Final Report
(January 2022)

Proficiencytesting@forensicfoundations

Biological Examination and DNA Analysis – 2 2021-6

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Introduction

Design

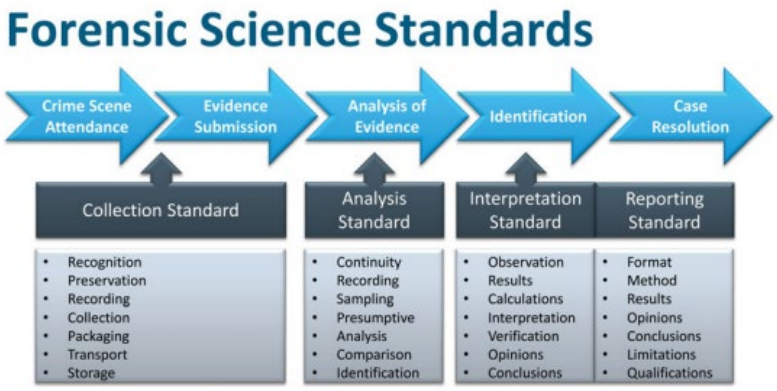
Forensic Foundations' Proficiency Tests are designed to address the following points:

- 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 2021 round of Proficiency Tests is publicly available via the 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

2021-6 Biological Examination and DNA Analysis - 2

The aim of this test was to review the end-to-end forensic examination, analysis, and reporting process. Thus, 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 provided a mechanism for participating laboratories to review their results and those of other laboratories to facilitate[2&3]:

- An evaluation and appraisal of their performance including
  - Comparison of a facility's performance with other facilities
  - monitoring of a facility's long-term performance;
- Continuous improvement including
  - staff education, training and competence monitoring
  - evaluation of methods, including the establishment of method precision and accuracy
  - contribution to a facility's overall risk management process
- Corrective action (where required)
- Confidence building with interested parties (e.g. customers, accreditation bodies, regulators, specifiers).

This proficiency test was distributed to 22 participants, 18 participants submitted results during this round of testing. The 18 participants represented three laboratories. Two laboratories submitted individual responses and one laboratory submitted 16 responses.

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

The participants were provided with one t shirt (Item 1), one bandana (item 2), one swab (Item 3) and three reference blood samples (Items 4-6).

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.

[3] NATA (2021) General Accreditation Criteria Proficiency Testing Policy

Report Summary

Continuity, receipt, and description of items

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 information provided by the participants concurs with the packaging, labelling and samples as distributed, however a number of participants described what they would do (their procedures) rather than what they actually did.

Examination / Analysis

Presumptive and confirmatory tests for both blood and saliva were utilised by the participating laboratories. The results indicate the usefulness of screening and confirmatory testing.

A range of commercial kits were used by the three participating laboratories during the examination and analysis.

The analytical results varied considerably between participants. It is not possible from the data to determine whether this variation is due to differing efficiencies of the extraction process, the PCR process or the thresholds used by individual participants in analysing the data. This will be addressed in subsequent proficiency tests.

A number of participants did not detect and subsequently test the saliva stain on the T-shirt (Item 1), thus were unable to associate one of the suspects with the T-shirt, indicating the importance of both preliminary and confirmatory tests for saliva.

Interpretation and Conclusions

The interpretation of the analytical results obtained from Items 2 and 3 presented some challenges to the participants, similar to those encountered in routine casework. The **reported** results were consistent with the expected results.

However, care should be taken in the situation where no result was obtained or a complex analytical result could not be interpreted.

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.

Participant responses are provided in an Excel spreadsheet (Table 1) attached to this report.

Forensic Foundations' comments

One participant (96150A) provided redacted case records which included photographs.

Two participants indicated that their continuity and receipt procedures complied with ISO17025:2017 but did not indicate what these procedures were. Legal (forensic requirements) for continuity and receipt are usually in addition to other basic requirements outlined in ISO17025.

Two of the three participating laboratories stated that they used a LIMS to track the items.

The information provided by the participants concurs with the packaging, labelling and samples as distributed

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.

Participant responses (for Items 1 & 2) from the proforma table and case notes where supplied, are provided in an Excel spreadsheet (Table 2) attached to this report. Tests appearing in the proforma table which were not applied by any participant laboratory have been removed. The shaded fields indicate that the participant did not undertake the specific test.

Item 3 (swab) was not examined in detail by any participant and proceeded directly to DNA extraction.

In addition, participants were encouraged to make any comments specific to the examination / analysis. These comments are shown below:

86826-1

- Saliva testing (both screening and confirmatory test) is not yet available in the laboratory.

86826-2,3,4,5,6,7,8,9,10,12,13,14 & 15

- In our laboratory does not have confirmatory testing for saliva. Its (sic) may lost an expected saliva stain on Item 2.
- Every steps (sic) of examination and analysis followed ISO/IEC 17025:2017.

86826-11

- Confirmatory test for saliva is not available in our laboratory

96150A

- Complete single source profiles were obtained for all three reference samples Item(s) - 3, 4, & 5 (sic)
- Complete single source profiles were obtained for two evidence samples from Item 1 (T-shirt)
- Item 2 was tested prior to sampling for the presence of saliva and a small area in the mid-section of the unfolded bandana gave a positive reaction to presumptive testing. The results obtained indicated that the samples taken from the bandana were degraded (cuttings of a phadebas positive stain, and a tapelift from the mid-section) or, a very low amount of cellular material was deposited. A profile suitable for upload was not obtained from this item.
- Item 3 (Swab of door handle) had very low levels of DNA detected and was not analysed further.

Forensic Foundations' comments

Examination Item 1 (T-shirt)

9 participants examined the item using an alternative light source. One laboratory (39376) obtained a positive detection under an orange filter and blue light, indicating that biological fluid may be located at the collar of the T-shirt.

One participant (96150A) undertook the Phadebas presumptive test for saliva with a positive result at the neckline of the T-shirt.

These results were correct, in that the stain originated from human saliva.

17 participants used Kastle Myer and one participant used Hemastix to test the reddish brown stain on the front of the T-shirt. All tests returned a positive result indicative of blood.

One participant (39376) undertook confirmatory testing for blood using both RSID and OBTI.

These results were correct in that the stain originated from human blood.

All participants used cutting as a means of subsampling, three participants also swabbed stains and one participant used tape lifts to collect sample material. The effectiveness of the subsampling techniques will be discussed later.

Examination Item 2 (Bandana)

Seven participants examined the item using an alternative light source. However, no positive results were obtained by any participant.

One participant (96150A) undertook the Phadebas presumptive test for saliva with a positive result. This participant also undertook confirmatory RSID Saliva testing of the stain with a positive result for saliva.

These results were correct, in that the stain originated from human saliva.

Eleven participants used cutting as a means of subsampling, 17 participants also swabbed areas of the bandana and one participant used tape lifts. Eight participants indicated that they cut out the centre of the bandana where saliva may be expected and swabbed the remaining area. The effectiveness of the subsampling techniques will be discussed later.

Examination Item 3 (swab)

No examination was undertaken

Examination Items 4, 5 & 6

No examination was undertaken

Analysis

The following systems were used by the three laboratories represented by the 18 participants.

	39376	86826	96150A
Extraction	Promega Casework Extraction Kit. Promega DNA IQ™ Casework Pro Kit, Maxwell® 16 Forensic Instrument.	1. QIAamp® DNA Investigator Kit 2. DNA IQ™ System	DNA-IQ™ System (Promega)
Quantification	Applied Biosystems™ Quantifiler™ Trio DNA Quantitation Kit, ABI 7500 Real-Time PCR System.	Quantifiler™ Trio DNA Quantification Kit	Quantifiler™ Trio DNA Quantification Kit is used in conjunction with the ABI PRISM® 7500 Sequence Detection System for DNA quantitation.
Amplification System(s)	Applied Biosystems™ GlobalFiler™ PCR Amplification Kit, ABI GeneAmp 9700 PCR System.	1. GlobalFiler™ PCR Amplification Kit 2. Yfiler™ Plus PCR Amplification Kit	GlobalFiler™ PCR Amplification Kit
Electrophoresis and detection method	Applied Biosystems™ 3500xL Genetic Analyzer, 3500 Series Data Collection Software 3. Analysis using Genemapper ID-x v1.5	1. 3500 xL Genetic Analyzer 2. Data Collection Software V3.0 3. GeneMapper ID-X v1.6	Applied Biosystems® 3500Genetic Analyzer Applied Biosystems GeneMapper™ ID-X Software

Analytical Results

Participants were requested to provide DNA typing information using a proforma table. This facilitated both consistent responses and the collation and comparison of responses.

Participant responses are provided in an Excel spreadsheet (Table 3) attached to this report.

Item 1 - T shirt

Complete profiles were obtained from the bloodstain by all participants.

Complete profiles were obtained from the saliva stain by 2 participants.
One participant reported a mixture consistent with at least one male contributor in the saliva stain (86826-1) however the profile provided appeared to be a partial single source profile from an unknown source.

Item 2 - Bandana

A complete single source autosomal profile and partial Y profile was obtained from the saliva stain by 1 participant (86826-12).

6 participants reported that there was insufficient DNA detected to continue with the analysis.

10 participants obtained partial profiles containing a varied number of alleles (both autosomal and Y). In addition to alleles matching those from the expected profile, alleles from at least 2 other donors were present. The source of these additional alleles is not known.

The results of the examination of the bandana (Item 2) reinforced the need for extreme care when interpreting low level DNA results.

Item 3 - Swab

Complete autosomal profiles were obtained by 3 participants, two participants also obtained a partial Y profile.

13 participants obtained partial profiles containing a varied number of alleles (both autosomal and Y).

2 participants reported that there was insufficient DNA detected to continue with the analysis.

Items 4, 5 & 6 Reference samples

Complete profiles were obtained by all participants from the reference samples.

A range of commercial extraction kits were used however all participating laboratories used the same quantification, amplification, electrophoresis and detection systems.

Some participants in laboratory 86826 also utilized the Yfiler Plus kit in addition to the autosomal STR kits.

Forensic Foundations comments

Item 1 and reference samples

No issues were encountered obtaining a DNA profile from the reference samples and the large bloodstain on the T-shirt (Item 1).

Saliva stain on the T-shirt (Item 1)

Three participants obtained a full (30376, 96150A) or partial (86826-1) profile from the saliva stain on the neck of the T-shirt (Item 1).

Participant 39376 used an alternative light source to detect the saliva stain, which was subsequently swabbed, whilst participant 96150A used Phadebas reagent to detect the stain which was then subsampled by means of cutting. It is not clear what mechanism for location of the stain, participant 86826-1 utilised, however the area was swabbed. Participants from laboratory 86826 indicated that 'Saliva testing (both screening and confirmatory test) is not yet available in the laboratory'. These results indicate the usefulness of such screening and confirmatory testing.

Item 2 & 3

The analytical results vary considerably between participants. It is not possible from the data to determine whether this variation is due to differing efficiencies of the extraction process, the PCR process or the thresholds used by individual participants in analysing the data. This will be addressed in subsequent proficiency tests.

The combined results from item 2 indicate the presence of DNA from at least three donors (the expected donor plus at least two others).

No comment may be made in relation to the effectiveness of either swabbing or cutting as a means of subsampling from this data.

Interpretation, Conclusions and Reported Results

Participant responses are provided in an PDF spreadsheet (Table 4) attached to this report.

The following table summarises the results reported

Item 1 T-shirt blood stain	Matched Chin	Full GlobalFiler profile (17 participants) Autosomal GlobalFiler profile (1 participant) Full Yfiler Plus profile (16 participants)
Item 1 T-shirt saliva stain	Matched Smyth	Full GlobalFiler profile (1 participant) Autosomal GlobalFiler profile (1 participant)
Item 2 Bandana	Matched Burke	Full GlobalFiler profile and partial Y profile (1 participant)
		Partial GlobalFiler profile (1 participant)
		Partial GlobalFiler / Yfiler Plus profile (1 participant)
	Mixture which includes Burke	Partial GlobalFiler profile (1 participant)
	Matches Burke	Full GlobalFiler profile (1 participant)
Item 3 Swab		Partial GlobalFiler profile (2 participants)
		Full GlobalFiler profile and partial Yfiler Plus profile (1 participant)
		Partial Gobalfiler and Yfiler Plus profile (7 participants)

The three participating laboratories used different forms of reporting.

39376 provided a report for intelligence purposes and therefore did not provide statistics.
86826 used posterior probability to indicate the significance of the result.
96150A used verbal equivalents to indicate the significance of the results and provided a table outlining the interpretation of the verbal scale.

<u>Forensic Foundations' comments</u>

The interpretation of the analytical results obtained from Items 2 and 3 presented some challenges to the participants, similar to those encountered in routine casework. The reported results were consistent with the expected results.

Care should be taken in the situation where no result was obtained or a complex analytical result could not be interpreted. The reader of the report should be clear whether no result was obtained from the items tested or an exclusion was obtained. A number of participants reported that 'The DNA profile from Reference sample-suspect 1–Kate Smyth (Item 5) did not match to other DNA profiles in the case'.

Whilst this is technically correct, DNA matching Kate Smyth was present but not detected. The above statement may imply to the reader, that Kate Smyth was excluded from the case.

APPENDIX A

EXAMINATION REQUEST AND ITEM SUBMISSION	EASTERN AUSTRALIAN POLICE SERVICE

OFFENCE:	Assault
DATE OF OFFENCE	Thursday 17th June 2021

BRIEF STATEMENT OF FACTS

Michael Burke has been assaulted, allegedly kidnapped and driven to an unknown location. During the assault he (Michael Burke) hit the male assailant and caused the assailant's nose to bleed. The female assailant also spat on him (Michael Burke).

In the car he was seated on the backseat behind the driver's seat and the male assailant sat next to him. He was gagged, using what he believed to be a bandana, and at one stage attempted to flee from the car by trying to open the back door by lifting the door lever.

He was ungagged and released a few hours later in a remote bush land area.

Police have located the suspect vehicle (XSB972).

ITEM SUBMITTED FOR EXAMINATION

Item 1 – T shirt from complainant – Michael Burke
Item 2 – Bandana located in vehicle registration number XSB972
Item 3 – Swab taken from doorhandle of the vehicle registration number XSB972
Item 4 – Reference sample – complainant – Michael Burke
Item 5 – Reference sample – suspect 1 – Kate Smyth
Item 6 – Reference sample – suspect 2 – Douglas Chin

EXAMINATION REQUESTED

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

APPENDIX B

forensic FOUNDATIONS™
ISO 9001 & ISO/IEC 17043

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PROFICIENCYTESTING@FORENSICFOUNDATIONS BIOLOGICAL EXAMINATION AND DNA INTERPRETATION 2

MANUFACTURER'S INFORMATION
Issued 26/10/2021
Revised 07/12/2021

This revision of the manufacturer's information has been issued following:

- The correction of an error in the manufacturer's information issued on 26/10/2021, highlighted where appropriate; and
- An internal review of proficiency test documentation. This version of the manufacturer's information contains information which was previously provided in the 'Program Plan"

Introduction

The test was designed to replicate an alleged assault with biological staining on clothing and potential gag and touch DNA points reported by the complainant.

Scenario

Michael Burke has been assaulted, allegedly kidnapped, and driven to an unknown location. During the assault he (Michael Burke) hit the male assailant and caused the assailant's nose to bleed. The female assailant also spat on him (Michael Burke).

In the car he was seated on the backseat behind the driver's seat and the male assailant sat next to him. He was gagged, using what he believed to be a bandana, and at one stage attempted to flee from the car by trying to open the back door by lifting the door lever.

He was ungagged and released a few hours later in a remote bush land area.

Police have located the suspect vehicle (XSB972).

Aim

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

Program design and assessment

The test was designed, coordinated and assessment conduct by Forensic Foundations.

Samples

- One set of matching male blood and saliva was sourced from Cardinal Bioresearch. The samples were received frozen and on dry ice and then stored upon receipt in a temperature monitored freezer and moved to a temperature monitored refrigerator to thaw prior to use. The blood was labelled ‘Sample A’ and the saliva labelled ‘Sample B’.

- One matching set of blood and saliva was sourced from a female donor. The blood was stored in a temperature monitored refrigerator prior to use. The saliva sample was used immediately. The blood was labelled ‘Sample C’ and the saliva was labelled ‘Sample D’.

- One sample of male whole blood was sourced from Red Cross Lifeblood. The blood was received on frozen blocks in an insulated container. The blood was stored upon receipt in a temperature monitored freezer and moved to a temperature monitored refrigerator to thaw prior to use. The sample was labelled ‘Sample E’
- Prior to test preparation, blood was removed from the 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 blood sample (A, C and E) was placed on swabs using a micropipette. Transfers were checked by a second scientist.

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

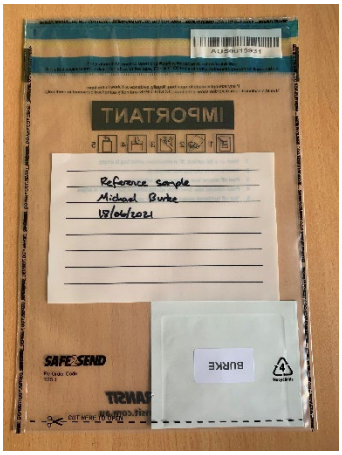
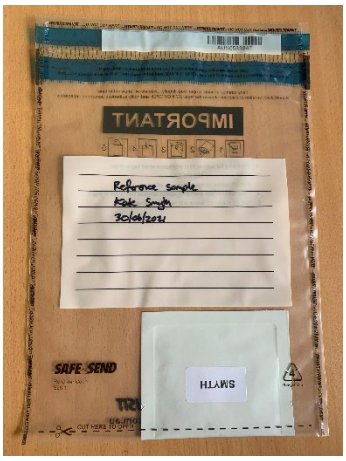
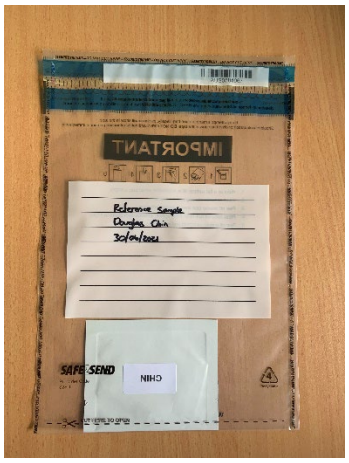
Test production

The tests were produced at Forensic Foundations’ facilities. Surfaces and equipment were cleaned prior to use and/or fresh bench coverings were used. Disposable consumables were used. All biological samples were agitated before and during production, to ensure homogeneity

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 4 – blood sample from complainant –Burke
Sample C	->	Item 5 – blood sample from suspect 1 –Smyth
Sample E	->	Item 6 – blood sample from suspect 2 –Chin

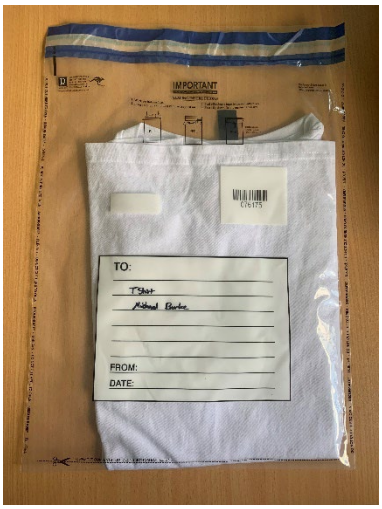
3 FTA cards (Items 4-6)			
	Item 4 Reference sample from Michael Burke	Item 5 Reference sample from Kate Smyth	Item 6 Reference sample from Douglas Chin

Item of Interest

Item 1 – T shirt belonging to the complainant

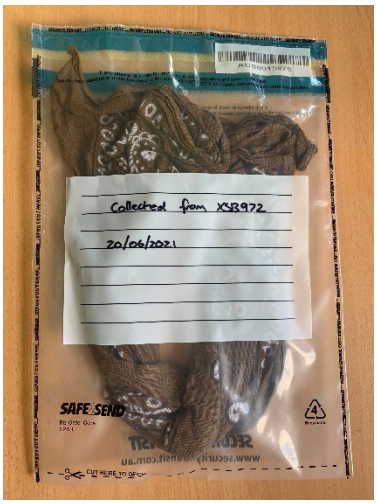
Approximately 50µl of blood from sample E was pipetted onto the upper front of the T shirt.

A diluted saliva sample (sample D 50:50 Saliva:PBS) was pipetted onto the collar stitching on the left side of the T shirt – marked with the arrow.

T shirt from complainant (Item 1)		

Item 2 – Bandana allegedly used to gag complainant. Located in vehicle registration number XSB972.

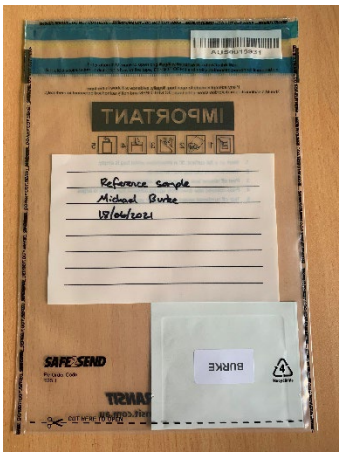
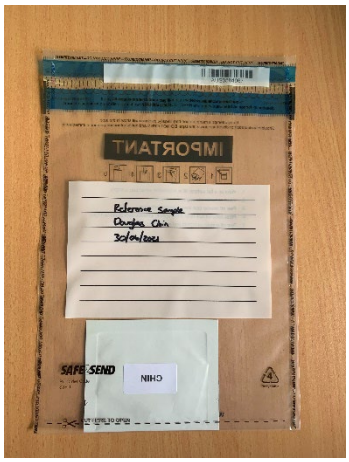
Each bandana was twisted and scrunched to replicate it being used as a gag and approximately 100µl of diluted saliva sample (sample B 50:50 Saliva:PBS) pipetted onto the centre of the scrunched bandana to simulate the location of the mouth.

Bandana found in the car (Item 2)		

Item 3 – Touch DNA swab from the car

Approximately 50 µl of diluted saliva sample (sample B 1:7 Saliva:PBS) to a swab. The swab was labelled ‘Door handle 20/06/2021’

Touch DNA swab from the car (Item 3)	

3 FTA cards (Items 4-6)			
	Item 4 Reference sample from Michael Burke	Item 5 Reference sample from Kate Smyth	Item 6 Reference sample from Douglas Chin

The major source of error during production included failure to properly disinfect workspace prior to sample preparation/ examination / analysis, sample mix-up

Tests were stored at room temperature prior to distribution using DHL Courier and receipt acknowledged by the recipients. Samples are stable and no use by date was provided.

Expected results

- Item 1: A human blood stain and a human saliva stain should be located on the T-shirt. DNA profile should be obtained from the both the bloodstain and the saliva stain.
- Suspect 2 (Chin)[4] cannot be excluded as the source of the bloodstain.
- Suspect 1 (Smyth)* cannot be excluded as the source of the saliva stain.
- Item 2: The DNA profile obtained from the biological material on the bandana cannot be excluded as originating from complainant (Burke).
- Item 3: The DNA profile obtained from the biological material on the touch DNA swab cannot be excluded as originating from complainant (Burke).

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.

The major source of error by the participant would be failure to correctly interpret DNA profiles, failure to properly disinfect workspace prior to sample preparation/ examination / analysis, sample mix-up.

[4] The details for the two matching profiles were transposed in the manufacturer's information dated 26/10/2021

Pretesting results

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

The tables containing the DNA profiles have been removed from this public document. If you require this information, please contact us.

END OF DOCUMENT

Biological examination and DNA Analysis 2021-6 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.
You can also complete this form at www.forensicfoundations.com.au/feedback

	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. You can also complete this form at https://www.forensicfoundations.com.au/proficiency-testing/proficiency-test-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.