 72 hours (3 days) at freezer temperature
3	Wednesday	Batch 3a - 48 hours (2 days) at fridge temperature
		Batch 3b - 48 hours (2 days) at freezer temperature
4	Thursday	Batch 4a - 24 hours (1 day) at freezer temperature
		Batch 4b - 24 hours (1 day) at fridge temperature
5	Friday	Batch 5 - 0 hours (0 days) no storage

Immediately after the pre-lysis was completed for Test Batch 5, all batches were extracted together using the QIAsymphony® SP instrument. The extraction was completed using the 'Extraction CW500 Trial HEIM' protocol (as accepted in Experiment 2).

All samples progressed through quantification, amplification, DNA fragment analysis and profile interpretation as per methods 5.4 – 5.7. All samples were quantified in duplicate.

Data Analysis

Results for Batches 1 to 4 (stored between 24 - 96 hours) were compared to those of Batch 5. This comparison was done by analysing the:

- Quantification DNA yield (SAT)
- Quantification degradation index (DI)
- Profile allele calls for any drop-out peaks which may indicate degradation

Acceptance Criteria

Storage timeframes and conditions were accepted where the results obtained for stored samples were comparable to or better than results obtained for samples which have been extracted immediately following the pre-lysis procedure (no storage).

6.4 Experiment 4: Sensitivity

Please note: This experiment was originally performed using pre-lysis Protocol 3. This experiment gave low DNA yields from both the QIAsymphony® and Maxwell®16. A review of the methodology for this experiment determined that nanopure water was used to prepare the buccal cell suspension instead of 0.9% saline solution (as per Method 5.2.6). The use of nanopure water has likely resulted in cell lysis of the cell suspensions, causing low DNA yields. Accordingly, this experiment was repeated, using a buccal cell suspension prepared as per Method 5.2.6, and using pre-lysis Protocol 4 (which was validated after the original labwork for Experiment 4 was conducted). The results for the original experiment can be located in the results spreadsheet (I:\Change Management\Proposal#168 - Validation of QIAsymphony\RESULTS\Sample Batches and results.xls).

Intent

The purpose of this experiment was compare the sensitivity of the QIAsymphony® instrument extraction using the QIAsymphony® DNA Investigator Kit to the Maxwell®16 instrument using Casework DNA IQ™ Pro Kit.

The results of this experiment were also used to assess the relative efficiency and recovery of DNA from the QIAsymphony®. This was done by comparing the known amount of input DNA with the amount of DNA extracted and detected at quantification.

Experimental Design

The cytobrush method as per method 5.1.2 was used to collect buccal cells for cell suspensions. A cell count of the cell suspension(s) was performed in the Evidence Recovery laboratory to estimate the number of cells present per μL and therefore, the number of picograms of DNA present per μL. This was performed as per method 5.2.5.

The cell count was performed and calculated using the following formula as per Section 9.2.3 QIS 25874 *Preparation of DNA Quantification Standards & In-house Quality Controls*:

Cell count = (number of cells counted / grids counted) x 90 x dilution factor

For this experiment a 1/5 dilution of Donor 14 was used to perform the cell count. An average of 63.2 cells was determined from five cell counts of ten grids. The cell count was determined as follows:

Cell number (cells/µL) = (average cell count / 10 grids) x 90 x 5

= (63.2 / 10) x 90 x 5

= 2844 cells/µL

It is estimated that 1 cell ≈ 6pg = 0.006 ng [4].

Therefore 2844 cells/µL = 17064 pg/µL = 17.064 ng/µL.

A dilution of the stock cell suspension was then performed (as per section 5.2.6) to ensure that an adequate amount of DNA (ng) was inoculated onto each swab. A 1:250 dilution of the stock cell suspension was chosen.

1:250 = 0.06826 ng/µL or 10:2500 = 0.06826 ng/µL

Swabs were inoculated in duplicate with the diluted cell suspension as per the volumes in Table 6 below.

Table 6 – Diluted cell suspension inoculated on swabs.

Inoculation Volume (µL)	ng/µL	pg/µL	Approximate number of cells
1	0.06826	68.26	~11 cells
2	0.13651	136.51	~23 cells
5	0.34128	341.28	~57 cells
10	0.68256	682.56	~114 cells
20	1.36512	1365.12	~228 cells
30	2.04768	2047.68	~341 cells
40	2.73024	2730.24	~455 cells

Two sample sets were required for this experiment (one each for the QIAsymphony® and Maxwell®16). Each sample set consisted of:

- one positive extraction control
- one negative extraction control
- 14 cells swabs with varying amounts of DNA input (as per Table 6)

One sample set was lysed using pre-lysis Protocol 4 (accepted in Experiment 5) and then extracted using the QIAsymphony® instrument using Protocol 4 (accepted in Experiment 2). A duplicate sample set was also extracted using the Casework DNA IQ™ Pro Kit for Maxwell®16 instrument for comparison. Table 7 shows the sample sets used in this experiment.

Table 7 – Sensitivity batches using diluted cell suspensions

Extraction Batches	
Swab	Sample – Cell Suspension Vol
1	Positive Control
2	Negative Control
3	1µL
4	1µL
5	2µL
6	2µL
7	5µL
8	5µL
9	10µL
10	10µL
11	20µL
12	20µL
13	30µL
14	30µL
15	40µL
16	40µL

All samples progressed through to quantification, as per methods 5.4. All samples were quantified in duplicate.

Data Analysis
The cell count for the cell suspension(s) (section 5.2.5) were used to determine the amount of DNA (ng) inoculated onto each swab in Table 7. This was compared to the amount of extracted DNA detected in DNA quantification. The extraction efficiency was determined and assessed as a percentage.

Sensitivity for the QIAsymphony® and Maxwell®16 was compared in terms of the SAT quantification results (comparing DNA yields at decreasing volumes of inoculated cell suspension).

Acceptance Criteria
The QIAsymphony® instrument using the DNA Investigator® Kit was accepted if it had an overall equivalent or higher sensitivity than the Maxwell®16 using the Casework DNA IQ™ Pro Kit based on DNA yields obtained at decreasing volumes of inoculated cell suspension.

6.5 Experiment 5: Verification of Additional Substrates

Intent
The aim of this experiment was to validate the QIAsymphony® for the extraction of a variety of substrates not tested in previous experiments. This was done by processing duplicate batches on both the QIAsymphony® instrument using the QIAsymphony® DNA Investigator® Kit and Maxwell®16 using the Casework DNA IQ™ Pro Kit and comparing DNA yields (based on average quantification values).

Experimental Design
Samples were prepared as per Section 5.1. Extraction batches 1 and 2 (see below) were extracted using the QIAsymphony® instrument.

Batches 3 to 6 (see below) were extracted using the Maxwell®16 instrument.

The results for the ten tapelift samples that were extracted using the Maxwell®16 instrument (Batch 6) were compared to the ten tapelifts that were extracted using the QIAsymphony® instrument in Experiment 2.

The extraction batches consisted of the following sample sets:

QIAsymphony® Batch 1: VALIQLYS20160111_01

- 1 positive extraction control
- 1 negative extraction control
- 5 fabric blood
- 5 FTA cells
- 5 cigarette butts
- 5 chewing gums

QIAsymphony® Batch 2: VALIQLYS20160111_02

- 1 positive extraction control
- 1 negative extraction control
- 5 tissue
- 5 female hair
- 5 male hair
- 5 fingernail

Maxwell®16 Batch 3: VALIQMAX20160111_01

- 1 positive extraction control
- 1 negative extraction control
- 5 fabric blood
- 5 FTA cells
- 4 cigarette butts

Maxwell®16 Batch 4: VALIQMAX20160111_02

- 1 positive extraction control
- 1 negative extraction control
- 1 cigarette butts
- 5 chewing gum
- 5 tissue

Maxwell®16 Batch 5: VALIQMAX20160202_01

- 1 positive extraction control
- 1 negative extraction control
- 5 female hair
- 5 male hair
- 4 fingernail

Maxwell®16 Batch 6: VALIQMAX20160202_02

- 1 positive extraction control
- 1 negative extraction control
- 1 fingernail
- 10 tapelift

An additional two extraction batches, one for each instrument were performed to re-test tapelift cell samples. The tapelift samples used in these extraction batches were prepared by inoculating 5µL of the buccal cell suspension directly onto the tape. This sample preparation change was made to remove any variability in sample collection during tape-lifting.

Due to decreased yields for tapelift samples extracted on the QIAsymphony® further troubleshooting and protocol modification testing was performed (see QIAsymphony® and Maxwell®16 batches 7 – 20 in experiment results I:\Change Management\Proposal#168 - Validation of QIAsymphony\RESULTS\Sample Batches and results.xls). These batches unsuccessfully tested a range of modified pre-lysis protocols. It was found at the completion of the testing that the poor DNA yield results for the QIAsymphony® was due to degradation of the Donor 14 cell suspension rather than sub-optimal performance of the extraction protocol. Therefore these experiments will not be included in this final report, but have been retained in the experimental results data located at I:\Change Management\Proposal#168 - Validation of QIAsymphony\RESULTS\ Sample Batches and results.xls

Extraction batches 21-22 consisted of the following sample set:

QIAsymphony® Test Batch 21: CQIALYS20160711_01

- 1 positive extraction control
- 1 negative extraction control
- 6 tapelift (Donor 14 new collection with 15µL of the buccal cell suspension)
- Pre-lysis Protocol 4 was used as per Section 6.1

Maxwell®16 Test Batch 22: VALIQMAX20160630_02

- 1 positive extraction control
- 1 negative extraction control
- 6 tapelift (Donor 14 new collection with 15µL of the buccal cell suspension)

All samples were sent for quantification, DNA fragment analysis and profile interpretation was performed as per methods 5.4 - 5.7.

Data Analysis
The DNA yields and average number of allele calls for samples extracted using the QIAsymphony® instrument were compared to the corresponding samples extracted using the Maxwell®16 instrument.

Acceptance Criteria
Extraction of each substrate type using the QIAsymphony® were accepted / validated if the DNA yields obtained from the QIAsymphony® were equal to or better than the yields obtained from the Maxwell®16.

6.6 Experiment 6: Inhibition

Intent
Forensic samples that are commonly submitted for DNA analysis may contain inhibitors. These inhibitors reduce the efficiency of a DNA extraction system by interfering with cell lysis or by nucleic acid degradation or capture, therefore manifesting as extraction inhibitors. Inhibitors can also co-extract with the DNA and inhibit downstream PCR amplification processes, therefore acting as PCR inhibitors [4].

This experiment was performed to assess whether the QIAsymphony® DNA Investigator Kit was able to effectively remove common inhibitors found in forensic casework and reference samples. Performance of the QIAsymphony® DNA Investigator Kit was compared to the validation results for the manual method for DNA IQ™ extraction[3].

This experiment also tested the ability for the QIAsymphony® DNA Investigator Kit and QIAsymphony® instrument to process a number of known difficult substrates.

Experimental Design
Fabric samples were prepared with approximately 0.5ng of input DNA (3.5µL of Donor 1 blood). Substrates that were considered to be difficult (in terms of pipetting) were also spiked with equal amounts of blood. The fabric samples were then spiked in duplicate with a range of concentrations of potential inhibitors (prepared as per section 5.2.12), see Tables 8-11 below.

Inhibitors were chosen for their known ability to inhibit PCR and their likelihood of appearing in routine casework samples. Difficult substrates that have been problematic in past automated extraction processes were also chosen. These included:

- Hematin – a known PCR inhibitor which is commonly found in degraded blood samples.
- Humic acid – known PCR inhibitor found in soil.
- Tannic acid – known PCR inhibitor found in leather.
- Indigo carmine dye – known PCR inhibitor found in denim fabrics.
- Motor oil – known PCR inhibitor.
- Urea – found in urine.

- Super Absorbent Polymer/Hydrogels – commonly found in sanitary pads and nappies. These products have caused issues with previous extraction protocols due to the viscosity of the lysate and instrument's inability to process these lysates due to issues aspirating the viscous liquid which causes tip blockages.
- Latex gloves – certain latex gloves also result in highly viscous lysates as per sanitary pads and nappies.

All samples were extracted using the QIAsymphony® DNA Investigator Kit and QIAsymphony® instrument. Tables 8 to 11 outlines the extraction batches, sample IDs and inhibitors at particular concentrations that were tested for this experiment.

Table 8 – Batch 1 - Inhibitors tested using the QIAsymphony® Protocol

Sample Number	Sample ID Number	Inhibitor Concentrations/ volumes	Instrument used for batch and Batch ID
1		Positive Control	QIAsymphony® instrument VALIQLYS20160203_01
2		Negative Control	
3		Nil inhibitor	
4		Nil inhibitor	
5		Hematin - 50µM	
6		Hematin - 50µM	
7		Hematin - 75µM	
8		Hematin - 75µM	
9		Hematin - 100µM	
10		Hematin - 100µM	
11		Hematin - 125µM	
12		Hematin - 125µM	
13		Hematin - 150µM	
14		Hematin - 150µM	
15		Humic Acid - 1% (w/v)	
16		Humic Acid - 1% (w/v)	
17		Humic Acid - 5% (w/v)	
18		Humic Acid - 5% (w/v)	
19		Humic Acid - 10% (w/v)	
20		Humic Acid - 10% (w/v)	
21		Humic Acid - 15% (w/v)	
22		Humic Acid - 15% (w/v)	
23		Humic Acid - 20% (w/v)	
24		Humic Acid - 20% (w/v)	

Table 9 – Batch 2 - Inhibitors tested using the QIAsymphony® Protocol

Sample Number	Sample ID Number	Inhibitor Concentrations/ volumes	Instrument used for batch and Batch ID
1		Positive Control	QIAsymphony® instrument VALIQLYS20160205_01
2		Negative Control	
3		Tannic Acid – 0.2M	
4		Tannic Acid – 0.2M	
5		Tannic Acid – 0.8M	
6		Tannic Acid – 0.8M	
7		Tannic Acid – 1.2M	
8		Tannic Acid – 1.2M	
9		Tannic Acid – 1.6M	
10		Tannic Acid – 1.6M	
11		Tannic Acid – 2.0M	
12		Tannic Acid – 2.0M	
13		Indigo Carmine Dye – 10mM	
14		Indigo Carmine Dye – 10mM	
15		Indigo Carmine Dye – 20mM	
16		Indigo Carmine Dye – 20mM	
17		Indigo Carmine Dye – 40mM	
18		Indigo Carmine Dye – 40mM	
19		Indigo Carmine Dye – 60mM	
20		Indigo Carmine Dye – 60mM	
21		Indigo Carmine Dye – 80mM	
22		Indigo Carmine Dye – 80mM	
23		Indigo Carmine Dye – 100mM	
24		Indigo Carmine Dye – 100mM	

Table 10 – Batch 3 - Inhibitors tested using the QIAsymphony® Protocol

Sample Number	Sample ID Number	Inhibitor Concentrations/ volumes	Instrument used for batch and Batch ID
1		Positive Control	QIAsymphony® instrument VALIQLYS20160205_02
2		Negative Control	
3		Motor Oil - 1µL	
4		Motor Oil - 1µL	
5		Motor Oil - 5µL	
6		Motor Oil - 5µL	
7		Motor Oil - 10µL	
8		Motor Oil - 10µL	
9		Motor Oil - 15µL	
10		Motor Oil - 15µL	
11		Motor Oil - 20µL	
12		Motor Oil - 20µL	
13		Urea – 0.2M	
14		Urea – 0.2M	
15		Urea – 0.4M	
16		Urea – 0.4M	
17		Urea – 0.6M	
18		Urea – 0.6M	
19		Urea – 0.8M	
20		Urea – 0.8M	
21		Urea – 1.0M	
22		Urea – 1.0M	
23		Humic Acid - 2% (w/v)	
24		Humic Acid - 2% (w/v)	

Table 11 – Batch 4 - Inhibitors tested using the QIAsymphony® Protocol

Sample Number	Sample ID Number	Inhibitor Concentrations/ volumes	Instrument used for batch and Batch ID
1		Positive Control	QIAsymphony® instrument VALIQLYS20160205_03
2		Negative Control	
3		Sanitary Pads (neat)	
4		Sanitary Pads (neat)	
5		Disposable Nappy (neat)	
6		Disposable Nappy (neat)	
7		Powdered Gloves (neat)	
8		Powdered Gloves (neat)	
9		Non - powdered Gloves (neat)	
10		Non - powdered Gloves (neat)	
11		Nitrile Gloves (neat)	
12		Nitrile Gloves (neat)	

All samples were progressed through quantification, amplification, DNA fragment analysis and profile interpretation as per methods 5.4 – 5.7. All samples were quantified in duplicate.

Data Analysis

DNA quantification results for all samples were assessed in terms of:

- Presence of the IPCCT flag used to identify samples which may be inhibited (IPPCT flag will indicate that the inhibitor has likely not been removed by the extraction).
- Number of alleles and profile quality – if the inhibitor has been successfully removed or difficult substrates have been overcome, all samples should result in a full DNA profile. Therefore the number of alleles and a qualitative assessment of DNA profile quality (drop out of large molecular weight loci) were used to assess results.

Results for the QIAsymphony® DNA investigator Kit were compared to the validation results for the manual method for DNA IQ™ extraction[3] for motor oil, tannic acid, urea, indigo dye and humic acid (N.B. comparisons took into consideration the different amounts of input DNA for each extraction method).

Acceptance Criteria

The QIAsymphony® DNA Investigator Kit and QIAsymphony® instrument was accepted if it performed as well or better than manual method for DNA IQ™ extraction for motor oil, tannic acid, urea, indigo dye and humic acid (taking into consideration the different amounts of input DNA for each protocol).

An assessment of the results for the remaining inhibitors (not included in the validation of the method for DNA IQ™ extraction) were used to inform sample workflows, including sampling and rework strategies for samples known to contain these inhibitors.

An assessment of the results for difficult substrates was used to inform sample workflows for samples of these substrate types.

6.7 Experiment 7: Degradation

Intent
This experiment was performed to test the ability of the QIAsymphony® DNA investigator kit and QIAsymphony® instrument to extract samples which were degraded samples using UV light.

Experimental Design
A cell suspension was prepared using buccal cells, collected with the cytobrush method as per method 5.1.2. A cell count was performed (as per method 5.2.5) on the cell suspension to estimate the number of cells and the concentration of DNA which was present in a µL of the suspension.

For this experiment, 25µL neat cell suspension of Donor 14 (approximately 207ng of DNA) of was used to inoculate fabric pieces as per method 5.2.8. These were then exposed to ultraviolet (UV) light as per times shown in Table 12.

Table 12 – UV exposure times

Sample	UV exposure time frame
1-2	Nil
3-4	15 minutes
5-6	30 minutes
7-8	45 minutes
9-10	1 hour
11-12	2 hours
13-14	4 hours
15-16	6 hours
17-18	8 hours
19-20	10 hours
21-22	15 hours
23-24	20 hours
25-26	24 hours

After UV exposure, all samples were extracted using the QIAsymphony® as per method 5.3 (using pre-lysis Protocol 3). All samples progressed through quantification, amplification, DNA fragment analysis and profile interpretation as per methods 5.4-5.7. All samples were quantified twice to obtain an average DNA yield.

Data Analysis
Quantification results including SAT, IPCCT and DI were tabulated along with the number of alleles obtained. These were used to make a qualitative assessment of the level of degradation and to establish if there is a relationship between DI and number of alleles obtained.

Assessment Criteria
An assessment of the results was used to determine if there was a relationship between DI and number of alleles obtained, so as to inform case management of degraded samples. As per the Quantifiler® HP and Trio DNA Quantification Kit user Guide [13], a DI value of between 1-10 indicates a slight to moderate degradation in a sample. A DI value of above 10 indicates significant degradation in a sample.

As written the intent of the experiment was to see the effectiveness of QIAsymphony in retrieving degraded (small fragment DNA). However due to the difficulties associated with artificially degrading DNA we used UV degradation and looked at profile outcomes. The UV has been effective replicating DNA degradation with corresponding reduction in allele numbers - however it does not effectively test the ability of QIAsymphony to retrieve (with efficiency) small DNA fragments. Given the resources and technology currently available the results of this experiment are an indication only and are not conclusive evidence of the performance of the QIAsymphony® instrument to extract degraded DNA.

6.8 Experiment 8: Pipetting Accuracy

Intent

The purpose of this experiment was to test the pipetting accuracy for the AS module which will be used to prepare quantification assay plates for samples that have been extracted using the SP Module.

Experimental Design

The Artel instrument and software, which is used to test pipetting accuracy, was used in this experiment according to QIS 26628 *Verifications using the Artel MVS*. The tips and volumes verified were those that will be routinely used during an assay setup using the AS module.

The volumes of 2µL and 18µL were verified for Thermo Fisher Scientific Quantifiler® Trio DNA Quantification Kit.

During this validation project, the Quantifiler® Human DNA Quantification Kit had been replaced with the Quantifiler® Trio DNA Quantification Kit. Initially a verification volume of 23µL was required, but due to the change of quantification kit, the volumes were amended to 2µL and 18µL.

Custom protocols developed by QIAGEN, outlined in Table 13 below, were used to check the pipetting accuracy of the AS module. These protocols will also be used to verify the AS module for routine three-monthly pipetting verifications.

Table 13 – Custom protocols for the AS module

Verification Batch	Volume to be tested	Protocol to be used	Tips to be used when testing
1	2µL	Artel verification 2µl	50µL
2	18µL	Artel verification 18µl	200µL

Verification Batch 1:

The Artel was set up as per QIS 26628 *Verifications using the Artel MVS*. The volume of 2µL using the 50µL tips was tested by the addition of 198µL of Diluent solution with the 2µL of Solution C in the positions using the channels illustrated below in Figure 1. An 8 data-point set for each pipette channel was set up through the ARTEL software protocol.

Verification Batch 2:
The Artel was set up as per QIS 26628 *Verifications using the Artel MVS*. The volume of 18µL using the 200µL tips was tested by the addition of 182µL of Diluent solution with the 18µL of Solution B in the positions using the channels illustrated below in Figure 1. An 8 data-point set for each pipette channel was set up through the ARTEL software protocol.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Channel 1	Channel 1	Channel 1	Channel 1	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
B	Channel 1	Channel 1	Channel 1	Channel 1	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
C	Channel 2	Channel 2	Channel 2	Channel 2	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
D	Channel 2	Channel 2	Channel 2	Channel 2	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
E	Channel 3	Channel 3	Channel 3	Channel 3	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
F	Channel 3	Channel 3	Channel 3	Channel 3	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
G	Channel 4	Channel 4	Channel 4	Channel 4	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
H	Channel 4	Channel 4	Channel 4	Channel 4	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank

Figure 1 - Verification layout of ARTEL plate.

Each verification plate was placed onto the Artel instrument and was analysed using the Artel Software.

Data Analysis
Once analysed, the microtiter plate readings were collected from the ARTEL MVS verification plate. The Data Manager software generated and displayed an Output Report, with a 'PASS' or a 'FAILED' result. The yellow or orange coloured data points represented dispensed volumes that exceeded the limits for Relative Inaccuracy and/or Coefficient of Variation. For the QIAsymphony, each individual channel was required to pass.

Acceptance Criteria
The pipetting of the AS module was deemed accurate if all tips collectively met the acceptance criteria set up using the Artel software.

The acceptance criteria for DNA Analysis POVAs (Piston Operated Volumetric Apparatus) used for routine verification is a %CV and %inaccuracy of +/- 5% (10% for volumes <10µL).

6.9 Experiment 9: Contamination Check

Intent
The aim of this experiment was to check the QIAsymphony® instrument for cross-contamination during the extraction protocol using the SP module and for quantification setup using the AS module.

Experimental Design

Part 1 – Contamination Check of SP module

The automated extraction Multiprobe® II platforms were assessed every 6-months for cross contamination using a soccerball pattern plate preparation method. This method (as per QIS 24012 *Miscellaneous Analytical Section Tasks*) was also used to test and identify any cross contamination issues using the QIAsymphony SP module run.

Soccerball plates were prepared by extracting blood swabs inoculated with neat blood in amongst negative controls. Twelve different blood donors were used to create blood swabs in this experiment. Four lysate batches were prepared as per Table 14.

Table 14 – Lysate samples and batches for 'Soccerball' batch that were run using the SP

Lysate Batch 1		Lysate Batch 2		Lysate Batch 3		Lysate Batch 4	
Position #	Sample Type	Position #	Sample Type	Position #	Sample Type	Position #	Sample Type
1-9	Blank	1-8	Blank	1-9	Blank	1-8	Blank
10	Pos Ctl (Donor 1)	9	Pos Ctl (Donor 4)	10	Pos Ctl (Donor 7)	9	Pos Ctl (Donor 10)
11-12	Blank	10-11	Blank	11-12	Blank	10-11	Blank
13	Pos Ctl (Donor 2)	12	Pos Ctl (Donor 5)	13	Pos Ctl (Donor 8)	12	Pos Ctl (Donor 11)
14-15	Blank	13-14	Blank	14-15	Blank	13-14	Blank
16	Pos Ctl (Donor 3)	15	Pos Ctl (Donor 6)	16	Pos Ctl (Donor 9)	15	Pos Ctl (Donor 12)
17-24	Blank	16-24	Blank	17-24	Blank	16-24	Blank

Once all four batches were lysed, the lysates were extracted using the QIAsymphony® instrument in batch order (1-4) to resemble a soccerball pattern (see Figure 2).

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	Blank	Blank	Pos Ctl (D 4)	Blank	Blank	Blank	Blank	Blank	Pos Ctl (D 10)	Blank
B	Blank	Pos Ctl (D 1)	Blank	Blank	Blank	Blank	Blank	Pos Ctl (D 7)	Blank	Blank	Blank	Blank
C	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
D	Blank	Blank	Blank	Blank	Pos Ctl (D 5)	Blank	Blank	Blank	Blank	Blank	Pos Ctl (D 11)	Blank
E	Blank	Pos Ctl (D 2)	Blank	Blank	Blank	Blank	Blank	Pos Ctl (D 8)	Blank	Blank	Blank	Blank
F	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
G	Blank	Blank	Blank	Blank	Pos Ctl (D 6)	Blank	Blank	Blank	Blank	Blank	Pos Ctl (D 12)	Blank
H	Blank	Pos Ctl (D 3)	Blank	Blank	Blank	Blank	Blank	Pos Ctl (D 9)	Blank	Blank	Blank	Blank

Figure 2 - Batch layout for soccerball (SP module)

All samples were progressed through to quantification, amplification and DNA fragment analysis as per methods 5.4 – 5.7.

Part 2 – Contamination Check of AS module

To test and identify any potential cross contamination using the QIAsymphony® AS module one quantification assay plate was prepared using samples from Part 1 – Contamination Check of SP module.

The batch layout for the contamination test of the AS module is shown in Figure 3. Positions 1 – 11 contained a set of standards and a reagent negative control as per routine quantification (as per QIS 33407 *Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit*).

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD 1	STD 5	Blank	Blank	Blank	Pos Ctl (D 6)	Blank	Blank	Blank	Blank	Blank	Pos Ctl (D 12)
B	STD 1	STD 5	Pos Ctl (D 3)	Blank	Blank	Blank	Blank	Blank	Pos Ctl (D 9)	Blank	Blank	Blank
C	STD 2	Neg Ctl	Blank	Blank	Pos Ctl (D 4)	Blank	Blank	Blank	Blank	Blank	Pos Ctl (D 10)	Blank
D	STD 2	Pos Ctl (D 1)	Blank	Blank	Blank	Blank	Blank	Pos Ctl (D 7)	Blank	Blank	Blank	Blank
E	STD 3	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
F	STD 3	Blank	Blank	Blank	Pos Ctl (D 5)	Blank	Blank	Blank	Blank	Blank	Pos Ctl (D 11)	Blank
G	STD 4	Pos Ctl (D 2)	Blank	Blank	Blank	Blank	Blank	Pos Ctl (D 8)	Blank	Blank	Blank	Blank
H	STD 4	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank

Figure 3 - Batch layout for soccerball (AS module)

This assay plate was then processed using the Applied Biosystems® 7500 Real Time PCR instrument and analysed as per method 5.4.

Data Analysis

Quantification results were analysed as per QIS 33407 *Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit.*

Acceptance Criteria

The SP of the QIAsymphony® instrument was accepted if:

- All blank controls gave an 'undetermined' quantification result and passed the acceptance criteria as per QIS 17130 *Capillary Electrophoresis Quality (CEQ) Check.*
- All positive control results gave the expected single source profile

The AS module of the QIAsymphony® instrument was accepted if all blank controls:

- Resulted in an 'undetermined' quantification result

6.10 Experiment 10: Integrated Runs

Background

The combined QIAsymphony® SP/AS instrument allows for integrated runs to be performed, whereby samples extracted using the SP module are immediately progressed to quantification assay setup using the AS module without the need for the DNA extracts to be removed from the instrument.

It was observed during the preliminary trials[1] that when an assay plate was left on the QIAsymphony® AS module overnight, a large amount of condensation formed on the cooling blocks that held the assay plate, reagents and sample rack. It was noted that this condensation caused an increase volume of approximately 10µL in each well across the assay plate. During the trial, the cooling block temperature was set at 4°C and room 3191 housing the QIAsymphony® was not air conditioned overnight which may have contributed to the condensation.

Intent

The purpose for this experiment was to perform a series of integrated runs to attempt to eliminate the condensation by modifying environmental factors and the assay setup protocol. The following modifications were tested:

- Modification of assay set-up protocol to increase the temperature of the cooling blocks from 4°C to 8°C
- The length of time plates are stored on the instrument following assay preparation
- Constant air-conditioning in Room 3191 at 21°C for between Monday mornings and Friday afternoons (air-conditioning only being switched off during the weekends).

Experimental Design

Four separate runs were performed where the lag time between the SP and AS protocols differed for each batch, as detailed in Table 15. Each sample set consisted of 10 blood swabs samples and one positive control and one negative control.

Table 15 – Integrated batches between the SP and AS modules.

Integrated Batch #	Sample types and numbers	Protocol and Instrument used
1.	NON - INTEGRATED RUN: SP Extraction immediately followed by non-integrated AS quantification assay setup Extraction Protocol: EXTRACTION CW500 HEIM (VALIQLYS20160429_01) Quantification Protocol: QUANT_TRIO DAY (4 ºC) (VALQUA20160504_02)	Extraction performed using the SP module followed immediately by assay setup using the AS module. Quantification reagents not loaded onto AS module until after extraction on SP module completed. Cooling adaptors on the AS module set to 4˚C. Room not air-conditioned overnight.
2.	INTEGRATED RUN – Overnight SP extraction and 2nd day quantification assay setup. Integrated Run Protocol: IR_Trio(8ºC) (VALIQLYS20160429_02 and VALQUA20160504_01)	Extraction commenced using the SP module in the afternoon and left to run overnight. Quantification reagents were prepared and placed onto the AS module the following morning and integrated protocol allowed to continue preparing the assay plate using the AS module. This batch used an integrated run protocol with the cooling adaptors on the AS module set to 8˚C. Room not air-conditioned overnight.
3.	INTEGRATED RUN – Overnight extraction and quantification assay prepared immediately following completion of extraction. Quantification assay plate stored on AS overnight at 8ºC. Integrated Run Protocol: IR_Trio(8ºC) (VALIQLYS20160429_03 and VALQUA20160510_01)	Extraction commenced using the SP module in the afternoon and left to run overnight. The assay setup automatically commenced using the AS module once the extraction was completed. This batch used an integrated run protocol. Batch was started at ~14:00hrs and allowed to continue to assay plate setup using the AS module automatically throughout the evening with the cooling adaptors set to 8˚C. The assay plate was removed from the cooling adaptor on the AS module the following morning at ~08:30hrs. Room not air-conditioned overnight.
4.	INTEGRATED RUN – Overnight extraction and quantification assay prepared immediately following completion of extraction. Quantification assay plate stored on AS overnight at 4 ºC. Integrated Run Protocol: IR_Trio(4 ºC) (VALIQLYS20160429_04 and VALQUA20160518_01)	Extraction commenced using the SP module in the afternoon and left to run overnight. The assay setup automatically commenced using the AS module once the extraction was completed. This batch used the integrated run protocol, with the cooling adaptors on the instrument set to 4˚C and the room temperature set and monitored to 21˚C overnight. The assay plate was removed from the cooling adaptor on the AS module the following morning at ~08:30hrs.

Acceptance Criteria
The results for batches 1, 2, 3 and 4 were assessed against the following acceptance criteria:

- Batch protocols were accepted if there was no condensation on the assay plates and cooling blocks (based on a visual assessment).
- In addition to the previous criteria, the volume of each eluate was measured and compared to expected volumes to determine if additional volume was present in the sample tube – only batch protocols with no additional volume were accepted.
- Batch protocols were accepted if quantification results were within passing criteria as per QIS 33407 *Quantification of Extracted DNA using Quantifiler® Trio DNA Quantification Kit.*
- Quantification results (IPCCT, SAT, LAT, DI) were assessed for gross outliers which may indicate additional volume in wells, or adverse effects of leaving quantification reagents and/or prepared assay plates on the AS module for extended periods.

Any batch protocols that passed these acceptance criteria were accepted as validated protocols.

6.11 Experiment 11: Repeatability and Reproducibility

Intent
The aim of this experiment was to assess the repeatability and reproducibility for the QIAsymphony® instrument.

Repeatability was assessed by ability of the QIAsymphony® instrument to produce the same results when one sample set was processed a number of times by the same operator under the same conditions.

Reproducibility was assessed by the ability of the QIAsymphony® instrument to produce the same results when one sample set was processed by different operators under different conditions.

Experimental Design
This experiment assessed the SP and AS modules independently.

One batch of samples for this experiment comprised of:

- one positive extraction control
- one negative extraction control
- 5 blood swabs
- 5 cells swabs

Repeatability – SP Module
Two separate batches of samples (Extraction A and Extraction B) were lysed (using pre-lysis Protocol 3) and extracted using the QIAsymphony® SP module on the same day by the same operator. Quantification assays were setup manually and then processed using the AB 7500 Real Time PCR System. All samples were quantified twice to obtain an average DNA yield.

The average quantification result for cell and blood swabs were calculated separately for Extraction A and Extraction B. These results were then compared against each other.

Repeatability – AS Module
Extraction A samples extracted for the repeatability of the SP Module, was placed onto the AS module and used to create two quantification assay plates (Quant A and Quant B) on the same day by the same operator. Both plates were processed using the AB 7500 Real Time PCR System and quantification results compared.

Quantification results from Quant A and Quant B were compared on a sample by sample basis.

Reproducibility – SP Module
One batch of samples (Extraction C) was lysed and extracted using the QIAsymphony® SP module by a different operator and on a different day to the Repeatability – SP Module experiment. The quantification assay was setup manually and then processed using the AB 7500 Real Time PCR System. All samples were quantified twice to obtain an average DNA yield.

An average quantification result for the cell and blood swabs was calculated for Extraction C. These results were then compared to the average quantification result from Extraction A and Extraction B.

Reproducibility – AS Module
Samples from Extraction A were placed onto the AS module and a quantification assay plate prepared (Quant C). This was performed by a different operator, and on a different day to that for the Repeatability – AS Module experiment. These samples were quantified twice to obtain an average DNA yield.

Quantification results for Quant C were compared to results from Quant A and Quant B on a sample by sample basis.

Acceptance Criteria
The QIAsymphony® SP module was accepted if the repeatability and reproducibility quantification results were comparable.

The QIAsymphony® AS module was accepted if the repeatability and reproducibility quantification results were comparable.

6.12 Experiment 12: Sample Recovery

Intent

The aim of this experiment was to test manual methods for the completion of the extraction process in the event of the QIAsymphony® SP instrument malfunctioning, stopping or accidently being cancelled during an extraction run.

Possible scenarios for instrument malfunction include but are not limited to:

- Power failure/outage in the laboratory
- Hardware failure on the QIAsymphony® instrument
- The opening of the hood of the SP module during stages of the run which would not allow the continuation of the run
- Tip arm colliding into the worktable and/or consumables (caused by damaged consumables or consumables not added to deck correctly)
- Tip arm colliding into the worktable and/or consumables if consumables added to the worktable had not been scanned when prompted

A manual method could also be used to extract samples already lysed if the instrument is offline for an extended period.

The QIAamp® DNA Investigator® Kit is the manual kit recommended by QIAGEN to be used in the unlikely event of an instrument malfunction or downtime. Methods that are currently in use within Forensic DNA Analysis were also tested and compared to the recommended kit.

Experimental Design

One batch of samples for this experiment comprised of the following samples, inoculated with single source DNA:

- one positive control
- one negative control
- 5 positive control blood swabs
- 5 tapelifts inoculated with 15µL of Donor 15 cell suspension

Four batches were used in this experiment. All batches were lysed using the pre-lysis Protocol 3 and samples were stored at 4°C. Each batch of samples was progressed through one of the extraction methods described below.

Batches 1 and 4 were used to simulate extended instrument downtime whereby a large number of samples have already undergone pre-lysis procedure and awaiting the final extraction using the QIAsymphony® instrument.

Batches 2 and 3 were used to simulate a malfunction or a cancelled run of the QIAsymphony® SP extraction on the instrument. These batches were compared to determine which manual method was best to progress samples which had commenced extraction but not finalised using the QIAsymphony® SP module.

All samples were quantified as per method 5.4.

Note: During the first attempt of this experiment it was noted that the contents of the sample prep cartridges from the QIAsymphony® was ~1600µL. The maximum volume that can be processed using a Maxwell® 16 instrument is

750µL. Therefore using the Maxwell instrument to complete a QIAsymphony® extraction is not possible.

Recovery Method Batch 1
Batch 1 tested the current method of extraction QIS 29344 *DNA IQ™ Extraction using the Maxwell® 16* from the lysate stage. Batch 1 samples were lysed using the QIAGEN pre-lysis method and the extraction completed as per QIS 29344 *DNA IQ™ Extraction using the Maxwell® 16,* section 7.1 from step 8 – the addition of 200µL of the Promega Lysis Buffer to each sample, vortex mixed for 10 seconds prior transferring to the Maxwell®16 instrument.

Recovery Method Batch 2
Batch 2 tested the effectiveness of the recovery using the current manual DNA IQ™ method of extraction (QIS 24897 *DNA IQ Method of Extracting DNA from Reference and Casework Samples*, section 7.1) as a potential recovery procedure from the QIAsymphony® SP work table.

Batch 2 samples were lysed using the QIAGEN pre-lysis method and then the extraction was commenced using the QIAsymphony® SP module. The extraction protocol was intentionally stopped and cancelled after the addition of the QSL3, QSW2, magnetic particles, carrier RNA and the lysate to the sample prep cartridge. These sample prep cartridges were then removed from the worktable, contents transferred into 2mL tubes and the extraction process continued and completed as per QIS 24897*DNA IQ method of Extraction from Reference and Casework samples,* section 7.1, step 13 – the shaking for 5 minutes and then continuing the process using the magnetic stand.

Recovery Method Batch 3
Batch 3 tested the QIAamp® DNA Investigator® Kit recovery procedure from the *QIAsymphony® SP Recovery Procedure for DNA Investigator® Application* method[5] to recover samples from the QIAsymphony® SP work table.

Batch 3 samples were lysed using the QIAGEN pre-lysis method and then extraction commenced using the QIAsymphony® SP module. The extraction protocol was intentionally stopped and cancelled after the addition of the QSL3, QSW2, magnetic particles, carrier RNA and the lysate to the sample prep cartridge. These sample prep cartridges were then removed from the worktable, contents transferred into 2mL tubes and the extraction process continued and completed using the QIAGEN manual method using the QIAamp DNA Investigator Kit.

Recovery Method Batch 4
Batch 4 tested the effectiveness of the current manual DNA IQ™ method of extraction (QIS 24897 *DNA IQ Method of Extracting DNA from Reference and Casework Samples*, section 7.1) as a potential manual extraction method from the lysate stage. Batch 4 samples were lysed using the QIAGEN pre-lysis method and extraction completed as per QIS 24879 *DNA IQ™ Method of Extracting DNA from Reference and Casework samples*, section 7.1 - step 11– the addition of 550µL of Lysis Buffer-DTT, the addition of 50µL of the resin beads, shaking on the shaker for 5 minutes and then continuing the process using the magnetic stand.

Table 16 shows a summary of the experiments performed.

Table 16 – Sample recovery and extraction

Batch	Extraction Protocol used	Kit reagents to be used	Consumables/Instrument to be used
1	DNA IQ™ Extraction using the Maxwell® 16	Casework DNA IQ™ Pro Kit for Maxwell®16	Maxwell®16 instrument
2	DNA IQ™ method of extraction (QIS 24897 section 7.1)	DNA IQ™ Kit	Magnetic stand
3	QIAsymphony® SP Recovery Procedure for DNA Investigator® Applications'	QIAamp® DNA Investigator® Kit	MinElute columns included in QIAamp® DNA Investigator® Kit
4	DNA IQ™ method of extraction (QIS 24897 section 7.1)	DNA IQ™ Kit	Magnetic stand

Acceptance Criteria
The Recovery method 1 or 4 with the greatest DNA yields was accepted as the method for finalising the extraction of samples from the lysate stage.

The Recovery method 2 or 3 with the greatest DNA yields was accepted as the method for processing samples which have commenced extraction on the QIAsymphony® SP but have not been completed due to instrument malfunction or error.

6.13 Experiment 13: Re-extraction of Substrates

Background
Currently for quality failed or sub-optimal automated DNA IQ™ extraction batches, the samples are re-extracted from the stored substrates (spin baskets) using the Maxwell® 16 instrument. These re-extractions typically give very low DNA yields. Re-extraction of spin baskets using the Maxwell® 16 instrument was investigated for quality failed or sub-optimal QIAsymphony® SP extractions.

Re-extraction using the Maxwell® 16 instrument was selected instead of re-extraction using the QIAsymphony® SP for the following reasons:

- It is easier to create and process small batches using the Maxwell® 16 instrument
- If the quality fail or sub-optimal extraction is due to possible reagent or instrument issue on the QIAsymphony® SP, then the use of the Maxwell® 16 instrument eliminates these issues.
- Re-extraction with the Maxwell® 16 instrument is consistent with current practice.

Intent
This experiment was added to the validation after the project proposal was finalised. The standard procedure for failed or sub-optimal extraction batches is to re-extract the extracted substrates. This experiment was included to test the ability to re-extract substrates using the Maxwell®16 that have been previously extracted using the QIAsymphony®.

Experimental Design
One batch of samples was used for this experiment which consisted of:

- one positive extraction control
- one negative extraction control
- 10 blood swabs
- 10 tapelift cells

The batch of samples extracted with Protocol 3 in Experiment 1 (see Section 6.1) was initially used for this experiment. Following Experiment 1, these samples were stored at 4°C. The samples were then re-extracted using the Maxwell®16 (as per QIS 29344 *DNA IQ™ Extraction using the Maxwell®16*). All samples were quantified in duplicate as per method 5.4.

After the findings of Experiment 5 in which the pre-lysis protocol was modified, additional assessment was required to determine if the modified pre-lysis protocol would affect re-extraction. The samples extracted in Experiment 5 QIAsymphony® Test Batch 21 CQIALYS20160711_01, were re-extracted using the QIAsymphony® SP (using the protocol accepted in Experiment 2) to determine the impact of the pre-lysis protocol modifications on the ability to re-extract samples. All samples were quantified in duplicate as per method 5.4.

Assessment Criteria
The DNA yields obtained from the re-extracted samples were assessed to determine whether this re-work strategy is viable, based on whether the quantification results would be likely to produce a DNA profile.

7 RESULTS AND DISCUSSION

7.1 Experiment 1: Modification of Pre-lysis Protocol

Please note that the results of this experiment should be read in conjunction with the results of Experiment 5, which involved further modification and testing of the pre-lysis protocol. Although a pre-lysis protocol was accepted at the completion of Experiment 1, ultimately Protocol 4 which was developed and tested in Experiment 5 gave better DNA yields and was accepted as the final preferred pre-lysis protocol.

Sample sets of blood swabs and tapelifts were lysed using Protocols 1, 2 and 3 and extracted using the QIAsymphony® SP module with the Extraction CW500 Trial protocol. Table 17 below gives average quantification results for each protocol.

Table 17: Average DNA yield for blood swabs and tapelifts

Incubation Protocol	Average DNA Yields for Blood Swabs (ng/µL)	Average DNA Yields for Tapelift (ng/µL)
1	1.8418	0.2143
2	1.0621	0.3824
3	1.4266	0.4877

Figure 4 below provides a graphical representation of the average DNA yields for each pre-lysis protocol for blood swabs and tapelifts, including standard deviation.

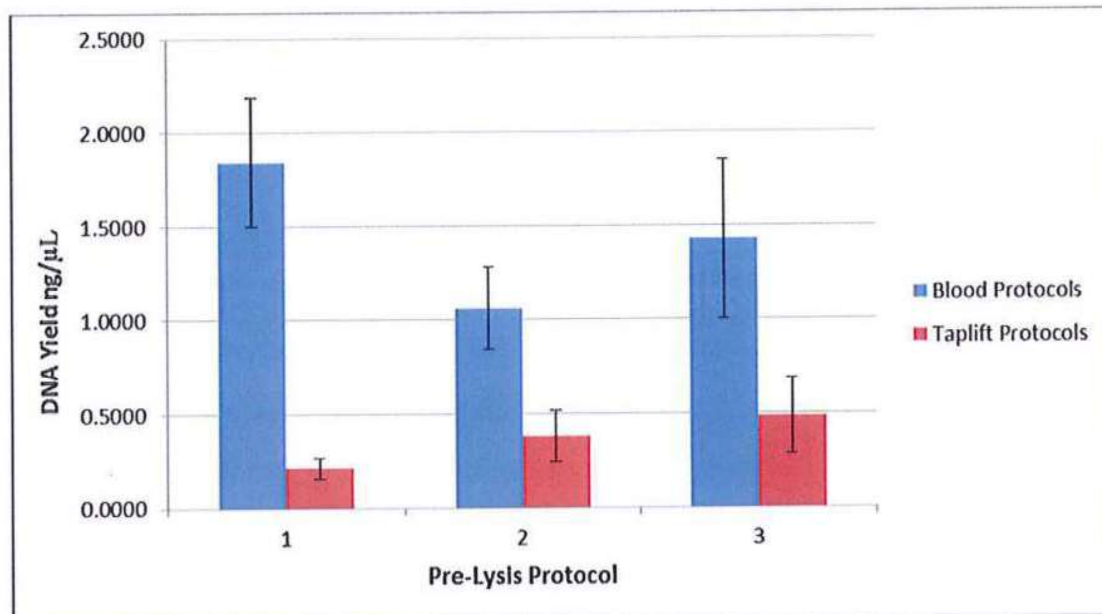

Figure 4: Average DNA yields with Y error bars showing ± one standard deviation for blood swabs and tapelifts

The comparison of DNA yields from the three pre-lysis protocols shows that Protocol 1 (QIAGEN's incubation method using the thermomixer to incubate samples at 56°C while shaking for 45 minutes at 900rpm) was found to achieve better DNA yields for blood swabs. This may be due to the continuous agitation of these samples for the entire incubation period which would assist in the release of cells contained on the swab into the suspension.

Protocol 3 (which used a vortex to manually mix each sample for 10 seconds before and after the incubation period) was found to achieve better DNA yields for tapelifts. This may be due to the vigorous vortex mixing resulting in more of the tapelift coming in contact with the buffer (when compared with shaking at 900rpm). It was noted during Protocols 1 and 2 (which use 900rpm and 1200 rpm respectively) that this level of agitation did not result in the entire tapelift being covered in buffer during incubation (i.e. not all areas of the tapelift were covered in buffer for equal times).

This meant the any cells at the top of the tapelift/tube are not exposed to the same amount of buffer as cells at the bottom of the tapelift/tube.

The results also showed that increasing the vigour of agitation increased DNA yields for tapelifts (i.e. Protocol 1 900rpm for 45mins, Protocol 2 1200rpm for 10 minutes and Protocol 3 (nil thermomixer agitation) vortex twice for 10 seconds). The increased agitation may have assisted in dislodging cells from the tapelift.

Blood swabs used for this verification were prepared by spotting blood onto the tip of the swab head. This means that when the swab heads were undergoing

pre-lysis 100% of the substrate was submerged in buffer for the duration of the lysis protocol regardless of the agitation speed. Protocol 1 gave the highest DNA yield for blood swabs, although based on the DNA quantification results, it would be reasonable to expect to achieve full DNA profiles for blood swabs extracted with any of the protocols.

Acceptance Criteria
Protocol 3 gave the highest yields for tapelifts, and comparable yields for blood swabs. As Protocol 3 gave the highest DNA yield for tapelifts (which are representative of routine crime scene samples and routinely have lower expected DNA yields than blood samples), Protocol 3 was accepted as the most effective protocol and implemented for the remainder of this project.

7.2 Experiment 2: Modification of Extraction Protocol

This experiment modified the QIAsymphony® Extraction CW500 Trial extraction protocol by increasing resin mixing and the addition of heating during the elution step.

The four batches processed with the four different protocols created by QIAGEN and then compared to each are shown in Table 18 below.

Table 18 – Modified Extraction Protocols

Test Batch	Protocol used and Batch ID	Modification made
1	Extraction CW500 Trial [redacted]	Custom protocol without modifications
2	Extraction CW500 trial IM [redacted]	Custom protocol with additional mixing of resin
3	Extraction CW500 trial HE [redacted]	Custom protocol with addition of heating at elution step
4	Extraction CW500 trial HEIM [redacted]	Custom protocol with addition of both modifications from Protocol 2 and 3

Protocol 4 was found to give the highest DNA yields for both blood swabs and tapelifts (see Table 19 below).

Table 19: Average DNA yield for blood swabs and tapelifts

Extraction Batch	Average DNA Yields for Blood Swabs (ng/µL)	Average DNA Yields for Tapelift (ng/µL)
1	1.5435*	0.1632
2	1.4449	0.2051
3	1.7567	0.2545
4	1.8552	0.2725

*one sample with a larger quantification value was excluded as an outlier

Figure 5 below displays the average DNA yield for each extraction protocol graphically, and includes standard deviation.

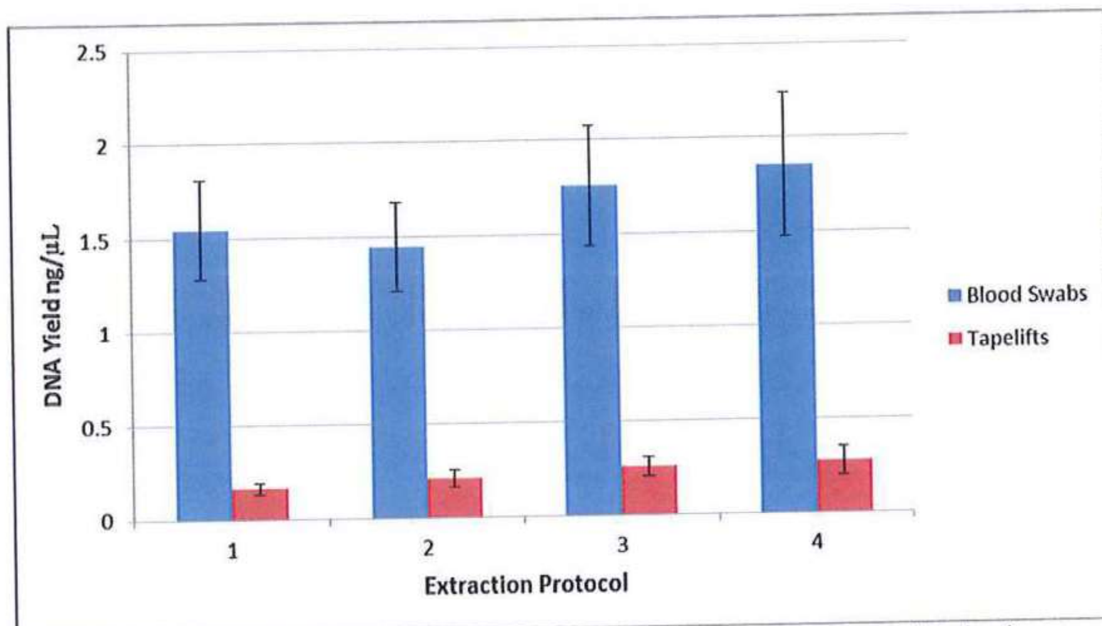

Figure 5: Average DNA yields with Y error bars showing ± one standard deviation for blood swabs and tapelifts

The additional mixing of the magnetic particles (resin beads) would ensure a homogenised resin solution before the addition of magnetic particles to the sample cartridge. This would ensure a consistent amount of magnetic particles would be added to each sample in the batch.

Heating during elution is used for the current DNA IQ™ extraction on the Maxwell® 16 (as per QIS 29344 *DNA IQ™ Extraction using the Maxwell® 16*) and has been shown in this experiment to be an effective means of increasing DNA yields.

The combination of additional mixing of magnetic bead particles and heating during elution gave the highest DNA yields for both blood swabs and tapelifts.

Acceptance Criteria
Protocol 4 gave the highest DNA yields for both sample types and was accepted as the pre-lysis method for the remainder of this validation.

7.3 Experiment 3: Lysate Storage

Lysate storage in the fridge (2°C -8°C) and freezer (-10°C – -20°C) were tested for intervals up to 4 days. Table 20 shows average results obtained for each time interval and storage condition tested.

Table 20 – Averages of DNA Yields, Degradation Index and allele calls

Storage Time	Storage Conditions	Average DNA Yields ng/µL	Average DI	Average Allele Calls*
0 hours	Room Temperature (~20 °C)	1.8475	0.8767	40
24 hours	Fridge storage (2°C – 8°C)	1.6476	0.8246	40
24 hours	Freezer storage (-10°C – -20°C)	1.9985	0.8468	40
48 hours	Fridge storage (2°C – 8°C)	1.8126	0.8216	40
48 hours	Freezer storage (-10°C – -20°C)	1.7014	0.8322	40
72 hours	Fridge storage (2°C – 8°C)	1.6258	0.7800	40
72 hours	Freezer storage (-10°C – -20°C)	2.0628	0.8928	40
96 hours	Fridge storage (2°C – 8°C)	1.6553	0.8585	40
96 hours	Freezer storage (-10°C – -20°C)	1.8978	0.8295	40

*excludes Amelogenin

Comparable DNA yields, degradation index (DI) results and average allele calls were observed between samples that were stored in the freezer and the fridge. The storage time results were assessed qualitatively and were not observed to be significantly different.

The average DNA yields for fridge and freezer storage at 96 hours in this experiment are within one standard deviation of the average DNA yields for batch 4 in Experiment 2.

Figure 6 below shows the peak height (RFU) for the D3S1358 locus (which is homozygous) obtained for samples stored in the fridge and freezer up to 96 hours.

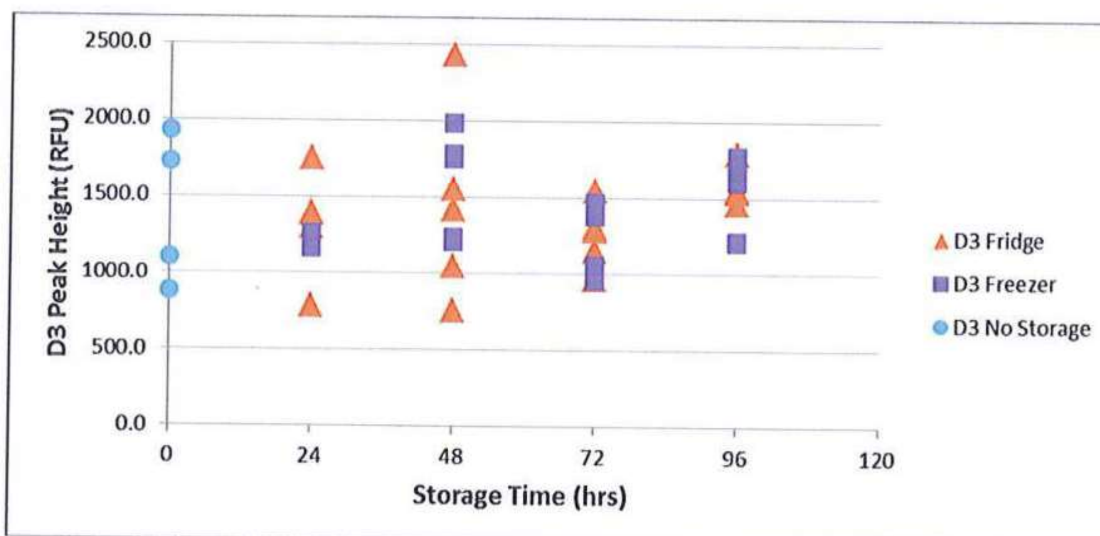

Figure 6 - D3S1358 peak heights for fridge and freezer storage

Figure 7 below shows the average peak height (RFU) for the FGA locus (which is heterozygous) obtained for samples stored in the fridge and freezer up to 96 hours.

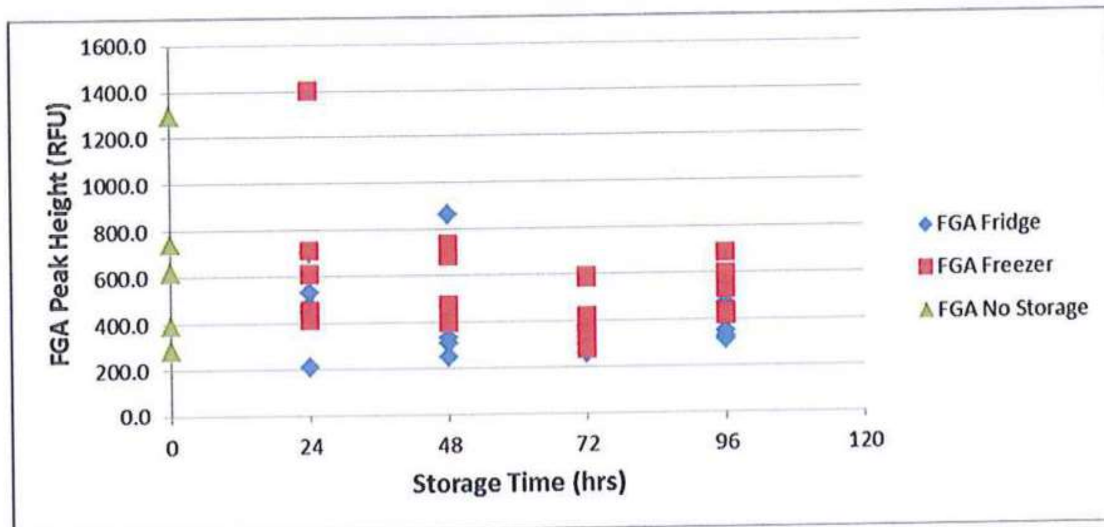

Figure 7 - FGA peak heights for fridge and freezer storage

Figures 6 and 7 above show that there was no significant reduction in peak heights for either D3S1358 or FGA over the time for which the lysates were stored. One small molecular weight locus (D3S1358) and one large molecular weight locus (FGA) were selected for analysis of DNA degradation. Fridge and freezer storage results were assessed qualitatively and were not observed to be significantly different.

Acceptance Criteria
Given that there was no observed significant difference between fridge and freezer storage (based on a qualitative assessment of peak height RFU, DNA yield and number of alleles obtained) both will be accepted.

Given that storage up to 96 hours did not show a detrimental impact on peak height RFU, DNA yield and number of alleles obtained, 96 hours will be accepted as the maximum lysate storage time.

7.4 Experiment 4: Sensitivity

For both QIAsymphony® SP and Maxwell®16 extractions, Donor 14 cell suspension was used. As per the experimental design (Section 6.4) Donor 14 cell suspension stock concentration was calculated to be 0.06826 ng/µL.

For both the QIAsymphony® SP and the Maxwell®16 samples, it was assumed that the total DNA extract volume after extraction was exactly 90 µL. This assumption was used in the calculation of the total DNA yields (ng) for each sample. The total DNA yields were calculated using the following formula:

DNA yield (ng) = Average DNA quantification result (ng/µL) x DNA extract volume (90 µL)

Table 21 and Figure 8 detail the sensitivity results for the samples extracted using the QIAsymphony® SP and the Maxwell®16.

Table 21 – QIAsymphony® and Maxwell®16 Sensitivity Results

DNA Input	Maxwell			QIAsymphony®		
	Average Quant (ng/µL)	Total Yield (ng) 90uL final volume	Extraction Efficiency %	Average Quant (ng/µL)	Total Yield (ng) 90uL final volume	Extraction Efficiency %
1µL of cells (0.06826ng)	0.0005	0.045	65.92	0.00145	0.1305	191.19
2µL of cells (0.13651ng)	0.000925	0.08325	60.99	0.001275	0.11475	84.05
5µL of cells (0.34128ng)	0.003275	0.29475	86.37	0.00275	0.2475	72.52
10µL of cells (0.68256ng)	0.00255	0.2295	33.62	0.007525	0.67725	99.22
20µL of cells (1.36512ng)	0.0051	0.459	33.62	0.009375	0.84375	61.81
30µL of cells (2.04768ng)	0.0062	0.558	27.25	0.0105	0.945	46.15
40µL of cells (2.73024ng)	0.00955	0.8595	31.48	0.014525	1.30725	47.88

- Higher average quantification result

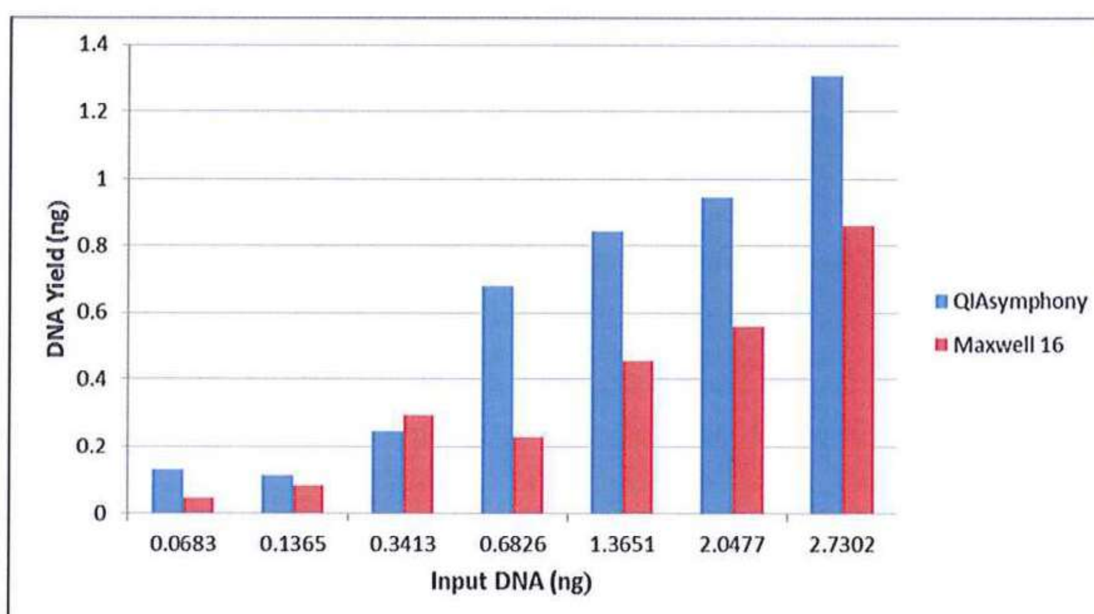

Figure 8 – QIAsymphony® and Maxwell®16 Sensitivity Results

As this experiment is testing the lower sensitivity range of both the QIAsymphony® SP and Maxwell®16 some run to run variation is expected from sample preparation, extraction and quantification.

The average DNA yields and extraction efficiency for all QIAsymphony® SP samples excluding for the 5 µL samples were higher than the corresponding samples processed on the Maxwell®16. The average for 5 µL Maxwell®16 sample was marginally higher than that obtained from QIAsymphony® SP.

The extraction efficiency was observed to increase for both the QIAsymphony® SP and Maxwell®16 as the amount of input DNA decreased, which is advantageous in forensic testing where often only small amounts of DNA are available for testing.

Acceptance Criteria
The QIAsymphony® SP was accepted because overall it gave higher DNA yields and better extraction efficiency results compared to the Maxwell®16 for the set of samples tested in this experiment.

7.5 Experiment 5: Verification of Additional Substrates

Table 22 below contains the DNA yield and allele call results for the additional substrates tested in this experiment for both the QIAsymphony® SP and Maxwell®16.

Table 22 –Average DNA yields and allele calls of various substrates

Substrate types	QIAsymphony		Maxwell	
	Average DNA yield (ng/µL)	Average Allele calls	Average DNA yield (ng/µL)	Average Allele calls
Fabric Blood	1.2750	40	0.0017	3.4
FTA Buccal Cells	2.5573	40	2.5999	40
Cigarette Butts	1.5027	37.2	2.2462	33.6
Chewing Gum	0.2118	39.4	0.6637	37.4
Tissue	24.5726	40	21.8417	40
Female Hair	1.5641	40	1.5906	39.8
Male Hair	5.6899	40	3.9415	40
Fingernails	0.6680	40	2.5281	39
Tapelifts	0.2725	40	0.4280	37.6

Figure 9 below is a graphical representation of the DNA yield data with error bars showing one standard deviation for all substrates excluding tissue. (N.B. tapelift results are initial results prior to pre-lysis protocol modification).

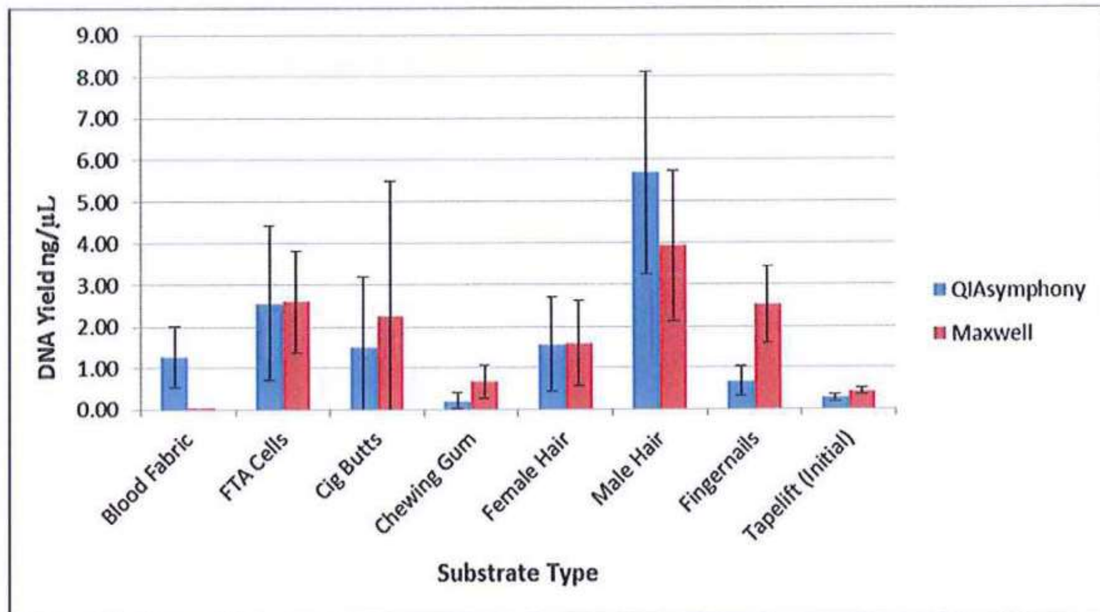

Figure 9: Average DNA yields with Y error bars showing ± one standard deviation for additional substrates

Figure 10 below is a graphical representation of the DNA yield data with error bars showing one standard deviation for tissue.

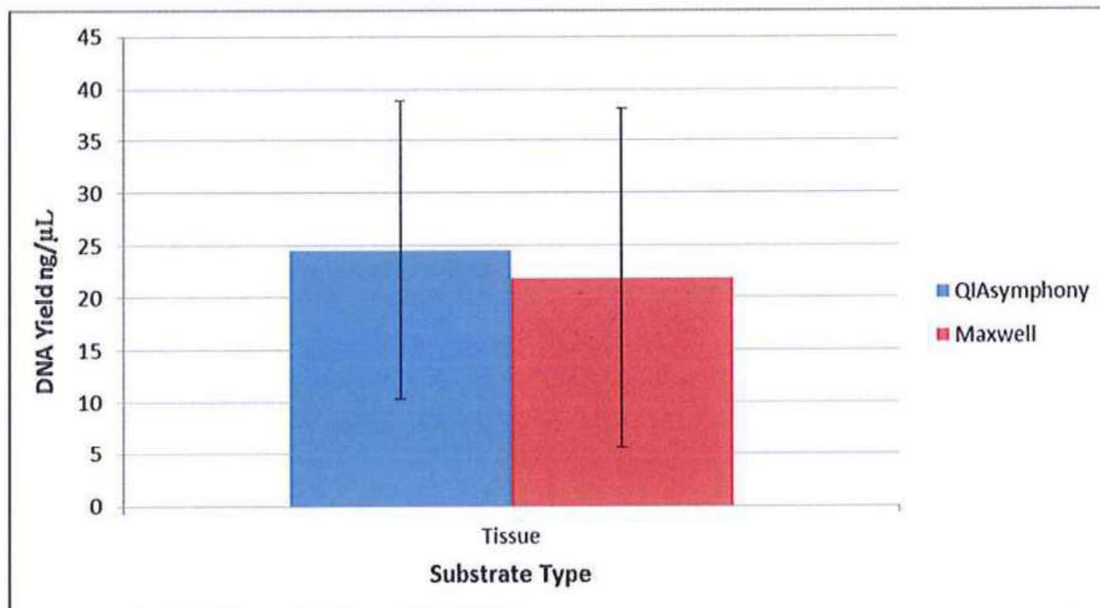

Figure 10: Average DNA yields with Y error bars showing ± one standard deviation tissue samples

Fabric Blood

The Maxwell®16 quantification results for Fabric Blood were significantly lower than those obtained from the QIAsymphony®. The average DNA yield for fabric blood extracted using the QIAsymphony® instrument was 1.2750 ng/µL, with each sample resulting in a full DNA profile. The average DNA yield for fabric blood extracted using the Maxwell®16 instrument was 0.0017 ng/µL which resulted in an average allele call of 3.4. This Maxwell®16 extraction was

completed with no performance issues noted and the positive extraction control yielded optimal results. A possible explanation for this result may be due to the presence of an inhibitor which may have been introduced into the fabric during the sterilisation method used. The fabric used for this validation was the remaining fabric from *Project # 109- Verification of two additional Maxwell 16 MDx Instruments (C and D).*

<u>FTA Buccal Cells</u>
The quantification and allele call results for the Maxwell®16 and QIAsymphony® were comparable as there was 1.6% difference in quantification results and all samples gave full DNA profiles.

<u>Cigarette Butts</u>
For cigarette butts, two of the five QIAsymphony® replicates gave full DNA profiles and the remaining replicates gave 32, 36 and 38 allele calls. For the Maxwell®16 only one of the five cigarette butts gave a full DNA profile and the remaining replicates gave 28, 29, 35 and 36 allele calls. The average quantification results for the Maxwell®16 were higher than those for the QIAsymphony®. The performance of both instruments was comparable, given the DNA profiling results.

<u>Chewing Gum</u>
For chewing gum, two of the five QIAsymphony® replicates gave full DNA profiles and each of the remaining three replicates gave 39 allele calls. For the Maxwell®16, two of the five replicates gave full DNA profiles and the remaining replicates gave 33, 35 and 39 allele calls. Average quantification results for the chewing gum samples processed on the QIAsymphony® were lower than for the Maxwell®16. Even so, the QIAsymphony® gave more allele calls when compared to the Maxwell®16. Chewing gum samples are expected to give variable DNA yield results given the lack of ability to control sample input. Based on allele calls, the performance of both instruments was comparable.

<u>Tissue</u>
Quantification and allele call results for both instruments was comparable (the QIAsymphony® gave a 12.5% higher DNA yield than the Maxwell®16). Both instruments gave full DNA profiles for all replicates.

<u>Male and Female Hair</u>
Quantification and allele call results for both instruments were comparable. DNA yields for female hair were 1.7% difference, whereas for male hair there was a 44.4% difference with the QIAsymphony® giving the greater DNA yield. For male hair, both instruments gave full DNA profiles for all replicates. For female hair, the QIAsymphony® gave full DNA profiles for all replicates, whereas the Maxwell®16 gave full DNA profiles for four of the five replicates (the fifth replicate gave 39 alleles).

<u>Fingernails</u>
A difference in DNA yields was observed for fingernail samples. The QIAsymphony® gave an average DNA yield of 0.6680 ng/µL, which was approximately 75% lower than the yields obtained for the Maxwell®16 (2.5281 ng/µL). This may be due to the difficulty in controlling the amount of DNA input

for this substrate type, resulting in sample to sample variation. The QIAsymphony® gave full DNA profiles for all five replicates. The Maxwell®16 gave full DNA profiles for three of the five replicates (the remaining two replicates gave 37 and 38 alleles). Although the quantification results for the QIAsymphony® were lower than for the Maxwell®16, the DNA profile allele designations were comparable. Amplification and quantification variation could account for not achieving full DNA profiles where quantification results are above 2 ng/µL.

One fingernail sample extracted using the QIAsymphony® instrument resulted in a mixture, with 6 extra peaks (above LOR). A mixture is not unexpected for this substrate type and could be due to a number of reasons. The collection method (see section 5.18) was performed in the donor's home and not in sterile laboratory conditions. The donor's personal nail clippers were used and it is possible that the clippers were not adequately decontaminated / sterilised. Also it is not possible to exclude an additional source of DNA present on the fingernails at the time of collection.

<u>Tapelifts</u>
The results for tapelifts on the QIAsymphony® were observed to be approximately 25% lower than the results for the Maxwell®16 (0.2725 ng/µL and 0.4280 ng/µL respectively). All tapelift samples extracted using the QIAsymphony® obtained full profiles. While those extracted using the Maxwell®16 obtained a full profile seven out of ten times.

The original tapelift samples were collected by spotting cell suspension onto fabric and then collecting, then tapelifting the fabric. Given that this introduces the potential for sample to sample variation all subsequent tapelifts were prepared by spotting cell suspension directly onto the tapelift.

Further testing was conducted to increase the DNA yields obtained for tapelift samples on the QIAsymphony®. The extra testing was performed as tapelifts are one of the most common substrate types received at Forensic DNA Analysis and thus it is critical that the best achievable results are obtained.

A modification to the pre-lysis protocol was tested, see Protocol 4 (Section 6.1 Experiment 1). For Protocol 4, the main incubation step was conducted on the ThermoMixer, shaking at 1400rpm at 70 °C. Table 23 below contains the results of the Protocol 4 testing.

Table 23 – Pre-lysis Protocol 4 results

	Sample Type	QIAsymphony®		Maxwell®16	
		DNA yield (ng/µL)	Allele count	DNA yield (ng/µL)	Allele count
	Pos Control	1.3517	(X,X) + 40	1.6491	(X,X) + 40
	Neg Control	Undet	NSD	Undet	NSD
1	Tapelift - 15µL cells	0.8549	(X,X) + 40	0.821	(X,X) + 40
2	Tapelift - 15µL cells	0.6705	(X,X) + 40	0.7159	(X,X) + 40
3	Tapelift - 15µL cells	0.8201	(X,X) + 40	0.8071	(X,X) + 40
4	Tapelift - 15µL cells	0.8963	(X,X) + 40	0.7834	(X,X) + 40
5	Tapelift - 15µL cells	0.8836	(X,X) + 40	0.8001	(X,X) + 40
6	Tapelift - 15µL cells	0.8377	(X,X) + 40	0.6569	(X,X) + 40

Figure 11 below is a graphical representation of the results of the Protocol 4 pre-lysis experiment with error bars showing one standard deviation.

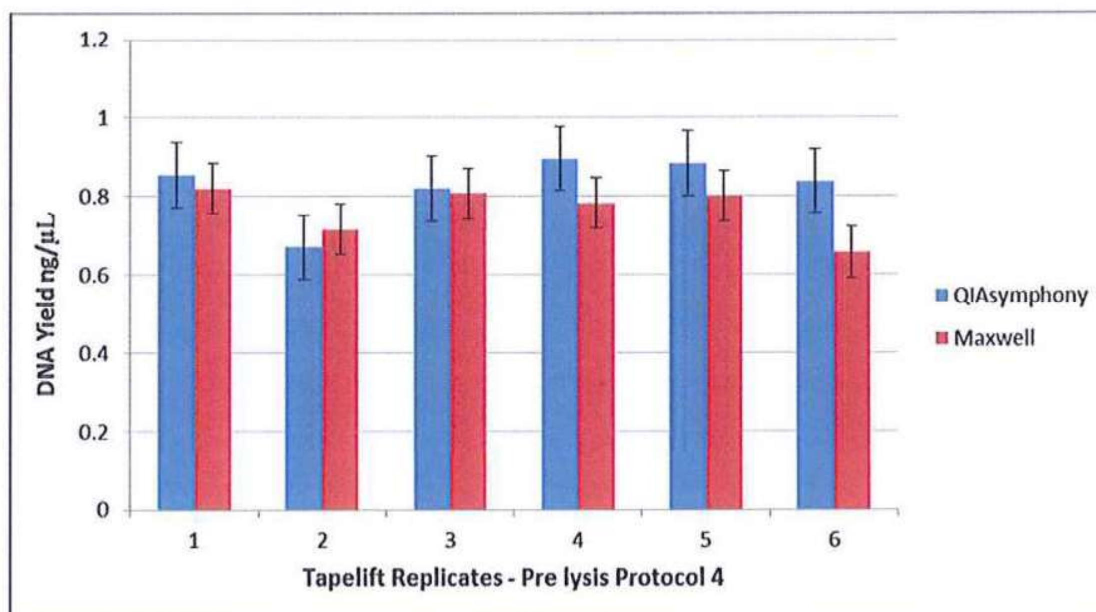

Figure 11: Average DNA yields with Y error bars showing ± one standard deviation for tapelifts (Protocol 4 pre lysis)

The results of Protocol 4 testing showed that this pre-lysis protocol increased the overall DNA yields for tapelifts processing on the QIAsymphony® to comparable levels with the Maxwell®16 (on average, the QIAsymphony® gave 7.6% higher DNA yields than the Maxwell®16). Increased mechanical agitation during the pre-lysis may have caused more cells to become dislodged from the tapelift. Also the increased agitation may cause more of the tapelift surface to be covered in the extraction buffer for more time during the incubation.

The temperature increase in combination with agitation on the ThermoMixer appears to have had a positive impact on the DNA yield. The 70 °C temperature was selected for testing as this is the temperature used in the lysis protocol for the Casework DNA IQ™ Pro Kit for Maxwell®16 as per QIS 29344 *DNA IQ™ Extraction using the Maxwell®16.*

Although Protocol 4 was only tested on tapelifts in this experiment, it is expected that it would give increased DNA yields for other substrate/sample types where differences between the QIAsymphony® and Maxwell®16 were observed based on the sensitivity results in Experiment 4.

Acceptance Criteria
For Tapelifts, Protocol 4 was accepted as the pre-lysis protocol which gave the best results (DNA yield and allele calls) for the QIAsymphony®.

The QIAsymphony® gave full DNA profiles for all Fabric Blood, FTA Buccal Cells, Tissue, Female Hair, Male Hair, Fingernails and Tapelift samples. The QIAsymphony® did not give full DNA profiles for all Cigarette Butts or Chewing Gum samples (37.2 and 39.4 average allele calls respectively). The Maxwell®16 gave full DNA profiles for only FTA Buccal Cells, Tissue and Male Hair substrate types.
Overall the results of this experiment were assessed qualitatively and there was no observed significant difference in performance between the two instruments and therefore the QIAsymphony® passed this experiment.

It is recommended that Protocol 4 be implemented as the preferred pre-lysis protocol for all sample types as it is expected to increase DNA yields for all substrate/sample types.

7.6 Experiment 6: Inhibition

Table 24 below contains the quantification and allele call results for each inhibitor and difficult substrate tested in this experiment.

Table 24 - Quantification results table of inhibitor samples.

Sample	IPC Ct Flag	IPC Ct Value (Mean)	Quant Value - SAT (Mean) ng/µL	# Alleles
NIL	No	27.135	1.500125	(X,Y) + 40
Hematin-50µM	No	27.425	0.262325	(X,Y) + 40
Hematin-75µM	No	27.165	0.2557	(X,Y) + 40
Hematin-100µM	No	27.29	0.1528	(X,Y) + 40
Hematin-125µM	No	27.165	0.296275	(X,Y) + 40
Hematin-150µM	No	27.245	0.10335	(X,Y) + 40
Humic Acid-1%	No	27.13	0.927	(X,Y) + 40
Humic Acid-2%	No	27.145	0.643375	(X,Y) + 40
Humic Acid-5%	No	27.32	0.49525	(X,Y) + 40
Humic Acid-10%	No	27.18	0.7894	(X,Y) + 40
Humic Acid-15%	No	27.095	0.33575	(X,Y) + 40
Humic Acid-20%	No	27.055	1.288	(X,Y) + 40
Tannic Acid-0.2M	No	27.425	0.2429	BB, PU, PP
Tannic Acid-0.8M	No	27.18	0.059075	BB, PU, PP
Tannic Acid-1.2M	No	27.415	0.275125	BB, PU, PP
Tannic Acid-1.6M	No	27.085	0.0741	BB, PU, PP
Tannic Acid-2.0M	No	27.305	0.29525	BB, PU, PP
Indigo Carmine–10mM	No	27.22	0.849	(X,Y) + 40
Indigo Carmine–20mM	No	27.155	0.867325	(X,Y) + 40
Indigo Carmine–40mM	No	26.99	0.53555	(X,Y) + 40
Indigo Carmine–60mM	No	27.06	0.97785	(X,Y) + 40
Indigo Carmine–80mM	No	27.27	0.287975	(X,Y) + 40
Indigo Carmine–100mM	No	26.88	0.87265	(X,Y) + 40
Sanitary Pads	N/A	N/A	N/A	N/A
Disposable Nappy	N/A	N/A	N/A	N/A
Powdered Gloves -1	No	27.81	1.5153	(X,Y) + 34
Powdered Gloves -2	No	27.63	1.6757	(X,Y) + 40
Non-powdered Gloves -1	No	27.52	1.7045	(X,Y) + 40
Non-powdered Gloves -2	No	27.35	1.4579	(X,Y) + 40
Nitrile Gloves - 1	No	27.38	2.009	(X,Y) + 31
Nitrile Gloves - 2	No	27.36	1.5665	(X,Y) + 40

(BB – bad baseline, PU – pull up, PP – partial profile)

The in-house validation of the manual method for DNA IQ™ extraction [3] included testing how effectively the protocol removed PCR inhibitors found in forensic samples. The validation showed that the DNA IQ™ system effectively removed motor oil, Humic acid, urea and indigo dye, but did not effectively remove Tannic Acid [3].

The results obtained from this experiment, demonstrated that the QIAsymphony® DNA Investigator® Kit and QIAsymphony® SP Module can effectively remove all inhibitor types used in this experiment possibly excluding

Tannic Acid. None of the samples in this experiment gave an IPCCt flag through the Quanitfiler® Trio would normally identify samples with inhibition. All samples resulted in expected quantification values and IPCCt values which were within the acceptable range of two units above the baseline (average of the ct values of the standards).

All inhibition samples for this experiment were progressed through to capillary electrophoresis and profile interpretation, and were found to have resulted in full DNA profiles with the exception of those containing the Tannic Acid. These profiles could not be adequately interpreted and were deemed as unacceptable due to the profile morphology containing excessive amounts of bad baseline and pull-up.

The results of the Tannic Acid experiments are inconclusive. The DNA profiles for these samples were partial and contained poor peak morphology and bad baseline, meaning that these DNA profiles could not be interpreted. The SAT quantification results indicate that there is extracted DNA present in the sample. The IPCCt results do not indicate the presence of a PCR inhibitor. However the capillary electrophoresis results (bad baseline, partial profile) may indicate that the Tannic Acid has inhibited DNA amplification with PowerPlex®21. Given that the Tannic Acid samples did not give full DNA profiles, it can only be assumed that the DNA extraction did not effectively remove the inhibitor.

It was also found that substrates (e.g. sanitary pads, nappies) that contained hydrogels (which were found to be problematic for the extraction Multiprobe® II), also became gelatinous / viscous during the QIAGEN lysate process (highlighted in red). An attempt to load these samples onto the QIAsymphony® SP Module for extracting, found that the instrument was able to detect the viscosity of the lysate by attempting to aspirate, but skipped the sample, leaving the lysate in its sample tube. The QIAsymphony instrument provides a 'clot detection function', which allows detection of clots and/or viscous and gelatinous samples aspirated during the sample transfer. After sample aspiration, the system checks if clots are located at the tip forefront. If a viscous sample is detected, the system dispenses the aspirated sample volume back into the sample tube and retries sample aspiration. This retry strategy is repeated up to three times. If the sample is still found to be too gelatinous / viscous, the sample will be ignored and flagged as invalid.

Although the instrument could not process these gelatinous samples, it showed that this function would allow the detection of such samples, and allowing the instrument skip these samples and continue the extraction process of all other lysates on the run without pausing or causing pipetting problems or contamination of the pipetting lines as experienced in the past with the Multiprobe® II. These samples would then be salvaged and extracted using the manual method of extraction.

For the glove samples (powdered, non-powdered and nitrile) the QIAsymphony® did not have any difficulty when aspirating or dispensing. All samples gave full DNA profiles except for one replicate each of the powdered and nitrile gloves (34 and 31 alleles respectively). Given that this test was

primarily a difficult substrate test, these DNA profile results were not interpreted further.

Acceptance Criteria

Inhibitors

The QIAsymphony® SP module along with the DNA Investigator® Kit was accepted as it successfully removed all DNA inhibitors, with the exception of Tannic Acid. The inability for the DNA Investigator® Kit to remove Tannic Acid is not grounds for failure of this experiment as the manual method for DNA IQ™ extraction was also shown to be incapable of removing Tannic Acid[3].

Difficult Substrates

The QIAsymphony® SP module, along with the DNA Investigator® Kit, was accepted. Although the instrument could not process the sanitary pads and disposable nappy samples, they were detected and skipped them without producing an error and/or stopping the batch.

7.7 Experiment 7: Degradation

Table 25 below contains the results of the degradation testing.

Table 25: Results table of degradation samples.

UV exposure time	Average Quant DNA yield	Degradation Index (DI)	IPCCT value	Average Allele Calls
Duplicate 1 – Nil mins	0.0319	4.5476	27.19	(X,X) + 40
Duplicate 2 – Nil mins	0.0497	3.9981	27.45	(X,X) + 40
Duplicate 1 - 15 mins	0.0121	32.5932	27.2	(X,NR) + 8
Duplicate 2 - 15 mins	0.0118	42.3752	27.22	(X,NR) + 5
Duplicate 1 - 30 mins	0.0118	15.3342	26.88	(X,X) + 7
Duplicate 2 - 30 mins	0.0166	39.6403	27.02	(X,NR) + 5
Duplicate 1 - 45 mins	0.0064	35.2531	27.28	(X,NR) + 6
Duplicate 2 - 45 mins	0.0076	31.2277	27.38	(X,NR) + 3
Duplicate 1 - 1 hour	0.0051	52.6524	27.19	(NR,NR) + 3
Duplicate 2 - 1 hour	0.0080	25.2671	27.37	NIL
Duplicate 1 - 2 hours	0.0016	No DI value	27.15	NIL
Duplicate 2 - 2 hours	0.0021	No DI value	27.51	(NR,NR) + 1
Duplicate 1 - 4 hours	0.0014	No DI value	26.89	NIL
Duplicate 2 - 4 hours	0.0016	6.1564	26.72	NIL
Duplicate 1 - 6 hours	0.0004	No DI value	27.22	NIL
Duplicate 2 - 6 hours	0.0008	No DI value	27.2	NIL
Duplicate 1 - 8 hours	0.0008	No DI value	27.25	NIL
Duplicate 2 - 8 hours	0.0008	9.3318	27.22	NIL
Duplicate 1 - 10 hours	0.0005	No DI value	27.15	NIL
Duplicate 2 - 10 hours	0.0002	No DI value	26.78	NIL
Duplicate 1 - 15 hours	0.0004	No DI value	27	NIL
Duplicate 2 - 15 hours	0.0005	No DI value	26.86	NIL
Duplicate 1 - 20 hours	0.0001	1.9575	27.16	NIL
Duplicate 2 - 20 hours	0.0001	No DI value	27.23	NIL
Duplicate 1 - 24 hours	0.0000	No DI value	27.39	NIL
Duplicate 2 - 24 hours	0.0003	No DI value	26.95	NIL

Fabric inoculated with cells used as the control sample (no exposure to UV) resulted in good quantification values resulting in full DNA profiles (X,X + 40). A low Degradation Index (DI) showed that there was minimal degradation.

Results showed that even after the first time interval of UV Exposure (at 15 minutes) there was a significant amount of degradation. Time intervals between 15 minutes – 45 minutes saw a substantial increase in the DI with a continuous decline in DNA yield and number of allele calls.

Thirteen of the sixteen samples with UV exposure of greater than 1 hour recorded no DI value through the quantification report and no allele calls (excluding duplicate 2 at 2 hours). Overall, samples that were exposed to even minimal UV exposure, showed that they had been degraded.

Assessment Criteria
This experiment showed that with increasing levels of UV exposure, the quantification results and allele calls declined as expected. Generally, the DI also increased as the UV exposure increased. This experiment was performed to assist case managers in the use of the DI for reworking of degraded samples, and was not used as a means of accepting/rejecting the QIAsymphony® SP.

As written the intent of the experiment was to see the effectiveness of QIAsymphony in retrieving degraded (small fragment DNA). However due to the difficulties associated with artificially degrading DNA we used UV degradation and looked at profile outcomes. UV has been effectively replicated DNA degradation with corresponding reduction in allele numbers - however it does not effectively test the ability of QIAsymphony to retrieve (with efficiency) small DNA fragments. Given the resources and technology currently available the results of this experiment are an indication only and are not conclusive evidence of the performance of the QIAsymphony® instrument to extract degraded DNA.

7.8 Experiment 8: Pipetting Accuracy

The purpose of this experiment was to test the pipetting accuracy for the AS module which will be used to prepare quantification assay plates for samples that have been extracted using the SP Module. The Artel instrument and software, which is used to test pipetting accuracy, was used in this experiment according to QIS 26628 *Verifications using the Artel MVS*.

During the validation QIAGEN made some minor modifications to the AS module's pipetting parameters to accommodate for the difference in the viscosity between the Artel reagents and in-use quantification master mix reagents.

The following results were obtained for the 2µL verification:

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.870	1.946	2.057	1.896	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
B	1.805	2.044	1.918	1.874	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
C	1.955	2.064	1.867	1.742	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
D	1.829	2.027	1.995	1.764	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
E	1.833	1.770	1.827	2.009	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
F	1.831	1.896	1.840	1.828	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
G	1.878	2.037	1.850	2.020	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
H	1.852	1.877	1.862	1.891	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank

Figure 11 - 2µL verification results

Acceptance criteria for DNA Analysis POVAs (Piston Operated Volumetric Apparatus) is a %CV and %inaccuracy of +/- 5% (10% for volumes <10µL).

Results for the 2µL verification for the QIAsymphony® AS module were the following:

- %CV = 4.78%
- %inaccuracy = - 5.07%

The following results were obtained for the 18µL verification:

	1	2	3	4	5	6	7	8	9	10	11	12
A	18.24	18.69	18.60	18.73	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
B	18.76	18.76	18.70	18.78	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
C	18.56	18.70	18.71	18.76	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
D	18.69	18.71	18.82	18.76	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
E	18.68	18.68	18.52	18.74	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
F	18.72	18.80	18.64	18.86	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
G	18.76	18.86	18.79	18.74	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
H	18.80	18.87	18.88	18.79	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank

Figure 12 - 18µL verification results

Acceptance criteria for DNA Analysis POVAs (Piston Operated Volumetric Apparatus) is a %CV and %inaccuracy of +/- 5% (10% for volumes <10µL).

Results for the 18µL verification for the QIAsymphony® AS module were the following:

- %CV = 0.65%
- %inaccuracy = 4.01%

Acceptance Criteria
The QIAsymphony® AS module was accepted as it passed the acceptance criteria for POVAs at both 2µL and 18µL.

7.9 Experiment 9: Contamination Check

The aim of this experiment was to check the QIAsymphony® instrument for cross-contamination during the extraction protocol using the SP module and for quantification setup using the AS module. Samples were first extracted on the SP module, which were tested for contamination through quantification using the Multiprobe® II for quantification assay setup. Once this test has passed, the samples extracted on the SP module were used to prepare a quantification assay using the AS module.

Part 1 – Contamination Check of SP module
Samples for the Contamination Check of SP module were processed on the quantification batches [redacted] All Positive Control samples have full expected DNA profiles. All blanks (excluding [redacted] gave undetermined quantification results and NSD profiles. 663643113 gave a quantification result of 0.0168 ng/µL, but gave a NSD profile. The quantification result for 663643113 may be explained by well contamination in the plate or contamination in this well position of the 7500 block. The amplification plot for batch [redacted] for this sample showed that there may have been a small bubble present in this well position. Even though a quantification result was obtained for this sample, the NSD profile indicates that no sample to sample contamination occurred. (N.B. this sample was included on [redacted] in the AS Module experiment and gave an undetermined quantification result).

Sample [redacted] gave a single peak at D1S1656 [8] with a peak height of 33RFU. None of the twelve positive control donors used in this experiment have a D1S1656 [8] allele. In a routine batch being processed in the Analytical laboratory, negative extraction controls containing less than three peaks in the profile would be dismissed as drop-in peaks according to the routine procedure in the acceptance criteria as per QIS 17130 *Capillary Electrophoresis Quality (CEQ) Check*. Given that the initial 'undetermined' result at the quantification stage, and that the observed peak is not consistent with any of the twelve positive control donors on this batch, this peak was considered to be a drop-in peak.

Overall, the results showed that there was no cross-contamination seen on the extraction performed using the SP Module.

The results for this contamination check of the SP module can be found in Tables 26-29.

Table 26 – SP Module Contamination Check Results

	Lysate Batch 1 VALIQLYS20160210_01		Quant #1 VALQUA20160212_02	Allele Calls VALGM20160304_01
Position #	Sample Type	Sample Number	Quant value	
1	Blank		undetermined	OK - NSD
2	Blank		undetermined	OK - NSD
3	Blank		undetermined	OK - NSD
4	Blank		undetermined	OK - NSD
5	Blank		undetermined	OK - NSD
6	Blank		undetermined	OK - NSD
7	Blank		undetermined	OK - NSD
8	Blank		undetermined	OK - NSD
9	Blank		undetermined	OK - NSD
10	Pos Ctl (Donor 1)		1.0189	OK – Full expected Profile Amel + 40
11	Blank		undetermined	OK - NSD
12	Blank		undetermined	OK - NSD
13	Pos Ctl (Donor 2)		2.4923	OK – Full expected Profile Amel + 40
14	Blank		undetermined	OK - NSD
15	Blank		undetermined	OK - NSD
16	Pos Ctl (Donor 3)		11.2441	OK – Full expected Profile Amel + 40
17	Blank		undetermined	OK - NSD (ART removed)
18	Blank		undetermined	OK - NSD
19	Blank		undetermined	OK - NSD
20	Blank		undetermined	OK - NSD
21	Blank		undetermined	OK - NSD
22	Blank		undetermined	OK - NSD
23	Blank		undetermined	OK - NSD
24	Blank		undetermined	OK - NSD

Table 27 – SP Module Contamination Check Results

VALIQLYS20160210_01 Lysate Batch 2			Quant #1 VALQUA20160212_02	Allele Calls VALGM20160304_01
Position #	Sample Type	Sample Number	Quant value	
1	Blank		undetermined	OK - NSD
2	Blank		undetermined	OK - NSD
3	Blank		undetermined	OK - NSD
4	Blank		undetermined	OK - NSD (spike removed)
5	Blank		undetermined	OK - NSD
6	Blank		undetermined	OK - NSD
7	Blank		undetermined	OK - NSD
8	Blank		undetermined	OK - NSD
9	Pos Ctl (DONOR 4)		2.1049	OK – Full expected Profile Amel + 40
10	Blank		undetermined	OK - NSD
11	Blank		undetermined	OK - NSD
12	Pos Ctl (DONOR 5)		3.967	OK – Full expected Profile Amel + 40
13	Blank		undetermined	OK - NSD
14	Blank		undetermined	OK - NSD
15	Pos Ctl (DONOR 6)		4.8132	OK – Full expected Profile Amel + 40
16	Blank		undetermined	OK - NSD
17	Blank		undetermined	OK - NSD
18	Blank		undetermined	OK - NSD
19	Blank		undetermined	OK - NSD
20	Blank		undetermined	PK @D1[8]
21	Blank		undetermined	OK - NSD
22	Blank		undetermined	OK - NSD
23	Blank		undetermined	OK - NSD
24	Blank		undetermined	OK - NSD

Table 28 – SP Module Contamination Check Results

	VALIQLYS20160210_03 Lysate Batch 3		Quant #1 VALQUA20160212_02	Allele Calls VALGM20160304_01
Position #	Sample Type	Sample Number	Quant value	
1	Blank		undetermined	OK - NSD
2	Blank		undetermined	OK - NSD
3	Blank		undetermined	OK - NSD
4	Blank		undetermined	OK - NSD
5	Blank		undetermined	OK - NSD
6	Blank		undetermined	OK - NSD
7	Blank		undetermined	OK - NSD
8	Blank		undetermined	OK - NSD
9	Blank		undetermined	OK - NSD
10	Pos Ctl (DONOR 7)		6.4483	OK - Full expected Profile Amel + 40
11	Blank		undetermined	OK - NSD
12	Blank		undetermined	OK - NSD
13	Pos Ctl (DONOR 8)		6.816	OK - Full expected Profile Amel + 40
14	Blank		undetermined	OK- NSD
15	Blank		undetermined	OK - NSD
16	Pos Ctl (DONOR 9)		3.9545	OK - Full expected Profile Amel + 40
17	Blank		undetermined	OK - NSD
18	Blank		undetermined	OK - NSD
19	Blank		undetermined	OK - NSD
20	Blank		undetermined	OK - NSD
21	Blank		undetermined	OK - NSD
22	Blank		undetermined	OK - NSD
23	Blank		undetermined	OK - NSD
24	Blank		0.0168	OK - NSD

Table 29 – SP Module Contamination Check Results

	VALIQLYS20160210_04 Lysate Batch 4		Quant #1 VALQUA20160212_02	Allele Calls VALGM20160304_01
Position #	Sample Type	Sample Number	Quant value	
1	Blank		undetermined	OK - NSD
2	Blank		undetermined	OK - NSD
3	Blank		undetermined	OK - NSD
4	Blank		undetermined	OK - NSD
5	Blank		undetermined	OK - NSD
6	Blank		undetermined	OK - NSD
7	Blank		undetermined	OK - NSD
8	Blank		undetermined	OK - NSD
9	Pos Ctl (DONOR 10)		3.5726	OK - Full expected Profile Amel + 40
10	Blank		undetermined	OK - NSD
11	Blank		undetermined	OK - NSD
12	Pos Ctl (DONOR 11)		3.9466	OK - Full expected Profile Amel + 40
13	Blank		undetermined	OK - NSD
			Quant #2 VALQUA20160212_03	
14	Blank		undetermined	OK - NSD
15	Pos Ctl (DONOR 12)		1.7876	OK - Full expected Profile Amel + 40
16	Blank		undetermined	OK - NSD
17	Blank		undetermined	OK - NSD
18	Blank		undetermined	OK - NSD
19	Blank		undetermined	OK - NSD
20	Blank		undetermined	OK - NSD
21	Blank		undetermined	OK - NSD
22	Blank		undetermined	OK - NSD
23	Blank		undetermined	OK - NSD
24	Blank		undetermined	OK - NSD

Part 2 – Contamination Check of AS module

All blanks across the quantification assay plate prepared using the AS Module (VALQUA20160503_01) resulted in an 'undetermined' quantification value. All positive control donor samples resulted in an expected quantification value. These results detailed in Table 30 shows that there was no evidence of cross-contamination during the assay set up of this batch using the AS Module.

Table 30 – AS Module Contamination Check Results

	VALIQLYS20160210_01-04 Lysate Batch 1-4		Quant #1 VALQUA20160503_01
Position #	Sample Type	Sample Number	Quant value
1	STD 1	N/A	N/A
2	STD 1	N/A	N/A
3	STD 2	N/A	N/A
4	STD 2	N/A	N/A
5	STD 3	N/A	N/A
6	STD 3	N/A	N/A
7	STD 4	N/A	N/A
8	STD 4	N/A	N/A
9	STD 5	N/A	N/A
10	STD 5	N/A	N/A
11	Reagent Blank	N/A	Undetermined
12	Pos Ctl (DONOR 1)		0.5579
13	Blank		undetermined
14	Blank		undetermined
15	Pos Ctl (DONOR 2)		1.6652
16	Blank		undetermined
17	Blank		undetermined
18	Pos Ctl (DONOR 3)		8.3341
19	Blank		undetermined
20	Blank		undetermined
21	Blank		undetermined
22	Blank		undetermined
23	Blank		undetermined
24	Blank		undetermined
25	Blank		undetermined
26	Blank		undetermined
27	Blank		undetermined
28	Blank		undetermined
29	Blank		undetermined
30	Blank		undetermined
31	Blank		undetermined
32	Blank		undetermined
33	Blank		undetermined
34	Blank		undetermined
35	Pos Ctl (DONOR 4)		1.4451
36	Blank		undetermined
37	Blank		undetermined
38	Pos Ctl (DONOR 5)		2.4788
39	Blank		undetermined
40	Blank		undetermined
41	Pos Ctl (DONOR 6)		2.5327
42	Blank		undetermined
43	Blank		undetermined
44	Blank		undetermined
45	Blank		undetermined
46	Blank		undetermined
47	Blank		undetermined
48	Blank		undetermined
49	Blank		undetermined
50	Blank		undetermined
51	Blank		undetermined
52	Blank		undetermined

53	Blank		undetermined
54	Blank		undetermined
55	Blank		undetermined
56	Blank		undetermined
57	Blank		undetermined
58	Blank		undetermined
59	Blank		undetermined
60	Pos Ctl (DONOR 7)		2.4399
61	Blank		undetermined
62	Blank		undetermined
63	Pos Ctl (DONOR 8)		3.3031
64	Blank		undetermined
65	Blank		undetermined
66	Pos Ctl (DONOR 9)		1.8971
67	Blank		undetermined
68	Blank		undetermined
69	Blank		undetermined
70	Blank		undetermined
71	Blank		undetermined
72	Blank		undetermined
73	Blank		undetermined
74	Blank		undetermined
75	Blank		undetermined
76	Blank		undetermined
77	Blank		undetermined
78	Blank		undetermined
79	Blank		undetermined
80	Blank		undetermined
81	Blank		undetermined
82	Blank		undetermined
83	Pos Ctl (DONOR 10)		1.6835
84	Blank		undetermined
85	Blank		undetermined
86	Pos Ctl (DONOR 11)		1.6432
87	Blank		undetermined
88	Blank		undetermined
89	Pos Ctl (DONOR 12)		0.7217
90	Blank		undetermined
91	Blank		undetermined
92	Blank		undetermined
93	Blank		undetermined
94	Blank		undetermined
95	Blank		undetermined
96	Blank		undetermined

Acceptance Criteria

The QIAsymphony® SP was accepted because:

- All blank controls (excluding one blank control) gave undetermined quantification results. One blank control did give a quantification result of 0.0168 ng/µL, however gave an NSD profile result and undetermined quantification result when re-quantified using the AS module, indicating that the sample had not been contaminated.
- All blank controls gave NSD profile results except for one blank control which gave a single peak at D1S1656 [8]. Blank controls with less than three peaks are accepted in routine sample processing as per QIS 17130 *Capillary Electrophoresis Quality (CEQ) Check*.
- All positive control results gave the expected single source profile.

The QIAsymphony® AS was accepted because:

- All blank controls gave undetermined quantification results.
- All positive control results gave an expected quantification result.

7.10 Experiment 10: Integrated Runs

This experiment found that by increasing the temperature of the cooling adaptors on the AS Module from 4 °C to 8°C and setting the room temperature at a constant 21 °C for 24 hours/day, condensation was dramatically reduced (from the approximately 10µL per quantification reaction well observed during the preliminary trials) to approximately 2µL during integrated runs. However, this condensation could not be completely eliminated for integrated runs where quantification plates were stored on the AS module overnight.

Batch 1
Batch 1 was a non-integrated run, however the quantification [redacted]) was commenced immediately following the completion of the extraction protocol [redacted]. Quantification reagents were not loaded onto the AS module until the extraction protocol was completed. Batch 1 found no condensation after the immediate removal of the quantification assay plate from the AS module. No condensation was found on any of the cooling adaptors, and the volumes contained in the extract tubes were as expected (between 85 and 90µL). This quantification batch passed all normal Quantifiler® Trio criteria thresholds for the slope, Y-Intercept and R^2 (as per QIS 33407 *Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit*) and no outliers were observed.

Batch 2
Batch 2 was an integrated run with AS cooling blocks increased to 8°C. The integrated run was started in the afternoon and left to run overnight (room temperature not controlled to 21 °C overnight), however the worktable on the AS module was left for the following morning to be then set up and the run continued (i.e. so that quantification reagents and consumables were not placed onto the AS overnight). As expected, no condensation was observed on the quantification assay plate as there was no time lapse between the continuation of the run (the transfer of samples across to the AS module to start preparing the assay plate). There was no condensation on the cooling adaptors, however, volumes contained in the extract tubes were lower than those in Batch 1 above, likely due to evaporation over night. The fluctuating room temperature was the most likely cause to this evaporation observed (this contributing factor is explored further for extraction and quantification batch 4 of this experiment). This quantification batch passed all normal Quantifiler® Trio criteria thresholds for the slope, Y-Intercept and R^2 (as per QIS 33407 *Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit*), and no outliers were observed.

Batch 3
Batch 3 was an integrated run with the AS cooling blocks set at an increased temperature of 8°C through the protocol. This run was started in the afternoon to begin the extraction process using the SP module and allowed to

automatically progress throughout the night to the AS module for the assay set up. The assay plate for this integrated run (which was stored on the AS module overnight) had approximately 2µL of extra volume in each well of the assay plate. As in the preliminary trials (where 10µL of condensation was found in each well with the cooling adaptors set at 4 °C), the 2µL of condensation observed in Batch 3 with cooling adapters set at 8°C resulted in minimal effect on the overall quantification batch parameters themselves. The Y-Intercept for the SAT was just outside the 3SD, but all other parameters passed our set thresholds.

Volumes in the extract tubes were slightly lower again than batch 1, which again demonstrates that leaving extracts on the SP overnight does result in slight evaporation with the fluctuating room temperatures. The increase in AS module cooling adapter temperature did somewhat improve condensation, it did not completely eliminate the issue.

Batch 4

Extraction and quantification Batch 4 was run as an integrated run using the protocol with the cooling blocks set at 4 °C, but run in a controlled environment of 21 °C. This run was started in the afternoon to begin the extraction process using the SP module and allowed to automatically progress throughout the night to the AS module for the assay set up. As observed in Batch 3, the assay plate for this integrated run also contained approximately 2µL of extra volume in each well regardless of the temperature of the controlled environment set at 21 °C. Although 2µL of condensation was present in each well on the assay plate, again there seemed to have been minimal effects on DNA yields with no outliers observed, and the overall quantification batch parameters themselves.

The extract volumes for Batch 4 were the same as for Batch 1, therefore keeping the room temperature at constant 21 °C appears to have removed the evaporation issue. Although keeping the room temperature at a constant 21 °C reduced the amount of condensation on the quantification assay plate, it did not eliminate the issue.

Table 31 below shows the results obtained for the Standard curves for each quantification Trio batch performed. The SAT Y-Intercept for quantification batch 3 was the only criteria which did not pass normal acceptance thresholds (highlighted in red), however for this batch the slope and R^2 passed. The SAT Y-Intercept being outside normal acceptance criteria may indicate sub-optimal performance of the quantification PCR reaction.

Table 31 – Standard curve results for Quantification batches performed

Quant batch	Quant Batch ID	SAT Slope	SAT Y-Intercept	SAT R2 value	LAT and Y-Target	Reagent Blank
1		-3.286	27.323	0.999	ok	undetermined
2		-3.335	27.98	0.999	ok	undetermined
3		-3.097	28.506	0.997	ok	undetermined
4		-3.142	28.064	0.994	ok	undetermined

Quantifiler® Trio criteria thresholds for Slope, Y-Intercept and R^2 are the following:

SAT Criteria	Allowable Thresholds
Slope	-3.0 to -3.6
Intercept	26.7952 – 28.1629 (2SD)
R^2	≥0.98000
LAT Criteria	Allowable Thresholds
Slope	-3.1 to -3.7
Intercept	24.5704 – 25.7360 (2SD)
R2	≥0.98000
Y-Target Criteria	Allowable Thresholds
Slope	-3.0 to -3.6
Intercept	26.1692 – 27.5003 (2SD)
R2	≥0.98000

Table 32 shows quantification values, extract and condensation volumes for the 4 integrated run batches performed.

Table 32 – Integration batches between the SP and AS modules.

Integrated Batch #	Sample ID	Sample type	Protocols used and Batch ID's	Quant value	Extract volume after quant	Volume of condensation
1.		Pos Ctl	NON - INTEGRATED RUN - One day extraction and assay set up Protocols used: Extraction CW500 Trial HEIM (VALIQLYS20160510_01) AND QUANT_TRIO DAY (4 °C) (VALQUA20160504_02)	2.0059	~90µL	0µL
		Neg Ctl		undetermined	~92µL	0µL
		Blood swab sample		2.5974	~85µL	0µL
		Blood swab sample		2.4762	~85µL	0µL
		Blood swab sample		2.5763	~85µL	0µL
		Blood swab sample		3.0109	~90µL	0µL
		Blood swab sample		2.2545	~88µL	0µL
		Blood swab sample		2.5301	~87µL	0µL
		Blood swab sample		2.5170	~86µL	0µL
		Blood swab sample		2.2866	~90µL	0µL
		Blood swab sample		2.3922	~90µL	0µL
		Blood swab sample		1.6348	~90µL	0µL
2.		Pos Ctl	INTEGRATED RUN – Overnight extraction and 2nd day quantification. Protocol used: IR_Trio(8 °C) (VALIQLYS20160429_02 and VALQUA20160504_01)	2.2865	~78µL	0µL
		Neg Ctl		undetermined	~80µL	0µL
		Blood swab sample		2.3608	~80µL	0µL
		Blood swab sample		2.2922	~80µL	0µL
		Blood swab sample		2.5690	~78µL	0µL
		Blood swab sample		2.9076	~78µL	0µL
		Blood swab sample		2.7049	~76µL	0µL
		Blood swab sample		2.8847	~78µL	0µL
		Blood swab sample		2.4507	~77µL	0µL
		Blood swab sample		1.9377	~80µL	0µL
		Blood swab sample		3.4526	~78µL	0µL
		Blood swab sample		2.4868	~78µL	0µL
3.		Pos Ctl	INTEGRATED RUN – Overnight extraction and assay performed overnight Protocol used: IR_Trio(8 °C) (VALIQLYS20160429_03 and VALQUA20160510_01)	1.5589	~81µL	~2µL
		Neg Ctl		undetermined	~82µL	~2µL
		Blood swab sample		3.0012	~83µL	~2µL
		Blood swab sample		2.4094	~83µL	~2µL
		Blood swab sample		2.7792	~82µL	~2µL
		Blood swab sample		3.3605	~82µL	~2µL
		Blood swab sample		4.0108	~82µL	~2µL
		Blood swab sample		4.2716	~80µL	~2µL
		Blood swab sample		1.8271	~82µL	~2µL
		Blood swab sample		4.0281	~82µL	~2µL
		Blood swab sample		3.1045	~83µL	~2µL
		Blood swab sample		4.4615	~81µL	~2µL
4.		Pos Ctl	INTEGRATED RUN – Overnight extraction and assay performed overnight Protocol used: IR_Trio(4 °C) (VALIQLYS20160429_04 and VALQUA20160518_01)	1.8827	~86µL	~2µL
		Neg Ctl		0.0006	~88µL	~2µL
		Blood swab sample		3.0010	~88µL	~2µL
		Blood swab sample		2.2110	~88µL	~2µL
		Blood swab sample		2.2794	~85µL	~2µL
		Blood swab sample		2.1237	~86µL	~2µL
		Blood swab sample		2.0817	~86µL	~2µL
		Blood swab sample		2.3060	~85µL	~2µL
		Blood swab sample		2.4886	~86µL	~2µL
		Blood swab sample		2.0744	~88µL	~2µL
		Blood swab sample		2.4497	~88µL	~2µL
		Blood swab sample		3.1338	~86µL	~2µL

Acceptance Criteria

The QIAsymphony® was assessed against the following criteria:

- Batch protocols were accepted if there was no condensation on the assay plates and cooling blocks (based on a visual assessment).
    - Quantification assays which were left on the AS module overnight were found to have condensation for each overnight test conducted. Therefore quantification assay plates may not be stored on the AS module overnight.
    - Quantification assay plates which were prepared and removed from the AS module as soon as practicable following completion of the run were not found to have condensation. Therefore this type of integrated run was accepted.
- In addition to the previous criteria, the volume of each eluate was measured and compared to expected volumes to determine if additional volume was present in the sample tube – only batch protocols with no additional volume were accepted.
    - No additional eluate volume was found in any sample tubes processed or stored on the SP module. Therefore sample tubes may be stored on the SP module overnight.
    - For sample tubes stored on the SP module overnight there was a reduction in volume noted, which was attributed to evaporation. The level of evaporation was significantly reduced by having the room temperature set at 21 °C. This room temperature will be accepted during operating hours.
- Batch protocols passed this experiment if quantification results were within passing criteria as per QIS 33407 *Quantification of Extracted DNA using Quantifiler® Trio DNA Quantification Kit.*
    - All tested protocols passed normal criteria as per QIS 33407 *Quantification of Extracted DNA using Quantifiler® Trio DNA Quantification Kit.*
- Quantification results (IPCCT, SAT, LAT, DI) were assessed for gross outliers which may indicate additional volume in wells, or adverse effects of leaving quantification reagents and/or prepared assay plates on the AS module for extended periods.
    - No gross outliers were identified for any of the protocols tested.

Based on the above acceptance criteria, the following protocol recommendations were made for QIAsymphony® Integrated Runs:

- Room temperature will set at 21 °C as this was shown to reduce the evaporation of extracts stored on the SP module overnight.
- Integrated runs can be performed during normal business hours with the block set to 4 °C, but the quantification assay plate must be removed from the AS Module for processing on the AB 7500 as soon as practicable following completion of the assay preparation.
- Integrated runs can be performed overnight, whereby the extraction protocol is processed and finalised outside business hours (with extract storage on the SP module overnight). However quantification assay plates and reagents are not permitted to be stored on the AS module overnight due to the inability to completely mitigate the condensation. The quantification assay setup must commence the following morning

(i.e. the operator will load reagents and commence assay setup the following morning).

- The AS Module cooling blocks are to be set at 4 °C, given that quantification assay plates will not be stored on the AS module overnight.

7.11 Experiment 11: Repeatability and Reproducibility

SP Repeatability:
Table 33 contains the results for the QIAsymphony® SP repeatability experiment.

Table 33 - QIAsymphony® SP repeatability experiment

SP Module	Extraction Batch A (VALIQLYS0160311_01)		Extraction Batch B (VALIQLYS20160311_02) Average DNA yields	
	Average DNA Yield (ng/µL)	**Std Dev**	**Average DNA Yield (ng/µL)**	**Std Dev**
Blood swabs	2.8132	0.470627	2.9226	0.452233
Cell swabs	0.0033	0.001854	0.0025	0.000766

The quantification results (average DNA yield and standard deviations) for both blood swabs and cell swabs were comparable, and within one standard deviation, for batches performed by the same operator on the same day using the QIAsymphony® SP module.

SP Reproducibility:
Table 34 contains the results for the QIAsymphony® SP reproducibility experiment.

Table 34 - QIAsymphony® SP reproducibility experiment

SP Module	Extraction Batch A (VALIQLYS0160311_01)		Extraction Batch B (VALIQLYS20160311_02)		Extraction Batch C (VALIQLYS20160311_03)	
	Average DNA Yield (ng/µL)	**Std Dev**	**Average DNA Yield (ng/µL)**	**Std Dev**	**Average DNA Yield (ng/µL)**	**Std Dev**
Blood swabs	2.8132	0.4706	2.9226	0.4522	3.1761	0.464084
Cell swabs	0.0033	0.0019	0.0025	0.0008	0.0032	0.002025

The average DNA yields obtained in Extraction C for both Blood and Cell swabs were within one standard deviation of the averages obtained for Extractions A and B. These results are therefore comparable.

AS Repeatability:
Table 35 contains the results for the QIAsymphony® AS repeatability experiment.

Table 35 - QIAsymphony® AS repeatability experiment

AS Module	Quantification Batch A (VALQUA20160509_01) Average DNA yields (ng/µL)	Quantification Batch B (VALQUA20160509_02) Average DNA yields (ng/µL)
Blood swabs	2.311 (Std Dev=0.320)	2.1915 (Std Dev=0.516)
Cell swabs	0.0024 (Std Dev=0.001)	0.0018 (Std Dev=0.001)
Small Autosomal		
Slope	-3.394	-3.338
Y-Intercept	27.48	27.579
R2	0.998	0.999
Large Autosomal		
Slope	-3.413	-3.496
Y-Intercept	25.42	25.458
R2	1.0	0.998
Y Target		
Slope	-3.314	-3.276
Y-Intercept	27.297	27.233
R2	0.998	0.996

The quantification results for both blood swabs and cell swabs were comparable, and within one standard deviation, for batches performed by the same operator on the same day using the QIAsymphony® AS.

These quantification batches passed all normal Quantifiler® Trio criteria thresholds for the slope, Y-Intercept and R^2 (as per QIS 33407 *Quantification of Extracted DNA using Quantifiler® Trio DNA Quantification Kit*), and no outliers were observed.

AS Reproducibility:
Table 36 contains the results for the QIAsymphony® AS reproducibility experiment.

Table 36 - QIAsymphony® AS reproducibility experiment

AS Module	Quantification Batch A and B Average DNA yields	Quantification Batch C (VALQUA20160510_02) Average DNA yields
Blood swabs	2.25127 (Std Dev=0.409)	1.8427 (Std Dev=0.466)
Cell swabs	0.0021 (Std Dev=0.001)	0.0019 (Std Dev=0.001)
Small Autosomal		
Slope		-3.284
Y-Intercept		27.511
R2		0.997
Large Autosomal		
Slope		-3.435
Y-Intercept		25.451
R2		0.999
Y Target		
Slope		-3.399
Y-Intercept		27.403
R2		0.995

The quantification results for both blood swabs and cell swabs were comparable, and within one standard deviation, for batches performed by a different operator on a different day using the QIAsymphony® AS. This quantification batch C passed all normal Quantifiler® Trio criteria thresholds for the slope, Y-Intercept and R^2 (as per QIS 33407 *Quantification of Extracted DNA using Quantifiler® Trio DNA Quantification Kit*), and no outliers were observed.

Acceptance Criteria
The QIAsymphony® SP was accepted as it was shown to produce repeatable and reproducible results.

The QIAsymphony® AS module was accepted as it was shown to produce repeatable and reproducible results.

7.12 Experiment 12: Sample Recovery

Recovery Methods 1 and 4
Recovery methods 1 and 4 were tested to determine the most effective protocol to process samples already at the lysate stage in the event of extended QIAsymphony® downtime. Table 37 below contains the results for recovery methods 1 and 4. These results show that Recovery Method 1 gave higher quantification results for both blood swabs and tapelifts, making it the preferred method.

Table 37– Average DNA yields obtained between Sample Recovery methods 1 and 4.

	Sample Recovery Method 1 Average DNA yields (ng/μL)	Sample Recovery Method 4 Average DNA yields (ng/μL)
Blood swabs	1.1374	0.9210
Tapelift Cells	0.0460	0.0201

Recovery Methods 2 and 3
Recovery methods 2 and 3 were designed for QIAsymphony® SP failure during an extraction, whereby the extractions would be finalised by manual extraction. Table 38 below contains the results for Recovery Methods 2 and 3.

Table 38 – Average DNA yields obtained between Sample Recovery methods 2 and 3.

	Sample Recovery Method 2 Average DNA yields (ng/μL)	Sample Recovery Method 3 Average DNA yields (ng/μL)
Blood swabs	3.2932	1.366
Tapelift Cells	0.2347	0.1179

Recovery Method 2 – the manual DNA IQ™ method of extraction (QIS 24897 *DNA IQ Method of Extracting DNA from Reference and Casework Samples*) gave higher quantification results that Recovery Method 3 (the QIAGEN QIAamp® Kit).

Although the recommended sample recovery method from QIAGEN is the QIAamp® Kit, we found that the DNA yields obtained for both blood swabs and tapelifts from this method was less than half of what was obtained using our current manual DNA IQ™ Kit method. Observations made during the use of QIAGEN's kit were made which could have contributed to the lower DNA yields:

- Precipitation of QIAGEN's reagents was noted for all manual recovery methods used in this experiment. It has been noted that QIAGEN's reagents have the tendency of precipitating at lower temperatures. This was an added issue during the processing of these samples manually whereby a warm water bath set at ~60ºC was required to submerge samples throughout the extraction to prevent this precipitation issue. This could be done for the samples processed with the manual DNA IQ method as they were contained in 2mL tubes. It was not possible with the QIAamp® Kit columns because they do not have an o-ring, and therefore there is a risk of contamination.
- The DNA IQ™ Kit method includes resin bead mixing steps which are not present in the QIAamp® Kit protocol which uses a column and centrifugation. Additional resin bead mixing was shown in this validation to increase DNA yields.

Acceptance Criteria
Recovery Method 1 gave higher DNA yields than Recovery Method 4 and was accepted as the preferred sample recovery method for samples at lysate stage, which cannot be processed using the QIAsymphony® SP due to extended instrument downtime.

Recovery Method 2 gave higher DNA yields than Recovery Method 3 and was accepted as the preferred sample recovery method for samples which have commenced extraction using the QIAsymphony® SP but needs to be finalised manually due to instrument malfunction.

7.13 Experiment 13: Re-extraction of Substrates

Table 39 below contains the results for substrates which had been previously extracted on the QIAsymphony® were re-extracted on the Maxwell® 16. Pre-lysis Protocol 3 was used for these samples.

Table 39 – Average DNA yields obtained after original extraction and re-extraction.

Substrate type	Original average DNA yield after QIAsymphony® extraction (ng/μL)	Average DNA yield after re-extract using the DNA IQ™ Maxwell® 16 (ng/μL)
Blood swabs	1.4266	0.9891
Tapelift	0.4876	0.0729

These results show that when substrates which have been previously extracted on the QIAsymphony® are re-extracted on the Maxwell® 16 that good DNA yields are obtained.

During Experiment 5, the QIAsymphony® pre-lysis protocol was altered and pre-lysis Protocol 4 was accepted and replaced Protocol 3. Due to this alteration, Experiment 13 was repeated using the sample substrates that were extracted using the pre-lysis Protocol 4. Two batches (Batch 19 and 21) from Experiment 5 were re-extracted, these results are shown in Table 40.

Table 40 – DNA yields obtained after extraction and re-extraction (using modified pre-lysis protocol, Batches 19 and 21)

Substrate type	Original DNA yield after QIAsymphony® extraction (ng/µL)	DNA yield after re-extract using the DNA IQ™ Maxwell® 16 (ng/µL)
Blood swabs	3.0615	0.0101
Tapelift*	1.3030	0.0868
Cell swabs	2.9311	0.0234

*average from both Batches 19 and 21

The DNA yield of the re-extracted tapelift samples (0.0868 ng/µL) is slightly higher than the original DNA yield (0.0729 ng/µL, Table 39). This increase is could be due to the different methods used to create the tapelift samples. Tapelift samples from Experiment 1 were created by inoculating fabric with 15µL of buccal cell suspension and then tape applied to the fabric to lift the cells from the fabric to the tape (Section 5.2.9). Whereas the tapelift samples from Experiment 5 were created by inoculating 15µL of buccal cell suspension directly onto the tape (Section 5.2.10).

The DNA yield of the blood swabs (0.0101 ng/µL) is significantly less than the previous result (0.9891 ng/µL). Since the method used to create the blood swabs had not changed, a possible explanation of the significant decrease is due to the modifications made to the QIAsymphony® pre-lysis protocol. It seems that these modifications have made the QIAsymphony® pre-lysis protocol more efficient in removing more of the cellular material from the substrates.

Assessment Criteria
This experiment has shown that substrates previously extracted using QIAsymphony® can be re-extracted using the Maxwell® 16 and provide DNA yields which may give DNA profile results. This should be considered as a reworking strategy as required.

8 CONCLUSIONS

The QIAsymphony® SP using the QIAsymphony® DNA Investigator Kit has been shown to give comparable DNA extraction results to the Maxwell® 16 using the Casework DNA IQ™ Pro Kit for Maxwell®16 for a range of cell types and substrates including:

a. Blood
b. Cells
c. Tissue
d. Fingernails
e. Hair
f. FTA Cards
g. Tapelifts
h. Cigarette butts
i. Chewing gum
j. Fabric (cotton)
k. Swabs

Based on the validation, the QIAsymphony® SP using the QIAsymphony® DNA Investigator Kit is considered suitable for the extraction of a range of substrate types not specifically validated, including paper, plastic, wood, straws and food. For the period January to September 2016 there were a total of 18,707 sample submissions to the Analytical Team of sample/substrate types a-k above. In this same period there were 54 sample submissions of other substrate types included including paper, plastic, wood, straws and food, which represents approximately 0.3% of all submissions

This project has validated a modified pre-lysis protocol (Protocol 4), which included increased incubation temperature and agitation on the ThermoMixer during the incubation step.

Lysate storage conditions were tested and it was found that storage at fridge (2°C – 8°C) and freezer (~20 °C) were both acceptable for periods up to 96 hours for the tested samples.

The pipetting accuracy of the AS module was tested. The AS module passed verification at 2µL and 18µL making it suitable for quantification assay setup.

Contamination check experiments were conducted on the SP and AS modules. Both modules passed this test, with no contamination identified.

Integrated runs were tested and validated but with the following operating conditions:

- Room temperature will set and maintained at 21 ºC from start of business Monday until close of business Friday, as this was shown to reduce the evaporation of extracts stored on the SP module overnight.

- Integrated runs can be performed during normal business hours with the block set to 4 ºC, but the quantification assay plate must be removed

from the AS Module for processing on the AB 7500 as soon as practicable following completion of the assay preparation.

- Integrated runs can be performed overnight, whereby the extraction protocol is processed and finalised outside business hours (with extract storage on the SP module overnight). However quantification assay plates and reagents are not permitted to be stored on the AS module overnight due to the inability to completely mitigate condensation. The quantification assay setup must commence the following morning (i.e. the operator will load reagents and commence assay setup the following morning).

- The AS Module cooling blocks are to be set at 4 ºC, given that quantification assay plates will not be stored on the AS module overnight.

The SP and AS modules of the QIAsymphony® were found to produce repeatable and reproducible results.

The QIAsymphony® was found to effectively remove a range of known PCR inhibitors, except for Tannic Acid. The performance of the QIAsymphony® in this regard was comparable to the Maxwell® 16.

When testing sanitary pads and nappies, the clot detection function identified these difficult substrates and notified the operator via error message. Once the operator had intervened to skip these samples the remainder of the extraction proceeded normally.

Sample recovery protocols were validated. Recovery Method 1 (extraction using the Maxwell® 16) was found to be the most effective for extraction of samples already at the lysate stage. Recovery Method 2 (the manual DNA IQ™ method of extraction) was found to be the most effective recovery method for samples already loaded to the QIAsymphony® SP worktable.

Re-extraction of substrates already extracted on the QIAsymphony® were tested. It was found that previously extracted substrates which were re-extracted using the Maxwell® 16, gave quantification results which would be likely to yield a DNA profile and therefore this could be a viable reworking strategy for suitable samples (i.e. success of re-extraction is dependent on the amount of DNA remaining on the substrate following the first extraction).

9 RECOMMENDATIONS

1. The QIAsymphony® with the QIAsymphony® DNA Investigator Kit be implemented for the extraction a wide range of cell/substrate types, including:
    a. Blood
    b. Cells
    c. Tissue
    d. Fingernails
    e. Hair
    f. FTA cards
    g. Tapelifts
    h. Cigarette butts
    i. Chewing gum
    j. Fabric (cotton)
    k. Swabs
    l. Other substrates types currently processed on the Maxwell® 16 (i.e. paper, plastic, wood, straws, food, other fabric etc).

    N.B. for the period January to September 2016 there were a total of 18,707 sample submissions to the Analytical Team of sample/substrate types 1a-1k above. In this same period there were 54 sample submissions of the substrate types included in 1l, which represents approximately 0.3% of all submissions

2. Further validation is completed at a future date to investigate the feasibility of implementing validation of additional cell/extraction types not within the scope of this validation:
    a. Bones
    b. Differential Lysis (including spermatozoa)
    c. Paraffin embedded tissue
    d. Retained supernatant
    e. Other substrate types as considered necessary (i.e. other fabric types)

3. Pre-Lysis Protocol 4 is implemented as the pre-lysis protocol for all validated cell types.
    a. Two ThermoMixers be purchased to enable Protocol 4 to be implemented for as the pre-lysis protocol for all validated cell and substrate types.

4. The "Extraction CW500 trial HEIM" QIAsymphony® SP extraction protocol is implemented as the extraction protocol for all extractions.

5. Lysates may be stored either in the fridge (2°C – 8°C) or freezer (~20 °C) for periods up to 96 hours.

6. Substrates containing super absorbent polymer/hydrogels (commonly found in sanitary pads and nappies) are not suitable for extraction on the QIAsymphony®.

7. Air conditioning in the room 3191 is set and maintained at 21°C from Monday start of business until Friday close of business.

8. AS module cold blocks to be set at 4 °C

9. Integrated runs are implemented with the following operating conditions:
    a. Integrated runs can be performed during normal business hours with the block set to 4 °C. However, the quantification assay plate must be removed from the AS Module for processing on the AB 7500 as soon as practicable following completion of the assay preparation.
    b. Integrated runs can be performed overnight, whereby the extraction protocol is processed and finalised outside business hours (with extract storage on the SP module overnight). However, prepared quantification assay plates and reagents are not permitted to be stored on the AS module overnight due to the inability to completely mitigate the condensation. The quantification assay setup must commence the following morning (i.e. the operator will load reagents and commence assay setup the following morning).

10. Recovery Method 1 (extraction using the Maxwell® 16 per QIS 29344 *DNA IQ™ Extraction using the Maxwell® 16*) is implemented for the

recovery of samples already at the lysate stage (if the QIAsymphony® is out of operation for an extended period).

11. Recovery Method 2 (the manual DNA IQ™ method of extraction QIS 24897 *DNA IQ Method of Extracting DNA from Reference and Casework samples*) is implemented for the recovery of samples already loaded to the QIAsymphony® SP worktable in the event of instrument error.

12. Where re-extract of substrates which have been previously extracted using the QIAsymphony® is required, the re-extraction is to be performed using the Maxwell® 16 as per QIS 29344 *DNA IQ™ Extraction using the Maxwell® 16.*

10 REFERENCES

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