

Forensic Biology/DNA Laboratory
 Forensic & Analytical Science Service
 PO Box 162 Lidcombe, NSW 1825
 ABN 49 382 586 535
NSWPATH-FASSDNA@health.nsw.gov.au



**Health
Pathology**

Expert Certificate

Section 177 Evidence Act 1995

RE: Alleged Murder of Ernest Allan HEAD

FASS Reference Number: FS760178 and FS055959

Police Reference Number: E206377694

- (1) I, David BRUCE, am employed at the NSW Health Pathology Forensic and Analytical Science Service, Joseph Street, Lidcombe.
- (2) I have a Bachelor of Science from the University of Sydney, a 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) Based on my specialised knowledge I can report as follows:

The report by P.A. CONNELLAN dated the 17th September 1976 details the examination of a number of submitted items, which underwent chemical and microscopic testing to identify body fluids and blood grouping tests for comparison with reference samples from the victim and others. Based on the information in this report on the initial testing, it may have been possible to carry out the following contemporary testing on these items if they were still available:

- i. Semen was detected on the penile (item 2) and anal (item 3) swabs and on a handkerchief (item 45). These samples may have been suitable for DNA testing to identify the source of the semen. In particular, tests of the semen from the handkerchief indicated a mixture of blood groups, one of which was consistent with Ernest HEAD's blood group. The DNA profile from the unknown contributor to this mixture may have been able to be determined and searched on the NSW or National DNA databases.



Accredited for compliance with ISO/IEC 17025 - Testing.
 This document shall not be reproduced, except in full.
The results apply to the sample(s) as received.

FASS Reference Number: FS760178 and FS055959
Police Reference Number: E206377694

- ii. Saliva was detected on several cigarette butts (items 29 to 32 inclusive). Saliva is a high yield source of DNA and these items may have been suitable for DNA testing. However, it should be noted that the cigarette butts were tested for the presence of amylase (an enzyme found in saliva) in 1976 which may have consumed the potential target areas for DNA testing on the items.
- iii. Human blood was detected on a number of the items tested including a radio (item 17), knives (items 18 and 19), trousers (item 27), bedding (items 20 to 23 inclusive) and a number of blood scrapings from the crime scene (items 37 to 44 inclusive) etc. The reported results for most of these give a blood group which is consistent with the victim; however, a few report an incomplete result due to insufficient sample or unsuccessful testing. DNA testing may have been able to be carried out on these stains to obtain a DNA profile for comparison with reference samples and uploading onto the database.
- iv. Hair samples recovered from the crime scene (items 33 to 36 inclusive) could have been examined microscopically to determine the species status and, if human, the suitability of the hair roots for nuclear DNA testing. Identified human hairs which were unsuitable for nuclear DNA testing may have been suitable for mitochondrial DNA testing.
- v. Reference blood samples collected from the victim and other individuals noted in the report could have been DNA profiled for comparison with DNA recovered from the crime scene items.
- vi. Clothing items from two individuals, [1454] (items 6 to 9 inclusive) and [1462] (items 47 to 50 inclusive) were received and examined for the presence of blood and semen. Testing of these items for the recovery of trace or low level DNA deposited by touch contact may have been possible; however, this is usually very low yield DNA and may not have been detectable after this considerable time period (see note below). Blood was detected on [1454]'s shoes and this may have been suitable for DNA testing.

N.B. The opinions given on the potential recovery of DNA from items in this case, if they were still available for testing, would depend greatly on the amount of sample available on the exhibit following the testing carried out in 1976. Grouping and other chemical testing required a very large amount of sample and may have totally consumed the biological stains available on the exhibits. In addition, it would be expected that any DNA recovered from these samples would have undergone some degree of degradation over time depending on the storage conditions of the items with freezing being the optimal method for the preservation of biological material. This would affect the quality of the DNA profiles recovered i.e. reduce the amount of information in the DNA profile. However, there may still be sufficient information for direct comparison to reference DNA profiles from nominated individuals or upload onto DNA databases for searching. Other methods such as Mitochondrial DNA sequencing can be used when the nuclear DNA component is too degraded for testing.

- (5) See the attached appendix for important information.



Reported By: David BRUCE

Date: 30th May 2023

FASS Reference Number: FS760178 and FS055959
 Police Reference Number: E206377694

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

Contents

1. Introduction.....	2
2. Analyses carried out in the Laboratory	2
3. Biological fluid testing	3
4. Differential DNA extraction.....	3
5. Unsuccessful results	3
6. DNA profile interpretation.....	3
7. Statistical overview	3
8. Interpretation software	5
9. Reports	6
10. Case review	7
11. DNA searchable databases	7
12. Further testing and/or interpretation.....	7
13. Transfer and persistence of DNA.....	8
14. Specialist DNA testing	9
15. Quality assurance	9
16. Quality system documentation.....	10
17. Secure storage	10
18. Sample retention.....	10

FASS Reference Number: FS760178 and FS055959
Police Reference Number: E206377694

1. Introduction

- 1.1 DNA (or deoxyribonucleic acid) is a molecule found in most cells of the body. Nuclear DNA is contained in the nucleus of the cells and it carries the code for the characteristics and functions of the body. DNA is inherited from the parents, half from the mother and half from the father. Barring mutation, body materials such as blood, semen, saliva, hairs, and skin cells from one person will all contain the same DNA.
- 1.2 While DNA in different individuals is largely the same, there are areas of the DNA that show considerable variability. Forensic DNA testing targets these areas so that, except for identical twins, the probability of discrimination between different people is extremely high.
- 1.3 While FASS has been using DNA testing since 1989, the processes used and the areas of DNA targeted have changed over the years. Since 1994, DNA analysis using PCR has been used in this laboratory. PCR (polymerase chain reaction) involves targeting specific areas of the DNA and copying (or amplifying) these targeted areas many millions of times. PCR allows a DNA profile to be developed from very small amounts of biological material. Since 1996, the forensic use of PCR for DNA analysis has involved determining the size variation that exists at specific DNA areas. Many scientific papers have been published demonstrating that this technology produces accurate, reliable, and robust results.
- 1.4 In the late 1990s, all Australian forensic laboratories introduced the Profiler Plus[®] system, which targeted nine highly variable areas (loci (singular: locus)) of the DNA and one area determining sex. In 2012, 18 loci were adopted to form the core comparison group for the National Criminal Investigation DNA Database (NCIDD). In 2013, the PowerPlex[®] 21 system was introduced at FASS. This system targets 20 highly variable areas and one area determining sex. Note: Profiler Plus[®] and/or Identifiler[™] results may still be reported for some historical cases.

2. Analyses carried out in the Laboratory

- 2.1 The reporting scientist takes responsibility for the scientific accuracy of the analyses and opinions expressed in the Expert Certificate. 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.
- 2.2 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 relevant competency-based training 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 relevant field.

FASS Reference Number: FS760178 and FS055959
Police Reference Number: E206377694

3. Biological fluid testing

- 3.1 The laboratory may employ a number of different confirmatory tests for blood and semen, as well as chemical screening tests for blood, semen, saliva, urine, and faeces. The circumstances of the case and condition of the evidence items will determine which tests may be indicated. Appropriate wording is used in reports to reflect the specificity of the tests performed. There is no screening or confirmatory test available for skin cells.

4. Differential DNA extraction

- 4.1 A differential DNA extraction is performed on samples where both spermatozoa (sperm cells) and non-sperm cells (for example, skin cells or blood) may be present, in an attempt to separate the spermatozoa from the other cell types. Under some conditions, DNA from the non-sperm cells may appear in the profile of the sperm cell fraction and/or DNA from spermatozoa may appear in the profile of the non-sperm cell fraction, resulting in mixed DNA profiles.

5. Unsuccessful results

- 5.1 A DNA result reported as 'unsuccessful' could indicate one of several outcomes, such as there was no DNA detected; or the amount of DNA recovered from the sample was below the laboratory threshold for routine further DNA testing. 'DNA testing was unsuccessful' will also be reported where routine further DNA testing has been carried out but no DNA profile was recovered; or a very limited amount of DNA profile information was recovered, and as such, the result is not suitable for meaningful comparison.

6. DNA profile interpretation

- 6.1 At the completion of testing, the raw DNA data are analysed independently by two scientists (or a scientist and an expert reading system) in the DNA laboratory. The analysed DNA profiles are then released to the Case Management Unit for interpretation by the reporting scientist. There is at least one technical review check by an appropriately trained scientist prior to the results being reported.

7. Statistical overview

- 7.1 If there are differences in the DNA profiles generated from good quality, high yield DNA samples, then these samples could not be from the same person. Where there are no differences between the DNA profiles of two samples, then these samples could be from the same person. A statistical calculation can be carried out to estimate the weight of this evidence. There may be occasions where the

FASS Reference Number: FS760178 and FS055959
Police Reference Number: E206377694

complexity and/or low yield of DNA prevents any interpretation from being conducted.

- 7.2 The standard statistic used by the FBL to evaluate the weight-of-evidence is the likelihood ratio. This approach is widely regarded by scientists and statisticians as being the most appropriate method to assess the value of evidence and provides a balanced, logical, transparent, and robust approach. The likelihood ratio considers the probability of the findings under two competing scenarios. These scenarios are typically chosen to reflect the positions of the prosecution and defence regarding the findings. For example, suppose a DNA profile has been recovered from a bloodstain left at the scene of a crime. The recovered DNA profile matches the DNA profile of the defendant. The prosecution may suggest that the DNA originates from the defendant. The defence may instead propose that the DNA originates from an unknown individual who is unrelated to the defendant. The likelihood ratio approach evaluates the probability of the findings under each of these scenarios.
- 7.3 The ratio of these probabilities is then calculated (hence, likelihood ratio). A likelihood ratio greater than 1 supports the proposition on the numerator (typically the prosecution proposition) while a likelihood ratio less than 1 supports the proposition on the denominator (typically the defence proposition). The greater the magnitude of the likelihood ratio, the greater the relative degree of support provided by the findings. A likelihood ratio of 1 provides equal support to both propositions and can be considered 'neutral' or 'inconclusive'.
- 7.4 Alternative statistical scenarios can be considered, if required. If it is proposed that the DNA originates from a close biological relative of the individual, it is preferable that a reference sample from the relative is collected and made available for analysis. If a reference sample is unavailable, a likelihood ratio can be calculated using the defence proposition that the DNA originates from a specified relative (for example, from a sibling of the person in question), rather than from an unrelated individual. It is advisable to contact the laboratory well in advance of any court proceedings if alternative scenarios need to be considered so that the necessary statistical calculations can be carried out.
- 7.5 In order to assess the probability of the findings, information regarding the relative proportion of each DNA type in the relevant population is required. Such information is contained in a population database of person samples in which the relative proportions of the different DNA types are listed.
- 7.6 There is much evidence to show there is little difference between the match statistics generated from different databases of similar racially comprised populations used in Australia and in other countries. All statistics are calculated using national Australian frequency databases specific for the Caucasian, Aboriginal, and Asian populations. Collectively, these three ethnicities account for the majority of people living in Australia.
- 7.7 In most cases, the race of the person who left the DNA is unknown and cannot be assumed. The likelihood ratio evaluates the probability of obtaining the DNA profile

FASS Reference Number: FS760178 and FS055959
Police Reference Number: E206377694

if the DNA originates from a specified individual rather than if the DNA originates from an unknown, unrelated individual in the Australian population. The most appropriate databases to use in this calculation are the national Australian frequency databases introduced above. However, calculations can be made using specific ethnic databases if the ethnicity of the offender (as opposed to the defendant) is known.

- 7.8 Adjustments are made to the calculation to account for sampling uncertainty, that is, the fact that the relative proportions of the different DNA types have been determined using a sample taken from the larger population. A co-ancestry correction (F_{ST} , also referred to as a theta correction) is also applied to account for the possibility that the defendant and the source of the DNA (if it did not originate from the defendant) may share common ancestry. The inclusion of these correction factors results in a more conservative likelihood ratio match statistic.
- 7.9 The laboratory incorporates a conservative cut-off of 100 billion when reporting the final likelihood ratio match statistic (where a billion is defined as 1000 million). The calculated likelihood ratio is often many times larger than this. The selection of 100 billion as the cut-off value was an arbitrary decision and was based on the perceived difficulties in comprehension of very large numbers by a non-scientific audience. A likelihood ratio of this magnitude provides extremely strong support for one proposition versus the alternative.

8. Interpretation software

- 8.1 In 2013, the FBL introduced STRmix™, a specialist probabilistic genotyping software that can aid in the interpretation of DNA profiles. STRmix™ is particularly useful in the interpretation of weak or complex DNA profiles. STRmix™ uses extensively validated methods to interpret forensic DNA profiles. The methods used by STRmix™ make better use of the quantitative data contained in the DNA profile, allowing for improved discrimination between true donors and non-contributors.
- 8.2 STRmix™ uses a statistical process called stratification to combine match statistics from the Caucasian, Asian, and Aboriginal populations and produce a single figure that is representative of the Australian population as a whole. The stratified likelihood ratio is essentially a weighted average that considers the proportion of each of these sub-populations within the wider Australian population.
- 8.3 In addition to accounting for sampling variation and co-ancestry, STRmix™ incorporates a number of additional factors that ensure the final figure reported is highly conservative. This includes consideration of the effect that run-to-run variation associated with the STRmix™ software would have on the likelihood ratio. Due to these factors, the reported figure can be considered to be close to the lower limit of the range of possible likelihood ratio values.
- 8.4 STRmix™ currently requires the user to make a determination regarding the number of contributors to the DNA sample and carry out the interpretation in

FASS Reference Number: FS760178 and FS055959
Police Reference Number: E206377694

STRmix™ under a specific assumption. For weak or complex DNA results, the number of contributors may not be able to be assigned with a high degree of confidence. Additional analyses may be carried out in STRmix™ to examine the effect on the statistical weight of evidence if the number of assumed contributors is varied. In many cases, varying the number of assumed contributors to a mixture has no effect on the reported figure. In those instances, the findings of these additional analyses may not be included in the report in order to aid comprehension but are retained in the case file and available upon request.

- 8.5 Sometimes, multiple typed individuals may be found to be possible contributors to a mixed DNA profile.
- 8.6 For example, a mixed DNA profile may be recovered that originates from at least two contributors. Reference samples have been submitted from two known individuals and both could be possible contributors to the DNA recovered. In this example, one could consider the following pairs of propositions (Note: other propositions may also be considered depending on case circumstances):
- A. The DNA originates from Individual 1 and an unknown, unrelated individual versus the DNA originates from two unknown, unrelated individuals.
 - B. The DNA originates from Individual 2 and an unknown, unrelated individual versus the DNA originates from two unknown, unrelated individuals.
 - C. The DNA originates from Individual 1 and Individual 2 versus the DNA originates from two unknown, unrelated individuals.
- 8.7 Commonly, the laboratory will evaluate match statistics for sets A, B, and C above. These sets of propositions evaluate the weight of evidence against each individual separately as well as together to confirm that both individuals could be co-contributors to the recovered DNA. The match statistics for set C, or for all three sets, may be included in the report.
- 8.8 Other statistical scenarios may also be appropriate and may be carried out and included in the report. Any assumptions made for the statistical calculations are stated in the report. Further statistical calculations may also be considered upon request. It is advisable to contact the laboratory well in advance of any court proceedings if alternative scenarios need to be considered so that the necessary statistical calculations can be carried out.

9. Reports

- 9.1 Reports are prepared in accordance with the National Association of Testing Authorities (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 in other records stored in the laboratory.

FASS Reference Number: FS760178 and FS055959
Police Reference Number: E206377694

- 9.2 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.
- 9.3 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 may use to support their opinions, it is difficult to provide a comprehensive list of reference materials. As an example, reporting a DNA statistic includes literature and other materials 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 materials that are routinely used in the laboratory. It is not complete and does vary but it does give a reasonable reference list on which the opinions reported by scientists are based.

10. Case review

- 10.1 All results undergo a technical review prior to release by a suitably qualified forensic biologist to check for scientific and technical correctness. Additionally, all case files are subject to an administrative review prior to completion. This is designed to check for consistency with laboratory policy and ensure the completeness and correctness of any reports issued.
- 10.2 All results presented in an Expert Certificate (or other form of expert statement) are independently reviewed by two senior scientists.

11. DNA searchable databases

- 11.1 DNA searchable databases are used for linking DNA profiles, either within NSW or, where permitted, between NSW and another State or Territory of Australia. All suitable DNA profiles from crime scene samples are uploaded to the database 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 and other crime scene profiles contained on the DNA database as permitted under the matching tables found within the NSW Crimes (Forensic Procedures) Act 2000.

12. Further testing and/or interpretation

- 12.1 In situations where limited amounts of DNA are present, where the DNA is degraded, or where there is DNA from multiple individuals (DNA mixtures), weak and/or complex DNA profiles may be recovered. These profiles can be particularly

FASS Reference Number: FS760178 and FS055959
Police Reference Number: E206377694

challenging and may require further testing before an interpretation can be progressed.

12.2 Where limited information regarding the case circumstances and the significance of the submitted items has been provided, the laboratory may not carry out statistical interpretation of these difficult profiles in favour of interpreting other results. This approach is taken to streamline the reporting process and is generally applied where it is considered that the lengthy interpretation of multiple complex results will not provide additional information.

12.3 If the exhibit is of particular importance to the case, the laboratory can be contacted with a request to carry out further testing and/or interpretation. It should be noted that some results may be considered unsuitable for further testing and/or interpretation. If further testing is carried out, some results may remain unsuitable for interpretation. It is advisable to contact the laboratory well in advance of any court proceedings if further testing and/or interpretation is required.

13. Transfer and persistence of DNA

13.1 The transfer and persistence of DNA is affected by a number of factors and there are several mechanisms by which DNA may be transferred.

13.2 Direct transfer (or 'primary transfer') involves the transfer of DNA on to an object, surface, or person directly from the source of that DNA. This may be via physical touch or contactless transfer of body fluids (for example, blood transferred following injury or droplets of saliva projected during conversation).

13.3 Indirect transfer (for example 'secondary transfer') involves the transfer of DNA indirectly via an intermediary. In this manner, it is possible for a person's DNA to be deposited on to an object, surface, or person that they have not had direct contact with.

13.4 The direct transfer of DNA is affected by many factors including, but not limited to: the amount of DNA available for transfer, the length and nature of the contact, and the nature of the surfaces and/or objects involved.

13.5 The indirect transfer of DNA is affected by many factors including, but not limited to: the number of transfer steps between the source of the DNA and the evidence item in question, and the time, and events, which may have occurred in between transfer steps.

13.6 In many situations, particularly when dealing with low-level DNA and where the DNA cannot be scientifically attributed to a particular body fluid, it may not be possible to provide an opinion regarding the probability of obtaining the observed results given a particular transfer scenario.

FASS Reference Number: FS760178 and FS055959
Police Reference Number: E206377694

- 13.7 Methods to 'age' DNA are not in widespread use within forensic laboratories. If exposed to adverse environmental conditions such as UV light, heat or humidity, DNA will degrade to such an extent that it may fail to be detected. Conversely, DNA may be successfully typed months or even years later if stored undisturbed in favourable conditions.
- 13.8 If 'how' or 'when' the DNA was transferred is at issue, it is advisable to contact the laboratory well in advance of any court proceedings to determine whether an opinion can be provided.

14. Specialist DNA testing

- 14.1 Y-STR testing targets areas on the Y chromosome only and is therefore male specific. All males along the same paternal line (for example, father, brother, son) are expected to have the same Y-STR profile, barring mutations.
- 14.2 Mitochondrial DNA testing targets DNA recovered from the mitochondria in a cell and is passed on the maternal line.
- 14.3 Separate appendices are available in relation to Y-STR and mitochondrial DNA testing.

15. Quality assurance

- 15.1 The FBL has been accredited by NATA (National Association of Testing Authorities, Australia) for Forensic Biology/DNA testing since 1999. The FBL is accredited to ISO 17025 standards.
- 15.2 The FBL has an extensive quality assurance programme in place 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 review of all methods and documents, full traceability of all exhibits, and formal and ongoing staff training programmes and competency assessments.
- 15.3 The laboratory participates in external and internal forensic proficiency testing programmes. NATA monitors the performance of accredited forensic science laboratories in external proficiency testing programmes.
- 15.4 There are many quality system checks within the FBL including DNA sample transfer system checks, contamination minimisation protocols, and the use of positive and negative controls where appropriate.

FASS Reference Number: FS760178 and FS055959
Police Reference Number: E206377694

16. Quality system documentation

16.1 Detailed policies, procedures and methods are held in the Laboratory and are available upon request.

17. Secure storage

17.1 The entire FBL 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.

18. Sample retention

18.1 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.