Project 13: Statement of Vojtech Hlinka

Given in Response to

A Notice to Give Information in a Written Statement (7.001), issued pursuant to section 5(1) (d) of the Commissions of Inquiry Act 1950 (Act), that sets out the matters you are required to address in your statement; and Identification

In Queensland

I, Vojtech Hlinka, formerly employed by Queensland Health Forensic and Scientific Service, Scientist, do solemnly and sincerely declare that:

On 19th of October, 2023, I was asked to provide a statement responding to Commission of Inquiry into DNA Project 13 (the Commission) to address the questions/matters below.

Identification
1. State:
(a) your full name;

Vojtech Hlinka

(b) your qualifications, skills or experience relevant to forensic science and DNA; and

Qualifications:
- PhD (Doctor of Philosophy) in Archaeology (2003, the University of QLD).
- BA Honours Class I in Anthropology (1997, the University of QLD).
- BA (Double major in Anthropology, 1996, the University of QLD).

Specialist in Ancient DNA analysis. Trained by Dr. Thomas Loy.

I worked as a Forensic Scientist through employment with QLD Health, undertaking significant training in forensics.

Relevant skills and experience include the following
* 2004-2006. Project leader of the DNA Processing Improvement Project at Forensic Biology, Queensland Health Scientific Services. The project aim was to improve the efficiency of processing DNA samples while minimising the costs and time involved. A large focus of this project was in reducing the reworking of precious and limited forensic samples. The main component of this work involved the optimisation and validation of a real-time PCR DNA quantitation method that enables DNA sample extracts to be worked at their most optimal DNA concentrations. The retrospective and prospective validation reports, published posters and Standard Operating Procedures (SOPS) were written.
* 2004-2008 (mainly 2006-2008). In Forensic Biology I was a member of the Automation Project Group. The function of this group was to automate a number of laboratory procedures by automating methods or components of methods of extraction, purification, quantitation and amplification of modern and old DNA samples.
* Robotics and Automation- Former member of the robotics and automation panel that was involved in the assessment and selection of robotics platform tenders in Forensic Biology, Queensland Health. Involved in the selection, validation and implementation of extraction and

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purification methods on Multiprobe II platforms.
* Method validation- Validation of methods to certification requirements (e.g. ISO 9001) and guidelines, including SWGDAM, NATA, the DNA Advisory Board and Queensland Government guidelines.
* LIMS- have worked with LIMS on a daily routine. I have had involvement into the development/implementation of Laboratory Information Management Systems including AUSLAB (Queensland Health Scientific Services).
* DNA quantitation using real-time PCR (e.g. Quantifiler system).
* Forensic and archaeological DNA profiling and profile analysis of STRs, including amplification of DNA using Profiler Plus and COfiler kits.
* Genetic analysis of DNA samples including DNA extraction, purification, profiling, PCR, and real-time PCR.

Completed:
Intensive Course in Forensic DNA Statistics (14-18th February, 2005).

Five-day course by John Buckleton (ESR, New Zealand) and Simon Walsh (University of Technology Sydney, Australia) at Queensland Health. The course provided practice and relevant coverage of the statistics involved in the genetic analysis of human DNA (STRs). This is directly applicable to statistical methods used in forensics, archaeology and in the courts.

Queensland Institute of Medical Research/Royal Brisbane Women's Hospital Statistics Unit Training Series Workshops, March-July (monthly workshops) 2007

Memberships
I am a Member of the Perkin Elmer Multiprobe II National User's Group.

(c) 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.

Role: Scientist
13th of May, 2004- 16th of May, 2013

Note:
October, 2011 onwards: Unable to work due to ongoing painful sciatica, allergies and other physical and mental health issues. Sciatica pain onset began during the automation project period around 2007/2008 so was struggling since then and unable to sit for long periods of time without suffering worsening pain.
Last attended work in October 2011.

2013 Retired from QLD Health and currently on Disability Pension. As such, I am unable to produce several relevant documents due to not being a current employee of Queensland Health and not having access to QLD Health documents and records. However, I have been able to give some relevant references.

The role above as a Forensic Scientist in QLD Health included the following subroles below:

* 2004-2006. Project Leader of the DNA Processing Improvement Project at Forensic Biology, Queensland Health Scientific Services (QHSS).

[redacted]

[redacted]

* 2004-2008 (mainly 2006-2008). In Forensic Biology, I was a member of the Automation Project Group (Automation Team). This is linked to my role in Robotics and Automation as a Former member of the robotics and automation panel that was involved in the assessment and selection of robotics platform tenders in Forensic Biology, Queensland Health. I was involved in the selection, validation and implementation of extraction and purification methods on Multiprobe II platforms.

Manual and Automated DNA Extraction Methods

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:

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[redacted]

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.

Tendered at the Commission of Inquiry into Forensic DNA Testing in Queensland as exhibit 129.95, FSS:0001.0084.1444: https://www.dnainquiry.qld.gov.au/public-hearings/exhibits.aspx#m4.

Manual Method

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

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The manual method (QHSS manual DNAIQTM method) referred to here, was based on both the CFS automated DNAIQ method and a manual Promega DNAIQ method on which the former was based. As such, it is a (ii), a modification of an existing manual method.

DNAIQ is a commercially available magnetic bead-based DNA extraction kit developed and sold by Promega (an organisation that manufactures certain biotechnological products). It is usually referred to as a system because there are several method available for using it.

The manual DNA IQTM system is an established forensic sample DNA extraction system developed and sold by the Promega Corporation.

It is a highly reliable, robust, validated and published extraction system used by forensic laboratories across the world. See, for example...

D. I. Komonski; A. Marignani; M. L. Richard; J. R. H. Frappier; J. C. Newman
Validation of the DNA IQ System for Use in the DNA Extraction of High Volume Forensic Casework
Canadian Society of Forensic Science Volume: 37 Issue: 2 Dated: June 2004 Pages: 103-109
The abstract is supplied as evidence.

In summary, the DNAIQTM kit works as follows:
(a) An area of interest is sampled;
(b) The sample has an extraction buffer added to break open cellular material;
(c) The sample substrate is spun and removed leaving a raw liquid material called lysate;
(d) A special resin containing magnetic beads is added to the lysate;
(e) A special buffer is added to allow the DNA to bind to the magnetic beads;
(f) The sample is added to a magnet to bind the beads and they are washed several times to remove non-DNA material and other chemical inhibitors; and
(g) The sample has a special buffer (elution buffer) added to allow the DNA to be released from the beads.

The details of the QHSS manual DNAIQTM method, with precision, are recorded in the Project 11 report, copied below...

"5.8
Extraction using the DNA IQTM System (Promega Corp.)
The manual DNA IQTM 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 IQTM protocol is described below:

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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 NuncTM 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 IQTM 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.

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7. Dispense 50µL of DNA IQTM 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 st and; 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 NuncTM tubes.

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21. Repeat step 17 to 20, transferring the supernatant to the appropriate NuncTM tube. The final

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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"

Section 18.4.2 of QIS 24897V3 likewise contains the manual protocol and the same Extraction Buffer volume at 300µL.

Reference
https://www.dnainquiry.qld.gov.au/public-hearings/assets/exhibits/module-4/EXH%20129.4%20WIT.0050.0003.0001%20WIT.0050.0003.0001_R.pdf

(ii) The QHSS manual DNA IQTM method used in Project 13 was based on an automated protocol developed by the Centre of Forensic Sciences (CFS) in Toronto, Ontario (PerkinElmer, 2004).

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

which is in turn based on the commercial Promega DNAIQTM manual method (the revision version we had in the laboratory for comparison was from 2006)...

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

The Perkin Elmer reference (2004) is a proprietary publication that is or was sold by Perkin Elmer as Perkin Elmer Part Number 8842157. I am aware that QLD Health obtained a copy of it but I am unable to have access to QLD Health documents and records because I am a retired former employee. Nevertheless, the document most likely would need to be released by Perkin Elmer directly.

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

A DNA IQ manual method was first investigated and evaluated in Project 9 against other DNA extraction chemistries and then subsequently validated in Project 11.

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.

I have no access to this document since I am no longer employed by Queensland Health. A copy may be obtained through Queensland Health.

In Project 9, the DNAIQTM system was found to generate results that were comparable or better than the current Chelex ® -100 protocol used at DNA Analysis, FSS (QHSS) (see abstract of Project 11).

Please refer to the Project 11 report on the validation method details...

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[redacted]

Project 11. Report on the Validation of a manual method for Extracting DNA using the DNA IQ™ System August 2008
https://www.dnainquiry.qld.gov.au/public-hearings/assets/exhibits/module-4/EXH%20129.94%20-%20FSS.0001.0084.1400.pdf

In summary, The QHSS manual DNAIQTM method was validated in Project 11 for extracting DNA from forensic samples, and incorporated studies on sensitivity and consistency, inhibition, substrate type, substrate size, and mixture studies. It was decided that this QHSS manual DNA IQTM method was suitable for verification on the automated MultiPROBE ® II PLUS HT EX extraction platforms.

(c) state whether, and if so how, the Manual Method differed from or otherwise modified the DNA IQTM 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);

I am aware of the following modifications:

1. The inclusion of a Lysis step using an Extraction Buffer (10mM Tris, 1mM EDTA, 100 mM NaCl, 20% w/v SDS) in the presence of Proteinase K, before incubation in the DNA IQTM Lysis Buffer.

2. Lysis incubation conditions were lowered to 37 degrees Celsius to broaden the range of samples that could be used. In particular, this change was made to accommodate the extraction of DNA from heat labile materials such as nylon and polyester. At high temperatures, heat labile materials can melt or breakdown and encase DNA, thus potentially lowering yield.

Both of these adaptations were present in the QHSS manual DNAIQTM and QHSS automated DNAIQTM protocols, but also appear present in the CFS Automated DNAIQTM Protocol. Other laboratories may have utilised a different approach during their verification or validation for use on the Multiprobe® II PLUS platform.

The Promega DNAIQTM manual method (2006) provides a modification of utilising a Lysis step with an Extraction Buffer for certain samples, prior to using the DNA IQTM Lysis Buffer.

Relevant quotes from the Promega DNAIQTM manual method (2006):
"Tissue masses including hair, bone, and sperm require a Proteinase K digestion to obtain reliable amounts of DNA."
"Samples Requiring a Proteinase K Digestion Prior to Addition of Twice the Recommend Volume of Lysis Buffer"

The modification made was to use the Lysis step with an Extraction Buffer for all samples, in order to accommodate for a broader range of sample types.

This is consistent with the Komonski *et al.* 2004 publication, where the abstract states "Modifications to this protocol were required to increase extraction efficiency from a range of sample types. These included: decreasing the incubation temperature; replacing the initial incubation in the kit's lysis buffer with an extraction buffer containing proteinase K; "
The abstract is supplied as evidence.

[redacted]

[redacted]

I note that the Extraction Buffer used at QHSS for the manual and automated methods differs to that found in Promega DNAIQTM manual method (2006). The QHSS version of the Extraction Buffer utilised came from Perkin Elmer (2004) (also known as the CFS Automated DNAIQTM Protocol) as stated in the quote from the Project 11 Report below.

"The manual DNA IQTM 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."

Modification 2 of lowering the Lysis incubation to 37 degrees follows the method from the Promega manual DNAIQTM method (2006) for heat-sensitive materials.
Quote
"Heat-sensitive fabrics (e.g, polyester and nylon): Extract without heating."
This refers to not heating the Lysis Buffer incubation to 95°C for 30 minutes during Extraction.

I believe Perkin Elmer (2004) (also known as the CFS Automated DNAIQTM Protocol) also used modification 2.

Modification 3
QHSS manual and automated DNAIQ methods both have a double elution of 50µL, the CFS Automated DNAIQTM Protocol had a smaller elution volume towards the lower amount recommended in the Promega DNAIQTM manual method (2006). QHSS originally used a single elution of 100µL which follows the recommendations in the Promega DNAIQTM manual method (2006) of using 25-100µL, but it was found carrying out a double elution of 50µL was more efficient.

Modification 4
The use of NUNC Bank-It tubes for storage of final extracts.

I am unable to fully answer the question or add further details due to not being currently employed by QLD Health and do not have access to the relevant records and documents.

(d) state when the Manual Method was so devised;

The manual DNAIQTM chemistry was first investigated in 2007 in Project 9 when it was evaluated against the performance of other available chemistries.

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.

I am unable to provide a copy of this document since I am no longer an employee of QLD Health and have no access to QLD Health documents and records.

The Manual QHSS DNAIQTM method is recorded in QIS Document 24897V3 (19th of March, Valid from 27th March 2008)
The Manual QHSS DNAIQTM method is recorded in Project 11 (August 2008) and also in Project 13. (August 2008).

[redacted] [redacted]

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

Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C., Ientile, V.

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

Reasons for the development and implementation of the QHSS manual DNAIQTM method (derived from the CFS Automated Protocol) that I am aware of...

1. Project 9 identified the Promega manufactured manual DNAIQTM (2006) chemistry as more efficient to the other chemistries investigated and compared in-house. In Project 9, the DNAIQTM system was found to generate results that were comparable or better than the current Chelex ® -100 protocol that was used at the time in DNA Analysis, FSS (QHSS) (see abstract of Project 11).

Reference:
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.

I am unable to provide a copy of this document since I am no longer an employee of QLD Health and have no access to QLD Health documents and records.

2. It is based on an automated DNA IQTM method validated by an external lab (CFS).
3. In theory, it should not matter if the "same" method is performed manually or via an automated process.
4. Management staff at the time wanted to have options of using a manual DNAIQTM method as similar as possible to the Automated DNAIQTM method in case the MultiProbe Automation machines were down for some reason.
5. Transition across the Manual and Automated methods is simpler for comparison reasons and for ease of staff training and understanding.

There are likely additional reasons that I am unable to get records for why the management staff of the time decided to implement the manual method.

CFS Automated Protocol

(g) describe, with precision, the "CFS automated protocol (PerkinElmer, 2004)" (CFS Automated Protocol) referred to in the seventh line of the second paragraph of the Introduction to the 2008 Report;

I am unable to fully answer this question due to not working for QLD Health any longer. I have no access to internal QLD Health documents or Perkin Elmer documents other than those available on the internet. Please contact QLD Health or Perkin Elmer to obtain a copy of the CFS automated protocol (Perkin Elmer 2004). This is a proprietary document that is legally protected and was or is sold by Perkin Elmer. It would most likely need legal permission to be publicly released.

Perkin Elmer supplied Queensland Health with the original CFS automated DNAIQTM program that was subsequently modified.

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The CFS Automated Protocol (Perkin Elmer 2004) is a validated and automated DNAIQTM method used for forensic samples developed by the Centre of Forensic Sciences (CFS) in Toronto, Ontario (PerkinElmer, 2004).

There are a 2 main modifications in the CFS Automated Protocol compared to the Promega DNAIQTM Manual method (2006).

1. A TNE Proteinase K - SDS Extraction Buffer being used for the initial Extraction Lysis before utilising the Promega DNAIQTM Lysis Buffer.
2. A lower incubation temperature of 37 degrees Celsius was used.

This is documented in the abstract below Komonski *et al.* 2004 publication, where the abstract states "Modifications to this protocol were required to increase extraction efficiency from a range of sample types. These included: decreasing the incubation temperature; replacing the initial incubation in the kit's lysis buffer with an extraction buffer containing proteinase K; "
The abstract is supplied as evidence.

I note that the Extraction Buffer used at by CFS (see Project 11) for their automated methods differs to that found in the Promega DNAIQTM manual method (2006). Quote from the Project 11 Report below.

"The manual DNA IQTM 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."

Modification 2 of lowering the Lysis incubation to 37 degrees follows the method from the Promega manual DNAIQTM method (2006) for heat-sensitive materials.
Quote
"Heat-sensitive fabrics (e.g, polyester and nylon): Extract without heating."
This refers to not heating the Lysis Buffer incubation to 95°C for 30 minutes during Extraction.

In other words, modification 2 in the CFS Automated DNAIQTM Protocol is actually a modification of the step in the Promega manual DNAIQTM method (2006) for heat-sensitive materials being applied to a broader range of sample types.

Manual DNA IQTM Protocol

(h) describe, with precision, the "manual DNA IQTM protocol" (Manual DNA IQTM 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; or
> (iii) was otherwise a modification of an existing Manual DNA IQTM protocol (and if so which method);

The manual method (QHSS manual DNAIQTM method) referred to here, was based on both the CFS automated DNAIQ method and a manual Promega DNAIQTM method on which the former was based. As such, it is a (ii), a modification of an existing manual method.

[redacted]

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DNAIQTM is a commercially available magnetic bead-based DNA extraction kit developed and sold by Promega (an organisation that manufactures certain biotechnological products). It is usually referred to as a system because there are several method available for using it.

The manual DNA IQTM system is an established forensic sample DNA extraction system developed and sold by the Promega Corporation.

It is a highly reliable, robust, validated and published extraction system used by forensic laboratories across the world. See, for example...

D. I. Komonski; A. Marignani; M. L. Richard; J. R. H. Frappier; J. C. Newman
Validation of the DNA IQ System for Use in the DNA Extraction of High Volume Forensic Casework
Canadian Society of Forensic Science Volume: 37 Issue: 2 Dated: June 2004 Pages: 103-109
The abstract is supplied as evidence.

In summary, the DNAIQTM kit works as follows:
(a) An area of interest is sampled;
(b) The sample has an extraction buffer added to break open cellular material;
(c) The sample substrate is spun and removed leaving a raw liquid material called lysate;
(d) A special resin containing magnetic beads is added to the lysate;
(e) A special buffer is added to allow the DNA to bind to the magnetic beads;
(f) The sample is added to a magnet to bind the beads and they are washed several times to remove non-DNA material and other chemical inhibitors; and
(g) The sample has a special buffer (elution buffer) added to allow the DNA to be released from the beads.

The details of the QHSS manual DNAIQTM method, with precision, are recorded in the Project 11 report, copied below...

"5.8
Extraction using the DNA IQTM System (Promega Corp.)
The manual DNA IQTM 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 IQTM 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 NuncTM tubes.

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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 IQTM 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.

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7. Dispense 50µL of DNA IQTM 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 st and; 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 NuncTM tubes.
Page 8 of 21
21. Repeat step 17 to 20, transferring the supernatant to the appropriate NuncTM 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"

[redacted]

[redacted]

Section 18.4.2 of QIS 24897V3 likewise contains the manual protocol and the same Extraction Buffer volume at 300µL.

Reference
https://www.dnainquiry.qld.gov.au/public-hearings/assets/exhibits/module-4/EXH%20129.4%20WIT.0050.0003.0001%20WIT.0050.0003.0001_R.pdf

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

To the best of my knowledge, the QHSS manual DNA IQTM protocol was based on the CFS automated version supplied by Perkin Elmer (2004), with modifications made at QHSS. Perkin Elmer is also the manufacturer of the MultiPROBE® II PLUS HT EX FORENSIC WORKSTATION platform.

(ii) was devised within the Laboratory; or

To the best of my knowledge, the QHSS manual DNA IQTM protocol was based on the CFS automated version supplied by Perkin Elmer, with modifications made at QHSS.

(iii) was otherwise a modification of an existing Manual DNA IQTM protocol (and if so which method);

To the best of my knowledge, the QHSS manual DNA IQTM protocol was based on the CFS automated version supplied by Perkin Elmer, with modifications made at QHSS. The CFS automated protocol is based on the Promega DNAIQTM manual method (2006).

(i) describe, with precision, the method by which the Manual DNA IQTM Protocol was validated;

A DNA IQ manual method was first investigated and evaluated in Project 9 against other DNA extraction chemistries and then subsequently validated in Project 11.

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.

I have no access to this document since I am no longer employed by Queensland Health. A copy may be obtained through Queensland Health.

In Project 9, the DNAIQTM system was found to generate results that were comparable or better than the current Chelex ® -100 protocol used at DNA Analysis, FSS (QHSS) (see abstract of Project 11).

Please refer to the Project 11 report on the validation method details...

Project 11. Report on the Validation of a manual method for Extracting DNA using the DNA IQ™ System August 2008
https://www.dnainquiry.qld.gov.au/public-hearings/assets/exhibits/module-4/EXH%20129.94%20-%20FSS.0001.0084.1400.pdf

[redacted]

[redacted]

In summary, the QHSS manual DNAIQTM method was validated in Project 11 for extracting DNA from forensic samples, and incorporated studies on sensitivity and consistency, inhibition, substrate type, substrate size, and mixture studies. It was decided that this QHSS manual DNA IQTM method was suitable for verification on the automated MultiPROBE ® II PLUS HT EX extraction platforms.

(j) state whether, and if so how, the Manual DNA IQTM Protocol differed from or otherwise modified the DNA IQTM 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);

I am aware of the following modifications:

1. The inclusion of a Lysis step using an Extraction Buffer (10mM Tris, 1mM EDTA, 100 mM NaCl, 20% w/v SDS) in the presence of Proteinase K, before incubation in the DNA IQTM Lysis Buffer.

2. Lysis incubation conditions were lowered to 37 degrees Celsius to broaden the range of samples that could be used. In particular, this change was made to accommodate the extraction of DNA from heat labile materials such as nylon and polyester. At high temperatures, heat labile materials can melt or breakdown and encase DNA, thus potentially lowering yield.

Both of these adaptations were present in the QHSS manual DNAIQTM and QHSS automated DNAIQTM protocols, but also appear present in the CFS Automated DNAIQTM Protocol. Other laboratories may have utilised a different approach during their verification or validation for use on the Multiprobe® II PLUS platform."

The Promega DNAIQTM manual method (2006) provides a modification of utilising a Lysis step with an Extraction Buffer for certain samples, prior to using the DNA IQTM Lysis Buffer.

Relevant quotes from the Promega DNAIQTM manual method (2006):
"Tissue masses including hair, bone, and sperm require a Proteinase K digestion to obtain reliable amounts of DNA."
"Samples Requiring a Proteinase K Digestion Prior to Addition of Twice the Recommend Volume of Lysis Buffer"

The modification made was to use the Lysis step with an Extraction Buffer for all samples, in order to accommodate for a broader range of sample types.

This is consistent with the Komonski *et al.* 2004 publication, where the abstract states
"Modifications to this protocol were required to increase extraction efficiency from a range of sample types. These included: decreasing the incubation temperature; replacing the initial incubation in the kit's lysis buffer with an extraction buffer containing proteinase K; "
The abstract is supplied as evidence.

I note that the Extraction Buffer used at QHSS for the manual and automated methods differs to that found in Promega DNAIQTM manual method (2006). The QHSS version of the Extraction Buffer utilised came from Perkin Elmer (2004) (also known as the CFS Automated DNAIQTM Protocol) as stated in the quote from the Project 11 Report below.

[redacted]

[redacted]

"The manual DNA IQTM 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."

Modification 2 of lowering the Lysis incubation to 37 degrees follows the method from the Promega manual DNAIQTM method (2006) for heat-sensitive materials.
Quote
"Heat-sensitive fabrics (e.g, polyester and nylon): Extract without heating."
This refers to not heating the Lysis Buffer incubation to 95°C for 30 minutes during Extraction.

I believe Perkin Elmer (2004) (also known as the CFS Automated DNAIQTM Protocol) also used modification 2.

Modification 3
QHSS manual and automated DNAIQ methods both have a double elution of 50µL, the CFS Automated DNAIQTM Protocol had a smaller elution volume towards the lower amount recommended in the Promega DNAIQTM manual method (2006). QHSS originally used a single elution of 100µL which follows the recommendations in the Promega DNAIQTM manual method (2006) of using 25-100µL, but it was found carrying out a double elution of 50µL was more efficient.

Modification 4
The use of NUNC Bank-It tubes for storage of final extracts.

I am unable to fully answer the question or add further details due to not being currently employed by QLD Health and do not have access to the relevant records and documents.

(k) state when the Manual DNA IQTM Protocol was so devised;

The manual DNAIQTM chemistry was first investigated in Project 9 when it was evaluated against the performace of other available chemistries.

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.

The Manual QHSS DNAIQTM method is recorded in QIS Document 24897V3 (19th of March, Valid from 27th March 2008)
The Manual QHSS DNAIQTM method was presented in Project 11 (August 2008) and also in Project 13. (August 2008).

(l) identify those within the Laboratory responsible for devising the Manual DNA IQTM Protocol;

Thomas Nurthen, Senior Scientist, Automation and Laboratory Information Management System (LIMS) implementation project
Vojtech Hlinka, Automation Team Scientist;
Breanna Gallagher, Automation Team Scientist;
Cecilia Iannuzzi, Automation Team Scientist;
Generosa Lundie, Automation Team Scientist;
Iman Muharam, Internal Auditor QIS 8227/Automation Team Scientist/Senior

[redacted]

[redacted]

Scientist Automation and LIMS implementation;

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.

Project 11. Report on the Validation of a Manual Method for Extracting DNA using the DNA IQTM System
Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C., Ientile, V. Automation/LIMS Implementation Project, DNA Analysis FSS (August 2008)

(m) state the reason(s) why the Laboratory chose to devise Manual DNA IQTM Protocol;
I believe it was for enabling direct comparison of a DNAIQTM manual method as similar as possible to the DNAIQTM automation platform protocol. I also believe management staff wanted the option of the DNAIQTM method to be performed either on or off the robot. Other reasons may have also existed that I currently cannot recall.

Automated DNA IQTM Protocol

(n) state whether the "automated DNA IQTM protocol" referred to in the first line of the third paragraph of the Introduction to the 2008 Report (Automated DNA IQTM 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;

I believe it is referring to the same protocol.

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

(i) the Manual Method;
(ii) the DNA IQTM 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 IQTM Protocol;

(i) the Manual Method;

Amendments in the QHSS automated protocol compared to the validated manual method in Project 11, according to project 13 (August 2008)
Minor modifications included
* Increasing the volume of Extraction Buffer to 500µL
* A SlicPrepTM 96 Device (Promega Corp, Madison, W, USA) was used for sample lysis;
* Incubation steps and any shaking steps were performed on the integrated DPC shaker.
* CRS toroid magnet (P/N) 5083175) was used for isolating the DNA IQTM resin.
* Instead of a single elution of 100µL, a double elution method (2 x 50µL) is used.

Note
The reference to the CRS toroid magnet (P/N) 5083175) is missing in the following documents below

[redacted]

[redacted]

Amendments in the QHSS automated protocol according to QIS document 24897V1 (23rd October, 2007, valid from the 24th of October, 2007) included
* The use of the SlicprepTM 96 device (Promega) for removing substrate from lysate.
* The increase of extraction buffer volume to 500µL for use with the SlicprepTM 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.

Amendments in the QHSS automated protocol according to QIS document 24897V3 (19/03/2008, Valid from 27/03/2008) included
* The use of the SlicprepTM 96 device (Promega) for removing substrate from lysate.
* The increase of Extraction Buffer volume to 500µL for use with the SlicprepTM 96 device.
* The use of tubes and spin-baskets for the off-deck lysis of samples prior to extraction on MPII. Use of a 96-deepwell plate for completion of extraction on MPII.
* The provision of initial incubation with TNE Buffer and retention of a portion thereof for further testing (retained supernatant testing).
* 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.

"manufacturer's instructions refers to

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

Lysis Buffer volume to 957µL + Lysis buffer (with DTT) 43µL=1000µL

The types of reagents used in the automated protocol were as per the manual method. Both QHSS Manual and Automated DNAIQTM contained the Double elution method.

I am unable to answer more regarding the question due to not working for QLD Health any longer. I have no access to QLD Health documents and records since I am no longer employed by QLD Health (other than to those I can find on the internet), or access to the protocols from other laboratories in order to be able to compare protocols.

(ii) the DNA IQTM 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);

At the time of the report, the laboratories that performed an automated DNAIQTM protocol included
1. PathWest (Western Australia),
2. Forensic Science South Australia (South Australia), and

[redacted]

[redacted]

3. Centre of Forensic Sciences in Toronto (Ontario)

I am unable to answer more regarding the question due to not working for QLD Health any longer. I have no access to QLD Health documents and records since I am no longer employed by QLD Health (other than to those I can find on the internet), or access to the protocols from other laboratories in order to be able to compare protocols.

(iii) the CFS Automated Protocol; and

I am unable to answer this question due to not working for QLD Health any longer. I have no access to QLD Health documents or Perkin Elmer documents (other than to those available on the internet). Please contact QLD Health or Perkin Elmer to obtain a copy of the CFS automated protocol.

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

The Perkin Elmer document is a proprietary publication that is or was sold by Perkin Elmer as Perkin Elmer Part Number 8842157. I am aware that QLD Health obtained a copy of it but I am unable to have access to QLD Health documents and records because I am a retired former employee. Nevertheless, the document most likely would need to be released by Perkin Elmer directly.

(iv) the Manual DNA IQTM Protocol;

Amendments in the QHSS automated protocol compared to the validated manual method in Project 11, according to project 13 (August 2008)
Minor modifications included
* Increasing the volume of Extraction Buffer to 500µL
* A SlicPrepTM 96 Device (Promega Corp, Madison, W, USA) was used for sample lysis;
* Incubation steps and any shaking steps were performed on the integrated DPC shaker.
* CRS toroid magnet (P/N) 5083175) was used for isolating the DNA IQTM resin.
* Instead of a single elution of 100µL, a double elution method (2 x 50µL) is used.

Note
The reference to the CRS toroid magnet (P/N) 5083175) is missing in the documents below

Amendments in the QHSS automated protocol according to QIS document 24897V1 (23rd October, 2007, valid from the 24th of October, 2007) included
* The use of the SlicprepTM 96 device (Promega) for removing substrate from lysate.
* The increase of extraction buffer volume to 500µL for use with the SlicprepTM 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.

[redacted]

[redacted]

Amendments in the QHSS automated protocol according to QIS document 24897V3 (19/03/2008, Valid from 27/03/2008) included
* The use of the SlicprepTM 96 device (Promega) for removing substrate from lysate.
* The increase of Extraction Buffer volume to 500µL for use with the SlicprepTM 96 device.
* The use of tubes and spin-baskets for the off-deck lysis of samples prior to extraction on MPII. Use of a 96-deepwell plate for completion of extraction on MPII.
* The provision of initial incubation with TNE Buffer and retention of a portion thereof for further testing (retained supernatant testing).
* 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.

"manufacturer's instructions" refers to

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

Lysis Buffer volume to 957µL + Lysis buffer (with DTT) 43µL=1000µL

The types of reagents used in the automated protocol were as per the manual method. Both QHSS Manual and Automated DNAIQTM contained the Double elution method.

(p) state when the Automated DNA IQTM Protocol was so devised;

23 October 2007 : First issue of QIS Document Number: 24897 R0
Automated DNA IQ™ Method of Extracting DNA

Source:
https://www.dnainquiry.qld.gov.au/public-hearings/assets/exhibits/module-4/EXH%20129.4%20WIT.0050.0003.0001%20WIT.0050.0003.0001_R.pdf

It has undergone several changes since, including...
R2 19 March 2008 M Harvey, B Andersen, C Iannuzzi, A McNevin
Addition of Off-deck Lysis procedure, Retention of fully automated method as Appendix, addition of reagent record tables into Appendix
R5 03 June 2009 M Aguilera, B Micic, C Iannuzzi, A. Cheng, V. Hlinka, I. Muharam, G. Lundie, C. Weber
Major changes to reflect new procedure. Updated to reflect changes in procedure as an outcome of internal and external audits. Created ver.6.4 ODL in MPII Platforms. Minor changes in procedure using 4titude and 4seal heat sealer to seal plates.
R6 29 June 2009 A McNevin, K Lancaster Removed references to retaining lysate and beads, fixed minor formatting errors. Created ver6.5 ODL in MPII Platforms. Substrates now to be retained in 2mL tube.

(q) identify those within the Laboratory responsible for devising the Automated DNA IQTM Protocol;

[redacted]

[redacted]

As recorded in QIS Document Number: 24897 R0

23 October 2007 On-deck lysis
Thomas Nurthen, Senior Scientist, Automation and Laboratory Information Management System (LIMS) implementation project
Vojtech Hlinka, Automation Team Scientist;
Breanna Gallagher, Automation Team Scientist;
Cecilia Iannuzzi, Automation Team Scientist;
Generosa Lundie, Automation Team Scientist;
Iman Muharam, Internal Auditor QIS 8227/Automation Team Scientist/Senior Scientist Automation and LIMS implementation;

The method relevant to the Project 13 Verification was the On-deck lysis method using WinPrepTM software protocol version "DNA IQ Extraction_Ver1.1.mpt as referred to in section 5.6 of the document and detailed in QIS Document Number: 24897 R0.

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

Some of the reasons I am aware of include:

* There is no standard automated DNA IQTM method since they vary across different laboratories. Methods must be adapted to materials that are available and best work in a laboratory's flow and work processes while maintaining chain of custody, integrity and quality of data.
* Changes that were made were minimal. For example, the introduction of the Extraction Lysis step with a 500µL volume was necessary to submerge samples as much as possible to maximise DNA yield. It also enabled processing of different sample types using the same robotics method, rather than create multiple programs for different sample types that would have needed multiple validations and verifications.
* Support was provided by Perkin Elmer to automate the process on the Multiprobe II device based on success in CFS and other labs.

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

3. State when the Laboratory received the Multiprobe II Device.

To the best of my knowledge in 2006 or 2007, but I am unable to give specific details since I have no access to QLD Health records and documents, other than those already released on the internet, since I am not a current QLD Health employee.

4. For each of the Manual DNA IQTM Protocol and the Automated DNA IQTM 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).

3 methods were used in early 2008.

Methods employed on the automated platform in early 2008
(a) On deck lysis (this protocol is largely performed on the MultiPROBE II);

(b) Off deck lysis (this protocol is performed on the MultiPROBE II instrument with some additional tasks performed off the instrument).
(c) Manual DNAIQ extraction (this protocol is a fully manual task without the use of the MultiPROBE II).

Project 13 employed the WinPrep program DNA IQ Extraction_Ver1.1, which was the On deck Lysis Version (see QIS Document 24897V1).

Amendments in the automated protocol included:
1. Increasing the volume of Extraction Buffer to 500µL (Volumes of 300, 350, 400, 450 and 500µL were tested and compared). Refer to Table 7 (Project 13 Report) for results.
2. A SlicPrepTM 96 Device (Promega Corp) was used for sample lysis.
3. Incubation steps and any shaking steps were performed on the integrated DPC shaker.
4. CRS toroid magnet (P/N 5083175) was used for isolating the DNAIQTM resin.
5. Instead of a single elution of 100µL, a double elution method (2 x 50µL) is used.
6. The increase of Lysis Buffer volume to 957µL proportional to the increase of Extraction Buffer volume, according to the manufacturer's instructions (Promega Corporation (2006). DNA IQTM System - Small Sample Casework Protocol [PN TB296, Rev. 4/06]. Promega Corporation: Madison, WI.).

Lysis Buffer volume to 957µL + Lysis buffer (with DTT) 43µL=1000µL

The types of reagents used in the automated protocol were as per the manual method.

5. State when the modifications were made.

The automated method was published internally on the 23rd October 2007, QIS Document 24897V1
23 Oct 2007 B. Gallagher, T. Nurthen, C. Iannuzzi, V. Hlinka, G. Lundie, I Muharam.
Automated DNA IQ™ Method of Extracting DNA

Source:
https://www.dnainquiry.qld.gov.au/public-hearings/assets/exhibits/module-4/EXH%20129.4%20WIT.0050.0003.0001%20WIT.0050.0003.0001_R.pdf

By the 23rd of October, 2007, the 6 changes above would have already been made.

QIS Document 24897V3 (19th of March (Valid from 27th of March, 2008)) states the following modifications:
"Addition of Off-deck Lysis procedure, Retention of fully automated method as Appendix, addition of reagent record tables into Appendix"
My understanding is that these modifications from the 19th of March were not used for Project 13.

6. Identify those within the Laboratory responsible for the modifications.

23 Oct 2007 B. Gallagher, T. Nurthen, C. Iannuzzi, V. Hlinka, G. Lundie, and I. Muharam.

7. State the reasons why the modifications were made.

1. Increasing the volume of Extraction Buffer to 500µL (Volumes of 300, 350, 400, 450 and 500µL

[redacted]

[redacted]

were tested and compared).

Reasons
Refer to Table 7 (Project 13 Report) for results. Better DNA yield was obtained at 500µL.

2. A SlicPrepTM 96 Device (Promega Corp) was used for sample lysis.

This device allows the simultaneous centrifugation of 96 samples and is designed so that both the digestion or lysis and centrifugation can be performed in the same device, thus avoiding the transfer step bottleneck and potentially reducing the risk of contamination.

Reference:
SlicprepTM 96 Device
September 2005
High-Throughput Processing of Samples on Solid Supports Using the SlicprepTM 96 Device
By Allan Tereba, Julia Krueger, Ryan Olson, Paraj Mandrekar and Bob McLaren
Profiles in DNA. Promega Corporation

3. Incubation steps and any shaking steps were performed on the integrated DPC shaker.

Avoided samples being manually taken off the automated platform for incubation and shaking.

4. CRS toroid magnet (P/N 5083175) was used for isolating the DNAIQTM resin.

The function of a magnet was to provide a highly efficient capture of the DNAIQTM resin, most likely to better match the shape of the equipment being utilised. I cannot recall any additional information or verify whether this was indeed the magnet used with the Automation DNAIQTM Protocol or whether this is an error in the report.

5. Instead of a single elution of 100µL, a double elution method (2 x 50µL) is used.

The most efficient extraction from validation was a double elution.

6. The increase of Lysis Buffer volume to 957µL proportional to the increase of Extraction Buffer volume, according to the manufacturer's instructions.

To maintain the correct proportions according to the manufacturer's instructions. Manufacturer recommends twice the amount of Lysis Buffer to Extraction Buffer.

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

Lysis Buffer volume to 957µL + Lysis Buffer (with DTT) 43µL=1000µL

7. NUNC tubes were the standard tubes utilised at the time for elution and storage within QHSS.

2008 Report

[redacted]

[redacted]

8. Describe your role in the preparation of the 2008 Report.

I was away in June, 2008 and part of July, 2008 for a job interview in Kiel, Germany, so was not involved much in the writing of the report. I believe I viewed the report after coming back and asked some questions about it.

However, I was one of the key scientists involved in the development of the WinPrepTM software protocol for the Automated DNAIQTM method, including version "DNA IQ Extraction_Ver1.1.mpt as referred to in section 5.6 of the document and detailed in QIS Document Number: 24897 R0.

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.

I believe I was asked by Thomas Nurthen to read the report after coming back from my job interview in Germany.

10. 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.

I believe I was asked by Thomas Nurthen to read the report after coming back from a job interview in Germany.

I vaguely remember a discussion about some of the sample dilutions being possibly affected by a phenomenon known as "clumping," as mentioned in the Project 13 report. The discussion would have occurred in late July or early August 2008. I am unsure now if I discussed it with Iman Muharam or Breanna Ghallagher or another Automation Team scientist involved.

11. Identify the persons to whom was the 2008 Report was distributed.

To the best of my knowledge, after being read and approved by members of the Automation Team, it was initially distributed to management staff of the time, that most likely included Vanessa Ientile or Cathie Allen, and Thomas Nurthen. Our Automation Team Leader, Thomas Nurthen, would be more relevant at answering who the report was distributed. I believe it was eventually made available to all forensic DNA staff at QHSS who were working there at the time. I am unable to verify this or the names of all the relevant staff members since I am no longer an employee of QLD Health and have no access to records regarding this.

12. In relation to the matters stated in the 2008 Report, state:
(a) 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, including:

> (i) 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

I am unable to provide any documents regarding communications at my former workplace, due to not being a current employee of QLD Health. I have no access to records and documents at QLD

[redacted]

[redacted]

Health.

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

My understanding is that the sentence on page 1 of the 2008 Report

"Data indicate that results from the automated procedure are comparable to those from the manual procedure"

refers to the correct profiling results being produced with both the Manual DNAIQTM and Automated DNAIQTM. In other words, it refers to the positive cell and positive blood samples yielding the correct DNA profile results in both the Zebra and Checkerboard tests. It does not refer to the yield and sensitivity results from Project 13 in section 6.4.
The recommendations made in the conclusion on page 18 were also based primarily on obtaining the correct profile results in the study.

Both manual and automated methods gave sufficient quality DNA profiles, although yield and sensitivity appeared significantly lower for the Automated DNAIQTM method. My interpretation was that this could have been potentially partly attributed to sample "clumping" during preparation and dilutions of some of the samples as described in the Project 13 report, potentially skewing results for some samples in favour of the manual DNAIQTM.

Quote from Project 13...
"Cell clumping may have occurred with the cell dilutions, therefore causing inaccurate dilutions as can be observed in the ratios between each dilution. "

Of most importance to me, was the recommendation to continue development to improve the Automated DNAIQTM method in order to obtain higher yields.

Quote of recommendation from Project 13...
"*Ongoing development of the automated extraction program to increase the efficiency of the extraction"

Development and improvement of the method progressed since the Project 13 Report was written, as recorded in further projects and QIS Document Number: 24897V3.

I have confidence in the profiling reliability, accuracy and robustness of both the manual and automated DNAIQTM methods, despite lower yields for the latter in Project 13.

I am unable to provide detailed information regarding this since I am no longer employed by QLD Health and have no access to records and documents at QLD Health.

(b) how the recommendations summarised on page 18 of the 2008 Report were decided, including by identifying:

(i) your role in the decision;

It is my current understanding that my role in deciding the recommendation was minimal. Most of

[redacted]

[redacted]

the recommendations were most likely made by management staff like Thomas Nurthen (the leading author), particularly in pressure and response to get the Automated DNAIQTM method up and running as soon as possible to clear the immense backlog of forensic samples.

I believe I read the report in either late July or early August, 2008 and approved its publication, given that the data were presented and described as accurately as possible in order for management staff to make a decision on implementation, taking yield and sensitivity differences of the methods into account.

I am unable to provide detailed information regarding this since I am no longer employed by QLD Health and have no access to records and documents at QLD Health.

> (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.

The communications/discussions regarding Project 13 by me would have been primarily with members of the Automation Team in late July, 2008, early August 2008, including the Automation Team Leader, Thomas Nurthen, and others including B. Gallagher, C. Iannuzzi, G. Lundie, and I. Muharam.

I am unable to provide any documents regarding communications at my former workplace, due to not being a current employee of QLD Health. I have no access to records and documents at QLD Health. I also have only a vague recall of what was said in discussions from the time.

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. I make this solemn declaration conscientiously believing the same to be true and by virtue of the provisions of the Oaths Act 1867.

SWORN

TAKEN AND DECLARED before me at Ipswich this 24th day of October 2023

[redacted]

Dr. Vojtech Hlinka

[redacted]

Witness

JUSTICE OF THE PEACE (QUALIFIED) Reg.No.: [redacted] DEPT OF JUSTICE & ATTORNEY-GENERAL

[redacted]

[redacted]

Exhibits

1. QIS 24897V1

Automated DNA IQ™ Method of Extracting DNA
23rd October 2007 QIS Document 24897V1
B. Gallagher, T. Nurthen, C. Iannuzzi, V. Hlinka, G. Lundie, I Muharam.

Source:
https://www.dnainquiry.qld.gov.au/public-hearings/assets/exhibits/module-4/EXH%20129.4%20WIT.0050.0003.0001%20WIT.0050.0003.0001_R.pdf

2. QIS 24897V3

DNA IQ™ Method of Extracting DNA from Casework and Reference Samples
19th of March (Valid from 27th of March, 2008)
M Harvey, B Andersen, C Iannuzzi, A McNevin

Source:
https://www.dnainquiry.qld.gov.au/public-hearings/assets/exhibits/module-4/EXH%20129.4%20WIT.0050.0003.0001%20WIT.0050.0003.0001_R.pdf

3. Promega DNA IQTM System—Small Sample Casework Protocol
Promega Corporation (2006). DNA IQTM System - Small Sample Casework Protocol [PN TB296, Rev. 4/06]. Promega Corporation: Madison, WI.

Source:
https://www.dnainquiry.qld.gov.au/public-hearings/assets/exhibits/module-4/EXH%20129.4%20WIT.0050.0003.0001%20WIT.0050.0003.0001_R.pdf

4. Project 11. Report on the Validation of a manual method for Extracting DNA using the DNA IQ™ System August 2008

Source:
https://www.dnainquiry.qld.gov.au/public-hearings/assets/exhibits/module-4/EXH%20129.94%20-%20FSS.0001.0084.1400.pdf

5. 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)

Source:
https://www.dnainquiry.qld.gov.au/public-hearings/assets/exhibits/module-4/EXH%20129.95%20-%20FSS.0001.0084.1444.pdf

6. SlicprepTM 96 Device
September 2005
High-Throughput Processing of Samples on Solid Supports Using the SlicprepTM 96 Device
By Allan Tereba, Julia Krueger, Ryan Olson, Paraj Mandrekar and Bob McLaren

[redacted]

[redacted]

Profiles in DNA. Promega Corporation

7. Abstract of

Validation of the DNA IQ System for Use in the DNA Extraction of High Volume Forensic Casework
D. I. Komonski; A. Marignani; M. L. Richard; J. R. H. Frappier; J. C. Newman
Canadian Society of Forensic Science Volume: 37 Issue: 2 Dated: June 2004 Pages: 103-109
https://www.ojp.gov/ncjrs/virtual-library/abstracts/validation-dna-iq-system-use-dna-extraction-high-volume-forensic

Abstract
A modified DNA IQ™ System (Promega) protocol was validated for the rapid processing of a range of samples, including cigarette butts, gum, dried nasal secretions on tissue, swabbed drink containers and blood samples. Promega's standard Database Protocol was primarily designed for DNA extraction from blood stains. Modifications to this protocol were required to increase extraction efficiency from a range of sample types. These included: decreasing the incubation temperature; replacing the initial incubation in the kit's lysis buffer with an extraction buffer containing proteinase K; using a 25 µL elution volume; decreasing the number of resin washes from 3 to 1. Complete 9-locus STR (short tandem repeat) profiles were generated in most instances without the need for additional sample purification prior to amplification. In addition to being a fast and effective method of DNA extraction, the use of a single protocol for a range of samples makes the procedure amenable to automation.

References unable to be obtained:

1. PerkinElmer (2004). MultiPROBE II Liquid Handling Forensic Workstation Application Guide: Automated DNA IQTM System for Mixed Casework Sample DNA Isolation [PN 8842157]. PerkinElmer Life and Analytical Sciences: Downers Grove, IL, USA.
(Also referred to as the CFS Automated DNAIQTM Protocol/Method)

This is a proprietary publication that is or was sold by Perkin Elmer as Perkin Elmer Part Number 8842157. I am aware that QLD Health obtained a copy of it but I am unable to have access to QLD Health documents and records because I am a retired former employee. Nevertheless, the document would most likely need to be released and approved for public viewing by Perkin Elmer directly to avoid legal conflicts.

2. Project 9 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 (QHSS), Queensland Health: Brisbane, QLD, Australia.

Internal QLD Health Document. I am no longer employed by QLD Health and have no access to this document.

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