STATEMENT OF PAULA MICHELLE BRISOTTO

I, Paula Michelle Brisotto, care of Queensland Health Forensic and Scientific Service, Team Leader Evidence Recovery and Quality Team, Forensic DNA Analysis, do solemnly and sincerely declare that:

1. I am employed by Queensland Health Forensic and Scientific Service ('QHFSS').

2. I hold a Bachelor of Science from Griffith University, and a Master of Science in Forensic Science from Griffith University.

3. This statement is in response to Notice 2022/00338.

In an email from Cathie Allen to Lindon Smallwood of 18 June 2008 (Attachment 1), Ms Allen advised that 'either distilled water or 70% ethanol would be a suitable solution to collect blood'.

a. *Advise whether the DNA Laboratory had conducted any validation or verification of using water or 70% ethanol as a solution to collect blood, and if yes, attach those validations/verifications.*

b. *Advise whether there was other evidence or studies relied upon to advise that 70% ethanol was an appropriate wetting agent, and if yes, attach that evidence / those studies.*

4. In the limited time permitted to respond to Notice 2022/00338, I have not been able to locate any validation or verification of using water or 70% ethanol as a solution to collect blood.

5. From information I can locate in the time given and with limited access to where this information may be held, I believe that the practice of using distilled water and/or 70% ethanol moistened swabs was in place since December 2001, according to standard operating practice (**SOP**) 17142 "*Examination of Items*" (version 1) which says:

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"15. EXAMINATION OF SUSPECTED BLOODSTAINS

...

- *A swab moistened with 70% EtOH can be used to sample very small or weak suspected bloodstains from items. This is instead of using a swab moistened with distilled water.*

16. EXAMINATION FOR EPITHELIAL CELLS OR SALIVA

- *Suspected epithelial cells on items can be collected for DNA analysis by scraping the area with a scalpel blade or by using small pieces of sticky tape and extracting these tapelifts. A swab moistened with 70% EtOH may also be used..."*

6. A copy of SOP 17412 is exhibited as **PB170** to this statement.

7. I commenced employment as a temporary technician with QHFSS in September 2001, so am not aware of what information was used to inform this process.

8. I cannot locate information prior to SOP 17142 relating to validation or verification of this practice.

In an email from Allan McNevin to Lyza-Jane McMenz and David Neville of 26 March 2009 (Attachment 2), Mr McNevin states 'We have considered the rayon swabs that David brought out for us suitable for use, we do not consider it necessary to perform any testing, as the rayon swab appears to be identical to a product we have used for various processes within the laboratory with the single exception that the swab head on the medical wire sample appears to be not as tightly wound as the brand that we use, however this is not a problem.'

a. *Advise whether the DNA Laboratory had conducted any validation or verification on the use of the rayon swabs referred to above for collecting evidence for DNA testing; and if yes, attach those validations/verifications.*

9. In the limited time permitted to respond to Notice 2022/00338, I have located:

   a. a document titled "*Batching Validation Samples Cells*" which indicates that rayon swabs were used in 2007 during what appears to be an automation

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validation project (refer to **PB171** to this statement). I do not know what project this would have been or who wrote these notes but it may have been Cecilia Iannuzzi, Breanna Gallagher and Generosa Lundie who appear to be the project officers listed for Project 11;

b. "*Project 11 Report on the Validation of a manual method for Extracting DNA using the DNA IQ™ System*" which appears to have been completed in August 2008 (refer to **PB172** to this statement). Project 11 seems to validate a manual method for DNA extraction of blood and cell stains on forensic samples, which included using rayon swabs. The recommendations made were:

> *"Based on the findings of this validation report, we recommend:*
> 1. *To enable processing of cell and blood samples using the validated manual DNA IQ™ protocol, except for samples on tapelift substrates.*
> 2. *To design and verify an automated protocol of the validated DNA IQ™ method for use on the MultiPROBE® II PLUS HT EX platforms, for processing blood and cell samples."*

c. SOP 25874 "*Preparation of Quality Controls for Extraction Processes*", which appears to have been drafted in 2008 and then active from February 2009, refers to rayon swabs being used to create positive extraction controls (refer to **PB173** to this statement);

d. a "*BSAG Method Instrument details 2019*" which indicates a number of other jurisdictions use rayon swabs (refer to **PB174** to this statement);

e. a working draft document titled "*Recognition, recording, recovery and storage of physical material for forensic purposes*" (refer to **PB175** to this statement). I am not sure who prepared this document. I note that it provides under Appendix H: Packing Guidelines:

DNA	*Any material that requires DNA analysis should not be sealed in plastic bags for long term storage unless completely dry.*

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	Objects may be themselves collected or may be swabbed in the field with 70% ethanol or distilled water. Tapelifts may be a suitable alternative to swabs.

10. The "*Copan 4N6 swab trial report*" dated 22 January 2009 compared 4N6 swabs to Copan rayon swabs. This project commenced in 2008 by Allan McNevin and Chiron Weber. A copy of this report is exhibited as PB154 of my statement dated 9 November 2022.

11. I have located a draft version of that report dated May 2008 (refer to **PB176** to this statement) which says:

"

a. *two swab types that are currently in use for the collection of material for forensic DNA testing. The swabs would be compared on two criteria:*
    i. *The ability to extract DNA from each swab type and,*
    ii. *The ability of each swab type to uptake DNA.*

b. *The two swab types chosen to compare against the 4N6 swab were a spun cotton swab with a small swab head and paper shaft (Copan, product code 164C) and a spun rayon swab with a medium sized swab head and plastic shaft (Copan, product code 155C)."*

c. *It appears, therefore, that the Rayon swabs were in use prior to May 2008.25874 - V1.0 active Feb 2009 details the preparation of positive extraction controls using rayon swabs."*

12. I also refer to paragraphs 4-14 of my statement dated 9 November 2022 which references the replacement of 4N6 swabs.

b. *Advise whether the DNA Laboratory had conducted any validation or verification on the use of the rayon swabs referred to above, with 70% ethanol as a wetting agent, for collecting evidence for DNA testing; and if yes, attach those validations/verifications.*

....
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13. In the limited time permitted to respond to Notice 2022/00338, I have not been able to locate any validation or verification specifically testing rayon swabs with a wetting agent of 70% ethanol to swab and collect evidence for DNA testing.

14. I have located Management Meeting Minutes dated 7 May 2009 (refer to **PB177** to this statement), which I attended, which state:

QPS rayon tubes - swab casing. ***(PT)***	*QPS wish to move from 4N6 Swabs to Rayon. Example of tubes and size of holes in top were shown.* *Agreed that definite decision cannot be made on these as yet. PT to draft a response to Dave Neville QPS (will be circulated to Mgt Team for input). 70% ethanol to be used instead of water by SOCOs.*	*PT*

15. I have located a draft document called 'swab casings' which I prepared in 2009 and was last modified on 7 May 2009 (refer to **PB178** to this statement), which appears to be a draft communication to Inspector David Neville. The draft I have located says:

> *"Hi Dave,*
>
> *The Management Team has looked at the swab casings for the rayon swabs, and we are not able to make a definite decision on the most appropriate hole size. Basically, the green lidded tube is the preferred tube type, however in regard to the hole size, there are a lot of factors that need to be considered.*
>
> *The larger hole size may potentially allow more air to get into the casing, however we would need to conduct experiments to determine if the adhesive we stick over the hole will provide an appropriate seal during our processes, given the larger hole size. Also, the type of item in the tube itself would need to be considered – if the samples are hairs, scrapings or fibres, then these may potentially escape through the hole.*
>
> *The above mentioned issues will also apply to the medium size hole, and even the smaller hole.*
>
> *Any of the hole sizes may still not be enough to stop mould from forming on the swabs, especially in humid conditions when the swab is still wet.* ***The best advice we can give at this time is, regardless of the hole size, is to use 70% ethanol and dry the swabs completely before placing into the swab casings and then the envelope. If drying is not possible, immediate freezing (if practicable) and transportation would be another option.***
>
> *Some other avenues that could be considered in the future, all of which will involve further investigation and experiments to determine if they are appropriate, are:*

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- *a desiccant added to either the envelope outside of the swab casing, or in the lid of the swab casing*
- *some sort of transport medium*
- *a swab with similar properties to the FTA card, which would inhibit the formation of mould*
- *a membrane covering in the lid which would allow the swab to 'breathe', without letting any material to escape.*

All of the above are just discussion points and require a lot of further investigation, however if we want to minimise the chance of mould forming on the swabs, then it might be necessary to look at the alternatives, some of which are provided above."

16. I do not recall whether the above draft communication was sent to Inspector Neville and I am unable to confirm this as I do not have access to emails prior to mid-2012. In the time permitted to respond to Notice 2022/00338, I have not been able to locate any correspondence related to the draft communication above.

17. On reading the draft communication and the comments in the Management Meeting Minutes dated 7 May 2009, this appears to be a Management Team discussion regarding tube hole size rather than the verification or validation of rayon swabs with a wetting agent of 70% ethanol to swab and collect evidence for DNA testing.

Advise whether, at any time prior to 2011, the DNA Laboratory had conducted any validation or verification for using, in combination, rayon swabs with a wetting agent of 70% ethanol, to swab and collect evidence for DNA testing.

a. *If yes, attach those validations/verifications.*

b. *If no, explain why not, and attach any other evidence gathered or reports produced concerning the use of rayon swabs with 70% ethanol.*

18. In the limited time permitted to respond to Notice 2022/00338, I have not been able to locate any validation or verification specifically testing rayon swabs with a wetting agent of 70% ethanol to swab and collect evidence for DNA testing.

19. As indicated in paragraph 5 above, SOP 17142 (version 1) is the earliest reference I have been able to locate in the time permitting for the use of 70% ethanol as a standard technique for swabbing and collecting evidence.

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Advise whether, at any time since 1 January 2011, the DNA Laboratory has conducted any validation or verification for using, in combination, rayon swabs with a wetting agent of 70% ethanol, to swab and collect evidence for DNA testing. If yes, attach those validations/verifications.

20. In the limited time permitted to respond to Notice 2022/00338, I have not been able to locate any validation or verification specifically testing rayon swabs with a wetting agent of 70% ethanol to swab and collect evidence for DNA testing.

21. I intend to provide a further statement in response to Notice 2022/00338 once I have had an opportunity to consider the questions further and/or locate any relevant documentation. Without further time to search for documents that may be relevant to the questions given in Notice 00338, I cannot say that they do not exist.

TAKEN AND DECLARED before me at Cairns in the State of Queensland this 25th day of November 2022.

....

Paula Michelle Brisotto

....

Witness Erin Boland
Justice of the Peace (Qualified)

EXHIBIT INDEX

Exhibit	Document Title	Pages
PB170	SOP 17142 "*Examination of Items*" (version 1)	9-15
PB171	"*Batching Validation Samples Cells*"	16-17
PB172	"*Project 11 Report on the Validation of a manual method for Extracting DNA using the DNA IQ™ System*"	18-39
PB173	SOP 25874 "*Preparation of Quality Controls for Extraction Processes*"	40-48
PB174	"BSAG Method Instrument details 2019"	49-50
PB175	Working draft document titled "*Recognition, recording, recovery and storage of physical material for forensic purposes*"	51-99
PB176	Draft report for "*Copan 4N6 swab trial report*" dated May 2008	100-105
PB177	Management Meeting Minutes dated 7 May 2009	106-118
PB178	Draft document called 'swab casings'	119

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Examination of Items

1 PURPOSE

To describe the procedure for the initial examination of items by case examiners in Forensic Biology, John Tonge Centre.

2 SCOPE

This SOP is an adjunct to individual methods for particular screening tests. Interpretations and limits of reporting are to be found in each method.

3 REFERENCES

1. QSB-DQP-008 Product Identification and Traceability.
2. QSB-DQP-009 Process Control in Testing.
3. QSB-DQP-010 Inspection and Testing
4. Biological Methods Manual, September, 1989

4 ASSOCIATED DOCUMENTS

1. 17117 Procedure for Case Record Documentation and Evidence Management
2. 17167 Procedure for the Retention and Storage of Items
3. Methods for screening and confirmatory tests are listed throughout this procedure.

5 CASE FILE DOCUMENTATION AND EVIDENCE MANAGEMENT

- Refer to 17117 (Procedure for Case Record Documentation and Evidence Management) for details on case file components and item identification.
- All items must be uniquely identified.
- All notes must be legible and in ink. Diagrams and sketches may be in coloured pencil.

6 CONTINUITY OF EVIDENCE

- Refer to SSFB-S-0018 (Procedure for the Retention and Storage of Items) for further information.
- Record in the case file, the date and time items were removed from storage.
- Record details of the packaging in the case file.

- Examine only one item at a time ensuring the bench is clean before each examination. Benches can be covered with paper as long as this is changed when a different item is examined. Items should be repacked as soon as possible after examination. *These practices are in place to reduce the chance of cross contamination.*

- Items are not to be left on benches unattended for extended periods of time. Each case analyst has access to a secure cage in the exhibit room where items undergoing examination can be stored.

7 WORK ENVIRONMENT

- Health and Safety protocols must be followed. Appropriate PPE (personal protective equipment) must be worn when handling and examining items.

- Benches and large examination tables can be cleaned with sodium hypochlorite solution (500ppm available chloride, 0.7% v/v detergent). Items can be examined on clean paper placed on benches but this must be changed for each item.

- Scissors, forceps and punches must be cleaned thoroughly before removing pieces of suspected biological material and between cutting stains and controls. Flaming with alcohol is preferred but distorts the metal after repeated use. Use disposable scalpel blades instead, wherever possible.

- Use plastic formers for stretching items of clothing and for separating front and back surfaces. Wash the formers before and after use.

- Cap disposable tubes used for samples immediately after placing each sample inside. Check the tubes are properly labelled.

- Equipment not required or in use, should be put away and the work surface kept clutter-free.

- Heavily stained items should be handled carefully to minimize dusting; clean the work surfaces thoroughly afterwards.

8 CHARACTERISATION OF BIOLOGICAL MATERIAL

- NATA *Standards for DNA Analysis* requires biological material to be characterised before DNA analysis. Case examiners must adhere to this whenever possible. Where a case examiner elects <u>not</u> to perform a screening test on an item it <u>must</u> be because the screen test will consume the evidence and the case notes <u>must</u> indicate why characterisation was not performed.

9 GENERAL EXAMINATIONS

- Read the case record before starting to be certain of the circumstances of the case and the types of examination required. Contact the investigating officer for further information if necessary.

- Use standard examination forms found in Q-pulse.

- Include a description of each item examined. Record the condition of each item. Record any packaging and labelling details of each item.

- Forensic Biology does not examine fibres. If fibre examination is indicated, DO NOT OPEN THE BAG CONTAINING THE ITEM. Consult with Forensic Chemistry for processing of the item.

Note: The method of opening and checking items at the front counter mitigates against trace evidence examinations. Case examiners should bear this in mind when removing or testing hairs found on items.

- Physical hair examinations are performed as a screening tool to identify hairs suitable for DNA analysis, or prior to mtDNA analysis.
- Note damage to garments being examined: *e.g.* tears, mends, missing buttons *etc.* Document whether this is due to "fair wear and tear" or due to violence. Attempt to assess if the damage is old or recent.
- Each item must be labelled with its unique sample id.
- The digital cameras can be used to photograph items and stains for inclusion in case files.

10 ITEMS TO BE FINGERPRINTED

- Keep all handling to a minimum.
- Wear latex gloves at all times when touching the item.
- Avoid touching item where fingerprints are likely to be found.

11 REMOVAL OF EXTRANEOUS MATERIAL

- Exchange of material between two items may occur when they come into contact with each other and proof of contact may be of important evidential value. Extraneous surface material may include fibres, hairs, wood, vegetation, string, feathers. If this is to be examined further, it is important that any extraneous material is removed before further examination is begun.
- Pick off easily visible material, which may be large enough to see clearly, such as wood fragments, hairs, feathers, string and vegetation, with forceps and place in separate labelled plastic bags or petri dishes to await further examination. Record positions on the examination sheet.
- If examination of this extraneous material is not required, seal in small labelled plastic bags. Attach the bags to the item for return with the item at the end of the examination.

12 EXAMINATION OF STAINS

- Record all pertinent stains on items and include diagrams or photographs. In addition to body fluids, other important stains could include food stains, oil or grease stains, green stains from vegetation and white chalky stains.
- Where the stain is difficult to see or may be removed during examination, mark its position with felt pen or chinagraph. Do not mark the positions of irrelevant stains on the item, although they should be indicated on the diagram.
- Number stains selected for further examination.

- If the item is difficult to write on, use the clothing tag gun to attach a label next to the stain.
- Describe stains as accurately as possible with relation to;
  - (i) type (smear, spot *etc.*)
  - (ii) intensity
  - (iii) distribution
  - (iv) surface
- Be careful when handling items where stains are crusty and likely to flake off, ie. knives, shoes and other non-absorbent surfaces.
- Subsamples submitted for DNA testing must be placed in a sterile 5mL sample tube and labelled with the sample id. The DNA number must be recorded on the side label and the lid when the sample is registered in DNAmaster, (Refer to 17161, Use of DNAmaster spreadsheet). If the sample is very small, it can be placed directly in a sterile 1.5mL tube inside the 5mL sample tube.
- The size of the sample that is submitted for DNA testing is dependent on the intensity and type of the stain. The subsample placed in the 5mL sample tube should be an appropriate size for extraction.

13 EXAMINATION OF FINGERNAILS

Note: Fingernails or fingernail scrapings are examined to find blood, tissue, fibres or other contact traces on or under the nails. Clippings, loose scrapings, scrapings on swab sticks or complete nails may be submitted.

- Fingernails or fingernail scrapings can be examined under the low power microscope for fibres and flakes of blood etc if required.
- The usual practice is to submit these items directly for DNA testing. Examination or screening for blood or cells can lose valuable information.

14 EXAMINATION OF WEAPONS

- Handle any weapons with extreme care.

Note: Refer to 17135 (Handling and Analysis of Syringes and Needles) when examining these items.

Note: Care should be taken when removing metal objects from the freezer. Condensation as objects heat up, may cause surface material to be redistributed or lost.

- Record description and condition of items. Include measurements of the length and width of the blade, which may then be compared with any, cuts on clothing items.
- Examine for hair, blood and other biological material.
- If bloodstained, look carefully for directional splashing; photograph as necessary.
- Mark the position of bloodstains, particularly where the stain is likely to be swabbed off completely during testing.

15 EXAMINATION OF SUSPECTED BLOODSTAINS

- Record the position of all suspected bloodstains using diagrams or photographs.

Note: Dark items of clothing etc. can be examined using a fibre optic lamp, strong light source, or stereo microscope to scan over the whole surface.

- Test all suspect stains using tetramethylbenzidine (TMB). Refer to SSFB-M-0022 (Tetramethylbenzidine Screening Test for Blood).
- Record descriptions of the stains including type, intensity, distribution, size.

Note: In the description of the stain, note which side of the item the stain was deposited and whether or not it has soaked through the item to the other side.

- If bloodstained garments are wet, dry them immediately.
- If no stains are visible and the item is dark coloured, screen the entire item for the presence of blood using TMB. If any area gives a positive result, sub-divide it and re-test to locate the stain.
- The Takayama Confirmatory Test for blood (SSFB-M-0023) is used to confirm the presence of blood. This method is not used routinely. Suspected bloodstains, which give a positive result using TMB, are routinely submitted for DNA analysis.
- Suspected bloodstains may be required to be identified as human in origin. This is usually done by extracting the stain and quantitating. The Quantitation protocol uses a probe, which is complementary to a primate specific DNA sequence. Stains that give a positive result using the presumptive test (TMB) but do not give a quantitation result (where there is sufficient sample) are reported as being non-human. Alternatively, the Human/Primate Species Identification protocol can be used. Refer to SSFB-M-0016. This method is rarely used.
- A swab moistened with 70% EtOH can be used to sample very small or weak suspected bloodstains from items. This is instead of using a swab moistened with distilled water.

16 EXAMINATION FOR EPITHELIAL CELLS OR SALIVA

- Suspected epithelial cells on items can be collected for DNA analysis by scraping the area with a scalpel blade or by using small pieces of sticky tape and extracting these tapelifts. A swab moistened with 70% EtOH may also be used.
- Scrapings of an area can also be used to mount on a slide to examine for the presence of cells. Slides are dried, heat fixed and stained with H & E.
- Saliva stains are detected and characterized using the Phadebas Test (SSFB-M-0025).
- Saliva can also be identified using an alternative method where the supernatant from the first step of the extraction process is tested using Phadebas. Refer to SSFB-W-0046. This method is used when stains must be identified but testing using Phadebas will reduce the chances of obtaining a DNA profile.
- Cigarette butts, stamps and swabs from drink containers are submitted for DNA analysis without testing for saliva.

17 EXAMINATION FOR SEMEN

- *Fluid semen* is occasionally received in the laboratory either in the form of a vaginal aspirate or contained in a condom. Depending on the amount present, the semen is either swabbed or pipetted out with distilled water and examined in the same manner as vaginal swabs.
- Freshly ejaculated semen is in a gel-like state and liquifies within about 20 minutes. It is often easily detectable as visible staining on certain fabrics. It may form a hard crusty stain and may also be clearly visible when viewed by UV light. The location and identification of seminal stains relies on visual, tactile, chemical, biochemical and microscopical examination.
- *Examination of items for seminal stains:*
- Polilight examination can be used to detect dried seminal stains. Refer to 17187 for further information.
- Acid phosphatase (AP) screening is routinely used to detect seminal stains. Refer to 17186 for further information.
- All stains identified through acid phosphatase screening or polilight examination must be examined microscopically to look for spermatozoa prior to DNA analysis. Refer to 17189 for further information.
- When no spermatozoa are detected on AP positive areas; the Prostate Specific Antigen (PSA) test should be used to confirm the presence of seminal fluid. Refer to 17185.

Note: Where low numbers of spermatozoa are present, it is possible that AP testing could consume some spermatozoa.

18 EXAMINATION OF SEXUAL ASSAULT SWABS

- If serum coated or charcoal swabs or other unsuitable swabs/media are submitted, the client must be notified.
- Stain and read the corresponding smears first. Refer to SSFB-M-0021 (Examination of Spermatozoa).
- If no spermatozoa are found, perform AP tests on the swabs.

Note: If no spermatozoa are found and the smear is light, it may be necessary to make a new smear from the swab and examine this. This should be done if subsequent AP screening of the swab gives a positive result.

- For smear negative, AP positive swab scenarios, it may be necessary to perform a PSA test (17185).

19 URINE

- *Fluorescence*: Urine stains on fabric are colourless or pale yellow in visible light. The colour is due to the presence of urinary pigments, particularly urochrome. Under ultra violet light these pigments fluoresce.

- *Smell*: The characteristic smell of urine can be easily discerned if the stain extract is concentrated over a water bath.
- Refer to SSFB-M-0011 (Method for Suspected Urine Stains) to confirm the presence of urine.

20 FAECES

- Refer to SSFB-M-0024 (Identification of Mammalian Faecal Stains) for the protocol for the identification of suspected faecal stains.

21 VOMIT

- Refer to SSFB-M-0020 (Identification of Vomit) for the protocol for the identification of suspected vomit stains.

22 REFERENCE SAMPLES

- Case notes should include details of the reference samples, e.g. labelling on outer packaging and the sample itself and whether the sample was sealed when received.
- The envelopes and property tags of reference samples received are filed by F number in the exhibit room. The case analyst should collect these, record the details in the case notes and store them with other items from the case.
- Reference swabs - submitted directly for DNA testing.
- Reference hair samples are examined for suitable sheath material and submitted for DNA testing.
- Reference blood samples – a 3mm-hole punch is used to sample the blood card. This punch must be sterilised with alcohol and flamed before use.
- Buccal cells on FTA cards are not sampled by the case analyst, the sample request is entered in DNAmaster and the technician performing the test is responsible for sampling the card. Refer to SSFB-M-0030, Method for FTA Paper Purification and Amplification.

23 AMENDMENT HISTORY

0	17 Feb 1999	
1	26 Jun 2001	V Ientile

PB171

Batching Validation Samples Cells

Sensitivity Experiment

√20 Rayon Samples — Spotted
1 " Blank — Labelled
1 Positive Control
1 Negative Control — BD 2/7/07
VALC20070502_02
Extracted: BD/CI 11/7/07

√ 20 Cotton Samples — Spotted.
1 " Blank — Labelled
1 Positive Control
1 Negative Control — CI 3/7/07
VALC20070502_03
Extracted: CI/BG 12/7/07.

Inhibition Experiment

8 Indigo Samples — labels printed
1 " Blank
8 Urea Samples — labelled GSC
1 " Blank
1 Positive Control
1 Negative Control
VALC20070502_04
Extracted:

8 Humic Samples — labels printed
1 " Blank
8 Tannic Samples — labelled CI
1 " Blank
1 Positive Control
1 Negative Control
VALC20070502_05
Extracted:

8 Motor Oil Samples — labels printed
1 " " Blank
1 Positive Control — batched ready
1 Negative Control — labelled. GSC.
VALC20070502_06
Extracted:

Substrate Experiment

4 Denim Samples
1 " Blank
4 Cotton Shirt Samples
1 " " Blank
4 Wool Samples
1 " Blank
4 Lycra Samples
1 " Blank
1 Positive Control
1 Negative Control
VALC20070502_07
Extracted:

(Labelled BD GSC 20/7/07)

4 Nylon Samples
1 " Blank
4 Polyester Samples
1 " Blank
4 Gum 1 Samples
1 " Blank
4 Gum 2 Samples
1 " Blank
1 Positive Control
1 Negative Control
VALC20070502_08
Extracted:

(BD Labelled)

√4 Rayon Swabs
1 " Blank
4 Cotton Swabs — Labelled
1 " Blank — CI 5/7/07
4 FTA Donor 1 Samples
4 FTA Donor 2 Samples
1 FTA Blank
1 Positive Control — completed. CI
1 Negative Control
VALC20070502_09
Extracted: GSC/CI 13/7/07

4 Tape Donor 1 Samples
4 Tape Donor 2 Samples
1 Tape Blank
4 Cig Butt 1 Samples
1 " " Blank
4 Cig Butt 2 Samples
1 " " Blank
1 Positive Control
1 Negative Control
VALC20070502_10
Extracted:

(Labelled BD)

4 Leather Samples — labels printed.
1 " Blank
1 Positive Control — Labelled
1 Negative Control
VALC20070502_11
Extracted:

Mixture Experiment

4 1:1 F:M Samples labels printed.
4 1:2 F:M Samples
4 1:10 F:M Samples
1 " Blank
1 Positive Control
1 Negative Control
labelled.
VALC2007 0626-01
Extracted: GSL

✓ 4 1:25 F:M Samples
4 1:50 F:M Samples
4 1:100 F:M Samples
1 " Blank
1 Positive Control
1 Negative Control
Labelled CA 2/7/07
VALC20070626-02
Extracted: BG

Size Experiment

12 Cotton Shirt Samples labels printed.
1 " " Blank
1 Positive Control
1 Negative Control
Labelled
VALC20070502_12
Extracted: CA 17/07/07.

VALC20070724-01
GSL 25.07.07

23
23
20
20
11
22
22
21
21
7
15
15
15
235

235
x 7
1645

Need to spot:

4 Denim
16 Cottonshirt
4 Wool
4 lycra
4 Nylon
4 Polyester
4 leather
4 Rayon

4 b
→ Cotton Swab

24 cotton under different lab No

8 FTA
→ two donors.

235
− 29
206
x 7
1456

1645
+ 1456
3101

PB172

CaSS | Forensic and Scientific Services

A CLINICAL AND STATEWIDE SERVICE

Project 11. Report on the Validation of a manual method for Extracting DNA using the DNA IQ™ System

August 2008

Automation and LIMS Implementation Project Team,

DNA Analysis

Forensic And Scientific Services

Clinical and Scientific Services

Queensland Health

safe | sustainable | appropriate

Queensland Government
Queensland Health

CaSS | Forensic and Scientific Services
A CLINICAL AND STATEWIDE SERVICE

Project 11. Report on the Validation of a Manual Method for Extracting DNA using the DNA IQ™ System

Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C., Ientile, V.
Automation/LIMS Implementation Project, DNA Analysis FSS (August 2008)

1. Abstract

The DNA IQ™ system was found to be the most suitable kit for extracting cell and blood samples that are analysed in DNA Analysis FSS (refer to Project 9). This DNA extraction system, based on magnetic bead technology, was found to generate results that were comparable or better than the current Chelex®-100 protocol. We have validated a manual DNA IQ™ method for extracting DNA from forensic samples, and incorporated studies on sensitivity and consistency, inhibition, substrate type, substrate size, and mixture studies. This manual DNA IQ™ method is suitable for verification on the automated MultiPROBE® II PLUS HT EX extraction platforms.

2. Introduction

A previous evaluation of various DNA extraction systems that were designed specifically for forensic samples was performed in order to select a suitable extraction technology for extracting various sample types that are processed in DNA Analysis FSS. DNA IQ™ was identified as a suitable kit for extracting forensic samples, and was found to outperform both the current Chelex®-100 protocol and also all the other kits evaluated. The results of the evaluation are reported in Project 9 (Gallagher *et al.*, 2007a).

DNA purification with silica matrices, either in membrane- or bead-form, commonly uses the affinity of DNA for silica without the need for hazardous organic reagents. However, these systems tend to require extensive washing to remove the guanidium-based lysis buffer. The DNA IQ™ system uses a novel paramagnetic resin for DNA isolation (Promega Corp., 2006). The DNA IQ™ System's basic chemistry is similar to other silica-based DNA isolation technologies, except that the specific nature of the paramagnetic resin, coupled with the formulation of the lysis buffer, is unique. In the DNA IQ™ System, negatively-charged DNA molecules have a high affinity for the positively-charged paramagnetic resin under high salt conditions supplied by the lysis buffer. Once DNA is bound to the magnetic resin, and the resin is immobilised by a magnet, the sample can be washed using an alcohol/aqueous buffer mixture. The high alcohol content of the wash buffer aids to maintain the DNA-resin complex in low-salt conditions, while the aqueous component functions to wash away residual lysis buffer and any inhibitors or non-DNA contaminants such as cellular debris and protein residues. DNA is released from the resin by using a low ionic strength elution buffer, and the purified DNA can be used directly in downstream applications such as PCR.

For samples that are in excess (e.g. reference samples), DNA IQ™ resin will only isolate up to a total of approximately 100ng of DNA due to bead saturation (Huston, 2002).

3. Aim

To validate a manual method for DNA extraction of blood and cell stains on forensic samples using the DNA IQ™ system (Promega Corp., Madison, WI, USA).

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4. Equipment and Materials

- DNA IQ™ System (Promega Corp., Madison, WI, USA); 100 samples, Cat.# DC6701), which includes:
    - 0.9mL Resin
    - 40mL Lysis Buffer
    - 30mL 2X Wash Buffer
    - 15mL Elution Buffer
- TNE buffer (10mM Tris, 100mM NaCl, 1mM EDTA, pH 8.0)
- MagneSphere® Magnetic Separation Stand, 12-position (Cat.# Z5342) (Promega Corp., Madison, WI, USA)
- DNA IQ™ Spin Baskets (Cat.# V1221) (Promega Corp., Madison, WI, USA)
- Microtube 1.5mL (Cat.# V1231) (Promega Corp., Madison, WI, USA)
- 95-100% ethanol
- Isopropyl alcohol
- 1M DTT (Sigma-Aldrich, St. Louis, MO, USA)
- Proteinase K (20mg/mL) (Sigma-Aldrich, St. Louis, MO, USA)
- 20% SDS (Biorad, Hercules, CA, USA)
- 0.9% saline solution (Baxter Healthcare, Old Toongabbie, NSW, Australia)
- ThermoMixer Comfort (Eppendorf, Hamburg, Germany)
- Vortex mixer
- Bench top centrifuge
- Cytobrush® Plus Cell Collector (Cooper Surgical, Inc., Trumbull, CT, USA)
- FTA® Classic Cards (Whatman plc, Maidstone, Kent, UK)
- Rayon (155C) and cotton (164C) plain dry swabs (Copan Italia S.p.A., Brescia, Italy)
- Vacuette® K2EDTA blood collection tubes (Greiner Bio-One GmbH, Frickenhausen, Germany)
- Sticky tape (BDF tesa tape Australia Pty Ltd)
- Tannic acid $C_{76}H_{52}O_{46}$ FW1701.25 (Selby's BDH, Lab Reagent >~90%)
- Urea NH_2CONH_2 FW60.06 (BDH, Molecular Biology Grade ~99.5%)
- Indigo carmine $C_{16}H_8N_2Na_2O_8S_2$ FW466.35 PN 131164-100G (Sigma-Aldrich, St. Louis, MO, USA)
- Humic acid sodium salt PN H167520-100G (Sigma-Aldrich, St. Louis, MO, USA)
- Used car motor oil, SW20/SAE50 (Caltex)
- Various clothing materials, including:
    - Best & Less Pacific Cliff, White cotton shirt, XXL
    - Big W Classic Denim, Men's Blue denim jeans, 112
    - Private Encounters, off-white nylon cami, size 14
    - Clan Laird, blue 100% wool kilt
    - Millers Essentials, blue 100% polyester camisole, size 10
    - Unknown, teal green 100% lycra swimwear
    - Leather Belt, brown

5. Methods

5.1 Cell and blood collection

Buccal cells were collected using a modified Cytobrush® protocol (Mulot *et al.*, 2005; Satia-Abouta *et al.*, 2002). Four donors were chosen. Each donor was asked to brush the inside of one cheek for one minute. Then, with another Cytobrush®, the other cheek was also sampled. The cells collected on the brush where then resuspended in 2mL of 0.9% saline solution. Multiple collections were taken on different days.

Whole blood was collected from three donors by a phlebotomist as per standard collection procedures in EDTA tubes. Blood samples were refrigerated until spotting onto substrate and cell-counting step.

Table 1 lists the donor sample ID's.

Table 1. List of donor samples used for validating a manual DNA IQ™ method.

Donor ID
Cell samples
D1
D2
D3
D4
Blood samples
D1
D2
D3

5.2 Cell counting

Buccal cell suspensions were diluted using 0.9% saline solution to create a 1/10 dilution of the original sample prior to submitting for cell counting. All counts were performed by the Cytology Department, RBWH (QIS 15393).

Blood cell counting was performed on a 1mL aliquot of the original sample also by the Cytology Department, RBWH (QIS 15393).

5.3 Sensitivity, Reproducibility (Linearity) and Yield

Sensitivity and reproducibility of the DNA IQ™ kit was assessed using dilutions of cell and blood samples.

For cell samples, dilutions were made using a sample from donor 4, diluted in 0.9% saline solution. The dilutions used were:

- Neat
- $^1/_{10}$
- $^1/_{100}$
- $^1/_{1000}$

For blood samples, dilutions were made using a sample from donor 2, diluted in 0.9% saline solution. The dilutions used were:

- Neat
- $^1/_{10}$
- $^1/_{100}$
- $^1/_{1000}$

Mock samples were created from rayon and cotton swabs using the above dilutions. The swab heads were removed from the shaft using sterilised scalpel and tweezers. Swab heads were then cut into quarters and each quarter was then added to separate sterile 1.5mL tubes. To each quarter swab, 30µL of each neat sample or dilution was added to

create a total of five replicates. Samples were dried using a ThermoMixer set at 56°C over 2 hours in a Class II biohazard cabinet.

5.4 Inhibition challenge

Quartered cotton swabs in sterile 1.5mL tubes were spotted with 30µL of neat cell suspension and were dried after each addition on a ThermoMixer as described previously. Neat blood samples were also created using the same method.

All the inhibitors except for the motor oil were obtained in powder form. Before making any liquid solution of the powdered inhibitors, research was conducted to determine the likely level of each inhibitor normally encountered in the environment (Hlinka *et al.*, 2007). Each solution was made at concentrations based on the information obtained (Table 2).

Table 2. Concentrations of various inhibitors used in the inhibition study.

Inhibitor	Excess/Neat Solution	Mass	Volume H_2O	Final inhibitor concentration
Tannic acid	Excess	600mg	500µL	0.705M
	Neat	200mg	500µL	0.235M
Humic acid	Excess	1g	5mL	20% (w/v)
	Neat	0.1g	5mL	2% (w/v)
Indigo carmine	Excess	0.47g	10mL	100mM
	Neat	0.047g	10mL	10mM
Urea	Excess	0.06g	1mL	1M
	Neat	0.021g	1mL	0.33M

A total of 30µL of each solution containing specified concentrations of various inhibitors was applied to the buccal cell and blood swabs prepared above. The only exception was motor oil, where only 15µL was added to the cell and blood swabs respectively. Each inhibitor sample was replicated in quadruplicate and left to dry overnight in a Class II biohazard cabinet.

To another set of prepared cell and blood swabs, an excess of each inhibitor was applied in quadruplicate for each inhibitor and allowed to dry overnight. This process was achieved by applying another solution of inhibitor exceeding the normal level (Hlinka *et al.*, 2007).

5.5 Substrates

Swabs

Four cotton and four rayon swab quarters in sterile 1.5mL tubes were loaded with 30µL of neat cell or blood sample and were extracted once the sample had dried on the swab.

Tapelifts

Two donors were sampled using the tape most commonly used within the laboratory (BDF tesa tape). Strips of tape were firmly applied to the inside of the fore arm and lifted off. This process was then repeated until the tape was no longer adhesive. The tape was wrapped around sticky-side-in, forming a cylinder shape, and placed in a sterile 1.5mL tube. These samples were created in quadruplicate. Tape was not used as a substrate in the blood validation.

Fabric

The material types tested included:

- Denim jeans;

- White 100% cotton shirt;
- Blue 100% wool kilt;
- Teal green 100% lycra swimwear;
- White 100% nylon camisole;
- Blue 100% polyester camisole; and
- Brown 100% woven leather belt.

All material types except leather were sampled and ten 2.5cm x 2.5cm pieces were cut from each material and washed in 10% bleach following an in-house washing method to remove any contaminating DNA from outside the laboratory (Gallagher *et al.*, 2007b). As for the leather, one strand of the leather weave was cut from the belt and washed following the same method. Once dry, the material was then cut into 0.5cm x 0.5cm pieces using sterile techniques, placed in 1.5mL tubes and 30µL of both cell sample and blood was applied to separate pieces. Each substrate sample was created in quadruplicate and dried on a ThermoMixer set at 56°C over 2 hours in a Class II biohazard cabinet.

Gum
Two types of chewing gum were chosen: (1) Wriggley's Extra White (peppermint flavour) and (2) Wriggley's Extra Green (spearmint flavour). The donor was asked to chew the gum for 30 minutes and dispose of the gum into a clip-seal plastic bag. The gum was then air dried in a Falcon tube overnight before it was frozen for roughly an hour before cutting into 3mm x 3mm x 3mm pieces and placed into sterile 1.5mL tubes. Gum substrates were not assessed for blood samples.

Cigarette butts
Two brands of cigarettes were smoked all the way through and then the butts collected. The filter paper of the butt was cut into $0.5mm^2$ pieces and placed into sterile 1.5mL tubes. Cigarette butts were not assessed for blood samples.

FTA® Classic Card punches
Eight sterile 1.5mL tubes, each containing four 3.2mm FTA® Classic Card punches, were spotted with 30µL of cells or blood before being dried on a ThermoMixer. Four replicates contained sample from one donor, the other remaining four replicate tubes had a different donor sample added.

5.6 Mixture studies

Buccal cells and whole blood were obtained from a male and female donor. Dilutions were made using 0.9% saline solution to ensure that the cell concentration was equal. Dilutions were then performed on the male sample to obtain the correct ratios.

Mock samples were created using the following ratios of female to male:

- 1:1,
- 1:2,
- 1:10,
- 1:25,
- 1:50 and
- 1:100.

A total of 30µL of the female component was spotted first on to a quarter of a cotton swab in a sterile 1.5mL tube and dried on a ThermoMixer before adding another 30µL of the male component. Samples were created in quadruplicate for all ratios, for both cell and blood samples.

5.7 Substrate size

Various sizes of material were cut from a white cotton shirt:

- 0.5cm x 0.5cm,
- 1cm x 1cm,
- 2cm x 2cm.

Each piece of material was stored in individual, sterile 1.5mL tubes and 30µL of cell sample was added to the material and allowed to dry on a ThermoMixer. The same process was followed for blood samples. Five replicates were made for each sample type.

5.8 Extraction using the DNA IQ™ System (Promega Corp.)

The manual DNA IQ™ method used was based on an automated protocol developed by the Centre of Forensic Sciences (CFS) in Toronto, Ontario (PerkinElmer, 2004). A Proteinase K – SDS Extraction Buffer was made as per the recommended protocol. The 1x Extraction Buffer for one sample consisted of:

277.5µL TNE buffer
15µL Proteinase K (20mg/mL)
7.5µL 20% SDS

The TNE buffer consisted of:

1.211g Tris (10mM Tris)
2mL 0.5M EDTA (1mM EDTA)
5.844g NaCl (100mM NaCl)

The adapted manual DNA IQ™ protocol is described below:

1. Set one ThermoMixer at 37°C and another at 65°C.

2. Ensure that appropriately sized samples are contained in a sterile 1.5mL tube. For every sample, prepare three set of labelled tubes: spin baskets (for every tube except the extraction control), 2mL SSI tubes and Nunc™ tubes.

3. Prepare Extraction Buffer and add 300µL to each tube. Close the lid and vortex before incubating the tubes at 37°C on the ThermoMixer at 1000rpm for 45 minutes.

4. Remove the tubes from the ThermoMixer and transfer the substrate to a DNA IQ™ Spin Basket seated in a labelled 1.5mL Microtube using autoclaved twirling sticks. Then transfer the liquid to a labelled 2mL SSI sterile screw cap tube.

5. Centrifuge the spin basket on a benchtop centrifuge at room temperature for 2 minutes at its maximum speed. Once completed, remove the spin basket and collect the remaining solution and pool with the original extract in the 2mL SSI sterile screw cap tube, then vortex.

6. Add 550 µL of Lysis Buffer to each tube.

7. Dispense 50µL of DNA IQ™ Resin – Lysis Buffer solution (7µL Resin in 43µL Lysis Buffer) to each tube. Invert the resin tube regularly to keep the beads suspended while dispensing to obtain uniform results.

8. Vortex each tube for 3 seconds at high speed then place in a multitube shaker set at 1200rpm to incubate at room temperature for 5 minutes.

9. Vortex each tube for 2 seconds at high speed before placing the tubes in the magnetic stand. Separation will occur instantly.

   Note: If resin does not form a distinct pellet on the side of the tube, or if the pellet has accidentally mixed with the solution while in the stand, vortex the tube and quickly place back in the stand.

10. Carefully remove and discard all of the solution without disturbing the resin pellet on the side of the tube. If some resin is drawn up in tip, gently expel resin back into tube to allow re-separation.

11. Remove the tube from the magnetic stand; add 125µL of prepared Lysis Buffer and vortex for 2 seconds at high speed.

12. Return tube to the magnetic stand, allow for separation and then remove and discard the Lysis Buffer.

13. Remove tube from the magnetic stand; add 100µL of prepared 1X Wash Buffer and vortex for 2 seconds at high speed.

14. Return tube to the magnetic stand, allow for separation and then remove and discard all Wash Buffer.

15. Repeat Steps 13 to 14 two more times for a total of three washes. Be sure that all of the solution has been removed after the last wash.

16. In a biohazard cabinet, place the lids of the tubes upside down on a Kimwipe, in their respective order, and the tubes into a plastic rack, and air-dry the resin for 5-15 minutes at room temperature. Do not dry for more than 20 minutes, as this may inhibit removal of DNA. Once dry, screw on the lids.

17. To each samples then add 50µL of Elution Buffer very gently on the top of the magnetic pellet. Do not mix.

18. Close the lid and then incubate the tubes in the ThermoMixer at 65°C for 3 minutes with no shaking and another 3 minutes shaking at 1100 rpm.

19. Remove the tubes and vortex for 2 seconds at high speed. Immediately place the tube in the magnetic stand. Tubes must remain hot until placed in the magnetic stand or yield will decrease.

20. Carefully transfer the supernatant containing the DNA to the respective labelled Nunc™ tubes.

21. Repeat step 17 to 20, transferring the supernatant to the appropriate Nunc™ tube. The final volume after the second elution should be approximately 95µL.

    Note: DNA can be stored at 4°C for short-term storage or at -20 or -70°C for long-term storage.

5.9 DNA quantitation

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

5.10 PCR amplification

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

5.11 Capillary electrophoresis and fragment analysis

PCR product was prepared for capillary electrophoresis using the manual 9+1 protocol (refer to Project 15 and QIS 19978). Capillary electrophoresis was performed on an ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) under the following conditions: 3kV injection voltage, 10 sec injection time, 15kV run voltage, 100µA run current, and 45min run time. Data Collection Software version 1.1 was used to collect raw data from the ABI Prism® 3100 Genetic Analyzer. Fragment size analysis was performed using GeneScan 3.7.1. Allele designation was performed using Genotyper 3.7, with thresholds for heterozygous and homozygous peaks at 150 and 300 RFU respectively. The allelic imbalance threshold is 70%.

6. Results and Discussion

6.1 Donor sample cell counts

Aliquots of buccal cell samples were counted at Cytology Department (RBWH) to determine the concentration of viable cells, in order to better estimate the number of cells at any particular dilution. A white cell count was not performed on all the blood samples, and therefore an estimate on the number of nucleated cells could not be determined.

6.2 Sensitivity, consistency and yield

To ensure the reliability and integrity of results for samples containing small amounts of DNA, a sensitivity study was conducted to determine the lowest concentration of DNA that provides reliable results. A consistency study was combined into the sensitivity experiment to determine the maximum acceptable difference between the results obtained. All samples were extracted in identical conditions by the same operator at the same time to minimise variability.

The cell sample used for the experiments was from donor sample 4A, which was counted to be around 3,680 nucleated cells (x 10^6/L). The blood sample used was from donor 6A, which was counted to be around 2,540 nucleated cells (x 10^6/L). The estimated amount of DNA present in each dilution is outlined in Table 3.

Table 3. Amount of DNA in each dilution, as calculated from the cell count.

Sample type	Dilution factor	Number of cells (/µL)	gDNA (ng/µL)	Theoretical total DNA on swab (ng)
Cells	Neat	3680	23.552	706.56000
	1/10	368	2.3552	70.65600
	1/100	36.8	0.23552	7.06560
	1/1000	3.68	0.023552	0.07656
Blood	Neat	2540	16.256	487.68000
	1/10	254	1.6256	48.76800
	1/100	25.4	0.16256	4.87680
	1/1000	2.54	0.016256	0.48768

The DNA yields resulted from extracting the above cell dilutions using the DNA IQ™ System is outlined in Table 4. Blood samples produced higher yields compared to cell samples. On average, blood samples on cotton swabs generated the highest yields. Cell samples on rayon and cotton swabs generated similar yields. All blood dilutions down to 1/1000 produced quantitation results, but cell samples only produced reliable quantitation results down to 1/100 dilution, possibly due to the effects of cell clumping.

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Table 4. DNA quantitation data for diluted cell and blood samples on rayon and cotton substrates.

Sample type	Dilution factor	Theoretical Input DNA (ng)	Rayon swab yield (ng)	Alleles	Cotton swab yield (ng)	Alleles	Rayon average yield (ng)	Rayon Std Dev	Recovery Rayon (%)	Cotton average yield (ng)	Cotton Std Dev	Recovery Cotton (%)
Cells	Neat	706.56000	110.0000	18	117.0000	18	134.5400	41.30	19.04	95.2800	32.69	13.48
			130.0000	18	124.0000	18						
			160.0000	18	46.8000	18						
			83.7000	7	76.6000	18						
			189.0000	17	112.0000	18						
	1/10	70.65600	10.1000	18	12.8000	18	10.4520	1.44	14.79	10.4820	2.52	14.84
			12.7000	18	6.3100	18						
			9.5500	18	11.5000	18						
			9.0100	18	10.1000	18						
			10.9000	18	11.7000	18						
	1/100	7.06560	0.6350	0	0.0000	0	0.9254	0.64	13.10	0.1270	0.18	1.80
			0.4930	0	0.0000	0						
			1.4000	5	0.2770	0						
			1.7900	14	0.3580	0						
			0.3090	0	0.0000	0						
	1/1000	0.7656	0.0000	0	0.3630	0	0.0166	0.04	2.17	0.0726	0.16	9.48
			0.0000	0	0.0000	0						
			0.0000	0	0.0000	0						
			0.0831	0	0.0000	0						
			0.0000	0	0.0000	0						
Blood	Neat	487.68000	216.0000	18	718.0000	18	317.0000	102.36	65.00	447.0000	196.46	91.66
			447.0000	18	297.0000	18						
			215.0000	18	595.0000	18						
			383.0000	7	326.0000	18						
			324.0000	18	299.0000	18						
	1/10	48.76800	113.0000	18	126.0000	18	124.7800	28.10	255.86	97.6600	21.66	200.25
			107.0000	18	91.9000	18						
			145.0000	18	75.4000	18						
			95.9000	18	81.0000	18						
			163.0000	18	114.0000	18						
	1/100	4.87680	14.3000	18	15.9000	18	12.4800	1.62	255.91	16.7600	4.69	343.67
			12.5000	13	12.1000	18						
			13.2000	18	20.8000	18						
			9.9000	18	22.4000	18						
			12.5000	18	12.6000	18						
	1/1000	0.48768	0.7300	18	2.3700	18	0.8894	0.20	182.37	3.0200	0.85	619.26
			0.6990	18	3.1300	18						
			1.1800	18	3.6300	18						
			0.8670	18	1.9700	18						
			0.9710	18	4.0000	18						

The average yield observed within cell and blood samples on either rayon or cotton swabs were comparable (Figure 1). Some inconsistencies were present in cell samples at the lower dilutions of 1/100 and 1/1000 due to unreliable quantitation data at these low dilutions. Blood samples were found to generate higher average yields than cell samples and gave unexpectedly higher recovery values, despite the fact that the input DNA amount was 2-fold higher for cells compared to blood samples (Table 4). This discrepancy may have arisen from inconsistencies in cell suspension uniformity during dilutions of the original cell or blood sample, resulting in inaccurate estimates for average cell concentrations.

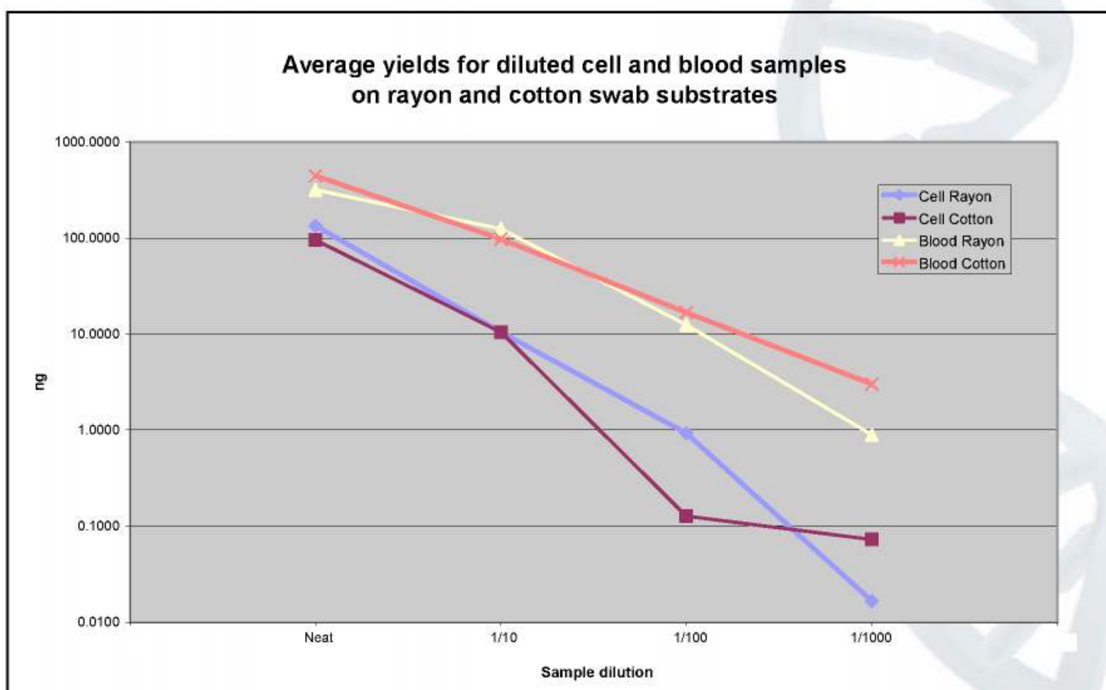

Figure 1. Average yields as observed in the sensitivity study. The yields for cell and blood samples, on two different swab types, were comparable as indicated by overlapping lines on the graph.

The dilution factor was, however, accurately reflected in the average yield for the various dilutions as displayed in Table 4 and Figure 2. An exception to this was the average yields for the neat dilutions (Figure 2). DNA IQ™ isolates a maximum of 100ng DNA as the resin is present in excess, and the system becomes more efficient with samples containing less than 50ng of DNA. Because the amount of DNA was in excess in neat samples, the observed yields varied from sample-to-sample. According to the manufacturer, the DNA IQ™ Database Protocol should be used for samples containing more than 100ng DNA to result in more consistent concentrations between the samples (Huston, 2002).

All five replicates for each neat dilution displayed the highest yields for each dilution series, as expected (Figure 2). For blood samples on rayon and cotton swabs, yields were still around 1ng for samples at the 1/1000 dilution (Figure 3).

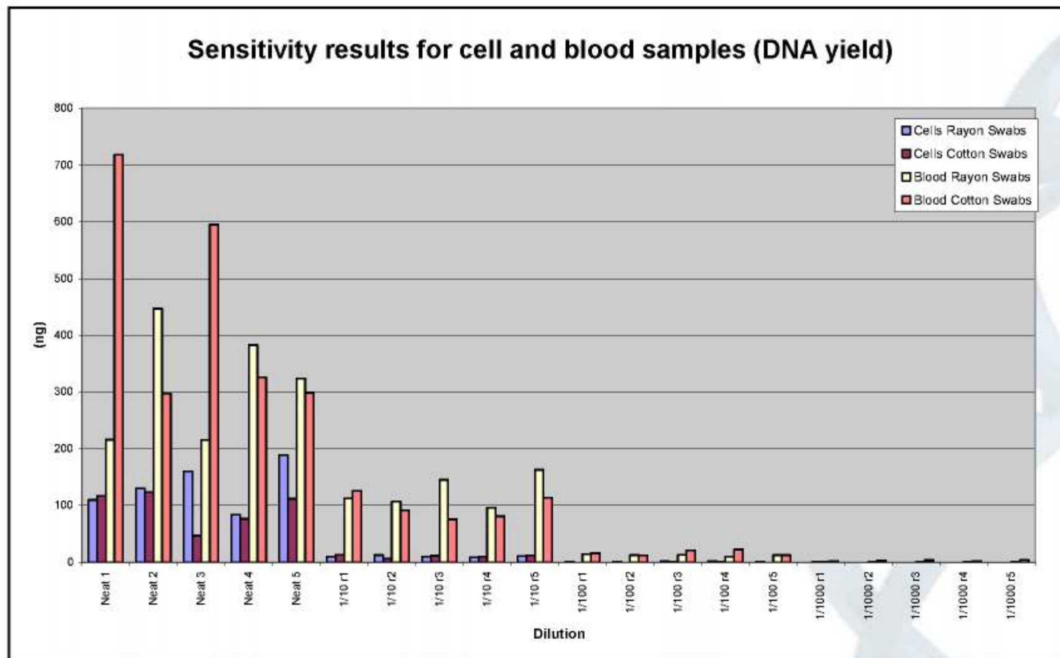

Figure 2. DNA yields (ng) observed for the sensitivity study. As expected, neat samples provided the highest yields. Yields were obtained down to 1/1000 for blood samples and 1/100 for cell samples.

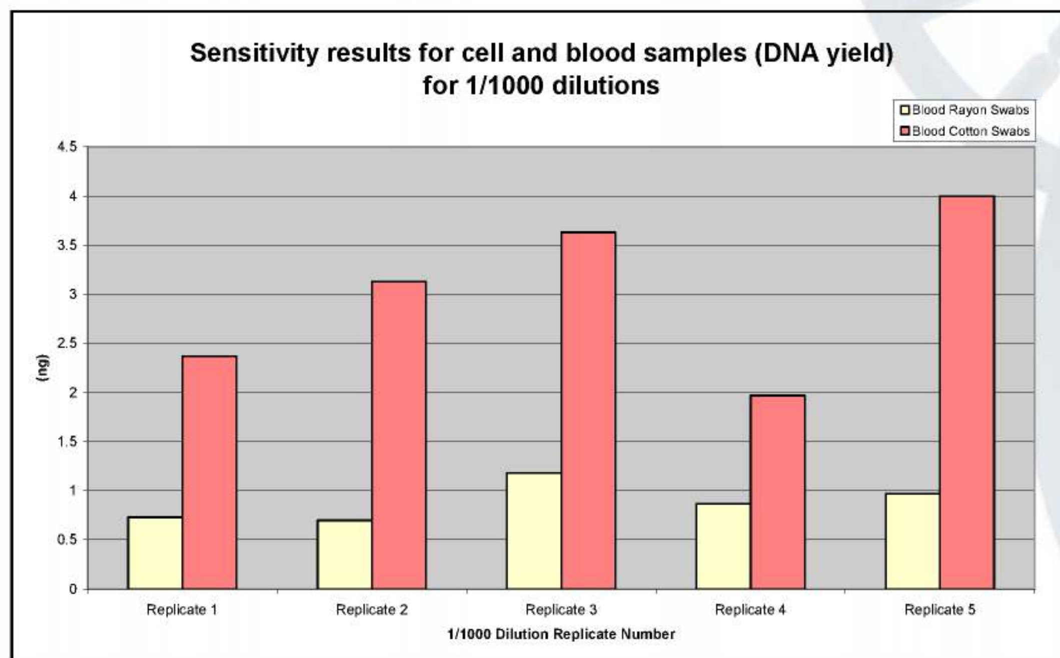

Figure 3. DNA yields (ng) observed for the sensitivity study, at the 1/1000 dilution.

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When amplified using a 9-locus STR system, all neat samples produced the expected full DNA profile (18/18 alleles), although one outlier was encountered for a cell rayon sample which produced a 7/18 partial profile (Table 4). For cell samples, full profiles could be obtained for samples that were diluted down to 1/10, with partial profiles generated from samples diluted to 1/100. For blood samples, full profiles were generally obtained from all dilutions down to 1/1000. Although two partial profiles were encountered in blood samples on rayon swabs, all blood cotton swabs produced full profiles at all dilutions.

The apparent discrepancy between the results for cell and blood samples can be attributed to inaccurate cell counts or non-uniform sample suspensions when creating the dilutions, as caused by cell clumping or cellular breakdown and precipitation.

For five replicates of each dilution, consistency was observed to vary depending on the dilution (Figure 4). Consistency, as an indication of reproducibility, was calculated as the percentage of the yield standard deviation over five replicates divided by the mean yield of all five replicates (%[SD_{yield} / $mean_{yield}$]). A value closer to 0% indicates minimal sample-to-sample variation and therefore the results are highly consistent. The mean combined reproducibility for all neat, 1/10, 1/100 and 1/1000 dilutions were 35.31%, 20.63%, 62.14% and 124.32% respectively (Figure 4), indicating that there was high reproducibility between the neat and 1/10 dilutions across the four sample types, and reduced reproducibility at the lower 1/100 and 1/1000 dilutions. Overall, the blood samples on rayon and cotton both exhibited high reproducibility across all dilutions at an average of 30.54% and 22.45% respectively (Figure 5). The cell rayon and cotton samples were more variable across all dilutions, producing lower reproducibility at an average of 84.23% and 105.19% respectively (Figure 5). The poor performance of the cell samples can be attributed to inconsistencies in quantitation data observed at the lower 1/100 and 1/1000 dilutions.

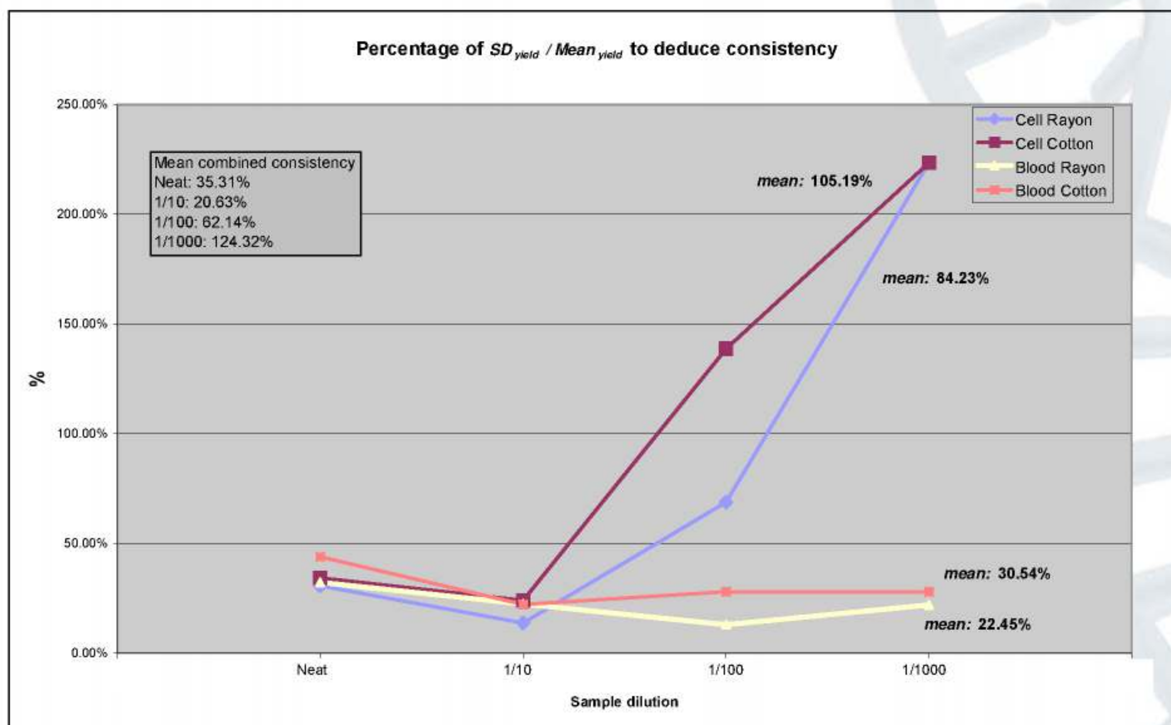

Figure 4. Reproducibility between replicates for cell and blood samples diluted down to 1/1000.

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6.3 Inhibition

Forensic samples that are commonly submitted for DNA analysis often contain inhibitors. These inhibitors may inhibit or significantly reduce the efficiency of a DNA extraction system, either by interfering with cell lysis or interfering by nucleic acid degradation or capture, therefore manifesting as extraction inhibitors (Butler, 2005). Inhibitors can also co-extract with the DNA and inhibit downstream PCR amplification processes, therefore acting as PCR inhibitors (Butler, 2005). For example, inhibitors such as hemoglobin and indigo dye likely bind in the active site of the *Taq* DNA polymerase and prevent its proper functioning during PCR amplification.

For the inhibition study, five substances were chosen for their known ability to inhibit PCR and their likelihood of appearing in routine casework samples:

- Indigo carmine: a component of the blue-dye encountered in denim jeans (Shutler, *et al.*, 1999).
- Tannic acid: a chemical used in the leather tanning process.
- Urea: a component of urine (Mahony *et al.*, 1998).
- Humic acid: a component found in soil and soil products (Tsai and Olson, 1992).
- Motor oil: contains various hydrocarbons and ethanolic compounds that can inhibit PCR.

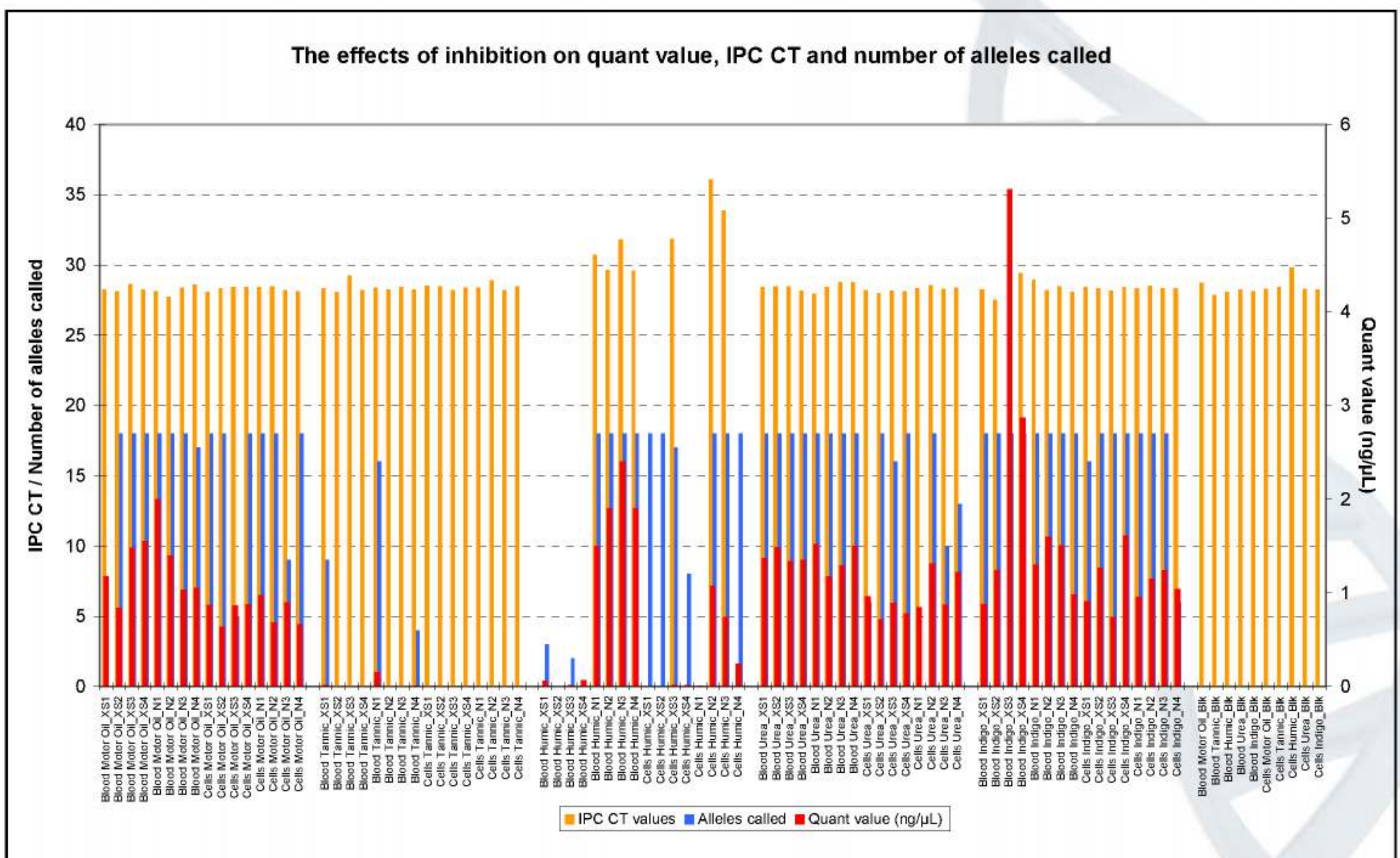

Figure 5. Effects of various inhibitors on quant value, IPC CT and number of resolved alleles.

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The observed effects of these inhibitors at neat and excess concentrations on the ability to extract, quantify and amplify various DNA samples are graphed in Figure 5. Samples were quantified using the Quantifiler™ Human DNA Quantitation Kit (Applied Biosystems) as this kit includes a built-in inhibition detector. Reaction efficiency and the presence of inhibitors can be assessed based on the performance of the internal positive control (IPC), which is known to be detected in this laboratory at around 28 cycles.

The observations that were made include:

- Samples that were spiked with motor oil, urea and indigo carmine dye did not show inhibition as determined by the IPC, and resulted in quantifiable DNA templates after extraction using DNA IQ™. The average DNA concentration observed for all samples was around 1ng/µL. The majority of samples yielded full DNA profiles, with the exception of several cell samples that were treated with urea (both at excess and neat concentrations).
- Blood and cell samples that were spiked with tannic acid did not show inhibition in Quantifiler™, as the IPC performed as expected. However, almost no amplifiable template DNA could be quantified and the majority of samples did not produce DNA profiles. This suggests that the original template DNA was degraded by application of tannic acid to the sample. It should be mentioned at this point that the tannic acid used was in the form of a yellow-brown paste substance that was applied directly to the sample swabs. The tannic acid paste, even at the neat concentration, may have been strong enough to severely fragment DNA to result in non-amplifiable templates. It was observed that three blood samples (1 with tannic acid in excess and 2 with tannic acid at neat concentration) yielded partial profiles (between 4-16 reportable alleles), and none of the cell samples produced reportable alleles. This may be caused by: (1) the concentration of viable cells in the buccal cell samples was lower than blood samples; (2) the drying of the blood stain on the substrate may have created a better barrier to protect the blood components from the degradative effects of the tannic acid.
- Blood and cell samples that were treated with humic acid in excess appeared to retain inhibition after extraction using DNA IQ™. However, at neat concentration, the effect of the humic acid inhibitor was overcome and amplifiable DNA template was purified as demonstrated by high DNA concentration yields. Residual inhibition was still present at neat concentration, as evidenced by higher CT values for the IPC (closer to 30), but full profiles were still produced. For some cell samples with humic acid in excess, the Quantifiler™ data suggested full inhibition (undetermined IPC CT and quantitation results), but two samples resulted in full DNA profiles.
- All reagent blanks were undetermined, indicating the absence of contamination in the results.

The results show that the DNA IQ™ system could be used to extract blood or cell samples that were spiked with motor oil, urea and indigo carmine at both excess and neat concentrations. Blood samples that contained humic acid in excess did not yield amplifiable template DNA, but 2 out of 4 cell samples with humic acid in excess appeared to produce full profiles. Samples that were exposed to tannic acid, at both neat and excess concentrations, resulted in non-amplifiable DNA, but the inhibitor was effectively washed out of the extract by DNA IQ™ as evidenced by the amplification of the IPC at the expected CT. Based on these results, we conclude that the DNA IQ™ system effectively removes inhibitors that are present in the original sample, resulting in a DNA extract that is of sufficient quality and is suitable for PCR amplification.

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6.4 Substrates

The substrate types examined included: swabs (cotton and rayon), tapelifts, fabric (denim, cotton, wool, lycra, nylon, polyester, leather), gum, cigarette butts, and FTA® paper. Cell and blood materials were spotted on to the substrates and extracted using DNA IQ™. The results for the two different sample types are presented in Figures 6 and 7 below.

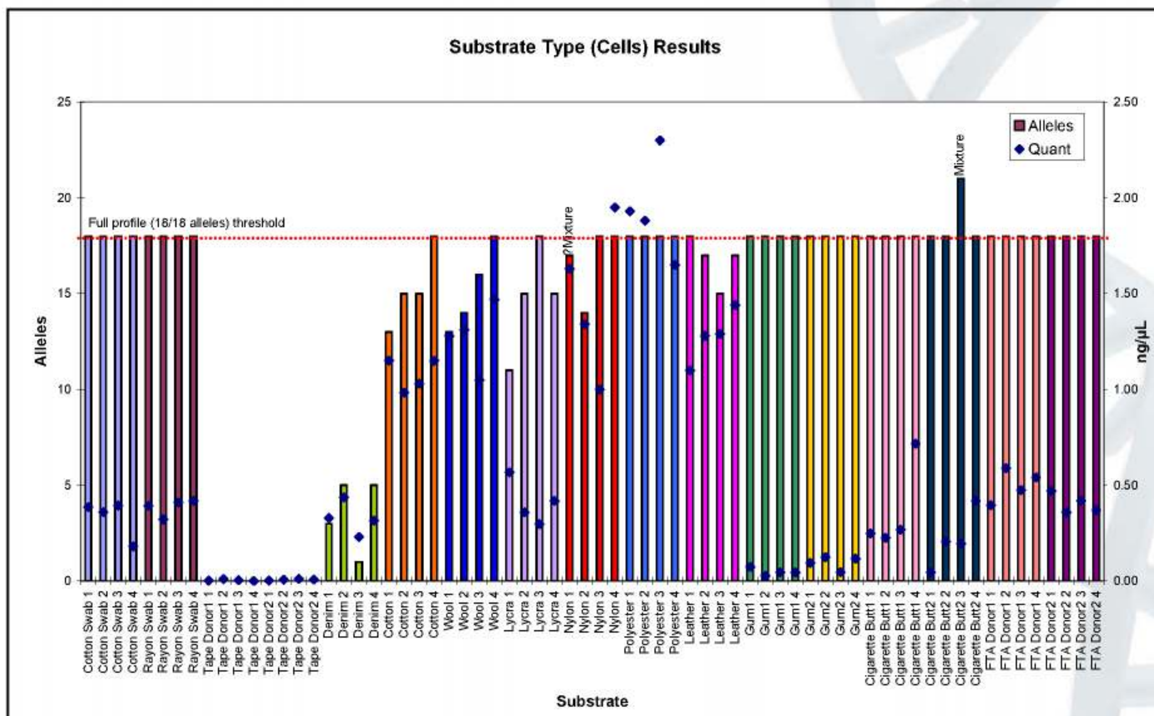

Figure 6. Number of reportable alleles and quantitation results for different substrate types containing cellular material.

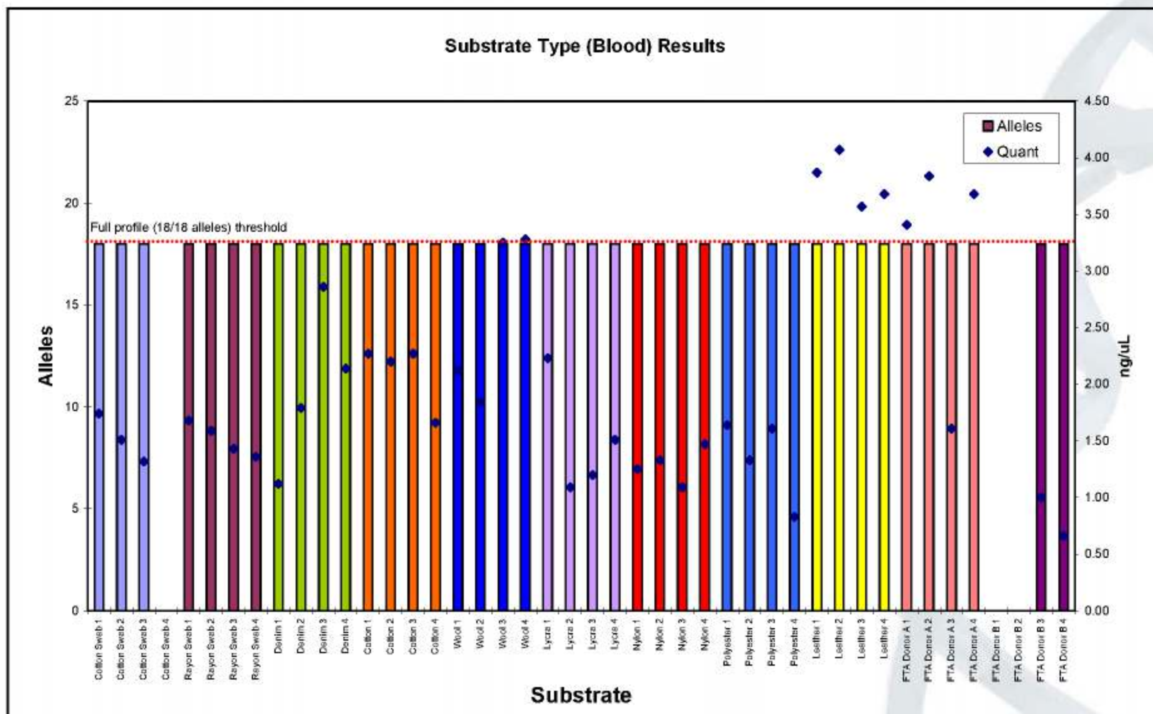

Figure 7. Number of reportable alleles and quantitation results for different substrate types containing blood material.

For cell samples:

- Full DNA profiles (18/18 alleles) were obtained from samples on cotton and rayon swabs, gum, cigarette butts and FTA® paper.
- The quantitation results for most of these samples were less than 0.5ng/µL. For gum samples, the average quantitation result was 0.072ng/µL, and therefore a PCR amplification at maximum volume (20µL) resulted in a total input DNA amount of 1.44ng which is sufficient to result in a full DNA profile.
- Tapelift samples gave an average quantitation result of 0.006ng/µL (just 0.002ng/µL higher than the observed background), and yielded no reportable alleles at all.
- The performance of clothing substrates was variable.
  - Cells on denim yielded quantitation results less than 0.5ng/µL but only partial profiles (maximum 5 reportable alleles), although Quantifiler™ results did not indicate any inhibition of the IPC. The poor performance of these samples may have been a result of sample preparation due to cell clumping.
  - Cells on cotton, wool and nylon resulted in higher quantitation values than lycra, but all substrates generated a similar number of reportable alleles (mean = 14 alleles). Only 25% of samples generated full DNA profiles.
  - Three out of four samples on polyester produced high quantitation results (~2ng/µL) but all samples resulted in a full profile.
  - Cells on leather displayed an average quantitation result of 1.3ng/µL and generated more than 15 reportable alleles.

For blood samples:

- All substrate types generated full DNA profiles.
- On average, the DNA quantitation results for all blood samples was greater than those resulted from cell samples. This is as per expected and was observed previously (see Project 9 report), because the concentration of nucleated cells in the blood samples were hypothesised to be higher than the concentration of buccal cell samples.
- Because of processing error, data was not available for the following samples: Cotton Swab 4, FTA Donor B 1 and FTA Donor B 2.

The results above are initial amplification results that do not take into account any reworking options.

We found that samples on tapelift substrates performed the worst; however this was probably due to the sampling method devised for this experiment, which did not adequately sample a sufficient number of cells.

6.5 Mixture studies

A mixture study was performed as part of the validation, however the results are not presented in this document because the mixture ratio was found to be inaccurate because cell counts were not performed on the saliva samples. Therefore, little information could be deduced from these results.

6.6 Substrate size

Blood on cotton swabs produced full DNA profiles for all sample sizes, ranging from 0.5 x 0.5cm to 2.0 x 2.0cm (Figure 8). Cells on cotton swabs did not perform as well (Figure 8), possibly due to the nature of the cells and difficulties in obtaining full DNA profiles from cell samples as observed in previous experiments.

Although the same starting amount of sample was used, it was observed that the 0.5 x 0.5cm samples generated higher quantitation results (therefore, also higher yields) compared to the 2.0 x 2.0cm samples (Figure 8). It appears that extraction efficiency decreases as the substrate surface area increases. This may be due to insufficient mixing and distribution of the lysis buffer over a larger substrate surface area, causing insufficient lysis of cellular material. This observation is in line with other reports that the DNA IQ™ system works more efficiently with smaller samples (Promega, 2006). The resulting IPC CT fell within the narrow range of 27.91 – 28.43 (mean = 28.10), indicating that both small and larger samples resulted in DNA extracts of similar quality, but the overall yield was lower for larger substrates (Figure 8 & 9).

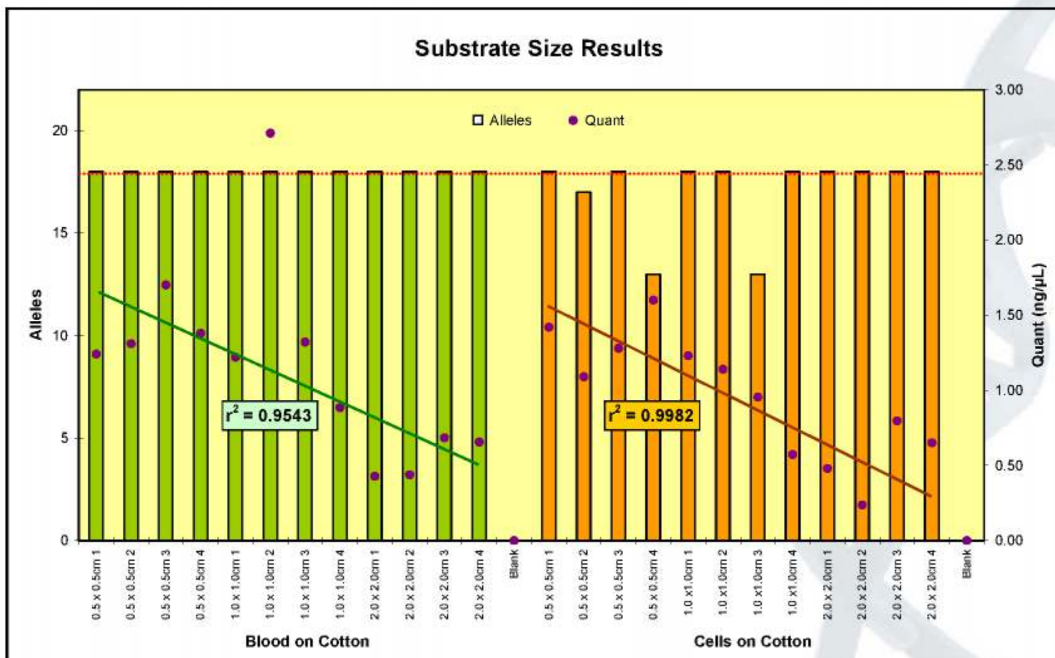

Figure 8. Results for blood and cell samples on cotton substrates of various sizes. All blood samples generated full profiles, but cell samples were more variable. The quantitation results for 0.5 x 0.5cm samples were higher than those for 2.0 x 2.0cm samples (blood r^2 = 0.9543*; cell r^2 = 0.9982; *Note: an outlier was removed from the calculation).

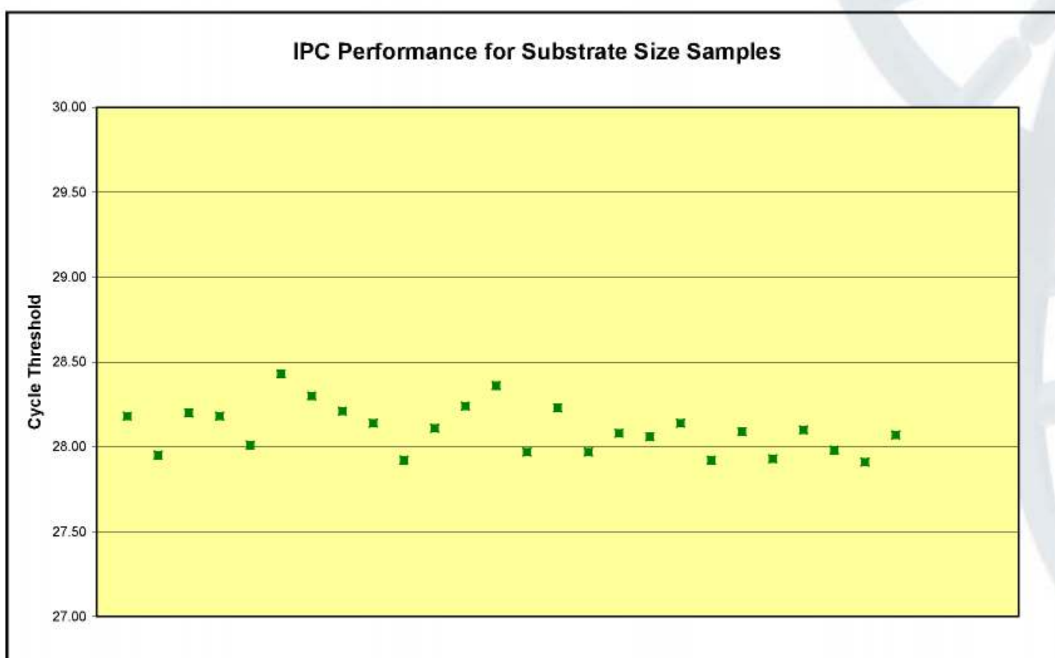

Figure 9. Various sample sizes resulted in similar CT values for the IPC, indicating that IPC performance is not affected by sample size, and that one sample size does not display a level of inhibition that is different to another sample size.

7. Summary and Recommendations

Based on the findings of this validation report, we recommend:

1. To enable processing of cell and blood samples using the validated manual DNA IQ™ protocol, except for samples on tapelift substrates.
2. To design and verify an automated protocol of the validated DNA IQ™ method for use on the MultiPROBE® II PLUS HT EX platforms, for processing blood and cell samples.

8. Acknowledgements

We wish to thank the Cytology Department at the Royal Brisbane and Women's Hospital for assistance with the cell-counting protocols.

9. References

Butler JM (2005). Forensic DNA Typing: Biology, Technology, and Genetics of STR Markers, 2nd Edition. Elsevier: Burlington, MA, USA.

Gallagher B, Hlinka V, Iannuzzi C, Lundie G, Muharam I, Nurthen T, Ientile V (2007a). Project 9: Report on the Evaluation of Commercial DNA Extraction Chemistries [Laboratory Report]. DNA Analysis, FSS: Brisbane, QLD, Australia.

Gallagher B, Hlinka V, Muharam I, Iannuzzi C, Lundie G, Nurthen T, Ientile V (2007b). Mock sample creation for cell and blood samples [Laboratory Report]. DNA Analysis FSS: Brisbane, QLD, Australia.

Hlinka V, Gallagher B, Iannuzzi C, Lundie G, Muharam I, Nurthen T, Ientile V (2007). Evaluation of the effect of inhibitors as assessed by a silica-based DNA extraction method and real-time PCR [in preparation].

Huston K (2002). DNA IQ™ System "Frequently Asked Questions". *Profiles in DNA* 5(1):11-12.

Mahony J, Chong S, Jang D, Luinstra K, Faught M, Dalby D, Sellors J and Chernesky M (1998). Urine specimens from pregnant and nonpregnant women inhibitory to amplification of *Chlamydia trachomatis* nucleic acid by PCR, ligase chain reaction, and transcription-mediated amplification: identification of urinary substances associated with inhibition and removal of inhibitory activity. *Journal of Clinical Microbiology* 36:3122-3126.

Muharam I, McNevin A, Iannuzzi C, Nurthen T, Ientile V (2007). Project 15: Report on the verification of automated capillary electrophoresis setup using the MultiPROBE® II PLUS HT EX platform [Laboratory Report]. DNA Analysis, FSS: Brisbane, QLD, Australia.

Mulot C, Stücker I, Clavel J, Beaune P, Loriot M-A (2005). Collection of human genomic DNA from buccal cells for genetic studies: comparison between Cytobrush, mouthwash and treated card. *Journal of Biomedicine and Biotechnology* 2005(3):291-6.

PerkinElmer (2004). MultiPROBE II Liquid Handling Forensic Workstation Application Guide: Automated DNA IQ™ System for Mixed Casework Sample DNA Isolation [PN 8842157]. PerkinElmer Life and Analytical Sciences: Downers Grove, IL, USA.

Promega Corporation (2006). DNA IQ™ System – Small Sample Casework Protocol [PN TB296, Rev. 4/06]. Promega Corporation: Madison, WI.

QIS 15396 R4 (2007). Method for bronchoalveolar lavage (BAL) cell count and differential cell count. [Standard Operating Procedure]. Anatomical Pathology, RBWH: Brisbane, Australia.

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QIS 19976 R1 (2007). Automated amplification of extracted DNA using the AmpFℓSTR® Profiler Plus® or AmpFℓSTR® COfiler® kit [Standard Operating Procedure]. DNA Analysis FSS: Coopers Plains, Brisbane, Australia.

QIS 19977 R1 (2007). Automated quantification of extracted DNA using the Quantifiler™ Human DNA Quantification Kit [Standard Operating Procedure]. DNA Analysis, FSS: Coopers Plains, Brisbane, Australia.

QIS 19978 R0 (2008). Capillary electrophoresis setup [Standard Operating Procedure]. DNA Analysis, FSS: Coopers Plains, Brisbane, Australia.

Satia-Abouta, King IB, Abouta JS, Thornquist MD, Bigler J, Patterson RE, Kristal AR, Shattuck AL, Potter JD, White E (2002). Buccal cell DNA yield, quality and collection costs: comparison of methods for large-scale studies. *Cancer, Epidemiology, Biomarkers & Prevention* 11:1130-3.

Shutler GG, Gagnon P, Verret G, Kalyn H, Korkosh S, Johnston E and Halverson J (1999). Removal of a PCR inhibitor and resolution of DNA STR types in mixed human-canine stains from a five year old case. *Journal of Forensic Sciences* 44(3):623-626.

Tsai YL and Olson BH (1992). Rapid method for separation of bacterial DNA from humic substances in sediments for polymerase chain reaction. *Applied and Environmental Microbiology* 58(7):2292-2295.

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PB173

Preparation of Extraction Quality Controls

1 PURPOSE AND SCOPE

This document describes the methods required to prepare Extraction Quality Controls for processes performed within the Analytical Section of DNA Analysis. The document applies to all DNA Analysis staff members required to prepare Quality Control samples.

2 DEFINITIONS

QC Quality Control

3 PRINCIPLE

QC (Quality Control) samples are included in processes to ensure and monitor the quality and integrity of the process. Positive controls are created with a known donor sample to produce an expected outcome. Negative controls should yield negative (i.e. no DNA detected) results. If the control does not produce the expected outcome, then there is evidence that the process has been compromised and this must be recorded and taken into account if reporting results, dependant actual QC result.

4 EQUIPMENT

4.1 Reagents

Table 1. Reagent storage locations.

Reagent	Device	Storage Location
10% v/v bleach	Bench	6120,6121, 6122
70% v/v ethanol	Bench	6120,6121, 6122
100% ethanol	Bench	6120,6121, 6122

4.2 Equipment

Table 2. Equipment used and location.

Equipment	Asset No.	Location
Rayon Swabs	N/A	6115, 6122
Scalpel	N/A	6120, 6121, 6122
Scalpel Blades	N/A	6120, 6121, 6122
QlickSmart scalpel blade remover	N/A	6120, 6121, 6122
Forceps	N/A	6120, 6121, 6122
Petrie dishes	N/A	6120, 6121, 6122
Bunsen Burner	N/A	6120, 6121, 6122
Metal Racks	N/A	6120, 6121, 6122
1.5mL non tethered tubes	N/A	6120, 6121, 6122
5mL tubes	N/A	6120, 6121, 6122
Ratek Dry Block Heater 40 position	30414940	6120
Ratek Dry Block Heater 60 position	508017195	6120
Ratek Dry Block Heater 60 position	508017201	6120
Whatman FTA Cards	N/A	6122, Blue cupboard, Analytical hallway
Foam swabs	N/A	Blue cupboard, Analytical hallway

Not Current

5 SAFETY

As per the procedures in the QIS document "*Operational Practices in the DNA Dedicated Laboratories*" (QIS 17120), PPE is to be worn by all staff when performing this procedure.

FTA cards must be cut with sterile scissors. To sterilize, dip the scissors into 10% (v/v) bleach, followed by 100% ethanol, then flame over a Bunsen burner. Allow the scissors to cool on a metal rack before cutting the FTA cards.

Blood tubes should be checked to ensure there is no damage or leakages. Glass stem pipettes are not to be used for the transfer of blood.

The Class II Biohazard cabinets are to be cleaned with 10% (v/v) bleach and 70% ethanol before and after use.

5.1 For large spills (approximately greater than or equal to 5x5cm in area)

1. Leaking samples shall be discarded into an appropriate waste container and a signed and dated notation made on the associated paperwork as to the reason for the action. The client should be asked to collect the sample again.
2. Cover the spill completely with Presept granules.
3. Leave for at least 2 minutes.
4. Remove granules with Rediwipe and dispose in Biohazard bin.
5. Wipe surface with damp Rediwipe.
6. Clean surface with 5% Decon 90 solution, followed by 10% (v/v) bleach and 70% ethanol.

5.2 For small spills (approximately less than 5x5cm in area)

1. Pour 10% (v/v) bleach solution over spill.
2. Use Rediwipe to soak up spill.
3. Dispose in Biohazard bin.
4. Clean surface with 5% Decon 90 solution, followed by 10% (v/v) bleach and 70% ethanol.

Not Current

6 SAMPLING AND SAMPLE PREPARATION

6.1 Blood

Blood is to be obtained from a consenting volunteer who has previously been profiled and who does not routinely perform Analytical Duties.

1. Two EDTA tubes are to be used when collecting blood and are to be clearly labeled with collection time and date, name of the volunteer, Date of Birth and the name of collector.

2. Once blood has been collected, each tube is to be given a barcode and registered in AUSLAB using the "FBOT" UR number assigned for blood positive controls for the given year and is not to be given a test code.

3. The blood tubes are to be stored at 4°C in the Walk in Freezer, separated from Reference and Casework samples. Blood should be kept no longer than 6 months.

6.2 Semen

Semen is to be obtained from a consenting volunteer who has previously been profiled and who does not routinely perform Analytical Duties.

1 Containers used when collecting semen and are to be clearly labeled with collection time and date, name of the volunteer and Date of Birth.

2 Once the semen has been received, each container is to be given a barcode and registered in AUSLAB using the "FBOT" UR number assigned for semen positive controls for the given year and is not to be given a test code.

3 The containers are to be stored at 4°C in the Walk in Freezer, separated from Reference and Casework samples. Semen should be kept no longer than 6 months.

6.3 Cells

Cells are to be obtained from a consenting volunteer who has previously been profiled and who does not routinely perform Analytical Duties.

If multiple collections are needed, perform only one collection per day in ensure maximum cell recovery.

1 A sterile 2mL tube containing 500µL of 0.9% saline are to be used when collecting the cells and are to be clearly labeled with collection time and date, name of the volunteer, Date of Birth and the name of collector.

2 Using a cytobrush, the volunteer is to brush the inside of one cheek for 1 minute. Once done, the cytobrush is washed by swirling the brush in the 500ul saline solution to remove the cells from the brush. Two cytobrushes are used for each cheek.

3 Once the cells have been collected, each 2mL tube is to be given a barcode and registered in AUSLAB using the "FBOT" UR number assigned for cell positive controls for the given year and is not to be given a test code.

4 The cells tubes are to be stored at 4°C in the Walk in Freezer, separated from Reference and Casework samples. The cells should be kept no longer than 6 months.

Not Current

6.4 Hair

Hair is to be obtained from a consenting volunteer who has previously been profiled and who does not routinely perform Analytical Duties.

6.5 FTA controls

The blood and cells used to prepare FTA controls are to be obtained from a consenting volunteer who has previously been profiled and who does not routinely perform Analytical Duties.

7 PROCEDURE

7.1 Preparation of Rayon Swabs for Blood, Cell and Differential Lysis Controls

The Rayon swabs used to create QC samples can be made ahead of time using the following procedure:

1. Set up a bench with the following equipment:
   - 1 Coplin jar of 10% v/v bleach
   - 1 Coplin jar of 100% ethanol
   - Bunsen burner
   - Forceps
   - Scalpel and scalpel blades
   - Box of Rayon (white lid) swabs
   - Petri dishes
   - Stainless steel rack
   - 1.5mL or 2mL screw-cap DNA free tubes

2. Dip the forceps and handle into the 10% v/v bleach, followed by 100% ethanol then briefly place into flame of Bunsen to burn off the ethanol. Place on a rack to cool.

3. In a clean Petri dish, use the scalpel to remove the swab bulb from the stem by cutting 5mm up the stem of the swab then discard the swab stem. Continue cutting and regularly change scalpel blade and Petri dish until all swabs are cut.

4. In a clean Class II Biohazard cabinet, transfer the swab bulbs from the Petrie dish into sterile 1.5mL or 2mL tubes. Once all bulbs have been placed into a tube, store in either room 6120, 6121 or 6122.

7.2 Preparation of Blood Extraction Controls

1. Locate an appropriate blood tube in the walk in freezer, ensure that the blood is within expiry date and all required details are on the tube. Allow the tube to come to room temperature and mix by inversion and low rpm vortex before use.

2. Wipe down a Ratek dry block heater (or other appropriate heating block/device) with ethanol before placing inside a clean Class II Biohazard cabinet. Set the dry block heater to 56 degrees.

3. Hand-label two clean 2mL tubes with the blood details and, within the Class II Biohazard cabinet, aliquot blood into the tubes, the volumes depending on the number of QCs to be made.

4. In the Class II Biohazard cabinet, slowly pipette 15µL of blood directly to the swab head, being careful of not pipetting blood to the side of the tube. Place tube lid up-side down onto a clean rediwipe.

5. Place the tube directly into the dry block heater and allow the blood to be absorbed by the swab while spotting the other swab heads. If a small batch of controls is being made, allow 2 minutes for the blood to absorb.

6. Remove each tube in turn from the dry block heater and repeat the previous two steps. (Therefore a total of 30µL of blood will have been added to each control).

Not Current

7 Allow the tubes to dry for between 2 and 4 hours (but no longer).

8 Whilst the tubes are drying, prepare 5ml tubes with QC-Blood labels. Using the B1-Lite program, create the labels using the QC-Blood label format shown in figure 1 below, where 123456789 is the barcode of the original blood tube.

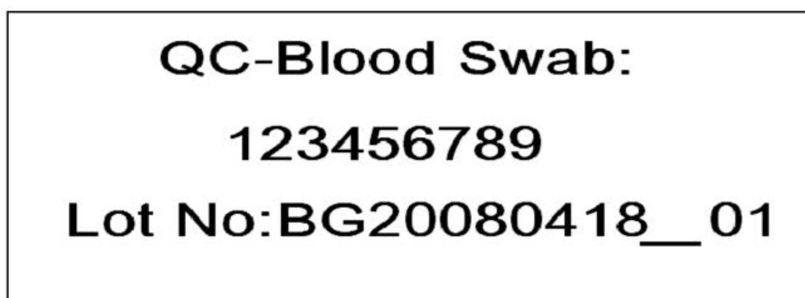

Figure 1. Example of Blood swab QC label.

9 Once the swabs are dry, recap the tubes and place into labeled 5mL tubes. Store in white foam racks in freezer 6117- 5 in the workflow area. Record details into electronic log (I:\AAA Analytical Section\Analytical Logs\QC Swab results log XXXX.xls – where XXXX represents the current year).

10 Register freshly made batches of controls for testing. The number of swabs to be tested is to be no less than 5% of the batch total e.g. 20 total – 1 swab tested, 50 total – 3 swabs tested, 100 total – 5 swabs tested, etc. Ensure that the swabs chosen for testing are taken at random from the batch.

11 Register the samples as a regular positive control with a priority of 1. In the "sample info" field enter batch test and batch ID comment e.g. "QC Batch Test, BG20080418_01". Enter "QC Batch Test" into the processing comment.

12 Record details into electronic log and follow up with results as well as notify Analytical HP4.

7.3 Preparation of Cell Extraction Controls

1 Locate an appropriate buccal cell collection in the walk in freezer, ensure that the cell suspension is within expiry date and all required details are on the tube. Allow the tube to come to room temperature and mix by inversion and low rpm vortex before use.

2 Wipe down a Ratek dry block heater (or other appropriate heating block/device) with ethanol before placing inside a clean Class II Biohazard cabinet. Set the dry block heater to 56 degrees.

3 Hand-label a clean 2mL tube with the cell suspension details and, within the Class II Biohazard cabinet, aliquot sufficient cell suspension into the 2mL tube, the volume depending on the number of QCs to be made. (This step is not necessary if the whole cell suspension is to be consumed).

4 In the Class II Biohazard cabinet, slowly pipette 15µL of cell suspension directly to the swab head, being careful of not pipetting to the side of the tube. Place tube lid up-side down onto a clean rediwipe.

Not Current

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5 Place the tube directly into the dry block heater and allow the cell suspension to be absorbed by the swab while spotting the other swab heads. If a small batch of controls is being made, allow 2 minutes for the cell suspension to absorb.

6 Remove each tube in turn from the dry block heater and repeat the previous two steps. (Therefore a total of 30µL of cell suspension will have been added to each control).

7 Allow the tubes to dry for between 2 and 4 hours (but no longer).

8 Whilst the tubes are drying, prepare 5ml tubes with QC-Cell labels according to the procedure for preparing QC-Blood labels in procedure above (NOTE: replacing the words "QC-Blood" with "QC-Cells").

9 Once the swabs are dry, recap the tubes and place into labeled 5mL tubes. Store in white foam racks in freezer 6117- 5 in the workflow area. Record details into electronic log (I:\AAA Analytical Section\Analytical Logs\QC Swab results log XXXX.xls – where XXXX represents the current year).

10 Register freshly made batches of controls for testing. The number of swabs to be tested is to be no less than 5% of the batch total e.g. 20 total – 1 swab tested, 50 total – 3 swabs tested, 100 total – 5 swabs tested, etc. Ensure that the swabs chosen for testing are taken at random from the batch.

11 Register the samples as a regular positive control with a priority of 1. In the "sample info" field enter batch test and batch ID comment e.g. "QC Batch Test, BG20080418_01". Enter "QC Batch Test" into the processing comment.

12 Record details into electronic log and follow up with results as well as notify Analytical HP4.

7.4 Preparation of Differential Lysis Extraction Controls

1 Locate the semen and cells in the walk in freezer, making sure that the specimens are within the expiry date and checking that all the details are on the tubes. Allow the tubes to come to room temperature and vortex before use.

2 Wipe down a Ratek dry block heater (or other appropriate heating block/device) with ethanol before placing inside a clean Class II Biohazard cabinet. Set the dry block heater to 56 degrees.

3 Within the Class II Biohazard cabinet, make a "stock solution" of semen and cells by combining equal volumes of semen and cell samples to an appropriately sized tube.

4 In the Class II Biohazard cabinet, slowly pipette 15µL of the stock solution directly to the swab head, being careful of not pipetting stock solution to the side of the tube. Place tube lid up-side down onto a clean rediwipe.

5 Place the tube directly into the dry block heater and allow the stock solution to be absorbed by the swab while spotting the other swab heads. If a small batch of controls is being made, allow 2 minutes for the blood to absorb.

6 Remove each tube in turn from the dry block heater and repeat the previous two steps. (Therefore 30µL of the stock solution in total will have been added to each control)

7 Allow the tubes to dry for between 2 and 4 hours (but no longer).

Not Current

8 Whilst the tubes are drying, prepare 5ml tubes with QC-Diff Lysis labels. Using the B1-Lite program, create the labels using the QC-Diff label format shown in figure 2 below.

Figure 2. Example of Differential Lysis QC label.

9 Once the swabs are dry, recap the tubes and place into labeled 5mL tubes. Store in white foam racks in freezer 6117- 5 in the workflow area. Record details into electronic log (I:\AAA Analytical Section\Analytical Logs\QC Swab results log XXXX.xls – where XXXX represents the current year).

10 Register freshly made QC for testing. The number of swabs to be tested is to be no less than 5% of the batch total e.g. 30 total – 2 swabs tested, 50 total – 3 swabs tested, etc. Ensure that the swabs chosen for testing are taken at random from the batch.

11 Register the samples as a regular positive control but with a priority of 1. In the sample info field enter batch test and batch ID comment e.g. "QC Batch Test, BG20080418_01". Enter "QC Batch Test" into the processing comment.

12 Record details into electronic log and follow up with results as well as notify Analytical HP4.

7.5 Hair

1 To 10 sterile 1.5mL tubes add 50µL of nanopure water and provide to volunteer inside a clip-seal plastic bag.

2 To each tube, the volunteer adds 2 plucked hairs (must contain macroscopically visible root material). Tubes are returned to the Analytical section as soon as practicable.

3 The 1.5mL tubes are added to 5mL tubes and these are then labeled as follows. The labels are created using the B1-lite program with the following information where AAA is the initials of the hair volunteer. (refer figure 3 below)

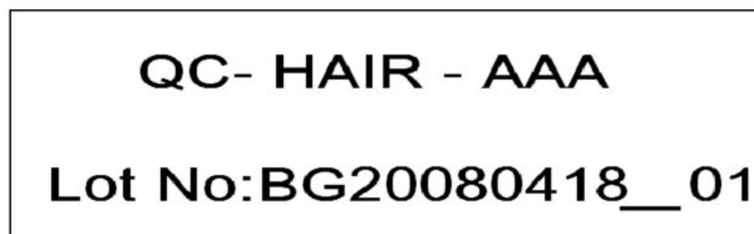

Figure 3. Example of a Hair QC label

4 Store the hair controls in a white foam rack in freezer 6117-5 in the workflow area. Record details into electronic log (I:\AAA Analytical Section\Analytical Logs\QC Swab results log XXXX.xls – where XXXX represents the current year).

Not Current

5 Register one control as a regular positive control but with a priority of 1. In the sample info field enter batch test and batch ID comment e.g. "QC Batch Test, BG20080418_01". Enter "QC Batch Test" into the processing comment.

6 Record details into electronic log and follow up with results as well as notify Analytical HP4.

7.6 FTA controls

7.6.1 FTA Blood Controls

FTA Blood controls are clothed in a similar manner as outlined in QIS Document 17153 Reference Blood Processing. Once dry samples are given an AUSLAB barcode, placed into individual envelope and kept in room 6127.

7.6.2 FTA Cell Controls

FTA Cell controls are prepared in the following method.

1 Whatman FTA cards are cut in half and are given to volunteer.

2 Using a large "lollypop" foam swab, the volunteer rubs the inside of one cheek with one side of the swab for 15 seconds followed by the other cheek with the other side for 15 seconds.

3 One side of the foam swab is then pressed against one circle of the FTA card and held there again for 15 seconds before turning the swab over and pressing the other side against the 2nd circle on the card.

4 The FTA card is then allowed to dry before being given an AUSLAB barcode, placed into individual envelopes and kept in room 6127.

7.7 Negative Controls

Negative controls are considered as reagent blanks, consisting of only the reagents and the empty tube or plate well used in each process except for FTA processing where the negative control consists of blank FTA card punches.

Not Current

8 QUALITY ASSURANCE/ACCEPTANCE CRITERIA

9 ASSOCIATED DOCUMENTS

16004 AUSLAB Users Manual – Forensic Biology

16028 Method for FTA Purification and Amplification (Blood)

17153 Reference Blood Processing (Blood Clothing)

17165 Receipt, Storage and Preparation of Chemicals, Reagents and Kits

17198 Method for FTA Purification and Amplification

24469 Batch Functionality in AUSLAB

24897 Automated DNA IQ Method of Extracting DNA from Blood and Cells Substrates

10 AMENDMENT HISTORY

Revision	Date	Author/s	Amendments
0	2008	Cecilia Iannuzzi, Breanna Gallagher, Allan McNevin	First Issue

Not Current

PB174

Lab	Robotic platform	Extraction casework	Differential method	Quant method casework	Amp volume casework	Amp amt casework	Instrument casework	Extraction reference	Quant method reference	Amp volume reference	Amp amt reference	Instrument reference	Thermal cyclers	Confirmatory/ presumptive testing eg Semen, Saliva, blood	Typing software (version)	Available kits	Interpretation software (version)	Currently validating/verifying
WA	**Extraction:** Perkin Elmer Janus 8 tip integrator (x1), Automate Express (x4) **Quant and PCR Setup:** Perkin Elmer Janus Mini (x4) **CE Setup:** INTEGRA Biosciences Viaflo 96 (x1, reference only) **FTA Punch:** BSD Duet (x2), BSD 600 Ascent (x1)	DNA-IQ (Janus) PrepFiler (Automate) Chelex (manual)	DNAIQ	Quant Trio	PP21: 25ul Yfiler: 25ul	0.4ng PP21 1.0ng YFiler	3500 x 2	Chelex/PrepFiler (manual), Direct amp on FTA	Quant Trio (PrepFiler and Chelex extracted samples only)	Direct Amp FTA: 12.5 ul; PP21 & Yfiler (manual extrated samples): 25ul	Extracted samples: 0.4ng PP21; 1.0ng Yfiler; NA direct Amp	3500 x 2	9700 (x1) ProFlex (x12)	KM, Hematrace (Blood); RSID (Saliva); AP, RSID, microscopy - Christmas tree stain (semen); Urine (Jaffe reaction); Faeces (Urobilinogen)	GMID-X V1.5	PP21 & Yfiler	STRmix V2.4	YfilerPlus; Verifiler; Robot tender underway; Diff extraction - Prepfiler and i-sep columns;
SA	**Extraction:** Hamilton AutoLys; **Quants & PCR Set Up:** Perkin Elmer Janus; 3500 run plates: Perkin Elmer Multiprobe	DNA-IQ (AutoLys) DNA-IQ (Manual)	DNA-IQ (Manual)	Quant Trio	GlobalFiler: 25uL YFiler Plus: 25uL	0.4ng GF; 0.5ng YF Plus	3500 x 2	Direct amp on FTA with BSD Ascent; DNA-IQ (manual)	Quant Trio (DNA-IQ manual extracts only)	GlobalFiler: 12.5uL; YFiler Plus: 25uL, moving to 12.5uL	Extracted samples: 0.4ng GF; 0.5ng YF Plus	3500 x 2	ProFlex x 6	Hemastix & HemaTrace (Blood); Spotty paper & RSID (Saliva); Acid Phophatase & ABA card-p30 (Semen)	GMID-X V1.4	GF & YFiler Plus	STRmix v2.6	2x Tecan Freedom Evo to replace PE Janus & PE Multiprobe; Argus 12 X-STR kit & Gnano for ancestry (31 SNP) both awaiting implementation
NSW	**Lysis:** Hamilton MicroLab AutoLys STAR **Extraction:** Tecan Freedom EVO 150 & AutoMate Express Quant & PCR Set Up: Tecan Freedom EVO 150 **CE Set up:** Tecan Freedom EVO 150	PrepFiler; Demineralisation method & QIAquick (manual) for compromised bone samples; PrepFiler BTA (AutoMate Express) for fresh bones	P. Gill Differential Extraction method manual to pellet, then PrepFiler automated extraction	Quant Trio (QuantStudio 5)	PP21: 25 ul; Yfiler Plus: 12.5ul	0.7ng PP21 (29 cycles); 0.7ng Yfiler Plus (30 cycles)	3500xL x 2	Direct amp on FTA with BSD 600 Plus; PrepFiler: extraction from hairs & old FTA card samples; Qiagen EZ1 (DNA Investigator) Mito refs	Quant Trio (PrepFiler & DNA Investigator extracts only); Bioanalyser (post PCR quant for Mito)	Direct Amp FTA PP21 (25 cycles) & Yfiler Plus (27 cycles): 12.5ul	Extracted samples: 0.7ng PP21; 0.7ng Yfiler; NA direct Amp	3500xL x 2; 3500 x 1 (Mito)	9700 (x5) ProFlex (x3) Mito: 9700 (x2) ProFlex (3 head x1)	O-tol & HemaTrace (Blood); RSID (Saliva); Acid Phophatase & ABA card-p30 (Semen); Microscopy & SpermFinder (Sperm) Urine (Urease); Feaces (Urobilinogen)	GMID-X V1.6	PP21; Yfiler Plus;	STRmix v2.6	Upgrading Tecan robot computers & software; verifying new tubes & racks for AutoLys robots; soon to start half reaction for Quantifiler Trio and Mitochondrial whole genome sequencing
VIC	Beckman-Coulter NxP (x5) To be replaced 2019 with Hamilton AutoLys (x2) Biomek i7 Series (x2) Biomek i5 Series (x2) Hamilton EasyPunch (x2)	DNA-IQ (Biomek NxP) DNA-IQ (Hamilton AutoLys & Biomek i7 Series) Organic/Chelex/DNA-IQ (manual)	DNA-IQ Organic	Quant Trio	PP21 - 25 ul YFP- 25ul	0.5ng PP21 0.5ng YFP	3500xL X 3	Organic/Chelex/DNA-IQ (manual) Automated DNA-IQ (Beckman Coulter NxP) Automated Direct amp (Hamilton EasyPunch) on EasyCollect Plus cards	Quant Trio only on Organic/Chelex/DNA-IQ (manual) Automated DNA-IQ (Beckman Coulter NxP) extracted samples	PP21 - 12.5 ul YFP - 25 ul (manual extraction) NxP YFP - 12.5 ul (Direct PCR EasyPunch)	0.25ng PP21 PP21 Direct PCR 0.5ng YFP YFP Direct PCR	3500xL X 3	Verity 96 well (x4) Verity 60 well (x4)	Hemastix (Blood) Hematrace (Blood) Luminol (Blood) AP (semen) P30 (semen) AP (semen) H & E (sperm) RSID (saliva) Jaffe reaction (urine) Ecoline test (urine) Urobilinogen (faeces) renin activity (vomit)	GMID-X V1.5	PP21 Y Filer Plus	STRmix V2.5	Hamilton EasyPunch and Direct PCR on easyCollect Plus cards Hamilton AutoLys, Biomek i7 and Biomek i5 Robotic validation STRmix V2.6.2
AFP	**Extraction:** QIAsymphony or Ez1XL **Quants & PCR Set Up:** QiaAgility (set-up) and Applied Biosystems 7500	Qiagen DNA Investigator (for both platforms)	Qiagen DNA Investigator (for both platforms)	Quant Duo	PP21: 25uL Globalfiler: 25uL YFiler Plus: 25uL	0.5ng, PP21 1.0ng GF & Yfiler plus	3500xL x 1	Qiagen DNA Investigator (EZ1 XL)	Quant Duo	PP21: 25uL Globalfiler: 25uL YFiler Plus: 25uL	0.5ng PP21, 1.0ng GF/Yfiler plus	3500xL x 1	ABI Veriti	Hemastix, RSID blood, RSID semen, RSID saliva, Acid Phophatase (semen), Starch Agar - Saliva, Ouchterlony - species testing	GMID-X V1.4	PP21, GF & YFiler Plus	STRmix v2.3.6	Quant Studio and Trio, STRmix 2.6, Genemapper ID-X v1.6, P1 swab (abf diagnostics) and MPS
TAS	**Quant & PCR Setup:** QiAgility	organic (manual)	organic (manual)	Plexor HY	PP21: 25ul Direct Amp: 13ul Y-Filer: 25ul	0.5ng PP21 0.5ng Yfiler	3130XL	Direct amp on FTA	Nil	PP21:13ul Yfiler: 12.5ul	NA	3130XL	ABI Veriti	Hemastix, Hematrace, haemochromogen, luminol (Blood), Acid Phosphatase, microscopy, Semenogelin (semen); Phadebas paper and tube (saliva)	GMID-X V1.4	PP21, YFiler; YFilerPlus validation work complete; close to implementation	STRmix V2.4.05	Argus X-12 kit; YFilerPlus close to implementation
NT	**Extraction:** Maxwell 16 (Promega) **Quant and Amp set up:** Nimbus (Hamilton)	DNA-IQ (Maxwell 16) Chelex (manual)	Chelex	Quant Trio	GlobalFiler: 25uL YFiler Plus: 25uL	1.0ng GlobalFIler	3500 x 2	Direct amp on FTA Chelex (Manual)	Quant Trio (Chelex extracted samples only)	Direct Amp FTA: 15 ul; GlobalFiler (manual extrated samples): 25ul	1.0ng GlobalFiler	3500 x 2	9700 (x3)	Otol, Luminol, NIR, Hematrace (Blood) RSID (semen and saliva) Phadabas (saliva) Crimelite (various)	GMID-X V1.5	GlobalFiler	STRmix V2.6	YfilerPlus;
QLD	QIAsymphony (x2) Maxwell 16 (x4)	DNA Investigator (QIAS) DNAIQ (Maxwell)	DNAIQ	Quant Trio	PP21: 25uL	0.5ng PP21	3130 x1	Direct amp on FTA, DNAIQ Extracted samples	Quant Trio (only extracted samples)	Direct amp FTA and Extracted samples: 12.5uL	Extracted samples: 0.5ng	3500 x2	9700 x6	AP/p30/micro (Semen), TMB (Blood), Phadebas (Saliva)	GMIDx v1.4	PP21	STRmix v2.6.2	Yfiler Plus, Verifiler Plus, DNA Investigator kit for Bones
VIC -VIFM	None	DNA investigator (QIAcube and manual) Chelex (manual)	None	Quant HP (nDNA) Bioanalyser DNA 1000 (mtDNA)	GlobalFiler: 25uL MiniFiler: 25uL mtDNA amp: 50 uL	0.5 ng GF	3500 x 2	DNA investigator (QIAcube and manual) Chelex (manual)	Quant HP (nDNA) Bioanalyser DNA 1000 (mtDNA)	GlobalFiler: 25uL MiniFiler: 25uL mtDNA amp: 50 uL	0.5 ng GF	3500 x 2	9700 (x3); Veriti (x1)	None	GMID-X V1.4 Sequencher V5.4	GF & MiniFiler		None
NZ	Hamilton Nimbus, Hamilton starlets	DNA IQ	differex/ DNA IQ	quant trio	Identifiler 25ul GF 25ul MF 25ul Y Filer Plus 12.5ul ID+ - LCN 12.5ul.	Identifiler 1.2ng GF 0.5ng MF 0.2ng Y Filer Plus 0.25ng ID+ - LCN 50pg.	3500 x 2 3130 x 1 retiring	direct PCR (Databank) chelex (blood refs manual,)	if needed quant trio	GF 13ul Y Filer plus 12.5 ul	GF 0.5ng Y Filer PLUS 0.4ng	3500 x 2	9700s and veritis	mRNA AP/P30/ micro TMB/KM/luminol hematrace (rarely) amylase paper and tube test	GMID-v 3.2.1 GMID ID X v1.4	Identifiler®, and MiniFiler® GlobalFiler, Yfiler® Plus and mRNA/CellTyper 2™ Identifiler+	STRmix v2.5.11	Identifiler +/ STRmix 2.7.0.37 (precommercial) /3500xl combo

MPS status Equipment/kits	STATS 1/07/18 - 30/06/19				Sampling devices used
	# EXHIBITS	# REFERENCE SAMPLES	# REPORTS	# FTE	
NIL	28,686	29,582	753 court reports; 2,328 Preliminary reports; 4,186 link reports; 10,200 results by spreadsheet	105.8 FTE (includes management, Admin, IT, R&D)	Trace DNA – BD BBL™ CultureSwab™ EZ (Sterile) + MilliQ Water Biological Fluid – Copan Rayon Swab (Sterile) + Water. Tapelift – Scotch Transparent Tape 3M
NIL					Trace DNA - IsoHelix swabs (DNA free) + isopropanol. Biological fluids - Copan cotton swabs (sterile 150C red and 164C green) + water. Tapelift - Lovell surgical supplies (DNA free)
Tender Underway	57,433 sub-samples and 8,746 exhibits	15,455	1655 court statements, and 61,860 intelligence reports	Establishment FTE 72 inclusive of management, adim and IT	Biological fluids and trace: Rayon Swabs + water (Dryswab, Medical Wire Equipment, EO treated via Lovell). Fingernail clippings: 4N6 FLOQswabs Genetics + water (Copan, subungual shape, sterile). Tapelift: 3M 9425 Tape (Lovell surgical supplies, EO treated)
NIL	Total - 8379 (volume crime - 4143) (major crime - 4236)	Total - 11,796 (Convicted Offenders - 8245) (Non-CO's - 3551)	Total Statements (for court) - 661 Total Case Result Summaries (CSR) - 1357 (CRS volume - 542) (CRS major - 815)	Total BSG FTE (inc admin) - 115.6 FTE on casework - 40.7 FTE on database - 7.8	Copan cotton swabs (sterile 150C) + water.
Illumia MiSeq, Verogen ForenSeq DNA Signature Prep Kit, MiSeq FGx Reagent Kit, Biomek NxP	Not able to provide	630	Not able to provide	16.5	Swabs - Fab-swab (Puritan), Dryswab MW102 and MW100 (MWE), Spin-Eze Push off swab (Fitzco) Tapelift - DNA tapelift (Lovell surgical supplies)
NIL	6506	4599 (but don't test duplicates)	Not able to provide	17	In the process of moving to Sarstedt DNA free swabs
NIL	733 cases (exhibits not counted)	1522	Approx. 230 (Court Reports)	11	Tapelift - Lovell surgical supplies (DNA free), Fitzco Pushoff Polyester Swabs (discontinued but trialling new Push-off swabs distributed by PathTech). Copan Plain Dry Swab (rayon).
Nil	27,349	15,159	628	63	Copan Plain Dry Swab (rayon) – in lab use, and Copan Plain Dry Swab (cotton) for SAIKs Majority of sampling materials as per QPS
Ion Chef; Ion S5; Converge; mtDNA panel (whole genome)					
Forenseq Panel B, miSeq FGx, writing up validation.	4500	databank samples = 7813 case reference samples= 765	884 full reports 1876 crime sample databank reports	38	Minitapes: standard work: Scotch brand 3M double-sided poster tape Swabs for LCN: Double ended, rayon, ETO treated, DNA free certified swab, Scenesafe. Catalogue number K4040-100ETO Swabs for standard work: Rayon Cultiplast swabs sterile, in pp tube, plastic shaft (5x100pcs/box) (Preferred Supplier], Thermo Fisher Scientific NZ Ltd, LPI111598

PB175

+Recognition, recording, recovery and storage of physical material for forensic purposes

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PREFACE

This Standard was prepared by the Standards Australia Committee CH-041, Forensic Analysis. This Standard is the result of a consensus among the representatives of the Committee to produce it as an Australian Standard.

This Standard provides a framework for the collection and storage of materials from crime scenes in order to preserve their integrity for forensic purposes and covers the processes:

Recognizing material of forensic interest;
Recording material at a scene;
Collection of material from a scene;
Containers and labelling;
Documentation;
Transport of material;
Storage of material;
Security of material in storage;
Retrieving material from storage; and
Documenting and tracking the location of material.

The Standard also extends to secondary scenes which may be associated with a crime scene. Persons involved in the crime, both living and deceased but no longer located at the primary crime scene, may be viewed as secondary crime scenes and their physical examination involves processes similar to those outlined above. Psychiatric examination of persons involved in criminal incidents is specifically excluded from this standard.
This Standard is part of a series which, when complete, will include:
AS
<#>: Recognition, Recording, Recovery and Storage of Physical Material for Forensic Purposes
<#>: Analysis of Material for Forensic Purposes
<#>: Interpretation of Results from the Forensic Analysis of Material
<#>: Reporting Results and Conclusions from the Forensic Examination of Material

The provisions of this document shall be restricted to qualified personnel, with demonstrated competence in the use of appropriate scientific procedures and techniques in a forensic context.
This Standard calls for the use of procedures that may be hazardous or injurious to health, if adequate provisions are not taken.

The term 'informative' has been used in this Standard to define the application of the annex to which it applies. An 'informative' annex is for information only.

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An Australian Government Initiative

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1. Scope

The purpose of this document is to set out the standard practice for recognition, recording, recovery, transportation and storage of physical evidence until its forensic examination is commenced in an appropriate facility such as laboratory, examination room or mortuary.
The principal focus of the document is the crime scene. That crime scene may be the actual location where the crime took place or a secondary location such as a car or dwelling which may yield physical evidence of value to the investigation or any subsequent judicial process. Persons involved in crime, whether perpetrator, witness or victim, shall also be regarded as possible "crime scenes" and therefore possible sources of physical evidence. Examples of such evidence would be samples from the sexual assault victim, post-mortem examination samples, blood spattered on a witness or broken glass on the clothing of the suspect.
Physical evidence encompasses any and all objects, gross or microscopic in size, living or inanimate, solid, liquid or gas and, importantly, includes the relationship between all such objects as they pertain to a crime. Impressions such as toolmarks are physical evidence in the same way as bloodstains, hairs and fibres, drug samples, broken glass and other more tangible objects.
This document does not address procedures for the recovery of digital material from storage media. It may, however, be important to remember that the storage medium itself may yield important physical evidence such as fingerprints or DNA. The forensic examiner should bear this in mind to maximize evidential recovery during the recording and handling of such evidence.
The response to a crime will depend on the seriousness and/or impact of that crime and the complexity of the crime scene. The resources and facilities required by and/or available to an investigator will vary both in quantity and quality. This standard provides the general principles that should be followed as far as practicable at all scenes.

The processes covered by this document include:

- Recognizing material of forensic interest;
- Recording material;
- Collection of material;
- Containers and labelling;
- Documentation;
- Transportation;
- Storage of material;
- Security of material in storage; and
- Documenting and tracking the location of material.

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2. Referenced and related documents

2.1 Referenced Documents

The following documents are referred to in this Standard:

AS	
2243	Safety in laboratories
3864	Medical refrigeration equipment - For the storage of blood and blood products
4757	Handling and destruction of drugs
ISO/IEC	
17025	General requirements for the competence of testing and calibration laboratories

Codes

Australian Code for the Transport of Dangerous Goods by Road And Rail (ADG Code), Seventh edition 2007, Commonwealth of Australia, ISBN 1 921168 57 9.
Australian Code for the Transport of Explosives by Road And Rail, Third edition 2009, Commonwealth of Australia, ISBN 978 0 642 32747 5.
National Pathology Accreditation Advisory Council, Requirements for the Facilities and Operation of Mortuaries, Second Edition, 2009.
National Directory for Radiation Protection, April 2010, Radiation Protection Series Publication No. 6, ARPANSA.
Intervention in Emergency Situations Involving Radiation Exposure, Radiation, 2004, Protection Series Publication No. 7, ARPANSA

Manuals

ISO/IEC 17025 Application Document, Supplementary Requirements for Accreditation in the Field of Forensic Science (including Parentage Testing), NATA December 2006.

2.2 Related Documents

Further information may be found in the following Standards:

AS	
1336	Recommended practices for occupational eye protection
1337	Eye protectors for industrial applications
1470	Health and safety at work—Principles and practices
1715	Selection, use and maintenance of respiratory protective devices
1716	Respiratory protective devices
1940	The storage and handling of flammable and combustible liquids.
2030	The verification, filling, inspection, testing and maintenance of cylinders for storage and transport of compressed gases (series)
2161	Occupational protective gloves (series)
2243	Safety in laboratories (series)
2375	Guide to the selection, care and use of clothing for protection against heat and fire
2675	Portable first aid kits for use by consumers

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2865	Safe working in a confined space
2931	Selection and use of emergency procedure guides for the transport of dangerous goods
3765	Clothing for protection against hazardous chemicals (series)
4360	Risk management
4501	Occupational protective clothing (series)

Codes

NOH&SC:2012 (1994): National Code Of Practice For The Labelling Of Workplace Substances, (National Occupational Health and Safety Commission).

Manuals

World Health Organisation, Laboratory Biosafety Manual, 3rd edition, Geneva 2004.
Crime Scene Investigation: A Guide for Law Enforcement, NIJ Research Report, January 2000.

3. Definitions and abbreviations

For the purpose of this Standard, definitions and abbreviations are included in Appendix A.

4. Underpinning principles

4.1 General

Physical material (i.e. real evidence) is available at most, and perhaps all, incident scenes*. Recognition, collection and subsequent management of physical materials shall be undertaken to ensure that:

- Recovery is maximised.
- The integrity of the physical material is not compromised.
- The potential for contamination is minimised.
- Evidence continuity is maintained
- The potential for analysis is maximised.

Evidence must be gathered legally (see Appendix B) by appropriately qualified persons. Examinations of persons should be carried out with consideration for the dignity and comfort of the person involved.

4.1.1 Evidence recognition

Forensic evidence depends upon investigators, forensic officers and health professionals recognising physical material at a scene as having potential probative value. This in turn relies on written protocols and the training and experience of the personnel involved. Information from the investigator, the complainant and/or witnesses may assist in forming an initial hypothesis of what material may be relevant but should not inhibit the formation of alternative hypotheses if an initial examination of the scene suggests them.

* Note: Scenes include secondary scenes which in turn include the physical examination of persons involved in an incident as well as the examination of any secondary locations.

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The success of analytical processes applied to physical material depends on recognition, recording and recovery of adequate physical material.

4.1.2 Preservation of material

From the point at which physical material is considered to be of potential evidentiary value, the preservation of the material becomes of paramount importance. Material shall be recovered and stored in such a way to minimise the risk of loss, deterioration, contamination or alteration, to ensure that the conclusions based on any scientific examination of the material are reliable and verifiable.
Forensic material shall be collected and stored as outlined in this Standard, prior to analysis, with consideration of the following variables:

- OH&S considerations for the examiner at the scene
- The type of material;
- The size or quantity of material required for analysis;
- The environment or matrix in which the material is situated;
- The persistence or volatility of the material;
- The longevity of the material;
- The storage requirements; and
- The preservation of material for re-examination.

5. Systematic approach to scene examination

5.1 First responses

First responders are usually police but may be paramedics or other emergency services. Preservation of life and safety, including that of the first responder, is paramount, but as far as possible physical evidence should be preserved.
If specialist police assistance is required, the first responder should:

- Keep track of any victims still alive and arrange any required emergency medical management;
- Make initial observations of the scene location and condition;
- Ensure the scene is clear of offenders and secured against unauthorised entry;
- Assess any possible hazards;
- Minimise any contact with potential evidence;
- Assess possible entry and exit points;
- Take appropriate notes of anything of possible interest to the informant;
- Start a scene log; and
- Brief the informant on their arrival.

Notes should be permanent.
The person in charge of the scene, at that stage, may then call forensic specialists and/or suitably qualified health professionals for assistance.

5.2 Scene examination

Scene examination (of a place or person) is the first step of the forensic science process and the treatment of an incident scene predetermines the quality and quantity of information available for the investigation and ultimately the information available as evidence in court.

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The different types of material encountered require different practitioner skill sets to ensure that recovery of material is appropriate to the circumstances. The overall approach to recovery shall be:

- Systematic;
- Thorough;
- Planned; and
- Documented.

Contemporaneous notes shall be taken when attending a scene. Access to any scene location should minimise scene disturbance. If possible, all known details of the incident should be obtained from first responders and investigating officers. This information shall be recorded and should include:

- The nature of the incident;
- The exact location, time and date of the incident;
- The location of any secondary scenes; and
- Information regarding who has been in the scene and for what purpose (and whether anything has been moved or touched in any way).

Details of any associated suspects, complainants or witnesses may be also be of relevance.

5.2.1 Examination plan

A plan should be developed for conducting the scene examination. Such a plan shall be fit for purpose. It should consider:

- Whether any existing cordons are adequate;
- Any specific OH&S hazards associated with the scene or material and the level of personal protective equipment to be used during the examinations. The protective equipment should prevent contamination of the scene as well as protect the examiner. See Clause 6 for further information on OH&S issues;
- Conducting a risk assessment of the situation and generating a safe entry and escape plan from the scene if the location so warrants;
- The areas within the scene that require examination;
- Whether extra coordination, specialist advice or specialist assistance will be required;
- What examinations are to be conducted in situ;
- Whether any special equipment is required;
- What types of material may need to be collected; and
- The specific requirements for collection of material:

Consideration should be given to prioritisation as follows:

- Collection or protection of any material at risk of deterioration or loss;
- The collection of any material that will enable safe access to any deceased remains or any other critical area of an incident scene along entry and exit paths;
- Any critical areas that may render the most relevant material or, once processed, enable removal of any deceased remains; and
- Assessment of any area or material that may provide an early indication as to the identity of the offender(s).

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Consideration should also be given to victims, property owners and others involved in the crime. Examinations shall be carried out in such a way that ensures that damage to property is minimised. Discarded or used equipment and material shall be collected, bagged and removed before leaving the scene and a location for such collection planned in advance.

Where the scene examination is an examination of a living person, the health and wellbeing of the person shall be considered and where the scene is a deceased person consideration should be given to the dignity of that person.

5.2.2 Scene Walk-through and Initial Examination

The crime scene examiner should conduct a preliminary walk-through of a scene location and prepare preliminary documentation of the scene as observed unless doing so may compromise safety or the evidence. The scene plan should then be reassessed and may need to take into consideration such things as new facts emerging, changes in the weather and new material located at the scene.

Further consideration may then be required of the resources needed to fully process the scene, the order of examinations and the order of evidence collection.

5.2.3 Scene Management

At complex scenes, whether a location or a person, the examiner may be required to coordinate a number of specialists and/or a number of functions. Appendix C lists possible functions and specialists that may be of assistance at crime scenes. The order in which specialists are utilised and functions carried out should be thoroughly planned to allow optimum recovery of items of possible evidential value.

At some scenes a forensic specialist may be in charge with generalist crime scene examiners as assistants. Examples include arson scenes and clandestine laboratory investigations, at least in some jurisdictions.

A crime scene examiner with appropriate expertise and experience, shall be appointed in charge of the examination of the scene location. That person must be satisfied that all aspects of scene recording, evidence collection and sample packaging have been satisfactorily completed, by all persons involved, before leaving the scene.
Where the scene is a person, coordination of these specialists and functions may be the responsibility of the physician or pathologist rather than a "crime scene examiner".

Crime scene recording and evidence collection may then begin as outlined below.

6. Occupational Health & Safety, Hazardous materials

6.1 General

Collecting material at a scene for forensic examination often involves dealing with hazardous materials. In all cases, local jurisdictional OH&S requirements shall apply. Unidentified materials shall be handled in such a manner as to avoid ingestion, inhalation and dermal exposure.

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Should the crime scene officer suspect the presence of any highly toxic, highly irritant or explosive material, all personnel shall retreat from the scene and appropriate experts notified (such as Hazmat or bomb/explosive experts).
There are many other situations where suitably qualified personnel may be required in order for the examiner to safely enter a scene. Examples include;

- Firearms shall be rendered safe by a person with appropriate expertise;
- Where buildings have structural damage, the building shall be declared safe by an approved building engineer or other suitable person;
- Where electrical hazards are suspected, the power supply shall be disconnected;
- When examining the scene of a train death the examiner should be escorted by railway personnel and the line closed;
- When attending the scene of an industrial death, any equipment or machinery involved shall be turned off and disconnected from the power supply and all hazards rendered safe by suitably qualified personnel; and
- When examining a sexual assault victim in a prison, security staff may be required.

Specific Standards apply when working in a confined space. Only trained and authorised personnel should work in confined spaces. Some aspects of working at heights may be impacted by jurisdictional regulation and require installation or operation of equipment by properly authorised persons.

6.2 Transport of dangerous goods

The ADG Code* is a reference document setting out detailed technical and procedural requirements, provisions and guidelines for the labelling, packaging and transportation of dangerous goods by either road or rail. The Code is supported by a legislative framework consisting of a Model Act, and a model set of regulations. Some dangerous goods including substances that are liable to explode or are flammable or corrosive shall not be carried on commercial aircraft.

6.3 Protective clothing

At scenes on or near roadways or railway lines, reflective or high visibility vests should be worn.

AS/NZS 2243 provides guidelines and requirements in the appropriate use of protective clothing when handling hazardous materials in a laboratory setting and shall also apply to handling hazardous material at an incident scene.
The level of PPE used at an incident scene shall be selected to protect the examiner from exposure to hazardous material, and minimise the risk of contamination of forensic material or infection of others by material from the examiner.
NOTE: See Clause 2 for a list of applicable Standards and codes that shall be applied in the provision and maintenance of clothing and equipment and as reference material for the preparation of operating procedures.

* Australian Code for the Transport of Dangerous Goods by Road And Rail (ADG Code), Seventh edition 2007, Commonwealth of Australia, ISBN 1 921168 57 9.

6.4 Biological Hazards

6.4.1 General

Any material that emanates from a person or animal (living or dead), such as body fluids or tissue, or material that has been in close contact with humans or animals, shall be considered as a potential biological hazard. Bacteria, fungi and moulds may also be encountered during botanical and other investigations. The universal precautions approach utilised with biological materials may, however, not be broadly practicable or warranted based upon risk assessment principles. The circumstances of the case will often provide information regarding potential hazards that may be present.

6.4.2 Handling biological material

AS/NZS 2243 provides guidelines and requirements in the appropriate use of PPE when handling biological material.

- PPE should include protective gloves, goggles and suitable overalls, aprons or gowns and masks;
- Appropriate use should involve careful removal of contaminated or damaged gloves, avoidance of contact with exposed areas of the body, no eating, drinking or smoking when wearing PPE and not handling equipment whilst wearing potentially contaminated gloves;
- The creation of droplets and aerosols shall be minimised;
- Contaminated materials or equipment shall be decontaminated prior to reuse;
- Hands shall be washed after removing protective clothing and gloves; and
- Persons with exposed cuts or abrasions shall not handle biological material without a protective dressing.

An emergency or contingency plan shall be developed at incident scenes or other unfamiliar environments.
Spills or accidental stains involving hazardous biological material shall be cleaned promptly with a suitable disinfectant. The disinfectant should also remove/denature DNA to a point where it is undetectable.
In the event of exposure to body fluids or a breach of PPE, the affected areas shall be suitably disinfected. First aid and safety personnel should be notified. If appropriate, medical attention should be sought and where possible, contaminated material should be tested for infectious agents, e.g. HIV, HepB or HepC.
Procedures for the management of biological spills and exposures shall be documented.

6.4.3 Mortuary safety

Forensic officers attending a post-mortem shall adhere to the procedures of the mortuary for handling hazardous biological materials and shall not conduct any examination without the permission of the mortuary staff.
Attendance and roles shall be documented.
All staff shall assume that all tissue, blood and bodily fluids are potential sources of infection, independent of diagnosis or perceived risk.
PPE shall be worn and used by forensic officers whilst attending a post-mortem examination, as outlined above in Clause 6.4.2 and shall be donned before entering the examination area.

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Before leaving the examination area, protective clothing shall be removed and placed in the receptacle(s) provided.

6.4.4 Biological decontamination

Contaminated equipment shall be disinfected as soon as possible. If necessary, the equipment manufacturer's advice should be sought regarding compatibility of disinfectants with surfaces or functions. Surfaces exposed to potential contamination should be decontaminated on a regular basis. Any contaminated equipment that requires service or repair shall be cleaned and disinfected before being sent to the maintenance service provider.

Decontamination procedures should also remove/denature any DNA present to a point where it is undetectable.

6.5 Chemical hazards

6.5.1 General

Where unknown or unidentified materials are collected for analysis, protective clothing and equipment shall be worn to limit possible hazards.

Where the identity of a chemical being used, collected or analysed is known, the relevant MSDS should be consulted prior to using or coming into contact with that chemical. If hazardous, appropriate PPE shall be used and procedures followed.

6.5.2 Handling of chemicals

AS/NZS 2243 provides guidelines and requirements for the appropriate handling of chemicals, along with precautions that shall be followed.

When handling hazardous chemicals, staff shall be appropriately trained and/or supervised and appropriate equipment employed.

All forensic facilities shall have documented procedures for the management of chemical spills at a scene. In the event of a spill, the documented procedures shall be followed.

If chemical exposure occurs, follow the appropriate procedures as outlined in the MSDS. Notify a first aid qualified person and if appropriate, seek medical attention at an emergency department as soon as possible and provide a copy of the relevant MSDS(s) to emergency staff.

6.5.3 Labelling of chemicals

Chemical solutions used in the field shall be stored in their original containers or in appropriate bottles with adequate labelling as per ISO/IEC 17025 and its accompanying NATA Field Application Document.

6.5.4 Storage of chemicals

Chemicals, including poisons and drugs, shall be stored in a manner that complies with any relevant statutory or legislative requirements and manufacturer recommendations. Relevant hazard warning and safety signs shall be used;

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Different classes of chemicals shall be stored separately, in suitable cabinets, as required.

Explosive materials (Dangerous Goods Class 1) shall be stored as required by legislation.

Chemical store rooms in a facility containing large volumes of volatile or gaseous chemicals should be fitted with a hazardous atmosphere alarm. This also applies to any forensic store used for the storage of large volumes of seized chemicals.

6.6 Physical hazards (sharps)

When handling sharp objects, the examiner should take necessary precautions as outlined in Appendix D.

WARNING: Due to the inherent risk, syringes should only be collected in major cases where their examination is considered essential to the investigation.

6.7 Radiological hazards

Radiological hazards may be encountered, for example, when examining bomb scenes or when investigating illicit or trafficked nuclear materials or radiation sources.
Note: There is a requirement to be licensed to possess some radiation sources.

Legislation and regulatory requirements shall be complied with.

Should radioactive material be encountered or suspected at an incident scene, ARPANSA shall be contacted for advice on managing the scene.

6.8 Electromagnetic radiation hazards

Appropriate eye protection is required when any high-intensity light source is employed, regardless of the wavelength of operation. When using ultraviolet light, protective UV-rated eyewear shall be worn by the operator and any other person in the vicinity; where possible exposed skin should be covered.

6.9 Disposal of waste

AS/NZS 2243 provides guidelines and requirements for the appropriate disposal of biological and chemical waste.

Bagged waste should be appropriately labelled. The use of a licensed disposal contractor should be considered.

7. Recording material in situ

7.1 General

Recording the condition, position and location of material prior to collection provides information and context.

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7.2 Notes

Notes should be contemporaneous. They should be uniquely identifiable and should include sufficient detail to enable the examiner to accurately report a scene location and description, who took the notes and when, who examined what and when, what material was collected and where it was transported, and any limitations to the examination or evidence collection.

It shall be apparent if any notes are altered or missing. Further guidelines are given in Appendix E.

Audio and audio-visual devices may be used to record observations and actions at a scene. The recording should include the same level of detail that would be included in written notes.

7.3 Diagrams and plans

The circumstances and positions in which material was located at a scene may be recorded with a scene diagram. Similarly diagrams may be useful in recording the positions of wounds on victims, damage on a car etc.
Such diagram may be used to specifically describe:

- The location of the material of possible interest in situ prior to collection or examination;
- The location of such material relative to other objects present or other material of interest; and
- The environment within which the material was located.

Diagrams may either be to scale, sketched without a complete scale or be pro-forma diagrams, and these can serve to enhance written or verbal notes.

Scale plans may be used to accurately convey the size, shape and position of significant objects and other features of an incident scene. Triangulation may be used as a method for recording the position of material at scenes. Accurate scale diagrams can also be produced using specialised imaging equipment such as laser systems or photogrammetry.

Sketch plans of the incident scene should enable all material of relevance to be located at the scene or on the body and the relation of such material to other objects and material of relevance to be investigated. Such plans will generally be designated as not to scale and contain a reference direction.

Where possible, the position of bodies and significant forensic material shall be recorded prior to removal, collection or alteration. If objects must be moved prior to measurement, they shall be photographed and their location shall be marked prior to displacement. The position of any fixed objects may be recorded at later time.

7.4 Photographic recording

7.4.1 Digital photography

Any digital format used shall be sufficient to allow accurate representation and recording of the scene according to the intended purpose.

It should be remembered that primary photographic records are evidence, may become exhibits and must be traceable at all times.
Where appropriate, the image quality should be determined and validation tests carried out to ensure suitability for the intended purpose. The digital image format (including any compression algorithm) should be fit-for-purpose.
The digital camera shall be fit for purpose.

7.4.2 Digital image capture

A primary photographic record of a scene should comprise a range of photographs taken over various distances. The photographs should which include reference points, show the position of material relative to the broader scene and/or relative to other objects within a scene, and contain close-up images that record the condition and detail of relevant material prior to collection. Similar considerations apply to photographs of persons.

NOTE: Markers for objects of interest should be used where required and an appropriate size scale should be placed within the field of view of photographic images where physical measurements are relevant to the scene examination.

Images should be of a quality fit for the purpose for which they are used which may include enlargement of the images. The use of a macro lens is recommended for photographing detailed images such as wounds, fingerprints and other marks. Images requiring accurate scale printing (such as 1:1 images for fingerprint identification purposes) shall include a visible scale within the frame.

After capture, images shall not be deleted or erased from the short-term media until after the Primary Images have been saved to long-term storage media (see Clause 7.4.3).

7.4.3 Digital image storage

To allow successive images to be captured, digital cameras store image data to an inbuilt drive or removable (short term) storage media.

Primary Images shall be saved to suitable long-term media as soon as is reasonably possible. When it is foreseen that there will be an extended period of time prior to saving Primary Images to long-term media, then the short-term storage media shall be stored appropriately to ensure its integrity until the long-term storage can be carried out. If this occurs then it shall be documented.

Where a CD or DVD is used as the Primary Image storage, the readability of the CD or DVD copy should also be checked prior to storage. The media shall be clearly identified and stored so as to ensure the integrity of the digital data.

Where a centralised hard disc drive (HDD) backup is used for primary image storage, access and levels of access to those images shall be strictly controlled.

Enhancement of a Primary Image shall not be carried out prior to the image being saved to long-term storage media. Image processing (including minor enhancements) should only be performed on working copies once Primary Images have been safeguarded.

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7.5 Video recording

The requirements for image recording specified in Clause 7.4 shall apply to video recording.

Precautions shall be taken to stop the overwriting of any video images.

8. Item collection

8.1 General

Collection of samples from a person is relatively well prescribed. The relevant samples from a sexual assault victim, for example, will depend on the assault but will generally comprise clothing and a number of biological samples from an accepted set of options. Appendix F is an example that outlines the processes used in one jurisdiction. A post mortem may lead to a cause of death in a relatively straightforward manner but on occasions, the cause may be quite elusive and require the taking of unusual specimens or the detection of rare substances. Similarly, recognition of what to collect from a homicide scene may be straightforward or may require considerable thought and experience.

Witness statements or information from the informant may be a good starting point for formulating hypotheses on what may have happened and therefore what to collect from where. Such information should never, however, be taken as certain and alternative hypotheses should always be considered.

The examiner (or specialist expert) should always look for anything that may be out of place, as well as collecting the material that is dictated by the initial hypothesis.

8.1.1 Collection

The examiner shall:

1. Collect a representative sample of the material that is sufficient for the examinations required (with provision for sufficient material, where possible, for re-analysis at a later time and testing on behalf of the defence). Where appropriate, the forensic facility should have written policies on what constitutes a representative sample;
2. Attempt to avoid examining, collecting or recording material that is not relevant to the investigation, or does not provide information relevant to the investigation (but see clause 8.1 above);
3. Place collected material in appropriate packaging;
4. Label material collected following the required guidelines (see below);
5. Record the collection of material in contemporaneous notes; and
6. Ensure continuity of the material to the site of analysis or hand the material to the investigating officer (or the person specified under the conditions of the search warrant, if applicable).

8.1.2 The use of enhancement techniques at crime scenes

Material of potential evidential value may be latent or poorly visible at some crime scenes. Enhancement techniques may be used to enable the material to be made more readily visible before sampling.

Visibility may be enhanced by the use of oblique lighting or light of particular wavelengths coupled with appropriate filters. When using complex equipment capable of emission over several wavelength ranges, the equipment shall be adequately maintained and suitable controls used to demonstrate correct operation.

Chemical enhancement reagents should be suitably labelled. Suitable control samples should be used to assure the efficacy of the reagents. In cases of reagent failure, that failure should be noted and sufficient details of the reagents and controls should be apparent to allow their manufacture to be traced.

8.1.3 Presumptive tests

Presumptive are usually inexpensive and transportable so they can be deployed into the field and applied by appropriate personnel without extensive training. Presumptive test results are not normally acceptable as reliable evidence in a judicial setting without confirmation. Nevertheless, appropriate quality assurance measures should be employed as in Clause 8.1.2 above.

8.1.3.1 Presumptive chemical or enzymatic tests

Tests for Biological Material

Presumptive enzymatic or chemical tests are routinely employed to detect the possible presence of biological material or where traces may be present, but not visible to the naked eye. Examples include the luminol test for bloodstains and acid phosphatase test for seminal staining. These tests react with the haem in blood to produce chemiluminescence and with an enzyme in semen to produce a purple colour respectively. Both are subject to interference from unrelated chemicals including bleach and/or some plant materials.

Further examples are the immunological kits for detection of biological warfare agents where kits may react with closely related bacteria as well as target organisms.

Tests for Chemical Material

Such tests are dependent on the presence of particular functional groups present in the chemical structure of the analyte and therefore chemical colour tests will often be specific for classes of drugs. In addition, the specificity of particular colour tests is affected by the type (and prevalence) of functional groups the test reacts to.

The actual colour observed when a chemical colour test returns a positive result depends on many factors, such as the concentration of the analyte, the chemical form of the analyte (salt or free base) or the presence of interfering substances.

8.1.3.2 Instrumental Techniques

Instrumental presumptive tests are either limited by their specificity (e.g. TLC, UV, IMS) and/or they are prone to interference when encountered as a mixture of compounds (e.g. IR or Raman Spectroscopy). Such tests are nevertheless invaluable for screening purposes in the field and may have similar laboratory use.

8.1.3.3 Presumptive Tests in Comparative Testing

A number of areas of forensic examination rely not on chemical or biological analysis but on pattern comparison by a trained expert. In these areas the equivalent of

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presumptive chemical or enzymatic tests is the quick exclusion on class characteristics. Examples include the elimination of the complainant's latent fingerprints at a scene and the assessment of a bullet as being a different caliber to a questioned weapon.

8.1.4 Items generated at the scene

Fingerprints, bloodstain patterns and two-dimensional shoeprints, for example, may be photographed where the photograph is of a sufficient quality to be examined in lieu of the material itself. In these instances, the photograph becomes the forensic evidence and continuity of the image must be maintained. The photograph shall be of a suitable quality and fit-for-purpose.

On the other hand fingerprints may be lifted, for example, onto adhesive tape and footwear impressions may be lifted using a gel lifter or by other means. Under those circumstances the lift becomes the forensic item.
Similarly three-dimensional shoeprints and toolmarks may be cast at the scene and the cast then becomes the forensic item.

8.1.5 Collection of physical material from the scene

To maximise preservation, if physical material (see Appendix A) has the potential to be lost, damaged or contaminated, it should be collected as soon as possible. To ensure that all physical material is properly collected, the type of examinations that the material will be subjected to shall be taken into consideration.

If practicable (or necessary) all of the evidential material present should be collected. In some cases, a sample (or samples) of the whole may be collected, provided that the samples are representative of the whole and sufficient for all the examinations that may be required.

The use of an appropriate sampling technique is particularly recommended when dealing with large quantities of hazardous materials, controlled substances or dangerous goods.

8.1.6 Minimising cross contamination

Care shall be taken to avoid cross contamination between involved persons, victims, physical material, facilities, equipment the collector and/or scenes. The following precautions shall be followed where there is potential for cross contamination:

- Gloves shall be changed between the collection of each item or sample;
- Masks shall be worn;
- Collection equipment and packaging shall be sterile and free of detectable DNA contamination;
- Collection equipment (disposable forceps, swabs, etc.) shall be discarded after each use. If this is not possible, then the collection equipment shall be cleaned and disinfected after each use;
- Victim and suspect samples shall be collected separately (i.e., separated by time and space); and
- PPE shall be changed between scenes (locations and/or persons).

Facilities in which examinations of persons are conducted should have adequate and documented cleaning policies and procedures, should minimise the number of persons accessing examination areas and should have secure areas for the storage and handling of equipment and, where necessary, samples.

8.1.7 Radiological material

In any situation where the presence of radiological material is suspected or confirmed, the recommendations contained within the ARPANSA publication "National Directory for Radiation Protection, April 2010, Radiation Protection Series Publication No. 6" shall be followed.

The recommendations contained within the ARPANSA publication "Intervention in Emergency Situations Involving Radiation Exposure, Radiation Protection Series Publication No. 7" shall be followed.

8.2. Sampling protocols

Depending on the type, physical materials may be collected using one or more of the following methods.

Note: If re-usable implements are used they must be thoroughly cleaned between each use.

8.2.1 Handpicking

Macroscopic material may be collected by handpicking using forceps.

8.2.2 Lifting

Tape-lifts may be used to collect trace material such as fingerprints, DNA, hairs or fibres. Gel lifts may be useful for shoe impressions. The lifting material shall be clean and contaminant free prior to use.

8.2.3 Sweeping

Sweeping may be used to collect material from inaccessible areas or where there is a mass of dispersed material. The brush shall be clean and contaminant free prior to use.

8.2.4 Vacuuming

Vacuuming may be used to remove trace material from inaccessible areas or where there is a mass of dispersed material. Where vacuuming is used, it shall be conducted using specialised equipment with a purpose built sample collection chamber. Equipment shall be clean and contaminant free prior to use.

8.2.5 Swabbing

Swabbing is generally the preferred sampling method for biological stains (e.g. blood, semen, saliva). Swab kits shall be contaminant free. Swabbing may be used for dry or wet stains. When swabbing dry stains or surfaces, the swab may need to be moistened with a sterile solution (e.g. distilled/deionised water).

Swabbing may not be the optimal method for sampling from absorbent substrates such as textiles.

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8.2.6 Cutting and scraping

A sample may be collected with a clean scalpel, by scraping the stain from the object into a paper envelope or sample tube.

For materials such as carpet and fabric, a piece of the stain may be cut out and placed in a suitable container.

8.2.7 Direct sampling

For larger absorbent surfaces, a piece or the whole of the surface bearing the material to be examined may be collected.

Where large amounts of fire debris are present at a scene, the relevant samples may be shovelled into an appropriate container.

Plants may be removed by hand directly from the soil or hydroponic cultivation.

8.3 Deceased remains

The examination of deceased remains may be at a scene, as part of an exhumation, or as part of a post mortem examination at a mortuary.

Deceased remains shall be examined in a systematic manner. The environment, position and state of the body, as well as physical damage and injuries, shall be recorded.

The deceased's head, jaw, hands and feet should be protected, as circumstances require, to preserve trace material and body parts from loss.

8.3.1 Post mortem attendance

The forensic examiner may be required to attend the post mortem examination performed by a forensic pathologist.

The forensic examiner shall maintain close liaison with the forensic pathologist prior to and during the post mortem examination, particularly for collection of specific samples or the carrying out of specific forensic procedures.

If required, fingerprint impressions shall be collected from the deceased remains using an appropriate method.

Photographs taken at the post mortem should be taken at a range of distances to show the entire body clothed (or as recovered from the scene) and unclothed. Injuries, marks or other material of interest on the deceased should be photographed to show any relationship between them. Close-up photographs of injuries, marks or other material of interest on the deceased should be taken. Appropriate scales should be included as required.

The forensic pathologist or other expert present may also request further photographs to record the post mortem process.

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8.3.2 Exhumations

Exhumations shall be conducted systematically, applying the same practices and considerations as apply with any forensic examination. Where appropriate, a grid should be applied to the area of the exhumation to aid in the systematic examination of the area and the systematic removal and examination of soil layers

8.4 Collection of material from a person

When collecting samples or material from a person, deceased or living, a strict protocol of use of PPE shall be followed to ensure protection from hazards and to minimise the risk of cross contamination.

Non-intimate samples shall be collected by authorised persons.
Where intimate samples, including photographs of intimate areas, are required from a person, these shall be taken in accordance with the relevant Forensic Procedures legislation in each jurisdiction.

8.4.1 Biological material

8.4.1.1 Sexual assaults

Examinations of victims of sexual assaults shall be carried out by qualified and suitably trained medical personnel. The associated forensic material should be collected as soon as possible.

Forensic facilities should follow written protocols and procedures.

All material shall be collected in accordance with these procedures and any jurisdictional legislation.

All equipment used in sample collection shall be clean and contaminant free prior to use. Where possible a forensic medical examination kit should be used.

8.4.1.2 Buccal swab

DNA reference samples should be collected using a buccal swab sampling kit where possible.

8.4.1.3 Fingernail trimmings and scrapings

Material such as skin or blood may be collected from under the fingernails of a victim, a suspect or a deceased person. If possible, fingernails may be trimmed and the cuttings collected. If trimming is not possible, scrapings or swabbings from underneath the nail may be collected.

Scrapings and trimmings from each hand shall be placed in separate containers.

8.4.1.4 Hair collection

Hairs may be of animal or human origin. Hairs are usually visible and should be collected by hand, noting their location and any special features such as how they may be physically retained. Short hairs or hair fragments, or hairs in difficult to access locations may be collected using a variety of recovery techniques including tape lifting and the use of specialist vacuum equipment.

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The value of hair examinations is often determined by how representative and adequate the known samples are. Sampling of a known donor for the purpose of hair comparisons should take into account visible variation in length and colour. As human hair has inherent variation at a microscopic level both within individuals and between individuals, known samples need to be adequate and include both combed and plucked hairs to ensure all growth phases are represented.

The roots of plucked hairs may also serve as a DNA reference sample where a buccal swab cannot be obtained.

8.4.2 Other material associated with a person

8.4.2.1 Collection of fingerprint impressions from a person

Fingerprint impressions collected from an individual should include one print taken from each available finger that has been rolled from one edge of the nail to the other, plain impressions of each of the four fingers of each hand, and plain impressions of each thumb. Palm impressions may also be taken, depending on jurisdictional practices. Fingerprints may be collected by digital scanning technology or by using ink on paper cards.

8.4.2.2 Entomological samples

Live and preserved samples of each maggot type should be collected. Samples from inside the body should not be collected until the post mortem (see Appendix G).

8.5 Packaging and labelling of physical material

8.5.1 General

The principles of preservation and minimising the risk of alteration or contamination shall be considered when packaging and labelling forensic material. The material shall not be packaged in a way that inhibits subsequent analysis and handling shall be minimised before analysis.

Note: If items are handed to the investigating officer as part of evidence collection under warrant, an extra contamination risk is entailed and consideration should be given to the use transparent packaging or packaging that contains transparent windows if possible (for information on powers of search and seizure see Appendix B).

8.5.2 Packaging of forensic material

Any hazardous samples collected shall be placed in a sample container appropriate for the material and shall be marked with an appropriate warning label to alert others of the potential hazard.
The procedures for the packaging of items are as follows:

- Each item shall be individually packaged in an appropriate container and sealed as soon as possible.

  Note: Items already mixed at the scene may be collected and packaged together as, for example, "Contents of rubbish bin";" Contents of ashtray" and so on.

- Each item package shall be individually labelled with a unique identifier;

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- A container used for packaging is properly sealed only if its contents cannot readily escape or become contaminated and only if the subsequent opening of the container is detectable due to alteration to a tamper-evident seal. Examples of how items may be sealed are as follows:
    - Tamper evident tape is placed over the container opening. The tape shall be signed/initialled and dated by the person sealing the item;
    - Items may be heat sealed in plastic with the signature/initials/date of the person sealing the item across the seal in indelible ink.
- Items shall not be handled unnecessarily.

Sealing large items (such as motor vehicles) may be impractical or inappropriate. In such cases, the area of the item that is subject to examination shall be protected from loss, deterioration or contamination by appropriate use of packaging and an appropriate seal or cover. Such items shall be stored in controlled access areas such as a locked garage or impound with logged access.

For packaging guidelines for different types of forensic material, refer to the relevant Clause in this Standard and Appendix H.

8.5.2.1 Inappropriate packaging

In cases where material is submitted for examination in inadequately sealed or in inappropriate packaging, the integrity of the evidence shall be assessed to determine whether it has been compromised. Any report produced shall include a description of the inappropriate packaging and any impact on the integrity of the evidence.

If accepted by the forensic facility for examination and/or analysis, the submitted material (along with the original packaging) shall be retained and sealed within new packaging.

8.5.3 Labelling of forensic material

The outermost packaging of all items shall bear a unique identifying label which allows the chain of custody to be tracked either by an electronic tracking system or by an item log or by recording transfers directly on the label. The chain of custody of all items shall be complete and unedited.

The label shall alert the handler to any hazards associated with the item and shall allow directly or indirectly through the tracking system, the following details to be retrieved:

- A description of the item;
- Whether the item contains any hazard (e.g. biohazard, sharps);
- The location the item was collected from;
- The date and time the item was collected;
- The name or identifier of the officer who collected the item; and
- The case file or investigation the item relates to

Labels shall not be placed directly on the item being collected.

Labels should be placed on the side of a container, not on a container's lid (unless the lid is permanently fixed to the body of the container).

Labelling may also include any specific storage or handling requirements.

9. Item storage and security

All forensic facilities shall have documented acceptance and rejection criteria in place that ensure that the integrity of forensic material is protected and maintained. The criteria shall cover the requirements for packaging, transport and storage of forensic material and shall specify under what circumstances an examination request is rejected.

9.1 Item documentation

All required documentation (whether digital or hardcopy) should be complete prior to submission and should include or be linked to the following information:

- The type of forensic examination being requested (include all if more than one examination is requested);
- A list of items submitted (and their respective item identifiers);
- Whether an item contains any hazard (e.g. biohazard, sharps);
- A unique case identifier;
- The case or investigation the item relates to;
- The date and time each item was collected;
- The location the item was collected from;
- The name or identifier of the officer who collected the item;
- The name or identifier of the requesting officer;
- The name or identifier of the submitting officer;
- Contact details for the officers involved; and
- The names of any suspects, victims or complainants.

9.2 Item continuity

Continuity of possession shall be maintained and recorded between sampling at the scene and acceptance for analysis or examination by the forensic facility. Where possible, items should be transported to that facility with a minimum of handling.

In addition to identifying and tracking items being submitted for examination, the movement of items after acceptance by the forensic facility shall be managed (and recorded) via an item management system. At any point in time, the location of an item shall be known by either:

- The storage location within the facility; or
- The forensic officer who has possession of the item.

NOTE: For the purposes of this Standard, a 'storage location' is defined as a secure area within a forensic facility, with controlled access, that is designated for the storage of forensic material. The purpose of a storage location may be for the storage of forensic material prior to (or after) examinations, or may be for the storage of forensic material while undergoing examination.

9.3 Security of items in storage

Access to forensic storage areas shall be by authorised personnel only and all access to forensic storage areas shall be logged and auditable.

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9.4 Specific storage requirements

See Appendix G for additional information.

9.4.1 Biological material

Biological material collected from a scene should be transferred as soon as possible to a securelocation with appropriate storage facilities.
Items that may deteriorate due to exposure to moisture shall be dried (in a secure area, see Clause 9.3) prior to storage.
Dry items may be stored at room temperature.
The following refrigeration and freezer parameters should be followed when storing wet items and tissue samples at a facility for forensic purposes (for deceased remains, see Clause 9.4.2).

- Short term storage (less than a week): +2 to +6°C.
- Medium term storage (less than a month): temperature at or below −20°C.
- Long term storage (greater than a month): temperature at or below −80°C.

All refrigerators, cool rooms and freezers used for biological tissue storage for forensic purposes shall either be:

- Monitored by a temperature recorder; or
- Equipped with a temperature alarm.

Further guidance is available in AS 3864.

9.4.2 Deceased remains*

Bodies shall only be held in a body storage facility for a period of time determined by jurisdictional legislation (subject to the policies of particular facilities).
A body storage facility shall be maintained at a temperature between +2 to +6°C.
If long-term storage is required, the body should be maintained at approximately −20°C or preserved by embalming. Skeletonised deceased remains may be stored in a secure room.

Jurisdictional requirements and legislation for the storage of biological specimens and tissues from a deceased person shall be followed, including specific procedures for the retention and storage of organs.

9.4.3 Botanical material

Botanical material should be stored away from direct light at room temperature.

9.4.4 Entomological material

Live material shall be stored in a ventilated container in a refrigerator (between 4 and 6°C) while awaiting transfer to a forensic entomologist.
Live larvae may need to be reared in a growth chamber at the forensic entomologist's laboratory. The growth chamber shall be located in a secure restricted access area.

*See also: National Pathology Accreditation Advisory Council, Requirements for the Facilities and Operation of Mortuaries, Second Edition, 2009.

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9.4.5 Chemical material

Due to the nature of the different samples likely to be submitted for examination, each requires special considerations with regards to storage conditions. In addition to the guidelines in Appendix G the following considerations apply:

- Representative samples should be collected when large quantities of corrosive material require analysis. The relevant facility should have written protocols to ensure adequate sampling; and
- All material of a volatile nature that is to go into medium or long term storage should be refrigerated as soon as possible.

9.4.6 Drug and clandestine laboratory seizures

AS/NZS 4757 provides a generic procedure for the seizure, handling, storage, disposal and destruction of drugs and drug related materials for law enforcement purposes.
Due to the portable and attractive nature of drugs the ability to account for drugs and drug related material in their entirety is paramount.

9.4.7 Fire debris

The following procedures shall apply:

- The storage environment shall be separate from possible sources of ignitable liquid contamination (such as motor vehicles or bulk fuel storage).
- The storage environment shall be ventilated to avoid the build up of volatile compounds.
- Fire debris samples and ignitable liquid samples that are to go into medium or long term storage shall be refrigerated on receipt.

9.4.8 Explosives

Explosives in greater than trace amounts shall be collected by authorised persons and transported and stored according to the relevant commonwealth and state legislation*.

9.4.9 Firearms

WARNING: Firearms shall be rendered safe prior to being packaged.
When receiving a firearm item, it shall be checked by an appropriately trained person prior to storage to ensure that the firearm is unloaded.

10. Analysis and examination of items

Analysis and examination of evidential items is dealt with in a separate standard. Further information on the security of material in storage, retrieving material from storage and documenting and tracking the location of material is also to be found in that Standard.

* Australian Code for the Transport of Explosives by Road And Rail, Third edition 2009, Commonwealth of Australia, ISBN 978 0 642 32747 5.

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APPENDIX A: GLOSSARY OF TERMS

(Informative)

Definitions

Acid phosphatase	An enzyme found in high concentration in human seminal fluid
Accreditation	Formal recognition by an independent recognised body that has assessed the technical competency of an organisation to meet the requirements of a predetermined standard
Algorithm	As applied to image compression, an algorithm is a process or set of rules to be followed to minimize the data file size. Two types of compression algorithms are (1) Lossless: in which a decompressed file is of exactly the same size as the original (uncompressed) file because no detail or content is lost in compression-decompression process. (2) Lossy: in which a decompressed file is smaller in size than the original file because some detail or content (which may not be obvious to the user) is lost in compression-decompression process.
Bloodstain	The stain left by wet blood contacting a surface.
Caliber	The diameter of a cylindrical body, esp the internal diameter of a tube or the bore of a firearm.
Compact Disc Recordable (CD-R)	Write once read many times storage media.
Chemiluminescence	The emission of light during a chemical reaction that does not produce significant quantities of heat.
Chromatography	A technique for separating the components of a mixture on the basis of differences in their affinity for a stationary and a mobile phase.
Class characteristic	Aspects of a pattern that enable the exclusion of some possible sources but do not permit the assignment of the pattern to a unique source.
Contamination	Foreign material from a source not related to the event being investigated, deposited at the scene or on an item to be examined. Contamination can also refer to contamination between incident scenes, or between items collected. Whether or not the material is or is not related to the event being investigated may be difficult to determine.
Control Sample	A sample required for comparison against a questioned sample. Samples are taken as a means of establishing the normal condition or composition of an object or area. A subsequent difference between a questioned sample and a control sample can then be established and interpreted.
Controlled substance	A drug or chemical substance whose possession and/or use are regulated under relevant jurisdictional legislation.
Crime Scene	The original location where the crime occurred and/or where the majority of forensic material is located.
Crime Scene Examiner	Person responsible for the systematic processing of the crime scene and any related locations. Examination may include the taking of some samples from persons as authorized by the

	relevant jurisdictional legislation.
Dangerous goods	Substances specified in the ADG Code as dangerous goods.
DVD-R	Digital Versatile Disk Recordable: a serial data storage media type that has a higher data density than CD-R.
Entomology (forensic)	The study of insects (entomology) as applied to legal issues. It is most commonly used to determine minimum post mortem interval.
Enzyme	A substance produced by a living organism that acts as a catalyst to bring about a specific biochemical reaction
Enzymatic	Of or relating to or produced by an enzyme.
Exhumation	The disinterment of a buried body from its resting place, generally in a cemetery.
Fingerprint	An impression on a surface formed by the ridges on a fingertip. Similar impressions are formed by skin on palms and the soles of the feet.
Forensic	To do with, or related to the law. Thus forensic science is the application of the principle and practice of science to the needs of the law.
Forensic facility	A location, other than in the field, at which sampling and/or analysis is carried out. The term includes laboratories, mortuaries, patient examination rooms and extends to public weighbridges, interview rooms and other places where evidence may be gathered, analysed or measured.
Forensic material	Any material that is collected for forensic purposes.
Functional group	A specific group of atoms within a molecule that is responsible for the characteristic chemical reactions of that family of molecules
Haem	A complex red organometallic pigment, found in blood, to which oxygen binds.
Hazardous material	Any material that, because of its quantity, concentration, or physical or chemical characteristics, may pose a real hazard to human health or the environment, as defined by the criteria of Safe Work Australia.
Immunological	Of or relating to immunology, the branch of biomedicine concerned with the structure and function of the immune system.
Infra-red	Pertaining to radiation that is just beyond the red end of the visible light
Infra-red spectroscopy	The spectroscopic study of the interaction of matter with infrared radiation; used as an analytical tool to identify (mostly organic) compounds
Intimate sample	A sample collected from an intimate part of the body of a person (as defined in the relevant legislation) as part of a forensic procedure. An intimate sample or a physical examination of an intimate part of the body may only be taken or conducted by a person authorized under the relevant legislation.
Ion mobility spectrometry	An analytical technique used heavily for military or security purposes, such as preliminary testing for drugs and explosives.

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Item	Physical thing that is collected for forensic purposes.
Latent	Present but not visible, apparent, or actualized.
Luminol	A white, water-soluble, crystalline compound that emits light when reacted under appropriate conditions with a number of substances including blood.
Macro lens	A lens specifically designed for close-up photography and capable of good optical performance when used very close to a subject.
Materials Safety Data Sheets	Material Safety Data Sheets (MSDSs) provide relevant and current information regarding the identity, physical characteristics, safe storage, use and disposal of substances that are handled in the workplace. They provide information on whether a substance is deemed hazardous and whether it is classed as a dangerous goods item. MSDSs also include information regarding first aid treatment and spill management. Manufacturers, importers or marketers of substances are required to prepare and disseminate MSDSs.
Original Image	The Primary Image or an exact binary copy.
Oviposition	The process of laying eggs.
Photogrammetry	The process of making scale drawings and maps from photographs.
Paper Boat	Paper folded in such a way as to securely contain a small sample. Term used to describe a piece of paper that has been folded into thirds and then in from each side and is sometimes referred to as the druggist's fold. Material can be safely collected and stored inside the folded paper.
Physical Material	Forensic material, encompassing any and all objects, gross or microscopic in size, living or inanimate, solid, liquid or gas, including the relationship between all such objects as they pertain to a crime.
Post mortem examination	An autopsy examination of a deceased person in order to determine the cause of death and sometimes the identity of the deceased.
Precursor	A compound that participates in the chemical reaction that produces another compound
Primary Image	The first instance in which a digital image is recorded onto any media that is a separate, identifiable object.
Radiation	Particles or waves of energy emitted from unstable atoms.
Raman spectroscopy	A method that extracts information about the vibrational structure of a molecule; a complementary technique to infrared spectroscopy that can reveal vibrations that are infrared-inactive.
Reaction	A chemical change that forms new substances.
Reagent	A chemical added to an unknown material to produce an expected response.
Reference Sample	A sample that is of known condition, composition, consistency or concentration.
Sample	A portion taken from something that was not collected as a whole or a portion taken from a collected item, on which a test

	or analysis is carried out.
Scale Plan	Plans or drawings that show dimensional relationships of a scene, area or objects in correct proportions.
Secondary scene	A location linked to the crime scene that may also contain material of forensic interest.
Seminal stain	The stain left by seminal fluid contacting a surface.
Sequestrene tube	Blood collection tube containing EDTA as anticoagulant.
Sharps	Objects that have sharp edges or points that have the potential to cut, scratch or puncture the skin.
Shoeprint	An impression on a surface made by features on a shoe sole.
Sketch Plan	Sketch plans are free-hand drawings of areas or objects used as an aid to handwritten notes.
Solvent	A liquid that dissolves a solid, liquid, or gaseous solute, to produce a solution
Spectra	Singular, spectrum. Originally The distribution of energy emitted by a radiant source, as by an incandescent body, arranged in order of wavelengths. Now more generally a range of values of a quantity or set of related quantities.
Spectroscopy	Originally, the science of measuring the emission and absorption of different wavelengths (spectra) of visible and non-visible radiation. Now more generally the production and analysis of spectra.
Storage location	A specified secure area within a forensic facility designated as a storage area for forensic material.
Sub-sample	A portion taken from a sample, on which a test or analysis is carried out.
Substrate	Material that underlies or serves as a basis or foundation upon which other substances lie.
Toolmark	The mark (or indentation) left by a tool (or implement) on the surface of an object or a structure, when force is applied.
Working Copy	A digital copy of the Original or Primary Image.

Abbreviations

ADG Code	Australian Code for the Transport of Dangerous Goods by Road And Rail
ARPANSA	Australian Radiation Protection and Nuclear Safety Agency
CD	Compact disc
CD-R	Compact Disc Recordable
DNA	Deoxyribonucleic acid
DVD	Digital Versatile Disc
DVD-R	Digital Versatile Disc Recordable
EDTA	Ethylenediaminetetraacetic acid, which is also known as edetate, versene, or sequestrene.
HDD	Hard Disc Drive
HDPE	High-density polyethylene
HepB	Hepatitis B
HepC	Hepatitis C
HIV	Human Immunodeficiency Virus

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IMS	Ion mobility spectrometry
IR	Infra-red
MSDS	Material Safety Data Sheet
NATA	National Association of Testing Authorities
OH&S	Occupational Health and Safety
PPE	Personal Protective Equipment
TLC	Thin layer chromatography
UV	Ultra-violet

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APPENDIX B: POWERS OF SEARCH AND SEIZURE

(Informative)
Where the crime scene is a location, items are often taken with the consent of the owner. If there is no such consent, however, search powers must be used. These powers vary from jurisdiction to jurisdiction but in general will fall into one of three categories:

- Search powers under common law, as modified be by relevant case law
- Search powers pursuant to specific state legislation
- Search powers pursuant to a warrant

Under English common law as derived from Lord Denning's judgment in Ghani v Jones (1970) 1 QB 683, in general, if:

- Police officers have reasonable grounds to believe a serious offence has been committed
- They have reasonable grounds to believe that an article is material evidence to prove the commission of the crime
- They have reasonable grounds to believe a refusal to allow the item to be collected is unreasonable and
- They retain the article for no longer than is necessary for evidential reasons

they may take an article without a warrant, without making an arrest without consent of the owner and retain it for the duration of any legal proceedings. The above criteria must be met in full at the time of the search and not justified by what might later happen.

In Australia some judicial discretion applies to evidence gathered according to the above criteria and Australian law now differs slightly from the English in this respect. In similar fashion case law may result in slight variation from jurisdiction to jurisdiction within Australia.

Statute law may vary more widely from jurisdiction to jurisdiction.

Where there is no applicable legislation, a may be issued warrant authorizing a member of the police to search a building, receptacle, place or vehicle for anything in respect of an indictable offence which has been committed and for which there are reasonable grounds to believe will afford evidence relevant to that offence. Instructions from the issuing officer, guidance from case law and local policy will then dictate how the evidence must be managed.

Where the scene is a person, again samples are often collected and photographs taken with the consent of the person being examined. If consent is withheld, an appropriate warrant shall be obtained. Again legislation varies somewhat between jurisdictions.

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APPENDIX C: SPECIALIST FUNCTIONS OF POSSIBLE ASSISTANCE AT THE CRIME SCENE

(Informative)

Examination of place

Arson expert
Ballistics/Firearms expert
Biologist
Bloodstain pattern analyst
Botanist
Building inspector
Clandestine laboratory chemist
Coordinator
DVI technician or other persons authorized to work in confined spaces
Entomologist
Explosives expert
Fingerprint expert
Firearms discharge residue expert
Fire brigade
Geologist
Graphic artist/plan drawer
Insurance investigator
Major collisions expert
Photographer
Railway personnel
Video technician

Examination of persons

Biologist, crime scene examiner or other person authorized to collect non-intimate samples

Entomologist
Mortuary technician
Nurse
Odontologist
Pathologist
Photographer
Physician

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APPENDIX D: PRECAUTIONS TO BE TAKEN WHEN HANDLING SHARPS

(Informative)

- Wear puncture-proof gloves where necessary.
- Not place hands where they cannot be seen when searching objects (e.g. pockets of clothing, underneath seats in vehicles, etc.).
- Not re-cap needles and should enclose needles and syringes in a puncture proof sharps container (such as a plastic tube with foam inserts and end caps). In the absence of a suitable container, enclose any sharp point or edge of the object until more appropriate packaging can be obtained.
- Not package shards of glass in paper or plastic bags without any protection from sharp points and edges;
- Transport sharps on the rear floor of the vehicle (not on the seat) or in the rear of the vehicle behind a cargo barrier;
- Use appropriate hazard warning labels (i.e. BIOLOGICAL HAZARD and/or SHARPS HAZARD) clearly visible areas on the external packaging containing sharps and mark the outer packaging where the sharp surface is (e.g. the tip of the knife.)
- Place any discarded sharps, used in examination, in a suitable, labelled container

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APPENDIX E: CRIME SCENE NOTES

Contemporaneous notes taken at an incident scene should include:

- A unique identifier.

  Note: At the time of examination this may be the location's address or the name of the person being examined;

- The specific location of the scene attended;
- Date and time of the scene examinations;
- For scene locations, the time of entry and exit if the scene is not guarded or there is no scene log being maintained;
- Experts/other persons accompanying the note taker;
- Any instructions received (areas to search, material to search for, material to collect, etc);
- A description of the scene;
- Any activities undertaken (searches, photography etc);
- The location of any object of interest;
- The collection of any material along with a unique identifying designation; and
- Any other observations that may be relevant to the scene or the condition of material present in the scene (general condition of the scene, weather, overall environment, unusual odours, etc).

Pro-formas such as a medical examination record may be used. Each page (or digital record) of notes shall be numbered and shall include an identification of the note taker (name or service number, etc), shall be dated individually or form a continuous narrative from the noted time and date of arrival at the scene to the noted time and date of departure from that scene.

A brief history of the alleged incident, if available, may also be useful later to help explain the course of action taken.

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APPENDIX F: INSTRUCTIONS FOR THE USE OF A FORENSIC MEDICAL EXAMINATION KIT IN THE EXAMINATION OF A SEXUAL ASSAULT VICTIM

(Informative)

Information regarding the use of the FMEK*

The FMEK has been assembled to facilitate the forensic medical examination of victims of sexual assault and should be used according to all of the instructions provided. For more detailed instructions and information, forensic medical examiners are referred to the **Forensic Specimens Clinical Practice Guidelines**.
The contents of this FMEK have been assembled in order to maximise use of the individual items. However, as forensic specimens will be collected according to the individual case circumstances there is no expectation that all contents must be used per examination or that the contents should limit the collection of specimens in each individual case.

All items within the FMEK enable the collection of forensic specimens. Each forensic specimens must be labelled, sealed, and packaged according to the FMEK instructions. This is a process to maintain the Chain of Custody, minimise the risk of contamination and ensure the integrity and security of specimens collected by the forensic medical examiner.

Items within the FMEK should be used in accordance with the following instructions and with reference to the Sexual Assault Clinical Practice Guidelines.

The following procedure should be followed when using an FMEK to conduct a Forensic Medical Examination on a victim of sexual assault. Gloves should be worn at all times when handling items within the FMEK and changed at several points during the examination where ensuring clean hands is appropriate.

1. Ensure FMEK seal is intact
2. Break seal and open FMEK
3. Record the FMEK barcode/individual number on your sexual assault proforma
4. Place couch protector on the examination bed or chair
5. Place the sterile 'dressing pack' on the examination trolley and open out to display items.
6. Remove the required items (swabs, slides, water) from within the kit (as indicated by the individual circumstances of the case) and place them on the dressing pack/trolley. If further items are required through the course of the examination they can be retrieved from the FMEK.
7. Use the measuring tape provided to assist in accurate documentation of injuries.
8. A speculum is available for the internal genital examination where indicated and should be disposed of following the examination. The used speculum is **NOT** to be returned to the FMEK.
9. Sterile water is provided to wet the swab tips where necessary

*Courtesy of the Victorian Institute of Forensic Science, April 2011.

10. Thirteen (x13) swabs are provided for sampling the following areas: Mouth, endocervix, high vagina, vulva, rectum, anus, penis, skin (wet and dry)
11. Specimens will be collected according to the allegations and individual case circumstances – there is no obligation to collect these specimens without indication.
12. Once each swab is used it is to be labelled, sealed and packaged in an individual sealed envelope. A wet and dry swab from the same site may be packaged in the same envelope.
13. Where a specimen is collected for the purposes of identifying semen, a corresponding slide should be made by dabbing the swab on the middle of the slide. Each slide should have the victims name, date of birth, site of collection and date of collection pencilled onto the frosted surface. Do not use pen. Place the slide in an individual slide holder which will be sealed with a label. The slide can be placed in the enveloped with the corresponding swab.
14. Using two swabs, collect buccal swabs for reference samples. These swabs are to be packaged together in the corresponding envelope but **will not be packaged in** the FMEK – please hand to police separately
15. The remaining swabs can be used to swab any area that may aid the investigation e.g. an area of genitalia that might be stained with menstrual blood, perineum, breasts.
16. If further swabs are required, **another FMEK** must be utilised and the process documented. FMEK components should remain in the kit that was opened for the purpose of the examination. This is so that barcodes can be adequately accounted for in process.
17. Ensure each specimen is individually labelled with the labels supplied and place each individually labelled and sealed forensic specimens into the FMEK container.
18. Place all unused items into the **UNUSED ITEMS** bag and **place into** the FMEK container.
19. Close the FMEK and seal with tamperproof tape on the three open sides of the kit (front, left and right)
20. Complete checklist of forensic specimens collected at the time of the examination.
21. Complete chain of custody requirements
22. Provide the police with all the appropriate information regarding integrity and security of the FMEK (storage, handling, tamperproofing etc).
23. Place the **WET CONTENTS** sticker (to instruct appropriate storage) on the front of the sealed FMEK if wet contents are sealed in the FMEK. **NB: Wet clothing should not be placed in the kit** (ie handed to police separately)

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APPENDIX G: ENTOMOLOGICAL SAMPLES*

(Informative)

Using known insect developmental data, in particular blowfly larvae (maggots), forensic entomologists may estimate the minimum post mortem interval (PMI) of a deceased person. Ambient temperature, rainfall and ability of insects to access the deceased remains all effect the time taken for insects to infest a body, therefore environmental factors that may influence the infestation and growth rates of fly eggs and larvae shall be considered and PMI shall be reported as a minimum time interval. Four main factors of incident scene evidence collection influence the accuracy of minimum PMI estimate. The following guidelines should be followed:

1. A sufficient number of specimen larvae from the scene and/or deceased remains should be collected. At least 5 maggots (and preferably 20) shall be collected to assess their age in a statistically robust manner.
2. Correct maggot preservation techniques should be used to avoid maggot shrinkage and discolouration.
3. The weather conditions at the scene (ambient temperature, and rainfall) shall be assessed (ambient temperature correction should be used where applicable†).
4. The availability of the deceased remains for oviposition immediately after death shall be considered (e.g. whether the deceased remains were buried, wrapped or moved).

Collection

At any scene where collection of samples for entomological examination is required, samples of eggs, larvae, pupae, puparia and adult insects should be collected from the body as soon as practicable. If this is not possible, they shall be collected from the body prior to its refrigeration at the mortuary. Where possible, entomological samples should be collected as follows:

- Assess whether there are any carrion insect infested animal remains or detritus within at least $30m^2$ of the body that could provide a source of evidence contamination. If so, collect entomological samples from this material and photograph it for the entomologist.
- Collect maggots from each maggot mass.
- Collect samples of wandering larvae (those moving away from the body).
- Collect empty puparia and store in a dry state. Collect pupae in ethanol (at least 70%). To locate pupae and puparia outdoors, dig transects several centimetres deep in the soil for three or more metres in several compass directions. If indoors, search for pupae and puparia under carpet, in cupboards and in other dark places.
- Collected larval samples should be representative of all types and sizes present on the body.
- Keep 'hairy' and 'smooth' maggots separated.
- Two samples of each maggot type and size should be collected:
    a. a live maggot sample that is placed into a ventilated container and stored in a refrigerator; and

* Byrd Jason H & Castner James L (Editors) (2001), *Forensic Entomology The Utility of Arthropods in Legal Investigations*, CRC Press, New York.

† Archer, Melanie *The Insects: An Outline of Entomology* (3rd Edition).

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   b. a maggot sample killed at the time of collection:
      i. First place it in boiling water for at least 30s.
      ii. Strain the sample and discard the water.
      iii. Transfer the sample to a container with 70-80% ethanol.
      iv. Label ethanol containers with waterproof pen or pencil.
- Record details of samples collected, including:
   a. Date and time the sample was collected.
   b. Location of the sample on the deceased.
   c. Condition of the body (decomposition/injuries).
   d. Weather conditions or windows open, heater on etc., if in a house; and
   e. Relevant temperature data (ambient temperature and maggot mass temperature).
- Do not collect samples from within the body until the autopsy.
- Record the location of maggots and pupae (check under objects 3-10 metres from deceased remains).
- It is essential that the samples are analysed immediately. Forward the samples to an approved expert as soon as possible and ensure that they are stored for transportation in a suitable cool container (around 4 - 6ºC is ideal). Adhere to chain of custody procedures when conveying evidence to the entomologist.
- Ask the entomologist as soon as possible whether they require ambient temperature to be measured at the scene with an automatic temperature logging device[*]. If so, obtain the device from the entomologist and leave it *in situ* for 5 - 10 days.

It is recommended that all case samples shall be kept by the forensic entomologist for at least two years, and this shall be specified in their report.

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[*] Archer, Melanie *The Insects: An Outline of Entomology* (3rd Edition).

APPENDIX H: PACKAGING GUIDELINES

(Informative)

The following table provides guidelines regarding appropriate containers and packaging that can be used for various material of forensic interest. When selecting packaging, consideration shall be given to preservation, protection against loss and minimisation of cross-contamination.

TABLE H.1 CONTAINER AND PACKAGING GUIDELINES FOR FORENSIC MATERIAL

Material	Packaging Guidelines
Abrasives	Collect a representative sample of the material. Use a plastic or glass container. If reference samples are available, collect them in their original container.
Accelerant	See 'Ignitable Liquid Residue' guidelines.
Acids	Collect a sample in a glass container and label 'Corrosive'. Collect a reference sample in its original container, if available. Note: Hydrofluoric acid will etch glass and if suspected, should be packaged in air-tight acid-resistant containers made of a suitable polymer such as HDPE.
Adhesive tape	Collect the entire sample and the source material. Avoid cutting the tape. If it is necessary cut the tape to obtain a sample, clearly indicate which cuts have been made as part of the sampling procedure.
Alkaline compounds	See 'Acids'.
Ammunition (unfired)	Collect a representative sample and pack in such a way that there is no contact between individual cartridge cases.
Anonymous letters	See 'Documents'.
Biological tissue	Collect with clean disposable forceps and place into a specimen container. Package the container in a press-seal plastic bag to contain possible leakage.
Blood (dry)	If possible, collect the material bearing the stain. If the blood is absorbed into a surface, use a clean scalpel or scissors to cut the stain from the object. If the blood is a surface stain - use a moist swab to collect a sample or, with a clean scalpel, scrape the stain from the object into a paper envelope or collect with a tape lift. Place the sample in a paper bag or paper envelope.
Blood (moist)	If possible, collect the material bearing the stain. Collect a blood sample with a cotton swab and allow to air dry. Package in a paper bag or paper envelope.
Blood (wet)	Collect a blood sample with a swab and allow to air dry. Package in paper bag or paper envelope. Alternatively, collect the sample using a disposable pipette or syringe and transfer the liquid into a tube containing EDTA.
Botanical material	Where appropriate, collect botanical material in paper bags. Do not package botanical material in plastic bags unless the material is completely dry.
Bullets (spent)	Wrap individually in tissue paper and place in individual containers.
Cannabis	See 'Botanical material.
Cartridge cases	See 'Ammunition'.
Cartridges (live)	See 'Ammunition'.
Caustic compounds & liquids	See 'Acids'.
Cheques	See 'Documents'.
Chewing gum	Collect whole and package in a capped plastic tube or specimen container.
Cigarette butts	Handpick and package in a paper envelope or if dry, plastic bag or specimen container.
Clothing	Package each piece separately in a paper bag. Do not seal clothing in plastic bags.
Condoms	Handpick and package in specimen container.
Cordage	See 'Rope'.
Corrosives	Collect a sample in a glass container and label 'Corrosive'. Collect a reference sample in its original container, if available. Some corrosives, for

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	example sodium hydroxide, will etch glass and if suspected, should be packaged in air-tight acid-resistant containers made of a suitable polymer such as HDPE.
Damp material	Use a container that allows airflow (to limit mould and micro-organism growth) such as wet-strength paper bags. Such material shall be laid out or hung to dry prior to storage or examination.
DNA	Any material that requires DNA analysis should not be sealed in plastic bags for long term storage unless completely dry. Objects may be themselves collected or may be swabbed in the field with 70% ethanol or distilled water. Tapelifts may be a suitable alternative to swabs.
Documents	Collect each item in a separate plastic sleeve or plastic bag. Keep documents flat at all times. Do not handle documents without gloves or tweezers. Include original envelopes (where relevant) and do not type or write on the outer container of the item once it has been packaged.
Drugs	Where a drug is seized in a packet or other container, the container as well as the drug should be collected for examination. Clip seal or heat sealed plastic bags should be used for collecting powders or drug impregnated paper. Glass containers with screw-top lids should be used for collecting liquid samples.
Explosives residue	Residue from bomb scenes should be packaged in either a polymer bag or an unlined paint tin.
Extortion notes	See 'Documents'.
Fingernail clippings/scrapings	Sample with clean scissors or a scraper and package in a paper bag or a paper envelope.
Fingerprints	Smooth, non-porous surfaces. Adhesive lifts are usually used to collect powdered fingerprints and these can be stored in paper bags, paper envelopes or plastic bags. Alternatively the enhanced fingerprint can be recorded photographically in situ.
Fire debris	See Table C2.
Firearm	Collect in a paper or cardboard container after ensuring that it has been made safe. Firearms shall be packaged separately to items containing ammunition. The packaging of firearms shall be guided by the potential requirements for the collection of further evidence types.
Firearm discharge residue	Samples should be collected using adhesive stubs. When collecting clothing or other material to be tested for firearm discharge residue, package by lying out and folding into paper and placing the wrapped object into a paper bag. Do not seal clothing in plastic bags. GSR sampling kits shall be separated from other possible sources of GSR.
Flammable liquids	Collect liquid in a glass container with an appropriate seal.
Food	If possible, collect the object bearing the stain. If this is not possible, swab the object with 70% ethanol or distilled water.
Fur	See 'Hair'.
Glass	Any packaging used shall be sufficient to prevent harm to any person handling the material. Items should be labelled appropriately if a hazard exists. Do not use glass containers to collect glass samples. Collect small particles in paper envelope or small plastic container. Collect large glass samples in a suitable container (e.g. a cardboard box).
Grease	Collect a sample and place in a glass or plastic container. If reference material is available, collect it in its original container.
Gun	See 'Firearm'.
Gunshot residue	See 'Firearm discharge residue'.
Hair	Collect hair in a paper envelope and seal the inside a plastic bag. Hairs from different areas should be placed into separate paper envelopes. Hair reference samples should be plucked to ensure the presence of the hair root and sheath material.
Handwriting	See 'Documents'.
Hazmat	Hazardous Material, usually in reference to an incident response.
Ignitable Liquid Residue	See Table H2.
Ink	Collect liquid ink in its original container or use a glass sample container.

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Insects/maggots	See Appendix G.
Knives	Use a knife tube or other impenetrable container. As a temporary measure should a suitable container not be available, make a sheath to place around the blade or use a cardboard box.
Liquid residues	Use an airtight container such as an unlined paint tin or Schott reagent bottle.
Liquor	Collect liquor in the container in which it was found or store the liquid in a clean glass container. Forward the original container with the sample.
LSD	Any suspected hallucinogen shall not be handled without wearing gloves as they can be readily absorbed through the skin. Package suspected hallucinogens in a plastic bag and place in an appropriate container to avoid any exposure to light.
Marine samples	Collect in a glass or plastic airtight container and consult and expert as soon as practicable for advice regarding further preservation strategies.
Matches	Collect in a container with cushioning material such as cotton wool or tissue paper.
Medicines	See 'Drugs'.
Metals	Package small samples in glass or plastic containers and wrap larger samples. Protect any edges that may require physical-fit examination.
Oil	Use glass, plastic or metal containers. Collect a reference sample in the original container if available.
Opium poppies	See 'Cannabis'.
Paint	For paint smears on objects, collect the entire object with suitable protection of the paint deposits. For paint flakes, carefully remove the flakes and place into folded paper or a clean glass container while avoiding physical damage to the flakes. For liquid samples, collect the paint container for examination.
Paper	See 'Documents'.
Pen	Collect pens in plastic bags.
Petrol	See 'Ignitable liquids'.
Plants	See 'Cannabis'.
Plastics and polymers	Use glass containers or folded paper. Multiple small pieces from the same site may be packaged collectively. Protect edges if physical fit is required.
Poisons	Each item shall be collected in a separate clean plastic or glass container. Care should be taken when handling these items as some poisons can be inhaled or absorbed through the skin.
Powders	See 'Drugs'.
Precursors	Precursors can be in liquid form, in solid form (powders) or can be in solution. For powders See 'Drugs'. For liquids and solutions, use a glass container with a screw-top lid to collect a sample. Collect or record as required by the local jurisdiction.
Reaction vessels	Once the reaction has been safely shut down, all reaction vessels and their contents should be collected or sampled and recorded. Collect or record as required by the local jurisdiction.
Rope	Protect the ends from fraying and package in a plastic or paper bag. Protect any knots.
Rubber stamps	Collect the rubber stamp and inkpads in separate plastic bags.
Saliva	As per blood.
Safe insulation or 'ballast'	Collect all available material and package in a plastic bag.
Semen	Package the material bearing the stain in a paper bag. Air dry wet stains thoroughly before packaging. Swab, tape lift or excise the whole stain if the object is not able to be removed from the scene.
Sharps	Use a sharps container or other impenetrable container. As a temporary measure should a suitable container not be available, make a sheath to place around the object or use a cardboard box.
Soils	For objects with soil deposits, (e.g. shoes) collect the entire object. For control samples, collect a sample in a clean container.
Solvents	Collect bulk or sample as required. Solvents should be collected using glass containers with a screw-top lid to collect a sample or bulk liquid.

Solid residues	Use an airtight container such as an unlined paint tin or well sealed glass bottle.
Stamps	See 'Rubber stamps'.
Syringes	Use a sharps container or other impenetrable container.
Tampons and sanitary pads	Handpick and allow to dry before packaging in a paper bag.
Tape	If practicable, collect the object to which the tape is adhering to. If it is necessary to cut the tape, ensure that the newly cut ends are clearly marked. If the original roll of tape is recovered, collect the entire roll. Package tape in plastic bags.
Threatening letters	See 'Documents'.
Tissue Samples	Plastic container with a screw-top lid or plastic bag.
Toolmarks	If possible, collect the object upon which the toolmark appears. When packaging and transporting items, care shall be taken not to damage surfaces that have a toolmark. Tools should be packaged in such a way that the blade of the tool is not damaged. Paper or plastic bags may be used. If this is not possible, prepare a cast of the toolmark using the required casting material. Package the cast in a manner that avoids damage.
Tools	Collect the implement (tool) in a bag. Protect the implement against any damage or loss of contact traces (such as paint, wood chips, etc.).
Trace material (non-biological)	Non-biological trace material collected from clothing (such as paint, glass, fibres etc.) should be contained in paper packaging that may then be sealed in a plastic bag or paper envelope.
Twine	See 'Rope'.
Typewriters	Collect the typewriter in a plastic bag, along with any typewriter ribbons, carbon paper, changeable type (e.g. 'daisy wheels' & 'typeballs') or any power cords.
Typewritten documents	See 'Documents'.
Vegetation	See 'Botanical material'.
Wax	See 'Grease'.
Wet objects	Use a container that allows airflow (to limit mould and micro-organism growth) such as wet-strength paper bags. These objects should be laid out to dry prior to storage or examination.
Wire	Protect the cut ends from further damage and mark any cuts made during sampling. Package in a plastic bag. If the source of the wire is recovered, collect intact if practicable.
Wood	Collect the entire object in a plastic container or plastic bag. If physical matching is required, ensure that all splintered ends are protected from damage or loss.

The following table provides guidelines for the collection and packaging requirements for ignitable liquids and residues. When selecting packaging, consideration shall be given to preservation, protection against loss and minimisation of cross-contamination.

TABLE H.2 CONTAINER & PACKAGING GUIDELINES FOR IGNITABLE LIQUIDS & RESIDUES

Sample Type	Amount of sample	Packaging	Comments
Liquid pool	At least 2ml	Sample using absorbent material and place in friction lid tin.	Maximum absorbent size = the equivalent of a cube of approx. 3 cm^3.
Fuel tanks, fuel cans, Jerry cans etc.	At least 2ml sample or the container if a sample cannot be taken.	Friction lid tin or original container sealed with a lid.	Ensure flammable liquid cannot spill.
Charred debris	Enough to half-fill a clean 2 litre metal can.	Friction lid tin.	May require further sealing if stored long term.

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Skirting boards, floor boards	Enough to half-fill a clean 2 litre metal can.	Friction lid tin.	May require further sealing if stored long term. Cut boards to fit into cans. **Do not use any type of petrol motor driven cutting device**.
Clothing	Each object should be packaged separately.	Sealed in polymer bags	Transport to laboratory for immediate analysis. Any ignitable residues on the clothing will generally only be present in small amounts.
Car fires	Foam from seating, debris from floor pan - enough to fill half a tin.	Friction lid tin.	May require further sealing if stored long term.
Soil	Fill half a tin	Friction lid tin.	May require further sealing if stored long term.
Trails on soil	If on dirt and grass, collect enough grass and soil to fill half a can.	Friction lid tin.	Transport to the lab immediately. Flammable fluids are rapidly decomposed by microbial agents in soil.
Trail	If on concrete and still wet, swab using clean absorbent material.	Friction lid tin.	May require further sealing if stored long term.

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Acknowledgements

Australian Federal Police, Forensic & Data Centres
Forensic Science South Australia
Northern Territory, Crime Scene Examination Section
NSW Police Forensic Services Group
Queensland Police, Forensic Services Branch
South Australia Police
Western Australia Police, Forensic Field Operations
Victorian Institute of Forensic Medicine

*** END OF DRAFT ***

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PREPARATION OF AUSTRALIAN STANDARDS

Australian Standards are prepared by a consensus process involving representatives nominated by organizations drawn from all major interests associated with the subject. Australian Standards may be derived from existing industry Standards, from established international Standards and practices or may be developed within a Standards Australia technical committee.

During the development process, Australian Standards are made available in draft form at all sales offices and through affiliated overseas bodies in order that all interests concerned with the application of a proposed Standard are given the opportunity to submit views on the requirements to be included.

The following interests are represented on the committee responsible for this draft Australian Standard:

Australia New Zealand Policing Advisory Agency,

National Institute of Forensic Science (ANZPAA NIFS)

Australian and New Zealand Forensic Science Society (ANZFSS)

Australian Federal Police (AFP)

Consumer Action

Expertise, Evidence & Law Program, School of Law, University of New South Wales

National Association of Testing Authorities, Australia (NATA)

New South Wales Police Force

Queensland Police Service

University of Canberra

Victoria Police Forensic Services Department

Victorian Institute Forensic Medicine (VIFM)

Western Australia Police

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Trial of Copan 4N6 flocked swab

23rd May 2008

Allan McNevin, Senior Scientist, Chiron Weber, Scientist

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Forensic and Scientific Services,
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Trial of Copan 4N6 flocked swab

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

Introduction

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

Figure 1. Flocked swab elutes specimen more efficiently.

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

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

AIMS

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

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

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

Experiment 1.

Method

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

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

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

Results

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

Table 1. Summary of results from Experiment 1

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

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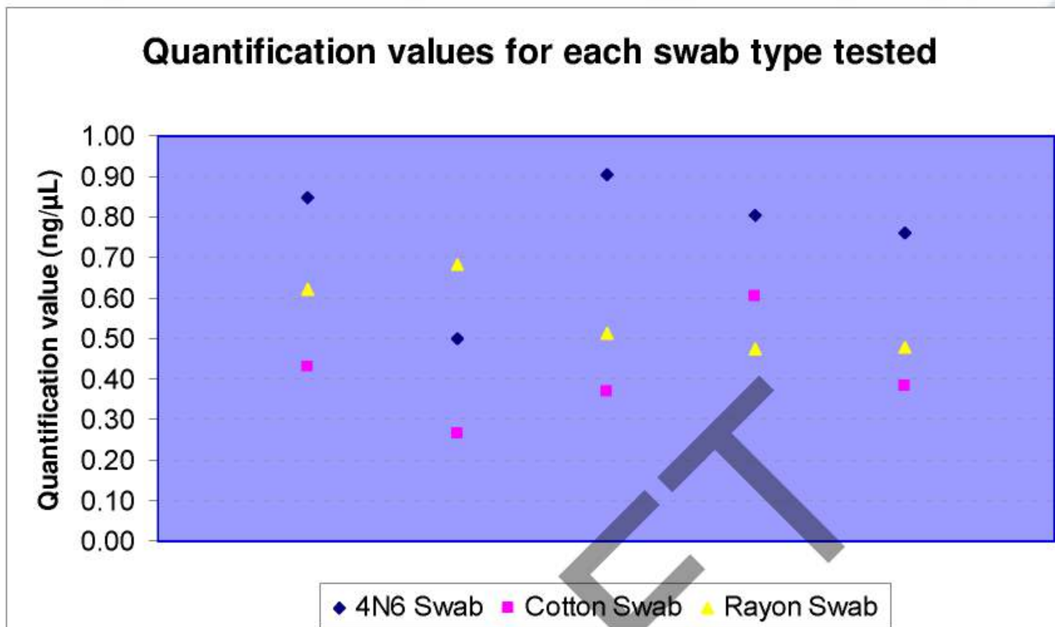

Figure 1. Quantification values for each of the 5 replicates for each of the 3 swab types tested

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

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Experiment 2

Method

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

Results

The results from these swabs are outlined in Table 2 and Figure 2 below.

Table 2. Summary of results from Experiment 2

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

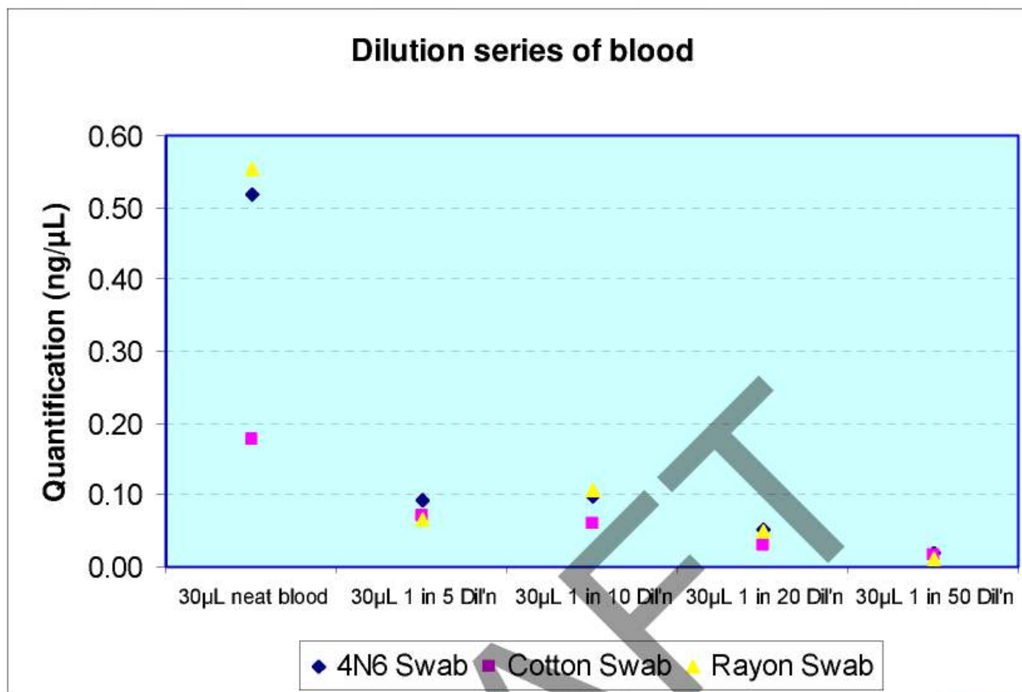

Figure 2. Quantification values from each dilution series for each of the 3 swab types tested

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

Results & Discussion

As can be seen from the two-sided students *t*-test results in Table 3 below, the 4N6 swab released more DNA when 30µL of whole blood was added and dried onto the swab head than either the cotton or rayon swabs.

Table 3. *t*-test results for experiment 1

Comparison	*p*-value*
4N6 v Cotton	0.0046
4N6 v Rayon	0.0388

* a *p*-value of <0.05 indicated a significant difference between the two data sets under test

The same can be shown for the dilution series where, across the dilution series, the results for each swab type were quite similar, with the cotton swab showing consistently lower quantification values. There was no evidence of inhibition or other effects on the extraction, quantification or amplification of the DNA extracts obtained from the 4N6 swabs using the methods currently employed within the laboratory.

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

Recommendations

Further testing is required to:

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- Determine if the results are homologous when cellular material is placed onto each of the swab types investigated,
- Testing with both blood and cells is required to investigate the uptake of material onto the 4N6 swab in comparison to the cotton and rayon swab types.

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

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DNA Analysis Management Team Minutes

PRESENT AND APOLOGIES			
Chairperson:	PT	**Date and Time:**	**2009 – 7 May**
Venue:	Conference Room 102	**Secretariat :**	WH
Attendees:	JAH, PT, EJC, LW, SMJ, IM, TEN, KS, CI, WAH		
Apologies:	CJA, AMc, AAP		
Guests:	AO, PAC		

1.0 PREVIOUS MINUTES ENDORSED

Minutes of previous meeting held on 23 April 2009 are endorsed by PT & WH without amendment or amended as follows:

Item	Topic	Discussion	Action required (inc: Officer, Due date)	Communications to go out
		2.0 STANDING AGENDA ITEMS		
2.1	**Training Update** *(Alice)*	▪ PAD sessions for Managers – scheduled for 22 May - 2 sessions planned for this day a) 10-12noon and b) 1-3pm. If both of these sessions are filled, then there might be a session held in the am on 15 May or on 21 May. (10 people required per session) ▪ 10675 forms have an expiry date of approx 3 months. Staff will therefore need to re-apply if form as expired. This ensures that Line Managers know when their staff are attending courses. ▪ If a staff members cannot attend a training course for which they are booked in to attend, please advise SSDU ASAP. SSDU have a standby list of staff to fill empty spots in most		

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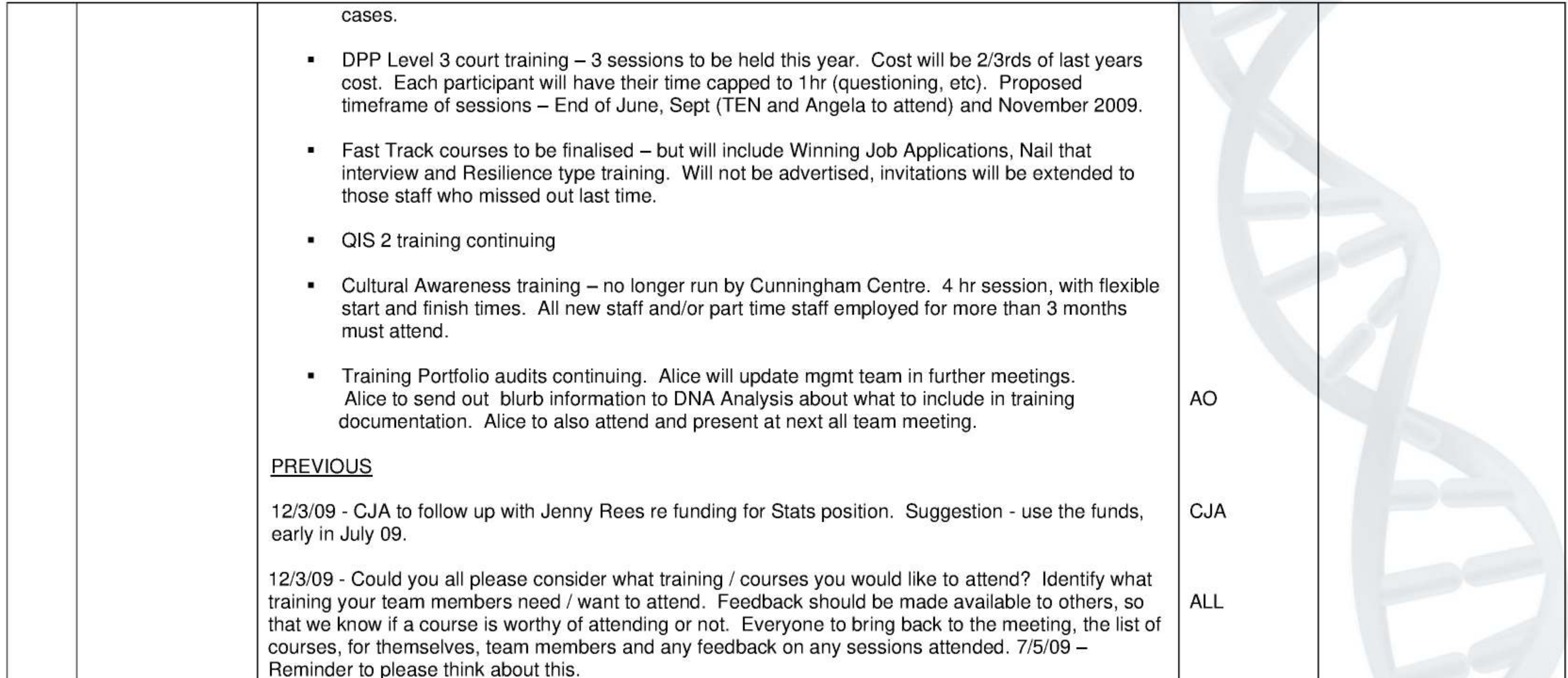

		cases. ▪ DPP Level 3 court training – 3 sessions to be held this year. Cost will be 2/3rds of last years cost. Each participant will have their time capped to 1hr (questioning, etc). Proposed timeframe of sessions – End of June, Sept (TEN and Angela to attend) and November 2009. ▪ Fast Track courses to be finalised – but will include Winning Job Applications, Nail that interview and Resilience type training. Will not be advertised, invitations will be extended to those staff who missed out last time. ▪ QIS 2 training continuing ▪ Cultural Awareness training – no longer run by Cunningham Centre. 4 hr session, with flexible start and finish times. All new staff and/or part time staff employed for more than 3 months must attend. ▪ Training Portfolio audits continuing. Alice will update mgmt team in further meetings. Alice to send out blurb information to DNA Analysis about what to include in training documentation. Alice to also attend and present at next all team meeting.	AO	
		PREVIOUS 12/3/09 - CJA to follow up with Jenny Rees re funding for Stats position. Suggestion - use the funds, early in July 09.	CJA	
		12/3/09 - Could you all please consider what training / courses you would like to attend? Identify what training your team members need / want to attend. Feedback should be made available to others, so that we know if a course is worthy of attending or not. Everyone to bring back to the meeting, the list of courses, for themselves, team members and any feedback on any sessions attended. 7/5/09 – Reminder to please think about this.	ALL	

2.2	**Automation Update**	Currently nothing happening due to BSD being down. New BSD should be installed today or tomorrow. When installed will run a checkerboard plate first. Cleaning procedure for BSD has been confirmed. When BSD is installed TEN to email all and advise of change of processes. PREVIOUS On Hold until New Year: Tom working on program for benchmarking extraction methods for all labs using Robots. Mock samples are being prepared (using blood samples first). TEN has spoken with WA, VIC, SA. Yet to speak with NSW & AFP. 12/12/09 Plan to roll together with proficiency tests. 28/8/08 – on hold. Project 24 sperm – IQ vs Chelex - quants. Need to establish if ProtK is the problem. TEN to carry out differex experiments.	TEN	
2.3	**GM IDX Update (Chiron)**	Printing has been checked – no fix available as yet. Process will remain as is for now. Peak shadowing HIDI appears to be cause – expiry dates for HIDI products has not been confirmed. Receiving conflicting information. Chiron chasing this up. PREVIOUS 23/4/09 - Need to think about who from our teams are next to attend the training after the Mgmt Team has had their session.	All Chiron / CJA	
2.4	**Change Proposals / Project Management** *(Tom)*	G:\ForBiol\Quality Assurance and NATA\Management Reports - Biology Management Team #53 Artel MVS System – arrived, software has been installed and testing done, it can't calibrate above 200 micro-litres. Greater than 200 to be done by Gravimetric balance as per current process. TEN to advise when Change Proposal is complete and ready for review. #52 GMIDX Mixture Package – waiting on calculation data. TEN to advise when Change Proposal is complete and ready for review. #51 Paperless project – still in development.	TEN TEN SMJ	

		Alice advised that NIFS use an improved version of popstats for mixtures. PREVIOUS 23/4/09 - JAH – THETA values proposal (SJC). Questions in Court and proposed responses. Workshop to be planned, this will bring us in line with other labs in Australia. 23/4/09 - Minor Change Register to be updated re new Upload Forms – LW **12/3/09** New change proposals – Needs Attn - #47 DNA IQ Clean Up – waiting on impact assessments please. JAH to update impact assessment for FRIT. #48 Fingerprint Testing – Melissa to undertake a literature review before we can progress with this. 23/4/09 - need to see if there are inhibitants in squirrel hair fingerprint brushes from QPS, Journals found, to be read. #49 Calculation Quants Standard Ranges – AMc to undertake phase 1 (timeframe 5 months). 23/4/09 - VH is sourcing information from overseas at present. #50 Nucleospin Clean Up Double elution – agreed 1st priority. 26/2/09 - #44 - NCIDD Bulk Upload – TEN has added data, please review this change proposal. ON HOLD - Evaluation processes required. Adverse Events SOP - Paula will put this SOP into draft. Individual SOPs referenced – at what point is investigation required. eg. Quants undetermined in neg control. SOP will detail process of what to check with regard to adverse event. Everyone to think of what things can go wrong and advise TEN asap. 20/12/07 - ON HOLD – (to be programmed) Suggestion to have photos on screen at Shift F9 and colour to indicate specimen notes. Tom agreed to look into this.	JAH LW JAH AAP AMc All	#44 - Please review prior to next mtg. 7/5/09

Item	Topic	3.0 NEW BUSINESS Discussion	Action	
3.1	**PPE in Exhibit Room (AAP)**	Agreed – gloves to be worn in exhibit room & walk in freezer. (Admin staff will not need gloves to put case files in ER Tray) TEN to advise Property Point staff of change. Environmental cleaning to continue in this room.	TEN	
3.2	**QPS rayon tubes - swab casing. (PT)**	QPS wish to move from 4N6 Swabs to Rayon. Example of tubes and size of holes in top were shown. Agreed that definite decision cannot be made on these as yet. PT to draft a response to Dave Neville QPS (will be circulated to Mgt Team for input). 70% ethanol to be used instead of water by SOCOs.	PT	
3.3	**PSA document (JAH)**	Rhys has completed this document. Please look at email sent some 2 weeks ago and feedback is requested please to JAH and Rhys.	All	
3.4	**Complex mixture wording (JAH)**	2 weeks ago a wordy preamble was distributed. Please review this and let JAH and PT know by COB 13 May. Meeting with QPS officer 14/5/09.	All	
3.5	**New EXR lines (JAH)**	Changing confirmatory to highly likely in Statements with respect to PSA. AP is always done first for SAIKs prior to PSA. A total review of all EXR lines is underway – some will be reworded. To be discussed with QPS officer on 14/5/09 as the information is to be clear and concise which transfers into Q Prime. No interpretation is to be made by FIRMU officers. KS to advise PT of any amendments re Evidence Recovery. Any other suggestions / additions to EXR lines to be forwarded to PT & JAH as well.	KS All	

3.6	**Calculation of Off ladder/ variant homozygote alleles in GeneMapper (TEN)**	If an unlabelled homozygous peak, use the stutter to calculate the allele designation. The sample must then be re-gene scanned to confirm. TEN to update relevant SOP.	TEN	
3.7	**Extraction/ Extraction preparation controls - use of, risks (TEN)**	Need to establish why (the purpose of) we have controls. What should we do when we find a failure in a control? TEN to put together a summary of discussion and forward to management team for review / thought.	TEN	
3.8	**Destruction Mask (TEN)**	Agreed AUSLAB details to remain on mask.		
3.9	**Review of outstanding agenda items**	Please review all of the outstanding agenda items in preparation for next meeting.	All	

	Topic	4.0 BUSINESS ARISING FROM PREVIOUS MINUTES Discussion	Action required (inc: Officer, Due date)	Communications to go out
	PROJECT	**STATUS, RISKS, ISSUES, COMMUNICATIONS**		
4.1	**FERRO and subsequent contact staff (AAP) 23/4/09**	FERRO and subsequent contact staff.doc Print Mask Department Name to be included on FERRO. AAP to supply contact list for FERROs to Property Point.	AAP	
4.2	**Barcode discrepancies with in tube samples (AAP / PT) 23/4/09**	Quality management a must – checking barcodes. Procedures in place to check PRIOR to opening packaging. If barcode discrepancy found – To be returned to originating station for correction to be made. If packaging opened, then a letter of advice must also be returned with the item. AAP and CJA to advise QPS of this new process.	All AAP / CJA	
4.3	**20/20.1 at D3 and how to report when it has been confirmed (LW) 23/4/09**	20.1 will be noted as NR in NCIDD. IP for Kinship - CJA to discuss with J.Rossiter. Needs to be resolved. Put in statement as full profile ~ but exclude loci in stat calcs. EJC to update pre-blurb SOP re partial profiles. CJA to advise QPS. Minor Change to be updated to reflect this EJC	CJA EJC CJA EJC	

4.4	**QPS / QHFSS Mtg 22/4/09**	**<u>NTR QPS advised</u>.** Agreed as follows. ▪ What is delivered by QPS will be tested, as priority should have been established by QPS prior to arrival here. ▪ Same deal for IQ cases. ▪ If Case has some tested and some NTR advice from QPS - Include all in statement if samples profiled. ▪ If advised by QPS to NTR and sample is less than Amp stage - Stop process, or continue if at Amp or after stage. ▪ If Case has all "N" – don't upload profile – no further work on case – cease work as above. JAH to coordinate guidelines – Statement all profiles interpreted. ▪ F# 1600 samples – in freezer – to be destroyed. Project / Business case to be established to work through these. Pre 2005 samples can be archived. To be discussed at FSLU issues meeting.	JAH PT	
4.5	**End of Financial Year – if funds are available - what can we purchase?**	Please give this some thought and send any ideas, suggestions through to CJA via email.	All	
4.6	**Processing Male and Female intimate cells on different batches (JAH)**	Think about this and discuss again next meeting 12/3/09 ▪ Pros and cons ▪ Urgent samples ▪ Workflow ▪ Risks	ALL	
4.7	**Allelic imbalance at Amelogenin (JAH) Post implementation review of AI and Pk heights 26/3/09**	Chiron, Ingrid and Emma to create a change proposal by 9 April, 2009 that outlines the plan and scope of the Project. Scope to include calculation methods. A fair amount of validation will be required. Suggestion – to seek research funding for the project. Huge benefit will be gained with respect to issuing statements and for every day analysis. Use of GeneMapper for mixtures. You select manually what is feasible. More beneficial than popstats, but can only do 2 person mixtures.	CW / EJC / IM	

4.8	**New print mask for photography uploaded to AUSLAB (AAP)** 26/3/09	Ingrid to contact FRIT members re JAVA software on computers and request to have it installed by IT, should it not be present. (to include PC barcode, name of staff member and location of the PC in list to IT). If staff cannot view annotations (shift F5) and the computer boots you out, then you don't have JAVA. Images are now bigger, easier to read. Identifies who examined the item etc. Images are linked to FBEXAM, so images don't work for tubes.	IM	
4.9	**Manual IQ of 4N6 swabs (JAH)** 26/3/09	Table distributed by Lisa. Not obtaining good profiles from blood swabs. Actions – Tom to continue data mining. Need to identify what our current results are for chelex. AMc to purchase some 4N6 swabs for a trial and look at nuclespin. Percentages of reportable profiles from 2769 samples is 26.94% (i.e. 1841 FSS – 28.79% and 928 QPST – 23.28%.	TEN / AMc	
4.10	**Retention of Substrates post extraction (AMc)** 12/3/09	Issue – we are steadily running out of space, however there has been a variety of uses for returning to the substrates at a later date in some circumstances. Property Point will move items around in the freezer to make some room. AMc to collate a breakdown of what is in storage (including slides) and the timeframes of such items. To be discussed further at a future meeting.	AMc	
4.11	**PSA Results and EXR/H reporting. (JAH)** 12/3/09	Need to move away from "confirmatory statement "in our statements as QPS do some testing and we don't. The change in AUSLAB could be made quite quickly. Agreed need to change statements where this has been made. ▪ Rhys to finalise report ▪ JAH to update words for EXR / H line and paragraph, pass this information to Paula. Paula will forward to AUSLAB. Relevant SOPs to be amended.	EJC / JAH / PT JAH	

4.12	**Proficiency Testing (TEN)** 12/3/09	2 SOPs have been combined into 1. Agreed to undertake CTS tests and internal tests the same. 2 samplers and 2 reporters paired up. Report to be peer reviewed, process to follow workflow of current work in the lab, i.e. collect file from exhibit shelf etc etc. Analytical staff would be included in testing; they could be tested on what they are signed off on. Blind testing – CJA to discuss with Michael Keller (QPS).	TEN CJA	
4.13	**Staff matches to complex mixtures (PT) 12/3/09**	Staff are wasting time on complex mixtures that are uninterpretable. Paula to update SOP to reflect that this is not required.	PT	
4.14	**Outstanding cell batches - 26/2/09**	Need to look at priorities for old Volume Crime samples that need to be processed. 40 Batches to be done. Need to ensure that old V/Crime samples are done in order as agreed. AMc to consider if newest samples can be done before pre July 01 2008 samples.	AMc	
4.15	**Duty Scientist 12/2/09**	26/2/09 – agreed this is good, and Snr Scientist to think about whom in their team could go on the roster and be paired by a Snr Scientist to answer enquiries. A reminder that correspondence should be in written form (email) and documented in the communications log, so there is a record of such conversations. Communication Log - G:\ForBiol\DNA Analysis Organisational Changes\Issues Log.xls	All	
4.16	**Meeting with DPP, QPS, Legal Aid & DNA analysis**	Legal Aid rep did not attend meeting. DPP where happy with process of statements. CJA to obtain a copy of a QPS statement.	CJA	
4.17	**In-Tube samples 12/2/09**	QPS will run out of 4N6 swabs and will have to revert back to Rayon swabs which will be delivered here in tubes. Screw caps will be tethered on the tubes. O Rings could be used both on automated platforms and with chelex. Yes O ring rubes can be produced in individual packs 50cents each.		

4.18	**Accuracy of Quants – round up vs round down (re 334727291)** (JAH)	Needs Attn - TEN to extract data on quants. Belinda will then look at the Data. Major change from 1.2 nanogram – Change proposal required. Verification to be done plate vs plate, a direct comparison. Agreed to start at 1.5 nanograms. Previously it has moved from 1.5 to 1.2 to 1 to 1.2. Data gathering to be done first. BUA and TEN. 30/1/09 - Update – On Hold for now – waiting on GeneMapper.	AMc / BUA AMc / BUA	Carried forward to next meeting 12/3/09
4.19	**Staff databases** (Tom)	QPS profiles in AUSLAB only. Staff check will occur prior to upload – will be flagged.		
4.20	**Discuss retention times for Staff/contractor reference samples** (Tom)	Update – 26/2/09 – TEN preparing a letter to LALU for legal advice. Profiles to be kept indefinitely. Vietnamese scientist, to visit. They will be swabbed on arrival. Currently no time limits in place. After 2 years is it really right to dispose of them? Agreed to seek maximum consent 12/02/09 – ▪ Obtain copy of QPS form, which is used by SOCOs, and has been through QPS Legal and QPS Union. ▪ Prepare process plan ▪ Forward to LALU TEN suggested that perhaps DNA samples should be taken from producers / manufacturers of the plastic consumables to assist in identification of contamination.	All TEN / PT	Ongoing
4.21	**D3S1358 20 and 20.1**	AMc and TEN to work on a request for research funding for Sequencing. <u>PREVIOUS</u> Appears to be a bin issue. Meaning the space between the bins differs between 4 and 4.1. This is	AMc / TEN	

		why manual calculations are irrelevant. Need to review previous minutes on this topic. Iman to source D3 kit. Approx. $400 Received PowerPlex kit and ladder – test to be carried out. 30/1/09 – Change Proposal to be prepared. 26/2/09 - Reply from Scott McLaren QPS – legislation is such that DNA Analysis is the only lab able to sample Ref Samples. Crime Scene samples need Minister approval to be sent elsewhere for testing, verification.	AMc	AMc to arrange for a staff member to complete this Change Proposal by 12 March 2009.
4.22	**Differential Lysis Extraction** (AAP)	SOP in draft which details that samples should not be spun too hard. Reader sheet to be scanned in. Yes new operators on diff lysis. – staff have not checked slides – this needs to be done. AAP AAP will continue thinking and liaising with staff regarding this topic. Needs Attn: Sample IDs to be investigated by BUA. Suggestion: look at all slides from that batch of Diff Lysis (2 steps) – to see if sperm present as a first step. Hasn't been anything unusual from the batch prior or after (nothing different noted). 1 difference is type of centrifuge used – some can spin faster at Max, so checking on that to ensure that the pellet is resuspended properly. LWC – give slides to Evid Rec to check. Slides have been checked – both slides had sperm but also some epgs. Method is currently being amended for the centrifugation speed – are in draft, will be activated on 27/02/09.	AMc	
4.23	**Methods Page (AUSLAB)**	11/9/08 – Screen mask not updated quickly. A word template similar to statement would be useful. Perhaps a checklist which can be scanned in. PT to follow up with Iman. 6/12/07 - Needs Attn: Could a rep from V/ C, M/C and Analytical please forward to Iman a list of ALL the methods used so that these can be added to AUSLAB. QIS document 17092 refers. The Methods Used in Casework page in AUSLAB is not up to date. Some methods are no longer used; some new ones are not listed eg. DNA IQ. 1. Review if required – PT 2. Use manual process – paper rather than AUSLAB – TEN 12/2/09 – Keep as a word document / PDF – include all the methods & yes and no fields.	PT / TEN	ONGOING

4.24	**Future Planning**	**Action** – Project Team to be established. Project – move towards electronic files – how do we get there? Evidence Recovery team working on paperless for in-tube samples, Intell working towards this for uploads.	CJA	

NEXT MEETING
The next meeting is scheduled for Thursday 21 May 2009.

PB178

Hi Dave,

The Management Team has looked at the swab casings for the rayon swabs, and we are not able to make a definite decision on the most appropriate hole size. Basically, the green lidded tube is the preferred tube type, however in regard to the hole size, there are a lot of factors that need to be considered.

The larger hole size may potentially allow more air to get into the casing, however we would need to conduct experiments to determine if the adhesive we stick over the hole will provide an appropriate seal during our processes, given the larger hole size. Also, the type of item in the tube itself would need to be considered – if the samples are hairs, scrapings or fibres, then these may potentially escape through the hole.

The above mentioned issues will also apply to the medium size hole, and even the smaller hole.

Any of the hole sizes may still not be enough to stop mould from forming on the swabs, especially in humid conditions when the swab is still wet. **The best advice we can give at this time is, regardless of the hole size, is to use 70% ethanol and dry the swabs completely before placing into the swab casings and then the envelope. If drying is not possible, immediate freezing (if practicable) and transportation would be another option.**

Some other avenues that could be considered in the future, all of which will involve further investigation and experiments to determine if they are appropriate, are:

- a desiccant added to either the envelope outside of the swab casing, or in the lid of the swab casing
- some sort of transport medium
- a swab with similar properties to the FTA card, which would inhibit the formation of mould
- a membrane covering in the lid which would allow the swab to 'breathe', without letting any material to escape.

All of the above are just discussion points and require a lot of further investigation, however if we want to minimise the chance of mould forming on the swabs, then it might be necessary to look at the alternatives, some of which are provided above.