Phase 1 Report- Verification of Promega DNA IQ™ for the Maxwell®16

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1 Abstract

Implementation of the Promega Maxwell®16 MDx instrument will provide an alternative to the current manual and automated (liquid handling units) DNA IQ™ methods of extracting DNA to supplement the existing high throughput and to improve workflow efficiency. Initially pre-lysis methods were tested to determine which method gave acceptable results and then would be used for the remainder of the verification. It was determined that the Promega recommended procedure with a few modifications was deemed to be the most suitable pre-lysis procedure. For the repeatability and reproducibility studies blood samples were found to have acceptable results, whereas cell samples were initially found to be variable when processed using the Maxwell®16 MDx instruments. This was due to a single batch that produced yields below expectation and further testing of cell substrates demonstrated reproducible, repeatable results. The Promega Maxwell®16 MDx instrument with a modified Promega procedure was comparable or outperformed the Manual DNA™ IQ method in the sensitivity studies. There was no evidence suggesting cross contamination occurred between any of the extraction batches performed for each experiment on either of the Maxwell®16 MDx instruments. The use of the Promega Maxwell®16 MDx instruments has been shown to be an acceptable alternative to manual DNA IQ™ method and is suitable for routine use in DNA Analysis Unit.

2 Background

The Promega Maxwell®16 MDx instrument is a pre-programmed, automated paramagnetic particle handler that is specifically designed for optimal DNA extraction of forensic casework samples using the Promega DNA IQ™ chemistry. Samples undergo a pre-processing step prior to DNA extraction and are then added to disposable cartridges containing pre-dispensed, ready to use extraction reagents. The Maxwell®16 MDx instrument can process up to 16 samples taking approximately 30 minutes.

3 Purpose

The aim of this study was to assess the suitability, reproducibility, repeatability, sensitivity and cross-contamination of the Maxwell®16 MDx instruments for the purpose of extracting DNA from blood and cell swabs. This study also aimed to verify the Maxwell®16 MDx instrument using Promega DNA IQ™ chemistry to provide a comparable alternative method to current in-use protocols for routine processing of casework and reference samples as per operational requirements of DNA Analysis Unit.

4 Equipment and Materials

- STORstar instrument (Process Analysis & Automation, Hampshire, UK)
- MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (PerkinElmer, Downers Grove, IL, USA)
- ABI 7500 Real Time PCR System (Life Technologies Applied Biosystems, Foster City, CA, USA)
- GeneAmp PCR system 9700 (Life Technologies Applied Biosystems, Foster City, CA, USA)
- ABI 3130*xl* Genetic Analyzer (Life Technologies Applied Biosystems, Foster City, CA, USA)
- Promega Maxwell® 16 MDx A and B Instrument (Promega Corp., Madison, WI, USA)
- 5804 centrifuge (Eppendorf,Germany)
- 5424 centrifuge (Eppendorf,Germany)
- Vortex (Ratek)
- Minifuge (Tomy)

- Miscellaneous consumables and labware (eg 1.5mL screw-cap tubes, pipettes, pipette tips, 96-well PCR plates, 2.0mL sterile screw-cap tubes)
- Sterile Conductive Filtered Roborack 175µl and 25µl disposable tips (PerkinElmer, Downers Grove, IL, USA)
- Cytobrush™ Plus Cell Collector devices (Cooper Surgical, Inc.,)
- Baxter 0.9% saline solution
- Sterile rayon swabs (Copan Italia SPA, Brescia, Italy)
- DNA IQ™ System Kit 400 sample kit (Promega Corp., Madison, WI, USA)
- DNA IQ™ casework Pro Kit for Maxwell® 16 (Promega Corp., Madison, WI, USA)
- Reagents
    - TNE
    - Proteinase K (20mg/mL) (Sigma)
    - DTT (Dithiothreitol) 1M (Sigma)
    - 5% v/v Trigene
    - 70% v/v and 100% v/v Ethanol
    - 5% v/v Bleach
    - 1% v/v Amphyl
    - 0.2% v/v Amphyl
    - 40% w/v Sarcosyl
    - Analytical Positive Control lot#29102010
    - Nuclease Free Water
    - Isopropyl Alcohol
- Quantifiler™ Human DNA Quantification kits (Life Technologies Applied Biosystems, Foster City, CA, USA)
- Promega Genomic Male DNA G147A (Promega Corp., Madison, WI, USA)
- AmpFℓSTR® Profiler Plus® PCR Amplification kits including 9947A control DNA (Life Technologies Applied Biosystems, Foster City, CA, USA)
- Hi-Di™ Formamide (Life Technologies Applied Biosystems, Foster City, CA, USA)
- 3130 POP-4™ Polymer (Life Technologies Applied Biosystems, Foster City, CA, USA)
- GeneScan™ 500 ROX™ Size Standard(Life Technologies Applied Biosystems, Foster City, CA, USA)
- Running Buffer (Life Technologies Applied Biosystems, Foster City, CA, USA)
- AmpFℓSTR® Profiler Plus® Allelic Ladder (Life Technologies Applied Biosystems, Foster City, CA, USA)
- GeneMapper-*IDX* ver. 1.1.1(Life Technologies Applied Biosystems, Foster City, CA, USA)

5 Methods

5.1 Sample Creation

5.1.1 Collection procedure for buccal cells

Buccal cells were collected from a donor using the Cytobrush™ method. 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.

5.1.2 Collection procedure for blood

A donor (different to the buccal donor) was selected and 10mL of blood was collected in EDTA tubes by a qualified phlebotomist and stored at 4°C until it was required for use.

5.1.3 Sample creation for swabs with buccal cells

Four collections of buccal cells were made and combined to ensure a uniform suspension. Pipetting of the buccal suspension and drying of swabs was performed in a Class II biohazard cabinet. Working areas were decontaminated using 10% v/v bleach and 70% v/v ethanol.

49 swabs were prepared for extraction, swab heads were cut away from the stick of the swab using a sterile scalpel and forceps. The swab heads were placed upside (end of swab head pointing up) into 2mL tubes ready for the cells to be spotted on.

The buccal cell suspension was resuspended by vortexing prior to dispensing onto swabs.

30µL of cell suspension was dispensed onto 49 swabs. Swabs were dried in an open 2mL tube at 56°C on a dry block heater for 2 hours.

Once dry, the swabs were inverted so the swab head was pointing down in the bottom of the tube, re-capped and stored at ≤-10°C.

5.1.4 Sample creation for swabs with blood

Pipetting of blood and drying of swabs was performed in a Class II biohazard cabinet. Working areas were decontaminated using 10% v/v bleach and 70% v/v ethanol.

77 swabs were prepared for extraction, swab heads were cut away from the stick of the swab using a sterile scalpel and forceps. The swab heads were placed upside (end of swab head pointing up) into 2mL tubes ready for sample creation.

The blood was resuspended by vortexing prior to dispensing onto swabs.

30µL of blood was dispensed onto 56 swabs. Swabs were dried in the open 2mL tube at 56°C on a dry block heater for 2 hours.

Once dry, the swabs were inverted so the swab head is pointing down in the bottom of the tube, re-capped and stored at ≤-10°C.

A series of seven samples each with a different amount of blood were created in triplicate (three swabs per volume) as per Table 1. The blood was resuspended by vortexing prior to pipetting onto swabs.

Table 1 Volume of blood added to swabs

Sample	Volume of blood
1	60µL
2	30µL
3	15µL
4	5µL
5	2µL
6	1µL
7	0.5µL

Swabs were dried in an open 2mL tube at 56°C on a dry block heater for 2 hours.

Once dry, the swabs were inverted so the swab head was pointing down in the bottom of the tube re-capped and stored at ≤-10°C.

5.2 Extraction

Samples were extracted using the Promega DNA IQ™ System kit according to either the current in house standard laboratory procedure (QIS 24897 DNA IQ™ Method of Extracting DNA from Casework and Reference samples) or to Technical Manual DNA IQ™ Casework Pro Kit for Maxwell®16 (Part# TM332 revised 10/10 - recommended procedure from the manufacturer). The latter protocol was revised during the course of this verification to include;

- combining Proteinase K and DTT into the initial extraction buffer before adding to each sample,
- an additional pulse spin after incubation and prior to the addition of lysis buffer and
- an increase in the final elution volume from 50µL to 100µL.

This revised method is referred to as the 'modified Promega method' in this report.

5.3 Quantification

All quantification reaction setups were performed using a MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform and quantified according to the standard laboratory procedure (QIS 19977 'Automated Quantification of Extracted DNA using the Quantifiler Human DNA Quantitation Kit').

5.4 Amplification

All samples were amplified with the Applied Biosystems AmpFℓSTR® Profiler Plus® PCR Amplification Kit at the volumes calculated from the quantification result. Approximately 1.2ng of DNA template was added for amplification reaction. The PCR reaction was set up using a MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform and amplified according to the standard laboratory procedures (QIS 19976 "Amplification of Extracted DNA using the AmpFlSTR® Profiler Plus® kit or AmpFlSTR® COfiler® Kit").

5.5 DNA Fragment Analysis and Profile Interpretation

All samples were sent for capillary electrophoresis and processed according to the standard laboratory procedure (QIS 15996 'Procedure for the use and Maintenance of the AB 3130xl Genetic Analyzers). All samples were analysed according to the standard laboratory procedure (QIS 17130 'CE Quality Check of Samples from the ABI Prism 3130xl Genetic Analyzers).

All sample results were interpreted using GeneMapper *ID-X* ver. 1.1.1 according to the standard laboratory procedure (QIS 17137 "Procedure for the Interpretation & Acceptance of Results using Profiler & COfiler systems').

5.6 Statistical Tests

Microsoft Excel was used to calculate averages, standard deviations, maximum and minimum values. It was also used to perform two-tailed t-tests to assess comparable data sets for significant difference, unless specified total DNA yield was used for this assessment. A *p*-value of <0.05 was considered to be significantly different.

6 Experimental Design

6.1 Experiment 1 – Suitability

Suitability studies were carried out to compare DNA yields (ng) between manual DNA IQ™ and DNA IQ™ extraction on the Maxwell®16 using both the current in-house pre-lysis method and the Promega pre-lysis method.

6.1.1 Pre-Lysis of samples for lysates to be extracted using DNA IQ™ Casework Pro Kit for Maxwell®16

Seven blood and seven buccal cell swab samples along with one positive and one negative control, were pre-lysed according to the current in-house pre-lysis procedure outlined in section 5.2.

Seven blood and seven buccal cell swab samples along with one positive and one negative control, were pre-lysed according to the Promega recommended pre-lysis procedure outlined in section 5.2.

6.1.2 Lysates to be extracted using DNA IQ™ Casework Pro Kit for Maxwell®16

Lysates obtained from the pre-lysis steps were extracted on both Maxwell®16 MDx instruments, using the recommended procedure from the manufacturer.

6.1.3 Samples extracted using Manual DNA IQ™

Seven blood and seven buccal cell swab samples along with a positive and negative control were extracted according to the in house procedure outlined in section 5.2.

6.2 Experiment 2 – Reproducibility and Repeatability

Reproducibility and repeatability studies were carried out to compare run to run variation and instrument to instrument variation. Note, due to an apparent failure of one batch of cell samples (see results and discussion), the entire experiment was repeated for the cell samples.

6.2.1 Reproducibility

The run to run variation was assessed by processing two further batches on each of the Maxwell®16 MDx instruments, using the modified Promega method outlined in section 5.2. Each batch consisted of seven buccal cell lysates, seven blood lysates, and a positive and negative control.

6.2.2 Repeatability

The instrument to instrument variation was assessed by comparing batches (using data from the reproducibility study) processed on one Maxwell®16 MDx instrument to batches processed on the other Maxwell®16 MDx instrument.

6.3 Experiment 3 – Sensitivity and DNA Yield

Sensitivity studies were carried out to show the difference in performance of the DNA IQ™ Casework Pro Kit for Maxwell®16 and the DNA IQ™ manual extraction using different volumes of blood applied to swabs.

6.3.1 Sensitivity testing

A sensitivity series with duplicate blood samples with volumes of 60µL, 30µL, 15µL, 5µL, 2µL, 1.0µL and 0.5µL and a positive and negative control were extracted using the modified Promega method on instrument Maxwell®16 A.

A further sensitivity series of blood samples with volumes of 60µL, 30µL, 15µL, 5µL, 2µL, 1.0µL and 0.5µL and a positive and negative control were extracted manually according to the current in house procedures outlined in section 5.2.

6.4 Experiment 4 – Cross-Contamination

Cross-contamination studies were carried out to determine whether any cross contamination occurs during the extraction process and to show no cross contamination occurred between extraction batches on the Maxwell®16 MDx instruments.

6.4.1 Cross- Contamination

Eight blank lysates and eight blood lysates containing DNA were placed on the Maxwell®16 MDx instrument in an alternating pattern and were extracted using the modified Promega method.

7 Results and Discussion

7.1 Suitability

The average DNA yields produced for blood and cell samples processed through DNA IQ™ extraction on the Maxwell®16 instruments using the current in-house pre-lysis method (DNA Analysis) and the Promega pre-lysis method and the manual DNA IQ™ process are shown in Figure 1 below.

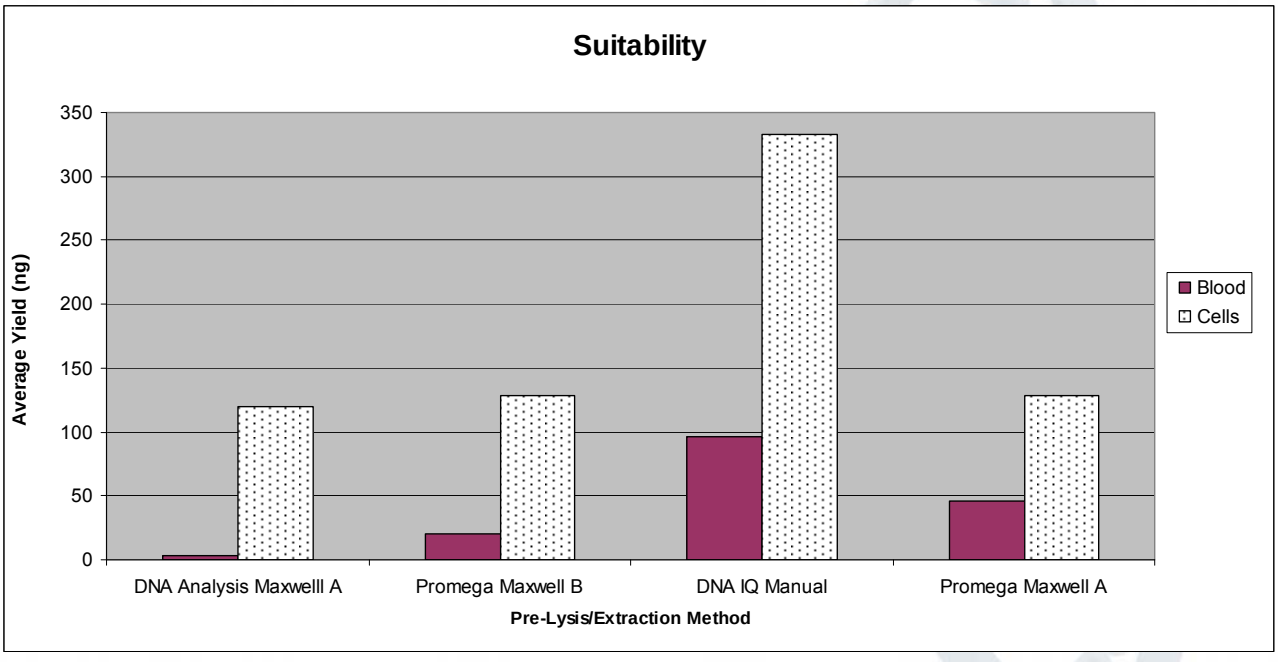

Figure 1 Comparison of in-house and Promega recommended pre- lysis procedure and Manual DNA IQ™.

A summary of the average yield, standard deviation, maximum and minimum yield values obtained for each method and sample type tested is outlined in Table 2 below.

Table 2 Summary of suitability results

Lysis / extraction Method	Sample type	Instrument	Average Yield (ng)	Standard deviation	Maximum yield (ng)	Minimum yield (ng)
DNA Analysis	Blood	A	3.63	1.90	7.10	1.01
DNA Analysis	Cell	A	120.21	42.79	183.00	76.50
Promega	Blood	B	20.63	6.28	26.75	11.65
Promega	Cell	B	128.29	44.67	198.00	80.50
DNA IQ Manual	Blood	N/A	96.56	28.78	136.00	46.80
DNA IQ Manual	Cell	N/A	332.57	87.30	489.00	238.00
Promega	Blood	A	45.76	11.47	59.00	27.20
Promega	Cell	A	128.14	23.81	172.50	104.00

The original validation of the manual DNA IQ™ chemistry gave an average yield of 317ng for blood swabs with a standard deviation of 102.36; cell swabs produced an average yield of 134.54ng with a standard deviation of 41.30 (Nurthen *et al*., 2007). The results of the manual DNA IQ™ in this verification showed a significantly lower yield with a lower standard deviation for the blood swabs

and a much greater yield for the cell swabs with an increased standard deviation when compared to the original validation of DNA IQ™ chemistry.

The average DNA yields for blood samples extracted using manual DNA IQ™ (refer section 6.1.3 above) were significantly higher than yields obtained using DNA IQ™ extraction on the Maxwell®16 instruments (refer section 6.1.2 above) using both the current in-house pre-lysis method (p = 0.000136345) and the Promega pre-lysis method (p = 0.000329464). The average DNA yields for cell samples extracted using manual DNA IQ™ were significantly higher than yields obtained using DNA IQ™ extraction on the Maxwell®16 MDx using both the current in-house pre-lysis method (p = 0.000299508) and the Promega pre-lysis method (p = 0.000383315).

The Promega pre-lysis procedure was repeated using the alternate Maxwell®16 instrument. The average DNA yields compared to manual DNA IQ™ also showed a significant difference for blood samples (p = 0.002593137) and cell samples (p = 0.000589507).

The Promega pre-lysis method outperformed the current in-house pre-lysis method and was subsequently deemed to be the most suitable for DNA Analysis' applications. This is most likely due to lack of DTT present in the buffer used with the current in-house pre-lysis method.

The relatively low yield noted with the Promega pre-lysis method coupled with extraction on the Maxwell®16 MDx compared with the routine manual DNA IQ™ procedure was possibly due to the difference in elution volume (the manual method uses a "double elution" method resulting in 100µL of eluent, the standard Maxwell®16 MDx protocol results in a 50µL elution).

To improve yield values and bring this process in line with manual DNA IQ method small modifications were made to the published protocol in the Promega Technical Manual (refer section 5.2 above). This protocol was revised to include;

- combining Proteinase K and DTT into the initial extraction buffer before adding to each sample and,
- an increase in the final elution volume from 50µL to 100µL.

Note: This revised method is referred to as the 'modified Promega method'. This was used for all subsequent experiments (refer sections 6.2, 6.3 and 6.4 above)

The average yields produced for blood and cell samples processed through DNA IQ™ extraction on Maxwell®16 A and B with the modified Promega method (refer section 5.2 above) compared to the manual DNA IQ™ method (refer to section 6.1.3 above) is shown in Figure 2 below.

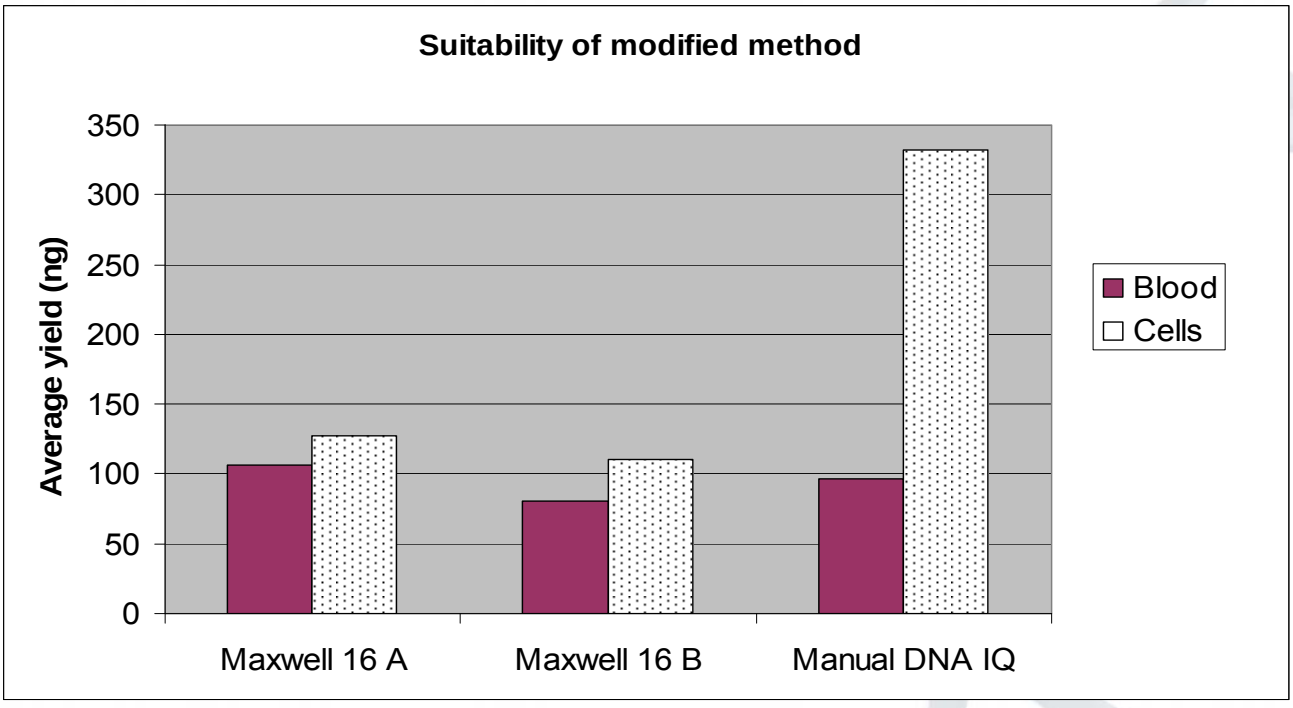

Figure 2 Average yield obtained with 100µL elution volume.

A summary of the average yield, standard deviation, maximum and minimum yield values obtained for the modified Promega method and sample type tested is outlined in Table 3. Also shown are the results from the manual DNA IQ™ extraction previously shown in Table 2.

Table 3 Summary of results for suitability of modified Promega method.

Lysis / extraction Method	Sample type	Instrument	Average Yield (ng)	Standard deviation	Maximum yield (ng)	Minimum yield (ng)
Promega (modified)	Blood	A	106.36	9.47	116	92.10
Promega (modified)	Cell	A	127.29	7.76	142	119.00
Promega (modified)	Blood	B	92.27	9.53	103	78.60
Promega (modified)	Cell	B	110.04	25.29	136	64.50
DNA IQ Manual	Blood	N/A	96.56	28.78	136	46.80
DNA IQ Manual	Cell	N/A	332.57	87.30	489	238

Using the modified Promega method the yield of the blood samples improved showing no significant difference when comparing manual DNA IQ™ to Maxwell®16 A (p = 0.419380318) and Maxwell®16 B (p = 0.719012613). The yields for the cell samples were significantly different when comparing manual DNA IQ™ to Maxwell®16 A (p = 0.000766146) and Maxwell®16 B (p = 0.000341129). This significant difference results from the manual DNA IQ ™ cell extraction producing much higher than expected yields. Differences in operators, shaking, incubation time, equipment used and preparation of mock samples could contribute to the difference in results. It is also possible that the binding capacity for the pre-dispensed resin had been reached in some cartridges therefore limiting the yields obtained from the extraction on the Maxwell®16 MDx instruments.

DNA profiles obtained from all the blood and cell samples gave the expected profile in all suitability studies with no evidence of cross contamination.

As a result of the suitability studies the modified Promega method was employed for the repeatability, reproducibility, sensitivity and cross-contamination testing (refer sections 6.2, 6.3 and 6.4 above).

7.2 Repeatability and Reproducibility

7.2.1 Run to Run Variation- Repeatability

The average yields obtained for blood and cell samples from each of the extraction batches performed on Maxwell®16 A and Maxwell®16 B are shown in Figure 3.

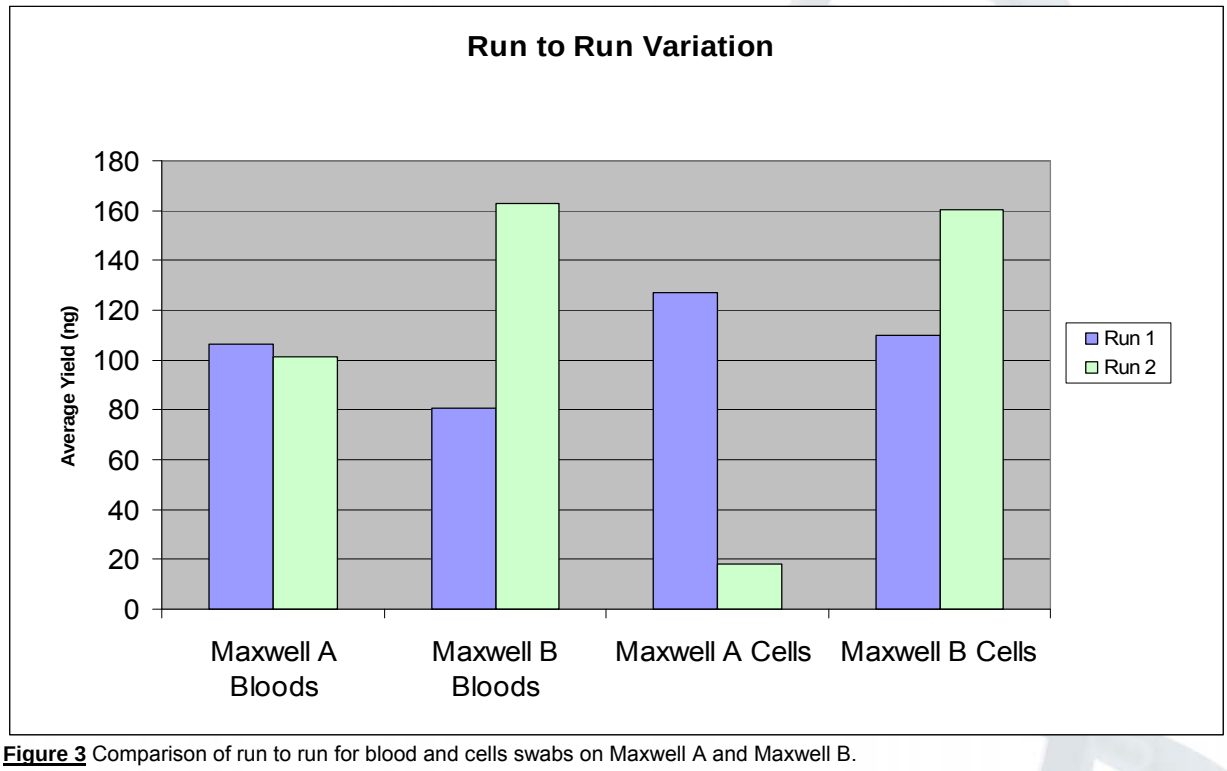

Figure 3 Comparison of run to run for blood and cells swabs on Maxwell A and Maxwell B.

A summary of the data for the repeatability studies, comparing run to run variation for each of the Maxwell®16 MDx instruments for blood and cells is outlined in Table 4.

Table 4 Summary of Repeatability Tests

Instrument	sample type	Run number	Average Yield (ng)	Standard deviation	Maximum yield (ng)	Minimum yield (ng)
A	Blood	1	106.36	9.47	116	92.10
A	Blood	2	101.31	41.14	134	19.00
B	Blood	1	92.27	9.53	103	78.60
B	Blood	2	163.00	41.41	221	119.00
A	Cells	1	127.29	7.76	142	119.00
A	Cells	2	18.32	46.60	124	0.32
B	Cells	1	110.04	25.29	136	64.50
B	Cells	2	160.14	34.46	207	117.00

This data shows average yield, the standard deviation and the maximum and minimum yields for each run. The standard deviation increased on batch 2 compared to batch 1 on Maxwell®16 A for blood samples due to a wide range of yields as seen in Table 4. This was also evident for blood samples on Maxwell®16 B. This range of standard deviation is similar to that observed with the original validation of the DNA IQ chemistry as outlined above and is similar to that observed with manual DNA IQ™ results obtained in this verification.

The cell data for run 2 on Maxwell®16 A was also variable, with one sample showing a yield of 124ng and the other 6 samples giving yields less than 1.1ng which were unexpectedly low. The approximate yield for this extraction was expected to be 100ng. The yield for the positive extraction control for this run (data not shown) was consistent with the yields observed for the positive extraction controls for the other runs shown in Table 4. Therefore, the inconsistency observed in the 2nd run of cells on Maxwell®16 A indicates that the instrument itself was not the cause of the low yield values, rather the cause was likely to be related to sample creation or the pre-lysis procedure (possible operator error).

The DNA yields from the first run compared to the second run on Maxwell®16 A for blood samples showed no significant difference (p = 0.761677182), indicating acceptable repeatability.

The DNA yields compared from the first run and second run on Maxwell®16 B for blood samples showed a significant difference (p = 0.003577971). The second batch outperformed the first batch as can be seen in Table 4. The improvement in yield for blood samples on the second batch from Maxwell®16 B may be due to a difference in mixing of the samples and centrifugation of the samples after incubation. This removed the liquid from the lids prior to the addition of lysis buffer allowing the lysis buffer access to all of the liquid containing DNA. These minor changes in technique improved the results and were utilised in later experiments. Additionally, the effect noted may also have been related to the wide standard deviation associated with the method relative to the average yield.

There was a significant difference (p = 0.000720208) in DNA yields from the first batch to the second batch on Maxwell®16 A for cell samples. The difference between the cell samples on the second batch on Maxwell®16 A gave unexpectedly low yields compared to the yields obtained on the first batch as discussed above.

The DNA yields compared from the first batch and second batch on Maxwell®16 B for cell samples also showed a significant difference (p = 0.010074). Cell samples on the second batch on Maxwell®16 B gave higher yields than the cell samples extracted on the first batch. The difference in yield between runs for the cell samples on Maxwell®16 B may be due to difference in mixing of the samples and centrifugation of the samples after incubation to remove lysate from the lids as discussed above. Additionally, the effect noted may also have been related to the wide standard deviation associated with the method relative to the average yield.

7.2.2 Instrument to Instrument Variation- Reproducibility

The average yields obtained for 14 blood swabs run on Maxwell®16 A compared to 14 blood swabs run on Maxwell®16 B and 14 cell swabs run on Maxwell®16 A and 14 cell swabs on Maxwell®16 B are shown in Figure 4.

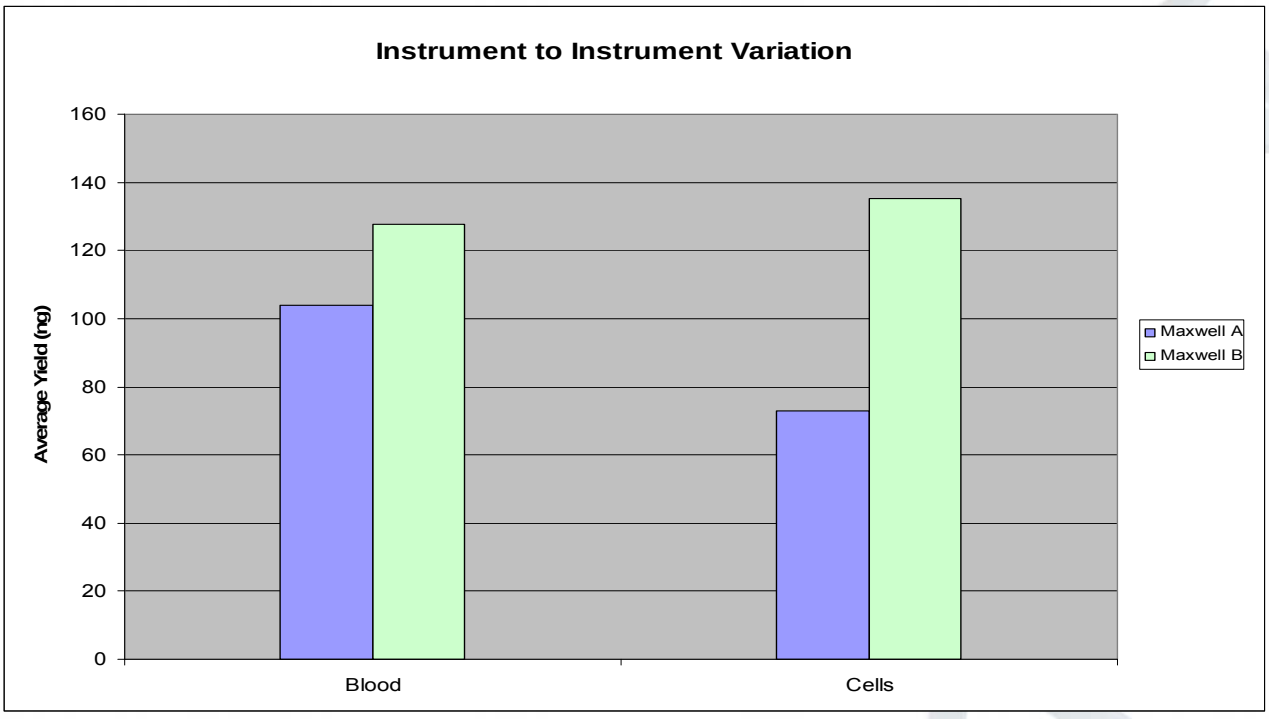

Figure 4 Comparison of Maxwell A to Maxwell B for blood and cell swabs.

The average yields, standard deviation and maximum and minimum for instrument to instrument comparison are displayed in Table 5.

Table 5 A summary of the Instrument to Instrument Testing

Instrument	sample type	Average Yield (ng)	Standard deviation	Maximum yield (ng)	Minimum yield (ng)
A	Blood	103.84	28.80	134	19.00
B	Blood	127.64	46.69	221	78.60
A	Cells	72.81	65.01	142	0.32
B	Cells	135.09	38.97	207	64.50

The DNA yields comparing blood samples between Maxwell®16 A and Maxwell®16 B showed no significant difference (p = 0.119016). The DNA yields comparing 14 cell swabs on Maxwell®16 A and 14 cell swabs on Maxwell®16 B was significantly different (p = 0.005689). This was due to the unexpectedly low yield results obtained from the second run on Maxwell®16 A as previously discussed.

Comparing the DNA yields for cell swabs between Maxwell®16 A and Maxwell®16 B excluding the low yield second batch results from Maxwell® 16 A, a t-test showed no significant difference (p = 0.481774). This suggested, as discussed above, that there had been a problem with the samples or the pre-lysis treatment rather than the instrument itself. Further testing was carried out, and these results are shown in 7.2.3 below.

7.2.3 Further testing

After a preliminary report was presented to the Management Team of the DNA Analysis Unit, it was decided the overall variability of the cell aspect of this verification was unacceptable and further testing was requested.

The reproducibility and repeatability experiments were repeated with new cell substrates created from a new collection of buccal cells from the same donor. Figure 5 shows the average yields from the further testing of cell samples.

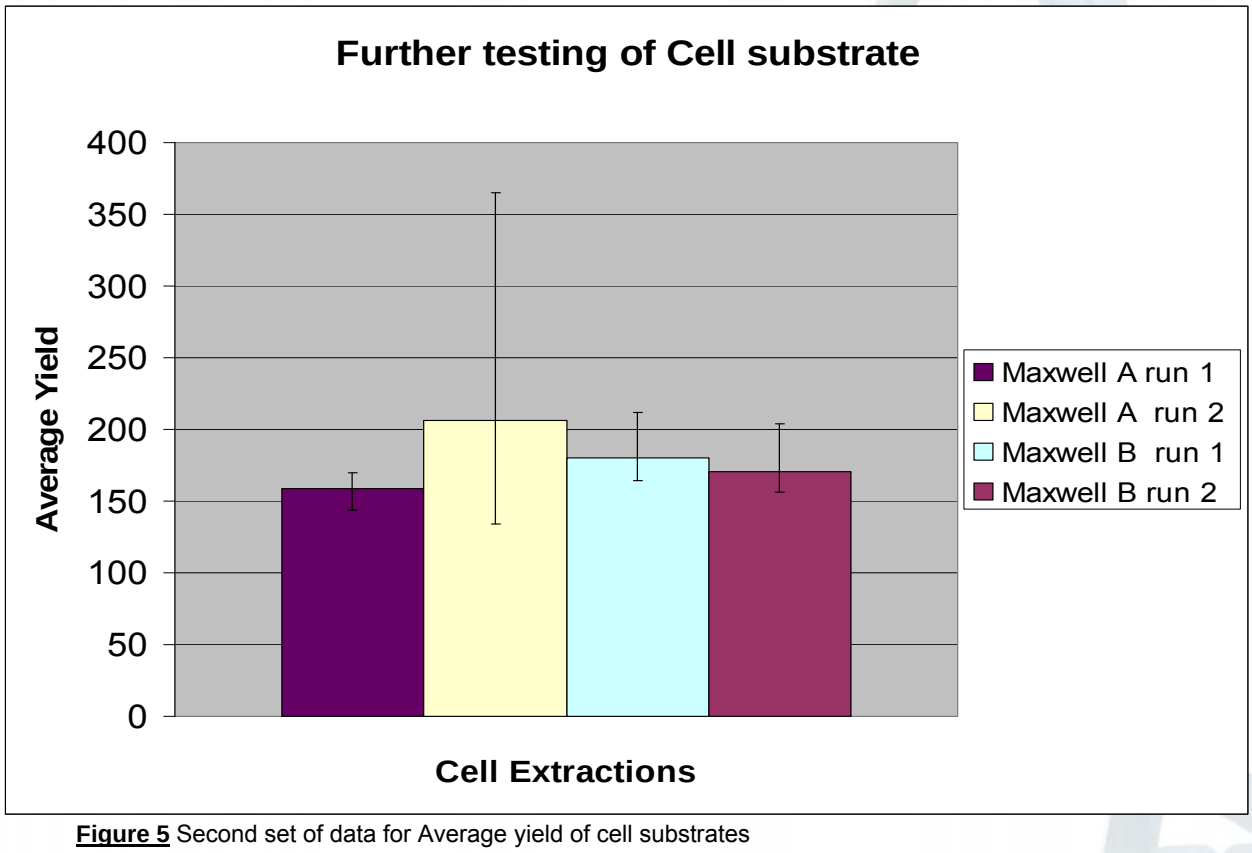

Figure 5 Second set of data for Average yield of cell substrates

Table 6 shows the average yields, the standard deviation and the maximum and minimum of the second set of cell data. The second batch of cells processed on Maxwell® A showed a higher standard deviation when compared to the other batches; this was due to a wide range of yield values.

Table 6 Summary of the second set of cell data

Instrument	Run number	Average Yield (ng)	Standard deviation	Maximum yield (ng)	Minimum yield (ng)
A	1	158.86	9.14	170	144
A	2	206.14	73.90	365	134
B	1	180.29	19.34	212	164
B	2	170.97	16.96	204	156

T-tests using the data from the further testing of cell substrates showed no significant difference between each run on Maxwell® A (*p*= 0.142182171) and each run on Maxwell® B (*p*= 0.357212553). There was also no significant difference in the instrument to instrument comparison (*p*=0.991410996).

The data from the further testing of cell substrate showed repeatability between batches processed on Maxwell® A and Maxwell® B and reproducibility between the instruments. This supports the premise the variability seen in earlier testing was due to the cell substrates and not the instruments.

DNA profiles obtained from all the blood and cell swabs gave the expected profile in all reproducibility studies with no evidence of cross contamination.

7.3 Sensitivity Testing and DNA Yield

For the 0.5µL and 1.0µL volumes both the manual DNA IQ™ method and the modified Promega method extraction on the Maxwell®16 MDx gave similar DNA yields. For the 2µL to 60µL volumes the extraction on the Maxwell®16 MDx gave better yields than the yields obtained with the manual DNA IQ™ method as shown in Figure 5.

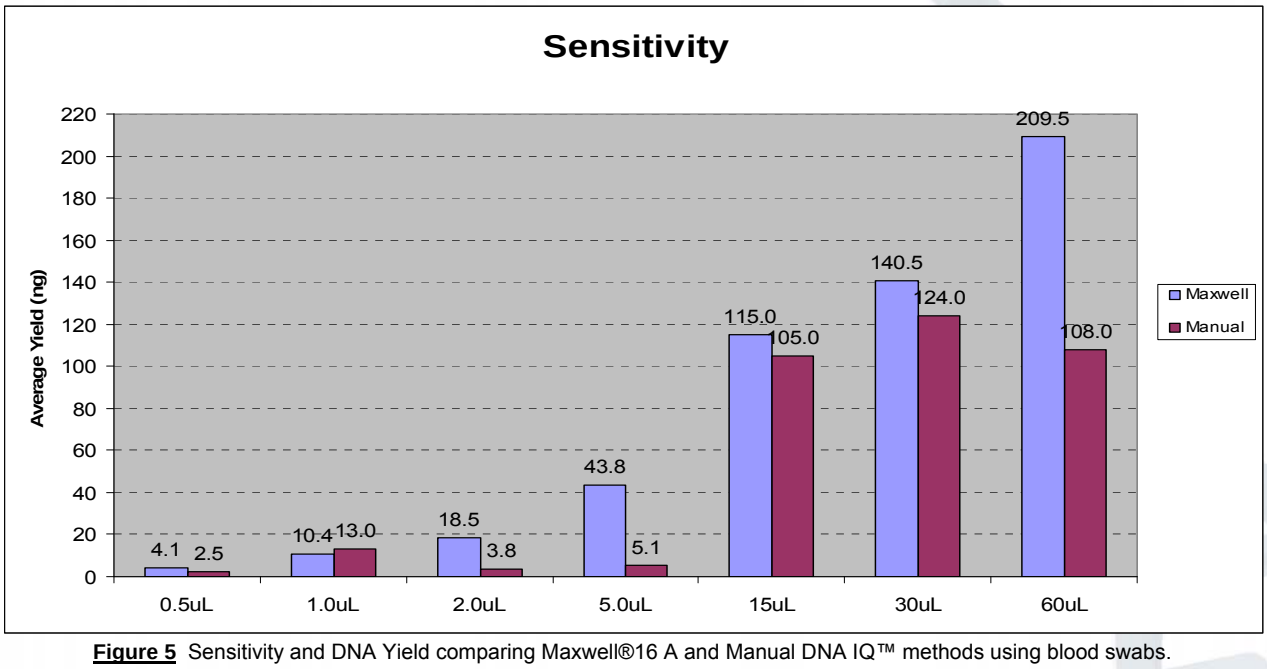

Figure 5 Sensitivity and DNA Yield comparing Maxwell®16 A and Manual DNA IQ™ methods using blood swabs.

7.4 Cross-Contamination

In an alternating pattern, eight blood samples and eight blank/negative controls were extracted using the modified Promega method on each of the Maxwell®16 MDx instruments. The quantification values, CT values and IPCCT values obtained for each of the blank controls and blood samples are shown in Table 5 and Table 6 below.

Table 5 Cross Contamination Results for Maxwell A

	Sample type	Instrument	Quant (ng/µL)	C_T	IPC
1	Negative Control	Maxwell®16 A	undetermined	undetermined	26.7
2	Positive Control	Maxwell®16 A	1.82	26.78	26.57
3	Blank Control	Maxwell®16 A	undetermined	undetermined	26.91
4	Blood swab	Maxwell®16 A	1.55	27	26.79
5	Blank Control	Maxwell®16 A	undetermined	undetermined	26.95
6	Blood Swab	Maxwell®16 A	2.49	26.34	26.69
7	Blank Control	Maxwell®16 A	undetermined	undetermined	26.89
8	Blood Swab	Maxwell®16 A	1.71	26.86	26.83
9	Blank Control	Maxwell®16 A	undetermined	undetermined	26.91
10	Blood Swab	Maxwell®16 A	1.61	26.95	26.66
11	Blank Control	Maxwell®16 A	undetermined	undetermined	26.82
12	Blood Swab	Maxwell®16 A	1.51	27.04	26.75
13	Blank Control	Maxwell®16 A	undetermined	undetermined	26.81
14	Blood Swab	Maxwell®16 A	2	26.65	26.65
15	Blank Control	Maxwell®16 A	undetermined	undetermined	26.84
16	Blood Swab	Maxwell®16 A	2.41	26.39	26.67

Table 6 Cross Contamination Results for Maxwell B

	Sample type	DNA IQ™ EXT method	Quant (ng/µL)	C_T	IPC
1	Negative Control	Maxwell®16 B	undetermined	undetermined	27.63
2	Positive Control	Maxwell®16 B	2.16	26.49	27.5
3	Blank Control	Maxwell®16 B	undetermined	undetermined	27.56
4	Blood swab	Maxwell®16 B	2.21	26.46	27.44
5	Blank Control	Maxwell®16 B	undetermined	undetermined	27.56
6	Blood Swab	Maxwell®16 B	1.82	26.73	27.42
7	Blank Control	Maxwell®16 B	undetermined	undetermined	27.72
8	Blood Swab	Maxwell®16 B	1.96	26.63	27.69
9	Blank Control	Maxwell®16 B	undetermined	undetermined	27.65
10	Blood Swab	Maxwell®16 B	2.2	26.47	27.48
11	Blank Control	Maxwell®16 B	undetermined	undetermined	27.6
12	Blood Swab	Maxwell®16 B	2	26.6	27.47
13	Blank Control	Maxwell®16 B	undetermined	undetermined	27.6
14	Blood Swab	Maxwell®16 B	1.25	27.26	27.53
15	Blank Control	Maxwell®16 B	undetermined	undetermined	27.73
16	Blood Swab	Maxwell®16 B	1.07	27.48	27.71

All blank/negative controls gave undetermined quantification values and all the blood samples gave quantification values consistent with results seen in the previous studies. The C_T (cycle threshold) values for all blank/negative controls gave values of undetermined indicating there was no DNA or not enough DNA to be amplified to reach the set cycle threshold. The blood samples gave C_T values within a range of 20-30 which is expected and is within normal range for samples with the presence of DNA.

The blank/negative controls and blood samples gave IPCCT (internal PCR control) values within a range of 20-30 which is within normal range indicating no presence of inhibitors in any of the samples. The blank/negative controls were profiled and analysed at 16RFU, which is the peak

detection threshold used for all negative controls processed in DNA Analysis Unit. The blood samples were profiled and analysed at 50RFU, which is the standard peak detection threshold for casework and reference samples. The blank/negative controls run on Maxwell®16 A displayed no DNA (NSD) profiles and the blood samples displayed excess (XS) sized peaks that were consistent with the expected profile. The blank/negative controls run on Maxwell®16 B displayed no DNA (NSD) profiles and the blood samples displayed profiles consistent with the expected profile with one sample displaying excess (XS) sized peaks.

There was no evidence suggesting cross contamination occurred between any of the extraction batches performed for each experiment on either of the Maxwell®16 MDx instruments. All blood and cell samples for the suitability, reproducibility, sensitivity and cross contamination studies obtained single source profiles with no presence of mixtures.

8 Conclusion and recommendations

This verification has determined the Maxwell®16 MDx instruments using the modified Promega method have produced repeatable, reproducible results and are suitable for routine processing of blood and cell swabs in the DNA Analysis Unit. It has also shown that this extraction procedure will give results comparable to the current routine manual DNA IQ™ method. It has also been shown that there is no evidence to suggest cross contamination between samples (between runs or between samples within a run) is likely to occur.

Therefore, it is recommended that the Maxwell®16 MDx instruments using the modified Promega method be introduced for the routine extraction of crime-scene swabs within the DNA Analysis laboratory.

It is also recommended that further investigation into the suitability of this procedure for the processing of other substrates - specifically tape-lifts and cigarette butts be carried out.

This would further enhance the workflow and throughput of DNA Analysis Unit as this technology would reduce the time taken for substrates in small batches to be processed, thereby improving current turn around times. The reduction in the amount of pipetting required compared with the labour intensive current routine manual DNA IQ™ method would also be of an occupational health and safety benefit to laboratory workers.

9 References

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