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ISO 9001 & ISO/IEC 17043

Final Report
(December 2019)

Proficiencytesting@forensicfoundations

Biological Examination and DNA Analysis 2019-3

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Introduction

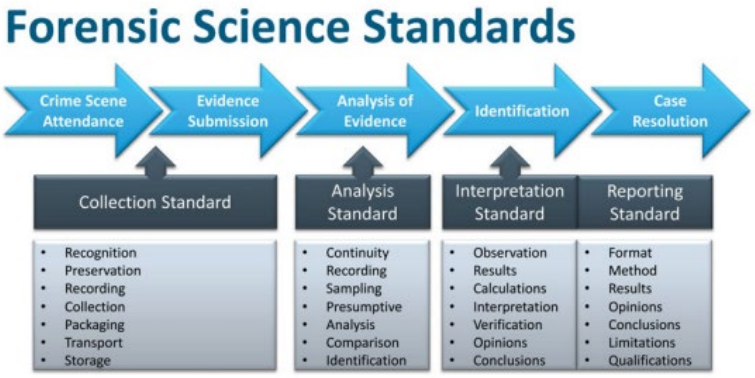
Design

Forensic Foundations' Proficiency Tests are designed to address the following points:

- Relevance to forensic science laboratories;
- Limitation of any potential context information;
- Knowledge of the 'ground truth' of samples;
- Importance of consistency between tests; and
- Cost affordability for the laboratories.

In addition to this exercise being a test of your laboratory procedures using controlled items, we also anticipated that it will enable participants to evaluate the quality of their analytical results against those from other laboratories and observe how other laboratories express their opinions or advice to clients. To enable this, we requested that participants submit the following:

- An outline of the methodology used; and
- Their opinion in the format that they would provide to court.



Forensic Foundations' Proficiency Tests are designed to test the end-to-end forensic examination process. The AS5388 and the ISO21043 series of Standards describe the forensic examination process from collection to reporting. This figure[1] illustrates the inter-relatedness of all steps in this process and was used as the basis of the Australian Standards' development. The figure is also used as the basis of the development of Forensic Foundations' Proficiency Tests.

Thus, all Forensic Foundations' Proficiency Tests commence with item collection and/or receipt and all the subsequent examination / analysis steps, culminating in the reporting, therefore reflecting actual forensic casework.

The Final Reports of this 2019 round of Proficiency Tests will be publicly available via Forensic Foundations web site. Participating laboratories may use the report as outlined in their respective laboratory policies.

[1] James Robertson, Karl Kent & Linzi Wilson-Wilde (2013) The Development of a Core Forensic Standards Framework for Australia, Forensic Science Policy & Management: An International Journal, 4:3-4, 59-67

Biological Examination and DNA Analysis 2019-3

This test was distributed to eleven laboratories, four laboratories submitted results during this round of testing.

The manufacture, distribution, assessment and reporting of this proficiency test has provided, and will provide, the basis for continuous improvement for both Forensic Foundations and the participating laboratories.

In addition to interpreting the results from known and unknown biological samples, testing generic issues such as receipt, triage and continuity of items for examination also formed part of the overall process.

In order to remove contextual bias in the interpretation, four suspects' reference samples were provided, however the actual number of 'offenders' was unknown.

Laboratory Responses

Continuity, receipt and description of items

Laboratory 96150B Full details of submission and continuity were provided by means of computer screen grabs. However, no details were provided regarding the description of any of the packaging (including seals), minimal generic descriptions of the items received were provided as part the data in the screen grabs. .

Laboratory 96150C Submission and receipt details of the items were provided. The case notes record that the exhibit identifiers match the Police paperwork. It was also noted that evidence tape was not used nor were the seals signed

The case notes include a detailed description of each item including a photograph, with scale when required.

The description provided by Laboratory 96150C concurs with the packaging, labelling and samples as distributed.

Laboratory 7307 Details regarding receipt to the laboratory were not provided, continuity was recorded by means of an ‘Exhibit Continuity Form’ and a ‘Return Seal Exhibits List’ . Each item and packaging was fully described in the case notes including the use of photographs with scale when required.

The descriptions provided by Laboratory 7307 concur with the packaging, labelling and samples as distributed.

Laboratory 54624 Details of receipt by the laboratory were recorded. The description of the packaging was recorded in written form and by means of photography. The sealed tamper evident bags were recorded as ‘sealed and labelled transparent plastic bags’. Seal numbers could not be identified from the photographs.

The description of the items was provided by means of annotated photographs.

The descriptions provided by Laboratory 54624 concur with the packaging, labelling and samples as distributed.

Examination / Analysis

Laboratory 96150B

Initial examination and results were outlined in the following table:

Exhibit	Sample	Method/Test	Result
Item 1 - Medical Samples	1.02 - High vaginal Swab	Microscopy – Christmas Tree Stain	Sperm were detected on a microscope smear prepared from the swab.
	1.03 - Low vaginal Swab	Microscopy – Christmas Tree Stain	Sperm were detected on a microscope smear prepared from the swab.
Item 2 - Singlet	1.04-01 – Cutting of blood-like stain on front left side (sample submitted for DNA analysis)	Siemens Hemastix® presumptive test for blood Acid Phosphatase (AP) presumptive test for semen	Hemastix® positive (HX:POS) AP Negative
	1.04-02 – area close to 1.04-01 (no sample for DNA analysis)	RSID™Saliva presumptive test for saliva	RSID Negative (RSID:NEG)
	1.04-03 – Cutting from upper right side	Presumptive tests for saliva – Phadebas® Press Test and RSID™Saliva	RSID Positive (RSID:POS) and Phadebas® positive
Item 3 - Underpants	1.05-04 – Cutting inner crotch	Acid Phosphatase (AP) presumptive test for semen Microscopy – Christmas Tree Stain	AP positive Sperm were detected on a microscope smear prepared from the swab.
	1.05-05 – area close to 1.05-04 (no sample for DNA analysis)	RSID™Saliva presumptive test for saliva	RSID:NEG
	1.05-06 – inner crotch towards rear (no sample for DNA analysis)	RSID™Saliva presumptive test for saliva Acid Phosphatase (AP) presumptive test for semen	RSID:NEG AP Negative

The following table summarises the methodology and results of further analysis:

Reference samples

Sample ID	Item 1.01	Item 4		Item 5		Item 6		Item 7	
Description	Reference Brown Saliva FTA card	Reference White Blood FTA card		Reference Nicholls Blood FTA card		Reference Smith Blood FTA card		Reference Greene Blood FTA card	
Extraction	DNAIQ								
Quant	Quantifiler Trio								
Amp	Globalfiler	Globalfiler	Yfiler Plus	Globalfiler	Yfiler Plus	Globalfiler	Y-filer Plus	Globalfiler	Y-filer Plus
FF Assessment of Results	Consistent with expected results	Consistent with expected results		Consistent with expected results		Consistent with expected results		Consistent with expected results	

Evidentiary samples

Sample ID	Item 1.02			
Description	Swab High Vaginal Semen			
Extraction	DNAIQ followed by differential extraction			
Faction	Epithelial		Sperm	
Quant	Quantifiler Trio			
Amp	Globalfiler	Yfiler Plus	Globalfiler	Yfiler Plus
FF Assessment of Results	Mixture consistent with expected female and male donors except at CSF1PO where a male donor allele was not present.	Profile consistent with expected male donor profile	Profile consistent with expected male donor profile	Profile consistent with expected male donor profile

Sample ID	Item 1.03			
Description	Swab Low Vaginal Semen			
Extraction	DNAIQ followed by differential extraction			
Faction	Epithelial		Sperm	
Quant	Quantifiler Trio			
Amp	Globalfiler	Yfiler Plus	Globalfiler	Yfiler Plus
FF Assessment of Results	Mixture consistent with expected female and male donors	Profile consistent with expected male donor profile	Profile consistent with expected male donor profile	Profile consistent with expected male donor profile

Sample ID	Item 1.04			
Description	Cutting singlet (Item2)			
Subsample ID	1.04-01 (Blood – Hx pos)		1.04-03 (Saliva – RSID pos)	
Extraction	DNAIQ			
Quant	Quantifiler Trio			
Amp	Globalfiler	Yfiler Plus	Globalfiler	Yfiler Plus
FF Assessment of Results	Profile consistent with expected male donor profile	Profile consistent with expected male donor profile	Profile consistent with expected male donor profile	Profile consistent with expected male donor profile

Sample ID	Item 1.05					
Description	Underpants (Item 3)					
Subsample ID	1.05-02 (Tape lift inner waistband Trace)		1.05-04 –Cutting Underpants Semen [AP:Pos]			
Extraction	PrepFiler BTA		Differential extraction			
Faction			Epithelial		Sperm	
Quant	Quantifler Trio					
Amp	Globalfiler	Yfiler Plus	Globalfiler	Yfiler Plus	Globalfiler	Yfiler Plus
FF Assessment of Results	Mixture of at least 3 individuals reported. Donor of semen not excluded. Female donor and other ‘suspect’ donors excluded	Mixture of 2 males. Donor of semen not excluded. Other ‘suspect’ donors excluded	Profile consistent with male donor profile	Profile consistent with male	Profile consistent with male donor profile	Profile consistent with male donor profile

Laboratory 96150C

The 'Item Examination' forms, included with report, contained information additional to that summarised below.
NB: LVS – Low vaginal swab, HVS – High vaginal swab.

Examination methods

Buccal reference sample
~ 10x10mm excised from sample area for DNA analysis.

FTA card reference samples
~10x10mm area of red/brown staining swabbed for DNA analysis.

Medical sample swabs
Acid phosphatase test (presumptive chemical test for semen) was conducted on a sample of the LVS and HVS items. RSID-Semen performed by excising a portion of the swab and used as a confirmatory test for human semen. Direct microscopy performed on HVS and LVS swabs, microscopy slides stained using the Christmas Tree staining method.

Singlet
Kastle Meyer (presumptive chemical test for blood) was conducted on the red/brown staining. The ABACard HemaTrace was performed by swabbing the stain and used as a confirmatory test for Human blood (inclusive of higher primate and ferret blood).
Trace sampling was conducted by tape-lift (avoiding visibly stained areas). A Crime-Lite was used to screen for areas of fluorescence- no fluorescent staining was observed.

Underpants
A Crime-Lite was used to screen for areas of fluorescence. The acid phosphatase test (presumptive chemical test for semen) was performed on areas of fluorescence identified by the Crime-Lite. RSID-Semen was performed by excising a portion of the fluorescent stain. Direct microscopy was performed on a sample of the fluorescent staining with the microscopy slides stained using the Christmas Tree staining method. A portion of the stain was excised for DNA analysis.

NOTE: As this test was being evaluated for suitability, a full suite of available screening and confirmatory tests were performed on these items. This is not indicative of routine casework processing by this laboratory, where a triage process is employed.

DNA Analysis methods

The following procedures were employed for DNA analysis within this case:

PrepFiler express BTA Pre-lysis and extraction using the Applied Biosystems AutoMate Express robotic platform: employed for tape-lifts.

PrepFiler Pre-lysis and extraction using the Applied Biosystems AutoMate Express robotic platform: employed for swabs and reference samples.

Differential extraction conducted via manual pre-lysis followed by DNA IQ Robotic extraction (sperm cell Faction) and QIAcube extraction (epithelial cell Faction).

All samples were quantified using the Applied Biosystems Quantifiler Trio DNA Quantification kit.

Amplification was conducted using the following kits: Promega PowerPlex 21 and ThermoFisher/Applied Biosystems AmpFLSTR Yfiler

Laboratory 7307

The 'Case Strategy / Instructions' form provided a framework for the examination and analysis of the items. Information and instructions were provided under the following headings:

- Case circumstances
- Points to prove / Expectations
- Items relating to Anne BROWNE
    - Item 1 ....
    - Item 2 ...
    - Item 3 ...
    - Item 4 ...
    - Item 5 ...
    - Item 6 ...
    - Item 7 ...

This form was annotated when tasks had been completed.

Details of the item examination and preliminary testing of evidentiary items were included in the case notes. The following testing regimes were used:
Item 1: Acid phosphatase & microscopy (Christmas Tree staining);
Item 2: Kastle-Myer, Acid Phosphatase, Phadebas;
Item 3: Visual searching, Acid phosphatase & microscopy (Christmas Tree staining);

Cutting and tape lifts were used as the means for subsampling.

The following samples were submitted to an external provider for DNA testing.

Item No.	Item
AB/1	1 x Buccal sample from ITEM1 medical samples from Anne BROWN
JW/1	1 x Blood sample from ITEM4 reference sample James WHITE
BN/1	1 x Blood sample from ITEM5 reference sample Billy NICHOLLS
KS/1	1 x Blood sample from ITEM6 reference sample Keaton SMITH
HG/1	1 x Blood sample from ITEM7 reference sample Hugo GREENE
ITEM2/1	1 x Blood stain from o/s front LHS from item number 2 singlet
ITEM2/3	1 x Saliva minitape from o/s front RHS from item number 2, singlet
ITEM1/1B	1 x Semen sample from ITEM1 LVS from Anne BROWN
ITEM1/2/A	1 x Semen sample from HVS swab from ITEM1, medical samples Anne BROWN 4/4/19
ITEM/3/3A	1 x Semen sample from I/S gusset of ITEM3 knickers

Details of the extraction, amplification and profiles obtained were not provided.

Laboratory 54624

The examination plan was outlined:

----- 1. Were there human saliva on evidence no. 261a/BIO/2019. ----------
----- 2. Were there human blood stain on evidence no. 262/BIO/2019, 264/BIO/2019 to
----- -- 267/BIO/2019. ----------
----- 3. Were there human semen stain on evidence no. 261b/BIO/2019, 261c/BIO/2019,
----- -- and 263/BIO/2019. ----------
----- 4. If human blood stain were found on evidence no. 262/BIO/2019, does it have a
----- -- matching DNA profile to evidence no. 261a/BIO/2019, 264/BIO/2019, 265/BIO/2019,
----- -- 266/BIO/2019, and 267/BIO/2019. ----------
----- 5. If human semen stain were found on evidence no. 261b/BIO/2019, 261c/BIO/2019,
----- -- and 263/BIO/2019, do they have matching DNA profiles with evidence no.
----- -- 264/BIO/2019, 265/BIO/2019, 266/BIO/2019, and 267/BIO/2019. ----------

Preliminary testing was undertaken:

A. Human saliva preliminary test result. ----------

Table 1. Saliva presence test result on evidence no. 261a/BIO/2019. ----------

Evidence	Test Method	
	Phadebas Test	Real Time PCR
261a/BIO/2019	Positive	Positive

Remarks: ----------
Phadebas Test: Preliminary test to check for saliva presence on evidence. ----------
Real Time PCR: Follow up test to check for human DNA presence on evidence by real-time PCR technique. ----------

B. Human blood stain preliminary test. ----------

Table 2. Blood stain presence test result on evidence no. 262/BIO/2019, 264/BIO/2019 to ---------- 267/BIO/2019. ----------

Evidence	Test Method	
	LMG Test	Real Time PCR
262/BIO/2019	Positive	Positive
264/BIO/2019	Positive	Positive
265/BIO/2019	Positive	Positive
266/BIO/2019	Positive	Positive
267/BIO/2019	Positive	Positive

Remarks: ----------
LMG (Leuco Malachite Green) Test: Preliminary test to check for blood presence on evidence. Real time PCR: follow up test to check for human DNA presence on evidence by real-time PCR technique. ----------

C. Human semen stain preliminary test result. ----------

Table 3. Semen stain presence examination result on evidence no. 261b/BIO/2019, ---------- ---------- 261c/BIO/2019 and 263/BIO/2019. ----------

Evidence	Test Method	
	Brentamine Test	Real Time PCR
261b/BIO/2019	Positive	Positive
261c/BIO/2019	Positive	Positive
263/BIO/2019	Positive	Positive

Remarks: ----------
Brentamine Test: Preliminary test to check for semen stain presence on evidence.
Real time PCR: follow up test to check for human DNA presence on evidence by real-time PCR technique.

The following table summarises the methodology and results of further analysis:

Reference samples

FF Sample ID	Item 1	Item 4	Item 5	Item 6	Item 7
Laboratory Sample ID	261a/BIO/2019	264/BIO/2019	265/BIO/2019	266/BIO/2019	267/BIO/2019
Extraction	Maxwell 16 Promega program				
Quantification	Quantifiler Duo				
Amplification	Globalfiler but not including Yindel				
FF Assessment of Results	Consistent with expected results	Consistent with expected results except at D12S391 where the lab reported 20,20. Pre-test results indicated the profile was 19.3, 20	Consistent with expected results	Consistent with expected results	Consistent with expected results

Evidentiary samples

FF Sample ID	Item 1	Item 3	Item 2	Item 3
Laboratory Sample ID	261b/BIO/2019	261c/BIO/2019	262/BIO/2019	263BIO/2019
Description	HVS	LVS	Blood stain singlet	Semen stain underpants
Extraction	Differential extraction followed by Maxwell 16 Promega program		Maxwell 16 Promega program	
Quantification	Quantifiler Duo			
Amplification	Globalfiler but not including Yindel			
FF Assessment of Results	Profile consistent with expected male and female donors	Profile consistent with expected male and female donors	Profile consistent with expected male donor profile	Profile consistent with expected male donor profile

Interpretation and Conclusions (please include the wording you would use in your report)

Laboratory 96150B

Reference sample(s):

Name	Exhibit
Anne BROWN	Item 1
James WHITE	Item 4
Billy NICHOLLS	Item 5
Keaton SMITH	Item 6
Hugo GREENE	Item 7

Evidence Samples: Autosomal STR Analysis:

Sample description		DNA profile description	Person	Hypothesis/Interpretation	Statistical weighting
Item 1: Medical samples relating to BROWN					
Sperm were observed on microscope smears prepared from the low vaginal and high vaginal swabs.					
1.02-01	Swab - high vaginal (sperm fraction)	Single source DNA profile	SMITH	**H1: SMITH is a contributor** H2: SMITH is not a contributor	> 100 billion (in favour of H1)
1.02-01	Swab - high vaginal (epithelial cell fraction)	Mixed DNA profile - two contributors including BROWN	WHITE	excluded	
			NICHOLLS	excluded	
			SMITH	**H1: SMITH is a contributor** H2: SMITH is not a contributor	> 100 billion (in favour of H1)
			GREENE	excluded	
1.03-01	Swab - low vaginal (sperm fraction)	Single source DNA profile	SMITH	**H1: SMITH is a contributor** H2: SMITH is not a contributor	10 000 (in favour of H1)
1.03-01	Swab - low vaginal (epithelial cell fraction)	Mixed DNA profile - two contributors including BROWN	WHITE	excluded	
			NICHOLLS	excluded	
			SMITH	**H1: SMITH is a contributor** H2: SMITH is not a contributor	> 100 billion (in favour of H1)
			GREENE	excluded	

Sample description		DNA profile description	Person	Hypothesis/Interpretation	Statistical weighting
Item 2: Singlet relating to BROWN					
Blood-like staining was detected on the front left side of the singlet. An area on the outer front right side gave a positive reaction to a presumptive test for saliva. Semen was not detected.					
1.04-01	Cutting - blood-like stain on front left singlet	Single source DNA profile	GREENE	**H1: GREENE is a contributor** H2: GREENE is not a contributor	> 100 billion (in favour of H1)
1.04-03	Cutting – stain on upper right side that gave a positive reaction to a presumptive test for saliva.	Single source DNA profile	SMITH	**H1: SMITH is a contributor** H2: SMITH is not a contributor	> 100 billion (in favour of H1)
Item 3: Underpants relating to BROWN					
Semen, confirmed by the presence of sperm, was detected on the inner crotch of the underwear. Saliva was not detected.					
1.05-01	Tapelift - outer waistband	Sample contains very low amounts or no DNA and was not analysed			
1.05-02	Tapelift - inner waistband	Mixed DNA profile - three contributors	BROWN	excluded	
			WHITE	excluded	
			NICHOLLS	H1: NICHOLLS is a contributor **H2: NICHOLLS is not a contributor**	> 100 billion (in favour of H2)
			SMITH	**H1: SMITH is a contributor** H2: SMITH is not a contributor	> 100 billion (in favour of H1)
			GREENE	excluded	
1.05-04	Cutting - inner crotch underpants (sperm fraction)	Single source DNA profile	SMITH	**H1: SMITH is a contributor** H2: SMITH is not a contributor	> 100 billion (in favour of H1)
1.05-04	Cutting - inner crotch (epithelial cell fraction)	Single source DNA profile	SMITH	**H1: SMITH is a contributor** H2: SMITH is not a contributor	> 100 billion (in favour of H1)

Evidence Samples: Y-STR DNA Analysis

Sample description		DNA profile description	Person	Hypothesis/Interpretation	Statistical weighting
Item 1: Medical samples relating to BROWN					
1.02-01	Swab - high vaginal (sperm fraction)	Single source DNA profile	SMITH	**H1: SMITH is a contributor** H2: SMITH is not a contributor	12 000 (in favour of H1)
1.02-01	Swab - high vaginal (epithelial cell fraction)	Single source DNA profile	SMITH	**H1: SMITH is a contributor** H2: SMITH is not a contributor	12 000 (in favour of H1)
1.03-01	Swab - low vaginal (sperm fraction)	Single source DNA profile	SMITH	**H1: SMITH is a contributor** H2: SMITH is not a contributor	12 000 (in favour of H1)
1.03-01	Swab - low vaginal (epithelial cell fraction)	Single source DNA profile	SMITH	**H1: SMITH is a contributor** H2: SMITH is not a contributor	12 000 (in favour of H1)
Item 2: Singlet relating to BROWN					
1.04-01	Cutting - blood-like stain on front left singlet	Single source DNA profile	GREENE	**H1: GREENE is a contributor** H2: GREENE is not a contributor	12 000 (in favour of H1)
1.04-03	Cutting - upper right side	Single source DNA profile	SMITH	**H1: SMITH is a contributor** H2: SMITH is not a contributor	12 000 (in favour of H1)

Sample description		DNA profile description	Person	Hypothesis/Interpretation	Statistical weighting
Item 3: Underpants relating to BROWN					
1.05-02	Tapelift - inner waistband	Mixed DNA profile - two contributors*	SMITH	**H1: SMITH is a contributor** H2: SMITH is not a contributor	12 000 (in favour of H1)
			WHITE	excluded	
			NICHOLLS	excluded	
			GREENE	excluded	
1.05-04	Cutting - inner crotch (sperm fraction)	Single source DNA profile	SMITH	**H1: SMITH is a contributor** H2: SMITH is not a contributor	12 000 (in favour of H1)
1.05-04	Cutting - inner crotch (epithelial cell fraction)	Single source DNA profile	SMITH	**H1: SMITH is a contributor** H2: SMITH is not a contributor	12 000 (in favour of H1)

* A major and minor component was interpreted from the Y-STR profile generated from sample 1.05-02.

Autosomal STR Analysis: DNA profile interpretation and Likelihood ratio calculation for DNA profile comparisons were performed using a continuous probabilistic genotyping program (STRmix™).

YSTR Analysis: DNA profile analysis was performed using binary interpretation methods using thresholds developed in house. Interpreted components were compared to the YHRD: Y Chromosome STR Haplotype Reference Database and a Likelihood ratio calculated using data from YHRD.

Where SMITH is favoured as a contributor to a DNA profile it was noted that in multiple DNA profiles raised n-4 stutter was observed at D2S1338:25. The n-4 stutter peak at D2S1338: 25 in the reference sample for SMITH was within the stutter threshold range

Laboratory 96150C

Please note that the "Case analysis, Interpretation and Conclusions" section has not been completed for this test.
As noted on the results sheet, due to the timing of the test being issued, and the size and complexity of profile interpretation and review required, the laboratory has not had the resources available to undertake the necessary analysis, interpretation and peer review of the casework results to submit them by the due date without impacting on routine caseload deadlines.
The results of the DNA analyses will be reviewed against the Manufacturer's Information when it is made available.

Laboratory 7307

Reference Samples

Name	Sample	Type	Comments
Anne BROWN	AB/1	Elimination	DNA 17 profile obtained
James WHITE	JW/1	Blood sample	DNA 17 profile obtained
Billy NICHOLLS	BN/1	Blood sample	DNA 17 profile obtained
Keaton SMITH	KS/1	Blood sample	DNA 17 profile obtained
Hugo GREENE	HG/1	Blood sample	DNA 17 profile obtained

Exhibits relating to Anne BROWN:

Exhibit:	Item 1	Description:	Low vaginal swab
Exhibit:	Item 1	Description:	High vaginal swab

These were examined for semen and the presence of semen was confirmed to both swabs. A sample from each swab was submitted separately for DNA analysis as ITEM1/1B and ITEM1/2/A.

Complete DNA profiles were obtained from both samples which matched the reference DNA profile of Keaton SMITH. In my opinion, these DNA profiles have originated from the semen tested. It was estimated to be a billion times more likely that the semen originated from Keaton SMITH rather than an individual unknown and unrelated to him.

There was an additional DNA component detected in the result from ITEM1/2/A, however, I understand that this finding was not suitable for robust comparisons to reference DNA profiles.

Additional material and the microscope slides created during the course of these examinations have been exhibited as ITEM1/1, ITEM1/2 and ITEM1/SLIDES.

Exhibit:	ITEM 2	Description:	Singlet

This was a grey sleeveless vest.

The outside front and back was examined for the presence of bloodstaining using overhead lighting. Blood was found to the outside front left side and to the outside back lower right side. Areas from this bloodstaining were taken as ITEM2/1 and ITEM2/2 respectively. Additional bloodstaining was found to the outside front lower right side. ITEM2/1 was submitted for DNA analysis.

A complete DNA profile was obtained which matched the reference DNA profile of Hugo GREENE. In my opinion, this DNA profile has originated from the blood tested. It was estimated to be a billion times more likely that the blood originated from Hugo GREENE rather than an individual unknown and unrelated to him.

The outside front and back was examined for the presence of saliva. An area of saliva was found to the front right of the vest with an additional area of probable saliva in close proximity to it. The saliva was sampled as ITEM2/3 and submitted for DNA analysis.

One possible DNA component was detected which was unsuitable for comparisons with reference DNA profiles.

The outside front and back was examined for the presence of semen. No semen was found.

Sample ITEM2/2 was not submitted for DNA analysis.

Exhibit:	ITEM 3	Description:	Underpants collected from Anne BROWN

The inside and outside of the left and right sides of the waistband were sampled as ITEM3/1 and the inside and outside front of the waistband was sampled as ITEM3/2 both in an attempt to recover cellular material containing DNA which could assist in determining who may have come into contact with the garment. However, these were not submitted for DNA analysis.

The inside and outside front and back of the knickers was examined for the presence of semen. Semen was confirmed to the inside front gusset. A sample of this semen was submitted for DNA analysis as ITEM3/3A.

A complete DNA profile was obtained which matched the reference DNA profile of Keaton SMITH. In my opinion, this DNA profile has originated from the semen tested. It was estimated to be a billion times more likely that the semen originated from Keaton SMITH rather than an individual unknown and unrelated to him.

Additional material and the microscope slides created during the course of this examination have been exhibited as ITEM3/3 and ITEM3/SLIDES.

FINDINGS AND INTERPRETATION:

Semen which could have come from Keaton SMITH was found on the low and high vaginal swabs and the inside front gusset of the knickers, all recovered from Anne BROWN. In my opinion, the findings are what I would expect if Keaton SMITH had vaginal intercourse with internal ejaculation with Anne BROWN in the 7 days prior to the swabs being taken. However, I can consider an alternative explanation should one be provided.

Blood which could have come from Hugo GREENE was found on the front left side of the vest belonging to Anne BROWN.

Should the bloodstaining in this case require an interpretation on how it was deposited or if it is necessary for the findings to be evaluated in relation to the specific scenario, then this would need to be carried out by a colleague with the relevant experience. This would be more effective carried out in advance of any trial.

Saliva was found on the front right of the vest belonging to Anne BROWN, however, I am unable to determine from whom this saliva could have originated.

Laboratory 54624

From the results of the criminalistics laboratory examination of the evidence, it could be concluded that. ----------

1. **It is correct** that the evidence reference buccal swab from the complainant **contains -- human saliva from a female individual (X, X)**. ----------
2. **It is correct that** the singlet evidence from the complainant **contains human blood stain -- from a male individual (X, Y)**. ----------
3. **It is correct that** the underpants evidence from the complainant **contains human semen -- stain from a male individual (X, Y)**. ----------
4. **It is correct that** the high vaginal swab evidence from the complainant **contains human -- semen stain and has more than one pair of alleles. Therefore, the biological materials -- on the high vaginal swab from the complainant came from more than one individual**.
5. **It is correct that** the low vaginal swab evidence from the complainant **contains human -- semen stain with more than one pair of alleles. Therefore, the biological materials on -- the low vaginal swab from the complainant came from more than one individual**. -----
6. The blood sample of **JAMES WHITE**'s was able to be analyzed, and it **came from a -- male individual (X, Y)**. ----------
7. The blood sample of **BILLY NICHOLLS'** was able to be analyzed, and it **came from a -- male individual (X, Y)**. ----------
8. The blood sample of **KEATON SMITH**'s was able to be analyzed, and it **came from a -- male individual (X, Y)**. ----------
9. The blood sample of **HUGO GREENE**'s was able to be analyzed, and it **came from a -- male individual (X, Y)**. ----------
10. The allele pairs on the twenty-three STR marker loci of the epithelial high vaginal swab -- from the complainant **matched** the allele pairs on the buccal swab's twenty-three STR -- marker loci of the complainant. **Therefore, both biological materials came from the -- same individual**. ----------
11. The allele pairs on the twenty-three STR marker loci of the epithelial low vaginal swab of -- the complainant **matched** with the allele pairs on the twenty-three STR marker loci of the -- buccal swab of the complainant. **Therefore, both biological materials came from the -- same individual**. ----------
12. The allele pairs on fourteen (out of twenty-three) STR marker loci of the epithelial high -- vaginal swab of the complainant **did not match** with the allele pairs of fourteen (out of -- twenty-three) STR marker loci of the blood sample of **JAMES WHITE**. **Therefore, both -- biological materials did not come from the same individual**. ----------
13. The allele pairs on thirteen (out of twenty-three) STR marker loci of the epithelial high -- vaginal swab of the complainant **did not match** with the allele pairs of thirteen (out of -- twenty-three) STR marker loci of the blood sample of **BILLY NICHOLLS**. **Therefore, -- both biological materials did not come from the same individual**. ----------
14. The allele pairs on the twenty-three STR marker loci of the epithelial high vaginal swab -- of the complainant **matched** with the allele pairs on the twenty-three STR marker loci of -- the blood sample of **KEATON SMITH**. **Therefore, both biological materials came -- from the same individual**. ----------
15. The allele pairs on fourteen (out of twenty-three) STR marker loci of the epithelial high -- vaginal swab of the complainant **did not match** with the allele pairs of fourteen (out of -- twenty-three) STR marker loci of the blood sample of **HUGO GREENE**. **Therefore, both -- biological materials did not come from the same individual**. ----------

16. The allele pairs on fourteen (out of twenty-three) STR marker loci of the epithelial low -- vaginal swab of the complainant **did not match** with the allele pairs of fourteen (out of -- twenty-three) STR marker loci of the blood sample of **JAMES WHITE**. **Therefore, both -- biological materials did not come from the same individual**. ----------
17. The allele pairs on the thirteen (out of twenty-three) STR marker loci of the epithelial low -- vaginal swab of the complainant **did not match** with the allele pairs of thirteen (out of -- twenty-three) STR marker loci of the blood sample of **BILLY NICHOLLS**. **Therefore, -- both biological materials did not come from the same individual**. ----------
18. The allele pairs on the twenty-three STR marker loci of the epithelial low vaginal swab of -- the complainant **matched** with the allele pairs on the twenty-three STR marker loci of the -- blood sample of **KEATON SMITH**. **Therefore, both biological materials came from -- the same individual**. ----------
19. The allele pairs on fourteen (out of twenty-three) STR marker loci of the epithelial low -- vaginal swab of the complainant **did not match** with the allele pairs of fourteen (out of -- twenty-three) STR marker loci of the blood sample of **HUGO GREENE**. **Therefore, both -- biological materials did not come from the same individual**. ----------
20. The allele pairs on fourteen (out of twenty-three) STR marker loci of the seminal high -- vaginal swab of the complainant **did not match** with the allele pairs of fourteen (out of -- twenty-three) STR marker loci of the blood sample of **JAMES WHITE**. **Therefore, both -- biological materials did not come from the same individual**. ----------
21. The allele pairs on thirteen (out of twenty-three) STR marker loci of the seminal high -- vaginal swab of the complainant **did not match** with the allele pairs of thirteen (out of -- twenty-three) STR marker loci of the blood sample of **BILLY NICHOLLS**. **Therefore, -- both biological materials did not come from the same individual**. ----------
22. The allele pairs on the twenty-three STR marker loci of the seminal high vaginal swab of -- the complainant **matched** with the allele pairs of twenty-three STR marker loci of the -- blood sample of **KEATON SMITH**. **Therefore, both biological materials came from -- the same individual**. ----------
23. The allele pairs on fourteen (out of twenty-three) STR marker loci of the seminal high -- vaginal swab of the complainant **did not match** with the allele pairs of fourteen (out of -- twenty-three) STR marker loci of the blood sample of **HUGO GREENE**. **Therefore, both -- biological materials did not come from the same individual**. ----------
24. The allele pairs on fourteen (out of twenty-three) STR marker loci of the seminal low -- vaginal swab of the complainant **did not match** with the allele pairs of fourteen (out of -- twenty-three) STR marker loci of the blood sample of **JAMES WHITE**. **Therefore, both -- biological materials did not come from the same individual**. ----------
25. The allele pairs on thirteen (out of twenty-three) STR marker loci of the seminal low -- vaginal swab of the complainant **did not match** with the allele pairs of thirteen (out of -- twenty-three) STR marker loci of the blood sample of **BILLY NICHOLLS**. **Therefore, -- both biological materials did not come from the same individual**. ----------
26. The allele pairs on the twenty-three STR marker loci of the seminal low vaginal swab of -- the complainant **matched** with the allele pairs of twenty-three STR marker loci of the -- blood sample of **KEATON SMITH**. **Therefore, both biological materials came from -- the same individual**. ----------

27. The allele pairs on fourteen (out of twenty-three) STR marker loci of the seminal low -- vaginal swab of the complainant **did not match** with the allele pairs of fourteen (out of -- twenty-three) STR marker loci of the blood sample of **HUGO GREENE**. **Therefore, -- both biological materials did not come from the same individual**. ----------

28. The allele pairs on twenty-two (out of twenty-three) STR marker loci of the blood stain -- on the undershirt from the complainant **did not match** with the allele pairs of twenty-two -- (out of twenty-three) STR marker loci of the blood sample of **JAMES WHITE**. -- **Therefore, both biological materials did not come from the same individual**. ----------

29. The allele pairs on eighteen (out of twenty-three) STR marker loci of the blood stain on -- the undershirt from the complainant **did not match** with the allele pairs of eighteen (out -- of twenty-three) STR marker loci of the blood sample of **BILLY NICHOLLS**. -- **Therefore, both biological materials did not come from the same individual**. ----------

30. The allele pairs on twenty-one (out of twenty-three) STR marker loci of the blood stain -- on the undershirt from the complainant **did not match** with the allele pairs of twenty-one -- (out of twenty-three) STR marker loci of the blood sample of **KEATON SMITH**. -- **Therefore, both biological materials did not come from the same individual**. ----------

31. The allele pairs on twenty-three STR marker loci of the blood stain on the undershirt from -- the complainant **matched** with the allele pairs of twenty-three STR marker loci of the -- blood sample of **HUGO GREENE**. **Therefore, both biological materials came from -- the same individual**. ----------

32. The allele pairs on fourteen (out of twenty-three) STR marker loci of the semen stain on -- the underpants from the complainant **did not match** with the allele pairs of fourteen (out -- of twenty-three) STR marker loci of the blood sample of **JAMES WHITE**. **Therefore, -- both biological materials did not come from the same individual**. ----------

33. The allele pairs on thirteen (out of twenty-three) STR marker loci of the semen stain on -- the underpants from the complainant **did not match** with the allele pairs of thirteen (out -- of twenty-three) STR marker loci of the blood sample of **BILLY NICHOLLS**. -- **Therefore, both biological materials did not come from the same individual**. ----------

34. The allele pairs on the twenty-three STR marker loci of the semen stain on the underpants -- from the complainant **matched** with the allele pairs of twenty-three STR marker loci of -- the blood sample of **KEATON SMITH**. **Therefore, both biological materials came -- from the same individual**. ----------

35. The allele pairs of fourteen (out of twenty-three) STR marker loci of the semen stain on -- the underpants from the complainant **did not match** with the allele pairs of fourteen (out -- of twenty-three) STR marker loci of the blood sample of **HUGO GREENE**. **Therefore, -- both biological materials did not come from the same individual**. ----------

Conclusion and Summary of the Test

The aim of this test was to examine the end-to-end forensic examination and analysis process. To minimise extraneous elements influencing the interpretation, limited contextual information was provided to the participating laboratories.

Items were sealed in tamper evident bags and included descriptors for continuity purposes.

The Forensic Science laboratories were provided with 4 reference blood samples, 1 singlet, 1 pair of underpants and 1 DNA Mini kit containing one FTA card and 2 swabs.

The singlet contained 100ul of suspect 4's blood (Greene) and 100ul of suspect 3's saliva (Smith)

The underpants contained 100ul of suspect 3's semen (Smith)

The DNA Mini Kit included:

- A FTA card contained saliva from the victim (Brown)
- A swab labelled High Vag contained 100ul of the victim's saliva (Brown) representing epithelial cells, and 50ul of suspect 3's semen (Smith)
- A swab labelled Low Vag contained 50ul of the victim's saliva (Brown) representing epithelial cells, and 10ul of suspect 3's semen (Smith)

NOTE: The following observations are based on the material provided; it is understood that the laboratories may hold additional material.

The table on the following page provides a 'snapshot' of the results provided by the four laboratories.

Laboratory	96150B			96150C			7307			54624		
Continuity and receipt	Screen grabs			✓			Continuity withing the laboratory and upon return from external provider			✓		
Description of items				✓			✓			Annotated photographs		
FF Item numbers	1	2	3	1	2	3	1	2	3	1	2	3
Preliminary testing of evidentiary samples	Microscopy (Christmas Tree)	Hemastix Acid Phosphatase RSID Saliva Phadebas	Acid Phosphatase Microscopy (Christmas Tree) RSID Saliva	Acid Phosphatase Microscopy (Christmas Tree) RSID Semen	ALS Kastle Meyer ABACard HemaTrace	ALS Acid Phosphatase Microscopy (Christmas Tree) RSID Semen	Acid Phosphatase Microscopy (Christmas Tree)	Kastle-Myer, Acid Phosphatase, Phadebas	Acid phosphatase Microscopy (Christmas Tree)	Brentamine Realtime PCR	Leuco Malachite Green Real time PCR	Brentamine Realtime PCR
Sample collection	Cutting & tape lift			Cutting & tape lift			Cutting & tape lift					
Extraction	DNAIQ followed by differential extraction	DNAIQ	DNA IQ only & DNAIQ followed by differential extraction	Differential extraction - manual pre-lysis followed by DNAIQ	Prepfiler BTA for tapelift	Differential extraction - manual pre-lysis followed by DNAIQ				Differential extraction followed by Maxwell 16 Promega method	Maxwell 16 Promega method	Maxwell 16 Promega method
Quantification	Quantifiler Trio			Quantifiler Trio						Quantifiler Duo		
Amplification	Globalfiler & Yfiler Plus			PowerPlex 21 & Yfiler			DNA 17			Globalfiler less Yindel		
FF Assessment of results	Expected results						Expected results			Expected results for blood and semen and epithelial faction. Saliva on T-shirt not detected.		
Statistical interpretation (Genomic)	LR: HVS > 100 billion LVS > 100 billion	LR: Blood > 100 billion Saliva > 100 billion	LR: Semen > 100 billion				LR: ~1 billion	LR: Blood ~1 billion Saliva ~1 billion	LR: ~1 billion			

Continuity, receipt, and description of items

This test was designed to test the end-to-end forensic process.

As the chain of custody for items, subject to forensic examination and analysis, is significant to the final outcome, information pertaining to receipt, continuity and a description of the items formed part of this test.

All of the laboratories participating in this test provided all or some of this information.

Examination / Analysis

Two laboratories provided an outline of the case examination approach prior to commencing the examination.

All laboratories employed the appropriate combination of:

- Acid Phosphatase / Brentamine
- Microscopy (Christmas Tree stain)
- RSID Semen
- Hemastix
- Kastle Meyer
- Leuco Malachite Green
- ABACard HemaTrace

Two laboratories used Phadebas or Phadebas & RSID Saliva to detect the saliva stain on Item 2 – Singlet.

One laboratory used Phadebas to detect saliva on Item 1 – Reference sample – Brown.

One laboratory used Real time PCR to confirm the presence of human DNA following preliminary testing.

Subsampling was undertaken by means of cutting or tapelifts.

The following methods were employed for the extraction of DNA from the evidentiary subsamples:

- Item 1 swabs
    - Differential extraction + DNAIQ or Maxwell 16 method
- Item 2 blood and saliva
    - Prepfiler BTA (Tapelifts) or DNAIQ or Maxwell 16 method (cuttings)
- Item 3 semen
    - Differential extraction + DNAIQ or Maxwell 16 method

Quantification was undertaken using either Quantifiler Duo or Quantifiler Trio.

The four laboratories each employed a different set of amplification systems:

- Globalfiler + Yfiler Plus
- PowerPlex 21 + Yfiler
- DNA17
- Globalfiler less Yindel.

However, as these systems all have loci in common, the laboratory results and the pretesting results can be compared.

Interpretation and Conclusions

In each case, where evidentiary samples were tested, the expected results were obtained and the correct conclusions made.

One laboratory reported an incorrect result at D12S391 for one of the reference samples – Item 4 White.

Two laboratories provided a statistical interpretation of the results using the Likelihood Ratio (LR) approach. The LR in each case appeared to be the laboratories' threshold reporting level rather than the LR specific to each sample.

Proficiency Test conclusions

The data provided by the four laboratories, in this round of proficiency testing demonstrate that although different methodologies were used, the testing enables the laboratory to provide the correct interpretation.

The only incorrect result was one mistyping in one reference sample.

Two laboratories did not detect the saliva on Item 2 – singlet.

APPENDIX A Form No: WEF-03-A

Proficiencytesting@forensicfoundations

PROGRAM PLAN

Program	Biological Testing and DNA		
Round	2019-3		
Program Coordinator /Technical Manager	Mrs. Anna Davey Director Forensic Foundations PO Box 2279 North Ringwood, 3134		
Discipline specific expert(s)	Dr Henry Roberts c/- Forensic Foundations PO Box 2279 North Ringwood, 3134		
Providers(s)	Initial sample collection & test production. Results interpretation.	Matched Semen/Blood/Saliva	DNA Profiling - Blood
		Cardinal Bioresearch Pty Ltd PO Box 1393 New Farm Qld 4005	DNALabs Level 1, 14 Giffnock Ave Macquarie Park NSW 2113
	Forensic Foundations PO Box 2279 North Ringwood, Victoria 3134	Blood	DNA Profiling saliva and semen
		Australian Red Cross Blood Bank 23-47 Villiers St North Melbourne, Vic 3051	Victorian Institute of Forensic Medicine 65 Kavanagh St Southbank Vic 3006
	Sample distribution to government facilities within Australia & NZ by ANZPAA-NIFS 637 Flinders St Docklands		
Aims/Objectives	The aim of the program is to assess the laboratories' ability to competently identify biological material and assess its significance and extract / amplify and interpret DNA profiles.		
Purpose	To assist the laboratories by ensuring their methods/procedures are performing adequately.		
Program Design			
Number of Rounds	1		
Number and type of samples	7- Clothing (t-shirt or similar, underpants) seeded with biological material (blood, saliva) Medical samples seeded with semen / epithelial cells Reference blood samples		
Hazard involved	Normal biohazard precautions should be taken when handling and disposing of blood and semen products.		
Scenario	A female complainant has been sexually assaulted. Participants will be provided with the complainant's clothing, medical samples		

	and a reference sample. Reference samples will also be provided from suspects
Sample size/ volume	The amount to biological material will range from 10-100ul.
Range of values/assigned values for reporting	The allelic values will be identified within the known allelic size range.
Traceability/origin of assigned values	1. Identification of biological material recorded upon collection. 2. DNA extracted by DNALabs/VIFM – continuity maintained. DNA profile obtained and independently interpreted by two individuals. 3. Samples seeded using masks and checked by second scientist 4. Alternate samples will be retained.
Design and Methods	Routine laboratory methods will be used to identify saliva, blood, semen and for DNA Profiling The scenario set up is designed to represent a typical sexual assault scenario
Selection Criteria	Selection of unknowns will be random.
Potential Major Sources of Error	Failure to identify biological stains, failure to correctly interpret DNA profiles, failure to properly disinfect workspace prior to sample preparation
Participants	Forensic Biology laboratories
Reporting Criteria, Accuracy	NA
Analysis	Correctly identify all biological material and interpret DNA profiles including mixtures and partial profiles.

Pre-testing	
Homogeneity Testing and criteria	Test samples will be set up, alternating with verification and retention samples. Verification samples will be tested prior to release to ensure homogeneity using positive presumptive tests of stained areas.
Stability Testing and criteria	NA – Dry staining remains stable in solution for periods in excess of the duration of the test. Historical data demonstrates the stability of dried stains.
Technical Review (internal)	
Participant Instructions	Provide copy of Instructions and evidence of Technical Review
Results Sheet	Provide copy of Results Sheet and evidence of Technical Review
Report	Include copy of Report and evidence of Technical Review

Sample Preparation	
Special conditions	Work area must be thoroughly cleaned before and after sample preparation using 0.5% Sodium Hypochlorite (NaOCl) (approximately 5000ppm free chlorine) or an alternative suitable disinfectant recommended by the facility. 0.5% NaOCl may be prepared by diluting household bleach (1 part) with water (9 parts)

Storage requirements	Biological samples 4°C Test samples Room Temperature
Distribution requirements	Distributed via Forensic Foundations
Sample checks	NA
Program Dates	
Invitation letter	August 2018
Sample distribution	First week May 2019
Results due	26th July 2019
Manufacturing Information to be sent	9th August 2019
Final report due date	First week October 2019
Statistical Analysis	
Homogeneity Testing	NA
Stability Testing	NA
Data Entry	Include evidence of data entry checks in file
	NA
Review by Statistician	NA
Reporting	
Report No:	2019-3
Master copy	Reports folder
Availability	Website

Program Coordinator signature: Anna Davey

Date: 31/1/2019

APPENDIX B

Proficiencytesting@forensicfoundations

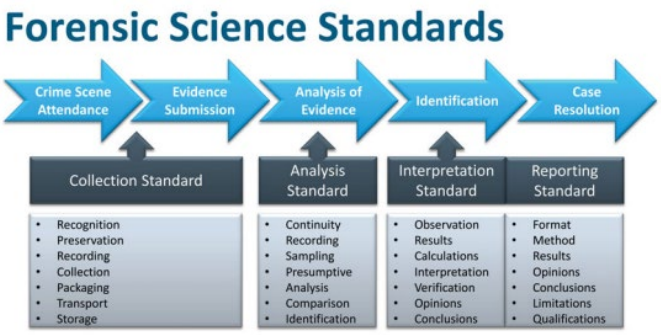
Forensic Biology
Biological examination and DNA
Sexual Assault
2019-3

Thank you for participating in this Proficiency Test. We hope that you find this test useful and welcome any feedback which can be used in the design of further tests.

In addition to this exercise being a test of your laboratory procedures using controlled items, we also anticipate that it will enable participants to evaluate the quality of their analytical results against those from other laboratories and observe how other laboratories express their opinions or advice to clients. To enable this, we request that participants submit the following:

- An outline of the methodology used; and
- Their opinion in the format that they would provide to court.

Forensic Foundations' Proficiency Tests are designed to test the end-to-end forensic examination process. The AS5388 and the ISO21043 series of Standards describe the forensic examination process from collection to reporting. This figure[2] illustrates the inter-relatedness of all steps in this process and was used as the basis of the Australian Standards' development. The figure is also used as the basis of the development of Forensic Foundations' Proficiency Tests. Thus, all Forensic Foundations' Proficiency Tests commence with item collection and/or receipt and all the subsequent examination / analysis steps, culminating in the reporting, therefore reflecting actual forensic casework.



Attached you will find the case 'Examination Request and Item Submission' form and the test commences with the receipt of the items followed by your routine processes- item description, examination, analysis and interpretation. The information submitted to the laboratory on the examination request form will direct what testing needs to be undertaken. Please use the attached results sheets. Additional pages may be added if required. An electronic copy of the results sheet can be downloaded from https://www.forensicfoundations.com.au/download/The results sheets should be returned to Forensic Foundations by Friday 26th July 2019. Hardcopy can be returned to PO Box 2279, Ringwood, Victoria, 3134, Australia or a soft copy can be uploaded to https://www.forensicfoundations.com.au/upload/

Qualitative feedback will be provided to participants. Feedback will be both participant-specific (i.e., whether a particular laboratory "got the right answer") and group specific (e.g., which techniques seemed to perform better than others).

Following the conclusion of the testing participants will be advised of the expected results and details regarding the production of the test.

[2] James Robertson, Karl Kent & Linzi Wilson-Wilde (2013) The Development of a Core Forensic Standards Framework for Australia, Forensic Science Policy & Management: An International Journal, 4:3-4, 59-67

APPENDIX C

EXAMINATION REQUEST AND ITEM SUBMISSION	EASTERN AUSTRALIAN POLICE SERVICE

OFFENCE:	Sexual Assault
DATE OF OFFENCE	Thursday 4th April 2019

BRIEF STATEMENT OF FACTS

Ms Anne Brown was walking in her local park, listening to music on her headphones. She was grabbed from behind. She believes that there were three or four offenders. She was held down and sexually assaulted. She believes:

- at least one of the offenders ejaculated;
- someone spat at her; and
- she hit one of the offenders, causing them to have a nose bleed.

Ms Brown attended the local hospital where she was forensically examined, and items of her clothes were collected.

ITEM SUBMITTED FOR EXAMINATION

Item 1 – Medical samples comprising:

- Reference buccal swab from complainant
- High vaginal swab collected from complainant
- Low vaginal swab collected from complainant

Item 2 – Singlet collected from complainant
Item 3 – Underpants collected from complainant
Item 4 – Reference sample – suspect 1 – James White
Item 5 –Reference sample – suspect 2 – Billy Nicholls
Item 6 –Reference sample – suspect 3 – Keaton Smith
Item 7 – Reference sample – suspect 4 – Hugo Greene

EXAMINATION REQUESTED

An examination of the medical samples and clothing of the complainant for the presence of biological material.
A comparison of any biological material located on the medical samples or clothing with reference sample obtained from the four suspects.
An evaluation of the weight which can be assigned to any match, if any match is found.

APPENDIX D

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ABN 23 839 112 155 ACN 130 236 618

PROFICIENCYTESTING@FORENSICFOUNDATIONS
BIOLOGICAL EXAMINATION AND DNA INTERPRETATION
2019-3
MANUFACTURER'S INFORMATION

Introduction

The test was designed to replicate blood, saliva and semen stains deposited as a result of a sexual assault.

Scenario

Ms Anne Brown was walking in her local park, listening to music on her headphones. She was grabbed from behind. She believes that there were three or four offenders. She was held down and sexually assaulted. She believes:

- at least one of the offenders ejaculated;
- someone spat at her; and
- she hit one of the offenders, causing them to have a nose bleed.

Ms Brown attended the local hospital where she was forensically examined, and items of her clothes were collected.

Test production

The tests were produced at Forensic Foundations' facilities.

Samples

- A sample of saliva was collected from a known female donor. The sample was stored in a temperature monitored refrigerator until use. The sample was labeled 'A'

- One set of matching blood, saliva and semen were sourced from Cardinal Bioresearch. The samples were shipped on dry ice and were stored temporarily upon receipt in a temperature monitored freezer and moved to a temperature monitored refrigerator to thaw prior to use. The samples were then labeled 'B' for the sample of saliva, 'C' for the blood sample and 'D' for the semen sample.

Matched blood, saliva and semen samples

- 1 Unit of whole blood from a male donor was sourced from Red Cross Australia. The blood was received and stored on frozen blocks in an insulated container during transport. This blood was used on the same day it was received. The Red Cross identification numbers were recorded and the item labeled ‘H’.

  The blood was removed from the blood bag using a syringe and placed into labeled EDTA vacutainers for further manipulations. Transfers were checked by a second scientist.

Pre-testing

Approximately 100µl of each biological material were placed on FTA cards (samples ‘C’ & ‘H’) or swabs (samples ‘A’, ‘B’ & ‘D’) using a micropipette. Transfers were checked by a second scientist. Swab Lot No: 1707166 with expiry 06/2020.

The samples were profiled by an independent ISO17025 accredited DNA laboratory.

Test production

Reference samples

- 2 units of whole blood, one each, from different male donors had been sourced previously from Red Cross Australia. The blood had been removed from the blood bags as described above. The blood was then aliquoted and stored at room temperature on FTA Cards (Lot No: 16806498). The FTA cards were labeled:

  Item 4 – blood sample from suspect 1 – James White
  Item 5 – blood sample from suspect 2 – Billy Nicholls

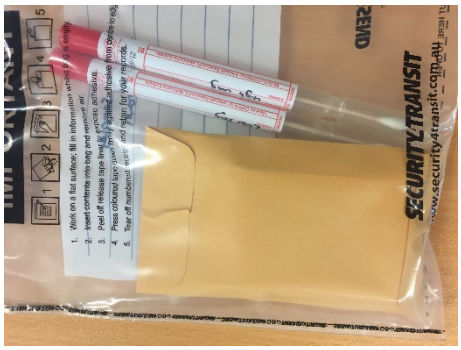
- Approximately 100µl of blood sample ‘C’ was pipetted onto FTA cards (Lot No: 16961695), using a micropipette. Transfers were checked by a second scientist. The FTA cards were labeled:
  Item 6 – blood sample from suspect 3 – Keaton Smith

- Approximately 100µl of blood sample ‘H’ was pipetted onto FTA cards (Lot No: 16961695), using a micropipette. Transfers were checked by a second scientist. The FTA cards were labeled:
  Item 7 – blood sample from suspect 4 – Hugo Greene

Items of Interest

- Item 1 - The DNA Mini Kit (Sexual Assault Sample Collection Kit) contained
  - one 'EasiCollect' Swab and FTA card; and
  - two swabs.

  Saliva from sample A was placed on the EasiCollect swab and the swab folded so that it came into contact with the FTA paper.
  The two other swabs were labeled High Vag and Low Vag.
  The 'High Vag' swab contained approximately 100µl of sample 'A' and 50µl of sample 'D'.
  The 'Low Vag' swab contained approximately 100µl of sample 'A' and 10µl of sample 'D'.



Item 1 - DNA Mini Kit 1

- Item 2 – Two biological samples were placed on the singlet using the seams as a guide so that all samples were placed in the same location on each replicate.
  Approximately 100µl of sample 'B' replicating the act of spitting.
  Approximately 100µl of sample 'H' which was smeared on the singlet simulating a bloody nose.

- Item 3 – Approximately 100µl of sample 'D' was place on the underpants using the seams as a guide so that all samples were placed in the same location on each replicate.

Item 3 - location of semen stain

Final product

The final test comprised:
- 1 DNA mini kit (Item 1);
- 1 singlet (Item 2);
- 1 pair of underpants (Item 3); and
- 4 FTA cards (Items 4-7)

Expected results

- Item 1 The DNA profile of the biological material on the 'High Vag' and' Low Vag' should match the profiles of the complainant and suspect 3 – Keaton Smith.
- Item 2 The DNA profile of the saliva stain should match suspect 3 – Keaton Smith. The DNA profile of the blood stain should match suspect 4 – Hugo Greene.
- Item 3 The DNA profile of the seminal stain should match suspect 3 – Keaton Smith.

The DNA profiles and the subsequent statistics obtained will vary due to the use of different amplification protocols and frequency databases. However, the final conclusions should not change.

Pretesting results

The following results were obtained from the pretesting of the samples.

		Single individual			Stored samples		BB
	Female saliva	Male Saliva	Male Blood	Male semen			Male Blood
Sample	A	B	C	D	4	5	H
	Xo1747_1.1.1	Xo1747_2.1.1	S05718	Xo1747_3.1.1			S05719
System							
D3S1358	16,18	16,17	16,17	16,17	14,16	15,15	15,17
vWA	16,18	16,16	16,16	16,16	14,16	16,18	17,17
D16S539	11,12	11,13	11,13	11,13	10,13	12,14	12,12
CSF1PO	12,12	11,12	11,12	11,12	10,12	12,12	10,11
TPOX	8,11	8,9	8,9	8,9	9,9	8,11	8,11
Yindel		2	2	2	2	2	2
AMEL	X,X	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y
D8S1179	10,14	12,12	12,12	12,12	13,13	12,13	13,15
D21S11	28,28	28,30	28,30	28,30	28,30	31.2,33.2	30,30
D18S51	12,14	15,15	15,15	15,15	13,17	14,14	15,16
DYS391		10	10	10	11,11	10,10	10
D2S441	11,14	11,12	11,12	11,12	11,13	10,11	10,14
D19S433	13,14	12,14	12,14	12,14	14,14	12,13	14,15.2
TH01	8,9	6,7	6,7	6,7	6,6	9.3,9.3	9,9.3
FGA	21,22	21,24	21,24	21,24	21,22	23,24	22,25
D22S1045	15,16	11,11	11,11	11,11	11,15	16,16	15,15
D5S818	11,11	9,12	9,12	9,12	11,12	11,12	12,12
D13S317	11,12	12,14	12,14	12,14	8,12	11,12	11,12
D7S820	8,11	10, 13	10, 13	10,13	10,10	10,10	8,8
SE33	18,28.2	29.2,31.2	29.2,31.2	29.2, 31.2	20,27.2	15,32.2	15, 17.3
D10S1248	14,17	14,15	14,15	14,15	13,15	13,14	13,16
D1S1656	12,16	15,15	15,15	15,15	12,17	12,17.3	12,17.3
D12S391	22,24	17,18	17,18	17,18	19.3,20	17.3,19.3	18,20
D2S1338	17,18	19,25	19,25	19,25	22,25	17,24	20,20

END OF DOCUMENT

Biological examination and DNA Analysis 2019-3 Feedback

Forensic Foundations prides itself in providing flexible fit-for-purpose forensic programs. This Challenge Test was the second developed by Forensic Foundations and thus the second undertaken by forensic laboratories. The manufacture, distribution and assessment and reporting of this test has provided and will provide the basis for continuous improvement for both Forensic Foundations and the forensic laboratories. To this end we would appreciate your comments to assist us to improve the tests.

Please tick the appropriate box and make any relevant comments.

	Strongly Agree	Agree	Disagree	Strongly Disagree	NA
1. The test was too basic for our facility	☐	☐	☐	☐	☐
…… …… …… ……					
2. The samples supplied were suitable	☐	☐	☐	☐	☐
…… …… …… ……					
3. The results required were not outlined sufficiently	☐	☐	☐	☐	☐
…… …… …… ……					
4. The final report provided suitable detail	☐	☐	☐	☐	☐
…… …… …… ……					
5. The tests involved should be more challenging	☐	☐	☐	☐	☐
…… …… …… ……					

Please comment briefly on the following:

5. Are there additional aspects which could be included in the test?

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……

6. Any additional comments

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7. Facility (optional)

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8. Would you like us to contact you to discuss your feedback?

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……
……

Forensic Foundations' Proficiency Tests are required to be fit-for purpose. To assist us to provide the relevant tests, please use the following form to suggest further tests for development.

Recommendation for Proficiency Test development

Contact	Name	
	Email	
	Phone	
Discipline/ subdiscipline		
Specific issues(s) to be addressed*. Note: The tests can be designed to be multidisciplinary.		
Suggested technical advisor (if known)		
Suggested manufacturer (if known)		

* All Proficiency Tests will include the end to end process (receipt & continuity, triage, description, examination, analysis, data generation, interpretation, reporting) but one aspect may be of particular interest/focus.

This form can be emailed to quality@forensicfoundations.com.au or you can discuss your suggestions on either 03 9018 8919 or 0429 966 012.