Final Report
(July 2021)

Proficiencytesting@forensicfoundations

Biological Examination and DNA Analysis – 1 2021-1

Authorised by Anna Davey, Director, Forensic Foundations, 08/07/2021

PO Box 2279, Ringwood North VIC 3134 Australia	Office: +61 (0) 3 9018 8919 Mobile: +61 (0) 429 966 012
quality@forensicfoundations.com.au www.forensicfoundations.com.au	ABN 23 839 112 155 ACN 130 236 618

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Introduction

Design

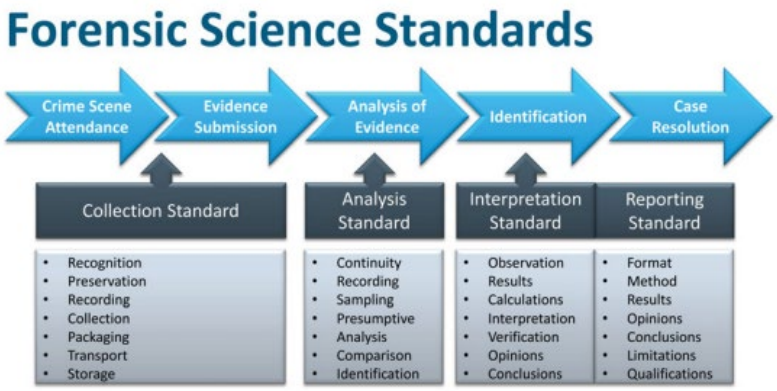
Forensic Foundations' Proficiency Tests are designed to address the following points:

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

In addition to this exercise being a test of 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 advise for their 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 the court.

Forensic Foundations' Proficiency Tests are designed to test the end-to-end forensic examination process. The AS 5388 and the ISO 21043 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 includes all the subsequent examination / analysis steps, culminating in the reporting of results, the process therefore reflects actual forensic casework.



Individual laboratory results remain confidential.

The Final Report of this 2020 round of Proficiency Tests is publicly available via Forensic Foundations web site. Participating laboratories may use the report as outlined in their respective laboratory policies. Any request to review and/or appeal the evaluation of a laboratory's performance should be made via the agency responsible for the distribution of the test or directly to Forensic Foundations.

[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

2021-1 Biological Examination and DNA Analysis - 1

This proficiency test was distributed to three laboratories, and all three laboratories submitted results during this round of testing.

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

In order to minimise contextual bias in the interpretation, the information relating to the 'offence' was minimal.

This test provides a mechanism for participating laboratories to review their results and those of other laboratories to facilitate[2]:

- An evaluation and appraisal of their performance
- Continuous improvement
- Corrective action (where required).

Disclaimer:

The data contained in this report and any observations made are based on the material provided by the participants; however, it is understood that the laboratories may hold additional material which supported the findings reached.

[2] ISO17025 (2017) General requirements for the competency of testing and calibration laboratories.

Laboratory Responses

Continuity, receipt, and description of items

Laboratories were requested to provide information with respect to the continuity, receipt, and description of each of the items received using a supplied proforma table. This facilitated both consistent responses and the collation and comparison of responses. Laboratories were requested to respond to each statement with a 'Yes' or 'No' and 'Comments' where appropriate. Where images of the items were included with the response, this was noted in the text below.

Item 1

Lab ID:		**96150A**		**92388**		**76700**
	Yes/No	**Comments**	**Yes/No**	**Comments**	**Yes/No**	**Comments**
Submission to the laboratory undertaken correctly, in accordance with laboratory procedures	Y		Yes	Item 1 – Reference sample – Robin Pike	Yes	
Sample description corresponds to accompanying paperwork	Y		Yes	Sealed and labeled transparent plastic bag.	Yes	
Security seals intact	Y		Yes		Yes	
Item fully described in case notes	Y		Yes	Reference sample – suspect 1 – Robin Pike	Yes	
Item recorded by means of photography	N		Yes	See Annex 1 for details.	Yes	
Description of any deviations from the expected	NA		No deviations from the expected.		None	

Item 2

Lab ID:		96150A		92388		76700
	Yes/No	Comments	Yes/No	Comments	Yes/No	Comments
Submission to the laboratory undertaken correctly, in accordance with laboratory procedures	Y		Yes	Item 2 – Reference sample – Lee Field	Yes	
Sample description corresponds to accompanying paperwork	Y		Yes	Sealed and labeled transparent plastic bag.	Yes	
Security seals intact	Y		Yes		Yes	
Item fully described in case notes	Y		Yes	Reference sample – suspect 2 – Lee Field	Yes	
Item recorded by means of photography	N		Yes	See Annex 1 for details.	Yes	
Description of any deviations from the expected	NA		No deviations from the expected.		None	

Item 3

Lab ID:		96150A		92388		76700
	Yes/No	Comments	Yes/No	Comments	Yes/No	Comments
Submission to the laboratory undertaken correctly, in accordance with laboratory procedures	Y		Yes	Item 3 – Reference sample – Nick Jackson	Yes	
Sample description corresponds to accompanying paperwork	Y		Yes	Sealed and labeled transparent plastic bag.	Yes	
Security seals intact	Y		Yes		Yes	
Item fully described in case notes	Y		Yes	Reference sample – suspect 3 – Nick Jackson	Yes	
Item recorded by means of photography	N		Yes	See Annex 1 for details.	Yes	
Description of any deviations from the expected	NA		No deviations from the expected.		None	

Item 4

Lab ID:		96150A		92388		76700
	Yes/No	Comments	Yes/No	Comments	Yes/No	Comments
Submission to the laboratory undertaken correctly, in accordance with laboratory procedures	Y		Yes	Item 4 – Reference sample – Tony White	Yes	
Sample description corresponds to accompanying paperwork	Y		Yes	Sealed and labeled transparent plastic bag.	Yes	
Security seals intact	Y		Yes		Yes	
Item fully described in case notes	Y		Yes	Reference sample – suspect 4 – Tony White	Yes	
Item recorded by means of photography	N		Yes	See Annex 1 for details.	Yes	
Description of any deviations from the expected	NA		No deviations from the expected.		None	

Item 5

Lab ID:		96150A		92388		76700
	Yes/No	Comments	Yes/No	Comments	Yes/No	Comments
Submission to the laboratory undertaken correctly, in accordance with laboratory procedures	Y		Yes	Item 5 - Medical Samples - Megan Cook • Reference sample - Megan Cook • Evidentiary sample - High vaginal swab collected from the complainant (Megan Cook) • Evidentiary sample - Low vaginal swab collected from the complainant (Megan Cook)	Yes	
Sample description corresponds to accompanying paperwork	Y		Yes	Sealed and labeled transparent plastic bag.	No	Notes do not make it clear that all three items were received in one package.

Lab ID:		96150A		92388		76700
	Yes/No	Comments	Yes/No	Comments	Yes/No	Comments
						This is probably because such items are not normally received in this fashion. These items would usually be submitted as three separate exhibits.
Security seals intact	Y		Yes		Yes	
Item fully described in case notes	Y		Yes	Reference sample - Megan Cook Evidentiary sample - High vaginal swab collected from the complainant (Megan Cook) Evidentiary sample - Low vaginal swab collected from the complainant (Megan Cook)	Yes	
Item recorded by means of photography	N		Yes	See Annex 1 for details.	Yes	Only reference sample, not vaginal swabs
Description of any deviations from the expected	NA		No deviations from the expected.		See sample description	

Forensic Foundations' comments

Items 1-4 The information provided by the laboratories concurs with the packaging, labelling and samples as distributed.

Item 5 No laboratory noted the difference in the dates given on the swabs (20/2/21) and the outer packaging (19/2/21). This inconsistency should be investigated and the correct date noted in the case notes. Issues relating to incorrect dating may lead to questions regarding continuity and time/sample integrity.

Examination / Analysis

Laboratories were requested to provide information with respect to the Examination and Analysis of the items received using a supplied proforma table. This facilitated both consistent responses and the collation and comparison of responses. A wide range of presumptive and confirmatory tests were included in the proforma table, to minimize the possibility of the contents being used for guidance (i.e. these were the type of tests expected to be performed) and contextual bias. In addition, a number of free text fields were provided. The following table summarises the results – tests appearing in the proforma table which were not applied by any laboratory have been removed

Items 1-4

Laboratory ID	96150A		92388		76700	
Confirmatory testing						
RSID Semen	N		Y	Negative	N	
RSID Saliva	N		Y	Negative	N	
RSID Blood	N		Y	Positive	N	
RSID Urine	N		Y	Negative	N	
Method of subsampling						
Cutting / excision	Y	Cutting from FTA Card	Y	Portion cut from FTA card	Y	
Method used for DNA extraction e.g. Chelex, organic, commercial kit (please specify), differential	Promega DNA IQ		Magnetic Bead Automated DNA Extraction Workstation (Changchun Bokun Biotechnology)		Qiagen EZ1Adv XL	
Method used for DNA quantification e.g. commercial kit (please specify), gel electrophoresis, spectroscopy	Quantifiler™ Trio ABI PRISM® 7500				Real time PCR - 7500 Fast Power Quant Kit	
Amplification System(s) used e.g. commercial kit (please specify), in-house method (please specify)	GlobalFiler™ PCR Kit (Applied Biosystems) Yfiler Plus™ PCR Kit (Applied Biosystems)		VeriFiler™ Plus & Yfiler Platinum Casework Kit		Promega ESI-17 Fast	
Electrophoresis and detection method	3500xl Genetic Analyzer (Applied Biosystems)		Capillary Electrophoresis (3500xL Genetic Analyzer Applied		Acrylamide capillary electrophoresis on a Applied	

Laboratory ID	96150A	92388	76700
e.g. DNA sequencer & associated software (please specify), agarose / acrylamide gel & staining	GenemapperID-X v1.6 (Applied Biosystems)	Biosystems) & GeneMapper ID-X v1.5	Biosystems 3500XL, laser detection

Item 5 (HV & LV Swabs)

Laboratory ID	96150A		92388		76700	
Presumptive testing						
Alternative Light Sources	N		Y	Positive	N	
Acid Phosphatase / Brentamine	N		N		Y	Positive HVS & LVS mod purple at 5 sec, strong purple at 2 mins
Confirmatory testing						
Hematoxylin & Eosin	N		Y	Sperm Observed		
Christmas tree stain	Y	Positive, High levels of sperm observed on HVS and LVS			Y	Pos HVS 4+ heads, LVS 4+ Heads
RSID Semen	N		Y	Positive	N	
RSID Saliva	N		Y	Positive	N	
RSID Blood	N		Y	Negative	N	
RSID Urine	N		Y	Negative	N	
Method of subsampling						
Cutting / excision	NA		Y	Swab heads cut-off into DNA extraction tube	N	
Method used for DNA extraction	Differential extraction Promega DNA IQ		Differential extraction kit (Changchun Bokun Biotechnology) Magnetic Bead Automated DNA Extraction Workstation (Changchun Bokun Biotechnology)		Semen/epithelial cells split using Pro K in lab before submission to DNA profiling Lab.	
Method used for DNA quantification	Quantifiler™ Trio ABI PRISM® 7500				Real time PCR - 7500 Fast Power Quant Kit	
Amplification System(s) used	GlobalFiler™ PCR Kit Yfiler Plus™ PCR Kit		VeriFiler™ Plus & Yfiler Platinum Casework Kit		Promega ESI-17 Fast	

Laboratory ID	**96150A**	**92388**	**76700**
Electrophoresis and detection method	3500xl (Applied Biosystems) GenemapperID-X v1.6 (Applied Biosystems)	Capillary Electrophoresis (3500xL Genetic Analyzer Applied Biosystems) & GeneMapper ID-X v1.5	Acrylamide capillary electrophoresis on a Applied Biosystems 3500XL, laser detection

Comments:

Laboratory: 92388
RSID Blood gave positive signals from Item 1-4.
RSID Saliva gave a positive signal from Item 5 - Reference sample from the complainant.

- Item 5 - High vaginal swab collected from the complainant
  RSID Semen and RSID Saliva gave positive signals.
  Sperm were observed on the microscope smear made of the swab.
- Item 5 - Low vaginal swab collected from the complainant
  RSID Semen and RSID Saliva gave positive signals.
  Sperm were observed on the microscope smear made of the swab.

Laboratory: 76700
Note that the body fluid examination is carried out in the xxx laboratory including splitting semen from any vaginal material. The semen sample along with reference samples is then submitted to an independent DNA profiling laboratory. The DNA results are analysed, using ESI-17 Fast, and then compared with reference samples. The results of the comparisons i.e. profile obtained matches suspect X, are returned to the xxx laboratory for incorporation into a statement along with activity assessments by xxx staff. xxx staff are not given the DNA results down to locus/allele level. Therefore alleles are not given in the results below

Forensic Foundations' comments –

All laboratories confirmed the presence of spermatozoa on the swabs.

Laboratory 92388 confirmed the presence of human semen using an RSID kit.

Laboratory 92388 also noted the presence of saliva on the 'Vaginal swabs'. This is consistent with the manufacture of the test but may raise questions if this were a real case.

DNA analysis
Several different commercial kits were used for the DNA Analysis. All were appropriate

Results

Laboratories were requested to provide DNA typing information using a proforma table. This facilitated both consistent responses and the collation and comparison of responses. The following table summarises the results – loci not analysed by any laboratory have been removed. Loci not analysed by a laboratory are shaded.

Item 1 (Pike) / Locus	**Expected genotype**	**96150A**	**92388**
Autosomal			
D1S1656	14,18.3	14, 18.3	14,18.3
D2S441	11,11	11, 11	11,11
D2S1338	18,20	18, 20	18,20
D3S1358	16,18	16, 18	16,18
D5S818	10,11	10, 11	10,11
D6S1043			12,19
D7S820	10,11	10, 11	10,11
D8S1179	9,13	9, 13	9,13
D10S1248	14,15	14, 15	14,15
D12S391	21,22	21, 22	21,22
D13S317	9,13	9, 13	9,13
D16S539	9,12	9,12	9,12
D18S51	14,17	14, 17	14,17
D19S433	14,15	14, 15	14,15
D21S11	30,30	30, 30	30,30
D22S1045	15,16	15, 16	15,16
CSF1PO	10,12	10, 12	10,12
FGA	21,26	21, 26	21,26
Penta D	12,12		12,12
Penta E	10,18		10,18
SE33	15,30.2	15, 30.2	
TH01	6,7	6, 7	6,7
TPOX	11,11	11, 11	11,11
vWA	14,19	14, 19	14,19
Non-autosomal			
AMEL	X,Y	X, Y	X,Y
Yindel	2	2	2
DYS19	14	14	14
DYS385 a/b	11,15	11/15	11,15
DYS389-I	13		13
DYS389-II	29		29
DYS390	25	25	25
DYS391	10	10	10
DYS392	14	14	14
DYS393	13	13	13
DYS437	15	15	15
DYS438	12	12	12

Item 1 (Pike) / Locus	Expected genotype	96150A	92388
DYS439	13	13	13
DYS456	15	15	15
DYS448	19	19	19
DYS449		29	29
DYS458	17	17	17
DYS460		10	10
DYS481		23	23
DYS518		37	37
DYS533		12	12
DYS570	17	17	17
DYS576	18	18	18
DYS627		22	22
DYS3891		13	
DYS38911		29	
DYS635	23	23	23
YGATAH4	12	12	12
DYF387S1		35/36	35,36
DYS549			12
DYS593			15
DYS645			8
DYS557			18
DYS522			11
DYS444			13
DYS643			12
DYS596			16
DYS527			21,23
DYS447			25
rs771783753			2
rs759551978			2
rs199815934			2

Forensic Foundations' comments

The typing provided by the laboratories concur with the expected and consensus genotypes.

Item 2 (Field) / Locus	Expected genotype	96150A	92388
Autosomal			
D1S1656	16,18.3	16, 18.3	16,18.3
D2S441	10,11	10, 11	10,11
D2S1338	19,26	19, 26	19,26
D3S1358	17,18	17, 18	17,18
D5S818	11,11	11, 11	11,11
D6S1043			13,19
D7S820	10,12	10, 12	10,12
D8S1179	13,13	13, 13	13,13
D10S1248	13,15	13, 15	13,15
D12S391	18,18	18, 18	18,18
D13S317	8,11	8, 11	8,11
D16S539	11,12	11,12	11,12
D18S51	12,17	12, 17	12,17
D19S433	12,13	12,13	12,13
D21S11	29,30	29,30	29,30
D22S1045	11,15	11,15	11,15
CSF1PO	11,12	11, 12	11,12
FGA	22,25	22, 25	22,25
Penta D	9,12		9,12
Penta E	7,18		7,18
SE33		15, 33.2	
TH01	6,7	6, 7	6,7
TPOX	8,9	8, 9	8,9
vWA	17,17	17, 17	17,17
Non autosomal			
AMEL	X,Y	X, Y	X,Y
Yindel		2	2
DYS19		14	14
DYS385 a/b		11/13	11,13
DYS389-I			13
DYS389-II			29
DYS390		25	25
DYS391	10	11	11
DYS392		14	14
DYS393		13	13
DYS437		15	15
DYS438		12	12
DYS439		13	13
DYS456		17	17
DYS448		18	18
DYS449		30	30
DYS458		17	17
DYS460		11	11

Item 2 (Field) / Locus	Expected genotype	96150A	92388
DYS481		25	25
DYS518		38	38
DYS533		12	12
DYS570		17	17
DYS576		18	18
DYS627		23	23
DYS3891		13	
DYS38911		29	
DYS635		23	23
YGATAH4		12	12
DYF387S1		36/37	36,37
DYS549			12
DYS593			15
DYS645			8
DYS557			16
DYS522			11
DYS444			12
DYS643			10
DYS596			16
DYS527			21,23
DYS447			24
rs771783753			2
rs759551978			2
rs199815934			2

Forensic Foundations' comments

The typing provided by the laboratories concur with the expected and consensus genotypes.

Item 3 (Jackson) / Locus	Expected genotype	96150A	92388
Autosomal			
D1S1656	15,15	15, 15	15,15
D2S441	11,12	11, 12	11,12
D2S1338	19,25	19, 25	19,25
D3S1358	16,17	16, 17	16,17
D5S818	9,12	9, 12	9,12
D6S1043			12,12
D7S820	10,13	10, 13	10,13
D8S1179	12,12	12, 12	12,12
D10S1248	14,15	14, 15	14,15
D12S391	17,18	17, 18	17,18
D13S317	12,14	12, 14	12,14
D16S539	11,13	11,13	11,13
D18S51	15,15	15, 15	15,15
D19S433	12,14	12, 14	12,14
D21S11	28,30	28, 30	28,30
D22S1045	11,11	11, 11	11,11
CSF1PO	11,12	11, 12	11,12
FGA	21,24	21, 24	21,24
Penta D			9,13
Penta E			10,11
SE33	29.2, 31.2	29.2, 31.2	
TH01	6,7	6, 7	6,7
TPOX	8,9	8, 9	8,9
vWA	16,16	16, 16	16,16
Non autosomal			
AMEL	X,Y	X, Y	X,Y
Yindel	2	2	2
DYS19		14	14
DYS385 a/b		13/16	13,16
DYS389-I			13
DYS389-II			30
DYS390		23	23
DYS391	10	10	10
DYS392		11	11
DYS393		12	12
DYS437		14	14
DYS438		9	9
DYS439		12	12
DYS456		15	15
DYS448		21	21
DYS449		27	27
DYS458		17.2	17.2
DYS460		11	11

Item 3 (Jackson) / Locus	Expected genotype	96150A	92388
DYS481		25	25
DYS518		41	41
DYS533		11	11
DYS570		18	18
DYS576		18	18
DYS627		21	21
DYS3891		13	
DYS38911		30	
DYS635		21	21
YGATAH4		11	11
DYF387S1		37/38	37,38
DYS549			13
DYS593			15
DYS645			8
DYS557			19
DYS522			14
DYS444			12
DYS643			9
DYS596			15
DYS527			22
DYS447			26
rs771783753			2
rs759551978			2
rs199815934			2

Forensic Foundations' comments

The typing provided by the laboratories concur with the expected and consensus genotypes.

Item 4 (White) / Locus	Expected genotype	96150A	92388
Autosomal			
D1S1656	12,15	12, 15	12,15
D2S441	11,12	11, 12	11,12
D2S1338	24,25	24, 25	24,25
D3S1358	17,18	17, 18	17,18
D5S818	11,12	11, 12	11,12
D6S1043			12,13
D7S820	7,12	7, 12	7,12
D8S1179	10,11	10, 11	10,11
D10S1248	13,15	13, 15	13,15
D12S391	16,23	16, 23	16,23
D13S317	12,12	12, 12	12,12
D16S539	9,11	9,11	9,11
D18S51	12,13	12, 13	12,13
D19S433	14,15	14, 15	14,15
D21S11	31.2, 31.2	31.2, 31.2	31.2,31.2
D22S1045	15,17	15, 17	15,17
CSF1PO	11,11	11, 11	11,11
FGA	24,25	24, 25	24,25
Penta D	9,12		9,12
Penta E	10,17		10,17
SE33		17, 28.2	
TH01	6,9.3	6, 9.3	6,9.3
TPOX	11,12	11, 12	11,12
vWA	14,14	14, 14	14,14
Non autosomal			
AMEL	X,Y	X, Y	X,Y
Yindel		2	2
DYS19		14	14
DYS385 a/b		14/15	14,15
DYS389-I			12
DYS389-II			28
DYS390		22	22
DYS391	10	10	10
DYS392		12	12
DYS393		13	13
DYS437		16	16
DYS438		10	10
DYS439		11	11
DYS456		15	15
DYS448		20	20
DYS449		28	28
DYS458		16	16
DYS460		9	9

Item 4 (White) / Locus	Expected genotype	96150A	92388
DYS481		25	25
DYS518		39	39
DYS533		12	12
DYS570		19	19
DYS576		16	16
DYS627		20	20
DYS3891		12	
DYS38911		28	
DYS635		22	22
YGATAH4		11	11
DYF387S1		37/38	37,38
DYS549			13
DYS593			15
DYS645			8
DYS557			16
DYS522			11
DYS444			13
DYS643			12
DYS596			15
DYS527			22
DYS447			23
rs771783753			2
rs759551978			2
rs199815934			2

Forensic Foundations' comments

The typing provided by the laboratories concur with the expected and consensus genotypes.

Item 5 (Cook) / Locus	Expected genotype	96150A	92388
Autosomal			
D1S1656	16,16.3	16, 16.3	16,16.3
D2S441	10,10	10, 10	10,10
D2S1338	19,20	19, 20	19,20
D3S1358	14,18	14, 18	14,18
D5S818	12,12	12, 12	12,12
D6S1043			11,12
D7S820	12,13	12, 13	12,13
D8S1179	8,13	8, 13	8,13
D10S1248	14,15	14, 15	14,15
D12S391	20,20	20, 20	20,20
D13S317	10,11	10, 11	10,11
D16S539	11,13	11,13	11,13
D18S51	13,14	13, 14	13,14
D19S433	11,13	11, 13	11,13
D21S11	28,29	28, 29	28,29
D22S1045	15,15	15, 15	15,15
CSF1PO	12,13	12, 13	12,13
FGA	20,22	20, 22	20,22
Penta D	9,11		9,11
Penta E	10,12		10,12
SE33		16, 18	
TH01	9.3,9.3	9.3, 9.3	9.3,9.3
TPOX	8,12	8, 12	8,12
vWA	14,18	14, 18	14,18
Non autosomal			
AMEL	X,X	X, X	X, X

Forensic Foundations' comments

The typing provided by the laboratories concur with the expected and consensus genotypes.

Item 5 HV / Locus	96150A		92388	
Fraction designation (As given in report)	sp	epi	Sperm	Epithelial
Autosomal				
D1S1656	14, 15, 18.3	14, 15, 16, 16.3, 18.3	15,15	15,16,16.3
D2S441	11, 12	10, 11, 12	11,12	10,11,12
D2S1338	18, 19, 20, 25	18, 19, 20, 25	19,25	19,20,25
D3S1358	16, 17, 18	14, 16, 17, 18	16,17	14,16,17,18
D5S818	9, 10, 11, 12	9, 10, 11, 12	9,12	9,12
D6S1043			12,12	11,12
D7S820	10, 11, 13	10, 11, 12, 13	10,13	10,12,13
D8S1179	9, 12, 13	8, 9, 12, 13	12,12	8,12,13
D10S1248	14, 15	14, 15	14,15	14,15
D12S391	17, 18, 21, 22	17, 18, 20, 21, 22	17,18	17,18,20
D13S317	9, 12, 13, 14	9, 10, 11, 12, 13, 14	12,14	10,11,12,14
D16S539	9, 11, 12, 13	9, 11, 12, 13	11,13	11,13
D18S51	14, 15, 17	13, 14, 15, 17	15,15	13,14,15
D19S433	12, 14, 15	11, 12, 13, 14, 15	12,14	11,12,13,14
D21S11	28, 30	28, 29, 30	28,30	28,29,30
D22S1045	11, 15, 16	11, 15, 16	11,11	11,15
CSF1PO	10, 11, 12	10, 11, 12, 13	11,12	11,12,13
FGA	21, 24, 26	20, 21, 22, 24, 25, 26	21,24	20,21,22,24
Penta D			9,13	9,11,13
Penta E			10,11	10,11,12
SE33	15, 29.2, 30.2, 31.2	15, 16, 18, 29.2, 30.2, 31.2		
TH01	6, 7	6, 7, 9.3	6,7	6,7,9.3
TPOX	8, 9, 11	8, 9, 11, 12	8,9	8,9,12
vWA	14, 16, 18, 19	14, 16, 18, 19	16,16	14,16,18
Non autosomal				
AMEL	X, Y	X, Y	X,Y	X,Y
Yindel	2	2	2	2
DYS19	14	14	14	14
DYS385 a/b	11, 13, 15, 16	11, 13, 15, 16	13,16	13,16
DYS389-I			13	13
DYS389-II			30	30
DYS390	23, 25	23, 25	23	23
DYS391	10	10	10	10
DYS392	11, 14	11, 14	11	11
DYS393	12, 13	12, 13	12	12
DYS437	14, 15	14, 15	14	14
DYS438	9, 12	9, 12	9	9
DYS439	12, 13	12, 13	12	12
DYS456	15	15	15	15

Item 5 HV / Locus	96150A		92388	
DYS448	19, 21	19, 21	21	21
DYS449	27, 29	27, 29	27	27
DYS458	17, 17.2	17, 17.2	17.2	17.2
DYS460	10, 11	10, 11	11	11
DYS481	23, 25	23, 25	25	25
DYS518	37, 41	37, 41	41	41
DYS533	11, 12	11, 12	11	11
DYS570	17, 18	17, 18	18	18
DYS576	18	18	18	18
DYS627	21, 22	21, 22	21	21
DYS3891	13	13		
DYS38911	29, 30	29, 30		
DYS635	21, 23	21, 23	21	21
YGATAH4	11, 12	11, 12	11	11
DYF387S1	35, 36, 37, 38	35, 36, 37, 38	37,38	37,38
DYS549			13	13
DYS593			15	15
DYS645			8	8
DYS557			19	19
DYS522			14	14
DYS444			12	12
DYS643			9	9
DYS596			15	15
DYS527			22	22
DYS447			26	26
rs771783753			2	2
rs759551978			2	2
rs199815934			2	2

Item 5 LV / Locus	96150A		92388	
Fraction designation (As given in report)	sp	epi	Sperm	Epithelial
Autosomal				
D1S1656	14, 15, 18.3	14, 15, 16, 16.3, 18.3	15,15	15,16,16.3
D2S441	11, 12	10, 11, 12	11,12	10,11,12
D2S1338	18, 19, 20, 24*, 25	18, 19, 20, 24*, 25	19,25	19,20,25
D3S1358	16, 17, 18	14, 16, 17, 18	16,17	14,16,17,18
D5S818	9, 10, 11, 12	9, 10, 11, 12	9,12	9,12
D6S1043			12,12	11,12
D7S820	10, 11, 13	10, 11, 12, 13	10,13	10,12,13
D8S1179	9, 12, 13	8, 9, 12, 13	12,12	8,12,13
D10S1248	14, 15	14, 15	14,15	14,15
D12S391	17, 18, 21, 22	17, 18, 20, 21, 22	17,18	17,18,20
D13S317	9, 12, 13, 14	9, 10, 11, 12, 13, 14	12,14	10,11,12,14
D16S539	9, 11, 12, 13	9, 11, 12, 13	11,13	11,13
D18S51	14, 15, 17	13, 14, 15, 17	15,15	13,14,15
D19S433	12, 14, 15	11, 12, 13, 14, 15	12,14	11,12,13,14
D21S11	28, 30	28, 29, 30	28,30	28,29,30
D22S1045	11, 15, 16	11, 15, 16	11,11	11,15
CSF1PO	10, 11, 12	10, 11, 12, 13	11,12	11,12,13
FGA	21, 24, 26	20, 21, 22, 24, 26	21,24	20,21,22,24
Penta D			9,13	9,11,13
Penta E			10,11	10,11,12
SE33	15, 29.2, 30.2, 31.2	15, 16, 18, 27.2, 29.2, 30.2, 31.2		
TH01	6, 7	6, 7, 9.3	6,7	6,7,9.3
TPOX	8, 9, 11	8, 9, 11, 12	8,9	8,9,12
vWA	14, 16, 18, 19	14, 16, 18, 19	16,16	14,16,18
Non autosomal				
AMEL	X, Y	X, Y	X,Y	X,Y
Yindel	2	2	2	2
DYS19	14	14	14	14
DYS385 a/b	11, 13, 15, 16	11, 13, 15, 16	13,16	13,16
DYS389-I			13	13
DYS389-II			30	30
DYS390	23, 25	23, 25	23	23
DYS391	10	10	10	10
DYS392	11, 14	11, 14	11	11
DYS393	12, 13	12, 13	12	12
DYS437	14, 15	14, 15	14	14
DYS438	9, 12	9, 12	9	9
DYS439	12, 13	12, 13	12	12

Item 5 LV / Locus	96150A		92388	
DYS456	15	15	15	15
DYS448	19, 21	19, 21	21	21
DYS449	27, 29	27, 29	27	27
DYS458	17, 17.2	17, 17.2	17.2	17.2
DYS460	10, 11	10, 11	11	11
DYS481	23, 25	23, 25	25	25
DYS518	37, 41	37, 41	41	41
DYS533	11, 12	11, 12	11	11
DYS570	17, 18	17, 18	18	18
DYS576	18	18	18	18
DYS627	21, 22	21, 22	21	21
DYS3891	13	13		
DYS38911	29, 30	29, 30		
DYS635	21, 23	21, 23	21	21
YGATAH4	11, 12	11, 12	11	11
DYF387S1	35, 36, 37, 38	35, 36, 37, 38	37,38	37,38
DYS549			13	13
DYS593			15	15
DYS645			8	8
DYS557			19	19
DYS522			14	14
DYS444			12	12
DYS643			9	9
DYS596			15	15
DYS527			22	22
DYS447			26	26
rs771783753			2	2
rs759551978			2	2
rs199815934			2	2

* 24 allele is in a stutter position of allele 25. We acknowledge the possibility of an elevated stutter product

Forensic Foundations' comments

DNA profiling results were only provided by two laboratories.

Both laboratories performed a differential extraction. In all instances there was retention of male cells in the epithelial fraction. Without peak height data it is not possible to estimate how much carryover of female material was demonstrated but some carryover was detected by Laboratory 96150A at the vWA locus.

Laboratory 96150A reported >2 alleles at many loci indicating a mixed profile in the 'sp' fraction.
Laboratory 92388 reported 1 or 2 alleles at all loci indicating a single source profile in the 'Sperm' fraction.

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

Laboratory 96150A

Sample description		DNA profile description	Person	Hypothesis/Interpretation	Statistical weighting
Medical samples - COOK					
Sperm were observed on a microscope smear prepared from the high vaginal and low vaginal swabs, confirming the presence of semen.					
1.06-01	Sperm fraction HV swab	Mixed DNA profile - three contributors including COOK	PIKE	H1: PIKE is a contributor H2: PIKE is not a contributor	> 100 billion (in favour of H1)
			FIELD	excluded	
			JACKSON	H1: JACKSON is a contributor H2: JACKSON is not a contributor	> 100 billion (in favour of H1)
			WHITE	excluded	
1.06-01	Epithelial fraction HV swab	Mixed DNA profile - three contributors including COOK	PIKE	H1: PIKE is a contributor H2: PIKE is not a contributor	10 billion (in favour of H1)
			FIELD	excluded	
			JACKSON	H1: JACKSON is a contributor H2: JACKSON is not a contributor	21 billion (in favour of H1)
			WHITE	excluded	
1.07-01	Sperm fraction of LV swab	Mixed DNA profile - three contributors including COOK	PIKE	H1: PIKE is a contributor H2: PIKE is not a contributor	> 100 billion (in favour of H1)
			FIELD	excluded	
			JACKSON	H1: JACKSON is a contributor H2: JACKSON is not a contributor	> 100 billion (in favour of H1)
			WHITE	excluded	
1.07-01	Epithelial fraction LV swab	Mixed DNA profile - four contributors including COOK	PIKE	H1: PIKE is a contributor H2: PIKE is not a contributor	6.8 billion (in favour of H1)
			FIELD	H1: FIELD is a contributor H2: FIELD is not a contributor	3100 (in favour of H2)
			JACKSON	H1: JACKSON is a contributor H2: JACKSON is not a contributor	> 100 billion (in favour of H1)
			WHITE	H1: WHITE is a contributor H2: WHITE is not a contributor	10 000 (in favour of H2)

Please note: The LR reported for sample 1.06-01 Epithelial fraction of HV swab, was calculated with the locus D1S1656 omitted due to an unresolved stutter peak.

Also please note: Sample 1.07-01 Epithelial fraction of LV swab, was assessed as being a four person mixture, including COOK, based on a putative allele at SE33 (27.2). This allele was not reproduced in a second amplification and is within drop in range as defined by xxx validation data.

Y-STR analysis

Sample description		DNA profile description	Person	Hypothesis/Interpretation	Statistical weighting
Medical samples - COOK					
Sperm were observed on a microscope smear prepared from the high vaginal and low vaginal swabs, confirming the presence of semen.					
1.06-01	Sperm fraction HV swab	Mixed DNA profile – two contributors	PIKE	H1: PIKE is a contributor H2: PIKE is not a contributor	20 000 (in favour of H1)
			FIELD	excluded	
			JACKSON	H1: JACKSON is a contributor H2: JACKSON is not a contributor	20 000 (in favour of H1)
			WHITE	excluded	
1.06-01	Epithelial fraction HV swab	Mixed DNA profile – two contributors	PIKE	not excluded	Not calculated
			FIELD	excluded	
			JACKSON	not excluded	Not calculated
			WHITE	excluded	

Y-STR analysis

Sample description		DNA profile description	Person	Hypothesis/Interpretation	Statistical weighting
Medical samples - COOK					
Sperm were observed on a microscope smear prepared from the high vaginal and low vaginal swabs, confirming the presence of semen.					
1.07-01	Sperm fraction of LV swab	Mixed DNA profile – two contributors	PIKE	H1: PIKE is a contributor H2: PIKE is not a contributor	20 000 (in favour of H1)
			FIELD	excluded	
			JACKSON	H1: JACKSON is a contributor H2: JACKSON is not a contributor	20 000 (in favour of H1)
			WHITE	excluded	
1.07-01	Epithelial fraction LV swab	Mixed DNA profile – two contributors	PIKE	not excluded	Not calculated
			FIELD	excluded	
			JACKSON	not excluded	Not calculated
			WHITE	excluded	

Please note: The haplotypes reported were unobserved when compared to all haplotypes currently stored on the Y Chromosome Haplotype Reference Database. The reported LR is based on the likely maximum frequency of this haplotype when sampling variation is considered.
Also please note: The epithelial fractions of 1.06-01 and 1.07-01 were not interpreted for the Y-STR analysis as no further evidentiary value would be gained.

Laboratory 92388

Item 5 – High vaginal swab collected from the complainant

- The allele pairs on 23 autosomal STR loci of sperm fraction which extracted from Item 5 - High vaginal swab collected from the complainant matched to the allele pairs on 23 autosomal STR loci of Item 3 - Nick Jackson. The LR calculation result of these alleles is 3.4361*10^32. (The allele frequency used for calculating LR is the allele frequency of xxx population).
- The allele pairs on 35 Y-STR loci of sperm fraction which extracted from Item 5 - High vaginal swab collected from the complainant matched to the allele pairs on 35 Y-STR loci of Item 3 - Nick Jackson.
- A Mixed DNA profile was obtained from epithelial fraction of Item 5 - High vaginal swab collected from the complainant, which matched to the mixed DNA profile of Item 3 - Nick Jackson and Item 5 - Reference sample from the complainant (Megan Cook). The mixed DNA profile is 3.9236*10^27 times more likely to occur if the DNA is a mixture of DNA from Megan Cook and Nick Jackson than if it originated from Megan Cook and another unrelated individual chosen at random from the xxx population (GenoProof Mixture 4).
- The allele pairs on 35 Y-STR loci of epithelial fraction which extracted from Item 5 - High vaginal swab collected from the complainant matched to the allele pairs on 35 Y-STR loci of Item 3 - Nick Jackson.

Item 5 – Low vaginal swab collected from the complainant

- The allele pairs on 23 autosomal STR loci of sperm fraction which extracted from Item 5 - Low vaginal swab collected from the complainant matched to the allele pairs on 23 autosomal STR loci of Item 3 - Nick Jackson. The LR calculation result of these alleles is 3.4361*10^32. (The allele frequency used for calculating LR is the allele frequency of xxx population).
- The allele pairs on 35 Y-STR loci of sperm fraction which extracted from Item 5 - Low vaginal swab collected from the complainant matched to the allele pairs on 35 Y-STR loci of Item 3 - Nick Jackson.
- A Mixed DNA profile was obtained from epithelial fraction of Item 5 - Low vaginal swab collected from the complainant, which match to the mixed DNA profile of Item 3 - Nick Jackson and Item 5-Reference sample from the complainant (Megan Cook). The mixed DNA profile is 7.2885*10^27 times more likely to occur if the DNA is a mixture of DNA from Megan Cook and Nick Jackson than if it originated from Megan Cook and another unrelated individual chosen at random from the xxx population (GenoProof Mixture 4).
- The allele pairs on 35 Y-STR loci of epithelial fraction which extracted from Item 5 - Low vaginal swab collected from the complainant matched to the allele pairs on 35 Y-STR loci of Item 3 - Nick Jackson.

Laboratory 76700

DNA profiling and interpretation for this case was performed by a Managed Service Provider, independent of the xxx. The results of this DNA profiling have been provided to me by a named forensic scientist from the Managed Service Provider. I did not undertake any of the DNA testing or analysis, but I have incorporated these DNA results into my statement and, where appropriate, used them to support my attribution of DNA to body fluids, evaluation and interpretation of the findings. Should any of these DNA results not be accepted, a statement regarding the DNA profiling process should be sought.

DNA profiles were obtained from the reference samples provided from the following people –
Megan COOK, Robin PIKE, Lee FIELD, Nick JACKSON, Tony WHITE

Note. In circumstances where semen is mixed with vaginal and/or other cells attempts are made to separate the semen from the vaginal and/or other cells, prior to DNA profiling the semen that is present. However, this separation may not be fully effective and some vaginal/other cells can remain mixed with the semen. In these instances the DNA result obtained will be a mixture of DNA from both the semen and vaginal/other cells present.

Examination of items relating to Megan COOK
Low vaginal swab (Item 5)
High vaginal swab (item 5)
Semen was found on both the high and low vaginal swabs. Semen from both the high and vaginal swabs were submitted, separately, for DNA profiling.

The samples from both the high and low vaginal swabs gave results containing a mixture of DNA from at least two people, in major and minor proportions. There was no trace of Megan COOK's DNA in either of these mixtures indicating that the semen/vaginal material separation was successful. Therefore, in my opinion, all of the DNA in these two mixtures originated from semen.

The results from both swabs were very similar and the findings described below apply to both the high and low vaginal swabs.

The major part of the DNA matched Nick JACKSON's DNA profile and therefore some of the semen could have come from him.

I have considered two propositions –
- The semen contributing the major part of the DNA came from Nick Jackson
- The semen contributing the major part of the DNA did not come from Nick Jackson but from an unknown man, unrelated to him.

I understand that the DNA results obtained are at least a billion times more likely if some of the semen came from Nick JACKSON, rather than an unknown man, unrelated to him.

The minor part of the DNA matched Robin PIKE's DNA profile and therefore some of the semen could have come from him.
I have considered two propositions –
- The semen contributing the minor part of the DNA came from Robin PIKE
- The semen contributing the minor part of the DNA did not come from Robin PIKE but from an unknown man, unrelated to him.

I understand that the DNA results obtained are at least a billion times more likely if some of the semen came from Robin PIKE, rather than an unknown man, unrelated to him.

There was no indication that Lee FIELD and Tony WHITE contributed any DNA to either of these results.

Evaluation of the results
When vaginal intercourse with ejaculation occurs semen can remain in the vagina for up to 7 days before drainage and/or degradation. However, vaginal intercourse can occur without ejaculation inside the vagina, for example ejaculation outside the body or

a condom may be worn. Therefore it is possible for vaginal intercourse to occur and semen not to be transferred.

As the results from both swabs were very similar the evaluation described below applies to both the high and low vaginal swabs.

A meaningful evaluation can only be carried out if two alternative propositions are considered in relation to the circumstances of this incident. I understand that Nick JACKSON, Robin PIKE, Lee FIELD and Tony WHITE has made no comment about the incident. It is unclear how many men are alleged to have had vaginal intercourse with Megan COOK. I have therefore assumed a reasonable alternative for each is that they have not had vaginal intercourse with Megan COOK. If other propositions are made available I can consider them instead.

In relation to Nick JACKSON
Semen was present within a mixture of DNA on Megan COOK's high and low vaginal swabs which could have originated from Nick JACKSON, and a likelihood ratio of at least one billion in favour of this proposition has been calculated.

In order to evaluate this finding I have assumed some of the semen did actually come from Nick JACKSON.

I have therefore considered the following propositions:
- Nick JACKSON had vaginal intercourse with Megan COOK
- Nick JACKSON did not have vaginal intercourse with Megan COOK

If Nick JACKSON had vaginal intercourse with Megan COOK and ejaculation occurred I have a high expectation of finding his semen on her high and low vaginal swabs, assuming the swabs were taken within a day or so of the alleged incident.

If Nick JACKSON did not have vaginal intercourse with Megan Cook I would not expect to find his semen present on her high or low vaginal swabs.

Therefore in my opinion, the findings support the view that Nick JACKSON had vaginal intercourse, with Mega COOK, rather than he did not have vaginal intercourse with her.

In relation to Robin Pike
Semen was present within a mixture of DNA on Megan COOK's high and low vaginal swabs which could have originated from Robin PIKE, and a likelihood ratio of at least one billion in favour of this proposition has been calculated.

In order to evaluate this finding I have assumed some of the semen did actually come from Robin PIKE.

I have therefore considered the following propositions:
- Robin PIKE had vaginal intercourse with Megan COOK
- Robin PIKE did not have vaginal intercourse with Megan COOK

If Robin PIKE had vaginal intercourse with Megan COOK and ejaculation occurred I have a high expectation of finding his semen on her high and low vaginal swabs, assuming the swabs were taken within a day or so of the alleged incident.

If Robin PIKE did not have vaginal intercourse with Megan Cook I would not expect to find his semen present on her high or low vaginal swabs.

Therefore in my opinion, the findings support the view that Robin PIKE had vaginal intercourse, with Megan COOK, rather than he did not have vaginal intercourse with her.

In relation to Lee FIELD and Tony WHITE
No DNA was detected on Megan COOK'S high and low vaginal swabs that could have originated from Lee FIELD or Tony WHITE.

I have therefore considered the following two pairs of propositions
- Lee FIELD had vaginal intercourse with Megan COOK
- Lee FIELD did not have vaginal intercourse with Megan COOK

- Tony WHITE had vaginal intercourse with Megan COOK
- Tony WHITE did not have vaginal intercourse with Megan COOK

If vaginal intercourse with either of these men occurred, as there is no information as to whether or not ejaculation took place, semen may or may not be present.

If no vaginal intercourse with either of these men occurred, I would not expect to find their semen.

As no semen that could be attributed to either Tony WHITE or Lee FIELD was detected, in my opinion the findings do not assist in addressing whether or not Lee FIELD and Tony WHITE had vaginal intercourse with Megan COOK.

Forensic Foundations' comments

The three laboratories reported using different formats.

All the laboratories reported using Likelihood Ratios using source level propositions. Laboratory 76700 also reported Likelihood Ratios using activity level propositions .

Laboratories 96150A and 76700 reported a mixed male DNA profile in the 'male' fraction obtained from the 'HV" & 'LV' swabs.

Laboratory 92388 reported a single source male DNA profile in the 'male' fraction obtained from the 'HV" & 'LV' swabs.

The following tables summarise the Likelihood Ratios reported by the participating laboratories.

High Vaginal swab (male fraction)

Laboratory ID	Proposition	Likelihood Ratio (autosomal)	Likelihood Ratio (Y-STR)
96150A	Pike is a contributor	>100 billion	20,000
	Jackson is a contributor	>100 billion	20,000
92388	Pike is a contributor		
	Jackson is a contributor	3.4361×10^{32}	Not calculated
76700	Pike is a contributor	>1 billion	Not calculated
	Jackson is a contributor	>1 billion	Not calculated

Low Vaginal swab (male fraction)

Laboratory ID	Proposition	Likelihood Ratio (autosomal)	Likelihood Ratio (Y-STR)
96150A	Pike is a contributor	>100 billion	20,000
	Jackson is a contributor	>100 billion	20,000
92388	Pike is a contributor		
	Jackson is a contributor	3.4361×10^{32}	Not calculated
76700	Pike is a contributor	>1 billion	Not calculated
	Jackson is a contributor	>1 billion	Not calculated

Conclusion and Summary of the Test

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

This test provides a mechanism for participating laboratories to use their results and those of other laboratories to facilitate[3]:

- An evaluation and review of their performance
- Continuous improvement
- Corrective action (where required)

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

The Forensic Science laboratories were provided with 4 reference blood samples (Items 1-4) and one set of medical samples (Item 5).

Continuity, receipt, and description of items

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

As the receipt and 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.

The three laboratories participating in this test provided all or some of this information.

The swabs contained in Item 5 were dated 20/2/21, whilst the outer packaging was dated 19/2/21. None of the laboratories reported this deliberate discrepancy. This discrepancy should have been noted and investigated. Issues relating to incorrect dating may lead to questions regarding continuity and time/sample integrity.

Examination / Analysis

One laboratory undertook confirmatory testing of the material contained on the reference FTA cards. As these samples were labelled as reference samples this was not strictly necessary. However, if testing of reference samples is a component of routine laboratory

[3] ISO17025 (2017) General requirements for the competency of testing and calibration laboratories.

procedure, this testing is appropriate. No adverse finding should be made where such testing was not conducted.

One laboratory conducted presumptive testing on the high and low vaginal swabs using Acid Phosphatase / Brentamine.

All laboratories confirmed the presence of spermatozoa using light microscope and one laboratory undertook RSID testing for Semen, Saliva, Blood and Urine. The tests for Semen and saliva returned a positive result. This is consistent with the test set up as saliva was used in lieu of vaginal secretions.

Different extraction, quantification and typing regimes were used by each of the laboratories, each regime was appropriate.

One laboratory did not provide the DNA typing results.
The typings provided by one laboratory were consistent with the expected results and the consensus profile.
The typings provided by the third laboratory were consistent with the expected results and the consensus profile with respect to the reference samples but only included one male contributor in the typings from the high and low vaginal swabs rather than a mixed male profile.

Interpretation and Conclusions

All the laboratories reported using Likelihood Ratios using source level propositions. Laboratory 76700 also reported Likelihood Ratios using activity level propositions .

Two laboratories appear to use a threshold reporting level rather than the specific LR.

One laboratory did not indicate what alternate propositions they used to calculate the Likelihood Ratio. The LR is meaningless without information indicating what ratio is being characterised or “what hypotheses are being compared”.

APPENDIX A

Proficiencytesting@forensicfoundations

PROGRAM PLAN

Program	Biological Examination and DNA			
Round	2021-1			
Advisory Group				
Program Coordinator /Technical Manager	Mrs Anna Davey Director Forensic Foundations PO Box 2279 North Ringwood, 3134			
Discipline specific expert(s)	Ms Pam Scott c/- Forensic Foundations PO Box 2279 North Ringwood, 3134			
Provider(s)	Test production. Results interpretation.	Matched semen / blood/ saliva	Additional blood	DNA Profiling
	Forensic Foundations Ms Pam Scott	Cardinal Bioresearch Pty Ltd	Red Cross Life Blood	DNA Solutions VIFM
Test set up location	Forensic Foundations			
	Sample distribution to government facilities within Australia & NZ by ANZPAA-NIFS			
Aims/Objectives	The aim of the program is to assess the laboratories' ability to competently identify biological material and assess its significance and then extract / amplify and interpret DNA profiles.			
Purpose	To assist the laboratories by ensuring their methods/procedures are performing adequately.			
Program Dates				
Invitation letter	August 2020			
Sample distribution	March 2021			
Results due	May 2021			
Manufacturing Information to be sent	June 2021			
Final report due date	July 2021			
Program Design				
Number of Rounds	1			
Number and type of samples	1. Sexual assault collection kit comprising high vag & low vag swabs, reference sample from the complainant 3 x reference samples from suspects.			

Hazards involved	Normal biohazard precautions should be taken when handling and disposing of blood, saliva and semen products.
Scenario	A female complainant has been sexually assaulted. Participants will be provided with the complainant's medical samples and reference sample. Reference samples will also be provided from three suspects.]
Sample size/ volume	The amount of biological material will range from 10-100ul.
Range of values/assigned values	The allelic values will be identified within the known allelic size range provided by the profiling system manufacturer.
Traceability/origin of assigned values	Allele values will be assigned by Genemapper software in reference to size standard and allelic ladder
Design and Methods	Biological material will be applied to either swabs or FTA cards according to the manufacturer's information.
Selection Criteria	Matching semen/saliva/blood will be provided by Cardinal Bioresearch. Additional blood samples will be sourced from the Red Cross Life Blood
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/ examination / analysis, sample mixup
Participants	
Criteria for participation	Forensic Biology laboratories
Expected number of participants	15-20
Reporting Criteria, Accuracy	NA
Analysis	Correctly identify all biological material and interpret DNA profiles including mixtures and partial profiles.

Pre-testing	
Homogeneity Testing and acceptance criteria	Liquid biological test samples will be agitated between the setup of each test, reference, retention and verification sample. Testing of verification samples will include testing for homogeneity.
Stability Testing and acceptance criteria	NA – Dry staining remains stable for periods in excess of the duration of the test. Historical data demonstrates the stability of dried stains.
Technical Review (internal)	
Participant Instructions	Provide evidence of technical review, may be emails
Results Sheet	Sample in file
Report	Sample in file, include review in file

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 -20ºC or 4ºC Test samples Room Temperature
Use by Date	NA dried biological samples are stable for long periods
Distribution requirements	Distributed via Forensic Foundations
Packaging requirements	NA
Sample checks	All samples will be checked by second operator
Statistical Analysis	
Homogeneity Testing and acceptance criteria	NA
Stability Testing and acceptance criteria	NA
Measurement Uncertainty	NA
Data Entry	Include evidence of data entry checks in file
Review by Statistician	NA
Reporting	
Report No:	2021-1
Master copy	Reports folder
Availability	Website
Additional Comments	NA

Program Coordinator signature: KAD

Date: 16/3/20

APPENDIX B

forensic FOUNDATIONS™
ISO 9001 & ISO 17043

Proficiencytesting@forensicfoundations

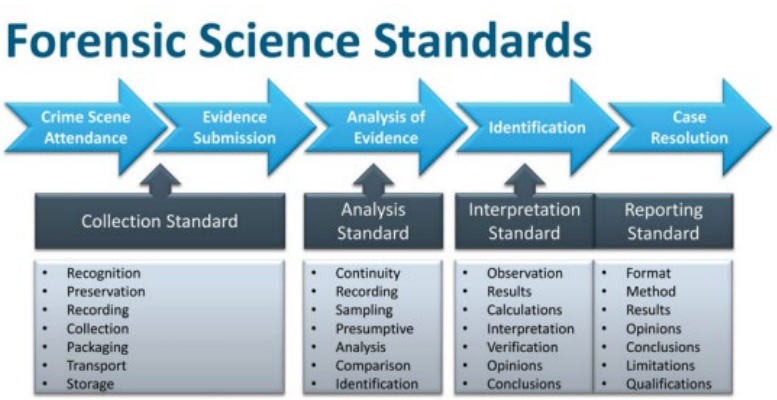
Forensic Biology
Biological examination and DNA - 1
Sexual Assault
2021-1

Thank you for participating in this Proficiency Test. We hope that you find this test useful and welcome any feedback which can be used to improve 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 advise for their 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 the court.

Forensic Foundations' Proficiency Tests are designed to test the end-to-end forensic examination process. The AS 5388 and the ISO 21043 series of Standards describe the forensic examination process from collection to reporting. This figure[4] 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 includes all the subsequent examination / analysis steps, culminating in the reporting of results, the process therefore reflects 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 **28th May 2021**. 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/

To meet the requirements of the National Privacy Principles, DNA Profiles of the donors must not be permanently uploaded onto a DNA database.

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 information regarding the production of the test.

[4] 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	Friday 19th February 2021

BRIEF STATEMENT OF FACTS

Ms Megan Cook presented at the emergency department of The Eastern Metropolitan Hospital. She reported that she had been sexually assaulted while at a night club. She immediately replaced her clothing and walked to the hospital. She is not sure who the assailants were but believes them to be friends of friends.

Ms Cook was examined by a forensic medical officer and swabs were taken for testing.

ITEM SUBMITTED FOR EXAMINATION

Item 1 – Reference sample – suspect 1 – Robin Pike
Item 2 – Reference sample – suspect 2 – Lee Field
Item 3 – Reference sample – suspect 3 – Nick Jackson
Item 4 – Reference sample – suspect 4 – Tony White
Item 5 – Medical samples comprising:

- Reference sample from the complainant
- High vaginal swab collected from the complainant
- Low vaginal swab collected from the complainant

EXAMINATION REQUESTED

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

APPENDIX D

forensic FOUNDATIONS™
ISO 9001 & ISO/IEC 17043

PO Box 2279, Ringwood North VIC 3134
Office: 03 9018 8919
Mobile: 0429 966 012

admin@forensicfoundations.com.au
www.forensicfoundations.com.au

ABN 23 839 112 155 ACN 130 236 618

PROFICIENCYTESTING@FORENSICFOUNDATIONS
BIOLOGICAL EXAMINATION AND DNA INTERPRETATION – 2020-1

MANUFACTURER'S INFORMATION
Issued 29 May 2021

Introduction

The test was designed to replicate the biological staining on intimate swabs collected from a female complainant who had allegedly been sexually assaulted.

Scenario

Ms Megan Cook presented at the emergency department of The Eastern Metro
Hospital. She reported that she had been sexually assaulted while at a night clu
immediately replaced her clothing and walked to the hospital. She is not sure wh
assailants were but believes them to be friends of friends.

Ms Cook was examined by a forensic medical officer and swabs were taken for
The doctor also reported that there were injuries in the genital area.

Test production

The tests were produced at Forensic Foundations' facilities. Samples
One fresh sample of saliva was sourced. The saliva sample was labelled 'Samp

Two set of matching blood and semen were sourced from Cardinal Bioresearch.
samples were shipped on dry ice and were stored upon receipt in a temperature
monitored freezer and moved to a temperature monitored refrigerator to thaw pr
The blood sample from the first matched set was labelled 'Sample B' and the se
sample 'Sample C'.
The blood sample from the second matched set was labelled 'Sample D' and th
sample 'Sample E'.

- Two units of whole blood were sourced from Red Cross Lifeblood, Australia. Both whole bloods were sourced from male donors. The blood was received and stored on frozen blocks in an insulated container during transport. The blood was stored upon receipt in a temperature monitored freezer and moved to a temperature monitored refrigerator to thaw prior to use. The Red Cross identification numbers were recorded, samples were labelled: 'Sample F' and 'Sample G'.
    - Prior to test preparation, blood was removed from each blood bag using a syringe and placed into labelled EDTA vacutainers for further manipulations. Transfers were checked by a second scientist.

Pre-testing

Approximately 100µl of each biological sample (A, B, D, F and G) were placed on swabs using a micropipette. Transfers were checked by a second scientist.

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

Test production

Reference samples

Approximately 100µl of each blood sample were placed onto FTA cards, using a micropipette. Transfers were checked by a second scientist. The FTA cards were labelled:

Sample B	->	Item 1 – blood sample from suspect 1– Pike
Sample D	->	Item 3 – blood sample from suspect 3 – Jackson
Sample F	->	Item 2 – blood sample from suspect 2 – Field
Sample G	->	Item 4 – blood sample from suspect 4 – White

Item of Interest

Item 5 - Sexual Assault Sample Collection Kit containing one Easi-Collect FTA card and two swabs.

Approximately 100µl of saliva from sample A was pipetted onto an Easi-Collect FTA cards. The Easi-Collect was labelled 'COOK 20/2/21'

The swabs were labelled 'Cook' & 'High Vag' or 'Cook' & 'Low Vag'. There was no collection date noted on the swabs.

The 'High Vag' swab contained approximately 50µl of sample 'A', 25µl of sample 'C' and 25µl of sample 'E'.

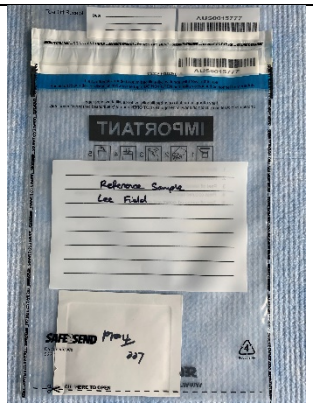
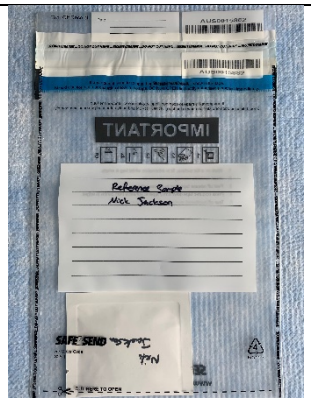
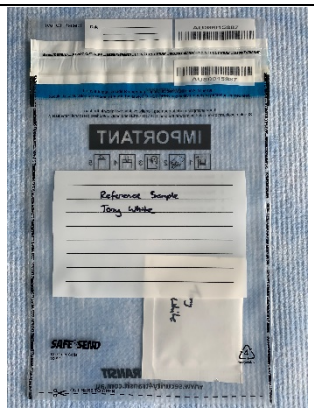
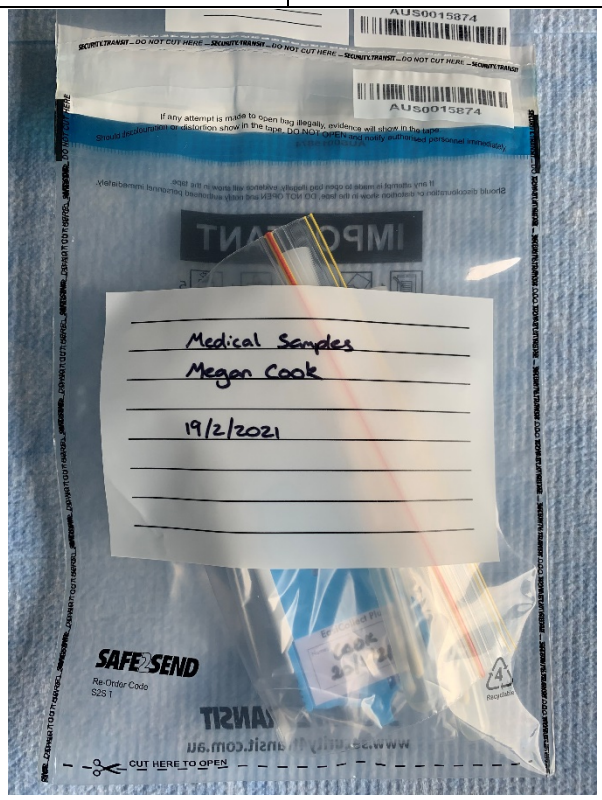
The 'Low Vag' swab contained approximately 25µl of sample 'A', 10µl of sample 'C' and 10µl of sample 'E'.

The packaging of Item 5 was labelled ‘Medical Samples Megan Cook 19/2/21’

The difference in the dates given on the swabs (20/2/21) and the outer packaging (19/2/21) should be noted by the participants.

Final product

The final proficiency test comprised:

4 FTA cards (Items 1-4)				
1 Sexual Assault Sample Collection Kit (Item 5)				

Expected results

- Item 1: The DNA profile obtained from the biological material on the ‘High Vag’ and’ Low Vag’ swabs should demonstrate a mixed DNA profile.
  - The complainant (Cook), suspect 1 (Pike) and suspect 3 (Jackson) cannot be excluded as contributors to this mixture.

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.

	Cardinal Bioresearch			Red Cross Lifeblood Service	
Label	Pike	Jackson	Cook	Field	White
System	PowerPlex Fusion / Globalfiler / Yfiler / Fusion 6C	Globalfiler	PowerPlex Fusion		
Autosomal					
D1S1656	14,18.3	15,15	16,16.3	16,18.3	12,15
D2S441	11,11	11,12	10,10	10,11	11,12
D2S1338	18,20	19,25	19,20	19,26	24,25
D3S1358	16,18	16,17	14,18	17,18	17,18
D5S818	10,11	9,12	12,12	11,11	11,12
D7S820	10,11	10,13	12,13	10,12	7,12
D8S1179	9,13	12,12	8,13	13,13	10,11
D10S1248	14,15	14,15	14,15	13,15	13,15
D12S391	21,22	17,18	20,20	18,18	16,23
D13S317	9,13	12,14	10,11	8,11	12,12
D16S539	9,12	11,13	11,13	11,12	9,11
D18S51	14,17	15,15	13,14	12,17	12,13
D19S433	14,15	12,14	11,13	12,13	14,15
D21S11	30,30	28,30	28,29	29,30	31.2, 31.2
D22S1045	15,16	11,11	15,15	11,15	15,17
CSF1PO	10,12	11,12	12,13	11,12	11,11
FGA	21,26	21,24	20,22	22,25	24,25
Penta D	12,12		9,11	9,12	9,12
Penta E	10,18		10,12	7,18	10,17
SE33	15,30.2	29.2, 31.2			
TH01	6,7	6,7	9.3,9.3	6,7	6,9.3
TPOX	11,11	8,9	8,12	8,9	11,12
vWA	14,19	16,16	14,18	17,17	14,14
Non- autosomal					
AMEL	X,Y	X,Y	X,X	X,Y	X,Y
Yindel	2	2			
DYS19	14				
DYS385 a/b	11,15				
DYS389-I	13				
DYS389-II	29				
DYS390	25				
DYS391	10	10		11	10
DYS392	14				
DYS393	13				
DYS437	15				
DYS438	12				
DYS439	13				
DYS456	15				

	Cardinal Bioresearch			Red Cross Lifeblood Service	
Label	Pike	Jackson	Cook	Field	White
System	PowerPlex Fusion / Globalfiler / Yfiler / Fusion 6C	Globalfiler	PowerPlex Fusion		
DYS448	19				
DYS458	17				
DYS570	17				
DYS576	18				
DYS635	23				
YGATAH4					

END OF DOCUMENT

Biological examination and DNA Analysis 2021-1 Feedback

Forensic Foundations prides itself in providing flexible fit-for-purpose forensic programs. 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:

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

……
……
……
……

2. Any additional comments

……
……
……
……

3. Facility (optional)

……
……
……
……

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

……
……
……
……

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.