In the matter of the *Commissions of Inquiry Act 1950*

Commissions of Inquiry Order (No. 1) 2023

Commission of Inquiry into DNA Project 13

STATEMENT OF DAVID HAROLD NEVILLE

I, **David Harold Neville**, c/- [redacted] Queensland state as follows:

1. I am providing this further information based on my review of the transcript from Day 1 of the *Commission of Inquiry into DNA Project 13*.

2. In response to a question from the Commissioner in relation to whether there had been a systemic lack of recovery of DNA, Mr Nurthen stated in part. "*I can only recall one time when there was what appeared to be a systemic problem and that was where the Queensland Police had changed the swab type. They had changed the swabs that they were using to collect the DNA and hadn't advised us, and I can recall that swabs that had been described as being blood positive weren't yielding any results at all, and that's the only time I can recall.*" (**emphasis added**).

3. I am assuming that Mr Nurthen was referring to the use of a 4N6 swab. The 4N6 swab was selected for use by QPS for the collection of DNA after joint research with QHFSS that was carried out in late 2008 or early 2009. The advantage of this swab was that it had a break point that allowed the swab head to be broken off and placed into a tube. QHFSS undertook testing on the efficacy of this swab to uptake and release DNA. They advised that the 4N6 swab was equivalent to other swabs in use at the time. A copy of their final report is attached and marked "**Exhibit 1**".

4. Ms Cathy Allen had raised this as a potential reason for the reduction in presumed blood swabs yielding a profile during a meeting on 27 May 2009. A copy of the Minutes are attached to my statement dated 31 October 2023. Queensland Police Service discontinued its use based on this advice.

5. These matters referred to above were included in my prior statement of 2 November 2022. provided to the *Commission of Inquiry into Forensic DNA Testing in Queensland* (QPS.0308.0002.0001). See paragraphs 11 to 13.

TAKEN AND DECLARED before me at Brisbane in the State of Queensland this

1st Day of November 2023

[redacted] ..... [redacted] .....

David Harold Neville Witness

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Commission of Inquiry into DNA Project 13

STATEMENT OF DAVID HAROLD NEVILLE

INDEX TO EXHIBITS

Exhibit no.	Description
1.	Report titled "Trial of Copan 4N6 flocked swab"

CaSS | Forensic and Scientific Services
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Trial of Copan 4N6 flocked swab

Allan McNevin, Senior Scientist, Chiron Weber, Scientist
DNA Analysis, Queensland Health Forensic and Scientific Services

Introduction

The examination of items for forensic DNA testing is a labour intensive and depending on the item, a time consuming process. For simple items such as swabs, laboratory efficiency could be improved by delivering items to the testing laboratory in a format that is suitable for analytical use. Such a format includes the supply of swab heads packaged in a tube suitable for testing in the analytical environment, i.e. suitable to be used directly in the DNA extraction procedure without the need for examination by a scientist. One such product available is the 4N6 DNA flocked swab (Copan). One format that the product may be purchased in is a kit containing a flocked nylon swab packaged with a 2mL tube (eppendorf) with a vented lid allowing for the drying of the swab head (catalogue number 3520CF). Figures 1 and 2 are reproduced from the 4N6 swab brochure (http://www.copanswabs.com/products/forensics/). Figure 1 is a representation of the ease with which the flocked swab will elute specimen as compared to a traditional swab in Figure 2.

Figure 1. Flocked swab elutes specimen more efficiently.

Figure 2. Traditional fiber (sic) swab elutes less specimen.

The website also provides a link to forensic studies. However, the information is based around testing for viral and bacterial pathogens rather than forensic testing. There does not appear to be any published papers that directly compare the 4N6 swab with other swabs currently used.

AIMS

The aim of the testing carried out was to compare the 4N6 DNA flocked swab (Copan, product code 3520CF) with two swab types that are currently in use for the collection of material for forensic DNA testing. The swabs would be compared on two criteria:

1. The ability to extract DNA from each swab type and,
2. The ability of each swab type to uptake DNA.

The two swab types chosen to compare against the 4N6 swab were a spun cotton swab with a small swab head and paper shaft (Copan, product code 164C) and a spun rayon swab with a medium sized swab head and plastic shaft (Copan, product code 155C). Initially five 4N6 swabs were received from Interpath for testing, deliveries of five and fifteen 4N6 swabs were received from Queensland Police Services (QPS) for further testing.

MATERIALS AND METHODS

Experiment 1 Release of DNA from blood

30µL of whole blood was spotted directly onto the surface of five swabs of each of the three swab types outlined above. This was done by cutting the head of the swab from the shaft into a sterile DNA-free tube using a pair of scissors sterilised by washing in 10% bleach, followed by 100% ethanol and flaming. 30µL of whole blood from a donor staff member (collected approximately 3 months previous and stored at 4°C) was added to each swab head and allowed to air dry for 1 hour at 56°C on a Thermomixer comfort (eppendorf) with no agitation.

The DNA was then extracted, quantified, amplified and profiles visualised according to standard laboratory procedures. Briefly, this entailed the lysis of cellular material in individual tubes (by incubation of substrate in 500µL of a buffer containing Proteinase K and Sarcosyl). The lysis solution was separated from the substrate by centrifugation and then added to a 96 deep-well plate using the *automate.it* STORstar system (Process Analysis & Automation Ltd. Hampshire. UK). The DNA was extracted from the lysed solutions using the DNA IQ™ kit (Promega Corp.) on a dedicated MultiPROBE® II PLUS HT EX with Gripper™ Integration platform (PerkinElmer). In the final staged of the extraction procedure, the DNA extracts are placed into individual tubes. The DNA extracts were then stored at -20°C between each of the following procedures.

The amount of DNA in each DNA extract was then quantified using the Quantifiler™ Human DNA Quantification kit (Applied Biosytems), prepared on a MultiPROBE® II dedicated to PCR set-up. The real-time PCR is then performed on an ABI Prism® 7500 Sequence Detection System (Applied Biosytems). Once the DNA quantification has been determined, an appropriate amount of DNA template is added to the STR amplification reaction. STR analysis is carried out by amplification with an AMPFℓSTR® Profiler Plus® PCR Amplification kit (Applied Biosytems), prepared on a dedicated MultiPROBE® II and amplified on a GeneAmp® PCR System 9700 thermalcycler (Applied Biosytems). Fragment analysis was performed by capillary electrophoresis on an ABI Prism® 3130*xl* Genetic Analyzer (Applied Biosytems), in combination with GeneScan® (version 3.7.2) and Genotyper® (version 3.7.1) software.

Experiment 2 Release of DNA from blood over dilution series

Dilutions of whole blood were made and spotted onto swab heads of each of the three swab types under test. This was done by firstly diluting whole blood from a donor staff member (collected approximately 3 months previous and stored at 4°C) in nanopure water in the following dilution series; 1 in 5, 1 in 10, 1 in 20, 1 in 50. 30µL of neat blood and one of each dilution series was added to each swab type under test as outlined in Experiment 1. DNA was extracted, quantified and amplified according to procedures outlined in Experiment 1.

Experiment 3 Release of DNA from cellular material

A suspension of buccal cells was made to perform testing. Buccal cells were collected from a female donor (a previously profiled staff member) using the Cytobrush™ method. Briefly, two Cytobrush™ Plus Cell Collector devices (Cooper Surgical, Inc., Trumbull, CT, USA) were used to collect buccal cells from each cheek for 1 minute, then collected into 500µL of 0.9% saline solution (Baxter Healthcare, Old Toongabbie, NSW, Australia) in a sterile 2mL tube. 30µL of buccal cell suspension was spotted directly onto the surface of five swabs of each of the three swab types outlined above. This was done by cutting the head of the swab from the shaft into a sterile DNA-free tube using a pair of scissors sterilised by washing in 10% bleach, followed by 100% ethanol and flaming. 30µL of buccal cell suspension from a donor staff member (collected the same day as testing) was added to each swab head and allowed to air dry for 1 hour at 56°C on a Thermomixer comfort (eppendorf) with no agitation. DNA was extracted, quantified and amplified according to procedures outlined in Experiment 1.

Experiment 4 Release of DNA from cellular material over dilution series

Dilutions of a buccal cell suspension were spotted directly onto the surface of five swabs of each of the three swab types under test. This was done by firstly diluting a buccal cell suspension from a donor staff member (collected approximately 1 week previous and stored at 4°C) in nanopure water in the following dilution series; 1 in 5, 1 in 10, 1 in 20, 1 in 50. 30µL of neat buccal cell suspension and one of each dilution series was added to each swab type under test as outlined in Experiment 3. DNA was extracted, quantified and amplified according to procedures outlined in Experiment 1.

Experiment 5 Uptake and release of DNA from blood and cellular material

30µL of whole blood (collected approximately 3 months previous and stored at 4°C) and 30µL of a buccal cell suspension (collected the same day as testing) was spotted directly onto the surface of new plastic Petri dish and allowed to dry overnight at room temperature in a class II biohazard cabinet. The dried area was marked on the underside of the Petri dish so that the area containing dried sample could later be identified. The dried samples were stored for approximately 15 weeks at approximately -20°C prior to sampling. Five swabs of each type under test were used to sample the dried blood stains using standard laboratory techniques employed within the laboratory for liberating dried blood stains from crime-scene exhibits. Briefly, this entailed wetting of the swab head with nanopure water and rubbing the stained area with the endmost area of the swab head. The procedure was repeated to sample dried cell suspensions. DNA was extracted according to procedures outlined in Experiment 1, with minor modifications to the automated extraction protocol resulting from process improvements incorporated between experiments. Briefly, these modifications included incubation of the substrate in 300µL of buffer during cellular lysis and improved mixing and pipetting steps during the automated protocol. DNA was quantified and amplified according to procedures outlined in Experiment 1. Additionally, capillary electrophoresis data was analysed using GeneMapper-*IDX* (version 1.0) software due to process improvements incorporated into the laboratory between experiments.

RESULTS

Experiment 1 Release of DNA from blood

Table 1 below shows quantitation values for each swab head tested and the average quantitation value for each swab type. The same data is also represented in Figure 1 below.

Table 1. Summary of results from Experiment 1

4N6 Swab		Cotton swab		Rayon Swab	
Sample	**Quantification value (ng/µL)**	**Sample**	**Quantification value (ng/µL)**	**Sample**	**Quantification value (ng/µL)**
1	0.8490	1	0.4290	1	0.6200
2	0.5000	2	0.2650	2	0.6810
3	0.9050	3	0.3690	3	0.5150
4	0.8050	4	0.6040	4	0.4740
5	0.7610	5	0.3810	5	0.4780
mean	*0.7640*	*mean*	*0.4096*	*mean*	*0.5536*

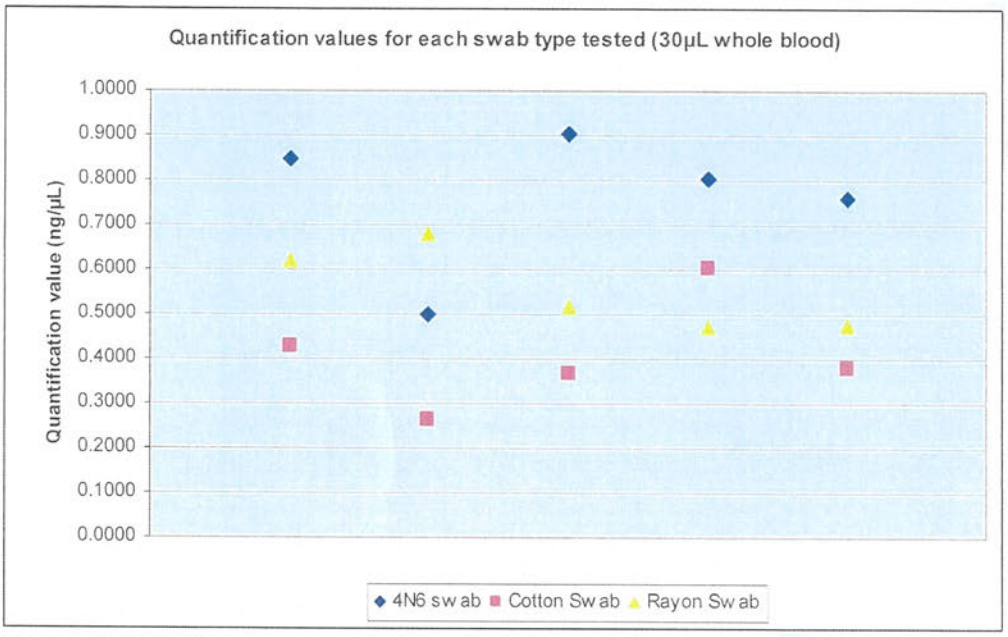

Figure 1. Quantification values for each of 5 replicates for each of 3 swab types tested

For every swab tested, full 9-loci DNA profiles were obtained, consistent with the expected profile.

Experiment 2 Release of DNA from blood over dilution series

Table 2 shows the quantification values obtained or the dilution series for each of the swab types tested. These results are also shown in Figure 2 below.

Table 2. Summary of results from Experiment 2

Dilution series	DNAQuantification (ng/µL)		
	4N6 Swab	Cotton Swab	Rayon Swab
Neat blood	0.517	0.177	0.555
1 in 5	0.0926	0.07	0.0665
1 in 10	0.0982	0.0598	0.107
1 in 20	0.0518	0.0303	0.0499
1 in 50	0.02	0.0177	0.0104

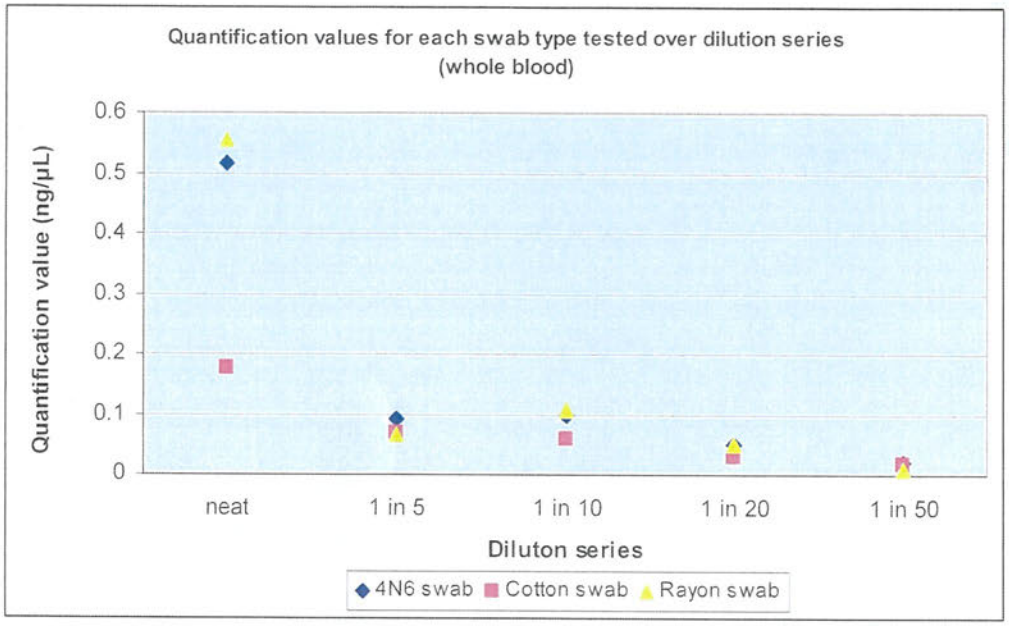

Figure 2. Quantification values from each dilution series for each of the 3 swab types tested

As with Experiment 1, all swabs yielded a full 9-loci DNA profile consistent with the expected profile.

Experiment 3 Release of DNA from cellular material

Table 3 below shows quantitation values for each swab head tested and the average quantitation value for each swab type. The same data is also represented in Figure 3 below.

Table 3. Summary of results from Experiment 3

4N6 Swab		Cotton swab		Rayon Swab	
Sample	Quantification value (ng/µL)	Sample	Quantification value (ng/µL)	Sample	Quantification value (ng/µL)
1	1.91	1	0.341	1	0.462
2	1.42	2	0.427	2	0.702
3	0.539	3	0.687	3	0.554
4	0.632	4	0.361	4	0.689
5	1.77	5	0.464	5	0.493
mean	1.2542	*mean*	0.4560	*mean*	0.5800

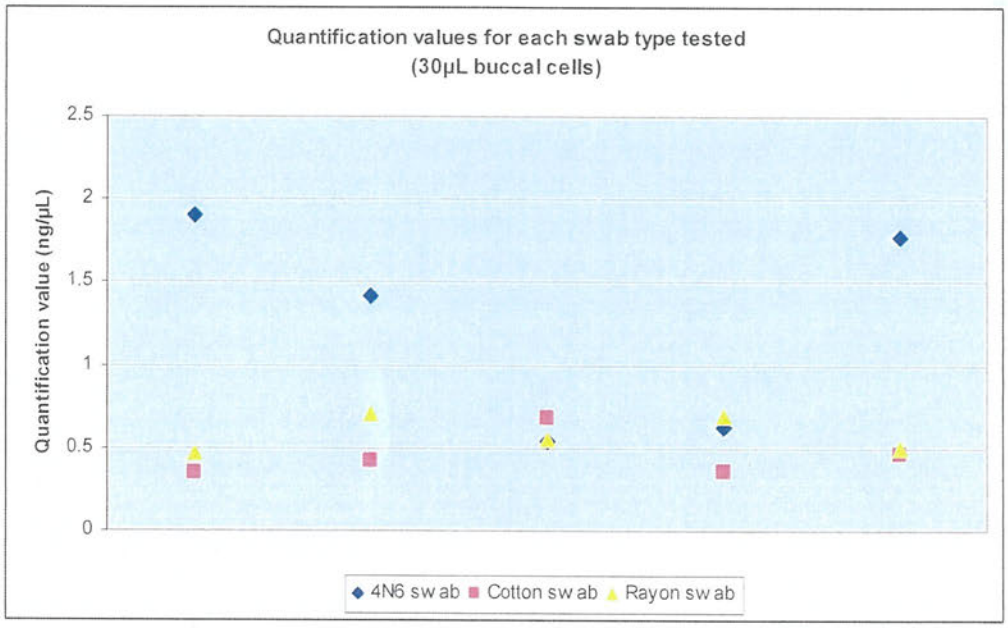

Figure 3. Quantification values for each of 5 replicates for each of 3 swab types tested

As with Experiment 1, all swabs yielded a full 9-loci DNA profile consistent with the expected profile.

Experiment 4 Release of DNA from cellular material over dilution series

Table 4 shows the quantification values obtained or the dilution series for each of the swab types tested. These results are graphically presented in Figure 4 below.

Table 4. Summary of results from Experiment 4

Dilution series	DNAQuantification (ng/µL)		
	4N6 Swab	Cotton Swab	Rayon Swab
Neat cells	1.19	1.06	1.14
1 in 5	0.227	0.257	0.266
1 in 10	0.132	0.101	0.12
1 in 20	0.0652	0.0317	0.0487
1 in 50	0.018	0.0226	0.0401

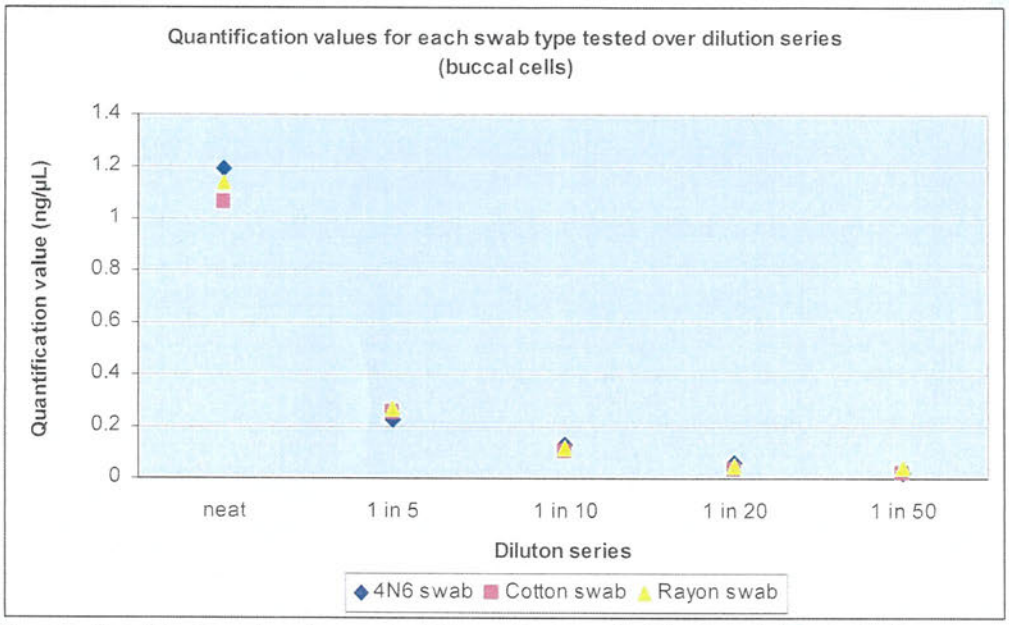

Figure 4. Quantification values from each dilution series for each of the 3 swab types tested

All swabs for samples containing 30µL of buccal cell suspension, as well as all swabs for each of the 1in5, 1in10 and 1in20 dilutions yielded full 9-loci DNA profiles consistent with the expected profile. However, the 1in50 dilution series yielded a full 9-loci DNA profile for the Rayon swab only, with the 4N6 swab and Cotton swab each producing partial DNA profiles (17/18 alleles and 12/18 alleles respectively).

Experiment 5 Uptake and release of DNA from blood and cellular material

Quantification values and profiles obtained for each swab type and sample type are shown in Table 5 below. Quantification values obtained for blood and cell sample types are graphically presented in Figures 5 & 6 respectively.

Table 5. Summary of results from Experiment 5

		4N6 swab		Cotton swab		Rayon swab	
Replicate		Quantification result (ng/µL)	Profile result (alleles)	Quantification result (ng/µL)	Profile result (alleles)	Quantification result (ng/µL)	Profile result (alleles)
Blood	1	0.507	18/18	0.869	18/18	0.785	18/18
	2	0.509	18/18	0.752	18/18	0.536	18/18
	3	0.561	18/18	0.506	18/18	1.12	18/18
	4	0.347	18/18	0.418	18/18	0.421	18/18
	5	0.432	18/18	0.307	18/18	3.39	18/18
	mean	*0.4712*	-	*0.5704*	-	*1.2504*	-
Cell suspension	1	0.298	13/18	0.326	11/18	0.42	10/18
	2	0.252	8/18	0.156	8/18	1.96	3/18
	3	0.26	5/18	0.321	12/18	0.732	4/18
	4	0.367	8/18	0.265	13/18	0.474	13/18
	5	0.456	13/18	0.449	13/18	0.36	13/18
	mean	*0.3266*	-	*0.3034*	-	*0.7892*	-

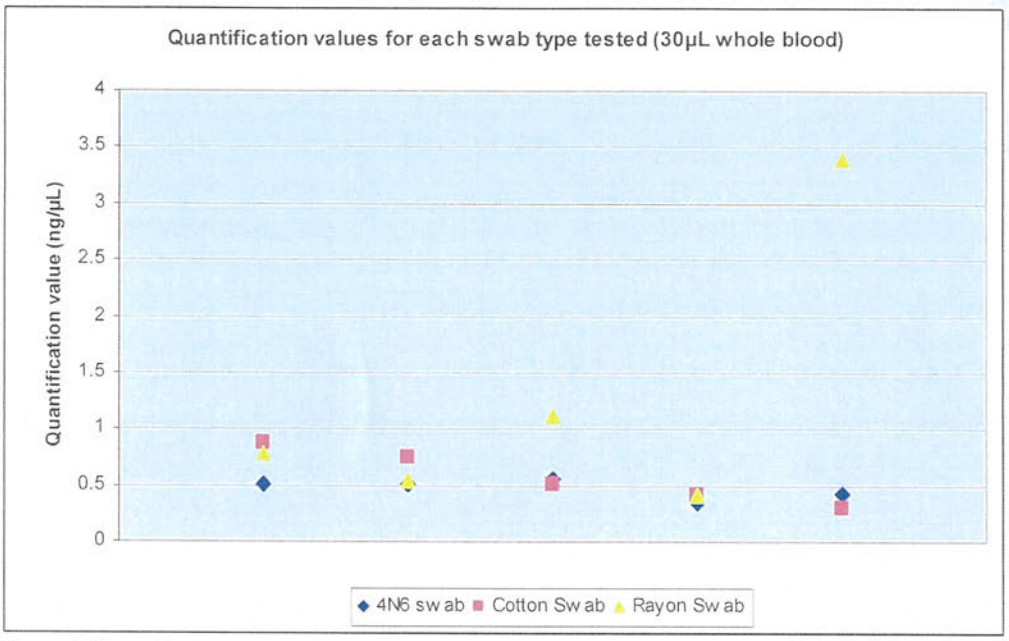

Figure 5. Quantification values from sampling of 30µL of dried blood for each of the 3 swab types tested

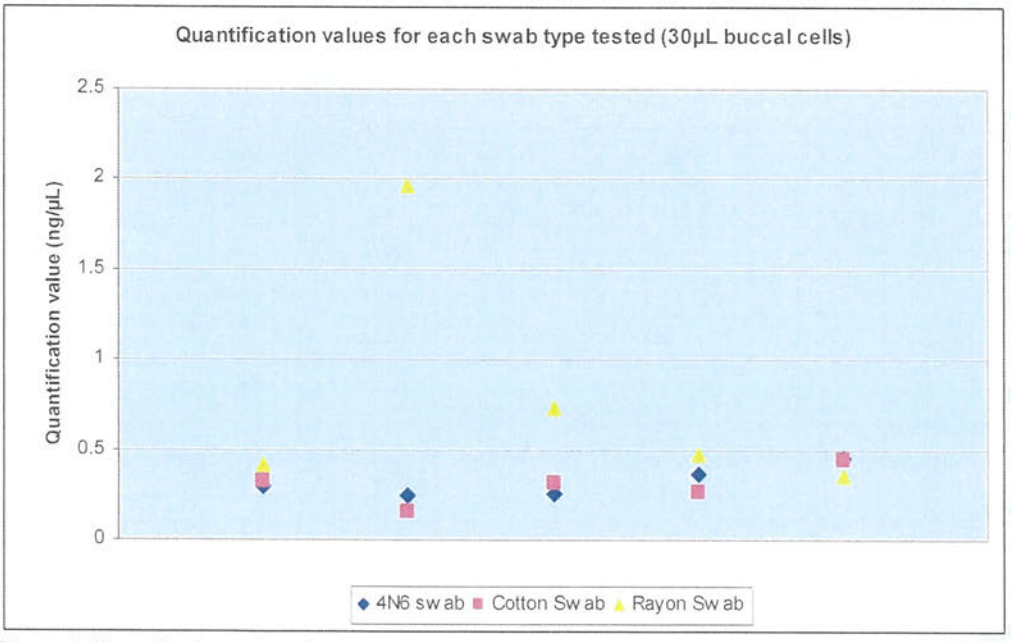

Figure 6. Quantification values from sampling of 30µL of dried buccal cell suspension for each of the 3 swab types tested

Although each swab that was used to sample dried buccal cells yielded partial DNA profiles, each of these samples contained sufficiently high quantification values to suggest that re-amplification with increased DNA template input would yield full 9-loci DNA profiles.

Statistical analysis for significant difference

Quantification values obtained from experiment 1 and from the "neat blood" sample from experiment 2 were compared for various combinations of swab types tested using a two-sided students *t*-test. Additionally, quantification valued obtained from experiment 3 and the "neat cells" sample from experiment 4 were compared using a two-sided students *t*-test. These results are shown in Table 6 below

Table 6. *t*-test results

Comparison**	*p*-value*	
	Blood	Cells
4N6 v Cotton	0.0034	0.0241
4N6 v Rayon	0.0560	0.0486
Cotton v Rayon	0.0234	0.4598

* a *p*-value of <0.05 indicates a significant difference between the two data sets under test when using a 95% confidence interval
**Results generated using Microsoft Excel formulae

Quantification values obtained from experiment 5 were compared for various combinations of swab types tested, using a two-sided students *t*-test. These results are shown in Table 7 below

Table 7. *t*-test results

Comparison**	*p*-value*	
	Blood	Cells
4N6 v Cotton	0.3978	0.7136
4N6 v Rayon	0.1939	0.1640
Cotton v Rayon	0.2577	0.1478

* a *p*-value of <0.05 indicates a significant difference between the two data sets under test when using a 95% confidence interval
**Results generated using Microsoft Excel formulae

DISCUSSION

As can be seen from the two-sided students *t*-test results in Table 6 above, the 4N6 swabs released more DNA (when 30µL of whole blood or 30µL of buccal cell suspension was added directly to the swab head) than cotton swabs. Since the *p*-value that is generated represents a probability of both data sets being not significantly different, and although the *p*-value for the comparison of 4N6 swabs against rayon swabs when 30µL of whole blood was added directly to the swab head falls slightly above the 0.05 threshold (*p*-value = 0.0560), the same can be held true for the comparison of 4N6 swabs and rayon swabs as for the comparison of 4N6 swabs and cotton swabs.

Over a dilution series for each swab type and for each sample type, quantification values and profiling quality obtained were quite similar. As the experiments were not duplicated, no statistical analysis could be performed. These results would support the proposition that, over a dilution series, the 4N6 swabs released DNA at least as well as either cotton or rayon swabs tested.

The two-sided students *t*-test results in Table 7 above show that the 4N6 swabs yielded comparable quantification values when both dried blood and dried cellular material was sampled according to standard laboratory practice. The DNA profile quality (alleles obtained) shown in Table 5 also supports the proposition that comparable DNA profiling was obtained when any of the three swab types were used to sample either dried blood or dried cellular material under controlled conditions. It may be possible that the 4N6 swab actually liberated less material from the dried stain than either of cotton or rayon swabs, but this was balanced by the superior ability of the 4N6 swab to release DNA, therefore resulting in comparable DNA profiling between all three swab types. However this cannot be accurately ascertained as quantification values between experiments 5 and previous experiments cannot be directly

compared as different buccal cell suspensions and slightly different extraction protocols were utilised for each experiment. Additionally, given the small sample size for these experiments (n=5), further testing is warranted to draw a clearer conclusion.

Partial profiles obtained for all swab types after sampling of dried buccal suspension may have been due to degradation of DNA from extended storage. It has been the experience within the laboratory that degraded DNA will tend to yield inflated quantification values with the Quantifiler™ Human DNA Quantification kit (Applied Biosytems). These samples were stored for approximately 15 weeks due to delays in testing caused by the demands of routine laboratory testing, and were not part of the testing regime.

There was no evidence of inhibition or other effects on the extraction, quantification or amplification of the DNA extracts obtained from the 4N6 swabs using the methods employed within the laboratory at the time of testing (these included updated methods to be implemented in the short term used in experiment 5).

The shaft of the 4N6 swab contains a breaking point, and with the laboratory procedures currently in place, this breaking point leaves an excessive amount of shaft making it unsuitable for easy processing (i.e. for each swab, it would need to be removed from it's tubing and have the shaft cut at the base of the swab head under sterile conditions, necessitating sterilisation of equipment and work area between each sample).

Recommendations

The testing carried in this trial has been on a small scale and represents some initial evaluation of the 4N6. The testing falls short of a validation or verification. All results should be viewed with caution given the small sample size for each experiment and the limited number of experiments performed, and as such no recommendation is made to either use or not use the 4N6 swab.