NSW GOVERNMENT Health Pathology

Certificate of Analysis

Section 177 Evidence Act 1995

Report 4

RE: Alleged Murder of William DUTFIELD

FASS Ref: FS052452

Police Ref: E904029103865

(1) I, David BRUCE, am employed at the Forensic Biology/DNA Laboratory of the Division of Analytical Laboratories, Joseph Street, Lidcombe.

(2) My scientific qualifications are Bachelor of Science from the University of Sydney, Postgraduate Diploma in Clinical Science from Riverina College of Advanced Education and a Doctor of Philosophy from the Open University, United Kingdom and I have specialised knowledge based on my training, study and experience.

(3) I acknowledge that I:

(i) have read the Expert Witness Code of Conduct in Schedule 7 of the NSW Uniform Civil Procedure Rules 2005; and

(ii) agree to be bound by the Code.

(4) The following items were received on:

Friday 18 June 2010 from S. HUNGERFORD of the Homicide Squad

1. Sticky tape dispenser (re-submitted)
11. Underpants - deceased (re-submitted)
12. Tea towel - kitchen floor (re-submitted)
17. Blue face washer (re-submitted)
18. DNA extract from bloodstain - tissue from waste bin
21. Cloth rag - kitchen sink (re-submitted)
22. Black cardigan (re-submitted)
23. Light blue shirt (re-submitted)
24. Blue shorts (re-submitted)
25. Right hand fingernails - deceased (re-submitted)
26. Left hand fingernails - deceased (re-submitted)
27. Dark brown cover from head of chair (re-submitted)
28. Light brown cover from head of chair (re-submitted)
29. Light brown cover from arm of chair (re-submitted)
30. Wig (re-submitted)

NSW Health Pathology
ABN 49 382 586 535

NSW Forensic & Analytical Science Service
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Friday 19 November 2010 from S. HUNGERFORD of the Homicide Squad

31. 2003 Diary - property of Arthur ASHWORTH
32. Passport - Arthur ASHWORTH

Thursday 13 January 2011 from the Forensic Science Services Branch with seal numbers 241281 and 241301

33. Various cards
34. Hairs from ashtray
35. Swab - stain inside ashtray
36. Swab - stain outside ashtray
37. Swab - stain on rim of ashtray
38. Swab - ashtray edges

For previous exhibit delivery details please see previous report(s).

The following reference sample(s) were received on:

Tuesday 14 September 2010 from S. VOSNIKIVOC of the Parramatta Police

Buccal sample from I83 (Barcode No. 52032173)

(5) Based on my specialised knowledge I can report as follows:

Item No	Item Description	Biological Fluid Testing	Results
1	Sticky tape dispenser (re-submitted)		Not examined.
1ii	Stored swab from tape dispenser	Blood not detected.	DNA testing was unsuccessful.
11	Underpants - deceased (re-submitted)		Not examined.
12	Tea towel - kitchen floor (re-submitted)		
12v	Stained area	Positive screening test for blood.	Due to the low levels of DNA recovered, an individual profile could not be determined.
12vi	Stained area	Positive screening test for blood.	The DNA recovered has the same profile as William DUTFIELD.
12vii	Stained area	Positive screening test for blood.	DNA testing was unsuccessful.
17	Blue face washer (re-submitted)		

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17i	Centre area on one side		The DNA recovered is a mixture that originates from more than two individuals. Individual 'A' cannot be excluded as a contributor to the mixture, however the significance of this finding cannot be determined as the DNA profile is too weak and complex for statistical calculations.
17ii	Centre area on opposite side		The DNA recovered is a mixture that originates from more than one individual. Due to the low levels of DNA the profiles of the individual contributors could not be determined.
18	DNA extract from bloodstain - tissue from waste bin		The DNA profile recovered using the Profiler Plus System is consistent with originating from an unknown male individual 'A' (reported FS 00/463, 25th July 2000). This profile could not have originated from I83 Additional testing using the Y-filer System was conducted. The Y-filer profile recovered matches the Y-filer profile of I83 Therefore, this profile could have originated from from a male relative of I83 on the paternal line.
21	Cloth rag - kitchen sink (re-submitted)		
21ii	Area of fluorescence		DNA testing was unsuccessful.
21iii	Area of fluorescence		DNA testing was unsuccessful.
21iv	Area of fluorescence		Due to the low levels of DNA recovered, an individual profile could not be determined.
21v	Area of fluorescence		DNA testing was unsuccessful.
22	Black cardigan (re-submitted)		
22i	Inside of the collar area		DNA testing was unsuccessful.
22ii	Inside of the left wrist		DNA testing was unsuccessful.
22iii	Area on abdomen	Blood not detected.	The DNA recovered is a mixture that originates from at least three individuals. Due to the low levels of DNA and the complexity of the mixture, the profiles of the individual contributors could not be determined.
22iv	Area on left sleeve	Blood not detected.	The DNA recovered is a mixture that appears to originate from two individuals. William DUTFIELD and Individual 'A' cannot be excluded as contributors to this mixture. It is approximately one million times more likely to obtain this mixed profile if it originates from DUTFIELD and Individual 'A', rather than from DUTFIELD and an unknown, unrelated individual in the general population.

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22v	Area on right chest	Blood not detected.	The DNA recovered has the same profile as William DUTFIELD. Traces of DNA from at least one other individual were also recovered but at levels too low to determine a profile.
22vi	Area on front centre	Positive screening test for blood.	The DNA recovered is a mixture that appears to originate from two individuals. William DUTFIELD and Individual 'A' cannot be excluded as contributors to this mixture. It is approximately one million times more likely to obtain this mixed profile if it originates from DUTFIELD and Individual 'A', rather than from DUTFIELD and an unknown, unrelated individual in the general population.
23	Light blue shirt (re-submitted)		
23vi	Stain - left shoulder of blue shirt	Positive screening test for blood.	The DNA recovered is consistent with originating from William DUTFIELD.
23xi	Stain - left shoulder of blue shirt	Positive screening test for blood.	The DNA recovered is consistent with originating from William DUTFIELD.
24	Blue shorts (re-submitted)		Not examined.
25	Right hand fingernails - deceased (re-submitted)		
25i	Right hand fingernails - deceased (re-submitted)		The DNA recovered is consistent with originating from William DUTFIELD.
25ii	Right hand fingernails - deceased (re-submitted)		The DNA recovered is consistent with originating from William DUTFIELD.
25iii	Right hand fingernails - deceased (re-submitted)		The DNA recovered is consistent with originating from William DUTFIELD.
25iv	Right hand fingernails - deceased (re-submitted)		The DNA recovered is consistent with originating from William DUTFIELD.
25v	Right hand fingernails - deceased (re-submitted)		The DNA recovered is consistent with originating from William DUTFIELD.
26	Left hand fingernails - deceased (re-submitted)		
26i	Left hand fingernails - deceased (re-submitted)		The DNA recovered is consistent with originating from William DUTFIELD.
26ii	Left hand fingernails - deceased (re-submitted)		DNA testing was unsuccessful.
26iii	Left hand fingernails - deceased (re-submitted)		The DNA recovered is consistent with originating from William DUTFIELD.
26iv	Left hand fingernails - deceased (re-submitted)		DNA testing was unsuccessful.
26v	Left hand fingernails - deceased (re-submitted)		DNA testing was unsuccessful.

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27	Dark brown cover from head of chair (re-submitted)	Not examined.
28	Light brown cover from head of chair (re-submitted)	Not examined.
29	Light brown cover from arm of chair (re-submitted)	Not examined.
30	Wig (re-submitted)	Not examined.
31	2003 Diary - property of Arthur ASHWORTH	
31i	Pages from 8th December - 14th December of 2003 diary	The DNA profile recovered is consistent with originating from unknown male individual 'A'.
31ii	Pages from 1st September - 7th September of 2003 diary	The DNA profile recovered is consistent with originating from unknown male individual 'A'.
32	Passport - Arthur ASHWORTH	Not examined.
33	Various cards	Not examined.
34	Hairs from ashtray	Not examined.
35	Swab - stain inside ashtray	DNA testing was unsuccessful.
36	Swab - stain outside ashtray	The DNA recovered has the same profile as William DUTFIELD.
37	Swab - stain on rim of ashtray	The DNA recovered has the same profile as William DUTFIELD.
38	Swab - ashtray edges	DNA testing was unsuccessful.

(6) See the attached appendicies for important information.

(7) Other scientific staff have assisted with the analysis and processing of items from this case.

Biologist's Signature: _____

Date: 18/12/13

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APPENDIX: Overview of Procedures and Methods used in the Forensic Biology/DNA Laboratory, NSW Forensic & Analytical Science Service

Introduction: In order to distinguish one person's blood, semen, hair or other body material from another person's, DNA tests are used. While the New South Wales Forensic Biology Laboratory (FBL) has been using DNA testing since 1989 the processes used and the areas of DNA targeted have changed markedly over the years.

DNA (or Deoxyribonucleic Acid) is a complex substance found in most cells. It carries the code for the characteristics and functions of the body and it is inherited by the offspring from the parents - half from the mother and half from the father. Body materials such as blood, semen, muscle, bone marrow, hairs and skin cells from the one person will all contain the same DNA.

While the DNA in different individuals is largely the same, there are areas of the DNA which show considerable variability. Forensic biologists target these areas so that, except for identical twins, the probability of discrimination between different people is extremely high.

Since 1994, DNA analysis by PCR has been the method of choice in this laboratory. PCR (or polymerase chain reaction) involves targeting specific areas of the DNA and copying or amplifying these targeted regions many millions of times. Since 1996, the forensic use of PCR for DNA analysis involved determining the size variation that existed at specific DNA sites. Many papers have been published over the years showing that this technology produces accurate, reliable and robust results.

In the late 1990's, all Australian forensic laboratories introduced the Profiler Plus™ system, which targeted nine highly variable areas (loci (sing: locus)) of the DNA and one area determining gender. In 2012, it was decided that 18 loci would form the core comparison group for the National Criminal Investigation DNA Database (NCIDD). In 2013, PowerPlex 21 was introduced into the FBL, a system that contains 20 highly variable loci and the gender locus. It is to be noted that there will be cases where Profiler Plus is still reported. A further DNA identification system that targets areas on the Y chromosome (male specific) is also validated for use in selected cases.

Statistical Overview: If there are differences in the DNA profile between good quality, high yield DNA samples then these samples could not be from the same person. However, increased sensitivity since the introduction of DNA testing has meant that perceived differences between two profiles may still mean they are from the same individual.

Where there are no differences between the DNA profiles of two samples, or the differences are considered insufficient to be considered an exclusion, a statistical estimate of this non-exclusion event may be possible. In some profiles, the complexity and/or low yield of DNA may prevent any statistical calculation being conducted.

For autosomal DNA, the frequency of the total DNA profile can only be determined if the frequency of each DNA type at each locus is known. Such information is contained in a population database of person samples from where the frequencies of the different types are derived. There is much evidence that shows there is little difference between the profile frequencies generated from a NSW database to those from databases of similar racially comprised populations used by other Australian States and worldwide. PowerPlex 21 statistics are calculated using a national frequency database specific that can be applied for Caucasians, Aborigines or Asians. Profiler Plus used the general database for population statistical reporting.

While simply multiplying together the different frequencies will allow the generation of a complete DNA profile frequency, alternate calculations are used in the FBL. Since the person contributing the evidence and the suspect may share a common ancestry, a correction factor for co-ancestry (F_{ST}) is used. A correction for sampling variation in creating finite size population databases may also be included in the calculation. The inclusion of these correction factors results in a more conservative estimate for the occurrence of a DNA profile in the population.

The laboratory incorporates a conservative cut-off of fewer than one in 10 billion (a billion is defined as a 1000 million) with Profiler Plus and fewer than one in 100 billion for PowerPlex 21 when reporting the final

statistical calculation. The actual result is usually many times larger than these cut-offs. The result is not incorrect although it is larger than the estimated seven billion inhabitants of the earth. It is based on a theoretical infinite population which includes every possible permutation of types at each of the DNA areas. It provides strong support for the hypothesis (without taking all other evidence into account) that the DNA from the evidence sample originates from the matched individual.

The profile frequency calculation does not apply to closely related individuals. Alternative calculations can be presented if it is suggested that the evidence sample comes from a close relative of the person. It is preferable that if such a suggestion is made, the relative's reference sample is made available for analysis.

Rather than report a profile frequency as 1 in x of the population it is commonplace that a likelihood ratio (LR) mixture calculation is used to determine the significance of the evidence. It is based on comparing the probability (or likelihood) of a crime stain and a reference sample having matching profiles given two competing scenarios. The value of the evidence is measured by how much the LR differs from 1. An LR less than 1 favours one scenario, a result greater than 1 favours the other scenario. Different scenarios can be suggested and the resulting LRs can vary significantly.

In many cases the race of the person who left the evidence sample is unknown. However, calculations can also be made from various ethnic databases if the ethnicity of the offender (as opposed to the defendant or suspect) is known.

Where a crime scene profile has been obtained for the Y chromosome (sex chromosome) a comparison is made to a person sample. Where the profile differs then in most situations this would be considered to be an exclusion. However, where an exclusion is not found a calculation is made based on the haplotype frequency. This is because the Y profile results are all from one chromosome and therefore considered to be a single haplotype. The haplotype frequency estimation is based on the counting method, that is how many times has the haplotype Y profile been seen in the database. The numerical result obtained is qualified with the comment that all males along the same paternal line will generally have the same Y profile.

Expert systems: Increases in sensitivity in DNA analysis has led to difficulties in interpretation of DNA profiles, in particular where mixtures of DNA from two or more individuals are present. In 2013, the FBL introduced STRmix, an expert system to be used to aid in the interpretation of PowerPlex 21 profiles. STRmix uses extensively validated interpretational software to apply a fully continuous approach to DNA interpretation. It uses the quantitative data available from the DNA profile results to make better use of the DNA profiling data so that important information is not simply discarded. This information may now aid in either providing support for one scenario, or may provide support for an alternative scenario.

Quality Assurance: The FBL has been NATA (National Association of Testing Authorities, Australia) accredited for Forensic Biology/DNA since 1999.

The FBL has an extensive Quality Assurance Programme in place that is used to ensure uniform and reliable testing and reporting, and to detect and prevent errors. This is achieved in a variety of ways including the control of all documents and forms, the bi-annual review of all methods and documents, full traceability of all exhibits, formal staff training programme for all the methods and procedures, participation in regular external and internal proficiency tests and regular internal audits of the laboratory.

There are also many quality system checks within the FBL including DNA analysis sample transfer system checks, contamination minimisation protocols, and the use of positive and negative controls, where appropriate.

Quality System Documentation: Detailed policies, procedures and methods are held in the Laboratory.

Secure Storage: The entire Forensic Biology laboratory is a secure area under restricted and controlled access. An extensive alarm system is in operation after hours. From the time of receipt until dispatch all items of evidence are stored within this secure facility. DNA extracts and person samples are retained indefinitely within this secure area.

External Proficiency Testing: The laboratory participates in external Forensic Proficiency Testing Programmes. NATA monitors the performance of accredited forensic science laboratories in the external proficiency testing programs.

Analyses carried out in the Laboratory: The Reporting Scientist takes responsibility for the scientific accuracy of the analyses and opinions expressed in the report. However, the receipt of exhibits, casework analyses, DNA testing and other related activities are usually carried out by numerous trained staff within the laboratory. This is standard practice in all types of scientific laboratories. All involvement of the staff in the processes and protocols is fully documented and their identities and details of their specific involvement can be provided, if required. All staff have undergone and passed the relevant competency based training pertaining to the task and are subject to ongoing review of their performance. The qualifications of the staff are appropriate for the tasks performed. For example, all scientists must have as a minimum a Bachelor of Science degree majoring in a field of biology. DNA is reported only by a senior scientist within the laboratory.

Peer Review: All Forensic Biology reports are subject to an Administrative review prior to release, which is designed to check for consistency with laboratory policy and ensure the completeness and correctness of the report. Additionally, most case files with court reports undergo a technical review by a peer forensic biologist to check for scientific and technical correctness.

DNA Profile Interpretation: All DNA profiles are read independently by two scientists or a scientist and an expert reading system. The reporting scientist is responsible for the interpretation of the test results. There is at least one additional peer review check by an appropriately trained scientist prior to the results being reported.

Sample Retention: Permitted person samples and DNA extracts from crime scene evidence, wherever possible, are retained indefinitely. Items from crime scenes are returned to the Police as soon as practicable.

Screening Tests: The laboratory employs a number of different chemical screening tests for blood, semen, saliva, urine and faeces. Appropriate wording is used in reports to reflect the specificity of the tests performed. Screening tests are not available for skin cells.

Differential DNA Extractions are performed on samples where both spermatozoa and epithelial (skin) cells may be present, in an attempt to separate the spermatozoa from the other cell types. Under some conditions, DNA from epithelial cells may appear in the sperm cell fraction DNA profile and/or DNA from spermatozoa may appear in the epithelial cell fraction DNA profile, resulting in mixed DNA profiles.

Reports: Reports are prepared in accordance with NATA requirements. They contain all the relevant information considered pertinent to the case. While they are, of necessity, a summary of the total analysis no important findings are intentionally omitted. Further details of the analyses performed are contained in the Case File and other information is available in the laboratory.

Reports also contain opinions of the reporting scientist. These opinions are based on the experience of the scientist, communication with peers, courses, scientific papers, attendance at conferences, and studies carried out within the FBL and by other laboratories. The reporting of forensic biology/DNA results covers many varied fields including biochemistry, immunology, molecular biology, statistics and genetics. Due to the varied nature of the fields, and the diverse range of materials that the reporting scientist uses to support their opinions, it is difficult to provide a list of all material to cover all fields. As an example, reporting a DNA statistic includes literature and other material related to population genetics, genetics, Bayesian theory, probability, sampling variation, etc. However, if required, the laboratory can provide a list of selected texts and other material that are used in the laboratory. It is not complete and does vary but it does give a reasonable reference list from where opinions used by reporting scientists are based.

DNA Searchable Databases are used for linking DNA profiles either within NSW or, where permitted, between NSW and another State or Territory. DNA profiles from crime scene samples are uploaded to the databases, unless the sample profile matches, or is strongly presumed to match, a volunteer or victim. Uploaded crime scene profiles are matched against DNA profiles from persons as allowed under the matching tables found in the NSW Crimes (Forensic Procedures) Act 2000.

APPENDIX: Overview of Y STR testing

Y STR testing (using Y-filer) employs the same technology as conventional DNA typing (using the Profiler Plus System). The difference is that the gender-determining chromosome of the male (the 'Y' chromosome) is targeted in the Y STR test. This can be particularly useful in a case where the DNA recovered from an item is a mixture of both male and female DNA. As females do not possess a Y chromosome (only X chromosomes) this difference is exploited in order to target only the male DNA in a male/female DNA mixture.

The mode of inheritance of DNA markers typed in conventional versus Y STR testing differs and hence, a different method is needed to interpret the statistical weight of a match. DNA markers, identified using the Profiler Plus System, are passed down to the child from both the mother and father, and the inheritance of each individual type is independent of each other. With Y STRs however, the DNA is passed down from the father to the son as a whole unit or 'haplotype', virtually unchanged (except for occasional mutations) from one generation to the next. Therefore the haplotype of a man should be the same as his biological brothers and sons (and all other males along the paternal lineage). A haplotype, using Y-filer, is composed of 17 markers.

In order to assign a statistical weight to a haplotype that matches a person, a counting method can be used as to the occurrence of this profile in population databases. A correction factor which takes into account the size of the sampled population is normally incorporated into this frequency estimate. At present the Division of Analytical Laboratories has a local population database which includes samples of Aboriginal, Asian, Middle Eastern and European individuals. A larger database –YHRD (Y Chromosome Haplotype Reference Database) is also available online at www.yhrd.org. An additional feature of the YHRD website is its ability to search for 'haplotype neighbours' (similar or related haplotypes that may have occurred from a mutational event).