Introduction of a Failed DNA Extraction Method at Queensland Health Forensic and Scientific Services DNA Analysis Unit

I, Dr Kirsty Melanie Wright, do solemnly and sincerely declare that:

1. Since 2019 I have been employed as a contractor by the Australian Defence Force to provide specialist forensic science expert services for capability development and project support.

2. I am employed by the Royal Australian Air Force to provide expert opinion on human identification based on DNA. I have held this position as a Specialist Reservist since 2010.

3. In 2017 I completed a Doctor of Philosophy in forensic DNA human identification, and in 2000 was awarded a Bachelor's in Biomedical Science (Honours).

4. I have previously held positions as a Senior Lecturer in Forensic Sciences at Griffith University, a Reporting Scientist in the Major Crime Team at Queensland Health Forensic and Scientific Services (QHFSS), Senior Scientist of Queensland Health's Skeletal Remains Project, Manager of the National Criminal Investigation DNA Database (NCIDD) at CrimTrac, Disaster Victim Identification DNA Team Leader with the Australian Federal Police, Senior Scientist of the Northern Territory Skeletal Remains Project, and Forensic Biology Expert for INTERPOL's FASTID Project.

5. This statement has been prepared upon request by Commissioner Bennett for the Commission of Inquiry to examine DNA Project 13 Concerns.

6. This statement prepared by me is true and correct to the best of my knowledge based on the information I have received.

Summary

1. This report outlines concerns arising from the introduction of a failed robotic DNA extraction method[1] at the Queensland Health Forensic and Scientific Services DNA Analysis Unit (QHFSS), used between October 2007 and November 2016 (nine years). The verification document (the Project 13 report) demonstrates the robotic method recovered up to 92% less DNA than the manual method prior to its implementation, and was one hundred times less sensitive. If the method was implemented with the knowledge crime scene evidence would fail, including trace evidence, visible blood stains, and tapelift evidence, then it is a matter of significant concern. Two further projects were conducted in 2008 after the method was

[1] The failed DNA extraction method is a coupling of a commercial chemical extraction kit (DNA IQ), and a robotic platform (MultiPROBE II).

implemented (Project 21 and Project 22) which demonstrated the method was continuing to fail.

2. The Senior Scientist in charge of Project 13, Mr Thomas Nurthen, provided a statement to the 2022 DNA Inquiry, however, did not disclose the yield failure.

3. The failed method could have been implemented to achieve faster results, and clear the much publicised backlog (the QHFSS 'Backlog Reduction Project') to meet a 2004 election pledge.

4. In 2011, a trial of a new DNA extraction robot (Maxwell) was reported in Project 70[2]. Comparisons were made against results from the failed method implemented in 2007, which showed clear differences between the two methods in DNA recovery. The new method was recovering up to eight times more DNA for trace blood samples. In my opinion, this shows QHFSS had an empirical awareness of the continued failure of the method. It is unclear what the authors of Project 70 did to report or investigate this further.

5. The senior author of Project 70, Mr Allan McNevin, was the Manager of the Analytical Section at this time, and provided a statement to the 2022 DNA Inquiry which discussed the failed method, however, did not disclose the yield failure.

6. Between 2007 and 2016 this method (and variations of it), in my opinion would have missed and wasted DNA evidence on a large scale, leading to provision of incorrect evidence to the courts and police, leading to incorrect judicial outcomes, and failure to identify violent offenders. Over nine years the failed method was likely used on well over 100,000 crime scene samples. The impacts of the failed method may be affecting cases currently before the courts, and may have impeded the resolution of serious criminal offences, including murder, that could be resolved if authorities were informed crucial evidence had been affected and could be re-tested.

7. This issue was not discovered by the Commission of Inquiry into Forensic DNA Testing in Queensland (CoI) in 2022, and in my opinion is more serious than the catastrophic 'Options Paper' extensively examined by the CoI.

Knowledge of the Failed DNA Extraction Method at the 2022 DNA Commission of Inquiry

8. The CoI engaged me to review the Blackburn DNA case file and other related documents to provide an opinion on the reliability of the DNA results produced by QHFSS. Two other independent experts, Dr Bruce Budowle and Jo Veth were also given this task, and were asked to review my report for the CoI. Our findings were included in Module 6 of the CoI hearings. The sequence of events that led to the discovery the method was systemically failing during 2012 to 2013 is outlined below.

[2] 'Phase 1 Report – Verification of Promega DNA IQ for the Maxwell 16'. Project 70. (FSS.0001.0001.0085)

9. This section discusses the initial absence of any knowledge about the failed DNA extraction method during the review of the QHFSS Blackburn DNA analysis.

10. By Module 6 of the CoI the failed DNA extraction method was still publicly unknown for the following reasons.

    i. Project 13 was an annexure in Professor Wilson-Wilde's expert report[3], however, the yield failure was not discussed in her report.

    ii. We were not provided with a copy of the Project 13 report and were unaware of its existence or importance.

    iii. Mr Nurthen was the Senior Scientist of the QHFSS Automation Project in charge of developing and implementing the failed method and lead author of the Project 13 report. He did not refer to the yield failure at all in a statement provided to the CoI which discussed issues with the failed method[4].

    iv. The CoI did not alert me, Dr Budowle, or Jo Veth of any yield failures in the automated DNA IQ method (referred to in Module 6 and this section as the MultiPROBE II method[5]). The information would have been crucial for our Module 6 analysis of the Blackburn DNA analysis.

    v. Mr McNevin was Manager of the Analytical Section (where DNA extraction occurs) and senior author of the Project 70 report which compared DNA recovery of the failed method with a new method being trialled in 2011. The report documents the yield issues of the failed method, however, Mr McNevin did not refer to the yield failure in a statement[6] he provided to the CoI.

    vi. Dr Bruce Budowle, Jo Veth, and I reviewed the results from the DNA extraction positive controls included on the batches containing the Blackburn crime scene samples, and initially considered them to pass.

11. Further investigations were conducted by the three of us only days before the Module 6 hearings which showed the MultiPROBE II method (the failed method) recovered significantly less DNA than a second automated extraction method (using the Maxwell robot) in 2012 and 2013. This analysis conducted by us is outlined below.

12. Dr Budowle and Jo Veth were provided with electropherograms of positive control samples included in each DNA extraction batch containing Blackburn crime scene samples. An electropherogram is the final graphical data output of the DNA profiling process. It used by scientists to interpret DNA profiles and evaluate the quality of the

[3] EXH 129.5 Expert Report Linzi Wilson-Wilde OAM.
[4] EXH 129.4 Signed statement Thomas Nurthen.
[5] In some e-mail communications referenced in this section, the term 'manual method' is used instead of the 'DNA IQ method' or the 'MultiPROBE II method'. This is because there was a second method used in 2013 which was fully automated (the Maxwell robot), compared to the DNA IQ method which had a manual first step.
[6] CoI EXH 129.3 Signed statement Allan McNevin.

result. If an electropherogram contains data for each available DNA location profiled (represented as a peak on the electropherogram), and if the peaks are tall, this indicates a large amount of good quality DNA was recovered during the DNA extraction process, and the positive control would be considered 'passed'. The electropherograms for the positive controls on the Blackburn batches contained tall peaks for each DNA location profiled, and therefore were passed by Dr Budowle and Jo Veth. I reviewed the same electropherograms and also considered them to represent a successful DNA extraction process.

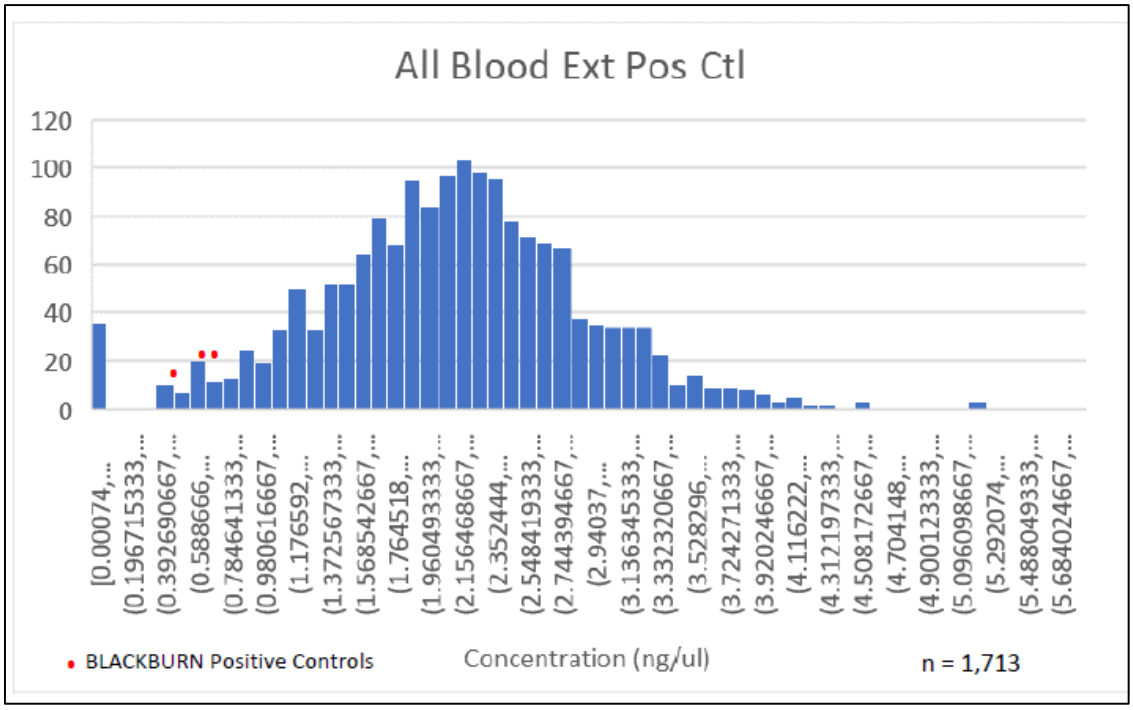
13. Still concerned by the lack of explanation for the failure to obtain profiles from some Blackburn crime scene samples, I requested the concentration values for each positive control from the Blackburn extraction batches, and positive control concentrations for 2012 to 2013 from the CoI on 11 November 2022[7]. The concentration value is obtained after the second step of the DNA profiling process. It is a reading of how much DNA is in a sample after it has undergone DNA extraction. The concentration is expressed as nanograms per microliter (ng/ul), that is, how much DNA is in a certain volume of liquid.

14. On 15 November 2022 I received the requested data and performed some analysis[8]. Blood extraction positive controls are made in a standard way by QHFSS using 30ul of blood dried on swabs. If DNA extraction methods are working successfully, it is expected that only a small variation in DNA concentration will exist across all positive controls. The purpose of my examination of the one year of blood extraction controls (n = 1,713 across 2012 to 2013) was to determine: a) concentration variation across all positive controls; b) a pass range for extraction batches; and c) the comparative success of the Blackburn extraction batches containing key crime scene evidence.

15. The analysis demonstrated an unexpectedly large variation of concentration values (Figure 1). There was a 7,945-fold difference between the lowest and highest concentration indicating significant failings in DNA extraction. The positive control concentration values from the three main Blackburn extraction batches were some of the lowest obtained in the twelve-month period.

[7] E-mail K.WRIGHT to L. REECE. 11 Nov 2022. Request for Concentration Values for Blackburn Sample Positive Controls.
[8] E-mail. K.WRIGHT to L.REECE. 15 Nov 2022. Request for Blackburn Positive Control Values & Notice of Data Provided.

Figure 1: Distribution of all blood extraction positive control DNA concentration values (both methods 2012-2013). The red dots are the positive controls used on the critical Blackburn evidence.



16. On 15 November 2022 I advised Laura Reece (counsel assisting for the CoI) that the concentration of the positive controls from three of the batches containing the Blackburn samples were lower than the mean concentration of positive controls for the 12 months of data over 2012 to 2013. I advised they were 3.6, 5.1 and 3.2 times lower. I asked the CoI if I could work with Dr Budowle and Jo Veth further on this issue (which was permitted), and wanted their opinion on these findings (Figure 1 was provided to them).[9]

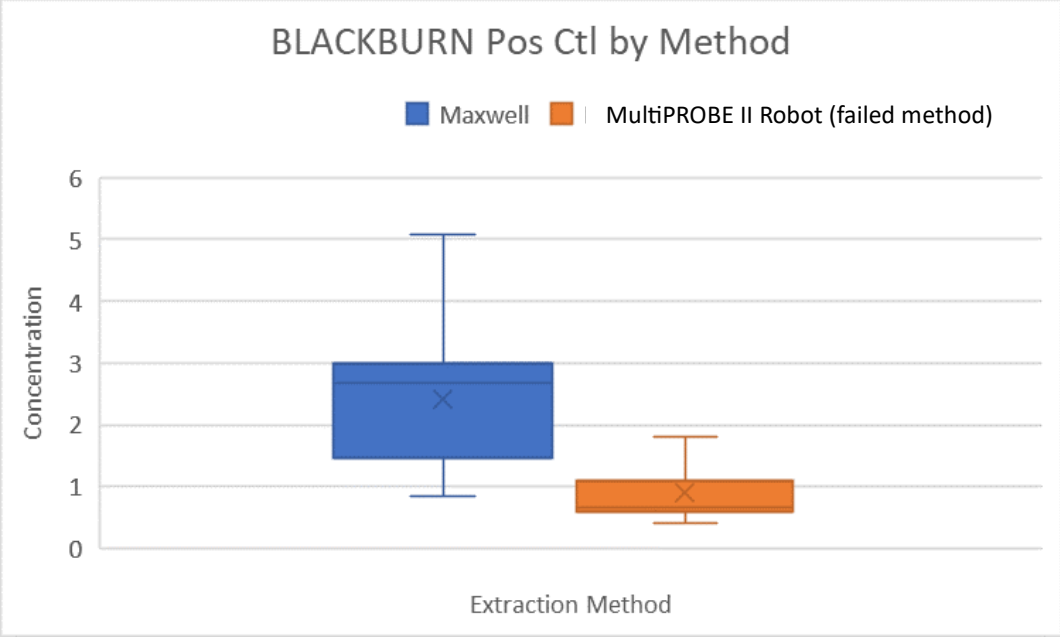
17. On 16 November 2022 a video conference was held with me, Dr Budowle, Jo Veth, Laura Reece (counsel assisting) and a representative from the CoI where we discussed the DNA extraction concerns further. There was no indication by the CoI during this meeting that concerns about the failed method had previously been raised with them.

18. After receiving my analysis the previous day, Jo Veth asked the CoI for more information about the extraction methods used on the Blackburn samples, and that morning had found the MultiPROBE II method was recovering significantly less DNA than the Maxwell method for the Blackburn positive controls. That is, Jo Veth found that it was one of the two extraction methods causing the poor DNA recovery.

[9] E-mail. K.WRIGHT to L. REEECE. 15 Nov 2022. Advice to CoI of Blackburn Positive Controls and 2012-2013 Data Analysis Findings.

19. Following this discovery, I performed further analysis on positive control data included in all Blackburn DNA extraction batches across both methods and found the MultiPROBE II method provided four times less DNA than the Maxwell robot[10] (Figure 2). At this stage it appeared there was a systemic issue with the MultiPROBE II method, but we needed more data to confirm this.

Figure 2: Comparison of blood positive control concentrations (ng/ul) between both DNA extraction methods at QHFSS used for the Blackburn matter.



20. As a group, we speculated that QHFSS were not aware of this because they had not been properly checking their positive control samples (ie checking the electropherogram only, and not checking the concentration value for each positive control).

21. On 18 November 2022 I provided the CoI with an addendum statement including my findings that the Blackburn positive controls appeared to be recovering only low amounts of DNA compared to the average concentration of positive controls from 2012 to 2013.[11,12]

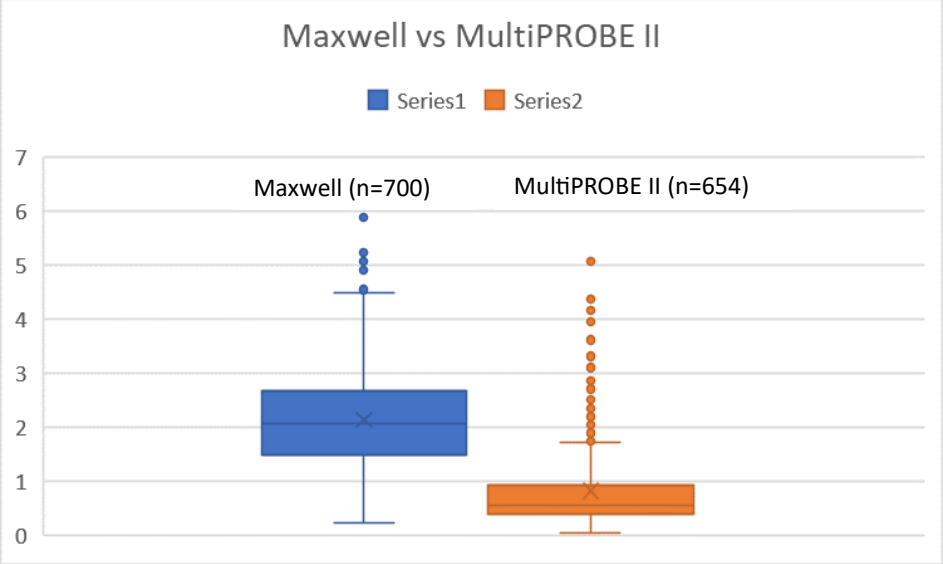
22. On 23 November 2022 the CoI provided me, Dr Bruce Budowle, and Jo Veth with further positive control data from 2012 to 2013 which included the extraction method used on each sample over those twelve-months[13]. The analysis of the larger data

[10] E-mail. K. WRIGHT to J. VETH. 16 Nov 2022. Blackburn Positive Control Boxplots.
[11] CoI EXH 221 Dr Kirsty Wright Addendum report.
[12] E-mail. K. WRIGHT to L. REECE. 18 Nov 2022. Addendum Report to CoI including Extraction Issues.
[13] E-mail. L. REECE to K. WRIGHT, B. BUDOWLE & J. VETH. 23 Nov 2022. Positive Controls from 2012 to 2013 Extra Data.

set was to confirm whether there was a systemic issue with the MultiPROBE II extraction method.

23. Jo Veth and I analysed this data, and it was clear the trend we saw for the Blackburn positive controls (that is, the MultiPROBE II method recovering far less DNA than the Maxwell method), was also seen in the 2012 to 2013 data. This now demonstrated there was a systemic problem with the MULTIPROBE II method throughout at least a twelve-month period. The average concentration for the Maxwell method was 2.065 ng/ul, versus 0.548 ng/ul for the MultiPROBE II, which represents a 3.76-fold difference (Figure 3). The discovery that the DNA recovery was systemic was found only two days before the Module 6 hearings (held on 25 November 2022). The CoI did not indicate they were aware of any long-term systemic failure of the MulitPROBE II method.

Figure 3: Comparison of blood positive control concentrations (ng/ul) between both DNA extraction methods at QHFSS between 2012 and 2013 (n = 1,354).



24. At this stage myself, Dr Budowle and Jo Veth were certain that QHFSS had not been properly checking their positive controls, otherwise we assumed they would have noticed the systemic failure and fixed it. The failure to properly check positive controls masked the failed method. QHFSS were automatically adding more DNA to positive controls with low yields during the amplification stage[14], which had the effect of artificially, and incorrectly passing failed DNA extraction batches.

25. Dr Budowle and Jo Veth described the poor performance of the MultiPROBE II method in their report to the CoI.[15]

[14] The amplification step artificially replicates DNA. It is step three in the profiling process.
[15] EXH 218 'Johann Veth and Dr Bruce Budowle, Review of DNA analysis undertaken in the Blackburn case'. Page 9, paragraphs 41. See also paragraphs 42 to 46.

"*The MultiProbe® II platform extraction positive controls had much lower quantification results than the Maxwell® extracted positive controls. This difference suggests that DNA was not being recovered effectively from the MultiProbe® II batches. Due to time constraints, this matter could not be investigated further and there may be other reasons for the lower positive control quantification results. However, this is a compelling indication that there was something about the MultiProbe® II extraction method that was resulting in a lower recovery of DNA when compared to the Maxwell® method*."

26. During the CoI (Blackburn module) it was reported the failed method was used on key evidence from the Blackburn case. Essentially, samples containing trace DNA from the Blackburn would likely not have provided DNA profiles using the failed method, but police and the courts were unaware of this at the time. Note the conclusion from Commissioner Sofronoff:

    "*..it's not possible to conclude that the offender's DNA was not on Ms Blackburn's body or clothing, and it's not possible to conclude that Ms Blackburn's DNA was not present in the car of the person who had been suspected of the killing. This is to say, it was not correct to say that there was no DNA. If would have been correct to say that having regard to the methods employed it was not possible to say whether or not there was any DNA in relevant places*."[16]

27. In December 2022 the CoI concluded, but what this left unanswered for me was:

    i. Why was the method failing?
    ii. When did it stop working?
    iii. How many cases and samples could this have affected?
    iv. How did the failed method affect the quantity and quality of DNA in the remaining available extract?
    v. Did anyone at QHFSS know it was failing but did nothing to fix it, noting it was used for a period of nine years?

28. My assumption at the end of the CoI was the method had stopped failing at some point after it was introduced, but no one at QHFSS noticed it because they were not properly checking positive controls. It did not occur to me, Jo Veth, or Dr Budowle that the method may have been failing during verification, but introduced regardless because, if so, this would have been a reckless and dangerous act. Conduct which we simply did not think was a possibility.

29. The Module 6 findings led to CoI Recommendation 105, which stated:

    "The laboratory should conduct a retrospective review of positive control extraction batches processed by the MultiProbe® II instrument to determine if this extraction method was performing sub-optimally, and if so, the period of time in which a sub-

[16] CoI transcript, 24 November 2022, page 14 paragraphs 5 to 13. https://www.dnainquiry.qld.gov.au/public-hearings/exhibits.aspx#m7 (Accessed 24 June 2023).

optimal method was used and whether there is utility in retesting or re-analysing any potentially affected samples."

30. Following the CoI I independently continued to examine documents available on the CoI website to understand why the method was failing. In 2023 I found the Project 13 report, immediately became aware of its failings, and formed the opinion the method was failing to sufficiently recover DNA prior to its introduction.

31. Based on the information in Project 13, I believe it was the implementation of this method, and variations of it, that led to key Blackburn samples failing, as well as samples from many thousands of other cases over nine years. These cases need to be identified, reviewed, and where needed, samples re-tested. Victims of violent crimes and their families also want to hear the truth about issues that may have affected their chances of justice. This is why I believe the Project 13 report (and documents related to this method), and why it appears the failed method was introduced and used over nine years without being fixed are significant issues to address.

Implementation of the Failed Method by QHFSS

32. In 2007 QHFSS introduced a DNA extraction method[17] using robots, which in my opinion and based on the Project 13 report, reveals it was known it would fail before implementation. The new method had two failings:

    i. it recovered 92% less DNA than the comparable manual method and was 100 times less sensitive[18]; and

    ii. the Project 13 report indicates the method caused contamination prior to its introduction, but does not appear to have been appropriately declared or investigated, causing large scale contamination and loss of crime scene evidence after implementation.

33. In 2006 QHFSS purchased two DNA extraction robots, called the MultiPROBE II. The intent was to replace the manual DNA extraction process with the robot, to save labour costs and process crime scene and reference samples faster. At this stage, QHFSS were one of the first forensic DNA laboratories in Australia to implement automated DNA extraction.

34. QHFSS needed to select and trial a commercial, off-the-shelf chemistry product compatible with the MultiPROBE II robot. The chemistry selected was called DNA IQ, from the manufacturer 'Promega'. It is the robot-chemistry coupling that comprised the DNA extraction method.

[17] Step 1 in the DNA profiling process.

[18] The poor DNA recovery from the new automated method reported in Project 13, indicates the method was also recovering approximately half as much DNA as the pre-existing chemistry (Chelex) that had been in use at QHFSS until 2007. The outdated chemistry could not be automated, hence the trial to find a new DNA extraction chemistry that could be used on robots.

35. The trial of the robot-chemistry coupling was conducted on mock blood and cell samples prior to its introduction in Project 13, 'Verification of an Automated DNA IQ protocol using he MultiPROBE II PLUS HT EX with Gripper integration platform'. QHFSS chose to conduct a 'verification' of the DNA extraction method, rather than a 'validation'. A verification is a scientific trial of a method that has been empirically demonstrated to work successfully by the manufacturer of a commercial product, or by another laboratory who originally developed the method by a laboratory. A validation is a larger, more stringent scientific trial of a method that has been developed in-house, or commercial methods that have been modified. The intent of a verification or validation is to demonstrate a method is producing reliable and accurate results, and is fit for purpose within a specific laboratory environment, and for the range of forensic evidence it is intended to test. It is also intended to determine any limitations of the method, and is an essential part of laboratory accreditation requirements.

36. At the completion of a verification or validation, a laboratory will review the results and if they are successful based on a pre-defined expectation, or comparison to a successful method, then the method is generally implemented. If poor results are obtained, a laboratory can either improve the method prior to implementation until it is producing expected results, or reject the method, and trial another chemistry / robot coupling. A forensic laboratory should never implement a method that provides poor results in a validation or verification.

37. In 2007 there were clear national guidelines, and professional standards that inform verification and validation processes[19]. The National Association of Testing Authorities (NATA) examines validation and verification reports produced by laboratories as part of their regular accreditation.

38. QHFSS referenced that the DNA IQ protocol has already been verified or validated for use on the MultiPROBE II robot by the Western Australia and South Australian forensic DNA laboratories, and a Canadian laboratory. QHFSS state they validated the manual DNA IQ method[20], but did not validate the automated DNA IQ method used on the MultiPROBE II, they instead chose to only verify the method (Project 13). The authors of Project 13 state the '*verified automated DNA IQ protocol is identical to the validated manual protocol*'[21]. Given QHFSS were adapting the manual method to the MultiPROBE II robot and adjustments needed to be made, a full validation should have been performed.

39. The Project 13 report clearly shows the new automated method was failing compared to the manual method during a trial before it was implemented. This should have resulted in the method being rejected, or optimised then validated. The report demonstrates the method would fail on trace samples, and would even struggle to recover DNA from visible blood stains. In my opinion, this data would have been obvious to any scientist reviewing it.

[19] ISO 17025: 'Testing and Calibration Laboratories'; Technical Note 17: 'Guidelines for the validation and verification of quantitative and qualitative test methods'; Scientific Working Group on DNA Analysis Methods (SWGDAM): 'SWGDAM Validation Guidelines for Forensic DNA analysis Methods'.
[20] Project 11.
[21] CoI EXH 129.95 Project 13, page 1 paragraph 4.

40. In my opinion, the Project 13 report shows some scientists at QHFSS must have known the method was going to fail on a large range of evidence, but recommended its implementation anyway.

41. The abstract of the Project 13 report incorrectly states the manual and automated methods were producing 'comparable results'[22]. Figures 9 to 12 within the report[23] and associated text clearly demonstrates this is incorrect.

Project 13. Report on the Verification of an Automated DNA IQ™ Protocol using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform

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

A manual method for extracting DNA from forensic samples using the DNA IQ™ system (Promega Corp., Madison, WI, USA) was validated for routine use in DNA Analysis (FSS). We have verified an automated DNA IQ™ protocol in 96-well format for use on the MultiPROBE® II PLUS HT EX Forensic Workstation platforms (PerkinElmer, Downers Grove, IL, USA). Data indicate that results from the automated procedure are comparable to those from the manual procedure. Contamination checks were performed using samples prepared in checkerboard and zebra-stripe format, and results were as expected. We recommend the use of the MultiPROBE® II PLUS HT EX platforms to perform automated DNA extraction using the DNA IQ™ system.

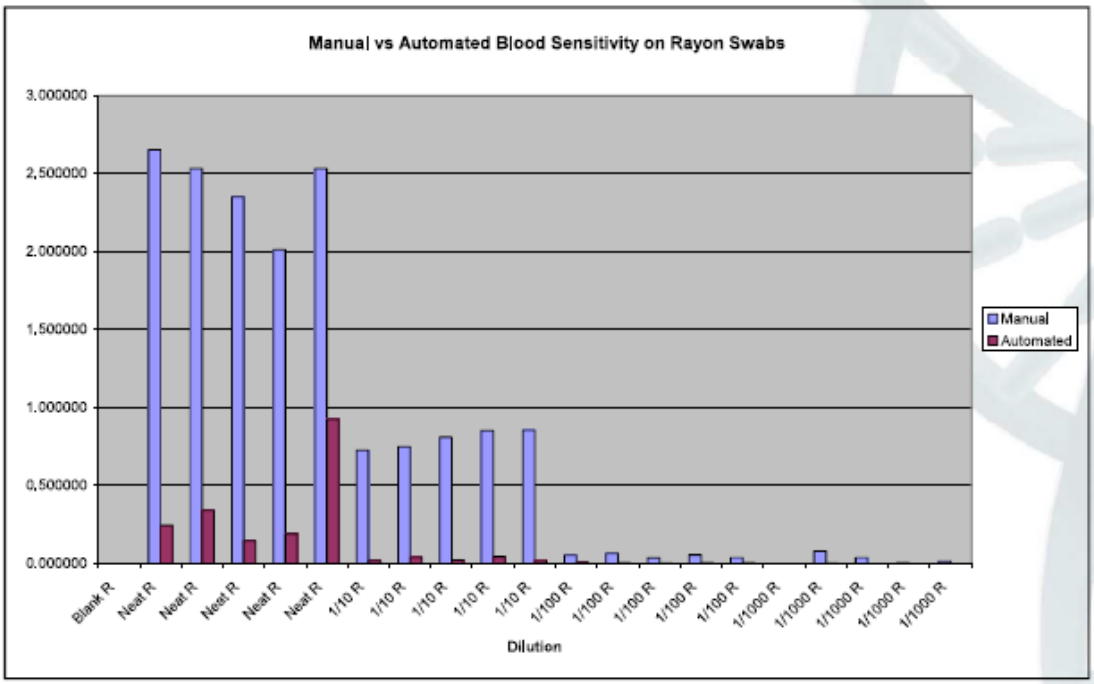


Figure 9. Comparison of sensitivity between the manual and automated DNA IQ™ methods for blood samples on rayon swabs.

[22] CoI EXH 129.5, page 1.
[23] CoI EXH 129.5, pages 13 to 16.

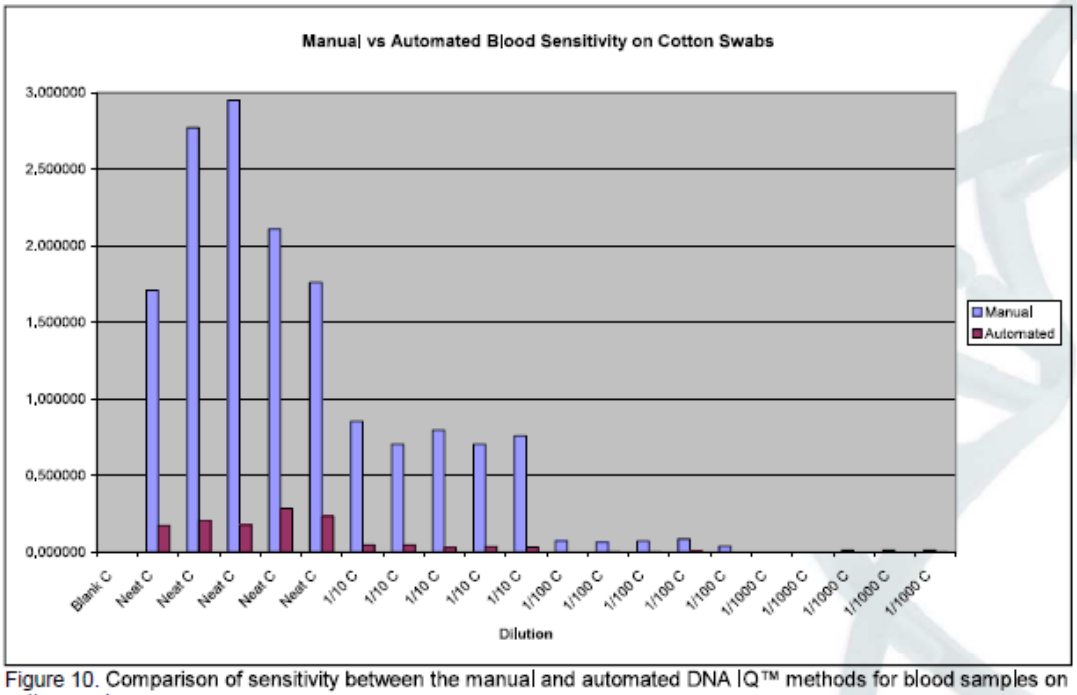


Figure 10. Comparison of sensitivity between the manual and automated DNA IQ™ methods for blood samples on cotton swabs.

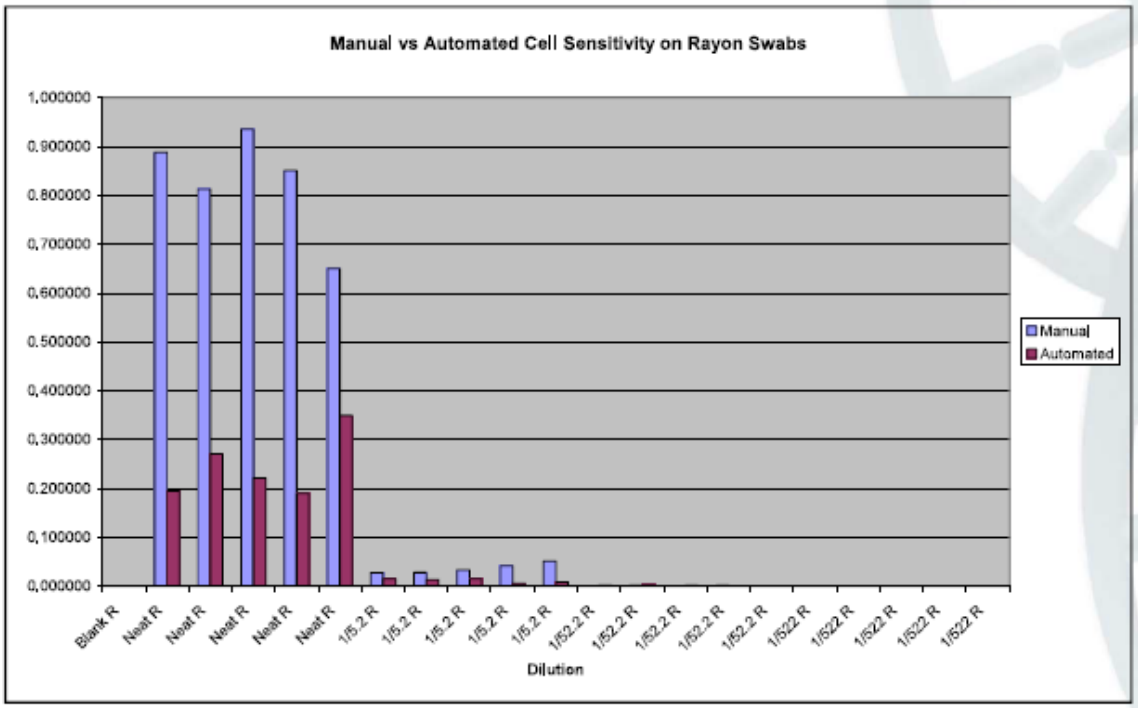


Figure 11. Comparison of sensitivity between the manual and automated DNA IQ™ methods for cells samples on rayon swabs.

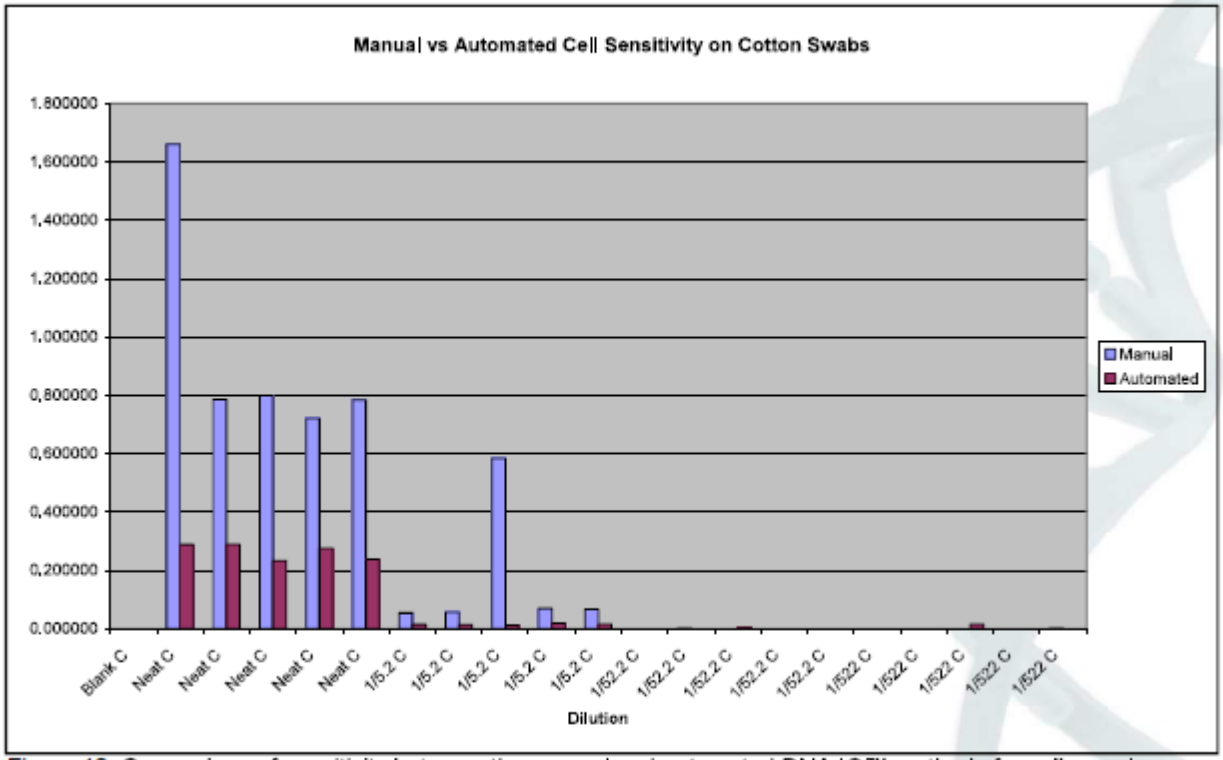


Figure 12. Comparison of sensitivity between the manual and automated DNA IQ™ methods for cell samples on cotton swabs.

42. The authors of the Project 13 report state in the summary: "*Data indicate that results from the automated procedure are comparable to those from the manual procedure*"[24], which is incorrect, and recommend its implementation. In my opinion, the summarising paragraph deceives the reader into believing the new robotic method produced the same quality of results as the comparable manual method. In my experience, it is typical for non-scientific people to just read the project summary and not read the entire technical report. When the technical data is scrutinised, in my opinion it clearly shows the method was failing prior to implementation.

43. In my opinion, the Project 13 report shows that QHFSS knew the method would fail to obtain DNA from thousands of violent crimes which should have provided profiles, and would have wasted critical evidence. QHFSS data shows the method was failing on a large range of samples, including trace DNA, and in an earlier evaluation it failed to provide DNA profiles from 50% of visible blood stains[25].

44. Tapelifts taken from volunteers' forearms failed on 100% of mock samples in another earlier evaluation, leading to a recommendation to not use the new method on

[24] CoI EXH 129.95, paragraph 1. The 'manual procedure' relates to the manual DNA IQ™ extraction method.
[25] 'Project 9 Report on the Evaluation of Commercial DNA Extraction Chemistries'. 2007. CoI EXH 129.93. Page 18, Table 7. See DNAIQ method, 2 out of 4 whole blood (visible blood) samples failed to provide a DNA profile. https://www.dnainquiry.qld.gov.au/public-hearings/assets/exhibits/module-4/EXH%20129.93%20-%20FSS.0001.0084.1462_R.pdf

tapelifts from crime scenes, however, this appears to have been ignored and tapelifts were processed on the failed method.

"Based on the findings of this validation report, we recommend: To enable processing of cell and blood samples using the validated manual DNA IQ™ protocol, except for samples on tapelift substrates."[26].

45. In my opinion, QHFSS scientists ought to have known that crucial evidence would be incorrectly reported to police and the courts as 'no DNA detected''. The Project 13 report still appears to be a draft, though this was the version submitted by QHFSS to the CoI. It is unclear whether sign off by all managers was needed to close Project 13.

46. Introducing robotic DNA extractions was the biggest change to QHFSS processes in its history and aimed to produce results faster and clear backlogs. Drastically reducing the amount of DNA evidence generated by QHFSS, to instead achieve faster results, is a major Queensland Health (QH) policy change in their service provision to police, justice stakeholders, and the community.

47. In a little over three months after the failed method was introduced, wide-spread contamination was detected in crime scene evidence (refer to Attachment 1 for a timeline of events). This was a catastrophic event and the main focus of remedy within QHFSS at the time. Gross unexplained contamination was also detected in Project 13[27], but does not appear to have been declared or properly investigated in the rush for implementation. The second catastrophic failing of the new method (poor DNA recovery) appeared to go unnoticed after implementation. Multiple internal audits, and an external audit of the new method only focused on the contamination issue. It appears the auditors were not given access to Project 13, and therefore did not discover the yield failure.

48. A memo from Vanessa Ientile to the DNA Analysis laboratory (QHFSS) dated 14 July 2008 informed them of the plan to address the contamination issue state:

> "*Automated DNA IQ extractions were introduced in October 2007, after an extensive validation process*"[28]

49. This statement is incorrect. Project 13 definitely was not an 'extensive validation'.

50. Significant contamination was detected in Project 13 with one out of five plates tested containing 12 instances of contamination. Rather than investigating the

[26] Project 11 'Report on the Validation of the manual method for extracting DNA using the DNA IQ™ System' (August 2008). Page 20, paragraph 1. CoI EXH 129.94. https://www.dnainquiry.qld.gov.au/public-hearings/assets/exhibits/module-4/EXH%20129.94%20-%20FSS.0001.0084.1400.pdf Note: Project 13 did not trial tapelifts, therefore, it appears the method was never fixed to enable reliable profiles from tapelift evidence prior to its implementation.

[27] Project 13, p9, paragraph 5 to p10 paragraph 1.
[28] CoI EXH 129.65, page 1, paragraph 4.

contamination and fixing it during Project 13, the results were 'invalidated[29]' (meaning they were excluded from the trial and conclusion). The abstract of Project 13 deceptively states:

> "*Contamination checks were performed using samples prepared in checkerboard and zebra-stripe format, and results were as expected*."[30]

51. Below is a progression of the DNA extraction methods used at QHFSS and the validation and verification projects involved in the failed robotic DNA IQ / MultiPROBE II method.

i. The original method (prior to 29 Oct 2007) was Chelex DNA extraction. This method could not be automated.

ii. Project 9: 'Report on the comparison of commercial DNA extraction chemistries'. This compared the Chelex method to four other off-the-shelf manual chemistries. QHFSS chose the DNA IQ manual protocol (developer's method) because it was the only one of the four chemistries that had been validated for use on the MultiPROBE II robot.

iii. Project 11: 'Report on the validation of a manual method of extracting DNA using the DNA IQ System'. QHFSS modified the DNA IQ developer's method, and validated the **manual** DNA IQ method. The manual method appeared to be working well.

iv. Project 13: 'Report on the verification of an automated DNAIQ™ protocol using the MultiPROBE® II PLUS HT EX with Gripper™ integration platform'. QHFSS adapted the manual DNA IQ method validated in Project 11, to a newly purchased robot (the MultiPROBE II). This report compared the manual DNA IQ method with the fully automated DNA IQ method.

v. On 29 October 2007 QHFSS starts using the **fully automated** DNA IQ method verified in Project 13.

vi. Project 21: 'A modified DNA IQ Method Consisting of Off-Deck Lysis to Allow Supernatant Retention for Presumptive Identification of a-Amylase'. In February 2008 QHFSS trialled a partially manual / robotic ('off-deck lysis') variation of the Project 13 method. The project led to the first step of the DNA extraction method (lysis) being performed manually, instead of on the MultiPROBE II robot. The Project 21 report discusses the change allowed for an increase in the range of sample types that could be processed, as samples such as fluffy swab heads or material with static attraction were not suitable to process on the robot. Importantly, the stated aims of this project did not include improving DNA yield.

[29] CoI EXH 129.95, page 9, Table 4.
[30] CoI EXH 129.95, page 1, paragraph 1.

vii. Project 22: 'A Modified DNA IQ Method for Off-Deck Lysis Prior to Performing Automated DNA Extraction'. This was a variation of the Project 13 method which trialled two different heat sources for the off-deck lysis and found both methods were comparable. Importantly, the stated aims of this project did not include improving DNA yield.

52. On 19 March 2008 QHFSS introduced the **manual / automated modification**.

53. I have checked exhibits available on the CoI site and have not found any documents that shows the failed method was fixed between its implementation in 2007 and its use in 2013 on the Blackburn samples, or until it ceased being used in November 2016.

54. These include:

i. EXH 129.96 Project 21;
ii. EXH 129.97 Project 22;
iii. EXH 129.67 Presentation -Audit 8227;
iv. EXH 129.68 Presentation-MPII Enhancements;
v. EXH 129.69 Presentation - Update on DNA Analysis Issues;
vi. EXH 129.15 Dr Sloots & Whiley external review;
vii. EXH 129.9 Analytical Issues Log;
viii. EXH 129.10 Audit 8227;
ix. EXH 129.11 to 129.14 standard operational procedures for the DNA IQ extraction methods (2007 to 2009);
x. DNA IQ Method for Extracting DNA from Casework and Reference Samples SOP (24897V8, 27 June 2012 and 24897V9 03 January 2014);
xi. EXH 129.52 Audit#9642;
xii. EXH 129.55 A review of DNA extraction control results obtained in the first six months of 2008;
xiii. EXH 129.56 A review of DNA extraction control results obtained in the second six months of 2008;
xiv. EXH 29.62 Report (Desley Pitcher) -DNA extraction modifications;
xv. EXH 129.63 Report (Desley Pitcher) – DNA extraction modifications; and
xvi. EXH 129.4 Tom Nurthen statement, (TN11 and TN12 'Testing of samples with different detergents in the extraction lysis buffer').

55. Further, the 'Validation' section in each standard operational procedure of the DNA IQ extraction method, does not list any further validations, verifications, or projects conducted on the method after Project 22[31], and the 'Amendment History' in each method does not list any significant changes to the method to fix the yield issue. Changes documented to the method were to fix the contamination issue.

[31] COI EXH 129.11. Automated DNA IQ Method for Extracting for Extracting DNA from Blood and Cell Substrates (SOP24987v1, 24/10/2007), p12. COI EXH 129.14 (SOP24897v6, 13/8/2009) p17; and (SOP24897v9, 3/1/2014) p22.

56. Table 1 has been compiled from QHFSS data and further demonstrates the failed method was not fixed after implementation in 2007.

Table 1: Performance Summary of Variations of the Failed Method

Method Variations	Avg Concentration (ng/ul)	Concentration Range (ng/ul)
Manual DNA IQ method (Project 11)[32]	3.17	2.16 to 4.47
Fully automated (Project 13)[33]	0.65	0.32 to 1.07
Fully automated (post-implementation early 2008)[34]	0.27	Not available
Project 21 (Man / Auto)[35]	0.08	0.07 to 0.10
Project 22 (Man / Auto) Data set 1[36]	1.22	0.85 to 1.3
Project 22 (Man / Auto) Data set 2[37]	0.66	0.47 to 0.77
2012 data from CoI (Man / Auto) (one year of data, n = 654)	0.82	0.03 to 5.07 Note: 82% of extraction controls were under 1.2
Shandee BLACKBURN batch positive control	0.59	
Shandee BLACKBURN batch positive control	0.42	
Shandee BLACKBURN batch positive control	0.68	

57. The DNA IQ manual method (Project 11) should be considered the target for DNA recovery at QHFSS given they had already achieved good results with this method (average 3.17 ng/ul).

58. Table 1 shows there was almost no improvement when the robotic method was changed to the hybrid manual / automated method in 2008. Of the three data sets from Project 21 and Project 22 that trial the manual / automated method only one data set shows an increase (1.22 ng/ul). Positive controls used on casework sample

[32] CoI EXH 129.94. Project 11. Data obtained from page 10, Table 4, row1, column 4 (neat blood). The five values for total yield were divided by 100 (because the DNA extraction elution volume was 100ul). The final concentrations were 2.16, 4.47, 2.15, 3.83, and 3.24.
[33] CoI EXh 129.96. Project 13. Data obtained from page 15, Table 6, (7ul resin volume). 0.701, 1.070, 0.319, and 0.499.
[34] CoI EXH 129.97. Project 22. Data obtained from page 5, paragraph 3 'QC blood swabs extracted since January 2008'.
[35] CoI EXH 129.96. Project 21. Data obtained from page 4, Table 2, row 3, 'QC Blood' concentrations were 0.0700 and 0.0991.
[36] CoI EXH 129.97. Project 22. Data obtained from page 4, Table 3, 'Mean Blood QC' row 7 concentrations 0.8506, 1.2030, 1.3298, 1.5500, and 1.1900.
[37] CoI EXH 129.97. Project 22. Data obtained from page 6, Table 6, '30ul blood rayon swab' row 12 concentrations 0.7352, 0.4698, and 0.7734.

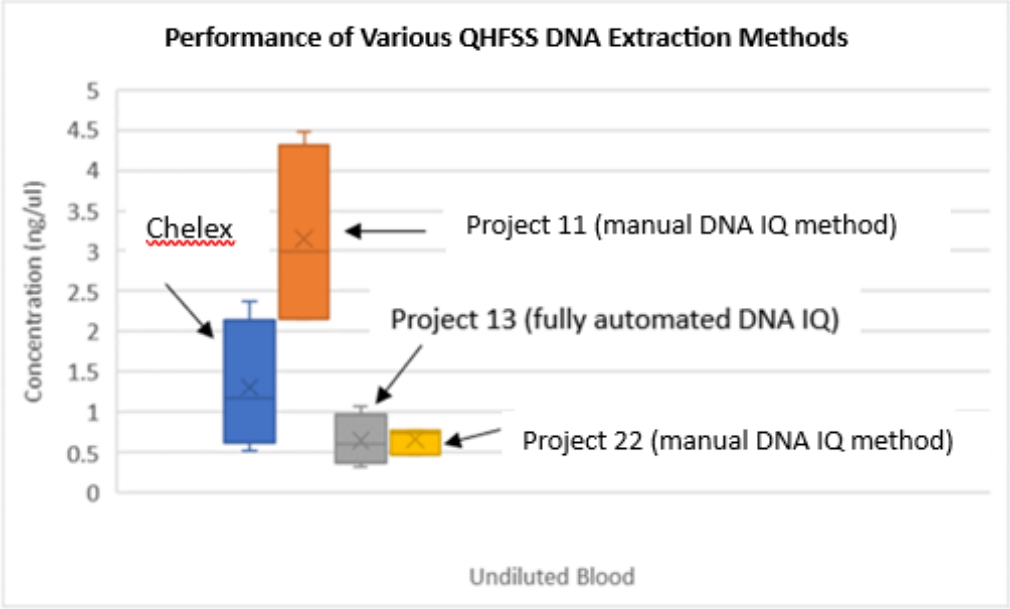
batches from just after initial implementation of the fully automated method in 2007 had an average concentration of 0.27 ng/ul.

59. The key figure is the 2012 data provided to me by the 2022 CoI. There were 654 positive extraction controls, and the average concentration was 0.82 ng/ul. This is only marginally better than the fully automated value in Project 13 (0.65 ng/ul). Therefore, the change of method from fully automated to manual /automated did not fix the recovery issue, it was still failing.

60. The 2012 data shows the manual / automated method was recovering 3.7 times less DNA than the manual method, and 4 times less DNA than a fully automated method (Maxwell robot) also used in 2012. Shandee Blackburn's key samples from PEROS' car, the knife, her body and clothing, and knives were all processed on the failed method and on failed batches. This was not disclosed when the evidence was presented in the trial. The samples were on separate extraction batches, and their respective extraction positive controls were 4 to 7 times less than the DNA recovery for the manual method.

61. Figure 4 further demonstrates the ongoing DNA recovery failure for each variation of the method (undilute blood only[38]). The failed automated method was recovering a fraction of DNA compared to the manual DNA IQ method, and on average about half as much DNA as the original Chelex method.

Figure 4: Comparison of DNA recovery for different QHFSS extraction methods.



[38] Different dilutions were used in the various projects which prevented direct comparison. Only data for neat blood could be used for direct comparison between the various methods.

Ongoing use of Failed Method at QHFSS

62. In 2011 QHFSS introduced a new DNA extraction robot, the Maxwell-16 MDX for blood and cell extractions using the 'DNA IQ Casework Pro Kit for Maxwell 16' chemistry kit. This was a fully automated DNA extraction process. A verification trial was conducted (Project 70: 'Verification of Promega DNA IQ for the Maxwell 16') which compared the Maxwell sensitivity with the MultiPROBE II sensitivity. Note QHFSS refer to the MultiPROBE II method as the 'manual' method, however, it is the hybrid manual / automated method that was introduced in 2008.[39]

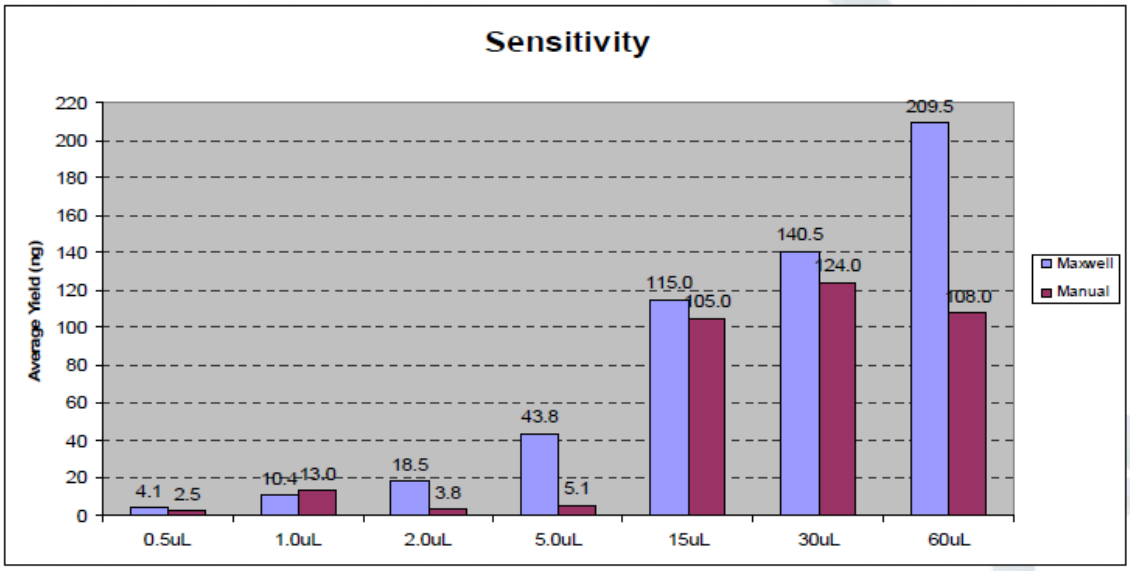


Figure 5 Sensitivity and DNA Yield comparing Maxwell®16 A and Manual DNA IQ™ methods using blood swabs.

63. When tested on decreasingly smaller volumes of blood, QHFSS discovered the Maxwell provided better DNA recovery for the 2.0ul to 60ul range than the MultiPROBE II. The largest differences between the methods were at the 2.0ul and 5.0ul blood volumes. The Maxwell recovered four times more DNA and eight times more DNA at these values respectively than the MultiPROBE II. At the lowest blood volume (0.5ul) the Maxwell recovered nearly twice as much DNA than the MultiPROBE II method. In my opinion, this is a significant finding which should have alerted the scientists to the failure of the MultiPROBE II method, particularly for smaller volumes of blood.

64. However, the conclusion stated the results from both methods were '*comparable*' which is incorrect based on the sensitivity studies illustrated in the Project 70 report.

'*It has also shown that this extraction procedure will give results comparable to the current routine manual DNA IQ method'.*[40]

[39] Of note, Project 70 does not list Project 13 in its list of references suggesting the author did not use, or did not have access to this document.
[40] Project 70: 'Verification of Promega DNA IQ for the Maxwell 16', page 16, paragraph 3.

65. Within the report it is documented the authors notices that the MultiPROBE II method was recovering more DNA from cell samples in the Project 70 trial than in the 2007 manual DNA IQ validation in 2007 (Project 11). Project 70 recovered an average concentration of 3.33 ng/ul and in Project 11 it recovered on average 1.34 ng/ul.[41] It is unclear whether this is due to a difference in the preparation of the cell samples between both projects, or due to an improvement in the method.

66. The authors also highlighted the average concentration for standard blood swabs using the MultiPROBE II method '*showed a significantly lower yield*' when compared to the original validation of the manual DNA IQ performed in 2007[42]. The authors document in the report that the 2007 manual validation (Project 11) recovered on average 3.17 ng/ul, while in the Project 70 study using the hybrid manual / automated method recovered on average 0.97 ng/ul and 1.2 ng/ul.[43] These values are similar to those observed in Project 22 indicating the method had not been fixed between 2008 and 2011.

67. In my opinion, the documented observation about the decrease in DNA yield for blood samples by the authors is critical. It shows they had an empirical awareness of the poor performance of the MultiPROBE II method on blood swabs, noticing a three-fold decrease in DNA recovery in 2011 compared to the 2007 study.

68. The difference between the two methods for blood sensitivity, and the lower blood yields documented by the authors between the 2011 MulitPROBE II results in Project 70 and the manual DNA IQ method results reported in Project 11, in my opinion, would have made QHFSS aware of the MultiPROBE II failures and should have led to further investigation and fixing of the method.

69. The senior author of the Project 70 report, Allan McNevin, was Manager of the Analytical Team (the team responsible for DNA extraction and profiling) from 2008 to 2014. It was therefore his responsibility to escalate this issue to the Laboratory Manager (in 2011 this would have been Cathie Allen). In my opinion, Mr McNevin should have been responsible for taking further actions to investigate and remedy the poor performance of the MultiPROBE II method.

70. I believe Mr McNevin should have worked with other QHFSS managers to:

    i. obtain all blood and cell positive extraction control concentration data since the implementation of the MultiPROBE II method in 2007 to perform a trend analysis, and verify whether the concentration ranges were failing. If so:

    ii. raise an Opportunity for Quality Improvement (OQI) report to thoroughly document the issue and perform a root cause analysis quality investigation;

[41] Project 70: 'Verification of Promega DNA IQ for the Maxwell 16', page 7, paragraph 3
[42] Project 70: 'Verification of Promega DNA IQ for the Maxwell 16', page 7, paragraph 3.
[43] Project 70: 'Verification of Promega DNA IQ for the Maxwell 16', page 7, Table 2, 'DNA IQ Manual Blood', column 4, row 6; and page 14, Figure 5, '30ul Manual'.

iii. evaluate which extraction batches had failed since 2007;

iv. issue notices, and where needed addendum statements, to QPs and members of the judicial system and meet with them to fully disclose the issue;

v. make a direction to stop using the MultiPROBE II method on crime scene evidence until the method was fixed or replaced; and

vi. determine whether affected samples could be retested.

71. A similar course of action was taken in 2008 when the MultiPROBE II method was found to have caused widespread contamination, and therefore was the benchmark for addressing such a significant issue at QHFSS.

72. Overall, I believe Project 70 was a clear opportunity for QHFSS to identify the DNA recovery failure of the MultiPROBE II method and to fix it. It is unclear what actions, if any, were taken by the authors of the Project 70 report to further investigate and fix the issue.

73. Mr McNevin provided a statement and oral testimony to the CoI relating to the failed MultiPROBE II method and the contamination issue, however, did not refer to the yield failure he observed in Project 70.

Review of the Project 13 Report and Method Variations

74. I have been asked by the CoI to review Project 13 and provide my opinion on its suitability. I have also reviewed the Project 21 and Project 22 reports to determine whether the failures observed in Project 13 were subsequently fixed. Table 2 lists documents and the multiple pieces of evidence contained within them that, in my opinion, clearly demonstrates the Project 13 failure and its severity.

Table 2: Review of Projects 13, 21 and 22.

Document	Evidence
Project 13. Report on the verification of an automated DNA IQ protocol using the MulitPROBE II PLUS HT EX Gripper integration platform.	1. In my opinion, Figures 9 to 12 clearly shows the automated method was systemically failing and recovered far less DNA than the manual method. 2. The automated method was failing to recover DNA at the 1/1000, 1/100 dilutions for blood on rayon swabs (Figure 9). 3. The automated method was struggling to obtain DNA from 1/10 dilutions and neat blood for both swab types (Figures 9 and 10). 4. The automated method was recovering far less DNA for cells on both swab types (Figures 10 and 11). 5. The report states: "*When dilutions of either blood or cells were applied onto either rayon or cotton swabs followed by extraction using the DNA IQ method, the results of the automated method were always lower in yield compared to the manual method*" Page 12, paragraph 2). In my opinion, the magnitude of the DNA recovery failure is clear in Figures 9 to 12. 6. The report states: "*For blood samples on rayon swabs, the automated method generated yields that were on average around 8% (SD 8.45%) of the automated* [manual] *method*". Page 13, paragraph 1. This means the automated method recovered 92% less DNA than the manual method for blood on rayon swabs. 7. The report states: *"For blood on cotton swabs, the yield from the automated method was also around 8% (SD 3.62%). The yields for cell samples were higher at around 33% (SD 16.29%) and 25% (10.32%) for cells on rayon and cotton swabs respectively*". Page 13, paragraph 1. This means the automated method recovered 92% less DNA than the manual method for blood on cotton swabs. For cells on rayon swabs the automated method recovered 67% less DNA than the manual method, and for cells on cotton swabs the automated method recovered 75% less DNA than the manual method.

	8. The report states: "*The manual method was found to be more sensitive than the automated method. Out of five replicates at the 1/100 and 1/1000 dilutions for blood on rayon swabs that were processed using the manual method, five and three replicates respectively were detected (and none from the automated method) (see Figure 9)*." Page 13, paragraph 2. This means that the automated method failed to recover detectable DNA on all 1/100 and 1/1000 dilutions of blood on rayon swabs. This represents all samples of trace blood found at crime scenes, which in my opinion, is a clear and significant failing. 9. Figure 5 to 8 also reveal the method was failing to consistently recover DNA. The figures show large variations between DNA recovery from blood samples and large variations between the recovery of DNA from cell samples. It also shows that some blood and cell samples failed (eg Figure 5, Column 3, Row G; Column 9, Row G; Figure 7, Column 7 Rows G and C). 10. In my opinion, the abstract clearly contradicts the results, stating: "*Data indicate that results from the automated procedure are comparable to those from the manual procedure*". Page 1, paragraph 1. 11. Significant contamination was detected in Project 13 with one out of five plates tested containing 12 instances of contamination. Rather than investigating the contamination and fixing it during Project 13, the results were 'invalidated'. Page 9, paragraph 4, page 10 Table 5. In my opinion, invalidating the failed plate in this instance was a way of dismissing unfavourable data so it would not be considered in the final evaluation of the method. 12. The abstract incorrectly states: "*Contamination checks were performed using samples prepared in checkerboard and zebra-stripe format, and results were as expected". Page 1, paragraph 1.* 13. The report is incomplete with large sections missing, even incomplete sentences and '???' within the body. 14. The date of the report is August 2008, which is ten months after the method was introduced (October 2007).
Project 21. A modified DNA IQ	1. 'QC Blood' (quality control blood) samples were trialled with the modified method, and both failed (0.0700 ng/ul, and 0.0991 ng/ul). Page 2, Table 4. These samples were made in the same way as those in Project 13 resulted in even <u>less</u>

method consisting of off-deck lysis to allow supernatant retention for presumptive identification of *a*-Amylase.	DNA. The average concentration for the manual method was 3.0 ng/ul and was therefore the target concentration for the automated method. 2. 'QC cells' (quality control cells) samples were trialled with the modified method, and both failed (0.1030 ng/ul, and 0.0582 ng/ul). Page 2, Table 4.
Project 22. A modified DNA IQ method for off-deck lysis prior to performing automated DNA extraction	1. Table 3 shows blood samples were still failing (concentration range 0.8140 ng/ul to 1.7400 ng/ul). Page 4. 2. Table 3 shows cell samples were still failing (concentration range 0.0999 ng/ul to 0.4530 ng/ul. Page 4. 3. A second data set for blood on rayon swabs shows samples were still failing (0.7352 ng/ul, 0.4698 ng/ul, and 0.2106 ng/ul). Page 6, Table 6. 4. The report states: "*For buccal cell samples, however, the Thermomixer samples (set 3) produced more undetermined results (0 ng/ul) compared to the hot block samples *st 1), but most results were generally low and close to the validated LOD of 0.00426 ng/ul and therefore indistinguishable from the background*." Page 7, paragraph 1. This means that the authors stated most cell samples they trialled with the modified method failed to produce DNA profiles. 5. Table 7 further demonstrates the systemic failure of the modified automated method to obtain DNA profiles from cells. Page 7. 'NRs' are not reportable alleles, that is, pieces of DNA that were not profiled. Set 1 has 52/64 NRs, Set 2 has 39/40 NRs, and Set 3 has 14/14 NRs. In my opinion, this is a clear failure of the method.

75. In my opinion, QHFSS should have conducted a full validation study on the new automated method, rather than a verification. Overall, the Project 13 verification was of a very poor standard and should not have been allowed to proceed. The results from Project 13 shows the method was failing to recover sufficient DNA, and significant contamination was detected. Based on the Project 13 results, I believe the method definitely should not have been implemented for use on case work or reference samples.

76. Analysis of Project 21 and Project 22 shows QHFSS made in-house changes to the method, and therefore, they should have been conducted as validations. I consider both projects were conducted to a very poor standard and should not have commenced.

77. While automated DNA extraction was relatively new for forensic science in 2007, quality assurance principles, and verification and validation of scientific methods were not. Sufficient guidelines and scientific knowledge existed within the forensic science community to inform: 1) the type of testing required (validation vs verification); 2) proper experimental design; 3) proper data analysis; 4) proper reporting and documentation of results; and 5) proper evaluation of the results. Therefore, the poor conduct of Project 13, and the decision to implement the method cannot be attributed to any deficiencies within the forensic science field at the time.

78. The cause of the method failure is still unclear. Table 3 outlines the changes QHFSS made to the method across each project.

Table 3: Key changes between DNA IQ methods.

Method	Changes	Comments
Project 9		The 'Promega DNA IQ System for Small Casework Samples' developer's method was used. Lysis Buffer was used in the pre-processing step (Option 1 in developer's protocol). This option did not require the Promega Casework Extraction Buffer Kit to be purchased.
Project 11	The Casework Extraction Buffer protocol was used in the pre-processing step (Option 2 in developer's protocol). The Promega Casework Extraction Buffer Kit was not purchased, instead an in-house Casework Extraction Buffer (CEB) was made with TNE buffer (10mM Tris, 1mM EDTA, and 100mM NaCl) Proteinase K (20mg.ml) and 20% SDS. No DTT was added to either CEB or Lysis Buffer. The first incubation step was 37ºC for 45 mins, instead of 56ºC for 30 mins. The final elution volume was 50ul. The developer recommended 25ul to 100ul, stating '*a lower elution volume ensures a higher final concentration of DNA*'[44].	This method was based on an automated protocol developed by the Centre of Forensic Sciences in Toronto, Ontario. I cannot access this protocol. The method was adapted from the manual DNA IQ method for use on the MultiPROBE II robot. This method produced good results.
Project 13	All steps, including the lysis steps, were performed on the MulitPROBE II robot.	This was the fully automated method which started producing poor results.

[44] DNA IQ System-Small Sample Casework Protocol. Promega Technical Bulletin (TB296, 11/12). Page 10, step 11.

	The final elution volume was 120ul (performed in two 60ul elution steps). This is over twice as much as the Project 11 protocol. Heat tiles supplied with the MultiPROBE II were modified to accept the SlicPrep 96 device.	
Project 21	The lysis steps were performed manually. The manual lysis included a final incubation at 65ºC for 10 minutes.	This was the hybrid manual / automated method. Extra CEB buffer was added, so a small sample of the lysate could stored in case *a*-amylase testing was required.
Project 22		This project trialled the use of different heat sources during the manual incubation step. Both heat sources were accepted.

79. The manual DNA IQ method from Project 11 produced good results and it was this protocol that was used in Project 13 to compare against results obtained from the fully automated DNA IQ method. It appears it was the adaptation of the manual DNA IQ method to the MultiPROBE II robot in Project 13 that led to the poor DNA recovery.

80. The poor results could be caused by: 1) changes made to the DNA IQ protocol from Project 11 to Project 13; 2) the functioning of the MultiPROBE II robot; or 3) a combination of changes made to the DNA IQ protocol and the functioning of the MultiPROBE II robot.

81. It is difficult to theoretically assign the failure to any specific change. However, the following should be considered:

    i. The increase in final elution volume from 50ul in Project 11 to 120ul in Project 13. The protocol developers warned this would result in a lower final concentration of DNA.

    ii. The correct heating of the sample during lysis and elution is key to firstly release DNA from cells, deactivate nucleases that could damage DNA, then elute DNA into the final tube for profiling. Modifications to the MultiPROBE II heat tiles were made by QHFSS, but verified in Project 13 as being reliable. Was the accuracy of the heating tiles regularly checked for accurate temperature?

    iii. The pipetting of chemicals and resin beads by the robots should be considered in terms of accuracy and non-disruption of resin beads.

    iv. The reduction of temperature during the lysis incubation step from 56ºC to 37ºC is a significant variation from the developer's protocol. While Proteinase K has a broad operating temperature, this change may have decreased the activity of the enzyme.

    v. QHFSS used an in-house CEB, rather than purchasing the Promega Casework Extraction Buffer Kit. This appeared to provide good results for the manual DNA IQ method in Project 11, however, may have needed optimisation for the automated protocol.

Retesting of Samples Affected by the Failed DNA Extraction Method

82. The DNA extract remaining from the failed method is unlikely to contain DNA of sufficient quality and quantity to retest using standard short tandem repeat (STR) technology if the sample has already been tested and failed to provide a DNA profile.

83. The exception to this could be the availability of mitochondrial DNA (mtDNA), however, this would only be suitable for samples not expected to contain more than one person's DNA[45].

84. QHFSS have retained substrates such as swabs and tapelifts which have been previously extracted, and are therefore available for further testing.

85. The original swab (either cotton or rayon) used to collect the biological sample is likely to contain cells that were not released during initial DNA extraction process. For example, rayon swabs have been shown to release less than 20% of available DNA during the extraction process.[46]

86. It is possible the original tapelifts may also contain cells. A range of other substrates may also exist (eg fabric, paper from cigarette butts, etc), however, it is unknown whether these may contain sufficient cells after initial DNA extraction.

87. In my opinion, simply retesting the original swab or tapelift with a standard DNA extraction method should be avoided also, as it will not successfully release the trapped cells. There are examples in the literature of methods being developed specifically to increase cell release from swabs. This includes up to 80% increased yield for cotton swabs[47], and in other research, a three-fold increase for cotton swabs[48].

88. A research project targeted at trialling methods designed to increase cell release from swabs should be conducted on mock samples containing cells and blood. If successful, the method should be validated and used on swabs affected by the failed DNA extraction method. Investigation is needed to determine if a similar approach would be beneficial for tapelifts and other common substrate types.

[45] DNA mixtures of two or more people cannot be routinely interpreted when using mtDNA technology.
[46] Bruijns B, Tiggelar R, Gardeniers H. The extraction and recovery efficiency of pure DNA for different types of swabs. J For Sci. 2018 Sep;63(5):1492-99. doi: 10.1111/1556-4029.13837.
[47] Gray, A., Kuffel, A., & Nic Daeid, N. An improved rapid method for DNA recovery from cotton swabs. Forensic Science International: Genetics. 2023; (64). https://doi.org/10.1016/j.fsigen.2023.102848
[48] Adamowicz M, Stasulli D, Sobestanovich, Bille T. Evaluation of methods to improve the extraction and recovery of DNA from cotton swabs for forensic analysis. PLoS ONE. 2014 Dec;9(12):e116531. doi:10.1371/journal.pone.0116351.

QHFSS Backlog Reduction Project

89. The flawed method may have been rushed into service because it was a key deliverable of the QHFSS 'Backlog Reduction Project' ($11 million over 3 years from 2004 to 2007), an election commitment of the incoming 2004 Beattie government. The Backlog Reduction Project was in response to media and public pressure to eliminate the DNA backlog and reduce DNA testing times, enabling results to be provided faster to the police and courts. The key outcomes were stated: '*The backlog in forensic biology as at February 2004 will be eliminated by 30 June 2005*'. The total backlog of cases at that time was 12,843. Implementation of the robots were key to ensuring the goals of the project were met, as the manual method was significantly slower and would result in the government's pledge being broken.

90. The 2004 Project Proposal states: '*The Beattie* Government *will allocate an extra $5 million this year and $3million in each of the following two years to help the John Tonge Centre clear the profiling backlog and cater for future profiling*.' This project is directly in response to the "law and order" election commitment made by the incoming government in February 2004. The commitment was to '*Provide an additional $11 million in new funding over three years to clear the DNA profiling and crime scene samples backlog at Queensland Health Pathology and Scientific Services (John Tonge Centre)*'.

Dr Kirsty Wright 23 October 2023

Attachment 1: Timeline

23 March 2004: QHFSS Forensic Services Backlog Project proposal.

2004: Vanessa Ientile replaces long-time Managing Scientist Leo Freeney.

January 2006: Automated platforms (robots) procured.

June 2007: Project 9. 'Report on the evaluation of commercial DNA extraction chemistries'. DNA IQ manual method chosen'. Note: the lab finds this is not the best performing method, but it is the only chemistry they tested which was validated for use on the MultiPROBE II robot by another laboratory (therefore saving QHFSS time to implement the new chemistry-robot coupling).

2007: Project 11. 'Report on the validation of a manual method for extracting DNA using the DNA IQ System. The manual DNA IQ method appears to be working well.

Jan to Oct 2007: Project 13. Verification of DNA IQ chemistry on extraction robots. QHFSS recklessly changed key manufacturer settings which likely caused the poor performance.

29 Oct 2007: Implementation of MultiPROBE II robots and use on crime scene samples. Note: The Project 13 Report is dated August 2008, which is ten months after the introduction of the failed extraction method.

6 February 2008: Contamination event on MultiPROBE II robot. Note: Unknown contamination events should be very, very rare in a forensic lab.

25 February 2008: Contamination detected on MultiPROBE II robot.

27 February 2008: Contamination event on MultiPROBE II robot.

19 March 2008: Project 21. Introduction of 'off-deck' lysis protocol for the MultiPROBE II robots.

2008: Project 22. 'A modified DNA IQ method for off-deck lysis prior to performing automated DNA extraction.

20 March 2008: Contamination event on MultiPROBE II robot.

4 April 2008: Contamination event on MultiPROBE II robot.

9 April 2008: Contamination event on MultiPROBE II robot.

16 April 2008: Contamination event on MultiPROBE II robot.

21 April 2008: Contamination event on MultiPROBE II robot.

29 April 2008: Contamination detected on MultiPROBE II robot.

1 Mary 2008: Contamination event on MultiPROBE II robot.

2 May 2008: Contamination detected on MultiPROBE II robot.

7 May 2008: Contamination event on MultiPROBE II robot.

19 May 2008: Contamination event on MultiPROBE II robot.

26 May 2008: Contamination event on MultiPROBE II robot.

4 June 2008: Contamination event on MultiPROBE II robot.

14 June 2008: Contamination detected on MultiPROBE II robot.

19 June 2008: Contamination event on MultiPROBE II robot.

23 June 2008: Contamination event on MultiPROBE II robot.

1 July 2008: Two contamination events on MultiPROBE II robot.

11 July 2008: Contamination event on MultiPROBE II robot.

14 July 2008: Extraordinary management meeting to address contamination concerns with MultiPROBE II robots (run by Vanessa Ientile)[49]. The cause of the contamination cannot be identified. Memo from Vanessa Ientile stating it is not feasible to stop all robotic extractions, one of two robots will be used on crime scene samples, the other one will not be used. The memo also states there was no evidence of contamination in the validation of the robots (Project 13), which is untrue having regard to the content of the Project 13 report.

23 July 2008: Contamination detected on both robots.

28 July 2008: Extraordinary management meeting to discuss contamination. Suspension of all robotic DNA extractions. All extractions to be done manually.

30 July 2008: Contamination detected on MultiPROBE II robot (run on a batch prior to the suspension).

July 2008: Vanessa Ientile leaves QHFSS, and Cathie Allen becomes the Acting Managing Scientist.

October 2008: Cathie appoints Tom Nurthen (Project Lead for the catastrophic Project 13), the Forensic Biology Quality Manager.

14 November 2008: Sloots and Whiley external audit report of the DNA extraction issues (contamination events) is released. The report also highlights an issue in the DNA extraction method that may cause systemic false negative results (ie failed results, even from high quality samples). The Quality Manager at the time (Tom Nurthen) cannot remember anyone following up on rectifying this issue.

20 August 2009: Volume crime samples to resume with one robot (~ 1 year after suspension).

19 January 2010: Crime scene and reference samples to resume with other robot (~1.5 years after suspension).

2010: A second DNA extraction robot is introduced (Maxwell). This was the better performing extraction method used on the Blackburn samples.

From February 2013: Critical crime scene samples from the Blackburn case are processed using flawed method (MultiPROBE II robot with QHFSS-modified protocol).

November 2016: The MultiPROBE II ceased being used to extract DNA (~ 9 years after implementation).

[49] EXH 129.65 Memorandum – Vanessa Ientile – DNA IQ extractions.