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Project 9. Report on the Evaluation of Commercial DNA Extraction Chemistries

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Automation and LIMS Implementation Project Team,

DNA Analysis

Forensic And Scientific Services

Clinical and Scientific Services

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Table 1. Extraction kits that were evaluated by Forensic Biology FSS.

DNA extraction kit and manufacturer	Technology type
DNA IQ™ (Promega Corp., Madison, WI, USA)	Novel paramagnetic beads
QIAamp® DNA Micro (Qiagen GmbH, Hilden, Germany)	Silica-based membrane
ChargeSwitch® (Invitrogen, Carlsbad, CA, USA)	Magnetic beads
*forensic*GEM™ (ZyGEM, Hamilton, NZ)	Thermophilic proteinase incubation
NucleoSpin® 8 Trace (Macherey-Nagel, Düren, Germany)	Silica-based membrane

Magnetic bead technology is based on the use of magnetic resin that has the capability to bind DNA when subjected to a particular environmental pH or ionic strength. Therefore, by using buffers with different pH values or different ionic components, the binding and elution of DNA can be controlled. Furthermore, whilst the DNA is bound to the resin, the resin-DNA complex can be washed using an alcohol-containing buffer in order to remove inhibitors and residual proteins. A magnetic force is applied during the washing procedure to immobilise the resin-DNA complex and ensure no DNA is lost during washing. Membrane technology is based on a similar principle, except the DNA is immobilised in a thin silica-based membrane within the column.

*forensic*GEM™, the recently-released one-tube proteinase incubation system, uses a thermostable enzyme to digest nucleases in order to yield a crude DNA extract. The enzyme digest method does not incorporate any washing steps, however, and therefore inhibitors are not removed from solution.

3. Aim

To evaluate several commercial DNA extraction kits (as per Table 1) that were specifically designed for forensic DNA samples, using the manufacturer's recommended manual protocols, and compare against the current in-house Chelex® protocol, in order to select a suitable kit for manual validation and automated verification.

4. Equipment and Materials

- Chelex®-100, P/N 143-2832 (Biorad, Hercules, CA, USA)
- DNA IQ™ System, P/N DC6701 (Promega Corp., Madison, WI, USA)
- QIAamp® DNA Micro Kit, P/N 56304 (Qiagen GmbH, Hilden, Germany)
- ChargeSwitch® Forensic DNA Purification Kit, P/N CS11200 (Invitrogen, Carlsbad, CA, USA)
- *forensic*GEM™ (ZyGEM, Hamilton, NZ)
- NucleoSpin® 8 Trace, P/N 740 722.1 (Macherey-Nagel, Düren, Germany)

For preparation of buffers and reagents specific for each kit, see the Methods section that is relevant for that kit.

5. Methods

5.1 Mock sample creation

Refer to document "Mock sample creation for cell and blood samples" (Gallagher *et al.*, 2007) for the detailed protocol.

8. Vortex supernatant, then pour back into original extract tube.

For all sample types

9. Vortex, then spin in centrifuge for 3min at maximum speed (~15,800g or the applicable centrifuge's maximum setting).
10. Carefully remove all but 50µL of supernatant. Leave substrate in tube with pellet.
11. Add 150µL of 20% Chelex® to each tube and vortex.

Note: When pipetting Chelex, the resin beads must be distributed evenly in the solution. Use magnetic stirrer in beaker of Chelex and wide bore pipette tips.

12. Add 4µL of Proteinase K (10mg/mL) to cells and mix gently by vortexing.
13. Incubate in 56°C water bath for 30min for blood and cell samples.
14. Vortex until mixed, then incubate in boiling water bath for 8min.
15. Vortex until mixed, then centrifuge for 3min at maximum speed (~15,800g or the applicable centrifuge's maximum setting).
16. Transfer supernatant to new labelled 1.5mL screw-capped tube leaving Chelex® beads behind.
17. Samples are stored at -20°C.

5.2.2. DNA IQ™ System (Promega Corp.)

Principle

The Promega DNA IQ™ system for small casework samples incorporates two distinct steps. The first step provides an easy, rapid, efficient and almost universal cell lysis method to extract biological materials off stains on solid supports. The second step utilised a specific paramagnetic resin that purifies DNA without extensive washing to remove the lysis reagent. The DNA IQ™ system is designed to purify DNA samples approximately 100ng or less, and is more efficient with samples containing less than 10ng of DNA.

Equipment and Materials

- DNA IQ™ System (100 samples, Cat.# DC6701) containing:
  - 0.9mL Resin
  - 40mL Lysis Buffer
  - 30mL 2X Wash Buffer
  - 15mL Elution Buffer
- MagneSphere® Magnetic Separation Stand, 12-position (Cat.# Z5342)
- DNA IQ™ Spin Baskets (Cat.# V1221)
- Microtube 1.5mL (Cat.# V1231)
- 95-100% ethanol
- Isopropyl alcohol
- 1M DTT
- 65°C heat block
- 70°C heat block
- Vortex mixer

Preparation of Buffers

- *Preparing 1X Wash Buffer*
  i. For DC6701 (100 samples), add 15mL of 95-100% ethanol and 15mL of isopropyl alcohol to 2X Wash Buffer.
  ii. Replace cap and thoroughly mix by inversion.

14. With lid open, air-dry the Resin in the MagneSphere® Magnetic Separation Stand for 5min to 15min.
15. Add 25-100µL Elution Buffer, depending on how much biological material was used. A lower elution volume ensures a higher final concentration of DNA.
16. Close the lid, vortex the tube for 2s and incubate at 65°C for 5min.
17. Remove the tube from the heat block and vortex for 2s. Immediately place on the MagneSphere® Magnetic Separation Stand.
18. Transfer the solution to a fresh tube.
19. Store the DNA extract at 4°C for short-term storage or at -20 or -70°C for long term storage.

5.2.3. *QIAamp® DNA Micro (Qiagen)*

Principle

The QIAamp® DNA Micro kit combines selective binding properties of a silica-based membrane with flexible elution volumes that is suitable for a wide range of sample materials such as small volumes of blood, blood cards, small tissue samples and forensic samples. The basic procedure consists of 4 steps:

- Lysis: the sample is lysed;
- Bind: the DNA in the lysate binds to the membrane of the QIAamp® MinElute column;
- Wash: the membrane is washed;
- Elute: DNA is eluted from the membrane.

Equipment and Materials

- QIAamp® DNA Micro kit containing:
  - QIAamp® MinElute Columns;
  - collection tubes (2mL);
  - Buffer ATL;
  - Buffer AL;
  - Buffer AW1 (concentrate);
  - Buffer AW2 (concentrate);
  - Buffer AE;
  - carrier RNA (red cap);
  - Proteinase K.
- Ethanol (96-100%)
- 1.5mL or 2mL microcentrifuge tubes (for lysis steps)
- 1.5mL microcentrifuge tubes (for elution steps)
- Pipette tips
- Thermomixer
- Microcentrifuge with rotor for 2mL tubes
- Scissors
- Blood collection cards or FTA® card
- Sterile cotton swabs
- DTT

<u>Important points before starting</u>

- Perform all centrifugation steps at room temperature (15-25°C).
- Check whether carrier RNA is required; for purification of DNA from very small amounts of sample, such as low volumes of blood (<10µL) or forensic samples, it is recommended to add

thoroughly mixed to yield a homogeneous solution. A white precipitate may form when Buffer AL is added to buffer ATL. The precipitate does not interfere with the QIAamp® procedure and will dissolve during incubation in step 5. Note: if carrier RNA is required, add 1µg dissolved carrier RNA to 300µL buffer AL.

5. Place the tube in the thermomixer or heated orbital incubator, and incubate at 70°C with shaking at 900rpm for 10min. If using a heating block or water bath, vortex the tube for 10s every 3min to improve lysis
6. Centrifuge the tube at full speed on a bench top centrifuge (20,000g; 14,000rpm) for 1min.
7. Carefully transfer the supernatant from step 6 to the QIAamp® MinElute column without wetting the rim. Close the lid, and centrifuge at 6,000g (8,000rpm) for 1min. Place the QIAamp® MinElute column in a clean 2mL collection tube, and discard the collection tube containing the flow-through.
8. If lysate has not completely passed through the membrane after centrifugation, centrifuge again at a higher speed until QIAamp® MinElute column is empty.
9. Carefully open the QIAamp® MinElute column and add 500µL Buffer WA1 without wetting the rim. Close the lid and centrifuge 6,000g (8,000rpm) for 1min. Place the QIAamp® MinElute column in a clean 2mL collection tube and discard the collection tube containing the flow-through.
10. Carefully open the QIAamp® MinElute column and add 500µL Buffer AW2 without wetting the rim. Close the lid and centrifuge at 6,000g (8,000rpm) for 1min. Place the QIAamp® MinElute column in a clean 2mL collection tube, and discard the collection tube containing the flow-through. Contact between the QIAamp® MinElute column and the flow-through should be avoided. Some centrifuge rotors may vibrate upon deceleration, resulting in the flow through, which contains ethanol, coming into contact with the QIAamp® MinElute column. Take care when removing the QIAamp® MinElute column and collection tube from the rotor, so that flow-through does not come into contact with the QIAamp® MinElute column.
11. Centrifuge at full speed (20,000g; 14,000rpm) for 3min to dry the membrane completely. This step is necessary, since ethanol carryover into the eluate may interfere with some downstream applications.
12. Place the QIAamp® MinElute column in a clean 1.5mL microcentrifuge tube and discard the collection tube containing the flow through. Carefully open the lid of the QIAamp® MinElute column and apply 45µL Buffer AE (equilibrated to room temperature) to the centre of the membrane to ensure complete elution of bound DNA. QIAamp® MinElute columns provide flexibility in the choice of elution volume.
13. Close the lid and incubate at room temperature (15-25°C) for 1min. Centrifuge at full speed (20,000g; 14,000rpm) for 1min. Incubating the QIAamp® MinElute columns loaded with Buffer AE or water for 5min at room temperature before centrifugation generally increases DNA yield.

5.2.4. *ChargeSwitch® (Invitrogen)*

Principle

ChargeSwitch® uses a novel magnetic bead-based technology known as ChargeSwitch Technology® (CST®). CST® provides a switchable surface charge, which is switched on and off by changing the pH. With a low pH buffer, the negatively charged DNA backbone binds to the positively charged beads and with a high pH buffer, DNA is eluted by neutralising the charge on the beads.

10. Allow beads to form a tight pellet by placing tube in MagnaRack™ and remove supernatant completely, without removing from rack or disturbing the pellet and discard.
11. Repeat steps 9 and 10 again.
12. Remove tube from rack, ensuring that supernatant has been completely removed and add 150µL ChargeSwitch® Elution Buffer (E5). Mix by pipetting up and down 10 times.
13. At room temperature, incubate for 1-5min then resuspend pellet and mix like in step 12.
14. Place tube in MagnaRack™ for 1min or until a tight pellet forms. Without removing tube from rack, aspirate DNA supernatant and place in a clean, sterile 1.5mL microcentrifuge tube, ensuring that the pellet is not disturbed. If elution is discoloured repeat steps 12 to 14 again.
15. Discard beads once extraction process is finished and either quantify immediately or store at -20°C.

5.2.5. *forensicGEM™ (ZyGEM)*

Principle

*forensic*GEM™ is a novel thermophilic proteinase developed as a rapid, cheap and effective DNA extraction solution for forensic laboratories that was recently released. It is a simple closed tube forensic DNA extraction method using a thermostable proteinase.

Protocols are available for blood and cell samples.

Equipment and Materials

- *forensic*GEM™ buffer
- *forensic*GEM™
- Heat block or water bath set at 75°C and 95°C
- 20µL sterile Aerosol Resistant Tips
- 0.5-10µL pipettor
- 300µL sterile Aerosol Resistant Tips
- 20-200µL pipettor
- 1mL sterile Aerosol Resistant Tips
- 50µL-1mL pipettor

Method

DNA extraction from buccal swabs using *forensic*GEM™

1. Add buccal swab to tube.
   Note: 1/4 head of swab specified but can utilise up to whole swab.
2. Add 200µL of *forensic*GEM™ buffer.
   Note: if more than 1/4 head of buccal swab is used need to add more *forensic*GEM™ buffer. Moss *et al.* (2003) added 200µL more of the *forensic*GEM™ buffer for trace samples.
3. Add 2µL of *forensic*GEM™.
   Note: *forensic*GEM™ buffer and *forensic*GEM™ can be added as a mastermix.
4. Incubate at 75°C for 15min.
5. Incubate at 95°C for 5min.
6. Remove supernatant to a new tube for storage.

DNA extraction from FTA® containing blood or salive using *forensic*GEM™

1. UV irradiate plasticware for 5min.

Method

1. Premix 25µL Proteinase K and at least 125µL buffer FLB and add to sample. Incubate the sample at room temperature for 3 hours.
2. Insert spacers "MTP/Multi 96 plate" into the vacuum manifold. Place the waste container inside the vacuum manifold and insert a MN Wash Plate into the notches of the spacers. Close the manifold with the lid.
3. Place a NucleoSpin® Trace Binding Strips inserted in Column Holder A into the rubber seal of the vacuum manifold's lid and apply the samples to the wells of the plate.
4. Add 1 volume isopropanol to 2 volumes of lysate, mix three times and transfer to NucleoSpin® Trace Binding Strips.
5. Bind genomic DNA by applying vacuum until all lysates have passed through the columns (-200mbar 2min; -600mbar 10s). Ventilate the vacuum manifold.
6. Wash silica membrane by adding 900µL Buffer B5 to each well of the NucleoSpin® Trace Binding Strips. Apply vacuum (-200mbar 1min) until all buffer has passed through the columns. Ventilate the vacuum manifold.
7. Repeat the wash procedure once.
8. After the final washing step, close the valve, ventilate the vacuum manifold and remove the wash plate and waste container from the vacuum manifold.
9. Remove any residual washing buffer from the NucleoSpin® Trace Binding Strips. If necessary, tap the outlets of the NucleoSpin® Trace Binding Strips onto a clean paper sheet (supplied with the MN Wash Plate) or soft tissue until no drops come out. Insert the column holder with NucleoSpin® Trace Binding Strips into the lid and close the manifold. Apply maximum vacuum (-600mbar) for at least 10min to dry the membrane completely. This step is necessary to eliminate traces of ethanol. Close the valve and ventilate the vacuum manifold.
10. For elution, insert spacers "Microtube Rack" into manifold and rest rack with MN Tube Strips on spacers. Insert Column Holder A with NucleoSpin® Trace Binding Strips into manifold lid. Pipette 100µL Buffer BE directly to the bottom of each well and incubate for 5min at room temperature. Apply vacuum (-400mbar 2min).

5.3 DNA quantitation

All DNA extracts were quantified using the Quantifiler™ Human DNA Quantitation kit (Applied Biosystems, Foster City, CA, USA) as per QIS 19977. Reaction setup was performed on the MultiPROBE® II PLUS HT EX (PerkinElmer) pre-PCR platform.

5.4 PCR amplification and fragment analysis

DNA extracts were amplified using the AmpFℓSTR® Profiler Plus® kit (Applied Biosystems, Foster City, CA, USA) as per QIS 19976. Reaction setup was performed on the MultiPROBE® II PLUS HT EX (PerkinElmer) pre-PCR platform.

5.5 Capillary electrophoresis and fragment analysis

PCR product was prepared for capillary electrophoresis using the manual 9+1 protocol (refer to Project 15 and QIS 19978). Capillary electrophoresis was performed on an ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) under the following conditions: 3kV injection voltage,10 sec injection time, 15kV run voltage,100µA run current, and 45min run time. Data Collection Software version 1.1 was used to collect

with the kit manufacturer's assistance. This, however, would only be decided at the conclusion of the evaluation process.

Table 3. An assessment of available validated protocols for the various kits that were evaluated by Forensic Biology FSS.

Kit	Availability of validated forensic protocol	Availability of validated MPII test file
DNA IQ™	✓	✓
QIAamp® DNA Micro	✓	✗
ChargeSwitch®	✓	✗
*forensic*GEM™	✓	✗
NucleoSpin® 8 Trace	✓	✗

The results and discussion for each of the kits that were evaluated, in comparison to Chelex®, are provided in the following sections. Refer to Tables 4 and 5 for quantitation results for cell and blood samples respectively. Yield calculations for Chelex® samples assume a final elution volume of 150μL.

6.2 Evaluation of DNA IQ™

The DNA IQ™ system uses a novel paramagnetic resin for DNA isolation. It consist of two steps: (1) lysis of the biological material on solid support; (2) using the paramagnetic resin to bind DNA, which allows washing of the resin-DNA complex while the resin is immobilised by a magnetic force, in order to remove the lysis reagent and inhibitors in solution.

The manufacturer's method required the use of the MagneSphere® Magnetic Separation Stand. This magnetic stand is used for the separation of the magnetic pellet in 12 samples at a time. The time to process a batch of 12 samples using the DNA IQ™ system takes about 3 hours, including 30 minutes of incubation time.

Three controls were run with each extraction batch: (1) a negative extraction control (empty tube); (2) a positive extraction control (QC dot saliva or blood depending on the extraction); and (3) a substrate blank (the substrate with only saline).

Samples were extracted using the DNA IQ™ method as described in the Methods section, and eluted using 100μL Elution Buffer. Due to volume loss during pipetting, the final elution volume is actually around 95μL. The same set of samples was also extracted using the in-house Chelex® protocol for comparison. Tables 4 and 5 display the DNA concentration (ng/μL) and yield (ng) for all cell and blood samples, compared to the results generated by Chelex®.

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Table 5. Quantitation values for blood samples on rayon swab substrates after extraction by Chelex® and the evaluated DNA extraction kits.

Blood samples	Chelex		DNA IQ		QIAamp DNA Micro		ChargeSwitch		forensicGEM		NucleoSpin 8 Trace	
Sample ID	...tion	Yield* ng	Concentration ng/uL	Yield ng	Concentration ng/uL	Yield ng	Concentration ng/uL	Yield ng	Concentration ng/uL	Yield ng	Concentration ng/uL	Yield ng
		355.5	0.482	48.2	2.31	103.95	0.751	112.65	0.00833	1.68266	1.16	116
		213	0.078	7.8	3.58	161.1	0.754	113.1	0.0066	1.3332	2.61	261
		76.8	0.356	35.6	3.32	149.4	0.929	139.35	0.0046	0.9292	1.61	161
		140.1	0.467	46.7	2.46	110.7	0.916	137.4	0.00727	1.46854	2.18	218
		196.3500	*0.3458*	*34.5750*	*2.9175*	*131.2875*	*0.8375*	*125.6250*	*0.0067*	*1.3534*	*1.8900*	*189.0000*
		119.8085	*0.1871*	*18.7137*	*0.6270*	*28.2137*	*0.0983*	*14.7451*	*0.0016*	*0.3173*	*0.6361*	*63.6082*
		32.85	0.238	23.8	0.227	10.215	0.219	32.85	0.00211	0.42622	0.611	61.1
		12.675	0.198	19.8	1.72	77.4	0.101	15.15	0.000597	0.120594	0.3	30
		32.4	0.195	19.5	4.59	206.55	0.0673	10.095	0.00128	0.25856	0.251	25.1
		24.75	0.136	13.6	0.657	29.565	0.0787	11.805	0.00166	0.33532	0.227	22.7
		25.6688	*0.1918*	*19.1750*	*1.7985*	*80.9325*	*0.1165*	*17.4750*	*0.0014*	*0.2852*	*0.3473*	*34.7250*
		9.4262	*0.0420*	*4.2019*	*1.9639*	*88.3776*	*0.0698*	*10.4628*	*0.0006*	*0.1294*	*0.1784*	*17.8438*
		1032	0.0554	5.54	0.0936	4.212	0.094	14.1	0.0126	2.5452	0.154	15.4
		24.6	0.114	11.4	0.175	7.875	0.0735	11.025	0.00174	0.35148	0.148	14.8
		42.9	0.145	14.5	0.123	5.535	0.0521	7.815	0.00363	0.73326	0.178	17.8
		76.95	0.125	12.5	0.0151	0.6795	0.0939	14.085	0.00167	0.33734	0.0819	8.19
		294.1125	*0.1099*	*10.9850*	*0.1017*	*4.5754*	*0.0784*	*11.7563*	*0.0049*	*0.9918*	*0.1405*	*14.0475*
		492.4030	*0.0385*	*3.8501*	*0.0668*	*3.0066*	*0.0200*	*2.9991*	*0.0052*	*1.0517*	*0.0411*	*4.1145*
		6.075	0.0792	7.92	0.0349	1.5705	0.0347	5.205	0.00757	1.52914	0.0766	7.66
		1.56	0.0566	5.66	0.0454	2.043	0.027	4.05	0.00667	1.34734	0.0923	9.23
		5.055	0.0847	8.47	0.0386	1.737	0.0197	2.955	0.00544	1.09888	0.0588	5.88
		4.845	0.109	10.9	0.0276	1.242	0.021	3.15	0.00245	0.4949	0.874	87.4
		4.3838	*0.0824*	*8.2375*	*0.0366*	*1.6481*	*0.0256*	*3.8400*	*0.0055*	*1.1176*	*0.2754*	*27.5425*
		1.9577	*0.0215*	*2.1515*	*0.0074*	*0.3341*	*0.0068*	*1.0274*	*0.0022*	*0.4510*	*0.3993*	*39.9285*

Table 6. Comparison of DNA profiles for cell substrate samples extracted using either Chelex® or DNA IQ™.

CELLS **Method: Chelex**

Dilution	*FTA*	*Cotton swabs*	*Rayon swabs*		*Cotton*	*Denim*
	Profile	Profile	Sample#	Profile	Profile	Profile
Neat	X,X+18	X,X+18	R14	NSD	X,X+8	NR/NSD
			R15	NR/NSD		
			R16	NSD		
			R17	NSD		
Dil 1/4	X,X+18	X,NR+3	R10	NSD	NR+1	NR/NSD
			R11	NSD		
			R12	NSD		
			R13	NSD		
Dil 1/8	X,X+17	X,X+3	R6	NSD	X,NR+3	NR/NSD
			R7	NSD		
			R8	NSD		
			R9	NSD		
Dil 1/16	NSD	NSD	R2	NSD	NSD	NSD
			R3	NSD		
			R4	NSD		
			R5	NSD		

CELLS **Method: DNA IQ**

Dilution	*FTA*	*Cotton swabs*	*Rayon swabs*		*Cotton*	*Denim*
	Profile	Profile	Sample#	Profile	Profile	Profile
Neat	X,X+14	X,X+18	R14	X,X+16	X,X+18	X,X+18
			R15	AI@D13(68%)		
			R16	X,X+18		
			R17	X,X+18		
Dil 1/4	X,X+17	X,X+18	R10	X,NR+3	X,X+18	XX+18
			R11	NR/NSD		
			R12	X,X+6		
			R13	NR/NSD		
Dil 1/8	X,X+8	X,X+18	R6	NR/NSD	X,X+17	X,X+17
			R7	NR/NSD		
			R8	NR/NSD		
			R9	NR/NSD		
Dil 1/16	NSD	X,X+4	R2	NR/NSD	NR/NSD	NR/NSD
			R3	NSD		
			R4	NR/NSD		
			R5	NSD		

For blood samples, only rayon substrates were extracted using the DNA IQ™ system as these were deemed sufficient for observing the effects of heme inhibition (without the need to factor variable substrate types). Almost all samples generated full profiles or a sufficient number of reportable alleles for matching purposes (Table 7). For neat samples extracted by Chelex®, no profiles were resulted from the FTA®, cotton swab or denim samples, indicating possible heme inhibition that could not be removed by the Chelex® protocol. For rayon samples, 19% of those extracted by Chelex® did not generate a profile, whereas DNA IQ™ yielded full profiles for all dilutions except two neat samples. Reworks of the two failed samples were performed but yielded the same NSD results. These failed results appear to be outliers, as all other dilutions yielded the expected results. It was observed that results from blood samples on rayon swabs were more likely (32%) to exhibit allelic imbalance at Amelogenin when extracted using the DNA IQ™ system.

Table 7. Comparison of DNA profiles for blood substrate samples extracted using either Chelex® or DNA IQ™.

BLOOD **Method: Chelex** **Method: DNA IQ**

Dilution	*FTA*	*Cotton swabs*	*Rayon swabs*		*Cotton*	*Denim*		*Rayon swabs*	
	Profile	Profile	Sample#	Profile	Profile	Profile		Sample#	Profile
Neat	NSD	NSD	R14	X,Y+18	X,Y+18	NSD		R14	NSD
			R15	X,Y+18				R15	NSD
			R16	X,Y+18				R16	X,Y+18(AI@AMEL)
			R17	NR/NSD				R17	X,Y+18(AI@AMEL)
Dil 1/4	X,Y+18	X,Y+15	R10	Not Uploaded	X,Y+18	X,Y+18		R10	X,Y+18
			R11	X,Y+18				R11	X,Y+18(AI@AMEL)
			R12	X,Y+18				R12	X,Y+18
			R13	X,Y+18				R13	X,Y+18
Dil 1/8	X,Y+18(AI@I	X,Y+18	R6	X,Y+18	X,Y+18	X,Y+18		R6	X,Y+18(AI@AMEL)
			R7	X,Y+18				R7	X,Y+18
			R8	NR/NSD				R8	X,Y+18
			R9	X,Y+18				R9	X,Y+18
Dil 1/16	X,Y+18	X,Y+18	R2	X,Y+18	X,Y+18	X,Y+18		R2	X,Y+18(AI@AMEL)
			R3	X,Y+18				R3	X,Y+18
			R4	X,Y+18				R4	X,Y+18
			R5	X,Y+18				R5	X,Y+18

Comparison of DNA profiles

Cell samples that were extracted using the QIAamp® protocol showed profile results that were either comparable or worse than samples that were extracted using the Chelex® protocol (Table 8). Out of 32 samples, only one QIAamp® sample resulted in a full profile (X,X+18). QIAamp® samples failed to produce full profiles for all but one (n = 8) of the neat samples. Overall, QIAamp® resulted in 86 reportable alleles compared to 89 alleles resolved by Chelex®. Some of the QIAamp® allele calls are inconsistent, e.g. the result for 1/4 dilution on cotton cloth was slightly better than the neat sample. This is further exemplified by the denim substrate samples. The QIAamp® method did not appear to effectively overcome inhibition caused by the denim dye as observed from the resulting profiles.

Table 8. Comparison of DNA profiles for cell substrate samples extracted using either Chelex® or QIAamp® DNA Micro.

CELLS **Method: Chelex**

Dilution	*FTA*	*Cotton swabs*	*Rayon swabs*		*Cotton*	*Denim*
	Profile	Profile	Sample#	Profile	Profile	Profile
Neat	X,X+18	X,X+18	R14	NSD	X,X+8	NR/NSD
			R15	NR/NSD		
			R16	NSD		
			R17	NSD		
Dil 1/4	X,X+18	X,NR+3	R10	NSD	NR+1	NR/NSD
			R11	NSD		
			R12	NSD		
			R13	NSD		
Dil 1/8	X,X+17	X,X+3	R6	NSD	X,NR+3	NR/NSD
			R7	NSD		
			R8	NSD		
			R9	NSD		
Dil 1/16	NSD	NSD	R2	NSD	NSD	NSD
			R3	NSD		
			R4	NSD		
			R5	NSD		

CELLS **Method: QIAamp DNA Micro**

Dilution	*FTA*	*Cotton swabs*	*Rayon swabs*		*Cotton*	*Denim*
	Profile	Profile	Sample#	Profile	Profile	Profile
Neat	X,X+12	X,X+18	R14	NSD	NR,NR+2	NR/NSD
			R15	NR/NSD		
			R16	XNR+6		
			R17	NR/NSD		
Dil 1/4	X,X+14	X,X+15	R10	NSD	NR,NR+3	NR/NSD
	(AI@D18)		R11	NSD		
			R12	NR,NR+1		
			R13	NR/NSD		
Dil 1/8	NSD+2	X,NR+6	R6	NSD	NR/NSD	XNR+7
			R7	NSD		
			R8	NSD		
			R9	NSD		
Dil 1/16	NR/NSD	NR/NSD	R2	NSD	NSD	NSD
			R3	NSD		
			R4	NSD		
			R5	NSD		

Table 9. Comparison of DNA profiles for blood substrate samples extracted using either Chelex® or QIAamp® DNA Micro.

BLOOD **Method: Chelex** **Method: QIAamp DNA Micro**

Dilution	*FTA*	*Cotton swabs*	*Rayon swabs*		*Cotton*	*Denim*		*Rayon swabs*	
	Profile	Profile	Sample#	Profile	Profile	Profile		Sample#	Profile
Neat	NSD	NSD	R14	X,Y+18	X,Y+18	NSD		R14	X,Y+18
			R15	X,Y+18				R15	X,Y+18
			R16	X,Y+18				R16	X,Y+18
			R17	NR/NSD				R17	NR,Y+15
Dil 1/4	X,Y+18	X,Y+15	R10	Not Uploaded	X,Y+18	X,Y+18		R10	X,Y+18
			R11	X,Y+18				R11	X,Y+17
			R12	X,Y+18				R12	X,Y+18
			R13	X,Y+18				R13	X,Y+18
Dil 1/8	X,Y+18(AI@D	X,Y+18	R6	X,Y+18	X,Y+18	X,Y+18		R2	X,Y+18
			R7	X,Y+18				R3	X,Y+18
			R8	NR/NSD				R4	X,Y+18
			R9	X,Y+18				R5	X,Y+18
Dil 1/16	X,Y+18	X,Y+18	R2	X,Y+18	X,Y+18	X,Y+18		R2	X,Y+18(AI@D8,D18)
			R3	X,Y+18				R3	X,Y+18
			R4	X,Y+18				R4	X,Y+18
			R5	X,Y+18				R5	X,Y+18

swabs outperformed Chelex®. However, the ChargeSwitch® system was unable to overcome inhibition in denim samples, and did not yield any DNA profiles at all, despite displaying quantitation results for the neat and 1/4 dilution.

Table 10. Comparison of DNA profiles for cell substrate samples extracted using either Chelex® or ChargeSwitch®.

CELLS   Method: Chelex

Dilution	FTA	Cotton swabs	Rayon swabs		Cotton	Denim
	Profile	Profile	Sample#	Profile	Profile	Profile
Neat	X,X+18	X,X+18	R14	NSD	X,X+8	NR/NSD
			R15	NR/NSD		
			R16	NSD		
			R17	NSD		
Dil 1/4	X,X+18	X,NR+3	R10	NSD	NR+1	NR/NSD
			R11	NSD		
			R12	NSD		
			R13	NSD		
Dil 1/8	X,X+17	X,X+3	R6	NSD	X,NR+3	NR/NSD
			R7	NSD		
			R8	NSD		
			R9	NSD		
Dil 1/16	NSD	NSD	R2	NSD	NSD	NSD
			R3	NSD		
			R4	NSD		
			R5	NSD		

CELLS   Method: ChargeSwitch

	FTA	Cotton swabs	Rayon swabs		Cotton	Denim
	Profile	Profile	Sample#	Profile	Profile	Profile
Neat	X,X+17	X,X+18	R14	X,X+8	X,X+11	NSD
	AI@FGA		R15	X,X+15		
	AI@D13		R16	X,X+16		
			R17	X,X+8		
Dil 1/4	X,X+9	X,X+18	R10	X,X+NR's	NRNR+2	NSD
		AI@D13	R11	NR/NSD		
			R12	X,NR+2		
			R13	X,NR+NSD		
Dil 1/8	NR/NSD	X,X+14	R6	NSD	NR/NSD	NSD
			R7	NSD		
			R8	NR/NSD		
			R9	NSD		
Dil 1/16	NSD	NR/NSD	R2	NR/NSD	NSD	NSD
			R3	NSD		
			R4	NSD		
			R5	NSD		

For blood samples on rayon swab substrates, all ChargeSwitch® samples consistently yielded full profiles for all dilutions and therefore outperformed Chelex® (Table 11). Two replicates of the lower, 1/16 dilutions displayed allelic imbalance at two different loci: D3S1358 and D7S820, possibly due to stochastic effects that arise from amplifying low concentrations of DNA.

Table 11. Comparison of DNA profiles for blood substrate samples extracted using either Chelex® or ChargeSwitch®.

BLOOD   Method: Chelex   Method: ChargeSwitch

Dilution	FTA	Cotton swabs	Rayon swabs		Cotton	Denim	Rayon swabs	
	Profile	Profile	Sample#	Profile	Profile	Profile	Sample#	Profile
Neat	NSD	NSD	R14	X,Y+18	X,Y+18	NSD	R14	X,Y+18
			R15	X,Y+18			R15	X,Y+18
			R16	X,Y+18			R16	X,Y+18
			R17	NR/NSD			R17	X,Y+18
Dil 1/4	X,Y+18	X,Y+15	R10	Not Uploaded	X,Y+18	X,Y+18	R10	X,Y+18
			R11	X,Y+18			R11	X,Y+18
			R12	X,Y+18			R12	X,Y+18
			R13	X,Y+18			R13	X,Y+18
Dil 1/8	X,Y+18(AI@D	X,Y+18	R6	X,Y+18	X,Y+18	X,Y+18	R6	X,Y+18
			R7	X,Y+18			R7	X,Y+18
			R8	NR/NSD			R8	X,Y+18
			R9	X,Y+18			R9	X,Y+18
Dil 1/16	X,Y+18	X,Y+18	R2	X,Y+18	X,Y+18	X,Y+18	R2	X,Y+18
			R3	X,Y+18			R3	X,Y+18
			R4	X,Y+18			R4	X,Y+18(AI@D3)
			R5	X,Y+18			R5	X,Y+18(AI@D7)

Table 12. Comparison of DNA profiles for cell substrate samples extracted using either Chelex® or *forensic*GEM®.

CELLS — Method: Chelex

Dilution	FTA	Cotton swabs	Rayon swabs		Cotton	Denim
	Profile	Profile	Sample#	Profile	Profile	Profile
Neat	X,X+18	X,X+18	R14	NSD	X,X+8	NR/NSD
			R15	NR/NSD		
			R16	NSD		
			R17	NSD		
Dil 1/4	X,X+18	X,NR+3	R10	NSD	NR+1	NR/NSD
			R11	NSD		
			R12	NSD		
			R13	NSD		
Dil 1/8	X,X+17	X,X+3	R6	NSD	X,NR+3	NR/NSD
			R7	NSD		
			R8	NSD		
			R9	NSD		
Dil 1/16	NSD	NSD	R2	NSD	NSD	NSD
			R3	NSD		
			R4	NSD		
			R5	NSD		

CELLS — Method: forensicGEM

	FTA	Cotton swabs	Rayon swabs		Cotton	Denim
	Profile	Profile	Sample#	Profile	Profile	Profile
Neat	X,X+15	X,X+18	R14	X,X+17	X,X+18	X,X+18
			R15	X,X+13		
			R16	X,X+3		
			R17	X,X+ 15		
Dil 1/4	X,NR+3	X,X+18	R10	X,NR+NR/NS	X,X+18	X,X+18
			R11	NR/NSD		
			R12	NR/NSD		
			R13	X,NR+NR/NSD		
Dil 1/8	NSD	X,X+18	R7	NR/NSD	X,NR+10	X,NR+7
			R8	NSD		
			R9	NR/NSD		
			R6	NR/NSD		
Dil 1/16	NSD	NR/NSD	R5	NSD	NSD	NR/NSD
			R4	NSD		
			R3	NSD		
			R2	NR/NSD		

For blood samples on rayon swabs, only the 1/16 dilutions generated profile results (Table 13). This is indicatory of potential inhibition for higher blood sample dilutions as predicted by the quantitation data.

Table 13. Comparison of DNA profiles for blood substrate samples extracted using either Chelex® or *forensic*GEM®.

BLOOD — Method: Chelex / Method: forensicGEM

Dilution	FTA	Cotton swabs	Rayon swabs		Cotton	Denim	Rayon swabs (forensicGEM)	
	Profile	Profile	Sample#	Profile	Profile	Profile	Sample#	Profile
Neat	NSD	NSD	R14	X,Y+18	X,Y+18	NSD	R14	NSD
			R15	X,Y+18			R15	NSD
			R16	X,Y+18			R16	NSD
			R17	NR/NSD			R17	NSD
Dil 1/4	X,Y+18	X,Y+15	R10	Not Uploaded	X,Y+18	X,Y+18	R10	NSD
			R11	X,Y+18			R11	NSD
			R12	X,Y+18			R12	NSD
			R13	X,Y+18			R13	NSD
Dil 1/8	X,Y+18(Al@[	X,Y+18	R6	X,Y+18	X,Y+18	X,Y+18	R6	NSD
			R7	X,Y+18			R7	NSD
			R8	NR/NSD			R8	NSD
			R9	X,Y+18			R9	NSD
Dil 1/16	X,Y+18	X,Y+18	R2	X,Y+18	X,Y+18	X,Y+18	R2	X,Y+15(Al@D13)
			R3	X,Y+18			R3	X,NR+3
			R4	X,Y+18			R4	NR,NR+5
			R5	X,Y+18			R5	NR,NR+2

6.6 NucleoSpin® 8 Trace

The NucleoSpin® 8 Trace kit is designed for extraction of genomic DNA from forensic samples. Cell lysis is achieved by incubating samples in a solution containing chaotropic ions in the presence of proteinase K at room temperature. Adding isopropanol to the lysate creates the appropriate conditions for binding of DNA to the silica membrane, a process that is reversible and specific to nucleic acids. Inhibitors are removed by washing steps using an alcohol-containing buffer. Pure genomic DNA is eluted in a slightly alkaline elution buffer.

The evaluation of this kit was performed with slight alterations in the manual method to incorporate the use of the MultiPROBE® II PLUS PVM vacuum manifold, together with the

Table 15. Comparison of DNA profiles for blood substrate samples extracted using either Chelex® or NucleoSpin® 8 Trace.

BLOOD | **Method: Chelex** | **Method: NucleoSpin 8 Trace**

Dilution	*FTA*	*Cotton swabs*	*Rayon swabs*		*Cotton*	*Denim*	*Rayon swabs*	
	Profile	Profile	Sample#	Profile	Profile	Profile	Sample#	Profile
Neat	NSD	NSD	R14	X,Y+18	X,Y+18	NSD	R14	Al@Amel.&D18
			R15	X,Y+18			R15	NR,NR+2
			R16	X,Y+18			R16	X,Y+13
			R17	NR/NSD			R17	X,Y+18
Dil 1/4	X,Y+18	X,Y+15	R10	Not Uploaded	X,Y+18	X,Y+18	R10	X,Y+18
			R11	X,Y+18			R11	X,Y+18
			R12	X,Y+18			R12	X,Y+18
			R13	X,Y+18			R13	X,Y+18
Dil 1/8	X,Y+18(Al@D	X,Y+18	R6	X,Y+18	X,Y+18	X,Y+18	R6	X,Y+18
			R7	X,Y+18			R7	X,Y+15
			R8	NR/NSD			R8	X,Y+18
			R9	X,Y+18			R9	X,Y+18
Dil 1/16	X,Y+18	X,Y+18	R2	X,Y+18	X,Y+18	X,Y+18	R2	X,Y+18
			R3	X,Y+18			R3	X,Y+18
			R4	X,Y+18			R4	X,Y+18
			R5	X,Y+18			R5	X,Y+18

6.7 Summary

Findings from the evaluation of various forensic DNA extraction kits, compared to the in-house Chelex® protocol, is summarised in Table 16.

Out of all the chemistries tested, only the Chelex® method and *forensic*GEM™ protocols do not incorporate washing steps for the removal of inhibitors and residual proteins. This is because in these protocols, the DNA is free in solution and not immobilised on to a capture device such as magnetic beads, and therefore washing of the sample cannot be performed. Washing steps result in high quality, purified DNA extracts. As such, Chelex® and *forensic*GEM™ extracts are considered to be crude DNA extracts of suboptimal quality that may not yield the best DNA profiles due to the presence of inhibitors that can affect PCR amplification of multiple STR loci. Although the dye in denim material did not appear to result in inhibition for *forensic*GEM™ samples, only 25/288 alleles (8.7%) from blood samples could be resolved by this extraction method.

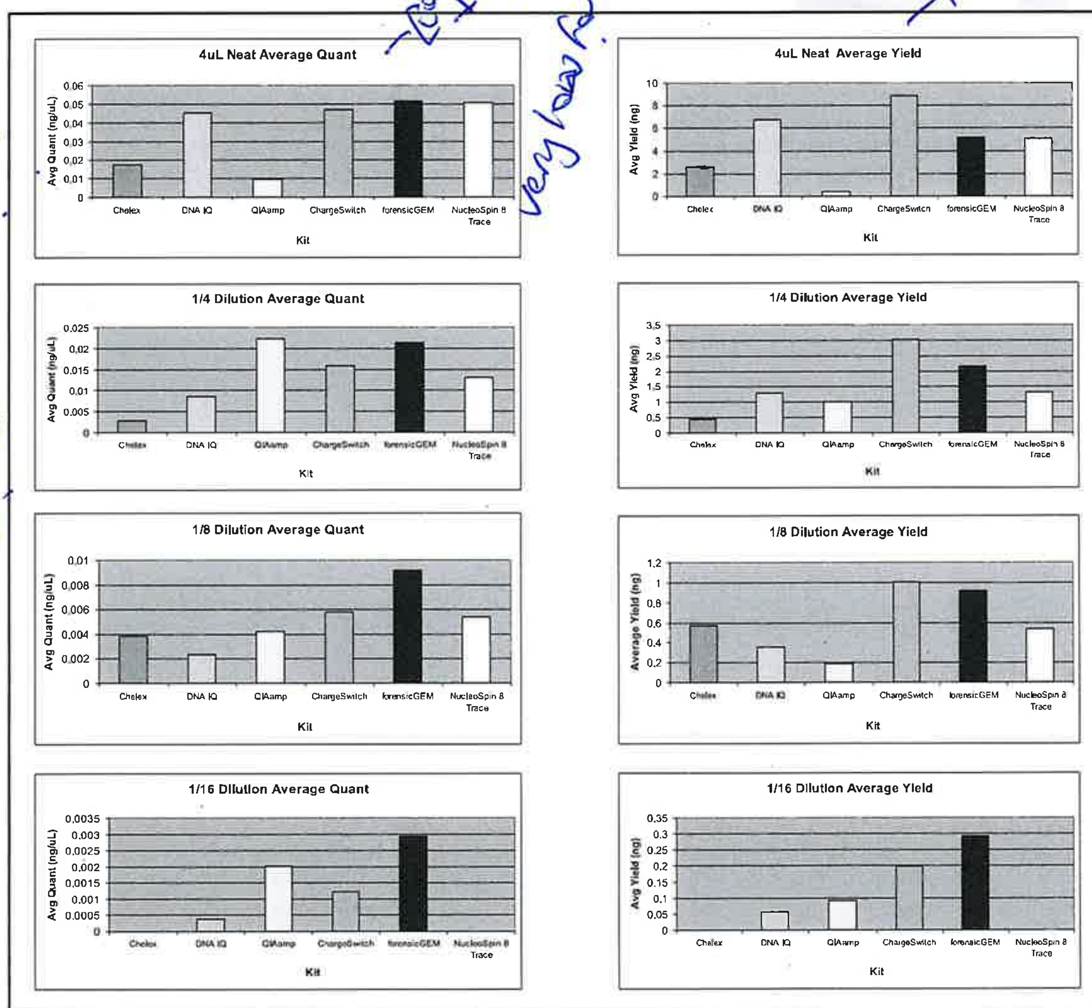

Figure 1. Average quantitation values (ng/µL) and yields (ng) for cell samples extracted using the various extraction chemistries tested, compared to Chelex®.

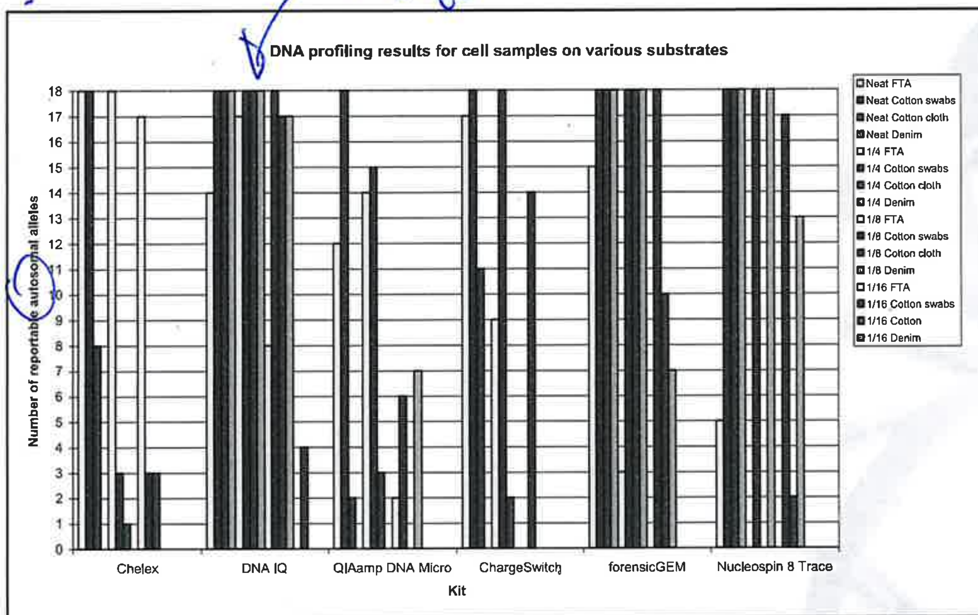

Figure 2. Total number of reportable alleles generated for cell samples on various substrates that were extracted using the various extraction chemistries tested, compared to Chelex®. The kit displaying the most number of full bars (i.e. most full profiles) was found to be DNA IQ™, indicating the superior performance of this kit over the other kits tested. The current in-house Chelex® method did not perform as well as several of the tested kits.

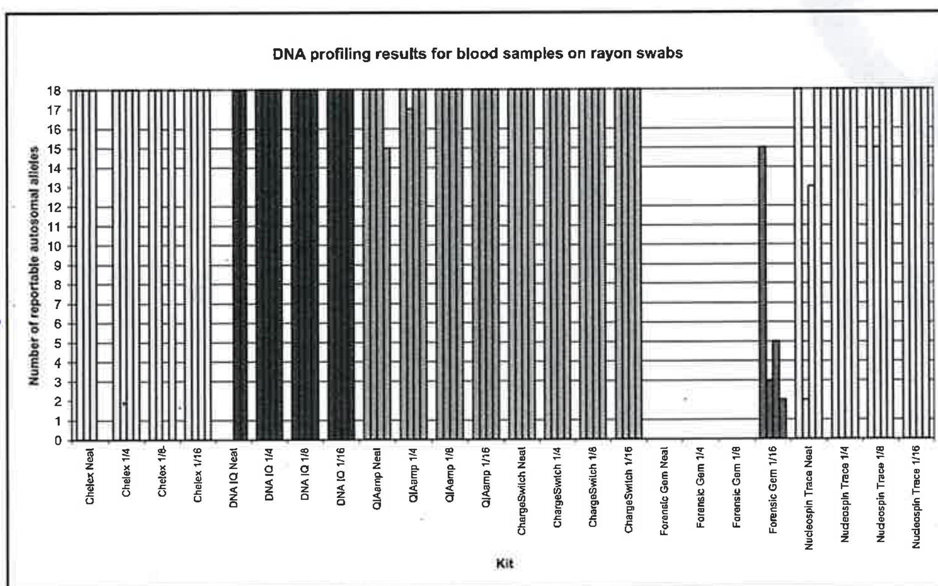

Figure 3. Total number of reportable alleles generated for blood samples on rayon swabs that were extracted using the various extraction chemistries tested, compared to Chelex®. All kits were able to resolve profiles from most dilutions, except *forensic*GEM™ which could only resolve alleles from the 1/16 dilution, indicating an inhibitory effect of heme on the *forensic*GEM™ system.

All of the forensic DNA extraction kits evaluated are amenable to automation, and automated protocols already exist for several kits. However, only the DNA IQ™ kit has been validated for use on the MultiPROBE® II PLUS HT EX platform and a validated protocol was developed by PerkinElmer (PerkinElmer, 2004).

why here?

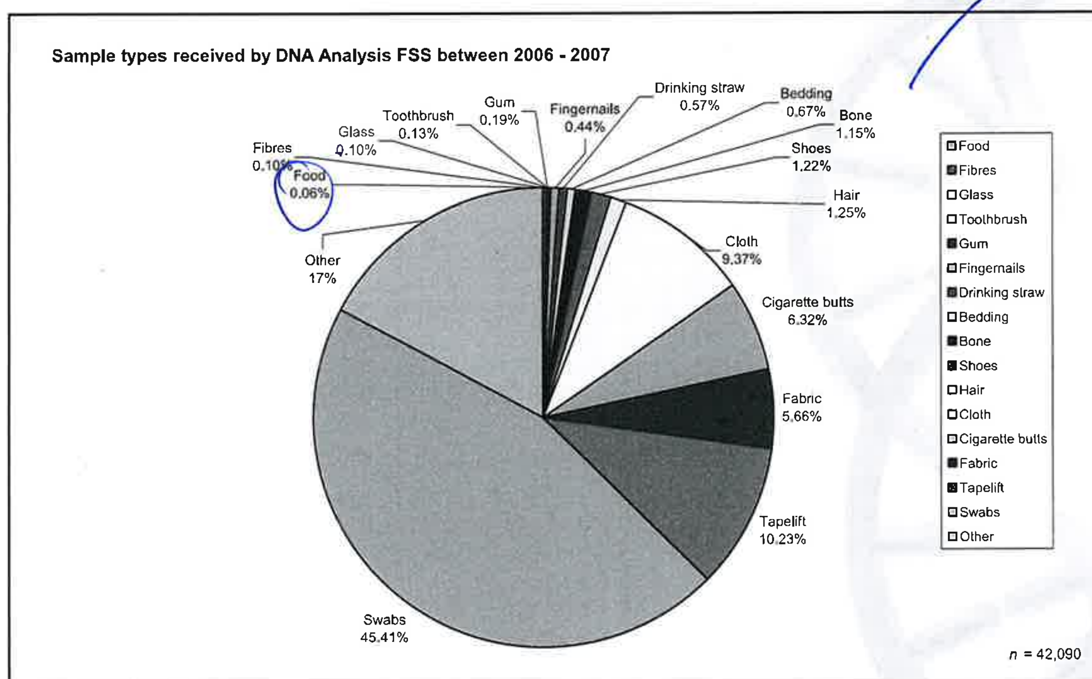

Figure 4. Pie chart of various sample types received by DNA Analysis FSS between 2006 and 2007. Around 45% of samples received for DNA analysis are swab substrates. Data was obtained from AUSLAB on 14 November 2007.

Some of the concerns raised regarding some of the kits tested include:

- QIAamp® DNA Micro involved multiple tube transfers that increased the risk of cross-contamination and also increased processing time to 5 hours for 12 samples.
- An increased risk of contamination was also prevalent in the NucleoSpin® 8 Trace method when coupled with the PVC vacuum manifold, because of the need to fit multiple adapters to ensure seals are maintained for a proper vacuum environment. If the plates and adapters were not assembled correctly, the vacuum environment would fail and possibly cause cross-contamination and, more alarmingly, loss of sample. Furthermore, even when assembled correctly, biohazardous contaminants (e.g. blood) are drawn down the manifold through the vacuum tubing and into the collection containers. Decontamination of the tubing and containers raises serious health and safety concerns.
- The *forensic*GEM™ system was the quickest protocol to perform and yielded crude DNA extracts that produced high allele counts for cell samples. However, the system could not deal with blood samples (and heme inhibition) effectively, therefore causing very low allele counts for blood samples.
- ChargeSwitch® was the alternative magnetic bead system to DNA IQ™. However, ChargeSwitch® did not produce results that were comparable or better than DNA

good candidate

Qiagen (2003). QIAamp® DNA Micro Handbook [Rev. 08/2003]. Qiagen GmbH: Hilden, Germany.
QIS 19171 (2007). Method for Chelex® Extraction [Standard Operating Procedure]. DNA Analysis FSS: Brisbane, QLD, Australia.
Scott S, Harbison S-A, Saul DJ (2003). Developmental validation of *forensic*GEM™: a Thermophilic Proteinase for Forensic DNA Extraction [oral presentation at the 18th International Symposium on the Forensic Sciences, Fremantle, WA, 2-7 April 2006]. Institute of Environmental Research Ltd: Hamilton, New Zealand.
Walsh PS, Metzger DA, Higuchi R (1991). Chelex®-100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* 10:506-13.
ZyGEM (2006). DNA extraction from buccal swabs using *forensic*GEM™. ZyGEM Corporation Ltd: Hamilton, New Zealand.

- report written appears biased.
- based on data made
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- don't agree for here.