Notice number: 9.001

COMMISSION OF INQUIRY INTO DNA PROJECT 13

Section 5(1)(d) of the *Commissions of Inquiry Act 1950*

STATEMENT OF BREANNA GALLAGHER

I, Breanna Lee Gallagher, care of Sara McRostie, MinterEllison, 1 Eagle Street, Brisbane, QLD 4000, Policy Officer at Australian Government Department of Health and Aged Care, do solemnly and sincerely declare that:

1. On 19 October 2023, I was requested to provide a statement responding to Notice 9.001 "Requirement to Give Information in a Written Statement" (**Notice 9.001**).

Identification

Question 1(a) - State your full name

2. My name is Breanna Lee Gallagher.

Question 1(b) - State your qualifications, skills or experience relevant to forensic science and DNA

3. In 2005, I graduated with a Bachelor of Applied Science majoring in Biotechnology from the Queensland University of Technology in Queensland, Australia.

4. I currently work in the field of genomics, in particular implementing genomic technology in the health care system as a Policy Officer for the Australian Government Department of Health and Aged Care.

Question 1(c) - State the period(s) of time you have been or were employed by or otherwise engaged with Queensland Health, Queensland Health Forensic and Scientific Services (QHFSS) and/or Forensic Science Queensland, and in what roles and when

5. On or around March 2006, I was employed as an Operational Officer with Queensland Health Forensic and Scientific Services (**QHFSS**). This was my first role after graduating from university.

6. Approximately six months after I commenced as an Operational Officer, QHFSS conducted an expression of interest for the role of a junior Project Scientist position, to be appointed to a team at QHFSS's discretion.

7. After providing my expression of interest for a Project Scientist position, I was employed as Project Scientist in the Automation/LIMS Implementation Project Team at QHFSS and remained in this role until my employment with QHFSS ended in approximately May 2008. During that time I was employed on numerous fixed term contracts for varying contract periods. I cannot recall the details about my employment contracts or when they were renewed.

8. My duties and responsibilities as Operational Officer consisted of work to assist other scientists conduct testing in the Laboratory, including:

(a) making up and dispensing basic reagents for laboratory use, such as bleach and ethanol for cleaning work surfaces and equipment;

(b) calibrating the pipettes in the Laboratory;

(c) administrative tasks such as filing for the scientists in the Volume and Major Crimes;

(d) recording temperature checks of fridges and freezes to comply with National Association of Testing Authorities (NATA);

(e) under the supervision of a scientist, logging DNA profiles into the National Criminal Investigation DNA Database (NICDD).

9. I have not been employed or otherwise engaged with Queensland Health and/or Forensic Science Queensland.

The Automation/LIMS Implementation Project Team

10. My recollection is the Automation/LIMS Implementation Project Team included:

(a) Project Lead – Thomas Nurthen;

(b) three scientists at that time - Cecilia Iannuzzi, Yojtech Hlinka and Iman Muharam;

(c) two junior Project Scientists, being Generosa Lundie and myself.

11. As I understood it the Automation/LIMS Implementation Project Team was tasked to develop an automated testing method for extracting DNA from samples for genomic and genetic sampling on an automated platform (**Project**), being one of the four devices manufactured by Perkin Elmer (**Automated Device**). I do not recall this project being specifically described as 'Project 13'.

12. The Laboratory received the four Automated Devices after I commenced employment with QFHHS and by the time I commenced my role as Project Scientist. I do not recall specific dates.

13. I recall the process of developing an automated testing method I have referred to in paragraph 11 involved the following:

(a) The analytical team at QHFFS conducted DNA extraction using a manual method, being the manual and human-led process. The results of testing for extracting DNA from samples using the manual method would be the comparator for the automated method the Automation/LIMS Implementation Project Team was developing. I do not recall if this manual method had a title.

(b) Conducting extraction processes for extracting DNA from samples on an Automated Device.

(c) The verification process and validation process of the working automated testing method. These processes are standard scientific methods employed by all laboratories to show accuracy and repeatability of results. Information relating to the verification and validation of the automated testing method would be provided

[redacted] ..... [redacted] .....

Breanna Lee Gallagher Witness

to National Association of Testing Authorities as evidence that the laboratory had complied with International Standards required for forensic laboratories.

(d) Comparing the results of conducting extraction processes on an Automated Device versus the manual method referred to at paragraph 13(a) above, to determine whether the laboratory could increase its capacity to test numerous types of samples and ensure that yield of DNA from samples was comparable to that of the manual method.

14. In relation to the process of developing an automated testing method as I recall it in paragraph 13:

(a) During the development of the automated extraction method, mock samples were created in order to test the method. Mock samples were created by adding a DNA sample – blood or buccal – in differing dilutions to commonly encountered "substrate" types, such as cloth, cotton and nylon swabs. Once the method was more fully developed in or around 2008 (rather than earlier in the project in 2007), samples from crime scenes, which I recall being predominately swabs and cigarette papers, were being processed on the Automated Devices.

(b) The automated testing method was initially based on methods provided by the manufacturers of the four automated platforms, Perkin Elmer. It was common for manufacturers of Automated Devices to provide protocols to apply an automatic testing approach on the Automated Device. Those settings could be adjusted. The scientist operating the Automated Device could then follow and apply an automated approach with the chosen settings, to process sample/s for DNA extraction on the Automated Device. This step could be described as 'trial and error' testing, in that we considered and applied manufacturer protocols to conduct extractions, tried to identify what factors in the Laboratory may be impacting extraction results, then made necessary changes to our automated approach to testing.

15. I do not recall the particular details of any protocol provided by the manufacturer of the Multiprobe II Device.

16. I do not know if:

(a) There was a designated period in which the Automation/LIMS Implementation Project Team was expected to complete the project to develop the automated testing method I have referred to in paragraph 11 above; and

(b) there were stages to the Project.

17. My duties and responsibilities as Project Scientist were:

(a) assisting with the work described at paragraph 13(b) and 13(c) including considering protocols for cleaning the Automated Devices;

(b) assisting the team lead, Thomas Nurthen, to try to determine how to implement the tracking of large batches of samples through the Auslab pathology system used by the Laboratory to record DNA results of processed samples;

[redacted] ..... [redacted] .....

Breanna Lee Gallagher Witness

(c) contributing to the drafting of a standard operational procedure in relation to the automated protocols developed, titled 'DNA IQ™ Method of Extracting DNA From Blood and Cell Substrates' dated in the footer as 'Valid From 11/01/2008', which is attached and marked as Exhibit **BG-1** to this Statement (**Standard Operating Procedure**).

18. On 24 October 2023, my legal representatives provided me a copy of the Standard Operating Procedure. I have never had a copy of my Standard Operating Procedure in my possession.

19. I was not solely responsible for drafting the Standard Operating Procedure. I helped to draft the parts of the Standard Operating Procedure relating to the elements of the automated testing method being developed and subsequently agreed within the Automation/LIMS Implementation Project Team as part of the Project as described at paragraphs 11, 13(b) and 13(c) above.

20. I do not recall any specific details in relation to when the Standard Operating Procedure was drafted or approved other than by reference to the Amendment History on page 17 of the Standard Operating Procedure.

21. From 24 October 2007 (the date referred to in the Standard Operating Procedure), I believe the Automation/LIMS Implementation Project Team followed the processes in the Standard Operating Procedure as updated from time to time.

22. I do not know if QHFSS continued to use the Standard Operating Procedure from the time my employment ended in May 2008, nor whether QHFSS used another automated method.

23. I do not know whether the Standard Operating Procedure is a part of, or is, the final method that was included within the 2008 Report.

24. My duties and responsibilities as Project Scientist did not include the work described at paragraph 13(a) above. I was not involved in it nor was I trained in a manual DNA method or protocol. In the absence of information or documents in relation to this method, I do not know if a manual method or protocol was able to be, or was, run on an Automated Device.

25. I cannot recall if my duties and responsibilities included the work described at paragraph 13(d) above, nor do I recall who was responsibilities for that comparison or the method for that comparison.

2008 Report

26. On 20 October 2023, my legal representatives provided a copy of the 'Project 13. Report on the Verification of an Automated DNA IQ™ Protocol using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform' dated August 2008 on page 1 (**2008 Report**) to me. A copy of the 2008 Report is attached and marked as Exhibit **BG-2** to this Statement. I do not recall having seen the 2008 Report prior to being provided with a copy on 20 October 2023.

[redacted] ..... [redacted] .....

Breanna Lee Gallagher Witness

27. I was not involved in writing the 2008 Report however I understand it relates to the work of the Automation/LIMS Implementation Project Team during my employment at QHFSS as Project Scientist.

28. I believe my name was included as an author of the 2008 Report because I was a member of the Automation/LIMS Implementation Project Team.

29. I do not know if the Standard Operating Procedure is the same as the 'automated procedure' or the 'automated DNA IQ™ Protocol' referred to in the 2008 Report.

Manual and Automated DNA Extraction Methods

Question 2 – In relation to the report being the "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) (2008 Report)1 and the abstract and introduction therein which state:

> 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.
>
> 2. Introduction
>
> The MultiPROBE® II PLUS HT EX FORENSIC WORKSTATION platforms (PerkinElmer, Downers Grove, IL, USA) are equipped to perform automated DNA extractions, as they include a DPC shaker and individual heat controllers to enable on-board lysis and incubation steps. Currently in DNA Analysis, the MultiPROBE® platforms allow walk-away operation of PCR setup protocols for DNA quantitation and amplification.
>
> The DNA IQ™ protocol has been verified or validated by various laboratories for use on the MultiPROBE® II PLUS platform. The laboratories that perform an automated DNA IQ™ protocol include PathWest (Western Australia), Forensic Science South Australia (South Australia) and Centre of Forensic Sciences in Toronto (Ontario). The MultiPROBE® II PLUS instrument comes pre-loaded with an automated DNA IQ™ protocol. Unlike the other laboratories, however, we did not validate the included protocol, but instead validated a manual DNA IQ™ protocol which was based on the CFS automated protocol (PerkinElmer, 2004), followed by verification of an automated protocol based on the validated manual method.
>
> The verified automated DNA IQ™ protocol is identical to the validated manual protocol used in-house: there are no differences in reagents or volumes. The adopted DNA IQ™ protocol differs slightly, however, from the manufacturer's protocol, as it includes a lysis step using Extraction Buffer (10mM Tris, 1mM EDTA, 100mM NaCl, 20% w/v SDS) in the presence of Proteinase K, before incubating in the DNA IQ™ Lysis Buffer. Furthermore, the lysis incubation conditions were lowered from 70°C to 37°C in order to accommodate extraction of DNA from heat labile materials such as nylon and polyester. In addition, the automated protocol utilises the SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA) for simultaneous processing of samples in a 96-well format.

Manual Method

[redacted] .... [redacted] .....

Breanna Lee Gallagher Witness

Question 2(a) describe, with precision, the "manual method" for extracting DNA from forensic samples using the DNA IQ™ system referred to in the first line of the Abstract to the 2008 Report (Manual Method), including whether the Manual Method:

(ii) was devised within the QFSS Forensic DNA Analysis laboratory (Laboratory); or

(ii) was otherwise a modification of an existing manual method (and if so which method),

30. I do not know, nor can I describe or recall, any information or knowledge about:

(a) a "manual method" for extracting DNA from forensic samples using the DNA IQ™ system referred to in the first line of the Abstract to the 2008 Report (**Manual Method**);

(b) the "DNA IQ™ system" referred to in the first line of the Abstract to the 2008 Report.

31. I was not involved in the devising of a "manual method" nor any modification of an existing manual method for extracting DNA from forensic samples.

32. During my employment at QHFSS:

(a) my duties and responsibilities were limited to work involving automated protocols as described at paragraph 17 above of this Statement; and

(b) I had no experience in the devising, modification or implementation of manual methods outlined at paragraph 13(a).

33. On 23 October 2023, my legal representatives provided me a copy of the *Project 11. Report on the Validation of a Manual Method for Extracting DNA using the DNA IQ System* (**Project 11 Report**) which includes my name on page 1, attached and marked as Exhibit **BG-3** to this Statement.

34. I did not draft the Project 11 Report and I have no knowledge of who was responsible for drafting the Project 11 Report.

35. During my employment at QHFSS, I was not aware of the Project 11 Report or of a 'project 11'.

36. I believe my name is on the Project 11 Report because I was a member of the Automation/LIMS Implementation Project Team.

Question 2(b) describe, with precision, the method by which the Manual Method's "routine use" in DNA Analysis (FSS) was validated

37. I do not know the method by which the Manual Method's "routine use" in DNA Analysis (FSS) was validated.

Question 2(c) state whether, and if so how, the Manual Method differed from or otherwise modified the DNA IQ™ protocol that was "verified or validated by various laboratories for use on the Multiprobe® II PLUS platform" (as stated in the second line of the second paragraph of the Introduction to the 2008 Report)

[redacted] .... [redacted] .....

Breanna Lee Gallagher Witness

38. I do not know if the Manual Method differed from or otherwise modified the DNA IQ™ protocol that was "verified or validated by various laboratories for use on the Multiprobe® II PLUS platform".

Question 2(d) state when the Manual Method was so devised

39. I do not know when the Manual Method was devised.

Question 2(e) identify those within the Laboratory responsible for devising the Manual Method

40. I do not know who within the Laboratory was responsible for devising the Manual Method.

Question 2(f) state the reason(s) why the Laboratory chose to devise and to implement the Manual Method

41. I do not know the reason or reasons why the Laboratory chose to devise and/or implement the Manual Method.

CFS Automated Protocol

Question 2(g) describe, with precision, the (CFS Automated Protocol) referred to in the seventh line of the second paragraph of the Introduction to the 2008 Report

42. I do not know, nor can I describe with certainty or recall, the "CFS automated protocol (PerkinElmer, 2004)".

Manual DNA IQ™ Protocol

Question 2(h) describe, with precision, the "manual DNA IQ™ protocol" (Manual DNA IQ™ Protocol)" referred to in the seventh line of the second paragraph of the Introduction to the 2008 Report, including whether it:

(i) was developed or otherwise supplied by the manufacturer of the MultiPROBE® II PLUS HT EX FORENSIC WORKSTATION platform;
(ii) was devised within the Laboratory;
(iii) was otherwise a modification of an existing Manual DNA IQ™ protocol (and if so which method)

43. I do not know, nor can I describe or recall, the "manual DNA IQ™ protocol" referred to in the seventh line of the second paragraph of the Introduction of the 2008 Report.

44. Accordingly, I do not know if a "manual DNA IQ™ protocol":

(a) was developed or otherwise supplied by the manufacturer of the MultiPROBE® II PLUS HT EX FORENSIC WORKSTATION platform;

(b) was devised within the Laboratory; or

(c) was otherwise a modification of an existing Manual DNA IQ™ protocol.

45. I repeat and refer to paragraph 24 above of this Statement.

[redacted] .... [redacted] .....

Breanna Lee Gallagher Witness

Question 2(i) describe, with precision, the method by which the Manual DNA IQ™ Protocol was validated

46. I do not know the method by which the Manual DNA IQ™ Protocol was validated.

Question 2(j) state whether, and if so how, the Manual DNA IQ™ Protocol differed from or otherwise modified the DNA IQ™ protocol that was "verified or validated by various laboratories for use on the Multiprobe® II PLUS platform" (as stated in the second line of the second paragraph of the Introduction to the 2008 Report)

47. I do not know how the Manual DNA IQ™ Protocol is different from or otherwise modified the DNA IQ™ protocol.

Question 2(k) state when the Manual DNA IQ™ Protocol was so devised

48. I do not know when the Manual DNA IQ™ Protocol was devised.

Question 2(l) identify those within the Laboratory responsible for devising the Manual DNA IQ™ Protocol

49. I do not know those within the Laboratory responsible for devising the Manual DNA IQ™ Protocol.

Question 2(m) state the reason(s) why the Laboratory chose to devise Manual DNA IQ™ Protocol

50. I do not know the reason or reason why the Laboratory chose to devise Manual DNA IQ™ Protocol.

Automated DNA IQ™ Protocol

Question 2(n) state whether the "automated DNA IQ™ protocol" referred to in the first line of the third paragraph of the Introduction to the 2008 Report (Automated DNA IQ™ Protocol) is the same as the automated protocol the subject of the 2008 Report. If it is not, then state the reasons why and describe any differences

51. I do not know if the "automated DNA IQ™ Protocol" is the same as the automated protocol the subject of the 2008 Report.

Question 2(o) state whether, and if so how, the Automated DNA IQ™ Protocol differed from or otherwise modified:

(i) the Manual Method;
(ii) the DNA IQ™ protocol that was "verified or validated by various laboratories for use on the Multiprobe® II PLUS platform" (as stated in the second line of the second paragraph of the Introduction to the 2008 Report)
(iii) the CFS Automated Protocol; and
(iv) the Manual DNA IQ™ Protocol

[redacted] ..... [redacted] .....

Breanna Lee Gallagher Witness

52. I do not know, nor can I describe or recall, the "automated DNA IQ™ Protocol" referred to in the second and third lines of the second paragraph of the Introduction to the 2008 Report.

53. Accordingly, I do not know if the Automated DNA IQ™ Protocol differed from:

    (a) the Manual Method and/or the Manual DNA IQ™ Protocol, because I do not recall either method or protocol, nor was I involved in manual sampling during my employment at QFHHS;

    (b) the DNA IQ™ protocol that was "verified or validated by various laboratories for use on the Multiprobe® II PLUS platform" (as stated in the second line of the second paragraph of the Introduction to the 2008 Report); and

    (c) the CFS Automated Protocol.

54. The protocol being developed as part of the Project may have differed from protocols that were used by other laboratories for use on the Multiprobe® II PLUS platform because the Laboratory needed to implement an automated protocol for testing numerous sample types whereas some other laboratory protocols were suited to testing of one sample type (e.g. blood only).

Question 2(p) state when the Automated DNA IQ™ Protocol was so devised

55. To the extent the Automated DNA IQ™ Protocol is a reference to the protocol being developed as part of the Project, I repeat and refer to paragraph 16 above of this Statement.

56. I do not know whether the development of an automated DNA IQ™ protocol continued after my employment ended.

Question 2(q) identify those within the Laboratory responsible for devising the Automated DNA IQ™ Protocol

57. I do not know who within the Laboratory was responsible for devising the Automated DNA IQ™ Protocol.

58. To the extent the Automated DNA IQ™ Protocol is a reference to the protocol being developed as part of the Project, no single person was responsible for devising the protocol being developed as part of the Project.

59. The Automation/LIMS Implementation Project Team occasionally had conversations with the following people in relation to the protocol being developed as part of the Project:

    (a) Allan McNevin, who I believe was the head of the analytical lab;

    (b) senior members of the heads of the major crime and volume teams at the time, Cathie Allen, Sam Cave and Sharon Johnstone because their sampling would flow into the analytical team.

60. I do not know who was formally responsible for making decisions regarding the operation of the Laboratory and approval of protocols.

[redacted] ..... [redacted] .....

Breanna Lee Gallagher Witness

Question(r) state the reason(s) why the Laboratory chose to devise the Automated DNA IQ™ Protocol rather than use the manufacturer method

61. I repeat and refer to paragraphs 11 to 25 above.

62. My answers below are provided on the understanding:

    (a) the Automated DNA IQ™ Protocol is a reference to the protocol being developed as part of the Project; and

    (b) the 'manufacturer method' is a reference to the protocols for an automated approach to sampling on the platforms, rather than the programming of the Automated Device itself .

63. The Laboratory chose to devise the protocol being developed as part of the Project rather than the manufacturer method because a manufacturer's method was not necessarily fit for the conditions of every laboratory. Those concerns arose for the Laboratory because the yield of DNA extracted from each sample was less than anticipated by the Automation/LIMS Implementation Project Team.

64. I cannot recall the reasons why the Automation/LIMS Implementation Project Team formed this view however I do recall there were various factors which may impact yield including:

    (a) the cleanliness of the original sample. If there were concerns about the cleanliness of the original sample, multiple extraction processes were required so that the sample was worthwhile testing and would yield some DNA from the extraction process;

    (b) the use of buffers in the extraction process, however I do not recall details about the particular issue/s caused by the buffers.

Multiprobe II PLUS HT EX with Gripper Integration Platform (Multiprobe II Device)

Question–3 - State when the Laboratory received the Multiprobe II Device

65. I repeat and refer to paragraph 12 above.

Question–4 - For each of the Manual DNA IQ™ Protocol and the Automated DNA IQ™ Protocol, describe, with precision and completeness, what, if anything was done to the device to modify it, including whether any of the manufacturer's factory settings were changed, and if so which ones and how (including but not limited to temperature settings, reagents and volumes)

Question–7 - State the reasons why the modifications were made

66. I do not know if any modifications were made to a device in relation to any manual DNA protocol. During my employment at QHFSS, I was not involved in any work in relation to manual sampling or manual protocol.

[redacted] ..... [redacted] .....

Breanna Lee Gallagher Witness

67. If the reference to 'manufacturer's factory settings' in Question 4 is a reference to the pre-loaded computer programming for the Multiprobe II Device, I am not aware of any changes being made to those settings.

68. If the Automated DNA IQ™ Protocol is a reference to the protocol being developed as part of the Project, the extent of my recollection is that the nature of amendments made to the suggested protocols in manufacturer's automated protocols related to:

    (a) The cleaning of samples. I recall the automated protocol being developed by the Automation/LIMS Implementation Project Team introduced an additional cleaning step to that in the manufacturer's automated protocol.

    (b) The use of buffers in the extraction process. I recall the Automation/LIMS Implementation Project Team observed that particular brands of buffers performed more suitably and optimally then buffers provided by manufacturers in an extraction kit.

    (c) The brand of reagent used (but not the type of reagent). I recall the Automation/LIMS Implementation Project Team noticed the DNA extraction results differed for the types of reagents, including that a *Qiagen* reagent was producing higher yield of DNA for a sample than *Promega*. I cannot recall further details regarding the reagents which were purchased or at what volume.

    (d) The length of time the pipette tips would hover over a plate containing samples during the extraction process. The purpose of this was to ensure there was a sufficient length of time to allow bubbles to drip off of pipette tips to avoid contamination risk.

    (e) Temperature of the plates using the heating device provided by the manufacturer of the Automated Device. The temperature of the heating device impacted the temperature of the sample with the plate. I recall issues with the yield of DNA extraction arose when we proceeded with extraction with the pre-set temperatures for the heating device outlined in the manufacturer's protocol because the samples would not increase to the necessary temperature.

69. I do not recall the specifics of each amendment or the final protocol.

70. Overall, the modifications were made after considering a sample outcome, identifying what was not working or what could be improved in the extraction process, and how that outcome was impacted by an element or elements of the protocol being developed as part of the Project.

71. The modifications in paragraph 68 informed, in part, the processes outlined the Standard Operating Procedure.

Question–5 - State when the modifications were made

72. To the extent the Automated DNA IQ™ Protocol is a reference to the protocol being developed as part of the Project, the Automation/LIMS Implementation Project Team made modifications to the protocol being developed as part of the Project over the course of approximately two years of running the program to obtain verification and validation. However, I do not recall specifics of each amendment or when they may have occurred.

[redacted] ..... [redacted] .....

Breanna Lee Gallagher Witness

Question–6 - Identify those within the Laboratory responsible for the modifications

73. No one person in the Automation/LIMS Implementation Project Team was responsible for identifying modifications, nor did a member of the team work in isolation from supervisors.

74. I recall the Automation/LIMS Implementation Project Team's general approach was to have conversations before implementing a suggested modification (e.g. temperature of a plate) to the protocol being developed as part of the Project for testing on an Automated Device.

2008 Report

Question–8 - Describe your role in the preparation of the 2008 Report

75. I refer and rely on paragraphs 26, 27, 28, 29 and 52 above of my Statement.

76. I had no role in the preparation of the 2008 Report.

Question–9 - Describe the directions you received in relation to the preparation of the 2008 Report, and identify the person or persons from whom you received those directions.

77. I did not receive any directions in relation to the preparation of the 2008 Report:

    (a) during my employment at QHFSS;

    (b) after my employment at QHFSS ended.

Question –0 - State the substance of the communications (including discussions) that occurred between you, any other authors of the 2008 Report and/or any supervisor or person in a position of management concerning the purpose(s) or intended purpose(s) of the 2008 Report, including by identifying with whom those communications took place and when.

78. I did not engage in any discussions with the other authors identified on page 1 of the 2008 Report, and/or any supervisor or person in a position of management concerning the purpose(s) or intended purpose(s) of the 2008 Report:

    (a) during my employment at QHFSS;

    (b) after my employment at QHFSS ended.

Question –1 - Identify the persons to whom was the 2008 Report was distributed.

79. I do not know the persons to whom the 2008 Report was distributed.

Question –2 - In relation to the matters stated in the 2008 Report, state

(ii) **Question 12(–) - how the conclusion on page 1 of the 2008 Report that "Data indicate that results from the automated procedure are comparable to those from the manual procedure" was reached, includini) any discussions or communications between any of the named authors of the 2008 Report and any supervisor or person in a position of management in relation to that conclusion or the referenced data; and**

[redacted] ..... [redacted] .....

Breanna Lee Gallagher Witness

(ii) how that conclusion can be reconciled with the data and figures outlined in part 6.4 of the 2008 Report.

80. I do not know how the conclusion on page 1 of the 2008 Report that "Data indicate that results from the automated procedure are comparable to those from the manual procedure" was reached.

81. I have no knowledge of:

(a) any discussions or communications between any of the named authors of the 2008 Report and any supervisor or person in a position of management in relation to that conclusion or the referenced data; nor

(b) how that conclusion can be reconciled with the data and figures outlined in part 6.4 of the 2008 Report.

(ii) **Question 12(–) - how the recommendations summarised on page 18 of the 2008 Report were decided, including by identifyini) your role in the decision;**

(ii) with whom and when you communicated (including by way of discussion) with any other person in connection with the recommendations and the decision to make them

82. I had no role in deciding how the recommendations summarised on page 18 of the 2008 Report were decided.

83. I had no communications or discussion with any other person in connection with the recommendations and the decision to make them.

84. During my employment as a Project Scientist, I do not believe I had the relevant experience (including in manual testing) to make recommendations in relation to any comparison between the effectiveness of automated methods versus manual methods to extract DNA.

[redacted] .... [redacted] .....

Breanna Lee Gallagher Witness

Declared by the deponent, Breanna Lee Gallagher, this 24th day of October 2023 at Canberra, Australia, and I say that:

1. I have not conferred or had any discussion with other witnesses in preparing my statement.
2. All the facts and circumstances declared in my statement, are within my own knowledge and belief, except for the facts and circumstances declared from information only, and where applicable, my means of knowledge and sources of information are contained in this statement.
3. I make this solemn declaration conscientiously believing the same to be true and by virtue of the provisions of the *Oaths Act 1867*.
4. The statement was made in the form of an electronic document.
5. The statement was electronically signed by the signatory.
6. The statement was made, signed and witnessed under Part 6A *Oaths Act 1867*.
7. I understand that it is an offence to provide a false matter in a statement.

Signature of deponent: [redacted].

And as a witness, I certify the following matters concerning the person who made this statement (the deponent):

1 I saw the face of the deponent.
2 I have confirmed the deponent's identity using the following identification document: 18+ Identification Card.
3 I have verified that the name of the deponent matches the name written on the statement.

Signature of witness: .. [redacted] ....

Blaire O'Loughlin-Mills

Australian legal practitioner, MinterEllison

Special witness under the *Oaths Act 1867*

I understand the requirements for witnessing a document by audio visual link and have complied with those requirements

[redacted] ..... [redacted] .....

Breanna Lee Gallagher Witness

EXHIBITS INDEX

Exhibits Index – Breanna Lee Gallagher Statement

Paragraphs	Exhibit	Document Title
17(c), 18, 19, 20, 21, 22, 23, 29	BG-1	DNA IQ™ Method of Extracting DNA From Blood and Cell Substrates
26, 27, 28, 29	BG-2	Project 13. Report on the Verification of an Automated DNA IQ™ Protocol using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform
33, 34, 35, 36	BG-3	Project 11. Report on the Validation of a Manual Method for Extracting DNA using the DNA IQ System

[redacted] ..... [redacted] .....

Breanna Lee Gallagher Witness

BG-1

WIT.0016.0188.0439

JH-41

CaSS | Forensic and Scientific Services
A CLINICAL AND STATEWIDE SERVICE

DNA IQ™ Method of Extracting DNA from Blood and Cell Substrates

1 PURPOSE AND SCOPE

This method describes the routine automated DNA extraction of cell and blood samples on the PerkinElmer MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms using the Promega DNA IQ™ system. The manual method has been included as a back-up method should it be required.

This method applies to all Forensic Biology staff that is required to extract cell and blood samples.

Reference samples and Casework samples must be extracted separately. If Casework and Reference samples are to be extracted on the same instrument, the instrument must be decontaminated between operations.

2 DEFINITIONS

Samples	Samples awaiting DNA extraction
DNA Extracts	Samples that had DNA extraction processes performed
DNA IQ™ Resin	Magnetic Resin Beads used to bind DNA
MP II	MultiPROBE® II Platform
DTT	1,4 Dithiothreitol
Pro K	Proteinase K
SDS	Sodium Dodecyl Sulphate
TNE	Tris, NaCl and EDTA buffer
EDTA	Ethylenediaminetetraacetate
EP-A	Extraction Platform A – back wall platform
EP-B	Extraction Platform B – side wall platform

3 PRINCIPLE

Sample Pre-lysis

The Extraction Buffer used in the pre-lysis of cellular material contains Proteinase K (Pro K), TNE (Tris, NaCl, EDTA) and SDS. TNE acts as a basic buffer with EDTA chelating ions in solution. SDS is a detergent that lyses open cell membranes. Proteinase K is added to digest protein and cellular material that interferes with the DNA binding capacity of the resin. It is also added to rapidly inactivate enzymatic activity that could potentially degrade DNA (e.g.nucleases).

Proteinase K (also known as Endoproteinase K) is a fungal enzyme from *Engyodontium album* (formerly *Tritirachium album*). It is a stable S8 family serine alkaline protease that has broad specificity and is inactivated by diisopropylfluorophosphate, phenyl-methane sulfonyl fluoride and Hg^{2+} ions. As a highly active endopeptidase, it cleaves peptide bonds adjacent/next to the carboxyl group of N-substituted (blocked alpha amino) hydrophobic, aliphatic and aromatic amino acids. Therefore, it actively hydrolyses proteins, glycoproteins, peptides and esters of amino acids. The smallest peptide that can be hydrolysed with Proteinase K is a tetrapeptide.

DNA IQ™ Kit

The DNA IQ ™ kit is designed to purify DNA from a number of different substrates and has been optimised for trace DNA samples. An in house validation was performed using a modified version of the PerkinElmer automated protocol. The protocol has been modified to incorporate a number of work practices used in Forensic Biology FSS. These are:

- The use of the Slicprep™ 96 device (Promega) for removing substrate from lysate.

- The increase of extraction buffer volume to 500µL for use with the Slicprep™ 96 device.
- The increase of Lysis Buffer volume to 957µL proportional to the increase of Extraction Buffer volume, according to the manufacturer's instructions.
- Double Elution step, with an Elution buffer volume of 60µL for a final volume of 100µL.
- The use of NUNC Bank-It tubes for storage of final extracts.

Cell lysis is performed with Promega Lysis Buffer containing Dithiothreitol (DTT). 1,4 Dithiothreitol is a reducing agent used in extractions to break disulfide bonds of proteins. The Lysis Buffer used is a proprietary buffer containing chaotropic salts required for binding of DNA to the magnetic beads. According to the MSDS, the lysis buffer contains 50-75% guanidinium thiocyanate, < 2.5% EDTA, < 2% 3-[(3-Choalamidopropryl)dimethylammonio] propanesulfonic and < 2% polyethylene glycol tert-octylphenyl ether.

The basis of the DNA IQ™ kit is a magnetic bead resin which contains novel paramagnetic particles covered with silica. The magnetic bead resin usually has a DNA binding capacity of 100ng but the addition of Pro K will increase the binding capacity. Samples with small amounts of DNA are more efficient than samples with large amounts of DNA, and as a result a small sample size is critical to ensure efficient recovery of DNA.

The magnetic beads with silica have a negative charge at basic and near neutral pH. The Lysis Buffer changes the pH and salt concentration of the solution and the silica on the magnetic beads becomes positively charged which is then able to bind the DNA.

Several washing steps are employed in the protocol to remove inhibitors. The first washing step is performed with Lysis Buffer. This wash ensures the DNA is bound to the magnetic bead resin and washes out inhibitors. The next three washing procedures are with a 1xWash Buffer. This buffer contains an alcohol/aqueous mixture which ensures the DNA is not eluted during washing by keeping the DNA dehydrated, and aqueous phase washes out the inhibitor.

Elution buffer removes the DNA from the magnetic bead resin. The Elution Buffer changes the salt content. Heating the complex to 65°C allows the DNA to be released from the magnetic beads. The Elution Buffer is a low ionic strength buffer that reduces the affinity of the DNA for the silica that covers the magnetic beads, by re-hydration of the phosphate backbone.

The DNA IQ™ kit isolates DNA greater than 80bp, smaller DNA is removed selectively to prevent PCR inhibition.

MultiPROBE® II HT EX Plus with Gripper™ Integration Platform

Within Forensic Biology, blood and cell extractions are performed using 2 MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms (Extraction Platform A, EP-A) and (Extraction Platform B, EP-B) perform casework or reference samples.
Each platform uses a computer – controlled Cartesian X-Y-Z liquid handling system designed for the efficient automation of sample preparation. Liquid transfer is performed by an 8-tip System with VersaTip® and VariSpan™ options. The VersaTip® option allows the use of fixed and/or disposable tips (both clear and conductive). The VariSpan™ option permits variable probe spacing between each of the individual probes so that a wide range of labware such as micro plates, tubes, vials and reagent troughs can be accessed. Each sample probe is capable of independent motion in the Z direction due to independent Z drives.

The 8-tip System is also capable of multichannel liquid-level sensing by utilising Accusense™ technology. This technology works by each probe detecting a change in capacitance within the liquid. This capacitive mode of detection is also possible when using conductive disposable tips. Accusense™ also permits the detection of low or non-ionic polar solutions and solvents. Pipetting on the platforms is driven by 8 individually controlled syringes via the positive displacement of the system liquid (nanopure water) when transferring liquid.

The Gripper™ Integration on all the platforms (except for the Post – PCR MPII) allows for automated identification of labware via the scanning of barcodes and also allows for automated transfer of plates from one position on the deck to another. To increase deck space and capacity, all platforms include a left deck extension.

In this program a platemap is utilised to provide the necessary information for correct volumes and locations for pipetting. It also contains information regarding positions where pipetting must take place at various locations, the number and IDs of samples. Without a platemap the program will fail to work.

4 REAGENTS AND EQUIPMENT

4.1 Reagents

1. DNA IQ™ System Kit – 400 sample Kit
    - Resin
    - Lysis Buffer (LB)
    - 2x Wash Buffer (2xWB)
    - Elution Buffer (EB)
2. Tris/Sodium chloride/EDTA Buffer (TNE)
3. Proteinase K (Pro K) 20mg/mL
4. Dithiothreitol (DTT) 1M
5. 5% TriGene
6. 70% Ethanol
7. 1% Amphyl
8. 0.2% Amphyl
9. Isopropyl alcohol
10. AnalR 100 %Ethanol
11. 20% SDS
12. Decon® 90 solution
13. Nanopure H_2O

Table 1. Reagent storage locations.

Reagent	Device	Storage Location
Pro K	Freezer	Room 6120
DTT	Freezer	Room 6120
20% SDS	Shelf	Room 6122
Isopropyl alcohol	Shelf	Room 6122
TNE pH 8 Buffer	Shelf	Room 6122
DNA IQ™ Kit	Shelf	Room 6122
Amphyl (1% and 0.2%)	Shelf	Room 6127
Nanopure Water	Shelf	Room 6127
5% TriGene	Shelf	Room 6127
AnalR 100 %Ethanol	Shelf	Room 6127

Please see Table 2 for the volume of reagents for a full plate or half plate. See QIS 17165 (Receipt, Storage and Preparation of Chemicals, Reagents and Kits) for preparation of the TNE buffer. All reagents, except for the Lysis Buffer with DTT (in fume hood), can be made on the bench in Room 6122. DNA IQ reagents are prepared by staff performing the DNA IQ method.

Table 2. Table of reagent volumes.

Reagent (volume per sample)	Constituent (volume per sample)	Volume req'd for 96 Samples (mL)	Volume req'd for 48 Samples (mL)
Extraction Buffer (500 µL/sample	TNE buffer 462.5µL	54	27
	Prot K (20 mg/mL)25.0 µL	2.9	1.5
	SDS (20 %) 12.5µL	1.5	0.7
Lysis buffer (with DTT) (1.127mL/sample)	Lysis buffer (no DTT)	130	66
	DTT (add to Lysis buffer)	1.3	0.66
Lysis Buffer (with DTT) Reagent Trough	As above	125	63
DNA IQ RESIN Solution (50µL/sample)	Lysis buffer (with DTT) (from above) 43µL	6	3
	DNA IQ RESIN 7µL	1	0.5
DNA IQ 1X Wash Buffer (300µl/sample)	See Below for preparation	35	18
DNA IQ Elution Buffer (120µl/sample)	Use directly from Kit	14	8

NOTE: For batches not equal to either 96 or 48 samples samples, refer to Appendix Reagents Calculation Tables. Table 1 for batches of <48 samples and Table 2 for <96 (but >48)

Extraction Buffer

Prepare the buffer just before extraction in a 100mL sterile glass bottle according to the table 2. Use one aliquot of 20mg/mL Proteinase K (1.5mL) for a 48 sample run and two aliquots of Proteinase K for a 96 sample run. Remove aliquot/s of Proteinase K from the freezer, defrost, vortex and centrifuge before use. Ensure that the 20% (v/v) SDS is completely dissolved (clear) in the stock solution before making the extraction buffer. If not dissolved invert the container a few times and leave longer at room temperature.

Lysis Buffer with DTT

Lysis buffer is supplied with the kit. Lysis buffer with DTT is prepared at the start of each run. Remove the DTT from the freezer, defrost, vortex and centrifuge before use. Into a sterilised glass bottle, add 1.3mL of DTT to 130ml of Lysis buffer for 96 samples. If 48 samples are to be run, use 660µl of DTT to 66ml of Lysis buffer, again, made up in a sterile glass bottle. Make up the Lysis buffer with DTT within the Laminar Flow and ensure that full PPE is worn, including face shield. Warning: Lysis Buffer and DTT are toxic.

DNA IQ™ Resin

DNA IQ™ Resin is supplied with the kit. The resin is prepared at the start of each run in a 10mL sterile tube. Ensure the resin is properly mixed by vortexing before pipetting. Look for calculations in table 2 for the correct volumes of resin and lysis buffer (with DTT). In the 10mL tube, mix by inversion before adding to column 4 in the 12-channel reagent plate.

1X Wash buffer

2X Wash buffer is supplied with the kit. Once a new kit has been opened, add 35mL of ***AnalR*** Ethanol and 35mL of Isopropyl alcohol to the 2X wash buffer. Once the reagents have been added, label the lid and side of the bottle with "1X Wash buffer," initial and date.

4.2 Equipment

The following equipment (Table 3) and consumables (Table 4) are required for the DNA IQ extraction.

Table 3. Equipment used and location.

Equipment	Asset No.	Location
STORstar (B system)	10238493	6122
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (Ext A Platform)	10076438	6127
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (Ext B Platform)	10076437	6127
DPC Shaker (Ext A Platform)	N/A	6127
DPC Shaker (Ext B Platform)	N/A	6127
Automated Temperature Controller, Heat Block tiles and heat block adapters (Ext A Platform)	N/A	6127
Automated Temperature Controller, Heat Block tiles and heat block adapters (Ext B Platform)	N/A	6127
Eppendorf 5804 centrifuge	10238416	6127
Vortex	30087015	6127
Fridge	30433424	6127
Micro centrifuge	None	6127
Decapper	None	6127

Table 4. Consumables used for extraction

Consumables	Location
175uL Clear Non-Conductive Filter Tips – Pre-sterilized	6127
MBP Pure 1000uL Tips – Pre-Sterilized	6127
SlicPrep™ 96 device plate	6122
Axygen 2mL Deep Well storage plate	6127
12 Channel plate	6127
Nunc tubes	6120
Nunc Caps	6127
Sterile 50mL Falcon tubes	6122
Sterile 10mL tubes	6122
Autoclaved 100mL glass bottles	6122
Autoclaved 250mL glass bottles	6122
Aluminium sealing film	6127

5 SAFETY

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

The automated platforms, labware and associated equipment should be wiped with 5% TriGene™ followed by 70% ethanol before and after use.

While the MPII is running a procedure, refrain from placing any part of the body inside the cabinet. Either pause the program or use the emergency STOP button located at the front of the instrument before touching anything within the cabinet or on the deck surface.

Warning: Tris base, EDTA, SDS and Lysis Buffer are irritants. DTT is a reducing agent that destroys disulphide bonds. Chaotropic reagents such as guanidinium thiocyanate (GuSCN) are toxic. Do not breathe alcohol fumes. Handle carefully and wear appropriate PPE.

Lysis Buffer contains guanidinium thiocyanate which can be harmful if inhaled, swallowed or comes in contact with skin. Any left over Lysis Buffer with DTT is to be disposed of in a brown Winchester bottle in the fume hood. Never dispose down the sink. If spillage occurs, absorb with liquid-binding material (such as sand, kitty litter, etc) and dispose in a biohazard bin. Again, handle carefully and wear appropriate PPE, including face shield.

6 SAMPLING AND SAMPLE PREPARATION

Samples waiting to be extracted are stored in freezers as described in Table 5.

Table 5. Sample storage locations.

Sample type	Storage Device	Storage Location
Urgent/High/Medium Priority Samples	Freezer	6117-2
Medium Priority Samples	Walk in Freezer or Freezer in 6117	6109 or 6117-5
Low Priority Samples	Walk in Freezer or Freezer in 6117	6109 or 6117-5

QC samples

All extraction batches require two controls to be registered. These controls, UR numbers and positions are listed in Table 6.

Table 6. Extraction Quality Controls

QC Name	UR Number	Description
Negative Control	FBOT33	Negative Extraction control – Empty well
Positive Control	FBOT35	Positive extraction control – Known Donor dried blood swab

Registration of QC

1. Log into the **AUSLAB Main Menu**.
2. Select **1. Request Registration**.
3. Select **2. Full Reception Entry**.
4. Scan in barcode of control.
5. Enter the UR number as per Table 4 and press **[Enter]**.
6. Enter the appropriate Specimen type (e.g. Blood for blood control).
7. Request a **9PLEX** test, when prompted to enter the processing comment, enter **EXTP** (Positive extraction control) or **EXTN** (Negative extraction control).
8. Enter **LAB** in the Billing code field.
9. Press **[F7]** ***Save*** to save the Billing details.
10. Press **[F4]** ***Save twice*** to save the registration details.

N.B Quality controls should not have a DNA priority allocated at time of registration to ensure they are included in the top positions of a batch

Create the Extraction Batch

1. Log into the **AUSLAB Main Menu**.
2. Select **5. Workflow management**.
3. Select **1. DNA workflow table**.
4. Highlight the appropriate Extraction batch type and press **[F5]** ***Batch Allocation***.
5. Press **[F6]** ***Create batch***.
6. Press **[F8]** ***Print menu***.
7. Press **[F6]** ***Print Batch label***. (print 7)
8. Press **[F7]** ***Print Sample Label***. (print 3 sets)
9. Press **[F8]** ***Print Worksheet***. (print 2)
10. Press **[SF5]** ***Main menu***.
11. Press **[SF11]** ***Print***.
12. Press **[SF6]** ***Accept batch***.
13. Press **[Pause/Break]** to exit to the **Main Menu**.
14. Obtain worksheets **(FBLASER3)** and labels **(FBLABEL13-16)** from the Analytical Section printing bench **(Room 6117)**.

Locating Samples

To locate samples refer to "*Analytical Sample Storage*" (QIS 24255).

Checking Samples

Check that appropriately sized portions of sample (eg swab, fabric, cigarette butts) have been submitted. If samples are not sized correctly they are to be sub-sampled please refer to *"Examination of Items"* (QIS 17142)
Label 1.5mL tubes removed from inside the original 5mL tube with sample labels if required. Label empty Nunc tubes ready for sequence checking.

Sequence Check the Sample substrates and Nunc Bank-It™ tubes

To sequence check sample substrates and storage tubes please refer to method "*Procedure for the Use of the STORstar unit for automated sequence checking*" (QIS 24256).

ENSURE the Slicprep™ 96 device is labelled, with the AUSLAB Batch ID label on the left side of the plate and the barcode on the right hand side of the plate.

ENSURE the Nunc tube rack is labelled with the AUSLAB Batch ID and barcode on the front of the plate.

7 PROCEDURE

Automated setup of DNA IQ extractions in 96-deep well format is performed using the MultiPROBE® II PLUS HT EX EP-A and EP- B platforms located in Room 6127.

Refer to "Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform" (QIS 23939) for instructions on the use and maintenance of the MultiPROBE® II PLUS HT EX platforms.

Summary of DNA IQ EXTRACTION winprep program (v 1.3)

1. ***Lysis of the biological material on solid support:*** Add prepared Extraction Buffer (500µL) to Slicprep plate wells. Cover the Slicprep plate & Spin baskets with Aluminium seal and incubate 45 min @ 37 °C. (this occurs at steps 8-12 of the protocol)

2. ***Remove the Slicprep plate & Spin baskets:*** add the collar and centrifuge for 2 min. Remove the collar and discard it. Remove the Spin baskets part and keep it in a clean container. Return the Slicprep plate to the deck. (this occurs at step 14 of the protocol)

3. ***Binding of paramagnetic resin to DNA and further Lysis:*** add Resin solution (50µL) and Lysis Buffer (957µL). Automated mixing and shaking @ room temperature for 5 min. (this occurs at steps 17-22 of the protocol)

4. ***Removing lysis reagents:*** Slicprep plate is moved to the PKI Magnet to separate beads. Removing of supernatant (1600µL) without disturbing resin, dispense this solution in the storage plate. (this occurs at steps 25-27 of the protocol)

5. ***Washing of the resin-DNA complex:*** To remove any inhibitors in solution.
The first wash is with Lysis buffer (125µL), shaking @ room temperature for 1 min. The plate is moved to the PKI Magnet and the supernatant is removed into the storage plate.

The next three washes are with 1X Wash buffer (100µL), shaking @ room temperature for 1 min. During each wash cycle, the plate is moved to the PKI Magnet and the supernatant is discarded. (this occurs at steps 30-68 of the protocol)

6. ***Removing any excess of 1X Wash buffer:*** air dry @ room temperature for 5 min. (this occurs at step 69 of the protocol)

7. ***Elution of DNA from the Resin-DNA complex:*** Add Elution buffer (60µL) and incubate @65 °C for 6 minutes (3 min no shaking and 3 min shaking). The plate is moved to the PKI Magnet. The eluted solution (supernatant) is removed to the NUNC tubes. Step 7 is repeated twice. (this occurs at steps 71-92 of the protocol)

8. ***Flushing of capillaries:*** The capillaries are washed with Amphyl and nanopure water.

Preparation of Reagents prior to extraction

1. Defrost Prot K and DTT

2. Refer to table 2 for reagent volumes to make up the required amount of Extraction Buffer, Lysis buffer (with DTT) and Resin solution. Also measure the required amount of 1X Wash buffer.

3. Record the Lot numbers of all the reagents used onto the AUSLAB worksheet.

Setting up the EP-A or EP-B MPIIs

These steps are to be carried out in the Automated extraction Room (Room 6127)

4. Turn on the instrument PC.

Figure 1 The WinPrep® icon.

5. Log onto the network using the **Robotics** login.

6. Double click the WinPrep® icon on the computer desktop (Figure 1).

7. Log onto the WinPrep® software by entering your username and password, then press **"Enter"**.

8. Ensure the **System Liquid Bottle Is FULL** before every run and perform a Flush/Wash.

9. Ensure that the daily/weekly start-up has been performed before running any program. If WinPrep® has been closed or been idle for a long period of time initialise the MP II platform as described in QIS 23939.

10. Open the Extraction setup MP II test file in WinPrep® by selecting:
    - **File**
    - **Open**, navigate to **C:\PACKARD\MULTIPROBE\BIN\QHSS PROTOCOLS**
    - Select **"DNA IQ Extraction_Ver1.3.mpt."**
    - Click the **"Open"** button

11. Check the whole program there are no bold fonts (e.g. plates may lose gripability and the program will not run). See the line manager.

12. Decontaminate the required labware by wiping with 5% TriGene followed by 70% ethanol before placing the labware on to the designated grid positions as outlined in the virtual deck view within WinPrep® (Figure 2).

- The white WALLAC Isoplates (catalogue #1450-514) that are used to support the SlicPrep™ 96 device plate must be placed into positions **E13, D16** and **C19**.

- Ensure that the PKI Magnet at **F16** is stable in its insert. This is critical so that beads and DNA are not lost during pipetting.

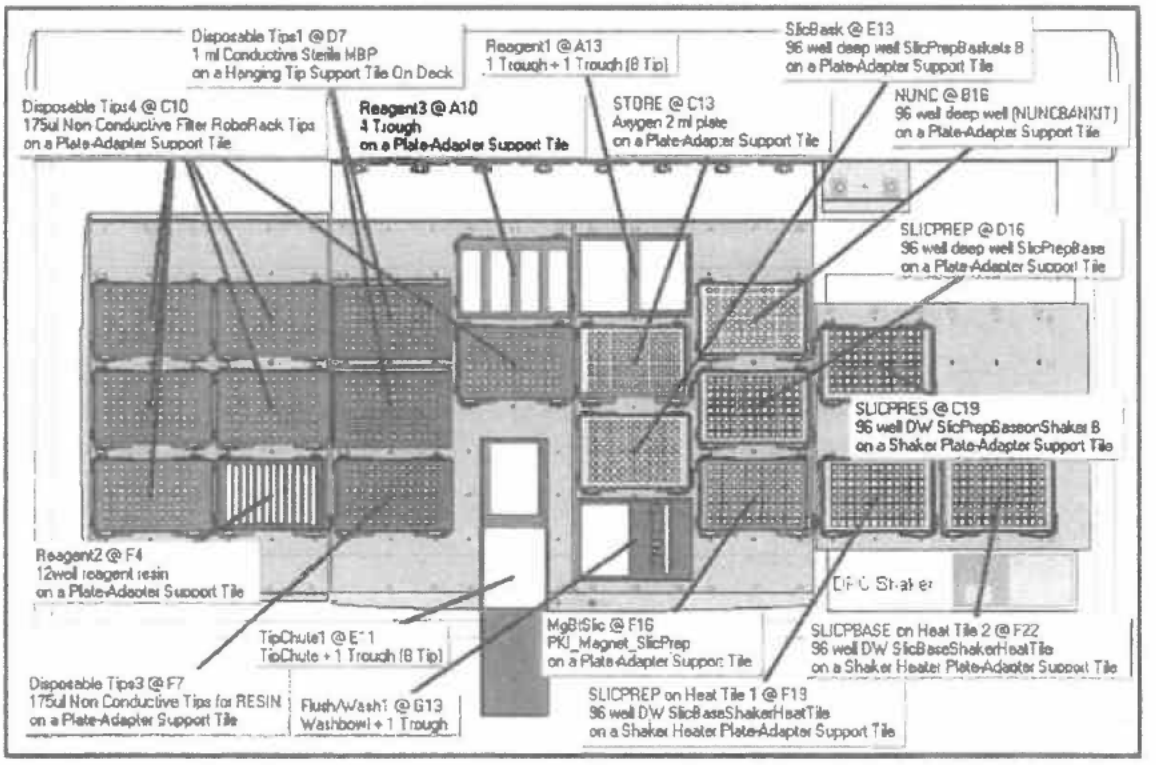


Figure 2. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

13. Ensure that the DPC Shaker and Heater Controller Box are switched on.
    For EP-A: Tile 3 should be at F19 (50°C), Tile 1 at F22 (85°C).
    For EP-B: Tile 1 should be at F19 (50°C), Tile 2 at F22 (85°C).
    Note: Press the start/stop button twice at the front of the DPC Shaker to ensure that it displays zero on the screen.

14. To the Amphyl wash station at **A10**, add fresh 1% Amphyl to the trough on the far left hand side, 0.2% diluted Amphyl to the middle left and place an empty reagent tough in the middle right position of the station. The nanopure water goes to position **G13** into a 160mL trough in the Flush-Wash station.

15. Pour the required amounts of Extraction Buffer and Lysis Buffer into the labelled 150mL reagent troughs. Place Lysis Buffer on the left hand side and the Extraction buffer on the right hand side of the 2 trough holder located in position **A13**.
    Note: Ensure that full PPE is worn, including face shield when handling these reagents

16. **Nunc tube rack**: Check that is the same Auslab batch number as the platemap to be run. Ensure that the barcode of the first (A1) and last tube (H12) are the respective ones. Attach batch label and batch barcode label to front side of rack. Add B1-Lite

generated "**NUNC**" barcode to the right side of the nunc tube rack. Then place nunc rack into position **B16**

17. On an Axygen 2ml deep well Storage plate, label the left side of the plate with both the Batch ID and barcode. Label the right side of the plate with a B1-Lite generated "**STORE**" barcode. Then place in position **C13**.

18. **Slicprep™ 96 device**: Gently remove septa mat from Slicprep™ 96 device and check that substrates are at the bottom of the Spin baskets, if not push them down with a sterile disposable tip and place the Slicprep™ 96 device into position **E13**.

19. In I drive from Extraction folder open the required plate map. Check that the plate map is complete. If there are blanks (samples that have been removed), make all volumes to 0. If it is not a full batch, delete the rows of blank samples at the end of the platemap, then save the changes. Copy the plate map to the following file path: **C:\PACKARD\EXT PLATE MAPS**

20. After ensuring that all the necessary labware has been positioned in the correct grid position as displayed within WinPrep®, click the "**EXECUTE TEST**" button. While the test is loading, record all run information in the Run Log book.

21. Click "**Reset Tip Boxes**" and ensure that the number of tips displayed in this window match those as available on the deck platform. Fill new tips if necessary. Click "**Close**" to accept the tip count, followed by clicking "**Next**"

22. Select the correct platemap by browsing to **C:\PACKARD\EXT PLATE MAPS**. Ensure that the platemap selected matches the batch ID affixed to the 96-well Slicprep™ 96 device in position **D16**. Once this has been done, click "**Start**", to continue.

23. After the barcodes have been read, a user prompt will appear as a reminder to:
"**Ensure**
1. Shaker and heat box are on.
2. Deck has been populated correctly.
3. The Lysis buffer is on the left side and Extraction buffer is on the right at A13."
Click "**OK**" to continue.

24. Once the extraction buffer has been added to the plate, a message will appear waiting for the heating tile to reach 50°C (real temp 37°C). When current temperature reaches 50°C click "**Continue**".

25. The next prompt that appears will request the following:
"Cover Slicprep with the Aluminium sealing film, then place in position F19. Press "OK."

26. After shaking, a User Prompt will appear with the following directions:
"Remove plate, add white plastic collar and centrifuge 5mins at 3021rpm, then in the cabinet, remove the spin basket part and place it in the empty 1 ml tip container."
Place the Slicprep™ 96 device into the plate centrifuge and ensure the correct balance plate is used. Once the plate has been centrifuged, carry the plate to the hood and remove the basket of the Slicprep™ 96 device, storing the basket in an empty 1mL tip box, discard the Collar. Complete the step by clicking "**OK**".
27. Add the Resin to the 12 Channel plate. Pipette mix thoroughly. Then dispense 50uL of resin into each well of the Slicprep™ 96 device.

28. Place the 12 channel plate into position F4 then add the Elution buffer to the plate by splitting the amount of elution buffer in half between channels 11 and 12.

29. Place the Wash buffer in the far right hand side of the 4 well reagent trough holder (Amphyl wash station at **A10**)

30. The next User prompt will appear with the following directions:
"**Place the Silcprep in position D16. Ensure wash buffer has been added. Manually add 50uL of Resin. Ensure Elution Buffer has been added.**" Press "**OK**" when steps 23-25 have been performed.

31. Once the lysis and wash steps have been completed, a message will appear waiting for the heating tile to reach 85^{0}C (real temp 65^{0}C). **DO NOT PRESS CONTINUE** it will continue automatically when temperature has reached 85^{0}C.

32. After the first elution step when the plate is moved to the PKI Magnet, a User message will appear requesting:
"**Check Nunc tubes are uncapped at position B16**
Push down the Silcprep on the PKI Magnet then press OK."
Note: Allow the plate to cool slightly before pushing down on all four corners of the plate to lock it into place on the PKI Magnet.

33. After the second elution step, the above prompt will appear again. **Note:** Allow the plate to cool slightly before pushing down on all four corners of the plate to lock it into place on the PKI Magnet

34. Once the program is completed, a final User Message prompt appears asking to:
"**Remove all the plates starting with the Silcprep plate, place the Spin Basket into the Silcprep plate.**
Cover the Storage plate with the aluminium sealing film."
Recap the NUNC tubes
Once all plates are removed from the deck and sealed, place into small clipseal plastic bags. Click "**OK**" to proceed to the Amphyl wash step to decontaminate the system tubing.

Finalising the MP II run

35. Discard the 12-channel plate used for Resin and Elution Buffer in the biohazard waste bin.
36. Remove Lysis buffer with DTT (wear face shield) and dispose of left over reagent into the glass bottle used previously. Bring this bottle to the room 6122 and dispose in the brown Winchester bottle located in the fume hood.
37. Empty the contents of the Amphyl wash station into the sink and rinse the troughs with nanopure H_2O upon completion of the run.
38. Remove all labware from the deck and clean with 5% TriGene™ followed by 70% ethanol, and setup for the next run if necessary.
39. Remove the tip chute and funnel, rinse with warm tap water to help loosen and remove any residue inside the chute before cleaning with 5% TriGene and 70% ethanol. Hang the chute to dry on the rack beside the sink. If running another extraction straight after, replace the tip chute with another spare, clean chute.
40. Move the platemap to C:\PACKARD\EXT PLATE MAPS to the "**Completed Extractions**" folder.

Recording Reagent Details and other information in AUSLAB

41. To record reagent lot numbers, log into the **AUSLAB Main Menu.**
42. Select **5.Workflow Management.**
43. Select **2. DNA Batch Details.**
44. Scan in the Extraction Batch ID.
45. Press **[F6]** ***Reagents.***
46. Press **[SF8]** ***Audit.***
47. Press **[F5]** ***Insert Audit Entry,*** enter the lot number details, operator name and Extraction platform the batch was run on and press **[Enter].**

Importing the MP II log file into AUSLAB

48. To extract the MP II log file, click on the Microsoft Access icon in the WinPrep® main menu to open the MultiPROBE log database.
49. Click on the relevant run Test ID in the Test Selection box. In the Report/Query/Action Selection dropdown menu, select "Report: Test Summary (Sorted by Destination Rack ID)"
50. In the Output Selection dropdown menu, select "File". Save the output file as *.csv format to **C:\PACKARD\EXT PLATE MAPS\EXT LOGS** with the same name as the AUSLAB batch ID and click "**Apply**". (refer to figure 4. below)

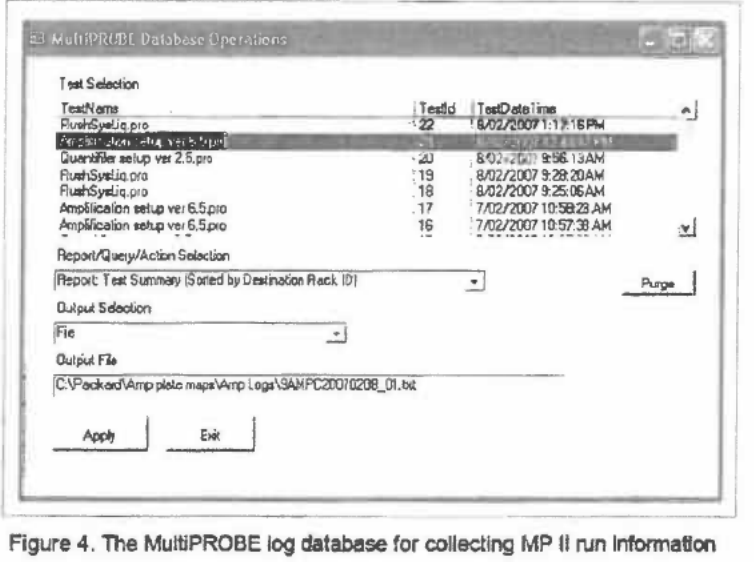


Figure 4. The MultiPROBE log database for collecting MP II run information

51. Open the log file and check for any errors that may have arisen during the Extraction process. Compare the listed errors to any that were encountered during the run. Report any critical errors to the line manager.
52. Copy the log file to **I:\EXTRACTION\EXT A MPII\LOGS** or **I:\ EXTRACTION\EXT B MPII\LOGS** for uploading to AUSLAB.
53. Log into the **AUSLAB Main Menu.**
54. Select **5.Workflow Management.**
55. Select **2. DNA Batch Details.**
56. Scan in the Extraction Batch ID barcode.
57. Press **[SF6]** ***Files.***
58. Press **[SF6]** ***Import Files.***
59. AUSLAB prompts "***Enter filename***"; enter the filename and extension and press **[Enter]**. (e.g. I:\EXTRACTION\EXT A MPII\Logs\CWIQEXT20071115_01.csv)
60. AUSLAB prompts "***Is this a result file Y/N?***" enter ***N*** and press **[Enter].**

61. Press **[Esc]**.

Importing Extraction "Results" Into AUSLAB

62. Log into the **AUSLAB Main Menu.**
63. Select **5. Workflow Management.**
64. Select **2. DNA Batch Details.**
65. Scan the Extraction batch ID barcode located on the worksheet.
66. Press **[SF6]** ***Files.***
67. Press **[SF6]** ***Import Files.***
68. AUSLAB prompts "***Enter filename***"; enter batch name and extension and press **[Enter]**.(e.g. CWIQEXT20071115_01.txt)
69. AUSLAB prompts "***Is this a results file y/n?***" enter "y" and press **[Enter]**.
70. The file will be imported into AUSLAB and appear in the DNA file table.
71. Highlight entry and press **[Enter]**, for access to the DNA results table.
72. Page down through the table and check that all sample results have been imported.
73. Press **[SF8]** ***Table Sort Order***, this sorts the table so any samples that have failed Autovalidation are sorted to the top of the table.
74. For all samples that have failed check the **Processing Comments**, by entering into the sample.
75. **a)** If processing comments state sample is to be sent to another batch type other than quant. Return the sample to the correct next batch type – e.g. microcon, nucleospin and pooling
    b) Press **[Esc]** to exit back to the DNA results table.
    c) Do not toggle accept.
76. **a)** If processing comment does not state next step for sample the sample will be processed as normal.
    b) Press **[Esc]** to exit back to the DNA results table.
    c) Highlight any entries to be changed and press **[SF7]** ***Toggle Accept***
77. Press **[F7]** ***Complete Batch***, all of the samples with a ***Yes*** in the Accept column will be transferred to the outstanding Quant Casework or Quant Reference lists.
78. File the Extraction worksheet into the relevant folder in Room 6117.

8 SAMPLE STORAGE

Please refer to "*Analytical Sample Storage*" (QIS 24255) for how to store the old original 5 mL sample tubes, the DNA extract NUNC tubes, Slicprep with Basket and Axygen store plates.

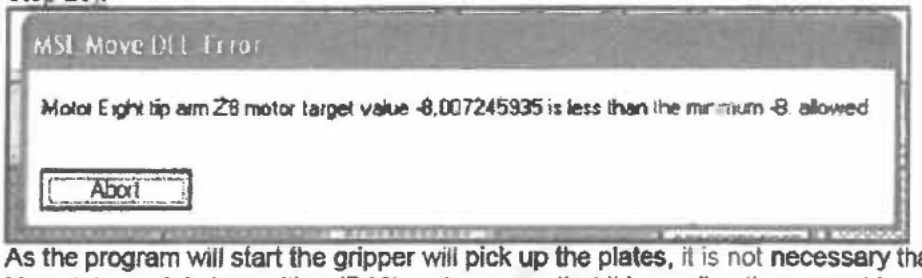
9 TROUBLESHOOTING

1. If the barcode reader is not reading the barcodes of the Nunc tube rack, or the Slicprep Plate or the Store plate, manually scan the appropriate barcodes.
2. When reading the Nunc tube rack barcode, if the Gripper is not picking up or placing the Nunc tube rack properly on the deck, just manually place the rack properly on the plate adapter support tile.
3. When reading the Store plate barcode, if the Gripper is not picking up or placing the Store plate properly on the deck, just manually place the plate properly on the plate adapter support tile.
4. When reading the Slicprep plate barcode if the Gripper is not picking up the plate properly :
    a. if the plate was not properly placed on the plate adapter support tile with the Wallac Isoplate, just manually place the plate properly.
    b. if the plate was properly placed on the plate adapter support tile with the Wallac Isoplate on it: it means that the gripper needs to be initialised. Abort the

run, Initialise the instrument and restart the run. If problem persists, shutdown the MPII and PC, restart and then initialise the whole instrument. Otherwise, contact your line manager.

c. Calibrate relevant labware using the SlicPrep Calibration plate. This has preset standardised positions that need to be the same on all labware where the Slicprep plate is being moved. The same plate is used on both extraction platforms A and B.
d. Check the calibrations against the run program DNAIQGripperTest.pro. This program moves the Slicprep across all the labware the gripper moves across. Start with the Slicprep at D16.

5. In steps 18 or 26, if a message is stating that the instrument is having a motor problem when picking up 1 mL tips and the only option is to Abort, abort, initialise and open program version **1.3a** (if the problem is in step 18) or version **1.3b** (if the problem is in step 26).



As the program will start the gripper will pick up the plates, it is not necessary that the Nunc tube rack is in position (B16), only ensure that it is reading the correct barcode. It is **important not** to place the Slicprep in the original position (E13) as the Slicprep plate has the Spin basket part removed (ie keep at D16), ensure it will scan the correct batch barcode. The Store plate remains in the original position. If the problem persists even after restarting, replace the rack of disposable 1 mL conductive sterile MBP tips for a new one.

6. If the program has already started step 18 and the previous message is appearing, you need to abort. Initialise the instrument and open program version **1.3a.**
Go to the plate map in C:\PACKARD\ EXT PLATE MAPS and change volume to 0 for all the samples that the Lysis Buffer have been dispensed (Column 6), ensure that the number of samples where the Lysis buffer was added is the same as the ones where the volume needs to be changed.
Save the changes and use this plate map for the restart of the program. Please read troubleshooting 5 for barcode reading of plates.
If the problem persists even after restarting, replace the rack of disposable 1 mL conductive sterile MBP tips for a new one.

7. If the program has already started step 26 and the previous message is appearing, you need to abort. Initialise the instrument and open program version **1.3b.**
Go to the plate map in C:\PACKARD\ EXT PLATE MAPS and change volume to 0 in all the samples that the Lysis Buffer and Ext buffer have been removed (Column 9), ensure that the number of samples where the solution was removed is the same that the ones the volume need to be changed.
Save the changes and use this plate map for the restart of the program. Please read troubleshooting 5 for barcode reading of plates.
If the problem persists even after restarting, replace the rack of disposable 1 mL conductive sterile MBP tips for a new one.

8. If a disposable tip gets stuck on the 8 tip arm during disposal of tips a user message will appear. Remove and press retry and then continue.

9. If the message:

has appeared, press OK and the program will be aborted automatically. Check that all the connections to the instrument (shaker, heater and computer) are properly plugged in. If everything is OK, you need to close WinPrep, shut down the instrument, shaker, heater and PC. After 2 min restart everything. Once Winprep has been opened, reinitialise the instrument and start the program (check version number according to which step the message has came up). Please read troubleshooting 5 for barcode reading of plates.

10 VALIDATION

- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 9. Automation: Report on the Evaluation of DNA Extraction Chemistries." June 2007.
- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 11: Report on the Validation of the Automated Extraction Chemistry Kit using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Casework Platform." 2007.
- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 13: Report on the Verification of the Automated Extraction Chemistry Kit using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Casework Platform." 2007.

11 QUALITY ASSURANCE/ACCEPTANCE CRITERIA

- A Negative control (also known as the reagent blank) is included with each batch of extractions. This reagent blank is processed as a normal sample through to completion. If any results are obtained from this sample, either at the quantitation step or the Genescan analysis step, then the possible source of the contamination is investigated. The samples extracted with this control are thoroughly checked and repeated if possible.
- Positive and negative controls are included in each extraction batch as per table 4.

12 REFERENCES

1. Boom, R., Sol, C.J.A., Salimans, M.M.M., Jansen, C.L., Wertheim-van Dillen, P.M.E., & van der Noordaa, J., Rapid and Simple Method for Purification of Nucleic Acids. J. Clin. Microbiol., 1990. 28: p. 495-503.
2. Chen., C.W.T.J., C.A., Recovery of DNA Segments from Agarose Gels. Anal Biochem., 1980. 101: p. 339-341.
3. Cowan, C., The DNA IQ™ System on the Tecan Freedom EVO® 100 Profiles in DNA. Profiles in DNA, 2006: p. 8-10.
4. Eminovic, I., Karamehić, J., Gavrankapetanović, F. & Helijić, B., A Simple method of DNA Extraction in Solving Difficult Criminal Cases. MEDARH, 2005. 59(1): p. 57-58.
5. Greenspoon, S.B., J., Robotic Extraction of mock sexual assult samples using the Biomek® 2000 and the DNA IQ™ System. Profiles in DNA, 2002. 5: p. 3-5.
6. Komonski, D.I., Marignani, A., Richard, M.L., Frappier, J.R.H., & Newman, J.C., Validation of the DNA IQ™ System for use in the DNA extraction of high volume forensic casework. Can.Soc.Forensic Sci.J., 2004. 37(2): p. 103-109.

7. Mandrekar, P., V., Flanagan, L., & Tereba, A., Forensic Extraction and Isolation of DNA Form Hair, Tissue and Bone. Profiles in DNA, 2002: p. 11.
8. Mandrekar, P.V., Kreneke, B. E., & Tereba, A., DNA IQ™: The Intelligent Way to Purify DNA. Profiles in DNA, 2001: p. 16.
9. Marko, M.A., Chipperfield, R., & Birnboim, H.C., A Procedure for the Large Scale Isolation of Highly purified Plasmid DNA using alkaline extraction and binding to glass powder. Anal. Biochem., 1982. 121: p. 382-387.
10. Melzak, K.A., Sherwood, C.S., Turner, R.F.B. & Haynest, C.A., Driving forces for DNA Adsorption to Silica in Percholorate Solutions. J. Colloid. Interface Sci., 1996. 181: p. 635-644.
11. PerkinElmer, Automated DNA IQ™ System for Mixed Casework Sample DNA Isolation. MultiPROBE II Liquid Handling - Forensic Workstation Application Guide, 2004: p. 1-25.
12. Promega, FAQs –DNA IQ™ System.
13. Promega, Protocols & Applications Guide. Chapter 9. rev. 7/06.
14. Promega, DNA IQ™ System -Small Casework Protocol. Promega Technical Bulletin #TB296 2006. Rev 4/06: p. 1-14.
15. Promega, DNA IQ™ System-Database Protocol. Promega Technical Bulletin #TB297, 2006. Rev 4/06: p. 1-14.
16. Promega, Tissue and hair Extraction Kit (for use with DNA IQ™) Protocol. Promega Technical Bulletin #TB307, 2006. Rev 5/06: p. 1-11.
17. Schiffner, L.A., Bajda, E. J., Prinz, M., Sebestyen, J., Shaler, R. & Caragine, T.A., Optimisation of a Simple, Automatable Extraction Method to Recover Sufficient DNA from Low Copy Number DNA Samples for Generation of Short Tandem Repeat Profiles. Croat Med J, 2005. 46(4): p. 578 -586.
18. Vandenberg, N., van Oorchot., R. A. H., & Mitchell, R. J., An evaluation of selected DNA extraction strategies for short tandem repeat typing. Electrophoresis, 1997. 18: p. 1624-1626.

13 STORAGE OF DOCUMENTS

All worksheets are stored in the Analytical area (Room 6117).

14 ASSOCIATED DOCUMENTS

QIS 17120 Operational Practices in the DNA Dedicated Laboratories
QIS 17142 Examination of Items
QIS 17171 Method for Chelex Extraction
QIS 17165 Receipt, Storage and Preparation of Chemicals, Reagents and Test Kits
QIS 23939 Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform
QIS 24255 Analytical Sample Storage
QIS 24256 Sequence Checking with the STORstar Instrument
QIS 24469 Batch functionality in AUSLAB

15 AMENDMENT HISTORY

Revision	Date	Author/s	Amendments
0	23 Oct 2007	B. Gallagher, T. Nurthen, C. Iannuzzi, V. Hlinka, G. Lundie, I Muharam.	First Issue
1	12 Dec 2007	M Harvey, C Iannuzzi, A McNevin	Reviewed and updated after initial training

16 APPENDIX

16.1 Reagents Calculation Tables

1. **Table for less than 48 samples** (note difference is in DNA IQ RESIN Solution)

Lysis-DTT buffer		**Volume (in mL)**
Lysis buffer	(Nx1.35)+0.75	
DTT (1M)	Lysis Buffer/100	
Extraction Buffer		
TNE buffer	Nx0.56	
Prot K (20 mg/L)	Nx0.03	
SDS (20 %)	Nx0.015	
DNA IQ RESIN Solution		
LYSIS buffer	0.054x(N+8)	
DNA IQ RESIN	0.009x(N+8)	
DNA IQ 1X Wash buffer	Nx0.36	
DNA IQ Elution buffer	Nx0.144	

N = number of samples on batch including positive and negative controls, round up to nearest multiple of 8)

2. **Table for more than 48 samples** (note difference is in DNA IQ RESIN Solution)

Lysis-DTT buffer		**Volume (in mL)**
Lysis buffer	(Nx1.35)+0.75	
DTT (1M)	Lysis Buffer/100	
Extraction Buffer		
TNE buffer	Nx0.56	
Prot K (20 mg/L)	Nx0.03	
SDS (20 %)	Nx0.015	
DNA IQ RESIN Solution		
LYSIS buffer	0.054x(N+16)	
DNA IQ RESIN	0.009x(N+16)	
DNA IQ 1X Wash buffer	Nx0.36	
DNA IQ Elution buffer	Nx0.144	

N = number of samples on batch including positive and negative controls, round up to nearest multiple of 8)

16.2 Manual method for extraction using DNA IQ™

16.2.1 Sampling and Sample Preparation

Samples waiting to be extracted are stored in freezers as described in Table 3.

Table 3. Sample storage locations.

Sample type	Storage Device	Storage Location
Urgent/High/Medium Priority Samples	Freezer	6117-2
Medium Priority Samples	Walk in Freezer	6109
Low Priority Samples	N/A	

QC samples

All extraction batches require two controls to be registered. These controls, UR numbers and positions are listed below in Table 4.

Table 4. Extraction Quality Controls

QC	UR Number	Extraction types
Neg Control	FBOT33	All
QC swab (blood)	FBOT35	Blood

1. Log into the **AUSLAB Main Menu**.
2. Select **1. Request Registration**.
3. Select **2. Full Reception Entry**.
4. Scan in barcode of control.
5. Enter the UR number as per Table 4 and press **[Enter]**.
6. Enter the appropriate Specimen type (e.g. Blood for blood extraction).
7. Request a **9PLEX** test, when prompted to enter the processing comment, enter **EXTP** (Positive extraction control) or **EXTN** (Negative extraction control).
8. Press **[F7]** Enter **LAB** in the Billing code field.
9. Press **[F4]** ***Save*** to save the Billing details.
10. Press **[F4]** ***Save*** to save the registration details.

N.B Quality controls should not have a DNA priority allocated at time of registration to ensure they are included in the top positions of a batch

Create the Extraction Batch

15. Log into the **AUSLAB Main Menu**.
16. Select **5. Workflow management**.
17. Select **1. DNA workflow table**.
18. Highlight the appropriate Extraction batch type and press **[F5]** ***Batch Allocation***.
19. Press **[F6]** ***Create batch***.
20. Press **[F8]** ***Print menu***.
21. Press **[F6]** ***Print Batch label***. (for the deep well plate)
22. Press **[F7]** ***Print Sample labels***. (print four sets of labels for all extractions)
23. Press **[F8]** ***Print Worksheet***.
24. Press **[SF5]** ***Main menu***.
25. Press **[SF11]** ***Print***.
26. Press **[SF6]** ***Accept batch***.
27. Press **[Pause/Break]** to exit to the **Main Menu**.
28. Obtain worksheets (FBLASER3) and labels (FBLABEL13-16) from the Analytical Section printing bench (Room 6117).

Locating Samples

Determine the storage locations of the required samples using the Batch Creation table/Batch details table print out. The columns ***Rack*** and ***Pos*** respectively identify the rack and the grid location where the sample has been stored. Remove the samples from the storage rack and place in an orange rack (12x8).

When all samples have been located:

1. Log into the **AUSLAB Main Menu**.
2. Select **2. Sample Processing**.
3. Select **7. Search Sample storage**.
4. Scan in the sample barcode that is affixed to the sample tube.
5. Press **[F6]** ***Remove Sample***.
6. AUSLAB prompts "***Are you sure you want to remove XXXX-XXXX? (Y/N)***", Enter Y and press **[Enter]**.
7. AUSLAB prompts "***Please enter remove comment***", No comment is required. Press **[Enter]**.
8. Press **[Scroll lock]** to clear.
9. Repeat steps 5 - 8 until all of the samples have been removed from their rack.

Sequence Check the tubes

1. Thaw samples at room temperature and label 1.5mL sample tubes.
2. Sequence check the tubes.
3. Add the sequence check details into AUSLAB.
4. Log into **AUSLAB Main Menu**.
5. Select **5. Workflow Management**.
6. Select **2. DNA Batch Details**.
7. Scan in the appropriate extraction batch ID barcode.
8. Press **[F5]** ***Sequence Check***.
9. Scan in the appropriate extraction batch ID barcode.
10. Press **[Pause/Break]** to exit to **Main Menu**.

16.2.2 Procedure

1. Enter the number of samples to be extracted (including controls) into the "No of Samples" column of the DNA IQ Reagent Calculations Table to calculate the volumes of each reagent to be measured out for the extraction. Aliquot regents into either 5ml tubes or 50ml Falcon tubes. **Note:** The volume of Lysis buffer calculated includes the volume used in the resin-lysis solution

2. Turn on the Eppendorf Thermo mixer and set the temperature to 37°C.

3. Remove 1.5ml tube and retain the 5mL tube. Prepare the Spin baskets by placing a DNA IQ™ Spin basket into a 1.5mL Microtube. Label the spin baskets (for every tube except Ext. control), 2mL SSI tubes and Nunc storage tubes (for every sample) with the sample barcodes. Have a second operator perform a sequence check of all tubes. This person must Press **[F5] Sequence Check** against the batch in AUSLAB

4. Using the Reagents table, prepare Extraction Buffer, Lysis buffer with DTT, & Resin Solution. Reagents need to be prepared fresh before each run.

5. Add 300 µL of Extraction buffer to each tube. Vortex each tube before incubating the tubes at 37°C on the Thermomixer at 1000 rpm for 45 minutes.

6. Remove the tubes from the Thermo mixer and add to a rack, increase the temperature on the Thermomixer to 65°C (for use in the Elution steps).

7. Transfer the substrate from the original tube to a DNA IQ™ Spin Basket using autoclaved twirling sticks. Centrifuge the Spin basket for 2 minutes at room temperature at its maximum speed. Once completed, remove the spin basket & retain in the original 5ml tube. Ensuring minimal contamination and transfer the extract to a labelled 2mL SSI sterile screw tube.

8. Transfer the remaining extract from the original tube to the corresponding 2mL tube. Vortex the tube gently.

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

10. Into a separate, clean 2mL SSI tube, aliquot the required amount of lysis buffer for the Resin solution. Ensure that the DNA IQ™ Resin solution has been thoroughly mixed by vortexing the resin bottle before adding the required resin volume to the lysis buffer. Pipette mix the solution to clear the tip of any lingering resin beads. Mix the solution by inverting the tube to prevent bubbles forming.

11. Add 50µL of DNA IQ™ Resin-Lysis solution into each tube. Invert the resin-lysis tube at regular intervals to keep the resin suspended within the solution to ensure uniform results.

12. Vortex each tube for 3 seconds at high speed before placing the tubes in the Multitubeshaker set at 1200 rpm to incubate at room temperature for 5 minutes.

13. Remove from the Multitubeshaker and vortex the tubes for 2 seconds at high speed before placing the tubes in the magnetic stand. Separation will occur instantly.

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

14. Carefully remove all of the solution in the tube into the original 1.5mL tube (i.e. the tube which originally contained the substrate), ensuring that the resin is not disturbed from its place on the side of the tube.

Note: If some resin is drawn up in tip, gently expel resin back into tube to allow re-separation.

15. Add 125µL of prepared Lysis Buffer and vortex for 2 seconds at high speed. Return the tubes to the magnetic stand and allow for separation to occur. Once separation has occurred again remove the Lysis Buffer into the original 1.5mL tube (i.e. the tube which originally contained the substrate).

16. Add 100µL of prepared 1X Wash Buffer and vortex for 2 seconds at high speed. Return tube to the magnetic stand and once separation has occurred remove and discard all Wash Buffer.

17. Repeat Step 16 another two times for a total of three washes. Ensure that all of the solution has been removed after the last wash.

18. Wipe down a Biohazard hood with bleach followed by ethanol. Uncap the tubes, placing the lids inside down onto a clean rediwipe in consecutive order and place the tubes in the same order into a clean plastic rack. Air-dry the resin in the hood for 15

minutes at Room temperature. Do not dry for more than 20 minutes, as this may inhibit removal of DNA during the elution phase.

19. Once the resin is dry, replace the lids on the corresponding tubes and remove from the hood. Add 50µl of Elution Buffer to each of the samples by carefully pipetting the liquid to the side of the tube, above the pellet. Do not mix.

20. With the lids on, incubate the tubes in the Thermomixer at 65°C for 3 minutes. After the three minutes are up, continue to incubate for a further 3 minutes shaking at 1100 rpm.

21. Remove the tubes and vortex for 2 seconds at high speed. Immediately place the tube in the magnetic stand while hot to ensure maximum DNA yield during elution.

22. Carefully transfer the DNA containing supernatant to the corresponding labelled Nunc tubes.

23. Remove tubes from the magnetic stand and add carefully another 50 µL of Elution Buffer above the magnetic pellet.

24. Repeat step 30 to 32. The final volume after this elution should be approximately of 95 µL of DNA solution.

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

16.2.3 Sample storage

1. Log into **AUSLAB Main Menu**.
2. Select **2. Sample Processing**.
3. Select **6. Sample Storage**.
4. Scan in Rack barcode.
5. Press **[SF5]** ***Fill Rack***.
6. Scan in sample barcode and place in rack in scanned position.
7. Repeat for all samples.
8. Press **[Esc]**.
9. Press **[Pause/Break]** to return to the **Main Menu**.
10. Select **3. Patient Enquiry**.
11. Scan in Rack barcode.
12. Tab down to the next blank ***DNA Batch No*** field and press **[F2]** ***Edit***.
13. Scan in the Batch ID of the samples stored.
14. Press **[Pause/Break]** to return to the **Main Menu**.

BG-2

CaSS | Forensic and Scientific Services
A CLINICAL AND STATEWIDE SERVICE

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.

2. Introduction

The MultiPROBE® II PLUS HT EX FORENSIC WORKSTATION platforms (PerkinElmer, Downers Grove, IL, USA) are equipped to perform automated DNA extractions, as they include a DPC shaker and individual heat controllers to enable on-board lysis and incubation steps. Currently in DNA Analysis, the MultiPROBE® platforms allow walk-away operation of PCR setup protocols for DNA quantitation and amplification.

The DNA IQ™ protocol has been verified or validated by various laboratories for use on the MultiPROBE® II PLUS platform. The laboratories that perform an automated DNA IQ™ protocol include PathWest (Western Australia), Forensic Science South Australia (South Australia) and Centre of Forensic Sciences in Toronto (Ontario). The MultiPROBE® II PLUS instrument comes pre-loaded with an automated DNA IQ™ protocol. Unlike the other laboratories, however, we did not validate the included protocol, but instead validated a manual DNA IQ™ protocol which was based on the CFS automated protocol (PerkinElmer, 2004), followed by verification of an automated protocol based on the validated manual method.

The verified automated DNA IQ™ protocol is identical to the validated manual protocol used in-house: there are no differences in reagents or volumes. The adopted DNA IQ™ protocol differs slightly, however, from the manufacturer's protocol, as it includes a lysis step using Extraction Buffer (10mM Tris, 1mM EDTA, 100mM NaCl, 20% w/v SDS) in the presence of Proteinase K, before incubating in the DNA IQ™ Lysis Buffer. Furthermore, the lysis incubation conditions were lowered from 70°C to 37°C in order to accommodate extraction of DNA from heat labile materials such as nylon and polyester. In addition, the automated protocol utilises the SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA) for simultaneous processing of samples in a 96-well format.

CaSS | Forensic and Scientific Services

3. Aim

To verify an automated DNA IQ™ protocol for use on the MultiPROBE® II PLUS HT EX platforms to allow extraction of DNA from various sample types.

4. Equipment and Materials

- MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (PerkinElmer, Downers Grove, IL, USA)
- Gravimetric Performance Evaluation Option with Mettler SAG285/L balance (Mettler-Toledo, Greifensee, Switzerland)
- DNA IQ™ System (Promega Corp., Madison, WI, USA)
- Extraction Buffer (10mM Tris, 1mM EDTA, 100mM NaCl, 20% SDS)
- SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA)
- Nunc™ Bank-It tubes (Nunc A/S, Roskilde, Denmark)
- 175µL non-conductive sterile filter RoboRack tips (PerkinElmer, Downers Grove, IL, USA)
- 1000µL Conductive sterile filter Robotix tips (Molecular BioProducts, San Diego, CA, USA)
- ABI Prism® 7000 SDS (Applied Biosystems, Foster City, CA, USA)
- Quantifiler™ Human DNA Quantification kits (Applied Biosystems, Foster City, CA, USA)
- AmpFℓSTR® Profiler Plus Amplification kits (Applied Biosystems, Foster City, CA, USA)
- GeneAmp® 9700 thermalcycers (Applied Biosystems, Foster City, CA, USA)
- ABI Prism® 96-well optical reaction plates (Applied Biosystems, Foster City, CA, USA)
- ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA)
- GeneScan™ 500 ROX™ Size Standard (Applied Biosystems, Foster City, CA, USA)
- Hi-Di™ Formamide (Applied Biosystems, Foster City, CA, USA)
- 3100 POP-4™ Polymer (Applied Biosystems, Foster City, CA, USA)
- Cytobrush® Plus Cell Collector (Cooper Surgical, Inc., Trumbull, CT, USA)
- 0.9% saline solution (Baxter Healthcare, Old Toongabbie, NSW, Australia)
- Stem digital tilting head thermometer
- For mock samples:
  - FTA™® Classic Card (Whatman Inc., Florham Park, NJ, USA)
  - Sterile cotton swabs (Medical Wire & Equipment, Corsham, Wiltshire, England)
  - Sterile rayon swabs (Copan Italia SPA, Brescia, Italy)

5. Methods

5.1 Gravimetric Evaluation of Pipetting Accuracy and Precision

Gravimetric analysis was performed by placing the SAG285/L balance on the platform deck and instructing the MP II to repeatedly pipette certain volumes of system liquid onto the balance pan. Readings were taken automatically by the software and compiled into a results table, which was then used to automatically generate an Excel-based results chart containing mean, %CV and %inaccuracy values. The mean values obtained were used to calculate R^2, slope and Y-intercept (offset) values to calibrate the system's pipetting.

Pipetting performance was assessed for various volumes using three different tips in order to calculate appropriate R^2, slope and Y-intercept (offset) values which were then added to the performance file. Values were calculated for both Blowout (single-liquid transfer) and Waste (multidispense) modes for the 1000µL conductive tips, and Blowout mode only for the 175µL non-conductive tips and fixed tips.

For the addition of resin, a specialised performance file was created based on the performance file for 175µL tips in blowout mode, except the "Blowout Volume" column values were set to 0 to allow pipetting performance that is similar to waste mode. Retesting was performed to confirm accurate and precise pipetting with these settings.

CaSS | Forensic and Scientific Services
A CLINICAL AND STATEWIDE SERVICE

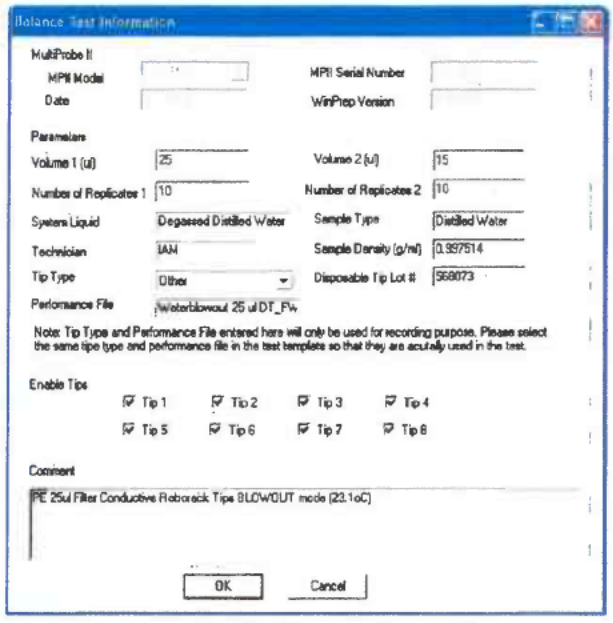


Figure { SEQ Figure * ARABIC }. The Balance Test Information Window .as present within the Balance Test DT program. All pipetting parameters are entered here and are subsequently transferred to the result output file.

All gravimetric testing was performed using the Balance Test DT test program within WinPrep®. Parameter values that needed to be entered into the Balance Test Information Window (Figure 1) included those as outlined in Table 1.

Table { SEQ Table * ARABIC }. Input values that are required for the various Balance Test Information parameters.

Parameter(s)	Value
Volume 1 and Volume 2	For 175µL tips: 175, 100, 50, 15µL For 1000µL tips: 1000, 700, 400, 100µL For fixed tips: 1000, 700, 400, 100µL
Number of Replicates	10
System Liquid	Degassed Nanopure Water
Sample Type	Nanopure Water
Technician	Initials of the operator performing the test
Sample Density (g/ml)	The density of water at environmental temperature*
Tip Type	Other
Disposable Tip Lot #	The lot number of the particular tips in use
Performance File	The appropriate Performance File for the tip (175µL, 1000µL or fixed tips) and pipetting mode (Blowout or Waste) in use
Enable Tips (checkboxes)	Select the actual tips (1 to 8) to be tested
Comments	Free text box to add additional information (eg. Tip type, mode, current environmental room temperature, etc).

* Water density values were obtained from http://www.simetric.co.uk/si_water.htm

Pipetting accuracy and precision were examined at four different volumes for each tip size: 175, 100, 50, 15µL for 175µL tips and 1000, 700, 400, 100µL for the 1000µL and fixed tips.

In order to calculate unbiased values for each set of volumes, the slope and offset values in the relevant Performance File were changed to the default 1 and 0 respectively prior to testing. The mean volumes that were pipetted by each tip (10 replicates per tip) at the four designated volumes were used to generate a standard curve. The slope and offset calculated from this curve was used to calibrate the relevant Performance File. The final Performance File settings were then tested at the highest and lowest volumes (as per Table 1) to confirm accurate and precise pipetting.

5.2 Blood Collection

Blood samples were collected from 2 staff donors (DJC/VKI) by a phlebotomist as per normal in three 4mL EDTA vials. Blood samples were stored at 4°C.

5.3 Cell Collection

Buccal cells were collected using a modified Cytobrush® protocol (Mulot *et al.*, 2005; Satia-Abouta *et al.*, 2002). The donor was instructed to brush the inside of one cheek for one minute using a Cytobrush®. Then, with another Cytobrush®, the other cheek was also sampled. Once each cheek was swabbed, the cells on the brush were suspended in 2mL of 0.9% saline solution. Buccal cell samples were stored at 4°C.

5.4 FTA cell Collection

Cells were collected from two staff donors (VKI/CJA) by using a "lolly-pop" swab to sample the inside of the donor's cheek for 15 seconds before pressing the swab onto the FTA™ paper to transfer the DNA. FTA™ cards were stored at room temperature.

5.5 Heater tile temperature verification

Heat tiles supplied with the MultiPROBE® II PLUS HT EX platforms were modified to accept the SlicPrep™ 96 Device. For testing, 1mL of nanopure water (at room temperature) was added to each well. The plate was then placed on a heater tile (controlled by the MP II heater controller) and allowed to reach temperature. The temperatures tested were 37°C and 65°C. Temperature readings for specific outer and inner wells (i.e. A1, A6, A12, D1, D6, D12, H1, H6, H12) were taken at regular intervals up to and including 45 minutes, using calibrated stem digital tilted head thermometer probes. The data were collated and means calculated to determine the distribution of heat over the tile.

5.6 Verification of automated DNA IQ™ Protocol

The automated DNA IQ™ protocol, based on the validated manual method (refer to Project 11), was programmed in WinPrep™ software. The final, optimised protocol was named "DNA IQ Extraction_Ver1.1.mpt". A screenshot of the Test Outline window for this protocol is depicted below in Figure 2. The deck layout is illustrated in Figure 3.

The automated DNA IQ™ protocol was designed to mimic the validated manual method, with minor modifications. Briefly, the changes include:

- Increasing the volume of Extraction Buffer to 500µL;
- A SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA) was used for sample lysis;
- Incubation steps and any shaking steps were performed on the integrated DPC shaker;

CaSS | Forensic and Scientific Services

A CLINICAL AND STATEWIDE SERVICE

- CRS toroid magnet (P/N 5083175) was used for isolating the DNA IQ™ resin.
- Instead of a single elution of 100µL, a double elution method (2 x 50µL) is used.

Reagents used in the automated protocol were as per the manual method.

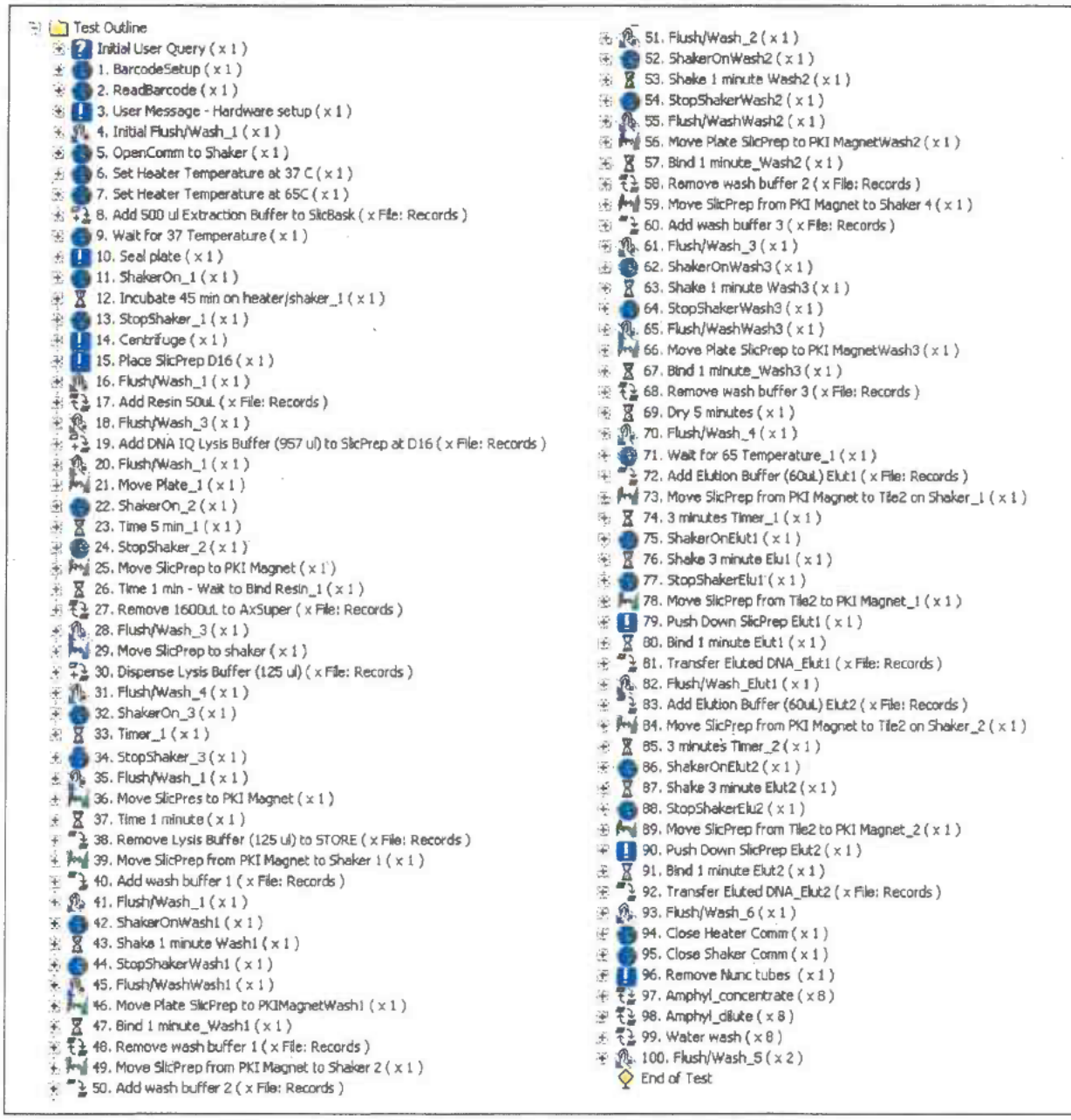


Figure 2. The Test Outline window displaying individual nodes within the DNA IQ Extraction_Ver1.1.mpt program test file.

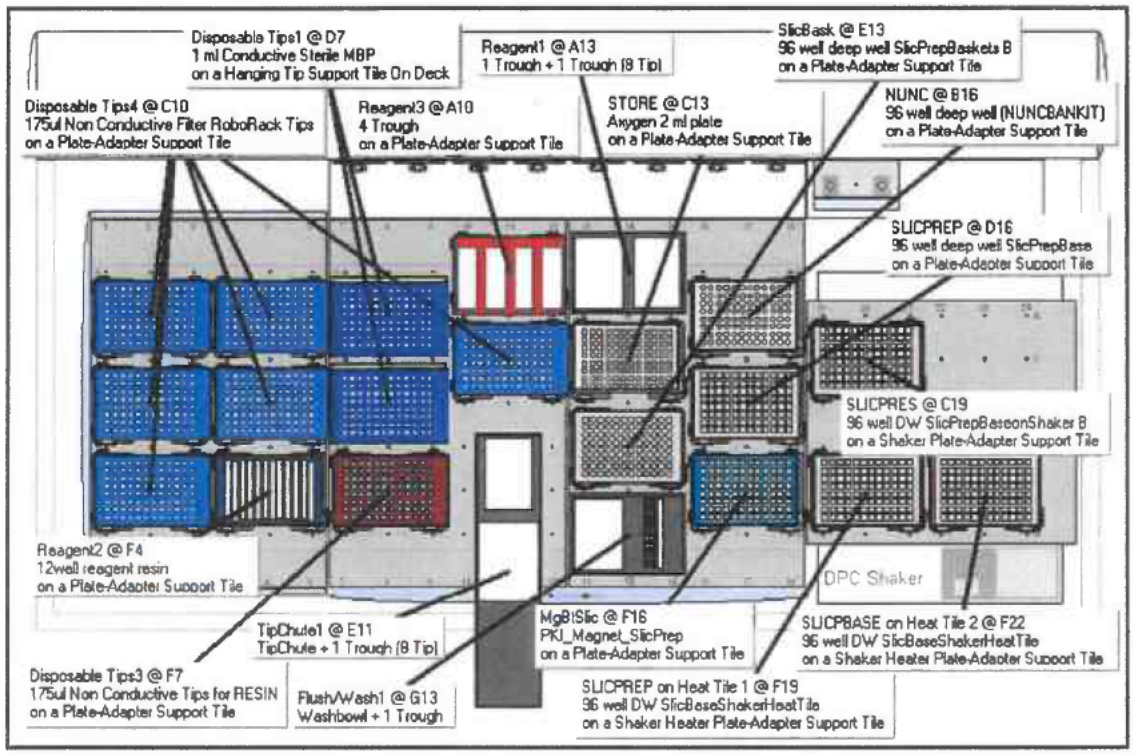
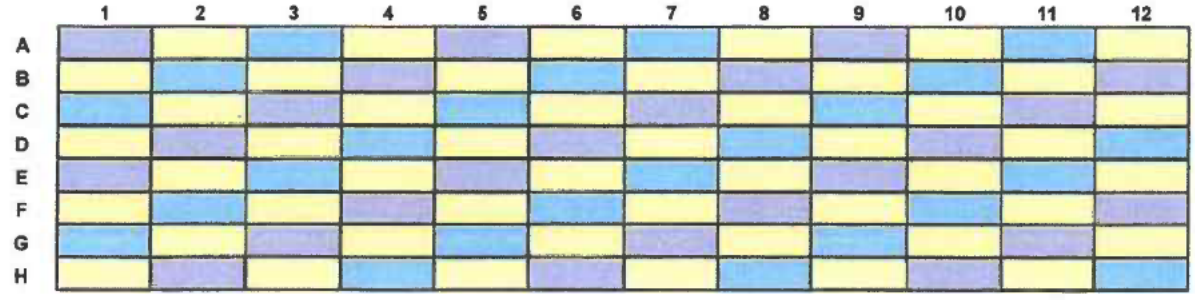


Figure 3. The deck layout for DNA IQ Extraction_Ver1.1.mpt, displaying the required labware on the platform deck.

The automated DNA IQ™ protocol was used to perform the following tests.

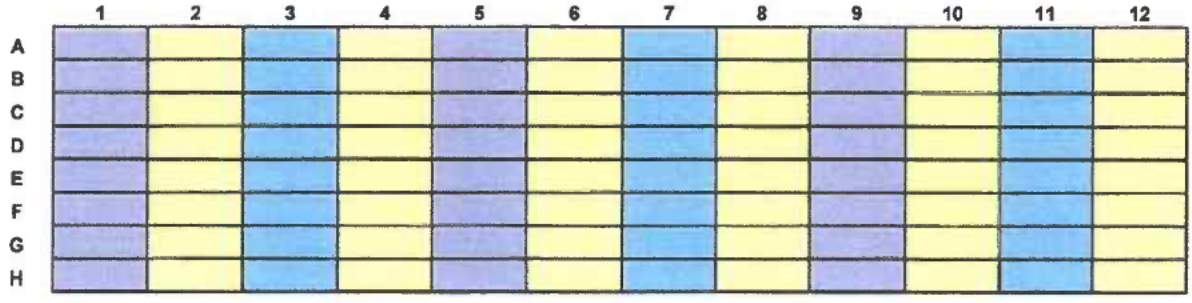
5.6.1. Contamination Check via Checkerboard and Zebra-stripe Patterns

Samples consisting of two 3.2mm FTA® discs (containing blood, buccal cells, or blank cards) were arranged in a checkerboard and zebra-stripe pattern (Figure 4) in SlicPrep™ plates using the BSD Duet 600 instrument (BSD Robotics, Brisbane, QLD, Australia) and extracted on the MultiPROBE® II PLUS HT EX platforms using the automated DNA IQ™ protocol. One checkerboard and one zebra-stripe plate was processed on each platform.



(a) Checkerboard Pattern

b) Zebra Stripe Pattern



Legend:

Blood FTA®
Blank FTA®
Buccal Cell FTA®

Figure 4. Checkerboard and zebra-stripe patterns utilised in the contamination check.

5.6.2. Comparisons with the manual DNA IQ™ method

Comparisons were made between results generated by the automated and manual methods to verify the performance of the automated DNA IQ™ protocol.

Verification samples consisted of different dilutions of blood and cells spotted in 30µL aliquots onto quartered cotton and rayon swabs. Four blood dilutions of neat, 1/10, 1/100 and 1/1000 and four cell dilutions of neat, 1/5.2, 1/52.2 and 1/522 were used to test the sensitivity of both the manual and automated methods. Dilutions were created using 0.9% saline solution for both sample types. Four replicates of each dilution were made up for each substrate and sample type.

The blood was collected using the same method as in 5.2. Four separate extractions were performed for the manual set based on the combination of sample type and swab type: Blood Rayon, Blood Cotton, Cell Rayon and Cell Cotton. For the automated verification, all sample types were extracted together after being transferred to a SlicPrep™ 96 Device to allow automated processing.

5.6.3. Resin volume

The performance of the automated DNA IQ™ protocol was assessed when either 7µL or 14µL of DNA IQ™ resin was used in the protocol to extract blood samples.

5.6.4. Modifying extraction volumes

The performance of the automated DNA IQ™ protocol was assessed for varying volumes of extraction buffer at 300, 350, 400, 450 and 500µL. In each case, the volume of DNA IQ™ Lysis Buffer was kept at 2x the volume of extraction buffer. Samples extracted were blood swabs, prepared as per 7?2.

5.6.5. Sensitivity of the automated DNA IQ™ protocol

The sensitivity of the automated DNA IQ™ protocol was assessed using dilutions of whole blood at neat, 1:10, 1:50, 1:100 and 1:1000.

6. Results and Discussion

6.1 Gravimetric Evaluation of Pipetting Accuracy and Precision

Pipetting on both automated platforms was assessed gravimetrically as per laboratory practice. Gravimetric results indicate that pipetting performance for five different pipetting behaviours using 500µL syringes on the instruments is accurate and precise to within the established threshold of ±5% (Table 2). The maximum CV at the maximum volume was 0.78%, whereas the maximum CV at the minimum volume was 1.1%. The CV for pipetting at lower volumes is expected to be slightly higher than the CV at higher volumes using 500µL syringes, because accuracy at small volumes is harder to achieve with larger syringe sizes. Nevertheless, pipetting on the extraction platforms is limited to a minimum of 50µL, which exhibited a CV of 0.36%.

Table 2. Gravimetric evaluation results for various performance files used on either MP II EXTN A or MP II EXTN B.

Performance File	Max. Vol. µL	Min. Vol. µL	Max. Vol. µL Mean	Max. Vol. %CV	Max. Vol. %Inac.	Min. Vol. µL Mean	Min. Vol. %CV	Min. Vol. %Inac.
EXTN A								
Water Blowout 175µL DT_FW _13112007RESIN.prf	50µL	N/A	49.98	0.36	0.0	N/A	N/A	N/A
Water Blowout 175µL DT_FW QHSS _13112007.prf	175µL	15µL	172.26	0.21	1.6	[redacted]	[redacted]	[redacted]
WaterWaste 1mL_FW_QHSS 12112007.prf	1000µL	100µL	999.11	0.24	0.1	99.22.	0.71	0.8
Water Blowout 1mL DT_QHSS _09112007.prf	1000µL	100µL	1001.02	0.27	0.1	100.65	0.63	0.7
Water Blowout Fixed Tips_08112007.prf	1000µL	100µL	995.97	0.31	0.4	99.6	0.71	0.4
EXTN B								
Water Blowout 175µL DT_FW_ 25102007RESIN.prf	50µL	N/A	50.12	0.36	0.2	N/A	N/A	N/A
Water Blowout 175µL DT_FW_ 25102007.prf	175µL	15µL	175.58	0.14	0.3	15.23	1.1	1.5
WaterWaste 1mLDT_FW_QHSS 24102007.prf	1000µL	100µL	1002.39	0.78	0.2	99.56	0.89	0.4
Water Blowout 1mL DT_QHSS 23102007.prf	1000µL	100µL	998.2	0.44	0.2	99.44	0.68	0.6

CaSS | Forensic and Scientific Services
A CLINICAL AND STATEWIDE SERVICE

Water Blowout Fixed Tips_FW 26102007.prf	1000µL	100µL	998.87	0.68	0.1	100.37	0.74	0.4

6.2 Heater tile temperature verification

Two heater tiles on each MP II platform was verified to reach either 37°C or 65°C, the optimum incubation temperatures for sample lysis and DNA elution respectively (using the DNA IQ™ kit). Each tile, upon completion of the verification process, could only be used for a specific temperature, and as such was labelled appropriately to ensure use of the correct tile for specific incubation steps (Table 3).

Table 3. Verified heater tiles for use in the automated DNA IQ™ protocol.

Extraction platform	Tile number	Heater Controller Setting	Average °C reached	Verified temperature	Incubation Step
EXTN A	3 (45W)	50°C	37°C	37°C	Sample Lysis
EXTN A	1 (45W)	85°C	[redacted]	65°C	DNA Elution
EXTN B	1 (45W)	50°C		37°C	Sample Lysis
EXTN B	2 (45W)	85°C	65°C	65°C	DNA Elution

A slight variation in the incubation temperature to achieve sample lysis is acceptable, because Proteinase K exhibits stable activity and broad specificity over a wide range of temperatures between 20-60°C, at which the serine protease still retains greater than 80% of its activity (Sweeney & Walker, 1993).

The efficiency of the elution step is dependent on heating the sample to 65°C in the presence of DNA IQ™ Elution Buffer (Huston, 2002). If the sample is not sufficiently heated, the extraction yield may be lower than expected. Two heater tiles were able to be verified for this crucial incubation step, with both tiles exhibiting minimal variation.

6.3 Contamination Check via Checkerboard and Zebra-stripe Patterns

Table 4 below lists the Extraction Batch ID's of the contamination checks.

Table 4. Extraction Batch ID's for the various contamination check plates that were processed on the MP II platforms using the automated DNA IQ™ protocol.

Type of plate	Extraction batch Id	Extraction Platform	Check passed
Checkerboard 1	VALB20070817_02	Extraction A	Invalidated
Checkerboard 2	VALB20070803_02	Extraction B	Yes
Zebra-Stripe 1	VALB20070803_03	Extraction A	Yes
Zebra-Stripe 2	VALB20070817_03	Extraction B	Yes
Checkerboard/Zebra	VALB20071022_01	Extraction A	Yes

Checkerboard 1

Position E3 (Sample Cells 6) was known to have been contaminated prior to the start of the extraction [redacted]. The result showed a mixed DNA profile, with contributing alleles originating from the expected wells (Table 5). In addition to this contamination event, eight of the designated blank samples (positions D3, A10, F1, H5, C4, E4, B7 and E6), two of the cell samples (A1 and B10) and two of the blood samples (F4

CaSS | Forensic and Scientific Services

A CLINICAL AND STATEWIDE SERVICE

and G7) all exhibited a partial DNA profile that was previously unknown (Table 5). This profile did not match any of the positive control samples present on the batch. The DNA profile was searched against the Staff Database and no matches were found. The source of this contaminating DNA profile could not be identified.

None of the other blank samples yielded any DNA profile. The rest of the cell and blood samples yielded the correct DNA profile. Although there is no evidence of well-to-well contamination, the unknown DNA profile obtained has invalidated this plate. A further checkerboard/Zebra-Stripe combination plate was performed to ensure...

Table 5. The DNA profile of the unknown contaminant that was observed in Checkboard-1.

Sample description	D3	vWA	FGA	Amel	D8	D21	D18	D5	D13	D7
Blk23-E6	14,17	14,17	22,24	X,Y	11,11	29,32.2	14,15	9,11	11,12	11,13
Blk25-B7	14,17	14,17	22,24	X,Y	11,11	29,32.2	14,15	9,11	11,12	
Blk15-E4	14,17	14,17	22,24	X,Y	11,11	29,32.2	14,15	9,11	11,12	11,13
Blk14-C4		14		X	11	32.2		9		
Blk20-H5	14,14	17,17	20,21	X,X	13,16	29,30	14,16	11,13	11,12	11,11
Blk3-F1	14	17		X	13	29,30	14		12	11
Blk10-D3	14,17	14		X,Y	11	29,32.2	14	9,11		11,13
Blk37-A10	14,17	14	22,24	X,Y	11	29	14	9,11		
Cells19-B10	14,17	14,17	20,21,22,24	X,Y	11,13,16	29,30,	14,15,16	11,15	11,12	11,11
Cells13-A1	14,17	14,17	20,21,22,24	X,Y	11,13,16	29,30,32,32.2,33	14,15,16	9,11,13	11,12	11,13
Blood14-G7	NR,17,18	NR,16	20,21	X,Y	NR,13,14	29,30,31,NR	NR,14	NR,12	10,10	10,NR,12
Blood8-F4	NR,17,18	NR,16,17	20,21,NR,24	X,Y	11,13,14	29,30,NR,NR	14,14	9,11,12	10,NR	10,NR,12
Cells 6-E3	14,17,18	16,17	20,21	X,Y	13,14,16	29,30,31	NR,14,16	11,12,13	10,11,12	10,11,12

Checkerboard 2

None of the blank samples yielded DNA profiles; all of the positive cell and positive blood samples yielded the correct DNA profile. Figure 5 illustrates the DNA quantitation results from this plate. DNA was not detected in any of the blank samples.

CaSS | Forensic and Scientific Services
A CLINICAL AND STATEWIDE SERVICE

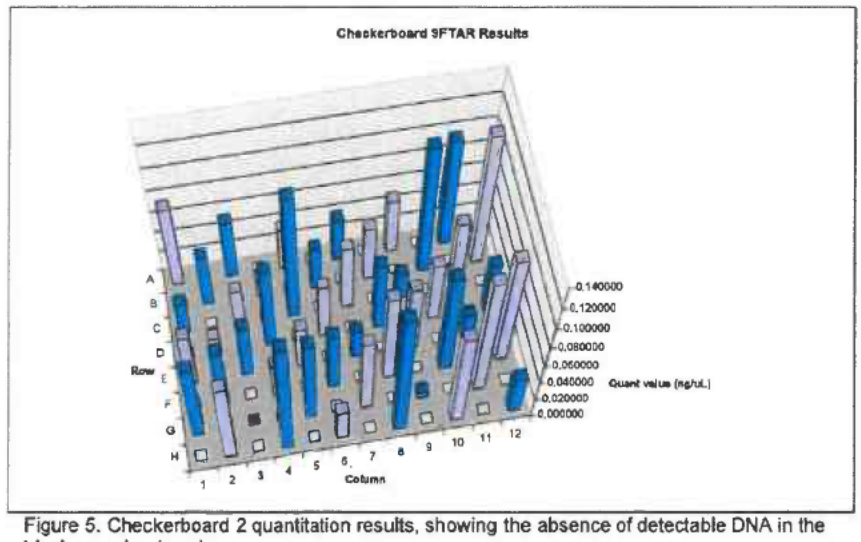


Figure 5. Checkerboard 2 quantitation results, showing the absence of detectable DNA in the blank samples (grey).

Zebra-Stripe 1

None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile. Figure 6 illustrates the absence of detectable DNA in the blank samples.

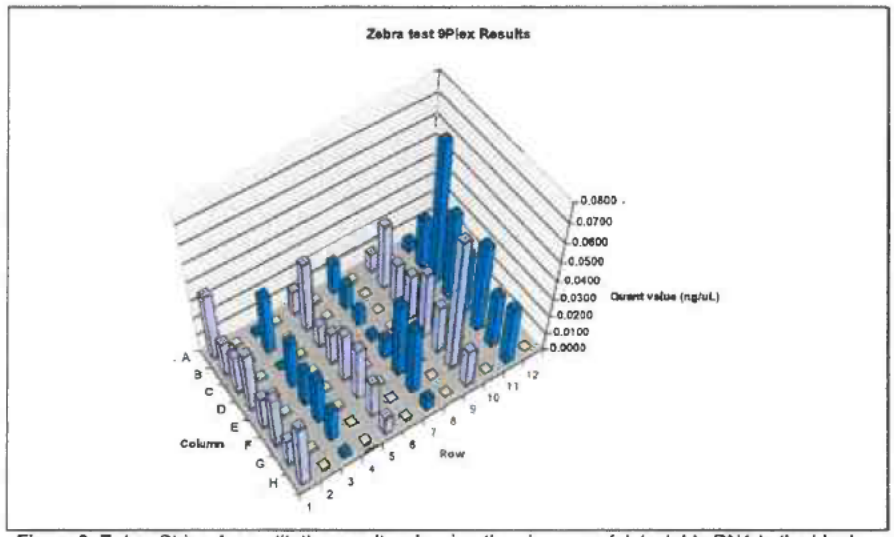


Figure 6. Zebra-Stripe 1 quantitation results, showing the absence of detectable DNA in the blank samples (grey).

Zebra-Stripe 2

None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile. Figure 7 shows the absence of detectable DNA in the blank samples.

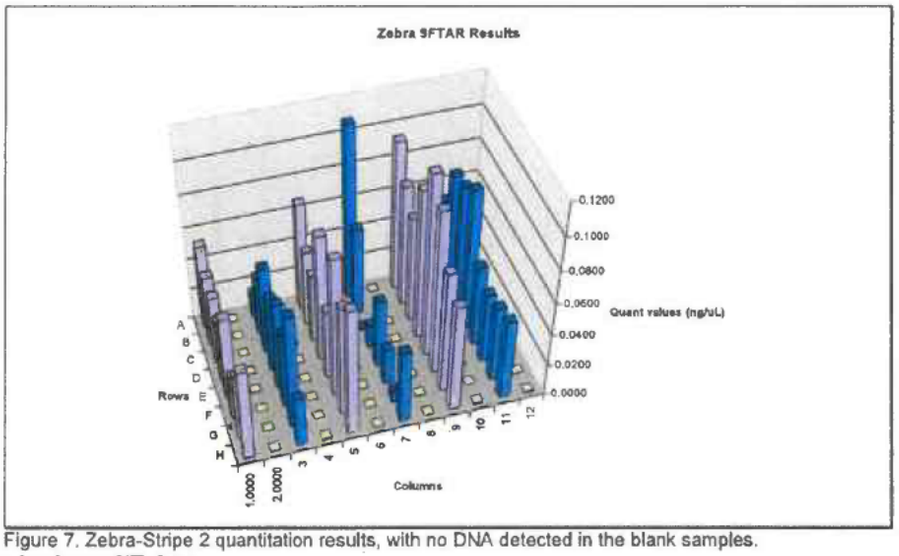


Figure 7. Zebra-Stripe 2 quantitation results, with no DNA detected in the blank samples.

Checkerboard/Zebra

None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile. DNA was undetected in the blank samples (Figure 8).

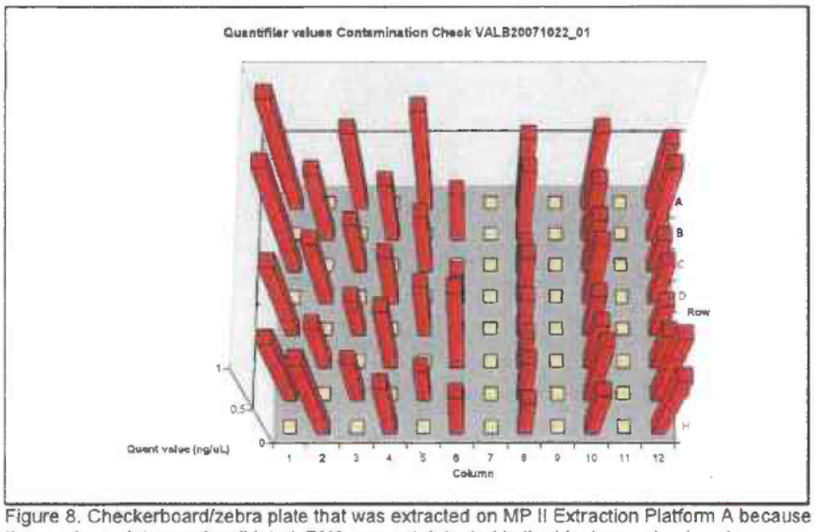


Figure 8. Checkerboard/zebra plate that was extracted on MP II Extraction Platform A because the previous plate was invalidated. DNA was not detected in the blank samples (grey).

6.4 Comparisons with the manual DNA IQ™ method

When dilutions of either blood or cells were applied on to 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. For blood samples on rayon

CaSS | Forensic and Scientific Services
A CLINICAL AND STATEWIDE SERVICE

swabs, the automated method generated yields that were on average around 8% (SD 8.45%) of the automated method. 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.

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). The trend is repeated for blood on cotton swabs (Figure 10). For cell samples on either rayon or cotton swabs, the automated method was found to be more sensitive as evidenced by detection of DNA at the 1/522 dilutions (Figure 11 and 12).

Cell clumping may have occurred with the cell dilutions, therefore causing inaccurate dilutions as can be observed in the ratios between each dilution.

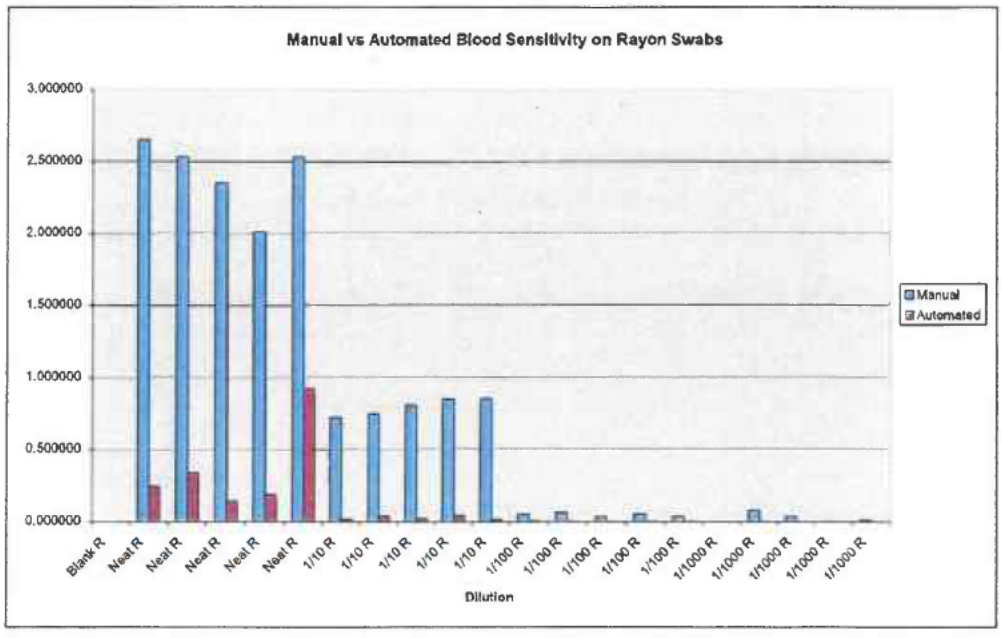


Figure 9. Comparison of sensitivity between the manual and automated DNA IQ™ methods for blood samples on rayon swabs.

CaSS | Forensic and Scientific Services

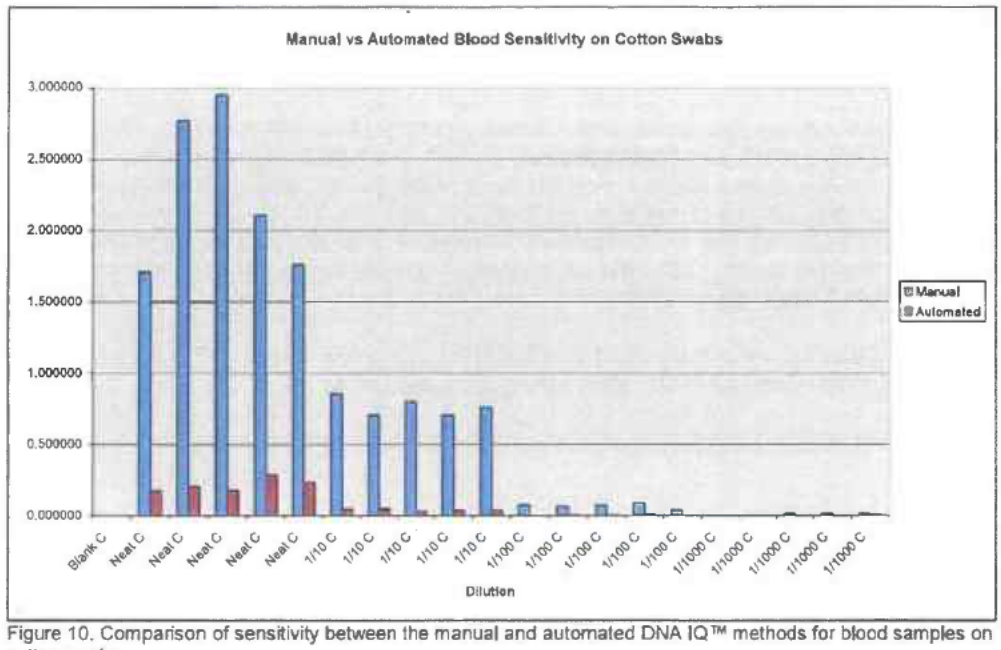


Figure 10. Comparison of sensitivity between the manual and automated DNA IQ™ methods for blood samples on cotton swabs.

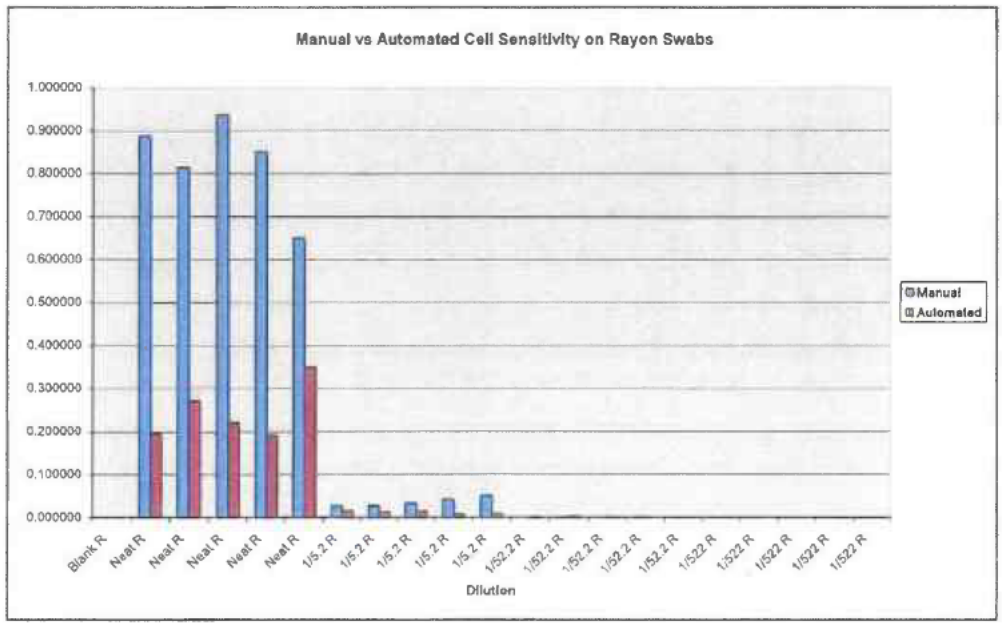


Figure 11. Comparison of sensitivity between the manual and automated DNA IQ™ methods for cells samples on rayon swabs.

CaSS | Forensic and Scientific Services

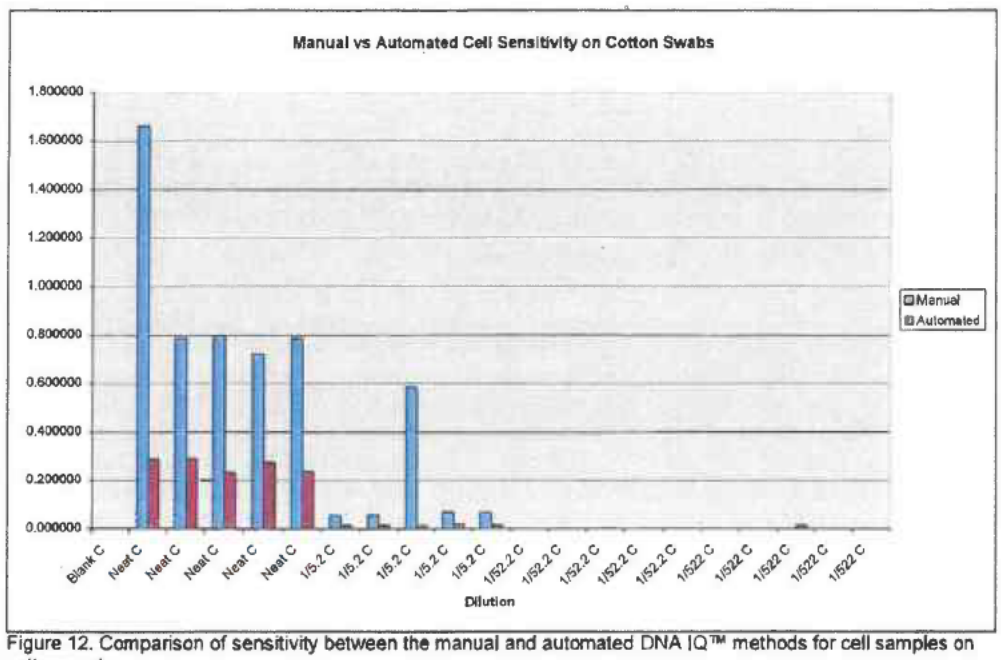


Figure 12. Comparison of sensitivity between the manual and automated DNA IQ™ methods for cell samples on cotton swabs.

6.5 Investigating resin volume

Promega recommends the use of 7µL of DNA IQ™ resin with their protocol. We investigated the performance of the protocol with double the amount of resin (14µL) in order to assess any benefits that may be gained in terms of the resulting yield and quality of the STR profile.

It was observed that doubling the resin resulted in a proportional doubling of the yield. On average, doubling the resin increased the yield by an additional 77.28% (*n*=4). The average yield from an extraction using 7µL of resin was 64.725ng (SD 32.21ng, *n*=4), whereas 14µL resin generated 114.75ng (SD 10.72ng, *n*=4) (Table 6). At the higher resin concentration, the amount of DNA isolated appears to be capped at around 100ng, indicating no change in the ability of the reaction to isolate more DNA due to saturation of resin.

Table 6. Comparison of the effects of doubling the amount of recommended DNA IQ™ resin.

Sample ID	Resin volume	[DNA] ng/µL	Reportable alleles
33383-4216	7µL	0.701	18/18
33383-4225		1.070	18/18
33383-4239		0.319	18/18
33383-4248		0.499	18/18
33383-4252	14µL	1.140	18/18
33383-4261		1.270	18/18
33383-4270		1.010	18/18
33383-4284		1.170	18/18

Samples extracted using either amount of resin generated concordant full DNA profiles (18/18 alleles). Samples processed using the 14µL method produced peaks that were slightly higher. The difference in peak heights between alleles within the same loci ranged from 59-86%, with a mean of 71%, indicating minimal difference between the two methods.

Doubling the amount of resin did not appear to provide any additional benefits compared to the original recommended protocol. More importantly, full DNA profiles were resolved using either method. Therefore, the costs associated with increasing the amount of resin cannot be justified at this stage.

6.6 Modifying extraction volumes

An investigation into optimising extraction volumes ranging from 300µL to 500µL was performed in order to ensure that buffer coverage over the samples was sufficient to enable optimal lysis and release of DNA. In addition, the use of an optimum volume of extraction reagents increases efficiency and economy, therefore potentially lowering laboratory costs.

Although the higher extraction volume generated higher yields when processed using the automated DNA IQ™ protocol (Table 7), DNA profile results were comparable across the various extraction volumes tested for eight replicates each (Table 8). Three instances of allelic imbalance were encountered in two samples from the 300µL and 450µL tests. In all instances, allelic imbalance was greater than 69%.

Table 7. DNA profile results for samples extracted using various volumes of Extraction Buffer, for 8 replicates.

Extraction Buffer Volume (µL)	Mean [DNA] (ng/µL)	SD
300	2.04	0.07
350	2.16	0.09
400	1.69	0.10
450	3.14	0.13
500	3.64	0.17

Table 8. DNA profile results for samples extracted using various volumes of Extraction Buffer, for 8 replicates.

Sample	Extraction Buffer Volume (µL)	DNA Profile Result
300-1 swab	300	OK
300-2 swab		OK
300-3 swab		OK
300-4 swab		OK
300-5 swab		OK
300-6 swab		OK
300-7 swab		AI D13
300-8 swab		OK

CaSS | Forensic and Scientific Services
A CLINICAL AND STATEWIDE SERVICE

350-1 swab	350	OK
350-2 swab		OK
350-3 swab		OK
350-4 swab		OK
350-5 swab		OK
350-6 swab		OK
350-7 swab		OK
350-8 swab		OK
400-1 swab	400	OK
400-2 swab		OK
400-3 swab		OK
400-4 swab		OK
400-5 swab		OK
400-6 swab		OK
400-7 swab		OK
400-8 swab		OK
450-1 swab	450	OK
450-2 swab		OK
450-3 swab		OK
450-4 swab		OK
450-5 swab		OK
450-6 swab		OK
450-7 swab		AI vWA, D18
450-8 swab		OK
500-1 swab	500	OK
500-2 swab		OK
500-3 swab		OK
500-4 swab		OK
500-5 swab		OK
500-6 swab		OK
500-7 swab		OK
500-8 swab		OK

6.7 Sensitivity of the automated DNA IQ™ protocol

DNA was detected from samples that were diluted down to 1:1000 (Figure 13).

CaSS | Forensic and Scientific Services

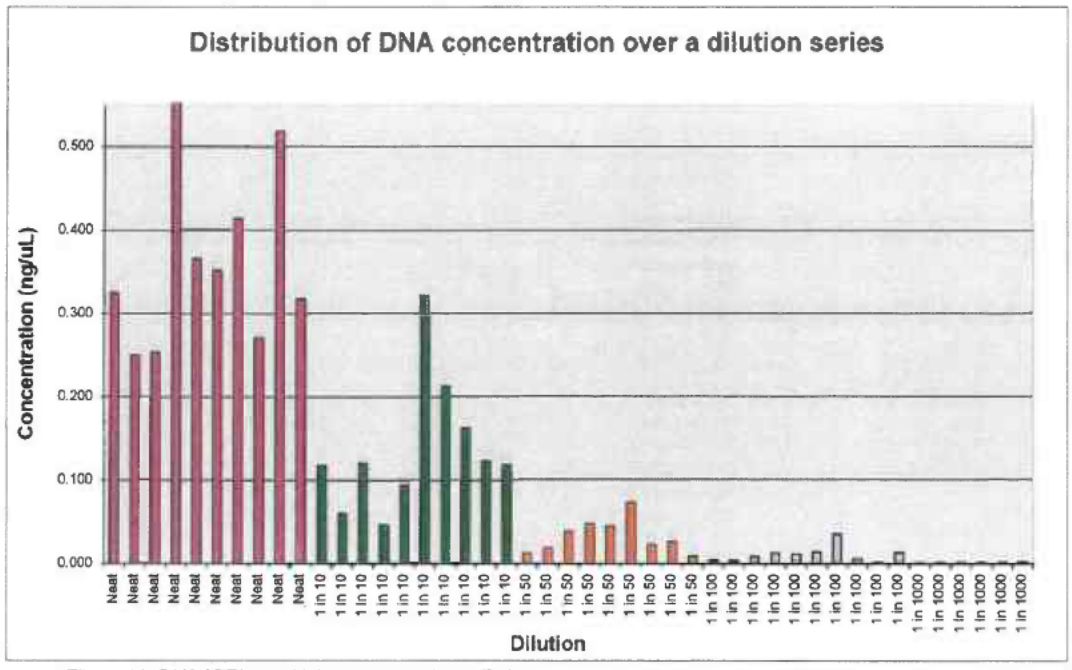


Figure 13. DNA IQ™ sensitivity across various dilutions

as

7. Summary and Recommendations

We recommend the following:

- Use of MPII for automated extraction of reference samples
- Use of MPII for automated extraction of casework samples
- Ongoing development of the automated extraction program to increase the efficiency of the extraction

Sweeney, P.J. and Walker, J.M., Burrell, M.M., Enzymes of molecular biology. *Methods Mol. Biol.* Towanam NJ , (1993) **16**, 306

BG-3

CaSS | Forensic and Scientific Services

A CLINICAL AND STATEWIDE SERVICE

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

August 2008

Automation and LIMS Implementation Project Team,

DNA Analysis

Forensic And Scientific Services

Clinical and Scientific Services

Queensland Health

Queensland Government
Queensland Health

CaSS | Forensic and Scientific Services

A CLINICAL AND STATEWIDE SERVICE

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

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

1. Abstract

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

2. Introduction

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

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

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

3. Aim

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

4. Equipment and Materials

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

5. Methods

5.1 Cell and blood collection

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

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

Table 1 lists the donor sample ID's.

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

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

5.2 Cell counting

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

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

5.3 Sensitivity, Reproducibility (Linearity) and Yield

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

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

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

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

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

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

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

5.4 Inhibition challenge

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

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

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

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

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

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

5.5 Substrates

Swabs

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

Tapelifts

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

Fabric

The material types tested included:

- Denim jeans;

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

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

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

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

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

5.6 Mixture studies

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

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

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

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

5.7 Substrate size

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

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

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

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

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

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

The TNE buffer consisted of:

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

The adapted manual DNA IQ™ protocol is described below:

1. Set one ThermoMixer at 37°C and another at 65°C.
2. Ensure that appropriately sized samples are contained in a sterile 1.5mL tube. For every sample, prepare three set of labelled tubes: spin baskets (for every tube except the extraction control), 2mL SSI tubes and Nunc™ tubes.
3. Prepare Extraction Buffer and add 300µL to each tube. Close the lid and vortex before incubating the tubes at 37°C on the ThermoMixer at 1000rpm for 45 minutes.
4. Remove the tubes from the ThermoMixer and transfer the substrate to a DNA IQ™ Spin Basket seated in a labelled 1.5mL Microtube using autoclaved twirling sticks. Then transfer the liquid to a labelled 2mL SSI sterile screw cap tube.
5. Centrifuge the spin basket on a benchtop centrifuge at room temperature for 2 minutes at its maximum speed. Once completed, remove the spin basket and collect the remaining solution and pool with the original extract in the 2mL SSI sterile screw cap tube, then vortex.
6. Add 550 µL of Lysis Buffer to each tube.

CaSS | Forensic and Scientific Services

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

5.9 DNA quantitation

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

5.10 PCR amplification

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

5.11 Capillary electrophoresis and fragment analysis

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

CaSS | Forensic and Scientific Services

6. Results and Discussion

6.1 Donor sample cell counts

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

6.2 Sensitivity, consistency and yield

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

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

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

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

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

CaSS | Forensic and Scientific Services
A CLINICAL AND STATEWIDE SERVICE

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

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

CaSS | Forensic and Scientific Services

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

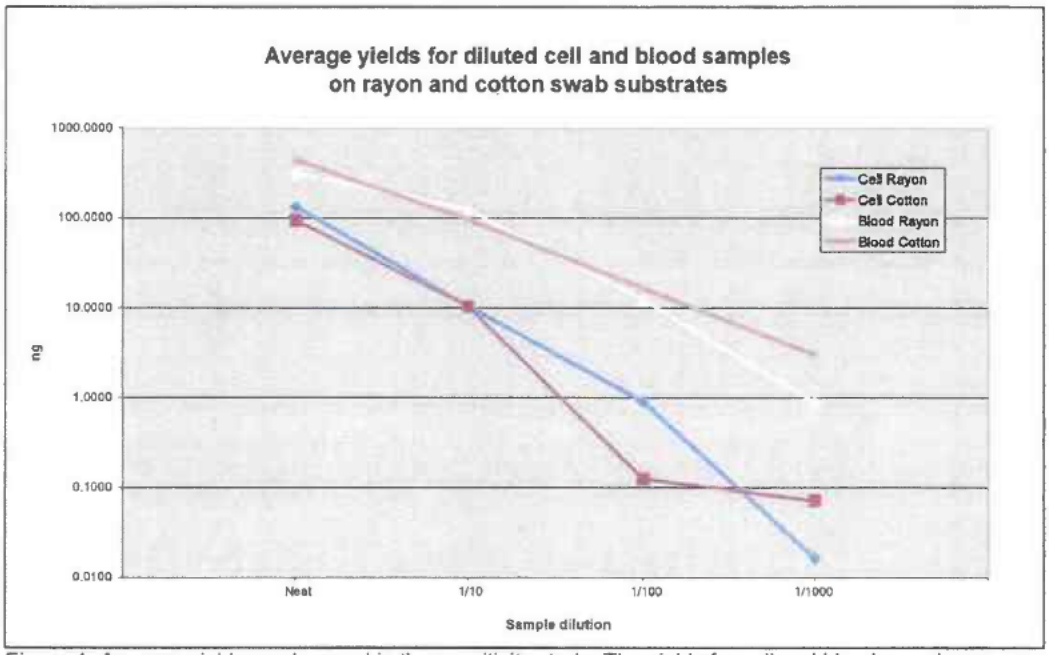


Figure 1. Average yields as observed in the sensitivity study. The yields for cell and blood samples, on two different swab types, were comparable as indicated by overlapping lines on the graph.

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

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

CaSS | Forensic and Scientific Services
A CLINICAL AND STATEWIDE SERVICE

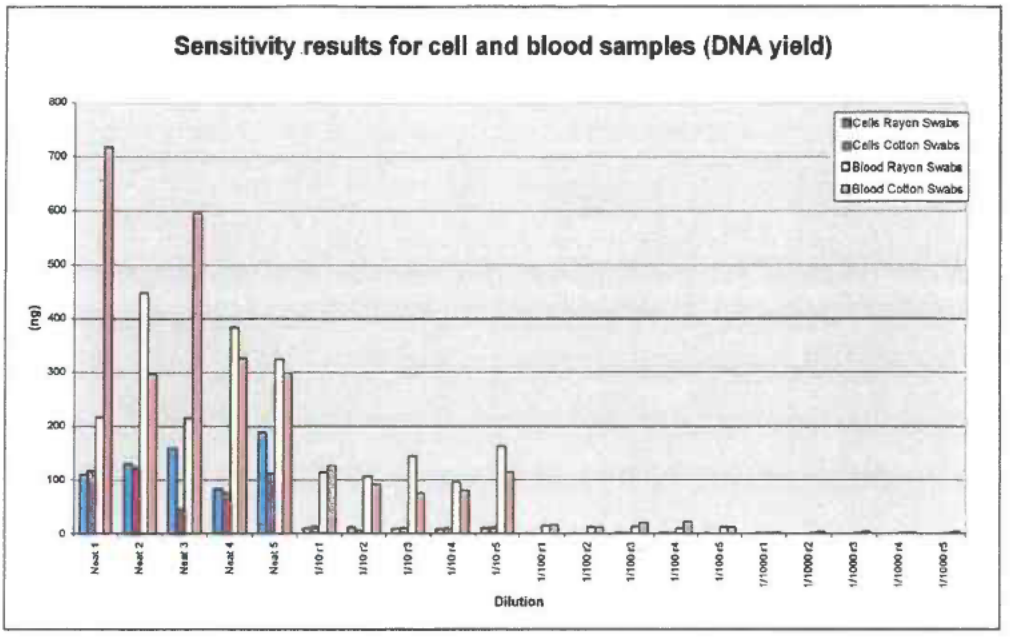


Figure 2. DNA yields (ng) observed for the sensitivity study. As expected, neat samples provided the highest yields. Yields were obtained down to 1/1000 for blood samples and 1/100 for cell samples.

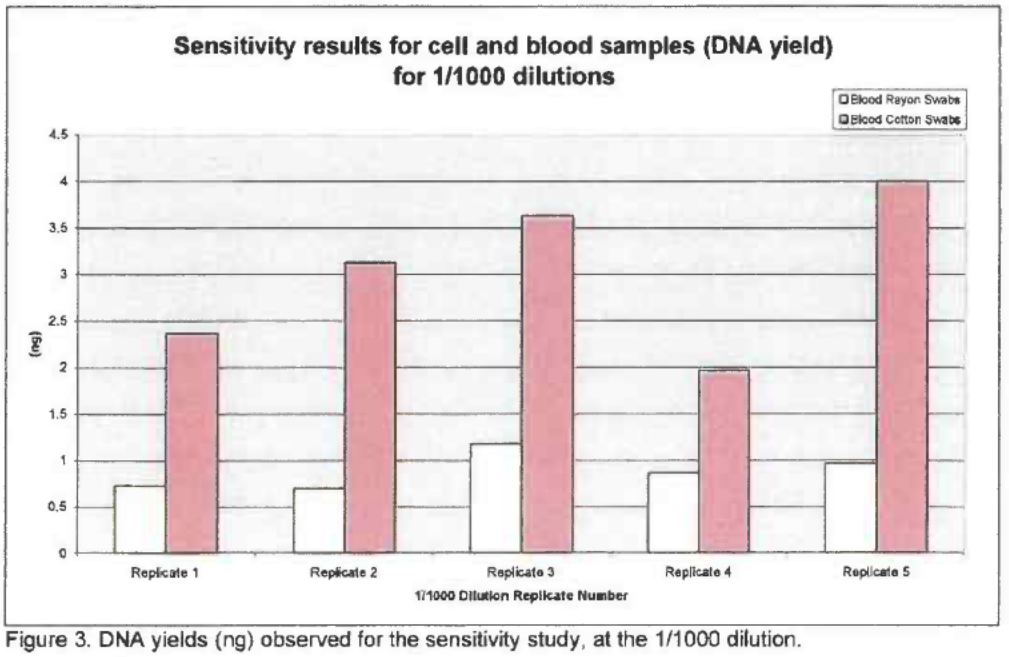


Figure 3. DNA yields (ng) observed for the sensitivity study, at the 1/1000 dilution.

CaSS | Forensic and Scientific Services

A CLINICAL AND STATEWIDE SERVICE

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

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

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

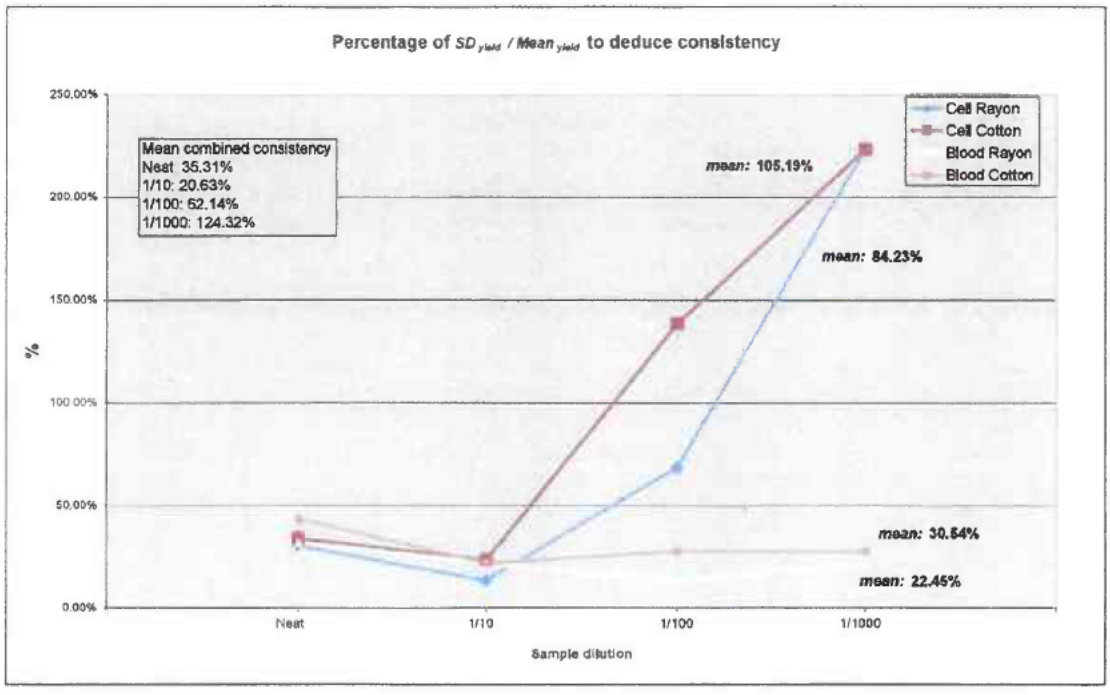


Figure 4. Reproducibility between replicates for cell and blood samples diluted down to 1/1000.

CaSS | Forensic and Scientific Services

6.3 Inhibition

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

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

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

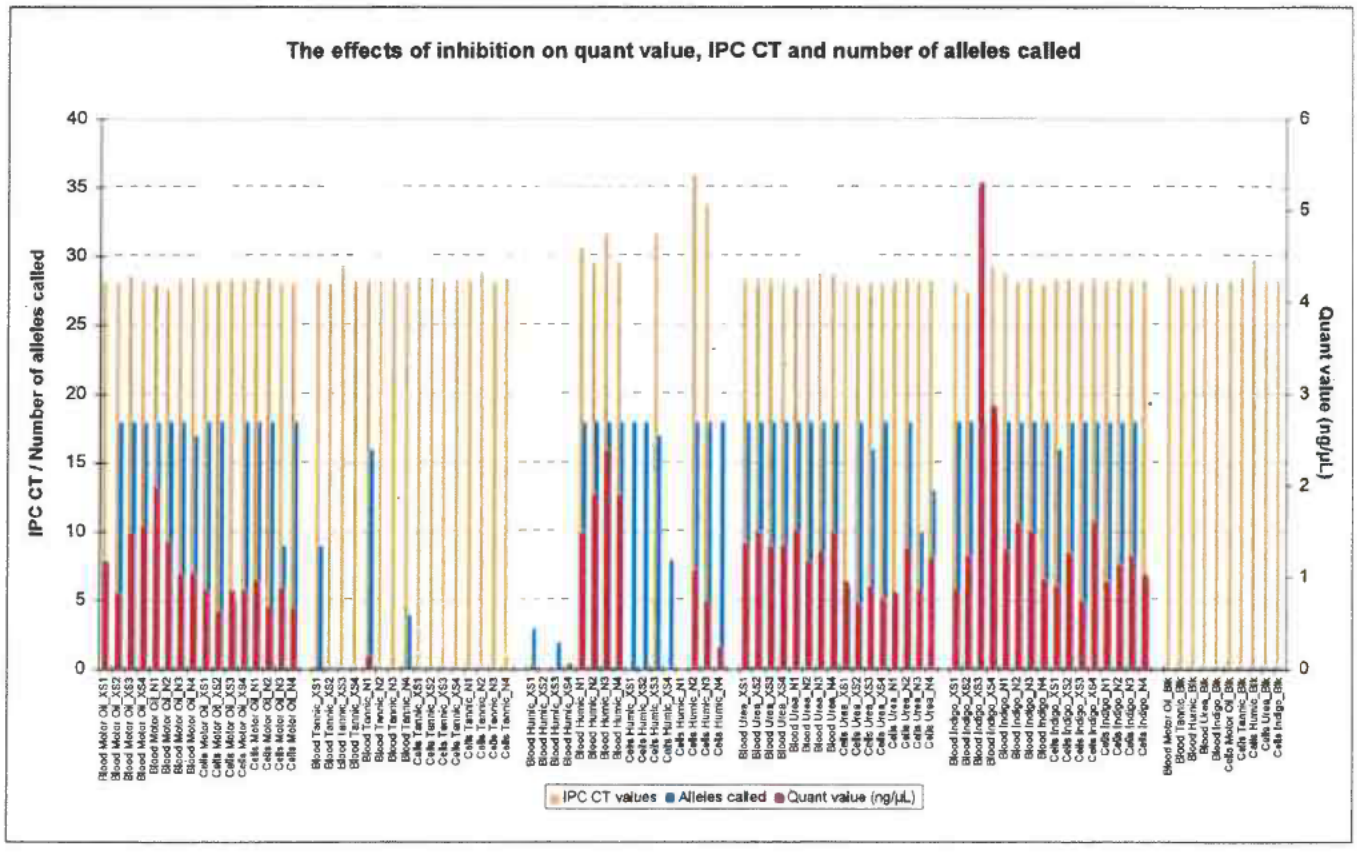


Figure 5. Effects of various inhibitors on quant value, IPC CT and number of resolved alleles.

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

The observations that were made include:

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

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

CaSS | Forensic and Scientific Services

A CLINICAL AND STATEWIDE SERVICE

6.4 Substrates

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

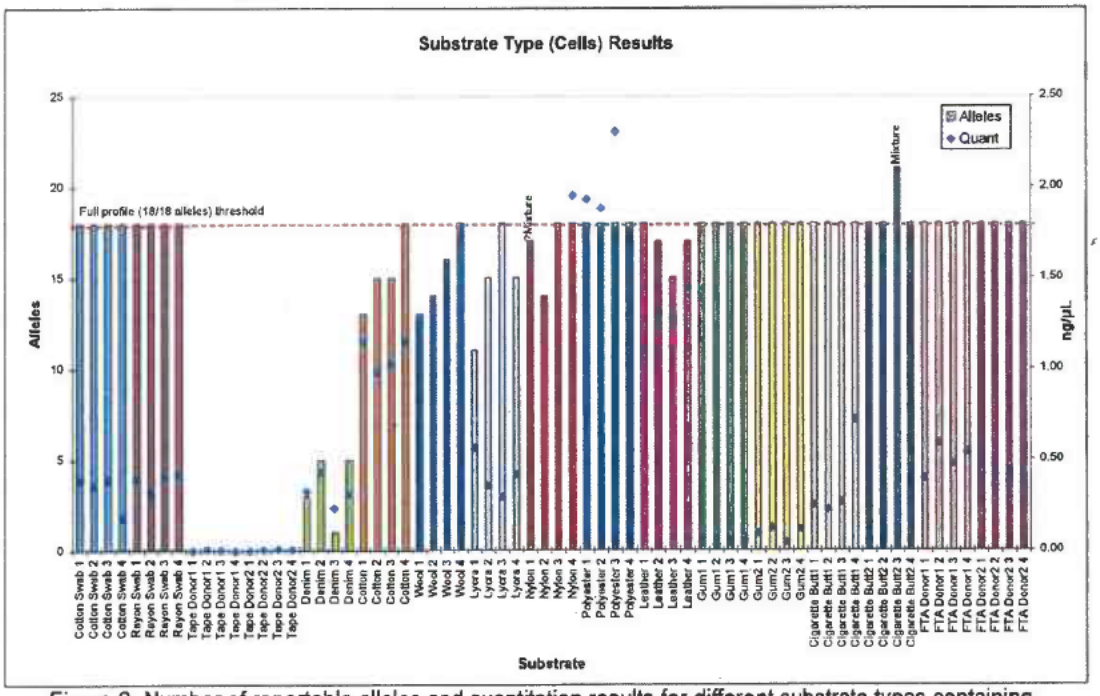


Figure 6. Number of reportable alleles and quantitation results for different substrate types containing cellular material.

CaSS | Forensic and Scientific Services

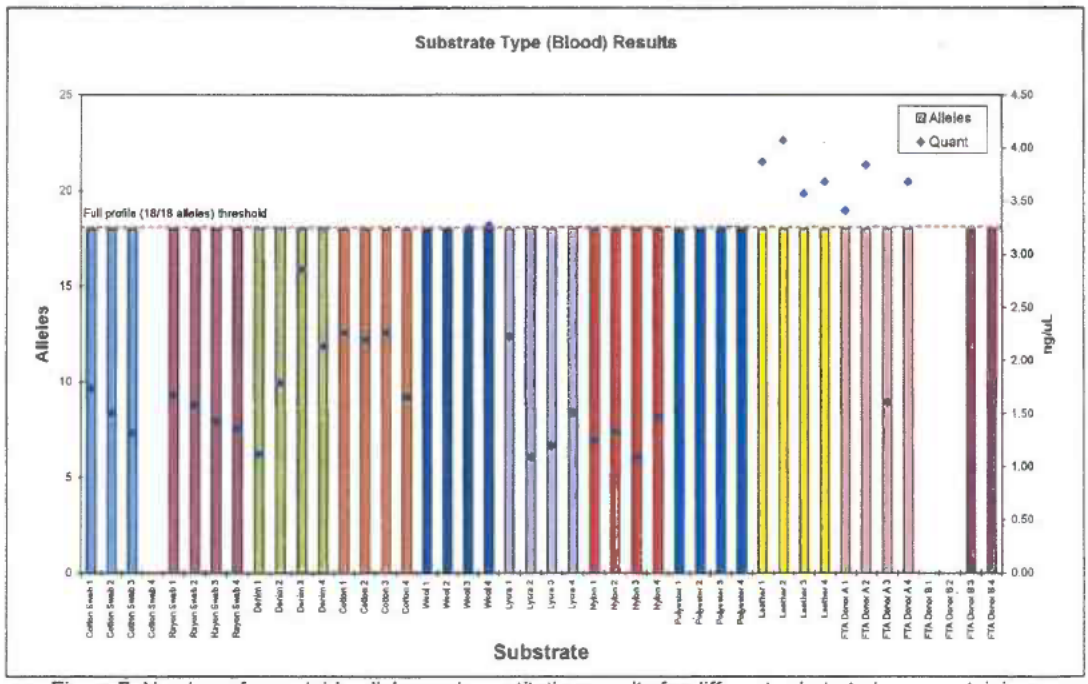


Figure 7. Number of reportable alleles and quantitation results for different substrate types containing blood material.

For cell samples:

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

For blood samples:

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

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

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

6.5 Mixture studies

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

6.6 Substrate size

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

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

CaSS | Forensic and Scientific Services

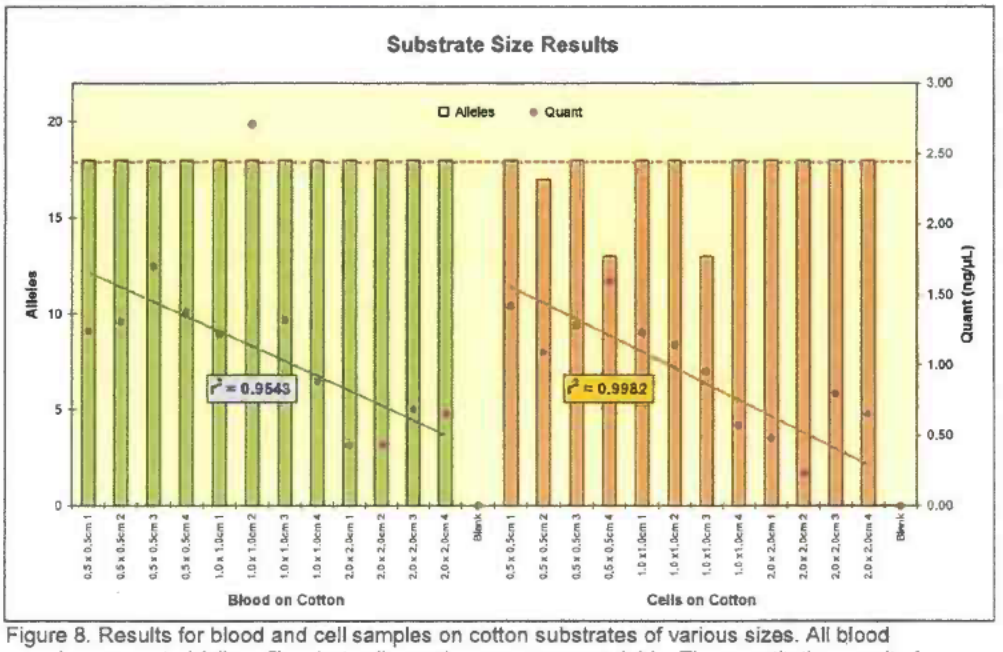


Figure 8. Results for blood and cell samples on cotton substrates of various sizes. All blood samples generated full profiles, but cell samples were more variable. The quantitation results for 0.5 x 0.5cm samples were higher than those for 2.0 x 2.0cm samples (blood r^2 = 0.9543*; cell r^2 = 0.9982;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

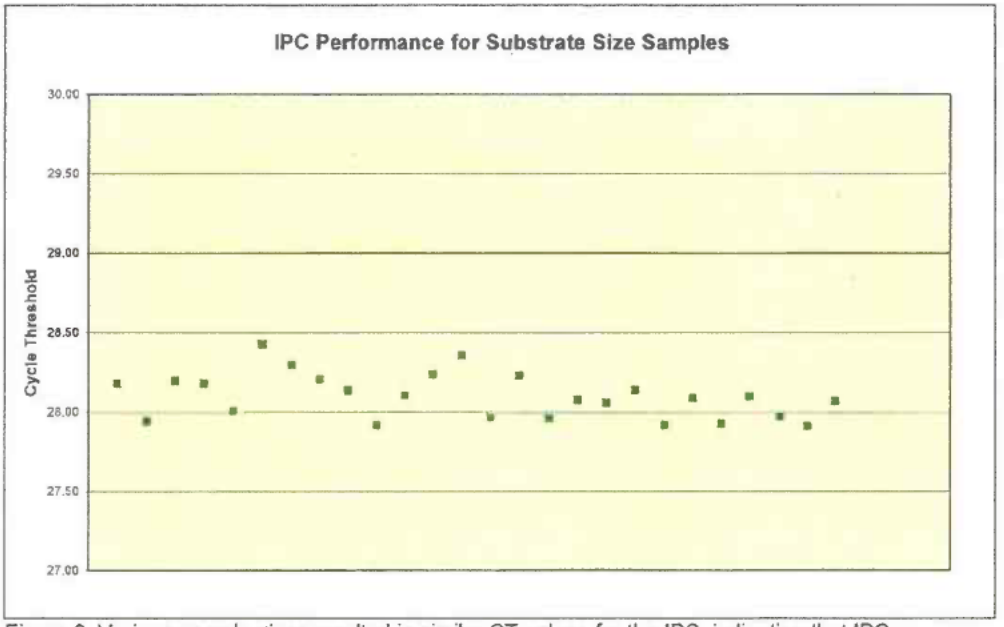


Figure 9. Various sample sizes resulted in similar CT values for the IPC, indicating that IPC performance is not affected by sample size, and that one sample size does not display a level of inhibition that is different to another sample size.

7. Summary and Recommendations

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

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

8. Acknowledgements

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

9. References

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

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

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

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

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

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

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

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

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

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

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

CaSS | Forensic and Scientific Services

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

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

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

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

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

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