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# Project 13. Report on the Verification of an Automated DNA IQ™ Protocol using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform

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## 1. Abstract

A manual method for extracting DNA from forensic samples using the DNA IQ™ system (Promega Corp., Madison, WI, USA) was validated for routine use in DNA Analysis (FSS). We have verified an automated DNA IQ™ protocol in 96-well format for use on the MultiPROBE® II PLUS HT EX Forensic Workstation platforms (PerkinElmer, Downers Grove, IL, USA). Data indicate that results from the automated procedure are comparable to those from the manual procedure. Contamination checks were performed using samples prepared in checkerboard and zebra-stripe format, and results were as expected. We recommend the use of the MultiPROBE® II PLUS HT EX platforms to perform automated DNA extraction using the DNA IQ™ system.

## 2. Introduction

The MultiPROBE® II PLUS HT EX FORENSIC WORKSTATION platforms (PerkinElmer, Downers Grove, IL, USA) are equipped to perform automated DNA extractions, as they include a DPC shaker and individual heat controllers to enable on-board lysis and incubation steps. Currently in DNA Analysis, the MultiPROBE® platforms allow walk-away operation of PCR setup protocols for DNA quantitation and amplification.

The DNA IQ™ protocol has been verified or validated by various laboratories for use on the MultiPROBE® II PLUS platform. The laboratories that perform an automated DNA IQ™ protocol include PathWest (Western Australia), Forensic Science South Australia (South Australia) and Centre of Forensic Sciences in Toronto (Ontario). The MultiPROBE® II PLUS instrument comes pre-loaded with an automated DNA IQ™ protocol. Unlike the other laboratories, however, we did not validate the included protocol, but instead validated a manual DNA IQ™ protocol which was based on the CFS automated protocol (PerkinElmer, 2004), followed by verification of an automated protocol based on the validated manual method.

The verified automated DNA IQ™ protocol is identical to the validated manual protocol used in-house: there are no differences in reagents or volumes. The adopted DNA IQ™ protocol differs slightly, however, from the manufacturer's protocol, as it includes a lysis step using Extraction Buffer (10mM Tris, 1mM EDTA, 100mM NaCl, 20% w/v SDS) in the presence of Proteinase K, before incubating in the DNA IQ™ Lysis Buffer. Furthermore, the lysis incubation conditions were lowered from 70°C to 37°C in order to accommodate extraction of DNA from heat labile materials such as nylon and polyester. In addition, the automated protocol utilises the SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA) for simultaneous processing of samples in a 96-well format.

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## 3. Aim

To verify an automated DNA IQ™ protocol for use on the MultiPROBE® II PLUS HT EX platforms to allow extraction of DNA from various sample types.

## 4. Equipment and Materials

- MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (PerkinElmer, Downers Grove, IL, USA)
- Gravimetric Performance Evaluation Option with Mettler SAG285/L balance (Mettler-Toledo, Greifensee, Switzerland)
- DNA IQ™ System (Promega Corp., Madison, WI, USA)
- Extraction Buffer (10mM Tris, 1mM EDTA, 100mM NaCl, 20% SDS)
- SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA)
- Nunc™ Bank-It tubes (Nunc A/S, Roskilde, Denmark)
- 175µL non-conductive sterile filter RoboRack tips (PerkinElmer, Downers Grove, IL, USA)
- 1000µL Conductive sterile filter Robotix tips (Molecular BioProducts, San Diego, CA, USA)
- ABI Prism® 7000 SDS (Applied Biosystems, Foster City, CA, USA)
- Quantifiler™ Human DNA Quantification kits (Applied Biosystems, Foster City, CA, USA)
- AmpFℓSTR® Profiler Plus Amplification kits (Applied Biosystems, Foster City, CA, USA)
- GeneAmp® 9700 thermalcycers (Applied Biosystems, Foster City, CA, USA)
- ABI Prism® 96-well optical reaction plates (Applied Biosystems, Foster City, CA, USA)
- ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA)
- GeneScan™ 500 ROX™ Size Standard (Applied Biosystems, Foster City, CA, USA)
- Hi-Di™ Formamide (Applied Biosystems, Foster City, CA, USA)
- 3100 POP-4™ Polymer (Applied Biosystems, Foster City, CA, USA)
- Cytobrush® Plus Cell Collector (Cooper Surgical, Inc., Trumbull, CT, USA)
- 0.9% saline solution (Baxter Healthcare, Old Toongabbie, NSW, Australia)
- Stem digital tilting head thermometer
- For mock samples:
  - FTA™® Classic Card (Whatman Inc., Florham Park, NJ, USA)
  - Sterile cotton swabs (Medical Wire & Equipment, Corsham, Wiltshire, England)
  - Sterile rayon swabs (Copan Italia SPA, Brescia, Italy)

## 5. Methods

### 5.1 Gravimetric Evaluation of Pipetting Accuracy and Precision

Gravimetric analysis was performed by placing the SAG285/L balance on the platform deck and instructing the MP II to repeatedly pipette certain volumes of system liquid onto the balance pan. Readings were taken automatically by the software and compiled into a results table, which was then used to automatically generate an Excel-based results chart containing mean, %CV and %inaccuracy values. The mean values obtained were used to calculate $R^2$, slope and Y-intercept (offset) values to calibrate the system's pipetting.

Pipetting performance was assessed for various volumes using three different tips in order to calculate appropriate $R^2$, slope and Y-intercept (offset) values which were then added to the performance file. Values were calculated for both Blowout (single-liquid transfer) and Waste (multidispense) modes for the 1000µL conductive tips, and Blowout mode only for the 175µL non-conductive tips and fixed tips.

For the addition of resin, a specialised performance file was created based on the performance file for 175µL tips in blowout mode, except the "Blowout Volume" column values were set to 0 to allow pipetting performance that is similar to waste mode. Retesting was performed to confirm accurate and precise pipetting with these settings.

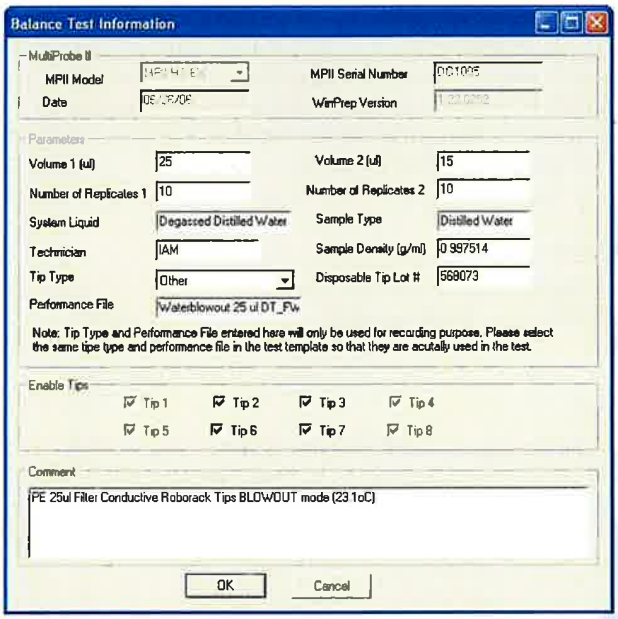


Figure { SEQ Figure * ARABIC }. The Balance Test Information Window as present within the Balance Test DT program. All pipetting parameters are entered here and are subsequently transferred to the result output file.

All gravimetric testing was performed using the Balance Test DT test program within WinPrep®. Parameter values that needed to be entered into the Balance Test Information Window (Figure 1) included those as outlined in Table 1.

Table { SEQ Table * ARABIC }. Input values that are required for the various Balance Test Information parameters.

| Parameter(s) | Value |
|---|---|
| Volume 1 and Volume 2 | For 175µL tips: 175, 100, 50, 15µL<br>For 1000µL tips: 1000, 700, 400, 100µL<br>For fixed tips: 1000, 700, 400, 100µL |
| Number of Replicates | 10 |
| System Liquid | Degassed Nanopure Water |
| Sample Type | Nanopure Water |
| Technician | Initials of the operator performing the test |
| Sample Density (g/ml) | The density of water at environmental temperature* |
| Tip Type | Other |
| Disposable Tip Lot # | The lot number of the particular tips in use |
| Performance File | The appropriate Performance File for the tip (175µL, 1000µL or fixed tips) and pipetting mode (Blowout or Waste) in use |
| Enable Tips (checkboxes) | Select the actual tips (1 to 8) to be tested |
| Comments | Free text box to add additional information (eg. Tip type, mode, current environmental room temperature, etc). |

* Water density values were obtained from http://www.simetric.co.uk/si_water.htm

Pipetting accuracy and precision were examined at four different volumes for each tip size: 175, 100, 50, 15µL for 175µL tips and 1000, 700, 400, 100µL for the 1000µL and fixed tips.

In order to calculate unbiased values for each set of volumes, the slope and offset values in the relevant Performance File were changed to the default 1 and 0 respectively prior to testing. The mean volumes that were pipetted by each tip (10 replicates per tip) at the four designated volumes were used to generate a standard curve. The slope and offset calculated from this curve was used to calibrate the relevant Performance File. The final Performance File settings were then tested at the highest and lowest volumes (as per Table 1) to confirm accurate and precise pipetting.

## 5.2 Blood Collection

Blood samples were collected from 2 staff donors (DJC/VKI) by a phlebotomist as per normal in three 4mL EDTA vials. Blood samples were stored at 4°C.

## 5.3 Cell Collection

Buccal cells were collected using a modified Cytobrush® protocol (Mulot *et al.*, 2005; Satia-Abouta *et al.*, 2002). The donor was instructed to brush the inside of one cheek for one minute using a Cytobrush®. Then, with another Cytobrush®, the other cheek was also sampled. Once each cheek was swabbed, the cells on the brush were suspended in 2mL of 0.9% saline solution. Buccal cell samples were stored at 4°C.

## 5.4 FTA cell Collection

Cells were collected from two staff donors (VKI/CJA) by using a "lolly-pop" swab to sample the inside of the donor's cheek for 15 seconds before pressing the swab onto the FTA™ paper to transfer the DNA. FTA™ cards were stored at room temperature.

## 5.5 Heater tile temperature verification

Heat tiles supplied with the MultiPROBE® II PLUS HT EX platforms were modified to accept the SlicPrep™ 96 Device. For testing, 1mL of nanopure water (at room temperature) was added to each well. The plate was then placed on a heater tile (controlled by the MP II heater controller) and allowed to reach temperature. The temperatures tested were 37°C and 65°C. Temperature readings for specific outer and inner wells (i.e. A1, A6, A12, D1, D6, D12, H1, H6, H12) were taken at regular intervals up to and including 45 minutes, using calibrated stem digital tilted head thermometer probes. The data were collated and means calculated to determine the distribution of heat over the tile.

## 5.6 Verification of automated DNA IQ™ Protocol

The automated DNA IQ™ protocol, based on the validated manual method (refer to Project 11), was programmed in WinPrep™ software. The final, optimised protocol was named "DNA IQ Extraction_Ver1.1.mpt". A screenshot of the Test Outline window for this protocol is depicted below in Figure 2. The deck layout is illustrated in Figure 3.

The automated DNA IQ™ protocol was designed to mimic the validated manual method, with minor modifications. Briefly, the changes include:

- Increasing the volume of Extraction Buffer to 500µL;
- A SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA) was used for sample lysis;
- Incubation steps and any shaking steps were performed on the integrated DPC shaker;

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- CRS toroid magnet (P/N 5083175) was used for isolating the DNA IQ™ resin.
- Instead of a single elution of 100µL, a double elution method (2 x 50µL) is used.

Reagents used in the automated protocol were as per the manual method.

Figure 2. The Test Outline window displaying individual nodes within the DNA IQ Extraction_Ver1.1.mpt program test file.

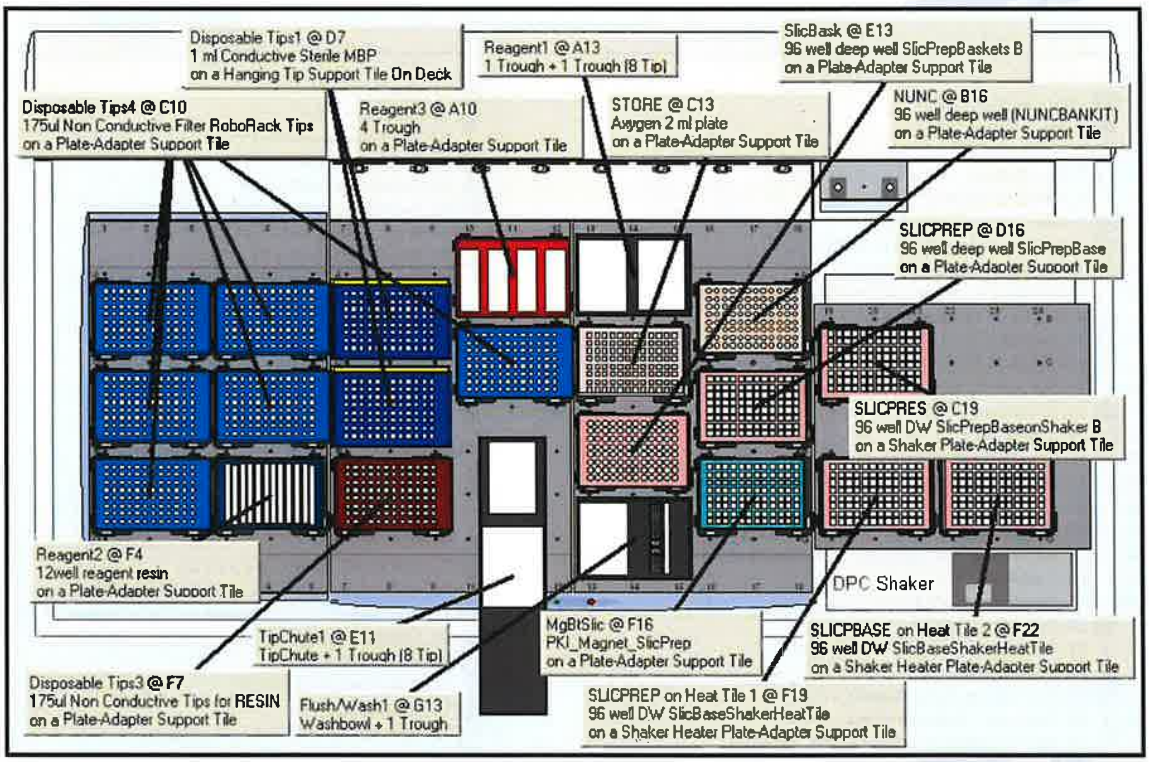
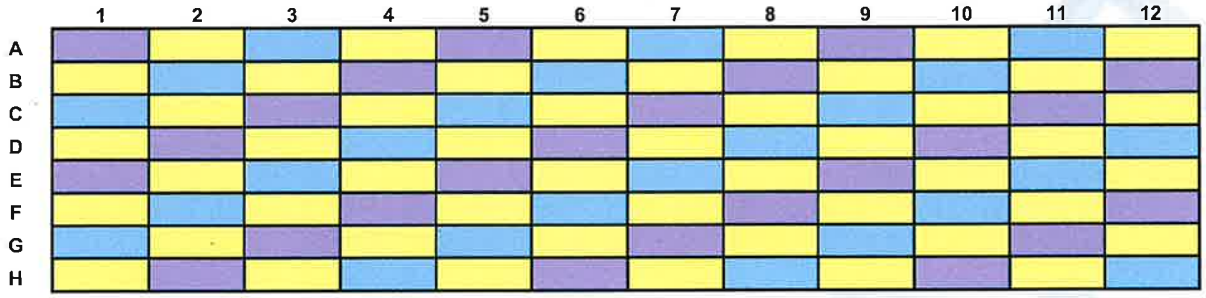



Figure 3. The deck layout for DNA IQ Extraction_Ver1.1.mpt, displaying the required labware on the platform deck.

The automated DNA IQ™ protocol was used to perform the following tests.

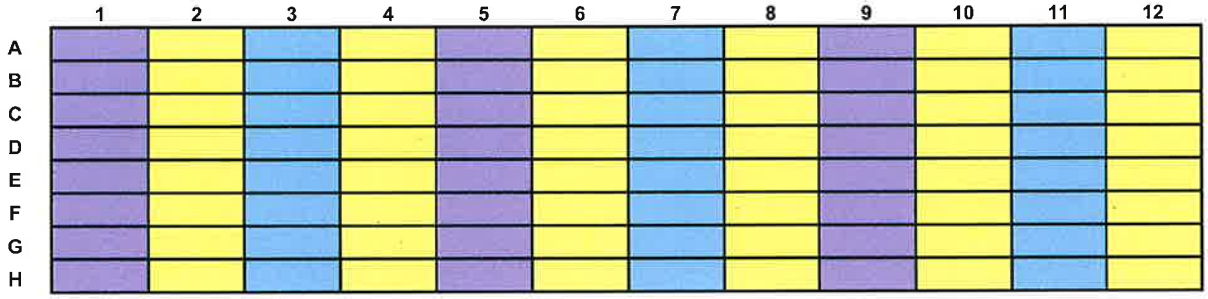
### *5.6.1.* Contamination Check via Checkerboard and Zebra-stripe Patterns

Samples consisting of two 3.2mm FTA® discs (containing blood, buccal cells, or blank cards) were arranged in a checkerboard and zebra-stripe pattern (Figure 4) in SlicPrep™ plates using the BSD Duet 600 instrument (BSD Robotics, Brisbane, QLD, Australia) and extracted on the MultiPROBE® II PLUS HT EX platforms using the automated DNA IQ™ protocol. One checkerboard and one zebra-stripe plate was processed on each platform.




(a) Checkerboard Pattern

b) Zebra Stripe Pattern




Legend:
Blood FTA®
Blank FTA®
Buccal Cell FTA®

Figure 4. Checkerboard and zebra-stripe patterns utilised in the contamination check.

### *5.6.2.* Comparisons with the manual DNA IQ™ method

Comparisons were made between results generated by the automated and manual methods to verify the performance of the automated DNA IQ™ protocol.

Verification samples consisted of different dilutions of blood and cells spotted in 30µL aliquots onto quartered cotton and rayon swabs. Four blood dilutions of neat, 1/10, 1/100 and 1/1000 and four cell dilutions of neat, 1/5.2, 1/52.2 and 1/522 were used to test the sensitivity of both the manual and automated methods. Dilutions were created using 0.9% saline solution for both sample types. Four replicates of each dilution were made up for each substrate and sample type.

The blood was collected using the same method as in 5.2. Four separate extractions were performed for the manual set based on the combination of sample type and swab type: Blood Rayon, Blood Cotton, Cell Rayon and Cell Cotton. For the automated verification, all sample types were extracted together after being transferred to a SlicPrep™ 96 Device to allow automated processing.

### *5.6.3.* Resin volume

The performance of the automated DNA IQ™ protocol was assessed when either 7µL or 14µL of DNA IQ™ resin was used in the protocol to extract blood samples.

### *5.6.4.* Modifying extraction volumes

The performance of the automated DNA IQ™ protocol was assessed for varying volumes of extraction buffer at 300, 350, 400, 450 and 500µL. In each case, the volume of DNA IQ™ Lysis Buffer was kept at 2x the volume of extraction buffer. Samples extracted were blood swabs, prepared as per ???.

### *5.6.5.* Sensitivity of the automated DNA IQ™ protocol

The sensitivity of the automated DNA IQ™ protocol was assessed using dilutions of whole blood at neat, 1:10, 1:50, 1:100 and 1:1000.

? swabs — how much did they add?

## 6. Results and Discussion

### 6.1 Gravimetric Evaluation of Pipetting Accuracy and Precision

Pipetting on both automated platforms was assessed gravimetrically as per laboratory practice. Gravimetric results indicate that pipetting performance for five different pipetting behaviours using 500µL syringes on the instruments is accurate and precise to within the established threshold of ±5% (Table 2). The maximum CV at the maximum volume was 0.78%, whereas the maximum CV at the minimum volume was 1.1%. The CV for pipetting at lower volumes is expected to be slightly higher than the CV at higher volumes using 500µL syringes, because accuracy at small volumes is harder to achieve with larger syringe sizes. Nevertheless, pipetting on the extraction platforms is limited to a minimum of 50µL, which exhibited a CV of 0.36%.

Table 2. Gravimetric evaluation results for various performance files used on either MP II EXTN A or MP II EXTN B.

| Performance File | Max. Vol. µL | Min. Vol. µL | Max. Vol. µL Mean | Max. Vol. %CV | Max. Vol. %Inac. | Min. Vol. µL Mean | Min. Vol. %CV | Min. Vol. %Inac. |
|---|---|---|---|---|---|---|---|---|
| EXTN A | | | | | | | | |
| Water Blowout 175µL DT_FW _13112007RESIN.prf | 50µL | N/A | 49.98 | 0.36 | 0.0 | N/A | N/A | N/A |
| Water Blowout 175µL DT_FW QHSS _13112007.prf | 175µL | 15µL | 172.26 | 0.21 | 1.6 | 12.47 | 3.35 | 16.19 |
| WaterWaste 1mL_FW_QHSS 12112007.prf | 1000µL | 100µL | 999.11 | 0.24 | 0.1 | 99.22. | 0.71 | 0.8 |
| Water Blowout 1mL DT_QHSS _09112007.prf | 1000µL | 100µL | 1001.02 | 0.27 | 0.1 | 100.65 | 0.63 | 0.7 |
| Water Blowout Fixed Tips_08112007.prf | 1000µL | 100µL | 995.97 | 0.31 | 0.4 | 99.6 | 0.71 | 0.4 |
| EXTN B | | | | | | | | |
| Water Blowout 175µL DT_FW_ 25102007RESIN.prf | 50µL | N/A | 50.12 | 0.36 | 0.2 | N/A | N/A | N/A |
| Water Blowout 175µL DT_FW_ 25102007.prf | 175µL | 15µL | 175.58 | 0.14 | 0.3 | 15.23 | 1.1 | 1.5 |
| WaterWaste 1mLDT_FW_QHSS 24102007.prf | 1000µL | 100µL | 1002.39 | 0.78 | 0.2 | 99.56 | 0.89 | 0.4 |
| Water Blowout 1mL DT_QHSS 23102007.prf | 1000µL | 100µL | 998.2 | 0.44 | 0.2 | 99.44 | 0.68 | 0.6 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Water Blowout Fixed Tips_FW 26102007.prf | 1000µL | 100µL | 998.87 | 0.68 | 0.1 | 100.37 | 0.74 | 0.4 |

## 6.2 Heater tile temperature verification

Two heater tiles on each MP II platform was verified to reach either 37°C or 65°C, the optimum incubation temperatures for sample lysis and DNA elution respectively (using the DNA IQ™ kit). Each tile, upon completion of the verification process, could only be used for a specific temperature, and as such was labelled appropriately to ensure use of the correct tile for specific incubation steps (Table 3).

Table 3. Verified heater tiles for use in the automated DNA IQ™ protocol.

| Extraction platform | Tile number | Heater Controller Setting | Average °C reached | Verified temperature | Incubation Step |
|---|---|---|---|---|---|
| EXTN A | 3 (45W) | 50°C | 37°C | 37°C | Sample Lysis |
| EXTN A | 1 (45W) | 85°C | | 65°C | DNA Elution |
| EXTN B | 1 (45W) | 50°C | | 37°C | Sample Lysis |
| EXTN B | 2 (45W) | 85°C | 65°C | 65°C | DNA Elution |

A slight variation in the incubation temperature to achieve sample lysis is acceptable, because Proteinase K exhibits stable activity and broad specificity over a wide range of temperatures between 20-60°C, at which the serine protease still retains greater than 80% of its activity (Sweeney & Walker, 1993).

The efficiency of the elution step is dependent on heating the sample to 65°C in the presence of DNA IQ™ Elution Buffer (Huston, 2002). If the sample is not sufficiently heated, the extraction yield may be lower than expected. Two heater tiles were able to be verified for this crucial incubation step, with both tiles exhibiting minimal variation.

## 6.3 Contamination Check via Checkerboard and Zebra-stripe Patterns

Table 4 below lists the Extraction Batch ID's of the contamination checks.

Table 4. Extraction Batch ID's for the various contamination check plates that were processed on the MP II platforms using the automated DNA IQ™ protocol.

| Type of plate | Extraction batch Id | Extraction Platform | Check passed |
|---|---|---|---|
| Checkerboard 1 | VALB20070817_02 | Extraction A | Invalidated |
| Checkerboard 2 | VALB20070803_02 | Extraction B | Yes |
| Zebra-Stripe 1 | VALB20070803_03 | Extraction A | Yes |
| Zebra-Stripe 2 | VALB20070817_03 | Extraction B | Yes |
| Checkerboard/Zebra | VALB20071022_01 | Extraction A | Yes |

**Checkerboard 1**

Position E3 (Sample Cells 6) was known to have been contaminated prior to the start of the extraction, during the STORstar process (???). The result showed a mixed DNA profile, with contributing alleles originating from the expected wells (Table 5). In addition to this contamination event, eight of the designated blank samples (positions D3, A10, F1, H5, C4, E4, B7 and E6), two of the cell samples (A1 and B10) and two of the blood samples (F4

and G7) all exhibited a partial DNA profile that was previously unknown (Table 5). This profile did not match any of the positive control samples present on the batch. The DNA profile was searched against the Staff Database and no matches were found. The source of this contaminating DNA profile could not be identified.

None of the other blank samples yielded any DNA profile. The rest of the cell and blood samples yielded the correct DNA profile. Although there is no evidence of well-to-well contamination, the unknown DNA profile obtained has invalidated this plate. A further checkerboard/Zebra-Stripe combination plate was performed to ensure...

Table 5. The DNA profile of the unknown contaminant that was observed in Checkboard-1.

| Sample description | D3 | vWA | FGA | Amel | D8 | D21 | D18 | D5 | D13 | D7 |
|---|---|---|---|---|---|---|---|---|---|---|
| Blk23-E6 | 14,17 | 14,17 | 22,24 | X,Y | 11,11 | 29,32.2 | 14,15 | 9,11 | 11,12 | 11,13 |
| Blk25-B7 | 14,17 | 14,17 | 22,24 | X,Y | 11,11 | 29,32.2 | 14,15 | 9,11 | 11,12 | |
| Blk15-E4 | 14,17 | 14,17 | 22,24 | X,Y | 11,11 | 29,32.2 | 14,15 | 9,11 | 11,12 | 11,13 |
| Blk14-C4 | | 14 | | X | 11 | 32.2 | | 9 | | |
| Blk20-H5 | 14,14 | 17,17 | 20,21 | X,X | 13,16 | 29,30 | 14,16 | 11,13 | 11,12 | 11,11 |
| Blk3-F1 | 14 | 17 | | X | 13 | 29,30 | 14 | | 12 | 11 |
| Blk10-D3 | 14,17 | 14 | | X,Y | 11 | 29,32.2 | 14 | 9,11 | | 11,13 |
| Blk37-A10 | 14,17 | 14 | 22,24 | X,Y | 11 | 29 | 14 | 9,11 | | |
| Cells19-B10 | 14,17 | 14,17 | 20,21,22,24 | X,Y | 11,13,16 | 29,30, | 14,15,16 | 11,15 | 11,12 | 11,11 |
| Cells13-A1 | 14,17 | 14,17 | 20,21,22,24 | X,Y | 11,13,16 | 29,30,32,32.2,33 | 14,15,16 | 9,11,13 | 11,12 | 11,13 |
| Blood14-G7 | NR,17,18 | NR,16 | 20,21 | X,Y | NR,13,14 | 29,30,31,NR | NR,14 | NR,12 | 10,10 | 10,NR,12 |
| Blood8-F4 | NR,17,18 | NR,16,17 | 20,21,NR,24 | X,Y | 11,13,14 | 29,30,NR,NR | 14,14 | 9,11,12 | 10,NR | 10,NR,12 |
| Cells 6-E3 | 14,17,18 | 16,17 | 20,21 | X,Y | 13,14,16 | 29,30,31 | NR,14,16 | 11,12,13 | 10,11,12 | 10,11,12 |

**Checkerboard 2**

None of the blank samples yielded DNA profiles; all of the positive cell and positive blood samples yielded the correct DNA profile. Figure 5 illustrates the DNA quantitation results from this plate. DNA was not detected in any of the blank samples.

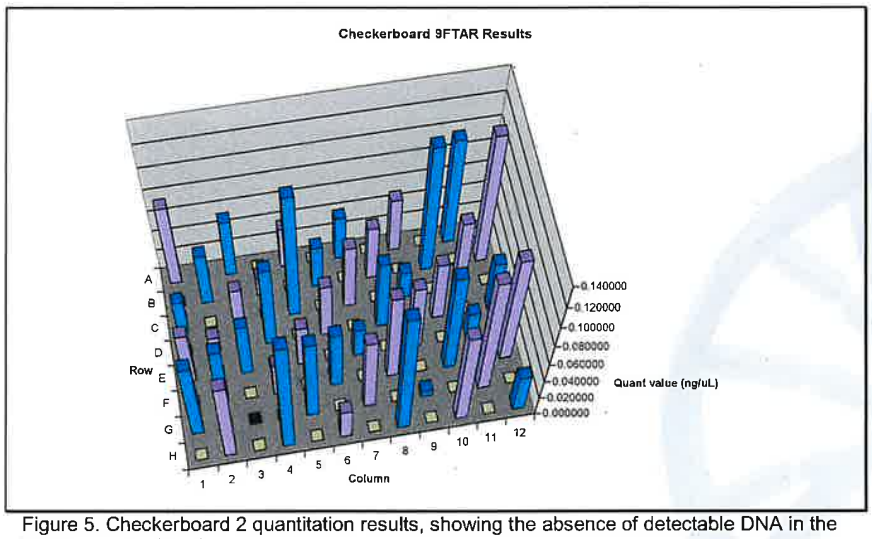



Figure 5. Checkerboard 2 quantitation results, showing the absence of detectable DNA in the blank samples (grey).

very low quants for these types of samples

**Zebra-Stripe 1**

None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile. Figure 6 illustrates the absence of detectable DNA in the blank samples.

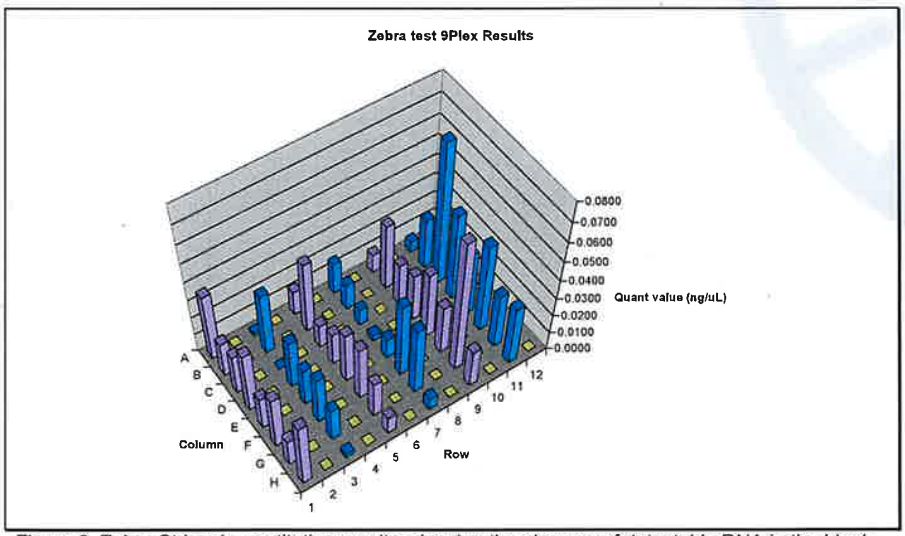



Figure 6. Zebra-Stripe 1 quantitation results, showing the absence of detectable DNA in the blank samples (grey).

**Zebra-Stripe 2**

None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile. Figure 7 shows the absence of detectable DNA in the blank samples.

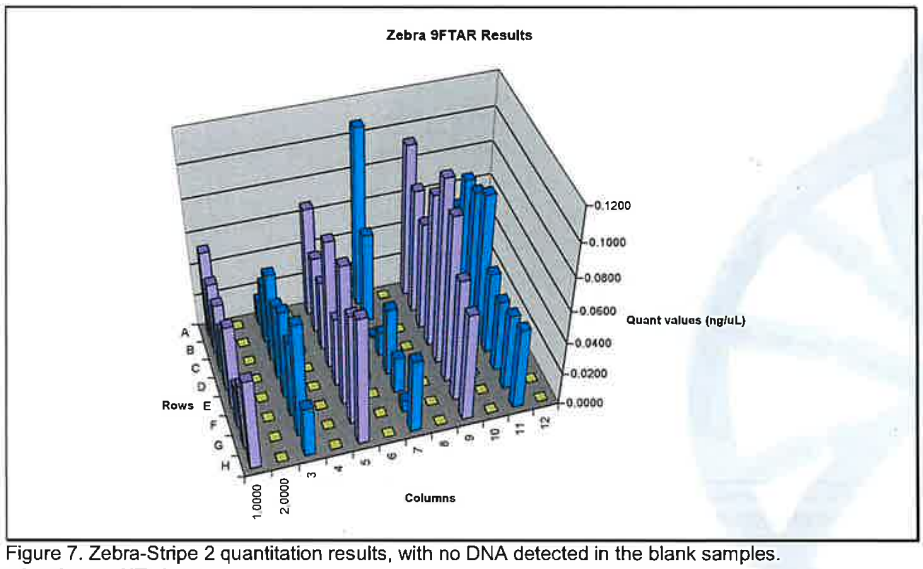



Figure 7. Zebra-Stripe 2 quantitation results, with no DNA detected in the blank samples.

**Checkerboard/Zebra**

None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile. DNA was undetected in the blank samples (Figure 8).

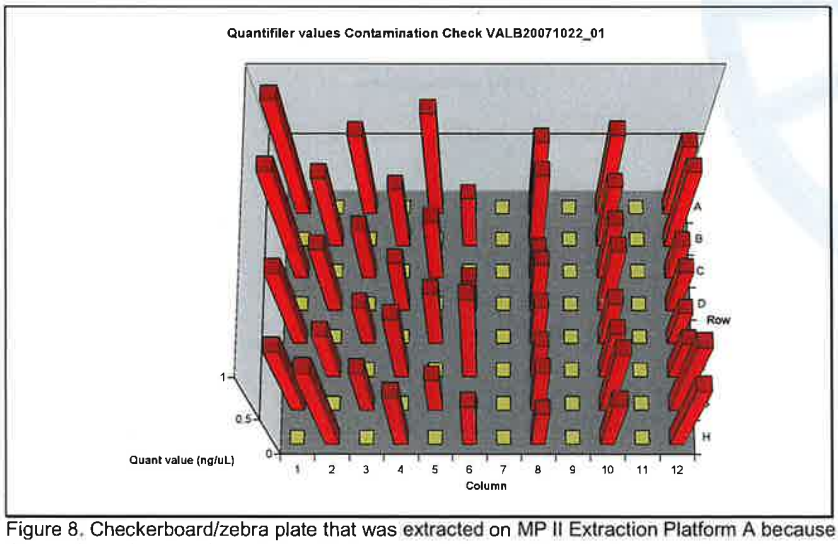



Figure 8. Checkerboard/zebra plate that was extracted on MP II Extraction Platform A because the previous plate was invalidated. DNA was not detected in the blank samples (grey).

## 6.4 Comparisons with the manual DNA IQ™ method

When dilutions of either blood or cells were applied on to either rayon or cotton swabs, followed by extraction using the DNA IQ™ method, the results of the automated method were always lower in yield compared to the manual method. For blood samples on rayon

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swabs, the automated method generated yields that were on average around 8% (SD 8.45%) of the automated method. For blood on cotton swabs, the yield from the automated method was also around 8% (SD 3.62%). The yields for cell samples were higher at around 33% (SD 16.29%) and 25% (10.32%) for cells on rayon and cotton swabs respectively.

The manual method was found to be more sensitive than the automated method. Out of five replicates at the 1/100 and 1/1000 dilutions for blood on rayon swabs that were processed using the manual method, five and three replicates respectively were detected (and none from the automated method) (see Figure 9). The trend is repeated for blood on cotton swabs (Figure 10). For cell samples on either rayon or cotton swabs, the automated method was found to be more sensitive as evidenced by detection of DNA at the 1/522 dilutions (Figure 11 and 12).

Cell clumping may have occurred with the cell dilutions, therefore causing inaccurate dilutions as can be observed in the ratios between each dilution.

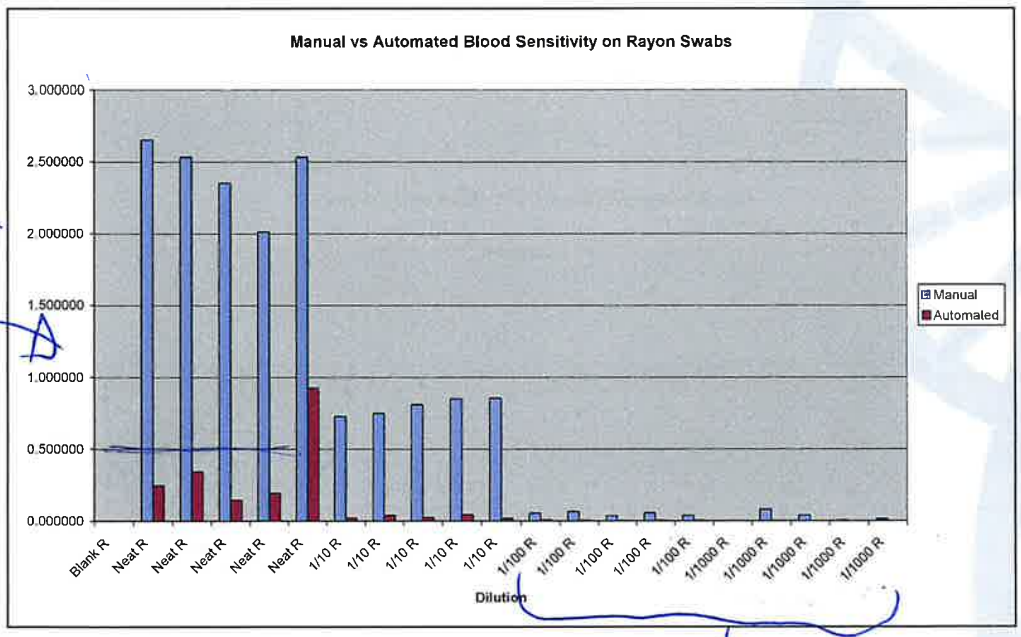


Figure 9. Comparison of sensitivity between the manual and automated DNA IQ™ methods for blood samples on rayon swabs.

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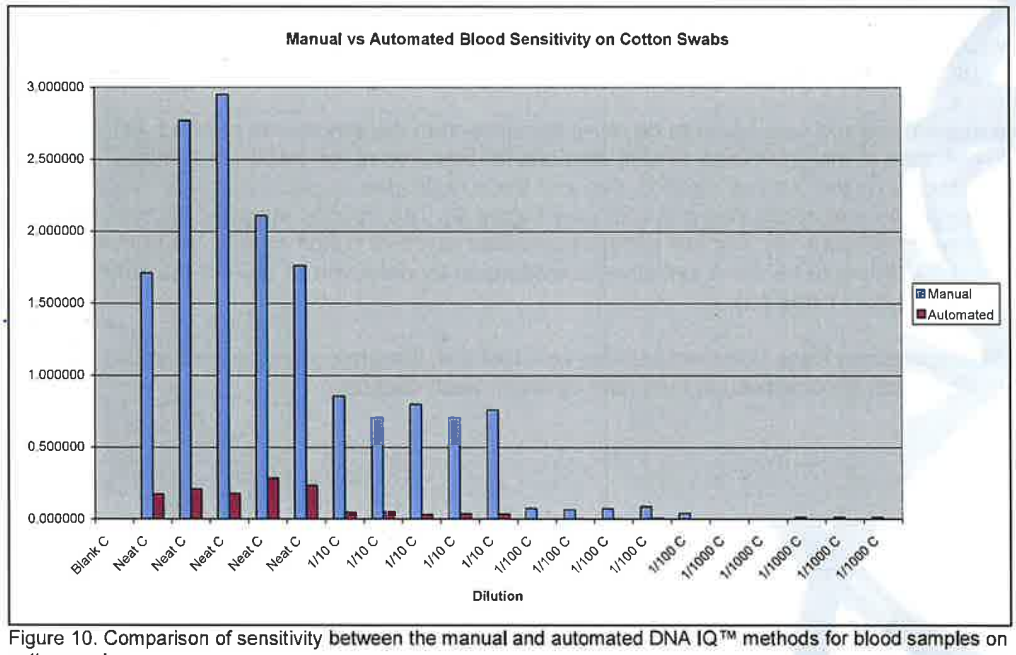


Figure 10. Comparison of sensitivity between the manual and automated DNA IQ™ methods for blood samples on cotton swabs.

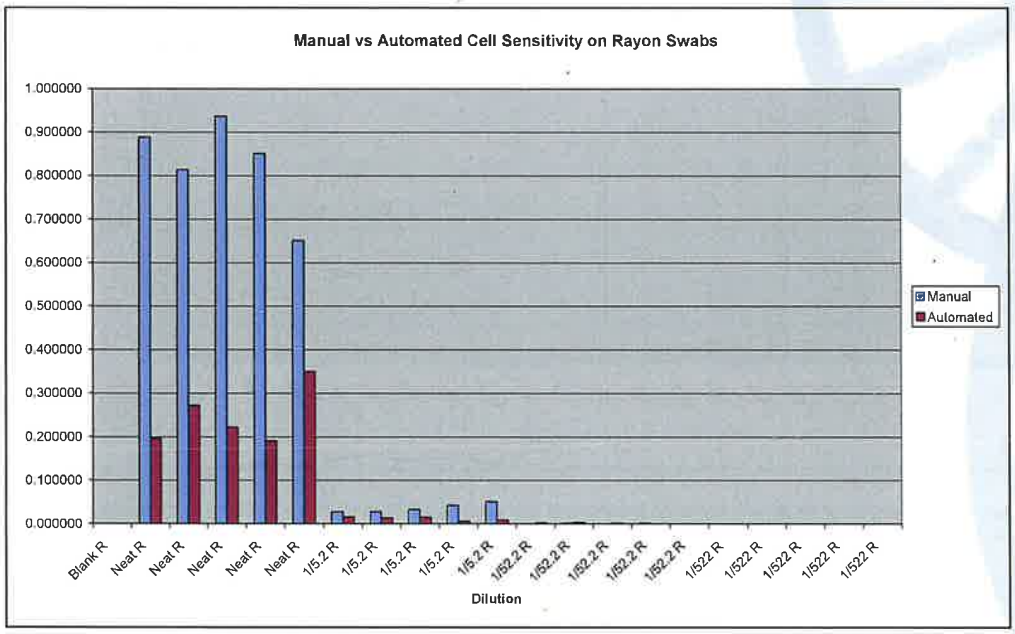


Figure 11. Comparison of sensitivity between the manual and automated DNA IQ™ methods for cells samples on rayon swabs.

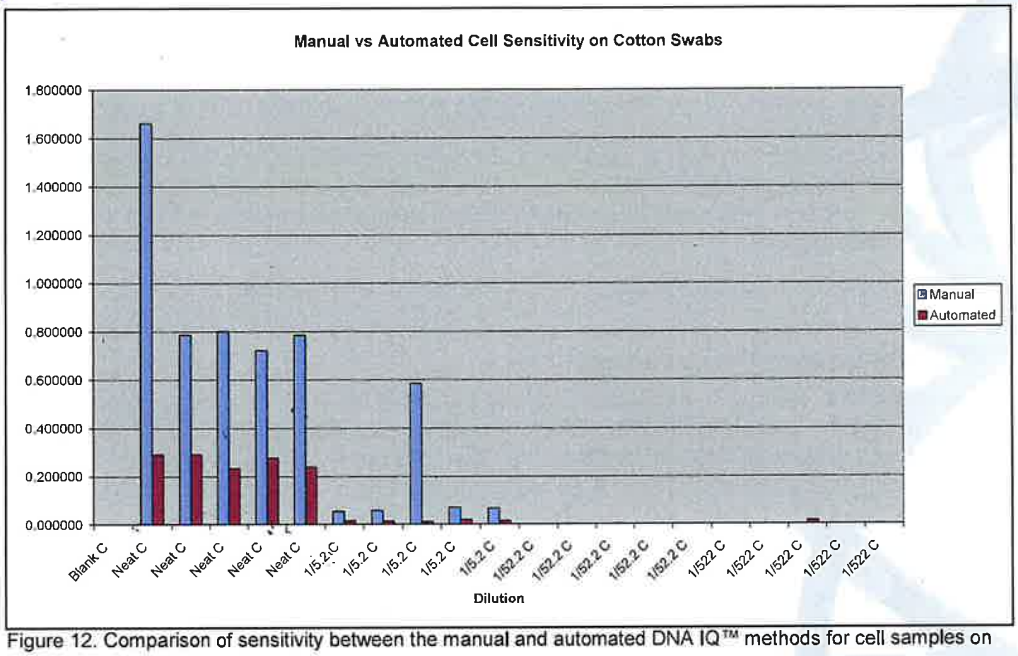


Figure 12. Comparison of sensitivity between the manual and automated DNA IQ™ methods for cell samples on cotton swabs.

## 6.5 Investigating resin volume

Promega recommends the use of 7µL of DNA IQ™ resin with their protocol. We investigated the performance of the protocol with double the amount of resin (14µL) in order to assess any benefits that may be gained in terms of the resulting yield and quality of the STR profile.

It was observed that doubling the resin resulted in a proportional doubling of the yield. On average, doubling the resin increased the yield by an additional 77.28% (*n*=4). The average yield from an extraction using 7µL of resin was 64.725ng (SD 32.21ng, *n*=4), whereas 14µL resin generated 114.75ng (SD 10.72ng, *n*=4) (Table 6). At the higher resin concentration, the amount of DNA isolated appears to be capped at around 100ng, indicating no change in the ability of the reaction to isolate more DNA due to saturation of resin.

Table 6. Comparison of the effects of doubling the amount of recommended DNA IQ™ resin.

| Sample ID | Resin volume | [DNA] ng/µL | Reportable alleles |
|---|---|---|---|
| 33383-4216 | 7µL | 0.701 | 18/18 |
| 33383-4225 | | 1.070 | 18/18 |
| 33383-4239 | | 0.319 | 18/18 |
| 33383-4248 | | 0.499 | 18/18 |
| 33383-4252 | 14µL | 1.140 | 18/18 |
| 33383-4261 | | 1.270 | 18/18 |
| 33383-4270 | | 1.010 | 18/18 |
| 33383-4284 | | 1.170 | 18/18 |

Samples extracted using either amount of resin generated concordant full DNA profiles (18/18 alleles). Samples processed using the 14µL method produced peaks that were slightly higher. The difference in peak heights between alleles within the same loci ranged from 59-86%, with a mean of 71%, indicating minimal difference between the two methods.

Doubling the amount of resin did not appear to provide any additional benefits compared to the original recommended protocol. More importantly, full DNA profiles were resolved using either method. Therefore, the costs associated with increasing the amount of resin cannot be justified at this stage.

## 6.6 Modifying extraction volumes

An investigation into optimising extraction volumes ranging from 300µL to 500µL was performed in order to ensure that buffer coverage over the samples was sufficient to enable optimal lysis and release of DNA. In addition, the use of an optimum volume of extraction reagents increases efficiency and economy, therefore potentially lowering laboratory costs.

Although the higher extraction volume generated higher yields when processed using the automated DNA IQ™ protocol (Table 7), DNA profile results were comparable across the various extraction volumes tested for eight replicates each (Table 8). Three instances of allelic imbalance were encountered in two samples from the 300µL and 450µL tests. In all instances, allelic imbalance was greater than 69%.

Table 7. DNA profile results for samples extracted using various volumes of Extraction Buffer, for 8 replicates.

| Extraction Buffer Volume (µL) | Mean [DNA] (ng/µL) | SD |
|---|---|---|
| 300 | 2.04 | 0.07 |
| 350 | 2.16 | 0.09 |
| 400 | 1.69 | 0.10 |
| 450 | 3.14 | 0.13 |
| 500 | 3.64 | 0.17 |

Table 8. DNA profile results for samples extracted using various volumes of Extraction Buffer, for 8 replicates.

| Sample | Extraction Buffer Volume (µL) | DNA Profile Result |
|---|---|---|
| 300-1 swab | 300 | OK |
| 300-2 swab | | OK |
| 300-3 swab | | OK |
| 300-4 swab | | OK |
| 300-5 swab | | OK |
| 300-6 swab | | OK |
| 300-7 swab | | AI D13 |
| 300-8 swab | | OK |

| | | |
|---|---|---|
| 350-1 swab | 350 | OK |
| 350-2 swab | | OK |
| 350-3 swab | | OK |
| 350-4 swab | | OK |
| 350-5 swab | | OK |
| 350-6 swab | | OK |
| 350-7 swab | | OK |
| 350-8 swab | | OK |
| 400-1 swab | 400 | OK |
| 400-2 swab | | OK |
| 400-3 swab | | OK |
| 400-4 swab | | OK |
| 400-5 swab | | OK |
| 400-6 swab | | OK |
| 400-7 swab | | OK |
| 400-8 swab | | OK |
| 450-1 swab | 450 | OK |
| 450-2 swab | | OK |
| 450-3 swab | | OK |
| 450-4 swab | | OK |
| 450-5 swab | | OK |
| 450-6 swab | | OK |
| 450-7 swab | | Al vWA, D18 |
| 450-8 swab | | OK |
| 500-1 swab | 500 | OK |
| 500-2 swab | | OK |
| 500-3 swab | | OK |
| 500-4 swab | | OK |
| 500-5 swab | | OK |
| 500-6 swab | | OK |
| 500-7 swab | | OK |
| 500-8 swab | | OK |

### 6.7 Sensitivity of the automated DNA IQ™ protocol

DNA was detected from samples that were diluted down to 1:1000 (Figure 13).

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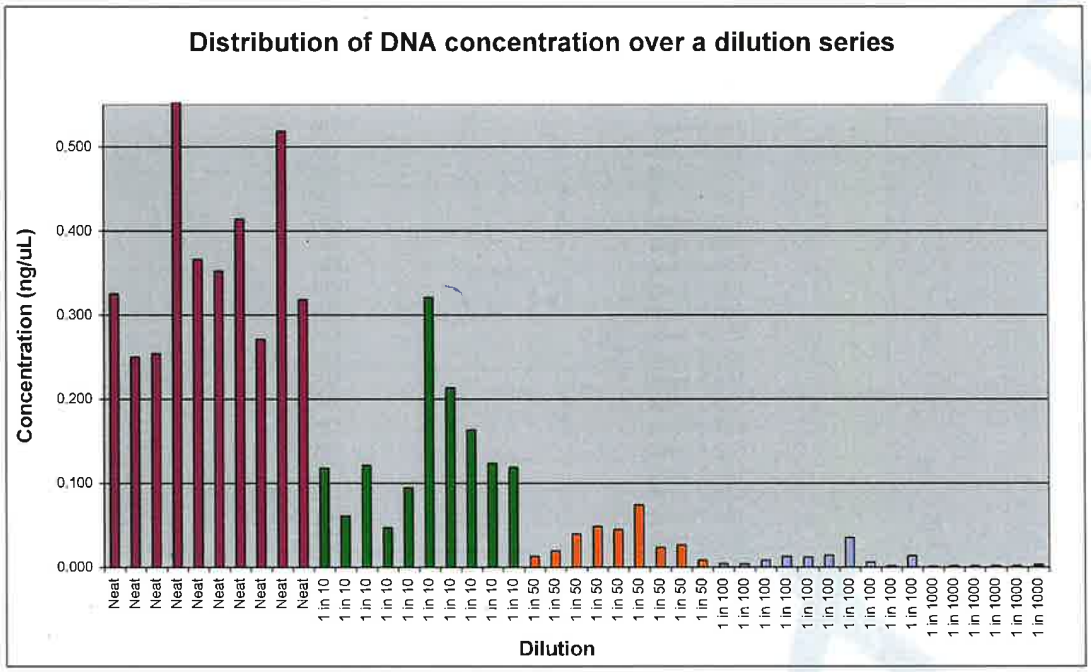


Figure 13. DNA IQ™ sensitivity across various dilutions

as

- results not consistent with fig 9 + 10.

## 7. Summary and Recommendations

We recommend the following:

- Use of MPII for automated extraction of reference samples
- Use of MPII for automated extraction of casework samples
- Ongoing development of the automated extraction program to increase the efficiency of the extraction

Sweeney, P.J. and Walker, J.M., Burrell, M.M., Enzymes of molecular biology. *Methods Mol. Biol.* Towanam NJ , (1993) **16**, 306