Professor Linzi Wilson-Wilde OAM PhD

ABN 18 568 796 588

REPORT

Report to: Walter Sofronoff KC, Commissioner
Commissioner of Inquiry into Forensic DNA Testing in Queensland

Report Date: 18 November 2022

Request: This report has been requested by the Commission of Inquiry into Forensic DNA Testing in Queensland.

The instructions to the expert provided by the Commission of Inquiry can be found at Appendix 1.

The main purpose of this report is to comment on whether the use of rayon swabs with 70% ethanol were properly validated before being implemented by QPS. If not, what are the consequences of that and what should be done to rectify that situation?

Information Reviewed: The index of information provided and considered as part of the development of this report can be found at Appendix 2.

Qualifications I commenced my career at Victoria Police in 1996 as a forensic biologist, attending crime scenes, with expertise in biological fluid identification and DNA analysis. In 2000 I joined New South Wales Police as a Forensic DNA Specialist working on legislative reform, policy development, the investigation of high-profile murder cases, cold case reviews and the highly publicised mass DNA screen in the town of Wee Waa, NSW. After moving to the Australian Federal Police (AFP) in 2002 as Team Leader of the Biology Team, I coordinated the DNA analysis of all samples involved in the disaster victim identification and criminal investigation of the Bali Bombing in October 2002 and advised on the associated legislative change. Whilst at the AFP I commenced my PhD at the University of Canberra in species identification of Diprotodontia for wildlife crime investigations, which I completed in 2011. I joined the National Institute of Forensic Science (NIFS) in 2008 and succeeded to Director NIFS in 2015. I am the Chair of Standards Australia committee CH041 and ISO committee TC272 – Forensic Sciences, developing forensic specific Australian and international Standards respectively. I am the current President of the International Forensic Strategic Alliance and represent them on the International Criminal Court Office of the Prosecutor Scientific Advisory Board. I am currently the Director of Forensic Science SA. My Curriculum Vitae has been previously provided to the Commission.

Comments and Opinions

Question Was the use of rayon swabs with 70% ethanol properly validated before being implemented by QPS? If not, what are the consequences of that and what should be done to rectify that situation?

Introduction

1. The method used to collect biological material from substrates is a critical element in the forensic DNA analysis process. If the biological material is not collected optimally, it can significantly affect the success of downstream DNA analysis processes.
2. There is no single method that is suitable for all biological sample types and substrates.
3. The success of the swabbing methodology is a factor of the substrate, biological material, swab type and wetting agent type.
4. Substrates can be porous or non-porous. Biological material includes blood, semen, saliva and trace DNA. The material on the end of swabs can include cotton, rayon, foam, divinised cellulose, polyester, nylon, viscose, Dacron, or paper. The swab handles can include wood or plastic. Wetting agents that have been suggested in the literature include sterile water, isopropanol, ethanol, lysis buffer, sodium dodecyl sulfate (SDS), and phosphate buffered saline (PBS). Please note these are not exhaustive lists but have been included to indicate the degree of variability.
5. Preferably swabs should be certified "forensic DNA grade" and compliant with ISO18385.
6. Sterile water is the most common wetting agent used for forensic DNA swabbing.
7. It is vital that the swabbing method is properly validated, consistent with the way it is intended to be used and includes prior testing that replicates casework.
8. Implementation of a method into casework should be preceded by an appropriately designed validation or verification study. Validation refers to an empirical study (according to international guidelines, see ANZPAA NIFS Empirical study design in forensic science 2019) that demonstrates that a method is fit for purpose and operates according to the intended use. The empirical study should demonstrate that the method is repeatable and reproducible, and the false positive and false negative rates (error rates) are understood. The empirical study should also be performed in conditions similar to operational casework, so that the results of the studies can be more accurately applied to casework.
9. If the method has been robustly validated and successfully implemented into another laboratory and the proposed method is unchanged from that validation, then the method only needs verification, which is an empirical study to demonstrate that the method operates as expected in the new laboratory.

Current method

10. For the collection of biological material via swabbing, Queensland Police Service (QPS) use a swab with 70% ethanol as the wetting agent (see CSE 101 Collection of Biological Evidence Crime Scene Examination, document number QPS.0020.0066.0001). The swab used is a rayon swab (see paragraph 13, Statement of David Neville dated 2 November 2022, document number QPS.0308.0002.0001).

Validation of current method

11. In 2009, QPS commenced the use of rayon swabs for the sampling of biological material, based on advice from Queensland Health Forensic and Scientific Services (QHFSS) (see Statement of David Neville dated 2 November 2022, page 3, paragraph 13 and attachment marked EXHBIT

222, relating to advice provided by Allan McNevin dated 26 March 2009). At the time, the rayon swabs were used in conjunction with sterile water as the wetting agent.

12. Once provided with advice regarding an appropriate swab type, it is appropriate that the swabs should have been validated or verified prior to implementation to ensure they were fit for purpose.

13. In 2010, stemming from an issue with mould, QPS investigated the use of ethanol as the wetting agent. This appears to be based on the advice from Adrian Pippia, QHFSS and earlier communication from Cathie Allen in an email dated 18 June 2008 (see the statement of David Neville dated 2 November 2022, page 3, paragraphs 14-15).

14. In the statement of David Neville dated 2 November 2022, I note in an attachment (page 102, labelled as EXHIBIT 223) a report entitled "Evaluation of Swab Drying Time" by Lyza-Jane McMenz. On page 1 of the report, it states:

    "…separate project studies have been undertaken to assess the ability to generate a DNA profile from dried blood stains collected using 70% ethanol and water as the solvent. Interim results indicated that water is more effective at generating a full profile than 70% ethanol. When collecting samples in both experiments it was noted that water was more effective at lifting the sample from the surfaces particular semiporous and porous surfaces. When collecting small blood stains this could affect the amount of DNA collected and therefore the ability to generate a full DNA profile."

    and

    "When using 70% ethanol moistened swabs it appeared that not as much of the stain is collected. This may prove to be critical in the case of small stains on semi-porous surfaces such as plasterboard."

15. In reference to a request for the study described above on the effectiveness of 70% ethanol versus water, David Neville states in his statement dated 14 November 2022 (page 3, paragraph 15) "*This work, if undertaken, occurred more than 10 years ago and the officer involved left the employment of the QPS several years ago. The paper refers to interim results only. A search of her records failed to find any information in relation to these studies or interim results*."

16. It should be noted that the rayon/70% ethanol combination should have been validated prior to implementation and the report of the validation study kept on file.

17. Inspector Neville lists some studies that involve the use of rayon and/or ethanol for sampling biological material. None of these studies include the rayon/70%ethnaol combination and no peer reviewed paper could be located in the literature that had this combination. Additionally, these studies are dated after the date of implementation of the rayon/70% ethanol swabbing method implemented in 2010 and therefore cannot support the implementation of the method in 2010. The studies listed, and any additional studies can however be used for support as part of a contemporary validation study.

18. Based on the above information, I can find no evidence to support an appropriate validation study was conducted by QPS for the swabbing methodology using the rayon swabs and 70% ethanol prior to implementation.

19. Further, based on the information in the report "Evaluation of Swab Drying Time" and the reference to the interim results (paragraphs 11-12), it is suggested that the use of 70% ethanol may compromise the results of the DNA analysis for samples collected with the rayon/70% ethanol combination.

20. It is my opinion that the implementation of the methodology currently used at QPS, for swabbing biological material for DNA analysis comprising a rayon swab combined with 70% ethanol as the wetting agent, does not constitute best practice.

21. It is also my opinion that there are better methods for swabbing biological material than the rayon/70% ethanol combination (see references in Appendix 2). There are other options for addressing the mould issues experience by QPS and QHFSS, such as using isopropanol as the wetting agent, or using a desiccant in the swab packaging. Whichever method is chosen, it should be based on a robust empirical validation study.

22. The implications of an inappropriately validated or unvalidated method is that the method may not produce optimal results, potentially leading to:

    - reduced sample collection efficiency,
    - compromised sample storage,
    - compromised DNA analysis and subsequent profile generation.

Next Steps

23. A robust validation should be undertaken comparing various swab types and various wetting agents. This should be undertaken as soon as possible and should assess the following using current processes used in Queensland (as an end-to-end process used by QPS and QHFSS):

    - Various swab types,
    - Various wetting agents,
    - Different types of biological material commonly encountered in case work,
    - Different types of substrates commonly encountered in case work,
    - A comparison of the collection efficiency of the swabs and wetting agents being tested,
    - A comparison of the extraction efficiency of the swabs and wetting agents being tested,
    - A comparison of the DNA analysis and profile generation efficiency of the DNA obtained from the swabs and wetting agents being tested,
    - A comparison of the performance of the of the swabs and wetting agents being tested on mock crime scene samples,
    - A comparison of the of the swabs and wetting agents being tested in various environmental, transport, and storage conditions commonly encountered in case work,
    - A comparison of the effect of different options for packaging commonly used in case work,
    - An analysis of microbial activity on the swabs and wetting agents being tested, and
    - The effect of enhancement chemicals for the detection of biological material used at crime scenes in Queensland (e.g. luminol, leuco crystal violet, acid phosphatase etc) on the swabs and wetting agents being tested.

24. References to the relevant validation and verification studies should be included as references in relevant Standard Operating Procedure (SOP). This will assist forensic practitioners to identify the relevant information regarding limitations, limits of detection, false positive rates etc. Whilst the references may be included in training material, practitioners do not go back to training manuals when SOPs are updated. Therefore, including appropriate references in SOPs, and updating the references as appropriate, ensures practitioners have contemporary information foe the methods they are using.

Preliminary Review of other Standard Operating Procedures

25. All biological sampling methods used must be checked to ensure they are sufficiently validated or verified and that reports of empirical studies are made available to those using the methods.

26. Any method, or critical equipment, used by QPS that has the potential to substantially impact the result obtained should be validated or verified prior to implementation. It is recommended

that all relevant methods be reviewed to ensure an appropriate validation/verification study has been conducted. This should include human-based methods (where the human is the 'instrument'), such as hair examination.

27. A preliminary review of the methods outlined in the SOPs provided and listed in Appendix 2 was conducted, as requested, to ascertain which elements should be validated/verified and if there was sufficient information in the SOP to identify if this had been completed. From the SOPs reviewed, the following methods were identified as requiring validation/verification:

    Analytical methods:

    - Combur test strips (for blood)
    - ABACard Hema-trace (for blood)
    - Tetramethylbenzidine (TMB) test (for blood)
    - Leuco Crystal Violet (LCV) staining (for blood enhancement)
    - Luminol test (for blood)
    - Harris's Haematoxylin stain (for identification of nuclear material in cells in hair follicles)
    - ABA Card p30 test (for seminal fluid)
    - Acid phosphatase (AP) test (for seminal fluid)

    Collection methods:

    - Swabs and wetting agents
    - Tapelift method
    - Vacuuming method
    - Swabs for fingernail scrappings

    Human-based methods:

    - Hair examination (where the human is the 'instrument')

    Critical Equipment:

    - Forensic Light Sources, such as the Rofin Polilight®, Rofin Polilight® Flares, Foster + Freeman Crime-lite® and Coherent TracER™ Laser

28. It should be noted that of the above methods, some have been robustly validated into practice in other laboratories and only evidence of verification is required. The SOPs for LCV staining, Combur test strips, TMB test, Luminol test, AP test, Forensic Light Sources, and Hair examination all included references that would provide the practitioner with some information about the performance of the method, but no validation information regarding how the method performed at QPS was included.

29. It is recommended that all methods and equipment listed under paragraph 27 have an appropriate validation/verification study and a reference to the relevant study is included in the appropriate SOP.

30. It is recommended that section 7 of the Quality Manual (see document number QPS.0013.0481.0001) is updated to provide additional guidance for verification and expressly state that all methods shall be validated or verified prior to implementation. It is also recommended that the Quality Manual expressly specifies that all critical equipment shall be validated prior to implementation.

[redacted]

Professor Linzi Wilson-Wilde OAM

Appendix 1 – Amended Instructions to expert

On 11 Nov 2022, at 5:43 am, Susan Hedge <[redacted] wrote:

Dear Linzi

I hope you are doing well. I am writing to see if you would be able to do another short piece of work for the Commission.

It relates to an issue identified by Heidi Baker about the QPS using rayon swabs with 70% ethanol as a wetting agent. Ms Baker raised an issue about literature suggesting that was not ideal for collecting DNA and suggesting it was worth considering whether that was properly validated by the QPS. We asked the relevant QPS officer, Inspector Neville, to outline the process of deciding to use rayon swabs with 70% ethanol as a wetting agent. We were hoping you might be willing to consider his response and advise whether the QPS have properly validated the use of rayon swabs with 70% ethanol. We think the parameters of the task would be:

1. Scope – was the use of rayon swabs with 70% ethanol properly validated before being implemented by QPS? If not, what are the consequences of that and what should be done to rectify that situation?
2. Mode of reporting – written report (2-3 pages)
3. Time – perhaps 3-5 hours? Here is a link containing inspector Neville's statement together with Ms Baker's email and a statement of Ms Allen and Ms Brisotto that in parts touch upon the decision making process, to assist in your estimating the time commitment: Brief (Swabs) – 11 November 2022
4. Due date – aiming for conversation with Counsel Assisting (Josh Jones) on Tues 15/11, report on Wed 16/11.

If you could let us know when you are able if you are able to take on this task, that would be great. Josh and I are available this afternoon if you would like to discuss.

Many thanks,
Susan

Susan Hedge
Counsel Assisting
[redacted]

Commission of Inquiry into Forensic DNA Testing in Queensland

Phone 07 3003 9722
enquiries@dnainquiry.qld.gov.au
PO Box 12028, George St Qld 4003
www.dnainquiry.qld.gov.au

Appendix 2 – Index of information provided and considered

No.	Document	Date	Inquiry Reference
1.	**Letter to Expert**		
1.1	Email instructions to Linzi Wilson-Wilde	11-11-2022	
2.	**Terms of Reference**		
2.1	Terms of Reference – Commission of Inquiry into DNA Testing in Queensland	10-06-22	
3.0	**Statements**		
3.1	Statement of Cathie Allen	09-11-2022	WIT.0019.0042.0001
3.2	Statement of David Neville	02-11-2022	QPS.0308.0002.0001
3.3	Statement of Paula Brisotto	09-11-2022	n/a
3.4	Statement of David Neville	14-11-2022	n/a
4.0	**Standard operating Procedures**		
4.1	QPS Operational Procedures Manual, Ch 2, Part 2.25	3 Jun 2022	QPS.0013.0028.0001
4.2	CSE 100 Crime Scene Examination	2 Aug 2021	QPS.0020.0025.0001
4.3	CSE 101 Collection of Biological Evidence Crime Scene Examination	3 Nov 2021	QPS.0020.0066.0001
4.4	CSE 104 Fingernail Scrapings Crime Scene Examination	4 Sep 2020	QPS.0089.0004.0001
4.5	CSE 111 Hair and Fibre Detection and Collection	26 Oct 2020	QPS.0089.0006.0001
4.6	HEX 100 Hair Examination and Comparison	17 Feb 2021	QPS.0089.0008.0001
4.6a	CSM 100 Crime Scene Coordination for Major Investigations	21 Mar 2016	QPS.0078.0003.000
4.7	CSE 105 Leuco Crystal Violet (LCV) Detection/Enhancement of Bloodstains	6 Jan 2021	QPS.0089.0005.0001
4.8	CSE 115 Use of Combur Test Strips	20 Jan 2022	QPS.0020.0223.0001
4.9	CSE 119 ABAcard Hema-Trace	21 Jul 2021	QPS.0089.0007.0001
4.10	PST 100 Tetramethylbenzidine (TMB) Screening Test for Blood	23 Mar 2022	QPS.0020.0271.0001
4.11	PST 101 ABAcard P30 Test for Seminal Fluid	1 Sep 2021	QPS.0020.0272.0001
4.12	PST 102 Luminol Detection and Enhancement of Bloodstains	21 Apr 2022	QPS.0020.0274.0001
4.13	PST 104 Seminal Fluid Screening and Sampling Protocols	30 Mar 2021	QPS.0020.0275.0001
4.14	PST 105 Forensic Light Sources v7	21 Apr 2022	QPS.0020.0277.0001
4.15	PFS 100 Forensic Services Group Quality Manual	Jun 2022	QPS.0013.0481.0001
4.16	SOC 100 Scenes of Crime Case File Procedure (Note: *appears to apply to Scenes of Crime Officers*)	19 Nov 2021	QPS.0078.0021.0001
4.17	SCI 116 Scientific Section Case File Management (Note: *appears to apply to Scientific Officers*)	4 Nov 2020	QPS.0078.0022.0001

No.	Document	Date	Inquiry Reference
5.0	**Correspondence**		
5.1	Email from Heidi Baker to Susan Hedge	01-11-2022	EXP.0007.0002.0001
6.0	**Miscellaneous**		
7.1	QPS List of Consumables	n/a	QPS.0089.0003.0001

References

1. Australia New Zealand Policing Advisory Agency, National Institute of Forensic Science, 2019. Empirical study design in forensic science https://www.anzpaa.org.au/forensic-science/our-work/products/publications.
2. Bonsu, D.O.M., Higgins, D. and Austin, J.J., 2020. Forensic touch DNA recovery from metal surfaces–A review. Science & Justice, 60(3), pp.206-215.
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10. Verdon, T.J., Mitchell, R.J. and van Oorschot, R.A., 2014. Swabs as DNA collection devices for sampling different biological materials from different substrates. Journal of forensic sciences, 59(4), pp.1080-1089.