STATEMENT OF KYLIE DALE RIKA

I, **Kylie Dale Rika**, of Queensland Health Forensic and Scientific Services, Forensic DNA Analysis, at 39 Kessels Road, Coopers Plains, do solemnly and sincerely declare that:

1. I have previously provided two statements to the Commission of Inquiry which primarily relate to the 'DIFP process' and the Options Paper.

The 'work list' system

2. The DNA Analysis Unit at the Forensic and Scientific Services (FSS) operates on a 'work list' based system. In short, this means that currently:

    a. a sample is received by the lab and processed by the evidence recovery and analytical teams;

    b. the quantification value is measured, and:

        a. if the quant value is below the threshold of 0.001 ng/µL the sample is given a reported result of 'no DNA detected'.

        b. if the quant value is between 0.001ng/µL and 0.0088 ng/µL the sample is put on hold. I believe this hold will continue until the QPS and QH reach an agreement about how to process these samples further. Prior to 6 June 2022, these samples were reported as DNA insufficient for further processing.

        c. if the quant value is above the threshold of 0.0088 ng/µL the sample is processed fully and moves to the reporting team's work list in the Forensic Register.

    c. The reporting team members work through the list of samples to report, one by one, oldest to newest.

    d. The initial interpretation of a sample is called a "profile data analysis" or a "PDA". Once this is complete, the sample moves to the review list to be reviewed by another staff member.

3. The sample in the work list has minimal case context and the samples are not linked or grouped in any way. A scientist in the reporting team can access some data behind

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a sample and any notes recorded by the Queensland Police Service (QPS) in relation to the sample. Whilst reporting scientists can look at other samples in the case when performing the PDA of a sample off the list, the work list system is not, in my opinion, conducive to that. This often means that reporting scientists are interpreting each sample as an abstract task.

The difficulties with the current system

Samples arriving 'in-tube' from the QPS

4. The work list system evolved from a change in the laboratory in 2008 when the QPS began performing the majority of the item examinations and submitting samples to the lab 'in-tube'. In my view, when this change occurred, the lab went from being a forensic biology lab to a DNA profiling facility.

5. The current arrangement means that scientists lose the ability to identify the most likely biological source origin of the DNA, which is important in some cases. It also means that as the scientist no longer has the whole item, they cannot see if there are other stains that may be tested upon evaluation of the results.

6. I believe that a better model is to have the laboratory more involved in the collection of samples. This was the way ESR in New Zealand operated when I was there, and I believe they still operate in this way.

7. The change in the model to have the QPS examine items and send the majority of samples to us 'in-tube' was, I believe, to reduce the backlog of samples and improve turnaround time by making our work as automated as possible. The 'in-tube' model allowed the samples to arrive at the lab 'robot-ready'. The most time-consuming part of the lab's work was the examination of items.

Limited information provided to scientists

8. We have limited case context and the information we are provided in the Forensic Register is restricted to what the QPS feel is relevant.

9. Prior to the introduction of the work list system, a reporting scientist was given an entire case to work on. The scientist would often case conference with other relevant experts

and parties to discuss examination strategies to best address the allegations including testing options, sample selection and sample prioritisation.

10. Previously, upon receipt of a case, the scientist would also receive QPS paperwork (QP127 – Submission of Articles for Forensic Examination) which provided a lot of information required by the scientist to undertake testing in a way that would best address the allegations. For example, the QP127 paperwork provided information about the allegations, who the suspect was, the relevance of the items, where they were located, whether ambulance officers arrived (which would help us to determine whether we should be expecting to see their DNA), and whether relatives might be involved (which might explain contribution from relatives). This meant that a scientist had the context of the case, knowledge of all the available samples and would be able to make forensic decisions based on that information.

11. While the QP127 paperwork still exists, reporting scientists ordinarily do not receive it. This means that we do not receive that additional useful information, unless the QPS have thought to provide additional information in that instance.

12. Regularly there is limited information available to us in the Forensic Register. We have access to offence type (burglary, weapons offence etc), sample type (swab, tape lift etc), complainant name, photograph of an envelope, sometimes a photograph of an envelope, sometimes a photograph of the item the sample was taken from and a brief item description. We are also able to see the biological fluid screening tests done by the QPS.

13. In sexual assault cases, we are often provided additional information through access to Sexual Assault Investigation Kit (SAIK) medical notes and the QP127. This is helpful as it often provides a brief summary of the offence which can aid in interpretation and provide context for why the sample is relevant to the case. Any information as to the reason the sample is important to the case could aid us with reworking the sample to get the best result.

14. One area that would be useful to have further information in most cases would be information on ownership of items. This means it would be noted if a shirt is taken from Suspect A or underwear worn by Complainant. This would allow us to condition these samples once a reference person sample is submitted. Known ownership can help

determine the number of contributors and conditioning on a sample can produce more information for the DNA profile contribution that is of interest to the QPS. Sometimes we are provided with ownership information and other times we are not. Sometimes even when we are provided ownership information, there can be confusion as to what the information means. For example, sometimes the QPS officer will tick the victim ownership box because they are hoping to find the victim's DNA on the item, but the reporting scientist interprets the ticked victim ownership box as meaning the item belongs to the victim.

15. Other members of the reporting team and I contact the QPS via the Forensic Register several times a week to ask for further information about certain samples and items, including the availability of reference samples for comparison and ownership of items for conditioning purposes.

16. The work list system results in scientists processing and analysing a large proportion of samples without the case context necessary to strategise and prioritise testing.

17. To address the issues that the work list system presents, some team members in the reporting team keep their eye out for large and/or complex cases and allocate all of the samples related to that case to themselves. This is an informal arrangement. Because the lab is alerted to the receipt of a Priority 1 case (which are usually large and/or complex) before it arrives, all Priority 1 cases are allocated from the outset to a reporting scientist and reviewing scientist. This means that Priority 1 cases have continuity of care and consistency in the case management and interpretations conducted.

Issues with the Forensic Register

18. There are some aspects of the Forensic Register that do not suit the laboratory.

19. As far as I am aware, we are not able to data mine from the Forensic Register without going through the QPS and Bdna (the company which owns Forensic Register). Previously, using AUSLAB, we were able to extract data to use to analyse our results and report back to the QPS about sampling methods and success. I know that I am not able to extract data from the Forensic Register. I am not sure if someone in a higher position is able to obtain this.

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20. If I was able to data mine the Forensic Register I would use it to improve practices both internally and collaboratively with all of our clients, including the QPS. I would be able to gather data around such things as: DNA profiling success rates from various items and samples, result amendments, client requests for external testing (demand for other services), specific and detailed turnaround times etc.

Internal database

21. There is an automatic internal database search function within the Forensic Register. When a scientist at FSS DNA Analysis interprets a DNA profile and puts the DNA profile of a contributor in the profile record table, the Forensic Register automatically searches an internal police database that is made up of person DNA Intel samples, staff elimination profiles from both QPS staff and lab staff. It also contains unknown profiles identified via contamination events.

22. It is my understanding that part of the reason this tool was added to the Forensic Register was so that QPS could see any links to people sooner than waiting for the proper process of links via the NCIDD which has many quality checks in place to ensure links are valid. I am uncertain if the QPS can see the internal link prior to results in the Forensic Register being validated, but I do recall one time having a meeting with Troy O'Malley where he informed us that he had seen an internal database link and wanted us to report it through the official NCIDD process urgently.

23. The internal database search makes me feel uncomfortable as the search only looks at the string of numbers in the profiles and does not compare the EPG of the probe profile with the EPG of the matching profile, which is best practice when comparing DNA profiles to ensure that the visual representations of the profiles match.

24. I raised my discomfort about the internal database search with Justin Howes in or around mid-2017, and he said words to the effect of "I know, but sometimes you have to accept that decisions are made at a higher level."

25. I recall there being an issue with the QPS having access to parts of the Forensic Register that should not have been available to them, particularly unvalidated results.

26. There was a meeting in or around mid-February 2020 that was held with Cathie Allen, John Doherty and the staff in the lab, where this was discussed. I recall that some staff

members were not happy and said that the possibility of the QPS having unauthorised access to our work should have been addressed earlier as part of implementation. Since the implementation of the Forensic Register, staff have had concerns about what the QPS has access to.

27. I remain concerned about whether the QPS have access to unvalidated results. What the QPS do and do not have access to has never been clearly explained to scientists, despite concerns and queries being raised with management.

Expectations of reporting scientists

28. Reporting scientists at FSS DNA Analysis do not have a quota to meet but there is an expectation that they will complete one profile data analysis (PDA) and one review per hour.

29. This expectation was delivered to staff by Justin in a FRIT meeting on 16 January 2019. Annexed and marked KR-01 is a copy of the meeting minutes.

30. In my opinion, this expectation would only be reasonable if:

    a. the sample and/or profile is simple,

    b. the scientist does not have other priority tasks for the day (such as court, urgent statement etc.), and

    c. the review discussions between PDA entry person and reviewer are not extensive.

31. In my opinion, there is a focus on quantity over quality by management at the FSS DNA Analysis. All processes are designed to ensure efficiency, rather than obtaining the best outcome for all clients. This is demonstrated by the work list system and expectations on scientists. Staff are often praised on their quick work and their individual tallies.

Validation at the laboratory and related issues

32. The lab uses a program, STRmix, which can be sensitive to changes in variables and so end to end validation is important when a process change can affect the final DNA

profile. If the validation is not performed correctly, STRmix may place undue weight on particular genotype combinations, especially if they involve peaks in stutter position.

33. I have seen management, at times, not do what I would consider be enough when validating/verifying new parts of the system. It is the responsibility of the management team to sign off on what will be validated. There are variables along the entire process that need to be taken into account before a new machine or model is implemented. Only examining the part of the process being altered results in a quicker validation but can also result in issues being discovered later.

34. I specifically recall there have been issues with:

    a. the PP21 validation,

    b. the verification of Proflex for PP21, and

    c. the introduction of DNA IQ.

PowerPlex 21 (PP21) validation 2012

35. I was on maternity leave when PP21 in combination with STRmix was validated and implemented. When I returned, I did not receive any formal training, but was given some PP21 profiles and was told to have a look at them and that it would take me some time to adjust. I was assigned a mentor to help me navigate the case management processes as part of a refresher training.

36. We previously used Profiler Plus. When we moved to PowerPlex 21 the profiling kit looked and behaved very differently to Profiler Plus. As a result, it took a long time for the reporting scientists to adjust to the new profiling system.

37. PP21 was validated in 2012 and the laboratory was still having issues with it in March 2014. The main issues at that time were around stability and what appeared to be significant stochastic variation which led to issues with profile interpretation. Other issues included repeatability, allelic drop out and reproducibility of minor contributors of profiles. All of these issues were making the intuitive check of DNA profiles by scientists very difficult and time consuming.

Verification of Proflex for PP21

38. When the lab replaced the thermal cyclers with a new model known as the Proflex, Luke Ryan and some of his staff prepared an Experimental Design for the verification of the Proflex's for PP21 and requested my feedback. In my view, we needed to do much more than what was proposed to validate the Proflex.

39. In my consideration of what we might be missing with this verification, I consulted our labs STRmix subject matter expert, Emma Caunt who suggested that we get advice from the STRmix support group to see if we needed to do Model Maker again.

40. Emma also told me that we probably should not just rely on intuitive qualitative comparisons, rather, we should perform some kind of statistical analysis. Further, she suspected that we may need to perform further work to re-do our baseline, stutter and assess locus efficiency. I included Emma's suggestions in my feedback to Luke in my email dated 23 March 2021, which is annexed and marked KR-02.

41. Luke asked me to co-ordinate obtaining the STRmix advice I suggested. At my request, Emma contacted STRmix, and obtained advice that we needed to do further work with Model Maker as a result of the implementation of the new Proflex. This would involve a considerable amount of further work.

42. The email chain regarding my feedback on the Experimental Design for the verification of the Proflex thermal cyclers for PP21 and the advice from STRmix is annexed and marked KR-03.

43. Justin and Paula both emailed me to suggest that the Model Maker work form part of the implementation rather than the verification. Annexed and marked KR-04 are the associated emails.

44. On 30 April 2021, I received an email from Luke with the final version of the experimental design for Proposal #199 - Verification of Proflex. This final version did not include the Model Maker work and in Luke's email he mentioned that STRmix trainers have recommended Model Maker be performed as part of implementation, separate to this verification.

45. On 12 January 2022, I emailed Justin to let him know I was going through the minor change register to make sure I had communicated all of the recent changes to my team and I noticed with Project 199 Proflex (implemented 10 January 2022), there

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appeared to be a gap in finalisation regarding Model Maker. I further mentioned that when looking at all of the project records for this project, I could not work out whether the Model Maker work had been done or not. Justin replied to me the same day to let me know that yes, this component of implementation had been missed. I then asked if we would be putting a hold on samples running through the Proflex until Model Maker was run and findings assessed. He did not think that was necessary. Annexed and marked KR-05 is a copy of the email thread.

46. This example demonstrates the danger in moving important components of testing to the implementation phase, as it can easily be missed. In my view, the management team wanted a quick sign off on the verification (although I am not sure why) but in doing so, missed an important element.

47. As a result, I believe we are using a system that hasn't been properly verified. Proper validation of procedures used in forensic DNA analysis is essential to ensure that reliable results can be obtained with a particular method and associated materials.

DNA IQ issue

48. In or around 2007 there was an issue with contamination in the lab, which seemed to coincide with when we started using DNA IQ extraction chemistry in combination with the Perkin Elmer MultiPROBE II PLUS HT EX with Gripper Integration Platform for routine processing in October 2007. There was a large and laborious investigation to determine the cause of the contamination. Eventually it was discovered that the seals and the resin mixing procedure relating to the automated DNA IQ extraction procedure were leading to the contamination.

49. Once the laboratory had discovered the issue, the scientists had to retrospectively assess all the samples that were processed with the relevant unsuitable seals and resin mixing procedure. The issue affected many batches of samples and there was a large amount of work to do to assess which samples had to be failed because we were not confident about the integrity of the results.

50. The first batch of samples affected were DNA extracted on the 25 February 2008. Further, samples run through automated DNA IQ extraction from October 2007 until processing was halted on 28 July 2008 were potentially affected.

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51. There were approximately 18 extraction batches affected, and if we assume that each plate was full, that is about 80 samples per plate excluding controls. So potentially up to 1500 samples were on batches that were known to be affected. There were many other batches that were unaffected but still had to be investigated.

52. There were 674 samples that were on NCIDD that had to be withdrawn. These were only made visible once each had been checked and cleared against their batches thoroughly.

53. In a management meeting on 28 July 2008, it was decided to pause processing using the MPII machines due to contamination concerns. In this meeting, the management team had decided to halt testing and investigate the potential cause of the contamination events.

54. Cathie asked each person in the meeting what they thought the cause was and what we thought a solution would be for those samples affected. She left the room for a period of time so we could think about options. She came back a while later and the management team had said they thought that re-extracting the spin basket was the best option. Cathie said that it would cost a lot of money and that if anything was mentioned from higher up in management regarding this, she would direct those people to us managers, as it was our decision. I remember being surprised and frustrated that cost seemed more important than quality.

55. On 10 September 2008, all DNA IQ results were put on hold. We were still expected to release results up until September 2008, even though testing had halted in July 2008.

56. The laboratory knew about the contaminations at least from early February 2008 to the end of June 2008 (by reference to OQIs around the time) without a comprehensive investigation occurring. It took until January 2010 for the lab to return to using DNA IQ extraction chemistry in combination with the Perkin Elmer MultiPROBE II PLUS HT EX with Gripper Integration Platform.

57. I recall that it was a large QPS operation, called Golf Alcove, that led to the discovery of the contamination.

58. The lab was required to send out communication to the QPS, the Office of the Director of Public Prosecutions and the Courts about the issue.

59. This issue may not have been picked up earlier because the new DNA IQ products were not adequately validated prior to implementation. The validation of DNA extraction using the DNA IQ extraction chemistry conducted on the Perkin Elmer MultiPROBE II PLUS HT EX with Gripper Integration Platform was not sufficient and needed to follow guidelines at the time described by J Butler (www.promega.com). A review of this procedure was conducted by external reviewers Dr Sloots and Dr Whiley and I agree with their recommendations. Annexed and marked KR-06 is a copy of that report.

Sperm microscopy

60. In 2016, some reporting scientists raised concerns to Amanda Reeves and myself of their observations of differences between microscopy slides prepared at the examination stage compared to those prepared during the DNA extraction process. In particular examples where nil or <1+ sperm were observed during item examination and 3+ or 4+ sperm were observed at the extraction stage microscopy.

61. From a reporting perspective, Amanda took the lead in raising the issue to the management team, I believe at least a couple of times. On one such time, I witnessed Allan McNevin slam his hands on the table, push himself back in his chair and yell at Amanda.

62. Ultimately, Amanda took a period of stress leave. When she returned, she was given a research task at a desk in the library, rather than return to sit with her team in Reporting.

63. The way that Amanda was treated after raising issues with sperm microscopy caused me significant concern. After witnessing what Amanda experienced, I have been hesitant in raising concerns that I have.

64. From what I saw, I do not think the issue of sperm microscopy was dealt with appropriately from a cultural point of view and further, from a scientific point of view, it took a long time for the issue to be addressed and resolved. Eventually, project 181

adequately addressed the issue from a scientific point of view due to the significant contributions and expertise from reporting scientists Matthew Hunt and Emma Caunt.

65. I have not seen the ESR report that the Commission of Inquiry has mentioned to me.

66. I am not sure if samples that may have been affected by the issue have been retrospectively investigated.

67. I believe it was in 2017 sometime that Paula Brisotto asked Matthew Hunt and me to help her with a data analysis she was doing to see what impact the issue may have had on cases in terms of evidential outcomes. The aim being to provide a report that may alleviate reporters concerns. I believe Paula wrote a draft report and Matthew and I provided a lot of feedback on it. The report went back and forth between Paula and Matthew and me. In the end, Matthew and I could not support the assertions and justifications being made in the report. I recall at some point that Paula suggested another set of eyes may help and asked Luke to review the report. I can't recall what happened after that, but I do not think any final report was produced.

Management of the laboratory and culture

68. In my opinion, there are some scientific issues as well as cultural and managerial issues within the FSS DNA Analysis Unit which cause difficulties in the workplace. In my experience, when staff members have raised issues or concerns, they have often been met with less than a positive response from FSS DNA Analysis management. It appears to me that as a consequence staff raise less issues with management.

69. I am aware that many staff fear reprisal action if they make a complaint about their dissatisfaction with a decision or with how something was handled. This relates to both human resources and scientific issues.

70. When I first met with staff at the Commission of Inquiry in July, I arranged to do so at a local council library on the weekend. I did this because I was concerned about the potential ramifications for me if management were to find out that I was speaking out about my concerns.

71. I have raised my concerns about the management of the laboratory and the cultural issues with senior management and human resources managers of FSS for many years.

72. I often feel isolated and disempowered by other staff in management. An example of unnecessary and inappropriate action towards me was when Cathie requested to meet with me to discuss "a workplace matter relating to compliance with workplace record keeping practices in which you may have further information and or have been involved". I found Cathie's email to be intimidating. Annexed and marked KR-07 is a copy of the email Cathie sent to me on 30 April 2018.

73. Given the toxic environment at the time, and after seeing what Amanda had endured, I felt the need to engage my Industrial Advocate, Brian Newman. Brian informed Cathie that he could not make it to the meeting on the date and time proposed and also requested a clear agenda be provided prior to the meeting. Despite the request, Cathie did not supply the requested agenda or provide particulars of what would be discussed. Annexed and marked KR-08 is a copy of the email chain between Cathie and Brian.

74. On 3 May 2018, the meeting took place with Brian present as my support person. Cathie and Therese O'Connor from Human Resources also attended. In the meeting Cathie asked me if I had seen anything go into the confidential bin that should not go in there on the day that Amanda packed up her belongings in the office space to leave FSS DNA Analysis. I said I did not recall seeing anything go in the bin that should not go in there. This was the only topic discussed in the meeting.

75. I believe the only two other staff members questioned on this were also friends of Amanda, Emma Caunt and Ingrid Moeller. To this day, I have had no follow up from Cathie on this matter, which has caused me stress.

76. The stress I experience at work has had a significant effect on my attendance at work, anxiety, confidence, and health.

Complaint to Michel Lok on 12 December 2017

77. On 12 December 2017, I sent an e-mail to Michel Lok, informing him about an incident involving me and Cathie on 7 December 2017. After Mr Lok's acknowledgment of my initial e-mail, I did not receive any further response from Mr Lok or anyone else in

relation to the incident I raised. Annexed and marked KR-09 is a copy of the email thread.

78. I raised this issue with Mr Lok as I had heard from Andria Wyman-Clarke (the Human Resources manager) that Mr Lok was a good man who would help our lab. I was hoping that Mr Lok could do something to bring about a better workplace culture in our lab. I thought that a first step would have involved meeting with me and Cathie to discuss the issue.

79. As far as I am aware, nothing was done in response to the incident I raised or this email I sent to Mr Lok.

Workplace Edge investigation

80. Between October 2017 and January 2018, an external investigation was conducted in the workplace by Workplace Edge. I was made aware of the investigation by an email from Mr Lok which explained that he had engaged Workplace Edge to assist us to restore usual business practices and maintain professional relationships within the DNA Unit.

81. I participated in an interview with Allan Holz from Workplace Edge sometime during the investigation. During this interview I was asked questions about Amanda Reeves and Allan McNevin, their character, my relationship with each of them and what I had witnessed in relation to the incident that occurred between them.

82. On 23 January 2017, Paul Csoban and Cathie delivered a presentation about the concerns discussed between interviewed staff and Workplace Edge. Prior to the presentation I met with Paul Csoban and Allan Holz where they explained to me what was going to be in the presentation. I was told that my 'nexus' with Amanda was a problem for staff. I found this meeting distressing and felt a lack of support and communication from Paul and Allan. I felt that they had not listened to me and other staff about the concerns we had raised, and they had not attempted to understand the cultural issues in the lab. This was the only 'one-on-one' style meeting I had with Paul while he was Executive Director.

83. Paul and Cathie's presentation to the reporting teams' staff detailed the themes gathered from Workplace Edge's investigation. They presented a plan to move forward

to "heal the team" and "gain better functioning". This was said to be based on the staff's feedback.

84. My impression of the presentation was that they had not listened to the concerns that had been raised by me and other staff. I recall during the presentation to staff that Paul said something along the lines of "be careful what you wish for". There was an implication that we needed to get back to work and stop complaining.

85. During the presentation it was said that someone would get in touch for the "plans for healing" but no one got in touch with me about this. I recall that I followed up with Justin a few weeks later about when this was going to happen, but I do not recall hearing anything further.

86. On 30 and 31 January 2018, I sent e-mails to HSQ executives (Michel Lok and Peter Bristow), informing them about the staff feedback I had received following the presentation on 23 January 2017. I did not raise these concerns with Paul Csoban because it had become clear to me that Paul was a firm supporter of Cathie and thought that the primary problem in the lab was Amanda.

Raising concerns to John Doherty

87. While John Doherty was Executive Director, I regularly raised concerns that I had about the lab with him. The main concerns I discussed with John were:

    a. The difference of opinion that there was in the lab about the Limit of Detection (LOD).

    b. Communication issues I was having with members of the management team, particularly Justin.

    c. The difficulties staff faced in having their flexible working arrangements approved.

88. I met with John in person or via teams on approximately 10 occasions to discuss the above issues and general cultural problems that existed in the lab. To arrange a meeting, I would usually send him an email forwarding concerns and request a time to chat.

89. At one point John explained to me that I had the option of putting in a grievance. I said that I was nervous about that because I was worried that things would be turned back on to me. John then explained that from his experience, it is a stressful process for the person that raises the grievance and often can come back on the side of the manager. I chose not to put in a grievance.

90. John was very receptive to the concerns that I raised. I felt that he tried very hard to improve the issues in the lab, but I ultimately got the impression that he felt that problems were unfixable.

91. When I would raise concerns about Cathie and Justin, John would often ask me if I was comfortable with him raising it with Cathie and Justin. I understand that he then did raise issues with Justin and Cathie because he would sometimes report back to me things such as "I haven't been able to get them (meaning Cathie and/or Justin) over the line but leave it with me".

Difference of opinion about the Limit of Detection

92. During the time that John was Executive Director, I had a disagreement with Allan McNevin and Justin Howes about the LOD at the lab. This arose when I provided feedback to Allan McNevin in my role as reviewer with regards to a DNA profile analysis (PDA) he had conducted. I disagreed with his call of "3-person mixture" as I argued that there was an extra peak just under the LOD, indicating the possibility of more than 3 people. I understand that Allan spoke to Justin about the disagreement in interpretation which eventually led to Justin directing me to not consider the peak below LOD in my review of Allan's interpretation.

93. It was not until I continued to follow up the matter with Justin and Allan that I was told that I was no longer required to be part of the interpretation of the sample. I later found out that Allison Lloyd reviewed the matter instead. Other than a response that we needed to follow the Standard Operating Procedure, it was not communicated to me why my views were no longer being considered or what could be done to resolve the disagreement. Annexed and marked KR-10 is a copy of the email threads and associated documentation.

94. Ultimately, the final DNA result reported on the sample was a "4-person mixture", which was different to Allan's initial interpretation that I disagreed with and more in line with what I had suggested. What happened with this interpretation was never explained to me by Justin

95. I felt that my views were not given as much weight as Allan's views, and I thought that there was a legitimate basis for them which at least warranted consideration.

96. I first raised this issue with John on 3 May 2020 when I sent an email to him about some concerns I had with Justin. The first concern listed relates to the difference in opinion about the LOD. I indicated to John that I felt bullied. John responded providing some assistance with how to deal with Justin. Annexed and marked KR-11 is a copy of the email thread.

97. John and I later met and spoke about the concerns I had raised in the meeting with him on MS Teams. He recommended that I keep talking to Justin about it and continue to challenge others where I thought that the science was out of date.

98. In June 2020 I sent a follow up email to John as I still held concerns about the way in which my review of Allan's interpretation was being handled by Justin and that lack of communication I had received from Justin about it. John and I arranged by email to meet to discuss the issue. Annexed and marked KR-12 is a copy of the email thread.

99. On 22 June 2020 I met with John to discuss the LOD and threshold issues. Following the meeting with John, I sent a paper to him in relation to the LOD that we had discussed. Annexed and marked KR-13 is a copy of the email and attachment.

100. During one of the meetings John said he was considering getting someone "like Kaye Ballantyne" (who is a well respected forensic scientist in Victoria) to do a "health check" on the science at the lab. I did not hear anything further about this.

101. I ultimately stopped pursuing a potential change to the way the lab handles peaks below the LOD, because I felt that I did not have the required support from the rest of the management team. I felt that others were not willing to support me or even give my concerns proper consideration. I sent an email from Justin on 26 August 2020 advising that I was no longer going to pursue the issue and that the experience had left me feeling unsafe to raise further issues and ideas. Justin responded assuring me that it

was a safe working environment. Annexed and marked KR-14 is a copy of the email thread.

Raising concerns to Lara Keller in 2022

102. In late 2021 and early 2022 I raised concerns with Lara Keller about the DIFP process and the general cultural problems that I believed existed in the lab. I was hoping that Lara would take action to improve the culture in the lab and potentially change or, at least, challenge the DIFP process.

103. I met with Lara approximately three times in person to discuss my concerns. We first met at the end of 2021. When we met to discuss the issues with the DIFP process I took the spreadsheet of examples that I had been collating where DIFP was processed and gave a good result. I handed this document to Lara and she also took some notes of things I raised.

104. Approximately two weeks later, I requested a further meeting with Lara. In this meeting I gave Lara all the documents I could find around project 184. Lara said she thought we should raise a PID (Public Interest Disclosure). I said that I was scared but I agreed that it was the right thing to do.

105. A number of weeks later, I met with Lara about a number of things, including further paperwork I had found about project 184. She let me know that Ethical Standards had assessed the information and decided that the issue did not meet the PID requirement. I felt disheartened and concerned that I had wasted Lara's time. Lara said that it would all come out in the review into the lab (which was planned at that stage – prior to the announcement of the Commission of Inquiry).

106. I do not know if anything else came of the concerns I raised with Lara.

All the facts and circumstances declared in my statement are within my knowledge and belief, except for the facts and circumstances declared from information only, and where applicable, my means of knowledge and source of information are contained in this statement.

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

TAKEN AND DECLARED before me at Brisbane in the State of Queensland this 6th day of October 2022.

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

Kylie Dale Rika Witness Eleanor Lynch

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

Kylie Dale Rika Witness [redacted]

KR-01

HealthSupport Queensland

Minutes

Forensic Reporting and Intelligence Team Meeting

Date: 16 January 2019
Time: 9 am
Venue: FSS CR102

Chair: Justin Howes (JAH)

Secretariat: Justin Howes (JAH)

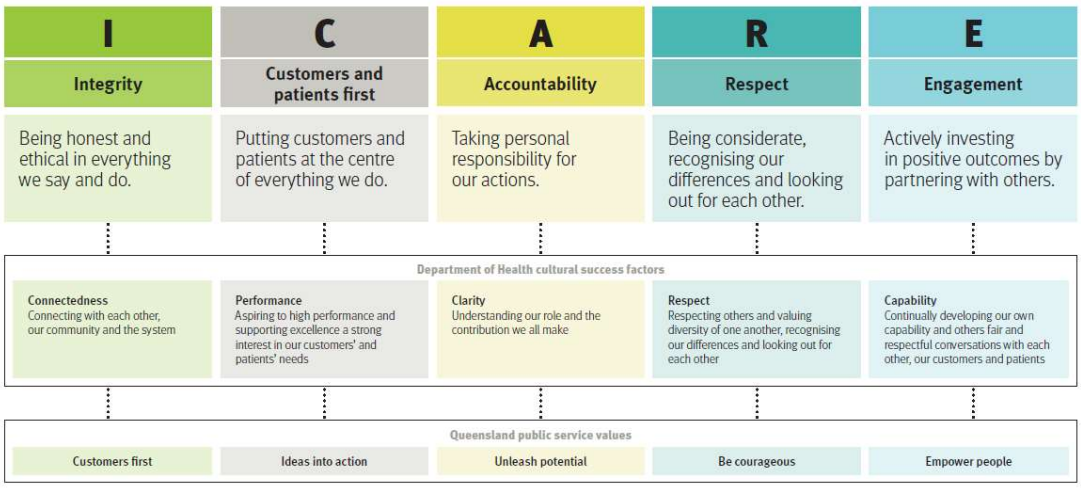
Attendees: Adrian Pippia; Alicia Quartermain; Allison Lloyd; Emma Caunt; Ingrid Moeller; Josie Entwistle; Jacqui Wilson; Cassandra James; Claire Gallagher; Angela Adamson; Penelope Taylor; Sharon Johnstone; Kylie Rika

Apologies: Hannah Pattison Anne Finch; Deborah Nicoletti; Rhys Parry; Thomas Nurthen; Matthew Hunt; Angelina Keller

Guests:

1.	
Item no	**Item**
1.1	Sad news Our thoughts are well and truly with Suzanne and her family, and specifically the family of Danielle. Condolence card has been sent from Forensic DNA Analysis and more things may come forward on how to help the family at this tough time. I am sure many of you knew Danielle as well. If anyone needs any help at this time, and for that matter any time, please remember you can contact Benestar to assist. https://qheps.health.qld.gov.au/hr/staff-health-wellbeing/counselling-support/department-of-health
1.2	**STRmix v2.6 validation and implementation** Follow up on some aspects to implementation of STRmix v2.6: ○ Thankyou for your efforts in completing your training. ○ Recall Kylie's email to staff yesterday and information at FRIT meeting 07 December that enquiries should go to line manager as first port of call.

	○ Samples are coming through now. Remember, you will get used to the new run times and if you come to any efficient work practices that could help others, please share. ○ Tom and Angelina's PCs have been upgraded, with Claire, Deb and Hannah's marked for upgrade with Erin. Spare PC in front bay will stay as v2.0 ○ Reminder to add justification to CMPU to the Sample Notes. ○ This will assist staff coming to the samples in the future to work out why the result was CMPU, including yourself leading to statement stage. ○ Staff are increasingly providing feedback on efficient processes to managing samples on the list. Several staff are taking the initiative to allocate the case to themselves (at PDA entry and review stages). This is encouraged by the senior group. ○ Promotes consistency in interps ○ Limits the amount of case familiarisation that occurs if taking random samples ○ Promotes variability as a case involves MIX and High Throughput ○ Leads to allocation of statement and decreases need for further interpretation at statement stage due to the fact that the interp has already been performed by themselves ▪ Reduces potential for 'incorrects' ▪ Facilitates the auto-statement approach ○ Gives ownership, responsibility and accomplishment when the case is finalised in the one hit. ○ Throughout standard profile management, it is accepted that there may be some reworks that you might not have ordered with STRmix v2.0. ○ Having said that, keep thinking on the point of some reworks. If you have a profile that is 2/3p and the probative component is the main component of the mixture (eg. Conditioned is in the minor component) then think about the value of a rework. ○ With 4p, we may need to rework what used to be CMPU. Hopefully you will learn what is working and what is not adding value when reworking these. Your feedback to others would assist here.
1.3	**<u>Targets for 2019</u>** <u>This applies to PDA entry and review only. All priority and excludes CW+Ref.</u> It is clear that having goals/targets will assist with meeting client demands, staff satisfaction and sense of completion. It also helps with taking responsibility for the work and being accountable for our actions including the work output. This is in line with feedback from staff surveys and the HSQ ICARE fundamental principles. I have had a look at the 2018 data in the FR (not the "batch, reviewed" section of FR, but a separate team output indicator that measures samples on lists and interps released) and discussed with the seniors. I think there is enough data now to get some measures around what we have been receiving for interpretation. In order to set up some goals/targets for everyone to work towards, we have looked at how many hours in the week we have available to work. ○ Factoring in hours per week (this way, p/t and f/t is accounted for), and assuming 60% of the time is allocated to PDA management (40% to statements, plate reading, other), we can see that we roughly have the same amount of hours available for the incoming samples for interpretation (that also need to be released): ○ approximately <u>300 per week for interp, and 300 for review</u>.

	○ It is known that what lands on a SS list may not be SS etc.. but gives an approximation that we can work with. There will be times when we will ask for more than meeting the incoming, but with STRmix v2.6 we have introduced a new factor that we will have to see how it goes. The workflow changes including interpreting with allele-specific stutter values, time to possibly transfer to v2.6, time to interpret and review 4p, but all balanced by the time saving approach with batching. Seniors will continue to allocate samples for the time being as this has received positive feedback. In doing so and in combination with the initiative in allocating the case to yourselves if other samples in the case could be released, we hope this 'ownership' approach transfers to the output. We will also be looking more towards the incoming at ER and through to AS as this will assist in predicting what might be landing on lists week to week, and then inform the plan for the next week. All of this together will help the work unit attempt to meet the client demands of 10 day TAT (that we have previously discussed). Further enhancements to statements to reduce the time in statement writing and reviewing are still with Forensic Technologies. Targets and goals are all about having something to strive for. If we think about what we might do over the course of say a month – first week targets not met but a blue folder case released, the next week targets exceeded because minimal work on statements etc, third week, targets not met due to court requirements etc…, fourth week targets met or exceeded due to a good run with minimal distractions. Over the month, it works out that weekly targets met. 
1.4	
1.5	

KR-02

Kylie Rika

From: Kylie Rika
Sent: Tuesday, 23 March 2021 2:40 PM
To: Luke Ryan
Subject: RE: Proposal #199 - Verification of Proflex for PP21

Hi Luke

Feedback as follows:

I think we need to do more than what is currently listed in the proposal. From the ENSFI guidelines I note that for a new thermal cycler model, it is suggested that a number of samples previously profiled are repeated using the kit in question. They further recommend that the selected samples will obviously allow examination of parameters such as reproducible results, including from mixtures and low DNA concentration samples. For a new thermal cycler that is of the same model as others already in use, a certificate from the manufacturer detailing a technical performance check done after installation in the lab and an internal sensitivity and homogeneity check would be sufficient.

I note from thermofisher that the ProFlex and 9700 are different models. Therefore, we need the following minimum parameters to be validated :

- Sensitivity (limit of detection) : a series of dilutions tested in three replicates.

- Repeatability : the three replicates of the same sample, distributed over the entire heating block can be used to evaluate the repeatability.

- Reproducibility : 3 repetitions of the amplification reactions used in the sensitivity test.

- Homogeneity of heating block : temperature control of the heating block or a comparison of the replicates allows the evaluation of the homogeneity of the heating block.

I also think we need to get advice from the STRmix support group to see if we need to do MM again etc..

I don't think we can rely just on intuitive qualitative comparisons, we need some form of statistical analysis. I suspect we may need to re-do b/line, stutter, assess locus efficiency (APH for each sample across each locus) etc

Thanks
Kylie

From: Luke Ryan <[redacted]>
Sent: Monday, 22 March 2021 12:23 PM
To: Allan McNevin <[redacted]>; Allison Lloyd <[redacted]>; Cathie Allen <[redacted]>; Justin Howes <[redacted]>; Kirsten Scott [redacted]; Kylie Rika <[redacted]; Paula Brisotto [redacted]>; Sharon Johnstone <[redacted]
Cc: Generosa Lundie <[redacted]>; Megan Mathieson [redacted]
Subject: Proposal #199 - Verification of Proflex for PP21

Afternoon All
Please see the attached Experimental Design for the verification of the Proflex thermal cyclers for PP21. Can you please provide feedback by COB Monday 29/03/2021.

Thanks
Luke

Luke Ryan
Senior Scientist – Analytical Team

Forensic DNA Analysis, Forensic and Scientific Services
Health Support Queensland, Queensland Health

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e [redacted] w www.health.qld.gov.au/healthsupport/businesses/forensic-and-scientific-services

Integrity | Customers and patients first | Accountability | Respect | Engagement

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

KR-03

Kylie Rika

From: Kylie Rika
Sent: Thursday, 1 April 2021 12:31 PM
To: Luke Ryan
Cc: Megan Mathieson; Generosa Lundie
Subject: RE: Proposal #199 - Verification of Proflex for PP21
Attachments: FW: [#3422] New instrumentation

Thanks Luke

Emma has access to the STRmix support site so I asked her to get some advice from them.

The advice is attached.

Thanks
Kylie

From: Luke Ryan <[redacted]>
Sent: Wednesday, 31 March 2021 3:09 PM
To: Kylie Rika <[redacted]>
Cc: Megan Mathieson [redacted]; Generosa Lundie [redacted]
Subject: RE: Proposal #199 - Verification of Proflex for PP21

Hi Kylie
I've put together responses for your feedback. I appreciate there is a lot of info so if any of this is unclear and you want to discuss let me know. The second revision of the Exp design will include a range of changes and will hopefully address your feedback.

Response – Validation vs Verification
We used the NATA 'General Accreditation Guidance – Validation and verification for quantitative and qualitative test methods" (January 2018) to determine whether a verification or validation was required. This NATA document is based on ENSFI and other relevant standards. See Appendix 1 for decision tree (and below). When we worked through the workflow it could go through two pathways (see below, I have highlighted the pathways I thought were relevant). I have explained our reasoning for the two paths we took below.

Yellow path:

1. Is the Candidate method…: Yes - PCR amplification has been previously validated internally and by Promega using the same thermal cycling parameters as will be used on the Proflex albeit on the 9700. Proflex will be used with 9700 emulation mode which mimics the ramping/thermal cycling characteristics of the 9700.
2. Is the method modified: Yes – Proflex is a new instrument (or analyte – based on definition of analyte in the NATA doc). N.B. this is the point where the yellow and blue paths diverge. I think you could justify a yes/no answer for this.
3. Is this a new analyte to the facility – Yes, Proflex is the analyte being tested and it is new to the facility.

Blue Path

1. Is the method modified – based on my reading of the document I don't think the method is modified. Thermal cycling parameters are not modified. Reagents are not modified. Thermal cycling conditions are not modified based on use of 9700 emulation mode. This position is justified given the next question allows you to indicate the Proflex is new to the facility (i.e. having a new instrument doesn't necessarily mean the method is modified).
2. Is this a new analyte to the facility: Yes – Proflex are a new instrument

The Yellow and Blue paths both lead to a verification. Based on this assessment the testing of the Proflex was as a verification and not validation.
The Experimental Design will be updated to include more details on the 9700 emulation mode on the Proflex and how this is designed to replicate the thermal cycling conditions (specifically the ramp rates) on the 9700s which were used for development and validation of PP21.

Appendix 1. Method validation and verification decision tree

Response - From the ENSFI guidelines I note that for a new thermal cycler model, it is suggested that a number of samples previously profiled are repeated using the kit in question.
Staff samples which have been previously run in PP21 on the 9700s will be analysed. We can determine allele call concordance however I think it would be difficult to determine whether other observed variation (i.e. peak height difference) is due to the Proflex vs 9700 or extract variation amp to amp (as observed in VFP validation testing of mixing), STARlet variation in amp preparation, STARlet variation in CE prep and 3500xL CE variation. It is possible to look at macro variations, i.e. amplifying at 0.5 ng template and getting partial/XS results which would indicate critical failure.

Response - Sensitivity
Based on your and other feedback a range of DNA template inputs will be included in the Casework plate run on each Proflex. This will simulate “real” casework samples which have a range of input templates. This will enable us to assess amplification at a range of inputs.

Response - Repeatability and Reproductivity
These are requirements for validations and so were not included in this verification. The same casework amp and will be run on each Proflex, and results can be compared.

The data analysis and acceptance criteria are being expanded to included among other things comparison of results between Proflex instruments. This does provide a form of repeatability/reproducibility assessment.

Response - Homogeneity of heating block
This is assessed during the Proflex PMs, last conducted on 22-09-2020. A report for each Proflex is in the FR against each respective Proflex. Assessing block accuracy and uniformity using the t-POD probe is the most accurate method as it measures the temperature of each well. This is more accurate than using CE results block performance as it would be difficult to determine whether observed variation is due to the Proflex, extract variation amp to amp, STARlet variation in amp preparation, STARlet variation in CE prep and/or 3500xL CE variation.

FDNA and/or BTS do not have the t-POD device and therefore we cannot assess this ourselves.

Response - I also think we need to get advice from the STRmix support group to see if we need to do MM again etc..
Can you please coordinate this advice as this would form part of your feedback.

Response - I don't think we can rely just on intuitive qualitative comparisons, we need some form of statistical analysis. I suspect we may need to re-do b/line, stutter, assess locus efficiency (APH for each sample across each locus) etc
We are expanding on data analysis and acceptance criteria to include more quantitative measures: assess stutter thresholds and compare the thresholds, assess AI, minus A, PCR arts, inter-locus balance etc.

Baseline – reassessing baseline is required after changes to the optical components of the 3500xL are made (i.e. laser, CCD camera) as it is measure of the background fluoresce produced by the genetic analyser optical components. I am not aware of any requirement to redo baseline for a new thermal cycler – or thermal cyclers. If you have a reference can you please provide and I will disseminate to the Management Team for consideration given this would be a large project to redo baseline for one or all of the new Proflex.

Stutter, assess locus efficiency – as per above we will include additional quantitative measures in the data analysis and acceptance criteria.

Thanks
Luke

From: Kylie Rika <[redacted]>
Sent: Tuesday, 23 March 2021 2:40 PM
To: Luke Ryan <[redacted]>
Subject: RE: Proposal #199 - Verification of Proflex for PP21

Hi Luke

Feedback as follows:

I think we need to do more than what is currently listed in the proposal. From the ENSFI guidelines I note that for a new thermal cycler model, it is suggested that a number of samples previously profiled are repeated using the kit in question. They further recommend that the selected samples will obviously allow examination of parameters such as reproducible results, including from mixtures and low DNA concentration samples. For a new thermal cycler that is of the same model as others already in use, a certificate from the manufacturer detailing a technical performance check done after installation in the lab and an internal sensitivity and homogeneity check would be sufficient.

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Thanks
Kylie

From: Luke Ryan <[redacted]>
Sent: Monday, 22 March 2021 12:23 PM
To: Allan McNevin <[redacted]>; Allison Lloyd <[redacted]>; Cathie Allen <[redacted]>; Justin Howes <[redacted]>; Kirsten Scott [redacted]; Kylie Rika <[redacted]>; Paula Brisotto [redacted]>; Sharon Johnstone [redacted]>
Cc: Generosa Lundie <[redacted]>; Megan Mathieson [redacted]>
Subject: Proposal #199 - Verification of Proflex for PP21

Afternoon All
Please see the attached Experimental Design for the verification of the Proflex thermal cyclers for PP21. Can you please provide feedback by COB Monday 29/03/2021.

Thanks
Luke

Luke Ryan
Senior Scientist – Analytical Team

Forensic DNA Analysis, Forensic and Scientific Services
Health Support Queensland, Queensland Health

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e [redacted] w www.health.qld.gov.au/healthsupport/businesses/forensic-and-scientific-services

KR-03-1

Kylie Rika

From: Emma Caunt
Sent: Thursday, 1 April 2021 12:19 PM
To: Kylie Rika; Cassandra James; Angela Adamson; Allan McNevin
Subject: FW: [#3422] New instrumentation

Hi all

Please see below response from STRmix support regarding the verification of Proflex.

Thanks

Emma

From: Zane Kerr <[redacted]>
Sent: Thursday, 1 April 2021 11:56 AM
To: Emma Caunt [redacted]
Subject: Re: [#3422] New instrumentation

Hi Emma,

I would recommend re-running Model Maker to see whether the new thermal cyclers have affected your peak height variance parameters. If there has been no substantial changes then you should be fine to keep using your existing STRmix parameters. If you do see significant differences then the best approach would be to set up a new kit within STRmix and use this whenever you interpret profiles that have been tested using the new instrumentation. If you do need to set up a new kit I would suggest retaining the existing kit for historic cases. It also means you will be able to interpret PCR replicates that have been tested using both instruments (you can use the multi kit functionality within STRmix to do this).

Regardless of whether you retain or update your peak height variance parameters, I would suggest carrying out a performance check using profiles that have been tested using the new thermal cyclers. I have outlined two approaches for this below:

- If you still have the DNA extracts of the mixtures used during your STRmix validation you could re-amplify a sub-set of these (20-30 mixtures of varying complexity/template amount) using the new thermal cyclers, interpret the resulting profiles in STRmix, then compare the LRs assigned for true donors & non-contributors back to the original result.
- If these extracts are no longer available, you could prepare some new mixtures, interpret in STRmix, and examine sensitivity (for true donors) and specificity (for non-contributors) as a function of per contributor average peak height.

The new thermal cyclers could also have an effect on your drop-in parameters. I think this would depend on the relative sensitivity between the new and old instruments. You might like to start keeping a drop-in register for the new thermal cyclers. Once you have sufficient data (maybe 3-6 months' worth of negative controls) you could re-assess your drop-in parameters (we would recommend that you periodically do this anyway, regardless of any changes to your protocols/instrumentation).

I hope this information helps, please let me know if you need anything else. [redacted]

Thanks,

Zane.

Ticket: https://support.strmix.com/helpdesk/tickets/3422

> On Wed, 31 Mar at 5:46 PM , Emma Caunt <[redacted]> wrote:
> Good morning We are performing a verification of new thermal cyclers - we are moving from the 9700 to Proflex. Can you please advise whether we will need to reassess any STRmix parameters, such as re-running Model Maker, as part of this verification or is it valid to retain our current settings? Many thanks

KR-04

Kylie Rika

From: Justin Howes
Sent: Tuesday, 13 April 2021 11:34 AM
To: Kylie Rika; Paula Brisotto; Luke Ryan
Subject: RE: Proposal #199 - Verification of Proflex for PP21

Hi
Thanks for this Kylie. This will be for me to ask of the current trainers to discuss. I think this element is not within a verification of the Proflex, more how its use fits within the system – so for me, more of an implementation task if anything.

I will put some questions to the trainers for consideration and recommendation.

Thanks
Justin

Justin Howes
Team Leader - Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Police Services Stream
Forensic & Scientific Services, Health Support Queensland, Queensland Health

Please note that I may be working from a different location during the COVID-19 Pandemic. The best contact method is via email.

p [redacted] m [redacted]
a [redacted]
e [redacted] w www.health.qld.gov.au/healthsupport/businesses/forensic-and-scientific-services

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From: Kylie Rika <[redacted]>
Sent: Tuesday, 13 April 2021 11:00 AM
To: Paula Brisotto <[redacted]>; Luke Ryan <[redacted]>; Justin Howes <[redacted]>
Subject: RE: Proposal #199 - Verification of Proflex for PP21

Hi all

Paula had a chat with me on Friday and here are some further thoughts:

Are we looking to validate just one Proflex then say that the rest are ok (if that validation is OK)? If yes, then maybe its OK to check the following on just the one proflex (I would be keen to get advice from our STRmix trainers):

Model Maker (peak height variance parameters)

Performance check using profiles that have been tested using the new thermal cyclers. Zane mentions two approaches for this below:

- If you still have the DNA extracts of the mixtures used during your STRmix validation you could re-amplify a sub-set of these (20-30 mixtures of varying complexity/template amount) using the new thermal cyclers, interpret the resulting profiles in STRmix, then compare the LRs assigned for true donors & non-contributors back to the original result.
- If these extracts are no longer available, you could prepare some new mixtures, interpret in STRmix, and examine sensitivity (for true donors) and specificity (for non-contributors) as a function of per contributor average peak height.

I would also be keen to see what the STRmix trainers think about this work as part of validation or at implementation stage.

Justin, do you want me to set up a meeting with the STRmix trainers or will you do that?

Thanks

Kylie

From: Kylie Rika
Sent: Wednesday, 7 April 2021 5:29 PM
To: Paula Brisotto <[redacted]>; Luke Ryan <[redacted]>; Justin Howes <[redacted]>
Subject: RE: Proposal #199 - Verification of Proflex for PP21

Hi all

Please bear with me as I write down some thoughts:

With the introduction of STRmix, the question of when to conduct end to end whole system verification is a more crucial consideration than ever before given the way STRmix uses the variances.

In the old days we could get away with just intuitive assessment of the profile results after verifying a new instrument. We can't do that anymore with STRmix.

The advice from Zane Kerr (see attached) pretty much sums up why we need to re-run MM. Whether we do it now or prior to implementation doesn't matter but other than for BCP purposes, I can't see the benefit of not doing it as part of the verification. Is there a rush because the 9700s are about to die?

It's a shame all the STRmix trainers are away this week but I am interested in their feedback on MM now or at implementation.

Thanks
Kylie

From: Paula Brisotto <[redacted]>
Sent: Wednesday, 7 April 2021 10:07 AM
To: Luke Ryan <[redacted]>; Kylie Rika <[redacted]>; Justin Howes <[redacted]>
Subject: RE: Proposal #199 - Verification of Proflex for PP21

Hi all,

I am wondering if this question re: MM is more for an implementation phase, instead of the verification of the instruments?

My thoughts are - the instrument verification can proceed as per the experimental design (including updates as an outcome of the combined feedback), and MM can be part of the implementation stage, as required.

With respect to running MM on each Proflex, I do not believe this is necessary as we have never done this previously, and should all Proflex's be assessed as fit for purpose, then my thinking is it will not matter which instrument MM is run on…. This is my understanding at present, so if advice has changed, happy for this to be a further discussion wrt implementation for these and as part of future verifications.

I think given all of the STRmix trainers are currently on leave, this part of the implementation can be put to that group next week for input, with the verification to proceed as is (with reference to any requirements for STRmix as part of the implementation).

If this seems a good way forward, Justin can you coordinate this discussion with the STRmix trainers for feedback to management team?

Thanks,
Paula

From: Luke Ryan <[redacted]>
Sent: Thursday, 1 April 2021 1:45 PM
To: Kylie Rika <[redacted]>
Cc: Paula Brisotto <[redacted]>; Justin Howes <[redacted]>
Subject: RE: Proposal #199 - Verification of Proflex for PP21

Hi Kylie
I sought your input on the proposed experiments because FRIT are the STRmix SMEs and better placed than me to design the STRmix experiments.

Given this external advice has the potential to change the way we validate/verify all instruments/techniques I'll refer this advice to JAH and PMB for further discussion with the Mgt Team.

Thanks
Luke

From: Kylie Rika <[redacted]>
Sent: Thursday, 1 April 2021 1:26 PM
To: Luke Ryan <[redacted]>
Cc: Paula Brisotto <[redacted]>; Justin Howes <[redacted]>
Subject: RE: Proposal #199 - Verification of Proflex for PP21

Hi Luke

This is your project and I have done what was asked – to provide feedback. You further asked me to coordinate the STRmix advice as this would form part of my feedback. I have done that.

If the feedback is not in line with where you were hoping the project would go, then it is now an item for mgmt. team discussion/decision.

I have fulfilled my role as reviewer of the experimental design

Thanks
Kylie

From: Luke Ryan <[redacted]>
Sent: Thursday, 1 April 2021 1:00 PM
To: Kylie Rika <[redacted]>
Cc: Megan Mathieson <[redacted]>; Generosa Lundie <[redacted]>; Paula Brisotto <[redacted]>; Justin Howes <[redacted]>
Subject: RE: Proposal #199 - Verification of Proflex for PP21

Hi Kylie
Excellent thanks. Do you support this advice – you didn't specify? If yes, as this may be a large body of work (i.e. potentially model maker runs for each new Proflex) I think this needs to go to Mgt Team for review. To enable this, can you please propose new experiments or modifications to existing experiments which you consider would satisfy this advice? Can you please be specific in your proposed experiments and include intent, experimental design and acceptance criteria so if approved it can be inserted directly into the Experimental Design. I will provide these experiments to the Mgt Team for discussion and feedback.

Thanks
Luke

From: Kylie Rika <[redacted]>
Sent: Thursday, 1 April 2021 12:31 PM
To: Luke Ryan <[redacted]>
Cc: Megan Mathieson <[redacted]>; Generosa Lundie <[redacted]>
Subject: RE: Proposal #199 - Verification of Proflex for PP21

Thanks Luke

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based on ENSFI and other relevant standards. See Appendix 1 for decision tree (and below). When we worked through the workflow it could go through two pathways (see below, I have highlighted the pathways I thought were relevant). I have explained our reasoning for the two paths we took below.

Yellow path:

1. Is the Candidate method…: Yes - PCR amplification has been previously validated internally and by Promega using the same thermal cycling parameters as will be used on the Proflex albeit on the 9700. Proflex will be used with 9700 emulation mode which mimics the ramping/thermal cycling characteristics of the 9700.
2. Is the method modified: Yes – Proflex is a new instrument (or analyte – based on definition of analyte in the NATA doc). N.B. this is the point where the yellow and blue paths diverge. I think you could justify a yes/no answer for this.
3. Is this a new analyte to the facility – Yes, Proflex is the analyte being tested and it is new to the facility.

Blue Path

1. Is the method modified – based on my reading of the document I don't think the method is modified. Thermal cycling parameters are not modified. Reagents are not modified. Thermal cycling conditions are not modified based on use of 9700 emulation mode. This position is justified given the next question allows you to indicate the Proflex is new to the facility (i.e. having a new instrument doesn't necessarily mean the method is modified).
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The Experimental Design will be updated to include more details on the 9700 emulation mode on the Proflex and how this is designed to replicate the thermal cycling conditions (specifically the ramp rates) on the 9700s which were used for development and validation of PP21.

Appendix 1. Method validation and verification decision tree

Response - From the ENSFI guidelines I note that for a new thermal cycler model, it is suggested that a number of samples previously profiled are repeated using the kit in question.
Staff samples which have been previously run in PP21 on the 9700s will be analysed. We can determine allele call concordance however I think it would be difficult to determine whether other observed variation (i.e. peak height difference) is due to the Proflex vs 9700 or extract variation amp to amp (as observed in VFP validation testing of mixing), STARlet variation in amp preparation, STARlet variation in CE prep and 3500xL CE variation. It is possible to look at macro variations, i.e. amplifying at 0.5 ng template and getting partial/XS results which would indicate critical failure.

Response - Sensitivity
Based on your and other feedback a range of DNA template inputs will be included in the Casework plate run on each Proflex. This will simulate "real" casework samples which have a range of input templates. This will enable us to assess amplification at a range of inputs.

Response - Repeatability and Reproductivity

These are requirements for validations and so were not included in this verification. The same casework amp and will be run on each Proflex, and results can be compared.

The data analysis and acceptance criteria are being expanded to included among other things comparison of results between Proflex instruments. This does provide a form of repeatability/reproducibility assessment.

Response - Homogeneity of heating block
This is assessed during the Proflex PMs, last conducted on 22-09-2020. A report for each Proflex is in the FR against each respective Proflex. Assessing block accuracy and uniformity using the t-POD probe is the most accurate method as it measures the temperature of each well. This is more accurate than using CE results block performance as it would be difficult to determine whether observed variation is due to the Proflex, extract variation amp to amp, STARlet variation in amp preparation, STARlet variation in CE prep and/or 3500xL CE variation.

FDNA and/or BTS do not have the t-POD device and therefore we cannot assess this ourselves.

Response - I also think we need to get advice from the STRmix support group to see if we need to do MM again etc..
Can you please coordinate this advice as this would form part of your feedback.

Response - I don't think we can rely just on intuitive qualitative comparisons, we need some form of statistical analysis. I suspect we may need to re-do b/line, stutter, assess locus efficiency (APH for each sample across each locus) etc
We are expanding on data analysis and acceptance criteria to include more quantitative measures: assess stutter thresholds and compare the thresholds, assess AI, minus A, PCR arts, inter-locus balance etc.

Baseline – reassessing baseline is required after changes to the optical components of the 3500xL are made (i.e. laser, CCD camera) as it is measure of the background fluoresce produced by the genetic analyser optical components. I am not aware of any requirement to redo baseline for a new thermal cycler – or thermal cyclers. If you have a reference can you please provide and I will disseminate to the Management Team for consideration given this would be a large project to redo baseline for one or all of the new Proflex.

Stutter, assess locus efficiency – as per above we will include additional quantitative measures in the data analysis and acceptance criteria.

Thanks
Luke

From: Kylie Rika <[redacted]>
Sent: Tuesday, 23 March 2021 2:40 PM
To: Luke Ryan <[redacted]>
Subject: RE: Proposal #199 - Verification of Proflex for PP21

Hi Luke

Feedback as follows:

I think we need to do more than what is currently listed in the proposal. From the ENSFI guidelines I note that for a new thermal cycler model, it is suggested that a number of samples previously profiled are repeated using the kit in question. They further recommend that the selected samples will obviously allow examination of parameters such as reproducible results, including from mixtures and low DNA concentration samples. For a new thermal cycler that is of the same model as others already in use, a certificate from the manufacturer detailing a technical performance check done after installation in the lab and an internal sensitivity and homogeneity check would be sufficient.

I note from thermofisher that the ProFlex and 9700 are different models. Therefore, we need the following minimum parameters to be validated :

- Sensitivity (limit of detection) : a series of dilutions tested in three replicates.

- Repeatability : the three replicates of the same sample, distributed over the entire heating block can be used to evaluate the repeatability.

- Reproducibility : 3 repetitions of the amplification reactions used in the sensitivity test.

- Homogeneity of heating block : temperature control of the heating block or a comparison of the replicates allows the evaluation of the homogeneity of the heating block.

I also think we need to get advice from the STRmix support group to see if we need to do MM again etc..

I don't think we can rely just on intuitive qualitative comparisons, we need some form of statistical analysis. I suspect we may need to re-do b/line, stutter, assess locus efficiency (APH for each sample across each locus) etc

Thanks
Kylie

From: Luke Ryan <[redacted]>
Sent: Monday, 22 March 2021 12:23 PM
To: Allan McNevin [redacted]; Allison Lloyd [redacted]; Cathie Allen [redacted]; Justin Howes [redacted]; Kirsten Scott [redacted]; Kylie Rika [redacted]; Paula Brisotto [redacted]; Sharon Johnstone [redacted]
Cc: Generosa Lundie [redacted]; Megan Mathieson [redacted]
Subject: Proposal #199 - Verification of Proflex for PP21

Afternoon All
Please see the attached Experimental Design for the verification of the Proflex thermal cyclers for PP21. Can you please provide feedback by COB Monday 29/03/2021.

Thanks
Luke

Luke Ryan
Senior Scientist – Analytical Team

Forensic DNA Analysis, Forensic and Scientific Services
Health Support Queensland, Queensland Health

p [redacted] m [redacted]
a [redacted]
e [redacted] w www.health.qld.gov.au/healthsupport/businesses/forensic-and-scientific-services

KR-04-1

Kylie Rika

From: Kylie Rika
Sent: Wednesday, 7 April 2021 5:29 PM
To: Paula Brisotto; Luke Ryan; Justin Howes
Subject: RE: Proposal #199 - Verification of Proflex for PP21
Attachments: FW: [#3422] New instrumentation

Hi all

Please bear with me as I write down some thoughts:

With the introduction of STRmix, the question of when to conduct end to end whole system verification is a more crucial consideration than ever before given the way STRmix uses the variances.

In the old days we could get away with just intuitive assessment of the profile results after verifying a new instrument. We can't do that anymore with STRmix.

The advice from Zane Kerr (see attached) pretty much sums up why we need to re-run MM. Whether we do it now or prior to implementation doesn't matter but other than for BCP purposes, I can't see the benefit of not doing it as part of the verification. Is there a rush because the 9700s are about to die?

It's a shame all the STRmix trainers are away this week but I am interested in their feedback on MM now or at implementation.

Thanks
Kylie

From: Paula Brisotto <[redacted]>
Sent: Wednesday, 7 April 2021 10:07 AM
To: Luke Ryan <[redacted]>; Kylie Rika <[redacted]>; Justin Howes <[redacted]>
Subject: RE: Proposal #199 - Verification of Proflex for PP21

Hi all,

I am wondering if this question re: MM is more for an implementation phase, instead of the verification of the instruments?

My thoughts are - the instrument verification can proceed as per the experimental design (including updates as an outcome of the combined feedback), and MM can be part of the implementation stage, as required.

With respect to running MM on each Proflex, I do not believe this is necessary as we have never done this previously, and should all Proflex's be assessed as fit for purpose, then my thinking is it will not matter which instrument MM is run on.... This is my understanding at present, so if advice has changed, happy for this to be a further discussion wrt implementation for these and as part of future verifications.

I think given all of the STRmix trainers are currently on leave, this part of the implementation can be put to that group next week for input, with the verification to proceed as is (with reference to any requirements for STRmix as part of the implementation).

If this seems a good way forward, Justin can you coordinate this discussion with the STRmix trainers for feedback to management team?

Thanks,
Paula

From: Luke Ryan <[redacted]>
Sent: Thursday, 1 April 2021 1:45 PM
To: Kylie Rika <[redacted]>
Cc: Paula Brisotto <[redacted]>; Justin Howes <[redacted]>
Subject: RE: Proposal #199 - Verification of Proflex for PP21

Hi Kylie
I sought your input on the proposed experiments because FRIT are the STRmix SMEs and better placed than me to design the STRmix experiments.

Given this external advice has the potential to change the way we validate/verify all instruments/techniques I'll refer this advice to JAH and PMB for further discussion with the Mgt Team.

Thanks
Luke

From: Kylie Rika <[redacted]>
Sent: Thursday, 1 April 2021 1:26 PM
To: Luke Ryan <[redacted]>
Cc: Paula Brisotto <[redacted]> Justin Howes <[redacted]>
Subject: RE: Proposal #199 - Verification of Proflex for PP21

Hi Luke

This is your project and I have done what was asked – to provide feedback. You further asked me to coordinate the STRmix advice as this would form part of my feedback. I have done that.

If the feedback is not in line with where you were hoping the project would go, then it is now an item for mgmt. team discussion/decision.

I have fulfilled my role as reviewer of the experimental design

Thanks
Kylie

From: Luke Ryan <[redacted]>
Sent: Thursday, 1 April 2021 1:00 PM
To: Kylie Rika <[redacted]>
Cc: Megan Mathieson <[redacted]>; Generosa Lundie <[redacted]>; Paula Brisotto <[redacted]>; Justin Howes <[redacted]>
Subject: RE: Proposal #199 - Verification of Proflex for PP21

Hi Kylie
Excellent thanks. Do you support this advice – you didn't specify? If yes, as this may be a large body of work (i.e. potentially model maker runs for each new Proflex) I think this needs to go to Mgt Team for review. To enable this, can you please propose new experiments or modifications to existing experiments which you consider would satisfy this advice? Can you please be specific in your proposed experiments and include intent, experimental design and acceptance criteria so if approved it can be inserted directly into the Experimental Design. I will provide these experiments to the Mgt Team for discussion and feedback.

Thanks
Luke

From: Kylie Rika <[redacted]
Sent: Thursday, 1 April 2021 12:31 PM
To: Luke Ryan <[redacted]
Cc: Megan Mathieson <[redacted]; Generosa Lundie
<[redacted]>
Subject: RE: Proposal #199 - Verification of Proflex for PP21

Thanks Luke

Emma has access to the STRmix support site so I asked her to get some advice from them.

The advice is attached.

Thanks
Kylie

From: Luke Ryan <[redacted]
Sent: Wednesday, 31 March 2021 3:09 PM
To: Kylie Rika <[redacted]
Cc: Megan Mathieson <[redacted]>; Generosa Lundie
<[redacted]
Subject: RE: Proposal #199 - Verification of Proflex for PP21

Hi Kylie
I've put together responses for your feedback. I appreciate there is a lot of info so if any of this is unclear and you want to discuss let me know. The second revision of the Exp design will include a range of changes and will hopefully address your feedback.

Response – Validation vs Verification
We used the NATA 'General Accreditation Guidance – Validation and verification for quantitative and qualitative test methods" (January 2018) to determine whether a verification or validation was required. This NATA document is based on ENSFI and other relevant standards. See Appendix 1 for decision tree (and below). When we worked through the workflow it could go through two pathways (see below, I have highlighted the pathways I thought were relevant). I have explained our reasoning for the two paths we took below.

Yellow path:

1. Is the Candidate method…: Yes - PCR amplification has been previously validated internally and by Promega using the same thermal cycling parameters as will be used on the Proflex albeit on the 9700. Proflex will be used with 9700 emulation mode which mimics the ramping/thermal cycling characteristics of the 9700.
2. Is the method modified: Yes – Proflex is a new instrument (or analyte – based on definition of analyte in the NATA doc). N.B. this is the point where the yellow and blue paths diverge. I think you could justify a yes/no answer for this.
3. Is this a new analyte to the facility – Yes, Proflex is the analyte being tested and it is new to the facility.

Blue Path

1. Is the method modified – based on my reading of the document I don't think the method is modified. Thermal cycling parameters are not modified. Reagents are not modified. Thermal cycling conditions are not modified based on use of 9700 emulation mode. This position is justified given the next question allows you to indicate the Proflex is new to the facility (i.e. having a new instrument doesn't necessarily mean the method is modified).
2. Is this a new analyte to the facility: Yes – Proflex are a new instrument

The Yellow and Blue paths both lead to a verification. Based on this assessment the testing of the Proflex was as a verification and not validation.
The Experimental Design will be updated to include more details on the 9700 emulation mode on the Proflex and how this is designed to replicate the thermal cycling conditions (specifically the ramp rates) on the 9700s which were used for development and validation of PP21.

Appendix 1. Method validation and verification decision tree

Response - From the ENSFI guidelines I note that for a new thermal cycler model, it is suggested that a number of samples previously profiled are repeated using the kit in question.
Staff samples which have been previously run in PP21 on the 9700s will be analysed. We can determine allele call concordance however I think it would be difficult to determine whether other observed variation (i.e. peak height difference) is due to the Proflex vs 9700 or extract variation amp to amp (as observed in VFP validation testing of mixing), STARlet variation in amp preparation, STARlet variation in CE prep and 3500xL CE variation. It is possible to look at macro variations, i.e. amplifying at 0.5 ng template and getting partial/XS results which would indicate critical failure.

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Thanks
Luke

From: Kylie Rika <[redacted]>
Sent: Tuesday, 23 March 2021 2:40 PM
To: Luke Ryan <[redacted]>
Subject: RE: Proposal #199 - Verification of Proflex for PP21

Hi Luke

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Thanks
Kylie

From: Luke Ryan <[redacted]>
Sent: Monday, 22 March 2021 12:23 PM
To: Allan McNevin <[redacted]>; Allison Lloyd <[redacted]>; Cathie Allen <[redacted]>; Justin Howes <[redacted]>; Kirsten Scott [redacted] Kylie Rika <[redacted]>; Paula Brisotto [redacted]; Sharon Johnstone <[redacted]>
Cc: Generosa Lundie [redacted]; Megan Mathieson <[redacted]>
Subject: Proposal #199 - Verification of Proflex for PP21

Afternoon All
Please see the attached Experimental Design for the verification of the Proflex thermal cyclers for PP21. Can you please provide feedback by COB Monday 29/03/2021.

Thanks
Luke

Luke Ryan
Senior Scientist – Analytical Team

Forensic DNA Analysis, Forensic and Scientific Services
Health Support Queensland, Queensland Health

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e [redacted] w www.health.qld.gov.au/healthsupport/businesses/forensic-and-scientific-services

KR-05

Kylie Rika

From: Justin Howes
Sent: Wednesday, 12 January 2022 2:46 PM
To: Kylie Rika
Subject: RE: Project 199

Hi
And here is some more anxiety-reducing stuff: I called Paula re holding (or not) and in talking it through, nothing would prevent samples going on the Proflex because the validations demonstrated its fit-for-use and it being comparable to 9700 (as effectively it is the same thing with the emulation mode). The only thing that may be affected is a setting in STRmix for samples that would be run in STRmix eventually. So it is only a setting that might change albeit unlikely at this point; an amplification process would not be affected.
I doubt any would be on lists yet and think it might be some time before they would even be looked at given the number of 2021 samples we have ready to go.

Hopefully that helps more!

Justin

Justin Howes
Team Leader - Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Police Services Stream, Forensic & Scientific Services
Prevention Division, Queensland Health

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a [redacted]
e [redacted] w www.health.qld.gov.au/fss

Please note that I may be working from a different location during the COVID-19 Pandemic. The best contact method is via email.

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.

From: Kylie Rika [redacted]>
Sent: Wednesday, 12 January 2022 2:33 PM
To: Justin Howes <[redacted]>
Subject: RE: Project 199

Thanks Justin

And thanks for following up so quickly and getting back to me. I have to admit, I was a little nervous raising the issue because I didn't want my intentions misunderstood. But your actions and reply eased my anxiety instantly 😊

Thanks
Kylie

From: Justin Howes <[redacted]>
Sent: Wednesday, 12 January 2022 2:25 PM
To: Kylie Rika <[redacted]>
Subject: Re: Project 199

Hi, I didn't discuss that element with Paula as I thought the following: Proflex uses the same program (emulation mode) as the 9700s, performed consistently as expected (due to emulation mode) and with that, figured it to be unlikely to get variances higher than what we have. I wouldn't think we have any samples on lists yet but I can chat to her again on that.
It was a good find there Kylie, I will say it again!

Justin

Justin Howes
Team Leader - Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Police Services Stream, Forensic & Scientific Services
Prevention Division, Queensland Health

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[redacted]
e [redacted] w www.health.qld.gov.au/fss

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From: Kylie Rika <[redacted]>
Sent: Wednesday, 12 January 2022 2:21 PM
To: Justin Howes <[redacted]>
Subject: RE: Project 199

Hi Justin

Will we be putting a hold on samples running through the proflexes until MM run and findings assessed?

Thanks
Kylie

From: Justin Howes <[redacted]>
Sent: Wednesday, 12 January 2022 2:10 PM
To: Kylie Rika <[redacted]>
Cc: Sharon Johnstone <[redacted]>
Subject: RE: Project 199

Hi both, FYI

I have spoken to Paula on this and yes, unfortunately it wasn't added to the implementation plan. We discussed a way to improve the implementation process, which is separate to the validation/verification, and one idea was that in drawing up the checklist, that the teams have spaces to add details similar to the project risk assessment that is performed at the start. But for now, we will need to get the component of MM tested as suggested by Angela (as summariser for the STRmix trainers). I will write to the trainers and ask them to draw on their previous experience and write up the findings as well.

Angela sent advice in April and I will send back to her and the others to co-ordinate time on this over the next week. It appears we have required samples available to use, so hopefully not much more than that time is required.

Thanks for remembering Kylie!

Justin

Justin Howes
Team Leader - Forensic Reporting and Intelligence Team
Forensic DNA Analysis, Police Services Stream, Forensic & Scientific Services
Prevention Division, Queensland Health
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e [redacted] w www.health.qld.gov.au/fss
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From: Justin Howes
Sent: Wednesday, 12 January 2022 10:46 AM
To: Kylie Rika <[redacted]>
Cc: Sharon Johnstone <[redacted]>
Subject: RE: Project 199

Hi, ok I will look into this through Paula/Kirsten.

Thanks
Justin

Justin Howes
Team Leader - Forensic Reporting and Intelligence Team
Forensic DNA Analysis, Police Services Stream, Forensic & Scientific Services
Prevention Division, Queensland Health
p [redacted] m [redacted]
[redacted]
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From: Kylie Rika <[redacted]>
Sent: Wednesday, 12 January 2022 9:56 AM
To: Justin Howes <[redacted]>
Cc: Sharon Johnstone <[redacted]>
Subject: Project 199

Hi Justin

I was just going through the minor change register to make sure I have communicated all of the recent changes to my team and I noticed with Project 199 Proflex (implemented 10 Jan 2022), there appears to be a gap in finalisation re Model Maker. If you look into

I:\Change Management\Proposal#151 to #200 (completed)\Proposal#199 - Proflex\2.0 Experimental Design\Feedback

You will see some comms/advice from our STRmix trainers about doing MM at implementation stage. I can't find this documented anywhere. I am also unsure if it was done or not.

Thanks
Kylie

Kylie Rika
Senior Scientist, Forensic Reporting and Intelligence Team
Forensic DNA Analysis, Police Services Stream, Forensic & Scientific Services
Prevention Division, Queensland Health
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Please note that I may be working from a different location during the COVID-19 Pandemic. The best contact method is via email.*

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CLEAN HANDS
SAVE LIVES
Wash your hands regularly
to stop the spread of germs.

Background

Following a request from the Director, Mr Greg Shaw, a review of procedures was conducted by Drs Sloots and Whiley (the reviewers) at the Forensic and Scientific Services laboratory, Clinical and State-wide Services, Coopers Plains, pertaining to the extraction of nucleic acids from samples submitted for analysis.

The reason for this review related to a previous episode in the laboratory which resulted in anomalous results and which appeared to be linked to the operation of robotic instrumentation utilised in the nucleic acid extraction process.

During their visit, the reviewers were made aware of the operations applied in the general laboratory from receipt of specimens to issuing of results, and then examined in detail the bench process relating to the pre-digestion of specimens and the extraction of nucleic acids using the Perkin Elmer MultiPROBE II PLUS HT EX with Gripper Integration Platform.

All aspects of these operations were scrutinised including staff input and instrument operation.

Findings

It was obvious to the reviewers that extensive measures were applied by all staff to prevent the misidentification or cross contamination of samples. There was appropriate use of personal protection equipment and other protective measures to prevent contamination of the work environment with extraneous nucleic acid.

The procedures currently in place for the Off-Deck Lysis and MPII extraction appeared to be adequate and specifically designed to prevent cross contamination of test samples.

We agree with the Forensic Services Management team that the previous issue of possible cross-contamination of samples most likely related to the use of adhesive film in sealing the deep-well plates used in the Off-Deck lysis procedure. The type of plate used, and the period of storage at reduced temperatures have in our experience caused similar problems in molecular diagnostics. The subsequent decision to change this procedure to the use of capped tubes has clearly solved this problem.

The use of robotic equipment for the extraction of nucleic acids has some considerable benefits for a busy laboratory, and prevents human error introduced as a result of repetitive actions. However, the efficient use of such instruments requires the proper maintenance and calibration be performed at the requisite time intervals. These appeared to be adequately performed at the time of review.

It may appear that the original issue concerning the cross-contamination of samples in the deep-well plates could have been prevented if this change in procedure had been fully validated against existing protocol when the new method was introduced. Although most

laboratories would have considered this change to be minor and therefore accepted without validation, it clearly demonstrates that all changes in procedure, no matter how minor, need to be validated according to a standardised protocol before their introduction as standard operating procedure.

Items for Further Consideration

During the review process some items were identified which may require further consideration by the management staff of the Forensic and Scientific Services laboratory.

These are:

1. Develop a standard validation protocol for each procedure based on the guidelines described by J Butler (www.promega.com; September 2006). Incorporate these into the Standard Operating Procedures for the laboratory.
2. We advise that the number of negative controls included in each batch of extractions be increased to comprise at least 10% of the total number of specimens tested. These controls should ideally be distributed randomly over the plate. Currently one negative control is included with 47 samples.
3. Quality assessment might be increased by testing a control plate once every 3-4 weeks on each of the MultiPROBE II PLUS platforms. We would suggest alternating between the soccer ball, zebra and checkerboard formats.
4. It was noted that the magnetic particles used for the nucleic acid extraction had a tendency to settle quickly, thereby blocking the filter tip and potentially producing a false-negative result. At the time of review this was not a problem as the attending operator was diligent in observing all stages of this process. We would like to reiterate however, that constant observation by the operator of all processes leading up to and including the addition of magnetic particle is necessary to ensure that failure of the robotic system does not occur.
5. Finally, it was noted that the laboratory design allowed traffic from the amplification/post-amplification area into the lysis/extraction areas. Presently this carries moderate contamination risk, as the amplification protocol is limited to 28-32 cycles. However, if this protocol is changed in the future to detect low copy nucleic acid (greater than 32 cycles) the risk of carrying post-PCR product into the extraction area would be high, and work-flow dynamics must then be carefully examined to minimise that risk. Likewise, sample cross-contamination during specimen handling and extraction processes will assume greater relevance when contemplating detection of low copy nucleic acid, and would necessitate stringent validation of all steps.

[redacted]

Theo P Sloots, PhD, Grad Cert Management.

[redacted]

David M Whiley, PhD,LLB, Grad Cert Law

KR-07

Kylie Rika

From: Cathie Allen
Sent: Monday, 30 April 2018 11:23 AM
To: Kylie Rika
Cc: Andrew Riddell
Subject: Meeting

Sensitivity: Confidential

Hi Kylie

I would like to meet with you on Wednesday, 2nd of May at 2.30pm in Conference Room 113, to discuss a workplace matter relating to compliance with workplace record keeping practices in which you may have further information and or have been involved. Before I decide how to proceed in this matter I would like to give you an opportunity to respond.

Andrew Riddell, Manager HR and Business Relationships, will be in attendance at the meeting. You may bring a support person with you. Your support person may be a union representative, friend or family member, your support person does not participate in the interview. If you cannot have a support person attend the interview face-to-face, arrangements can be made so that they can attend by telephone. Can you please advise Andrew of your support person to ensure appropriateness.

I reiterate I am only considering this matter at this stage. No decision has been made as to what, if any, further action will be taken in relation to this matter. If, after considering your response I determine further action is required, I will contact you again to provide details of the further action.

Lawful directions

Confidentiality

You are directed to keep the details of this matter confidential as far as possible. You may however discuss the matter with your support person, union, legal representative or employee assistance. If you need to discuss this matter with any staff member you should make this request through myself on telephone [redacted] in the first instance.

Employee assistance

Employee assistance offers a confidential counselling service which is free of charge to all employees of HSQ for up to six sessions per calendar year. Access to this service is by self-referral. If you wish to utilise this service, please contact Optum on 1800 604 640. More information on employee assistance can be found at http://qheps.health.qld.gov.au/eap.

Cheers
Cathie

Cathie Allen
Managing Scientist – Police Services Stream

Forensic & Scientific Services,
Health Support Queensland, **Department of Health**

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KR-08

Kylie Rika

From: Brian Newman <[redacted]
Sent: Thursday, 3 May 2018 7:40 AM
To: Kylie Rika; Emma Caunt
Subject: Fwd: Proposed meeting with Emma Caunt and Kylie Rika

Sent from my iPhone

Begin forwarded message:

From: Cathie Allen <[redacted]
Date: 2 May 2018 at 14:36:31 AEST
To: Brian Newman <[redacted]
Cc: Therese O'Connor <[redacted]
Subject: FW: Proposed meeting with Emma Caunt and Kylie Rika

Hi Brian

I propose the following meeting times:

11.00am to 11.30am – Kylie Rika
11.30am to 12.00pm – Emma Caunt

Please let me know if this is suitable for you.

Unfortunately Andrew Riddell, Manager HR and Business Relationships is unable to attend. Therese O'Connor, Senior HR Business Partner will be attending the meeting.

Cheers
Cathie

Cathie Allen
Managing Scientist – Police Services Stream

Forensic & Scientific Services,
Health Support Queensland, **Department of Health**

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a [redacted]
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From: Cathie Allen
Sent: Wednesday, 2 May 2018 12:30 PM
To: Brian Newman
Subject: RE: Proposed meeting with Emma Caunt and Kylie Rika

Hi Brian

I'm currently liaising with the People Performance and Excellence HR Business Partner for FSS regarding their availability for tomorrow. I will advise as soon as I have details of a suitable time.

I can confirm that the meeting will not proceed this afternoon.

I am unable to discuss with you confidential meetings that may be held with other staff members.

Cheers
Cathie

Cathie Allen
Managing Scientist – Police Services Stream

Forensic & Scientific Services,
Health Support Queensland, **Department of Health**

p | [redacted] m | [redacted]
a | [redacted]
w | www.health.qld.gov.au e | [redacted]

From: Brian Newman [redacted]
Sent: Wednesday, 2 May 2018 6:31 AM
To: Cathie Allen
Subject: Re: Proposed meeting with Emma Caunt and Kylie Rika

Cathie

Thank you for your email.

We have been retained by both Emma and Kylie to support them now, but as we discussed yesterday, I will not be available tomorrow due to a matter before the QIRC.

Both of our clients have expressed concerns in relation to this meeting and both have no other support person available and do not want to proceed with the meeting without a support person of their choosing present, such is their right.

With that said, I am available tomorrow morning.

I am aware that there was another staff member who had their meeting moved forward.

This was not an option you had discussed yesterday and had you done so, we may have been able to facilitate the meeting then.

Nevertheless, the fact remains that our clients feel threatened and they are not willing to attend without a support person of their choosing, nor should they be forced to.

Can you please confirm that you agree to move this proposed meeting to tomorrow (Thursday)?

Regards
Brian Newman
Workers First Pty Ltd

[redacted]

On 1 May 2018, at 16:04, Cathie Allen <[redacted]> wrote:

> Hi Brian
>
> We are available to meet tomorrow afternoon at 1630 if that assists. If you're unable to attend in person, we are able to set up a teleconference for the meeting, or include another representative from your organisation.
>
> Cheers
> Cathie
>
> <image001.jpg> **Cathie Allen**
> Managing Scientist – Police Services Stream
>
> Forensic & Scientific Services,
> Health Support Queensland, **Department of Health**
>
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> a | [redacted]
> w | www.health.qld.gov.au e | [redacted]
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>
> **From:** Brian Newman [redacted]
> **Sent:** Tuesday, 1 May 2018 12:14 PM
> **To:** Cathie Allen
> **Subject:** Re: Proposed meeting with Emma Caunt
>
> Cathie
>
> Thank you for the accommodation, however, as I am sure you may be aware, it would unlikely that I would get away from the QIRC prior to 1600.

On that basis, and given the significant concerns which are apprehended by our client with respect to this matter and recent workplace matters which have been witnessed and cause for serious concern of our client and her welfare, we respectfully request that this matter is postponed for another day.

For your additional consideration, we have also been contacted by other staff in the workplace to support them in what appears to at least be a similar meeting in the same workplace at a similar time and place with the same people and agenda.

Once we have taken formal instructions from those staff, we would be making the same or similar request for postponement of the proposed meeting.

Yours truly
Brian Newman JP(Qual)
Director of Investigations & Industrial Advocacy

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On Tue, May 1, 2018 at 11:52 AM, Cathie Allen [redacted] wrote:

Hi Brian

The purpose of the meeting is to bring clarification regarding workplace record keeping and to gain information that Emma may have regarding this. I'm unable to provide any further information until we meet to discuss it.

The meeting is scheduled for 1500 (not 1400) on Wednesday 2nd of May. We are able to schedule the meeting later in the afternoon, however the latest that the start time could be is 1600 on Wednesday.

Cheers

Cathie

<image002.jpg> **Cathie Allen**
Managing Scientist – Police Services Stream

Forensic & Scientific Services,
Health Support Queensland, **Department of Health**

[redacted]

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From: Brian Newman [mailto:[redacted]
Sent: Tuesday, 1 May 2018 9:33 AM
To: Cathie Allen
Subject: Proposed meeting with Emma Caunt

Dear Cathie

We have been requested to attend a proposed meeting with our client, Mrs Caunt on Wednesday 2 May 2018 at 1400.

Agenda and further and better particulars

The agenda for the meeting is unclear and the particulars outlined in your email are vague.

Would you please outline a clear agenda for the meeting and provide particulars which are to be discussed at the meeting.

Proposed date and time of meeting

I am committed to a Queensland Industrial Relations Commission (QIRC) conference in Brisbane city at the proposed time of the meeting and respectfully request that this meeting is moved to another time to enable my attendance as Mrs Caunt's support person.

We would appreciate your urgent reply to this request to move the meeting time and enable my attendance to support our client.

Kind regards

Brian Newman

Workers First Pty Ltd

(p) [redacted]

(f)

(e)

[redacted]

--

Yours truly
Brian Newman
Workers First Pty Ltd

Beenleigh Office
[redacted]

[redacted]

Office
[redacted]

Facsimile
[redacted]

Website
[redacted]

Email
[redacted]

[redacted]

#workersfirstNOTunionsfirst

[redacted]

KR-09

15

Kylie Rika

From: Michel Lok
Sent: Tuesday, 12 December 2017 3:00 PM
To: Kylie Rika; [redacted]
Subject: RE: further item for workplace edge

Hello Kylie,

I wanted to briefly acknowledge receipt of your email. I am sorry this event has caused you distress and we will certainly look into the issue. I have briefly spoken with Alan from Workplace Edge who will be in contact with you shortly.

I am committed to ensuring all employees have a safe and healthy workplace and our engagement of Workplace Edge is intended to assist us achieve this.

Regards

Michel

Michel Lok
General Manager

Strategy, Community and Scientific Support
Health Support Queensland, **Department of Health**

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w | Queensland Health e | [redacted]

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From: Kylie Rika
Sent: Tuesday, 12 December 2017 11:22 AM
To: [redacted]
Cc: Michel Lok
Subject: further item for workplace edge

Hello

I send this email as a further item to add to the information that I supplied at my interview on 22 November 2017.

Last week at the Forensic DNA Analysis Management meeting held on 7 December 2017, I left the meeting feeling humiliated and upset due to the following event:

When the WH&S agenda item was raised, I raised the topic of a number of staff having had recent RSI issues due to computer mouse use and that for a couple of staff, we have had great success in using a new roller mouse. I asked if the budget would allow for further purchases of this item should other staff require it.

Cathie Allen replied as follows:

I have heard that staff believe the RSI issues are due to the use of the Forensic Register. My question to you is has anyone put in an enhancement into VSTS for the Forensic Register to have adjustments made to allow less arm movements leading to RSI?

Cathie's tone of voice and body language (contempt, disrespect, condescending) was such that I was made to feel embarrassed and humiliated for asking the question.

All I could respond with therefore was "I don't know".

Cathie then continued her reply in the same tone and with the same body language as follows:

Well perhaps that would be a good place to start, getting your team together to map the processes and come up with some enhancements for the Forensic Register.

The subsequent feeling in the meeting was very unpleasant but no-one else in the meeting said anything.

Whilst at first thought, Cathie's point seemed valid, it is actually impractical given we are still working on obtaining core functionality in the FR and that any enhancements put forward can take a long time to implement. Time is critical when responding to WH&S issues.

I was so upset by the way she replied to me (especially in front of the rest of the management team) that I spoke to Paula Brisotto (Acting Team Leader of Forensic Reporting and Intelligence) about it immediately afterwards. I mentioned that I felt publicly humiliated. I don't think this information will be fed back to Cathie by Paula as I was told by Paula that the best way to deal with her is to stay calm and let her carry on inappropriately (as you can't control the way someone else behaves.)

Since this event, I have been teary every day and have felt panicky and sick driving into work in the mornings.

Whilst I love my job and the work that we do, I am bringing this to your attention as I hope something can be done about this unprofessional and distressing environment.

Kind regards

Kylie Rika Dip Mgt BSc PGrad Dip (Forensic)
Senior Reporting Scientist – Forensic Reporting and Intelligence Team

Forensic DNA Analysis | Forensic & Scientific Services,
Health Support Queensland, **Department of Health**

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KR-10

Kylie Rika

From: Kylie Rika
Sent: Tuesday, 2 June 2020 3:14 PM
To: Justin Howes; Allan McNevin
Subject: RE: [redacted] follow up
Attachments: STATSWG recommendations for the interpretation of DNA 03-03-2011_Final.doc

Hi both

I just wanted to follow up on this email I sent to you on the 14 May, as I have not yet had any replies.

Also, as you know, I have been working on the STRmix discussion paper (what to do with old versions of STRmix) and as part of my research for this, I came across the attached **STATSWG recommendations for the interpretation of DNA** (which I believe have been endorsed by BSAG).

I note Recommendation 13: LOD as follows:

Recommendation 13: LOD

We recommend that the limit of detection be set as a convenient round number above the average baseline plus 10 standard deviations (SD). Information down to the baseline plus 3 SD may be used for information purposes, for instance to guide on the number of contributors or on non-concordances.

Note: the term limit of detection is inappropriate and we are seeking an alternative. One suggestion is that the term 'limit of detection' refer to the average + 3SD and the term 'baseline' refer to average + 10SD

Thanks
Kylie

From: Kylie Rika
Sent: Thursday, 14 May 2020 12:08 PM
To: Justin Howes <[redacted]>; Allan McNevin <[redacted]>
Subject: RE: [redacted]

Hi both

I have used the relevant SOPs and guidelines to finalise the review of this sample.

There are many aspects that I consider when interpreting a DNA profile. One cannot simply "not see" other peaks that are below LOD and they do help build a picture of the profile as a whole, however I would never use this alone to make a call on a DNA profile. In fact with this DNA profile [redacted], if I was simply blind to peaks below LOD, my call on this DNA profile is that it is still complex unsuitable due to the followings reasons:

Degradation – is observed and according to the number of contributors guidelines, the sample should be reworked
Stochastic range – at least two of the contributors in this sample are within the stochastic range (below ~300 rfu) and according to the number of contributors guidelines, further investigation may be required in this scenario
Allelic Imbalance – this is observed within this DNA profile and according to the number of contributors guidelines, the sample should be reworked
Reworking – according to the number of contributors guidelines, this is the type of sample that should be reworked despite it being a P3 sample

As a reviewer it is my opinion that either this sample is reworked or called complex unsuitable.

Allan if you decide to not rework then you will have to get a third expert to give their opinion on this sample. If it turns out that the third person agrees with your interpretation, then my opinion as original reviewer will need to be documented including the grounds for the dismissal of my viewpoint. This could be included in the FR as a notation against the sample.

As a side discussion, I note that none of our SOPs actually list LOD as being 16rfu except for 33538 which is an information document, not a SOP. If the definition of LOD is mean + 3 SD, then that is 8 rfu.

I also note the following from the number of contributors guidelines:

The aim of these guidelines is to assist in the assessment of the number of contributors for mixed DNA profiles obtained using the PowerPlex 21 system. These guidelines should be used in conjunction with the training and experience of the scientist. There may be features within the DNA profile other than those detailed in this document that may inform the number of contributors. If the scientist observes information/behaviours within the DNA profile that override these guidelines, it is acceptable for these observations to be used in the determination of the number of contributors. There are also certain reworks that are required, for example for quality reasons, before a reasonable assessment of the number of contributors can be made and these should be performed separately to the guidelines provided. Background information has also been presented in this document as this has been considered in the development of the recommendations provided. References to stochastic effects relate to peaks which may drop out or be imbalanced.

Further, I would like to take this opportunity to formally challenge our SOPs and information documents in QIS as needing to be updated to better reflect what the validation data shows, what is suggested in current literature and what we have learned and experienced with DNA profiles over the last few years, in the interest of continuous quality improvement. Could you please advise on the most appropriate channel for me to request a review of the LOD and how we use it?

Thanks
Kylie

From: Justin Howes <[redacted]
Sent: Friday, 24 April 2020 4:33 PM
To: Kylie Rika <[redacted]>; Allan McNevin <[redacted]
Subject: RE:[redacted]

Hi both
Healthy discussion is great! Firstly, yes this is not a world of fully continuous interpretations yet so we need to use what we have developed and supported as a Management Team.

We have supported LOD=16RFU since the PP21 validation in 2012, and continually supported this value through a number of reassessments since then. Our PP21 training presentations support above LOD of 16 and our case mgt SOPs support this as well, including the intuitive exclusion workflow and no. contributor guidelines.

We don't have any SOPs or guidelines supporting below this value. We are supported in below LOR within our documentation for assistance in determining possible number of contributors.

We use, and have used for years the same value for assessment of controls; we are not supported in our quality system below this value.

We'll keep moving with the values we have supported and we can look forward to the assessments of these values with VFP in conjunction with the latest versions of GMIDx and STRmix.

Independently I have assessed this profile and am satisfied with the interpretation as entered. I don't think a rework will work here; the only option would be a microcon. I think this is suitable to review as it is.

Regards
Justin

Justin Howes
Team Leader - Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Police Services Stream
Forensic & Scientific Services, Health Support Queensland, Queensland Health

Please note that I may be working from a different location during the COVID-19 Pandemic. The best contact method is via email.

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e [redacted] w www.health.qld.gov.au/healthsupport/businesses/forensic-and-scientific-services

From: Kylie Rika <[redacted]>
Sent: Friday, 24 April 2020 10:41 AM
To: Allan McNevin [redacted]>
Cc: Justin Howes <[redacted]>
Subject: RE: [redacted]

Thanks Allan

In a world where we are not yet fully continuous, we have no choice but to assign thresholds. However, we must also take into consideration the experience and subjectivity of the analyst. We have DNA profile interpretation guidelines to assist in helping to group staff's interpretation approaches as closely together as possible, but the fact still remains, these are guidelines and interpretation is subjective.

I would be more confident explaining why I had considered the 21 peak at D2 (when it is only a few rfu less than the 25 and is clear from bassline and has good morph) than explaining why I ignored it because we have an arbitrary LOD of 16rfu (which isn't even the true LOD). 16rfu was decided on out of convenience after the laser change in 2017. I note that the # of contrib. guidelines talks about not using S/T peaks under LOD, but those guidelines were developed before the laser change. I guess what I am saying is that in the interests of continuous improvements in the way we interpret, we are probably overdue for a re-think on some things.

I also note the following from Taylor, Buckleton and Bright:

Does the use of probabilistic genotyping change the way we should view sub-threshold data?

Duncan Taylor, John Buckleton & Jo-Anne Bright

3. Conclusion

Continuous systems (at least STRmix as trialled here) can overcome the issues of missing low-level data with minimal effects on the outcome of the analysis. The effects of overestimation of the number of contributors may not be too severe as long as the system has been reliably validated for this policy. This situation should not be used to enable a reduction of valid quality practices such as replication and careful expert inspection of profiles and cannot be assumed to be conservative. However, any system, even one possessing the soundest theoretical basis, that cannot withstand the rigours of practical use, is destined to remain nothing more than a nice idea. We have discussed strategies to mitigate the effect of uncertainty in the number of trace contributors present when sub-threshold information is present in a DNA profile. We support replication and lowering the AT whenever practical. The use of sub-threshold data without lowering the AT may be useful in some cases. The effects of mis-assignment of N in either direction are relatively mild and restricted to LRs less than one when comparing known contributors and low LRs greater than one when comparing known non-contributors.
We believe that treating the number of contributors as an unknown nuisance variable is the best long-term solution. An even better solution would be to combine the treatment of number of contributors as a nuisance variable with an expert system that utilises fluorescent signal directly and has models for different known artefacts. In such a system all data would be treated probabilistically and the tyranny of thresholds would be completely abolished. We are not aware of any system that can perform at this level and so can provide no examples of how it would perform.
Last, we suggest that some profiles are simply too complex and should not be interpreted. Ultimately it is the role of the scientist to assess each profile on its own merits and the case context in order to determine if and how analysis will proceed.

New baseline work with VFP will be a good opportunity to re-consider the way we consider LOD and LOR (or just one AT). It doesn't matter where you put a threshold you will always have peaks under it that you need to decide what to do with.

Thanks
Kylie

From: Allan McNevin <[redacted]>
Sent: Thursday, 23 April 2020 2:43 PM
To: Kylie Rika <[redacted]>
Subject: RE: [redacted]

Hi Kylie,
Apologies for the delayed response. I have consulted with Justin, and his advice was that the inclusion of any possible peaks below the agreed upon 16RFU LOD limit is not a supported approach. There was a previous discussion and subsequent voting e-mail where the management team agreed to maintaining this LOD. Please discuss this with Justin if you would like more information or would like to discuss further the inclusion of sub LOD peaks.
Cheers
Al

Allan McNevin
Senior Scientist – Evidence Recovery

Evidence Recovery Team, Forensic DNA Analysis
Forensic & Scientific Services, Health Support Queensland, Queensland Health

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From: Kylie Rika <[redacted]>
Sent: Wednesday, 11 March 2020 11:38 AM
To: Allan McNevin <[redacted]>
Subject: RE: [redacted]

Hi Allan

In my opinion this profile is 4p or complex and for me I would say complex.

I can't justify calling the 25 peak at D2 sub thresh and then just ignoring the 21 peak just because it is below LOD when they both look very similar. In addition our actual calculated LOD is 8 not 16 as per:

I:\Change Management\Verification of Equipment (post part replacement)\Review Baseline 3130xl B post laser change January 2017/Summary Report 3130xl B laser change January 2017 vfinal

Feel free to ask another scientist for their opinion.

Thanks
Kylie

From: Allan McNevin <[redacted]>
Sent: Thursday, 6 February 2020 3:01 PM
To: Kylie Rika [redacted]>
Subject: RE: [redacted]

Hi,

I've uploaded a further zoom from GMIDX on the D2 locus, there is a 16,19 "major" 18,20 above threshold in the minor, 22 & 25 subthreshold, everything else is below LOD

Cheers
Al

Allan McNevin
Senior Scientist – Evidence Recovery

Evidence Recovery Team, Forensic DNA Analysis
Forensic & Scientific Services, Health Support Queensland, Queensland Health

p [redacted]
a [redacted]
e [redacted] w www.health.qld.gov.au/healthsupport/businesses/forensic-and-scientific-services

From: Kylie Rika <[redacted]>
Sent: Thursday, 6 February 2020 2:54 PM
To: Allan McNevin <[redacted]>
Subject: RE: [redacted]

D2

From: Allan McNevin <[redacted]>
Sent: Thursday, 6 February 2020 12:12 PM
To: Kylie Rika <[redacted]>
Subject: RE: [redacted]

HI,
I had another look. I'm still not seeing greater than 3P, can you point me in the direction of what you are seeing?
Thanks
Al

Allan McNevin
Senior Scientist – Evidence Recovery

Evidence Recovery Team, Forensic DNA Analysis
Forensic & Scientific Services, Health Support Queensland, Queensland Health

p [redacted]
a [redacted]
e [redacted] w www.health.qld.gov.au/healthsupport/businesses/forensic-and-scientific-services

From: Kylie Rika <[redacted]>
Sent: Thursday, 6 February 2020 11:37 AM
To: Allan McNevin <[redacted]>
Subject: [redacted]

Hi Allan

It is my opinion that this is >3p

Can you please have another look

Thanks
Kylie

Kylie Rika
Senior Scientist - Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Police Services Stream
Forensic & Scientific Services, Health Support Queensland, Queensland Health

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KR-10-1

Kylie Rika

From: Kylie Rika
Sent: Wednesday, 3 June 2020 8:23 AM
To: Justin Howes
Subject: RE: [redacted]

Thanks Justin

My full conversation (as original reviewer) with the PDA analyst is listed in the emails I sent on:

6 Feb 2020
11 March 2020
24 April 2020
27 April 2020
14 May 2020
2 June 2020

Please let me know if you don't have all of them and I will send to you so you can add in the FR notation.

Thanks
Kylie

From: Justin Howes [redacted]>
Sent: Tuesday, 2 June 2020 4:11 PM
To: Kylie Rika [redacted]>
Subject: RE: [redacted]

Hi Kylie
Thankyou for your information below. The information provided is part related to a particular sample and its reporting, and part related to process. This email is to address the sample component only and will complete this thread on this sample – I will add it to the FR as a sample notation in due course.

I will have the profile looked at by an additional person and through that process, they will naturally communicate with the PDA entry scientist to determine what path the sample could take from there. Thankyou for your information on this sample; your assistance on this sample is not required from here.

After the sample has progressed, I will add this thread to the FR so as not to influence the further interpretation. Please note that I have my reply here, and your last email, added to the last communication that I provided you on 1 May 2020 which was a reply to your email on 27 April 2020; this is to complete the entire thread on this sample.

Thanks
Justin

Justin Howes
Team Leader - Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Police Services Stream
Forensic & Scientific Services, Health Support Queensland, Queensland Health

Please note that I may be working from a different location during the COVID-19 Pandemic. The best contact method is via email.

p [redacted] m [redacted]
[redacted] qld.gov.au/healthsupport/businesses/forensic-and-scientific-services

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From: Kylie Rika <[redacted]>
Sent: Thursday, 14 May 2020 12:08 PM
To: Justin Howes <[redacted]>; Allan McNevin [redacted]>
Subject: RE: [redacted]

Hi both

I have used the relevant SOPs and guidelines to finalise the review of this sample.

There are many aspects that I consider when interpreting a DNA profile. One cannot simply "not see" other peaks that are below LOD and they do help build a picture of the profile as a whole, however I would never use this alone to make a call on a DNA profile. In fact with this DNA profile [redacted] if I was simply blind to peaks below LOD, my call on this DNA profile is that it is still complex unsuitable due to the followings reasons:

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in the development of the recommendations provided. References to stochastic effects relate to peaks which may drop out or be imbalanced.

Further, I would like to take this opportunity to formally challenge our SOPs and information documents in QIS as needing to be updated to better reflect what the validation data shows, what is suggested in current literature and what we have learned and experienced with DNA profiles over the last few years, in the interest of continuous quality improvement. Could you please advise on the most appropriate channel for me to request a review of the LOD and how we use it?

Thanks
Kylie

From: Justin Howes
Sent: Friday, 1 May 2020 4:07 PM
To: Kylie Rika [redacted]; Allan McNevin [redacted]
Subject: RE: [redacted]

Hi Kylie
I have considered the information you have provided further here.

To follow my email where I described where we are supported and non-supported, I direct you to use our Standard Operating Procedures and associated guidelines to complete the review interpretation of [redacted]

Thankyou
Justin

Justin Howes
Team Leader - Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Police Services Stream
Forensic & Scientific Services, Health Support Queensland, Queensland Health

Please note that I may be working from a different location during the COVID-19 Pandemic. The best contact method is via email.

p [redacted] m [redacted]
a [redacted]
e [redacted] qld.gov.au/healthsupport/businesses/forensic-and-scientific-services

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From: Kylie Rika <[redacted]>
Sent: Monday, 27 April 2020 10:48 AM
To: Justin Howes <[redacted]>; Allan McNevin <[redacted]>
Subject: RE: [redacted]

Thanks Justin

The LOD of 16 rfu which was calculated in 2012 was valid at the time with the data that was obtained. The number of contributors guidelines and intuitive exclusions guidelines were written based on this LOD and this data. The baseline was recalculated in 2017, after the creation of these guidelines. In this recalculation it was shown that the LOD was only 8 rfu. In order to maintain a smooth workflow (to avoid changing plate reading rules, STRmix settings etc) it was decided that since the LOR/LOD had not increased it would be ok to leave them as they are. This seemed to be the appropriate decision at the time and this is why the SOPs and training material reflect this value.

However, it has been noted over time that the baseline of the [redacted] is significantly lower than 16 rfu and therefore peaks are regularly distinct from baseline but below the implemented LOD of 16 rfu. This means that, as scientists we are regularly in the position as outlined in Talyor et al, where we have peaks below the threshold that we feel we cannot ignore. Maybe, if we had had this journal article when we agreed to keeping the thresholds the same, we would not have made this decision.

We are now in the position where some scientists are using their scientific knowledge and experience and looking below LOD and some scientists that don't want to go against the SOP and won't look below LOD even though this is against their scientific judgement. This is leading to samples being incorrected at statement stage. In fact, it is my opinion that if this sample is reported as it currently stands, it will be incorrected at a later date.

The scientific data and published journal articles support the use of peaks below 16 rfu (down to 8rfu in fact), and therefore I don't think I can ethically use the 16 rfu threshold and ignore these peaks.

Maybe it is time for all reporting scientists to discuss this topic and come to an agreement on the way forward – after all, it is the reporters that need to defend the interpretations in court.

Agree that it is good that we can have healthy scientific debate.

Thanks
Kylie

From: Justin Howes [redacted]
Sent: Friday, 24 April 2020 4:33 PM
To: Kylie Rika [redacted]; Allan McNevin [redacted]
Subject: RE: [redacted]

Hi both
Healthy discussion is great! Firstly, yes this is not a world of fully continuous interpretations yet so we need to use what we have developed and supported as a Management Team.

We have supported LOD=16RFU since the PP21 validation in 2012, and continually supported this value through a number of reassessments since then. Our PP21 training presentations support above LOD of 16 and our case mgt SOPs support this as well, including the intuitive exclusion workflow and no. contributor guidelines.

We don't have any SOPs or guidelines supporting below this value. We are supported in below LOR within our documentation for assistance in determining possible number of contributors.

We use, and have used for years the same value for assessment of controls; we are not supported in our quality system below this value.

We'll keep moving with the values we have supported and we can look forward to the assessments of these values with VFP in conjunction with the latest versions of GMIDx and STRmix.

Independently I have assessed this profile and am satisfied with the interpretation as entered. I don't think a rework will work here; the only option would be a microcon. I think this is suitable to review as it is.

Regards
Justin

Justin Howes
Team Leader - Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Police Services Stream
Forensic & Scientific Services, Health Support Queensland, Queensland Health

Please note that I may be working from a different location during the COVID-19 Pandemic. The best contact method is via email.

p [redacted] m [redacted]
e [redacted] w www.health.qld.gov.au/healthsupport/businesses/forensic-and-scientific-services

From: Kylie Rika <[redacted]>
Sent: Friday, 24 April 2020 10:41 AM
To: Allan McNevin [redacted]>
Cc: Justin Howes <[redacted]>
Subject: RE: [redacted]

Thanks Allan

In a world where we are not yet fully continuous, we have no choice but to assign thresholds. However, we must also take into consideration the experience and subjectivity of the analyst. We have DNA profile interpretation guidelines to assist in helping to group staff's interpretation approaches as closely together as possible, but the fact still remains, these are guidelines and interpretation is subjective.

I would be more confident explaining why I had considered the 21 peak at D2 (when it is only a few rfu less than the 25 and is clear from bassline and has good morph) than explaining why I ignored it because we have an arbitrary LOD of 16rfu (which isn't even the true LOD). 16rfu was decided on out of convenience after the laser change in 2017. I note that the # of contrib. guidelines talks about not using S/T peaks under LOD, but those guidelines were developed before the laser change. I guess what I am saying is that in the interests of continuous improvements in the way we interpret, we are probably overdue for a re-think on some things.

I also note the following from Taylor, Buckleton and Bright:

Does the use of probabilistic genotyping change the way we should view sub-threshold data?

Duncan Taylor, John Buckleton & Jo-Anne Bright

3. Conclusion
Continuous systems (at least STRmix as trialled here) can overcome the issues of missing low-level data with minimal effects on the outcome of the analysis. The effects of overestimation of the number of contributors may not be too severe as long as the system has been reliably validated for this policy. This situation should not be used to

enable a reduction of valid quality practices such as replication and careful expert inspection of profiles and cannot be assumed to be conservative. However, any system, even one possessing the soundest theoretical basis, that cannot withstand the rigours of practical use, is destined to remain nothing more than a nice idea. We have discussed strategies to mitigate the effect of uncertainty in the number of trace contributors present when sub-threshold information is present in a DNA profile. We support replication and lowering the AT whenever practical. The use of sub-threshold data without lowering the AT may be useful in some cases. The effects of mis-assignment of N in either direction are relatively mild and restricted to LRs less than one when comparing known contributors and low LRs greater than one when comparing known non-contributors.
We believe that treating the number of contributors as an unknown nuisance variable is the best long-term solution. An even better solution would be to combine the treatment of number of contributors as a nuisance variable with an expert system that utilises fluorescent signal directly and has models for different known artefacts. In such a system all data would be treated probabilistically and the tyranny of thresholds would be completely abolished. We are not aware of any system that can perform at this level and so can provide no examples of how it would perform.
Last, we suggest that some profiles are simply too complex and should not be interpreted. Ultimately it is the role of the scientist to assess each profile on its own merits and the case context in order to determine if and how analysis will proceed.

New baseline work with VFP will be a good opportunity to re-consider the way we consider LOD and LOR (or just one AT). It doesn't matter where you put a threshold you will always have peaks under it that you need to decide what to do with.

Thanks
Kylie

From: Allan McNevin <[redacted]>
Sent: Thursday, 23 April 2020 2:43 PM
To: Kylie Rika <[redacted]>
Subject: RE: [redacted]

Hi Kylie,
Apologies for the delayed response. I have consulted with Justin, and his advice was that the inclusion of any possible peaks below the agreed upon 16RFU LOD limit is not a supported approach. There was a previous discussion and subsequent voting e-mail where the management team agreed to maintaining this LOD. Please discuss this with Justin if you would like more information or would like to discuss further the inclusion of sub LOD peaks.
Cheers
Al

Allan McNevin
Senior Scientist – Evidence Recovery

Evidence Recovery Team, Forensic DNA Analysis
Forensic & Scientific Services, Health Support Queensland, Queensland Health

p [redacted]
e [redacted] w www.health.qld.gov.au/healthsupport/businesses/forensic-and-scientific-services

From: Kylie Rika <[redacted]>
Sent: Wednesday, 11 March 2020 11:38 AM
To: Allan McNevin <[redacted]>
Subject: RE: [redacted]

Hi Allan

In my opinion this profile is 4p or complex and for me I would say complex.

I can't justify calling the 25 peak at D2 sub thresh and then just ignoring the 21 peak just because it is below LOD when they both look very similar. In addition our actual calculated LOD is 8 not 16 as per:

I:\Change Management\Verification of Equipment (post part replacement)\Review Baseline 3130xl B post laser change January 2017/Summary Report 3130xl B laser change January 2017 vfinal

Feel free to ask another scientist for their opinion.

Thanks
Kylie

From: Allan McNevin <[redacted]>
Sent: Thursday, 6 February 2020 3:01 PM
To: Kylie Rika [redacted]>
Subject: RE: [redacted]

Hi,

I've uploaded a further zoom from GMIDX on the D2 locus, there is a 16,19 "major" 18,20 above threshold in the minor, 22 & 25 subthreshold, everything else is below LOD

Cheers
Al

Allan McNevin
Senior Scientist – Evidence Recovery

Evidence Recovery Team, Forensic DNA Analysis
Forensic & Scientific Services, Health Support Queensland, Queensland Health

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From: Kylie Rika <[redacted]>
Sent: Thursday, 6 February 2020 2:54 PM
To: Allan McNevin [redacted]>
Subject: RE: [redacted]

D2

From: Allan McNevin <[redacted]>
Sent: Thursday, 6 February 2020 12:12 PM
To: Kylie Rika <[redacted]>
Subject: RE: [redacted]

HI,
I had another look. I'm still not seeing greater than 3P, can you point me in the direction of what you are seeing?
Thanks
Al

Allan McNevin
Senior Scientist – Evidence Recovery

Evidence Recovery Team, Forensic DNA Analysis
Forensic & Scientific Services, Health Support Queensland, Queensland Health

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From: Kylie Rika <[redacted]>
Sent: Thursday, 6 February 2020 11:37 AM
To: Allan McNevin <[redacted]>
Subject: [redacted]

Hi Allan

It is my opinion that this is >3p

Can you please have another look

Thanks
Kylie

Kylie Rika
Senior Scientist - Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Police Services Stream
Forensic & Scientific Services, Health Support Queensland, Queensland Health

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KR-10-2

Kylie Rika

From: Kylie Rika
Sent: Wednesday, 3 June 2020 8:33 AM
To: Justin Howes
Subject: RE: [redacted]

Thanks Justin

I used the phrase "formally challenge" based on some advice given to me on the 13 May 2020.

I will follow up with an avenue to do this.

Thanks for you time with all of this.

Kylie

From: Justin Howes <[redacted]>
Sent: Tuesday, 2 June 2020 4:11 PM
To: Kylie Rika [redacted]>
Subject: RE: [redacted]

Hi Kylie
Thankyou for your information below. The information provided is part related to a particular sample and its reporting, and part related to process. This email is to address the process component only.

You have some options available to you and it will be for you to determine which path you take. I would suggest the starting point is to be clear on what it is exactly you would like to 'formally challenge'. I gather it is more of a review you are requesting rather than the strong word of 'challenge', and this is something that you could put to the Mgt Team to consider.

Regards
Justin

Justin Howes
Team Leader - Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Police Services Stream
Forensic & Scientific Services, Health Support Queensland, Queensland Health

Please note that I may be working from a different location during the COVID-19 Pandemic. The best contact method is via email.

p [redacted] m [redacted]
a [redacted]
e [redacted] w www.health.qld.gov.au/healthsupport/businesses/forensic-and-scientific-services

From: Kylie Rika <[redacted]>
Sent: Thursday, 14 May 2020 12:08 PM
To: Justin Howes <[redacted]>; Allan McNevin <[redacted]>
Subject: RE: [redacted]

Hi both

I have used the relevant SOPs and guidelines to finalise the review of this sample.

There are many aspects that I consider when interpreting a DNA profile. One cannot simply "not see" other peaks that are below LOD and they do help build a picture of the profile as a whole, however I would never use this alone to make a call on a DNA profile. In fact with this DNA profile [redacted], if I was simply blind to peaks below LOD, my call on this DNA profile is that it is still complex unsuitable due to the followings reasons:

Degradation – is observed and according to the number of contributors guidelines, the sample should be reworked
Stochastic range – at least two of the contributors in this sample are within the stochastic range (below ~300 rfu) and according to the number of contributors guidelines, further investigation may be required in this scenario
Allelic Imbalance – this is observed within this DNA profile and according to the number of contributors guidelines, the sample should be reworked
Reworking – according to the number of contributors guidelines, this is the type of sample that should be reworked despite it being a P3 sample

As a reviewer it is my opinion that either this sample is reworked or called complex unsuitable.

Allan if you decide to not rework then you will have to get a third expert to give their opinion on this sample. If it turns out that the third person agrees with your interpretation, then my opinion as original reviewer will need to be documented including the grounds for the dismissal of my viewpoint. This could be included in the FR as a notation against the sample.

As a side discussion, I note that none of our SOPs actually list LOD as being 16rfu except for 33538 which is an information document, not a SOP. If the definition of LOD is mean + 3 SD, then that is 8 rfu.

I also note the following from the number of contributors guidelines:

The aim of these guidelines is to assist in the assessment of the number of contributors for mixed DNA profiles obtained using the PowerPlex 21 system. These guidelines should be used in conjunction with the training and experience of the scientist. There may be features within the DNA profile other than those detailed in this document that may inform the number of contributors. If the scientist observes information/behaviours within the DNA profile that override these guidelines, it is acceptable for these observations to be used in the determination of the number of contributors. There are also certain reworks that are required, for example for quality reasons, before a reasonable assessment of the number of contributors can be made and these should be performed separately to the guidelines provided. Background information has also been presented in this document as this has been considered in the development of the recommendations provided. References to stochastic effects relate to peaks which may drop out or be imbalanced.

Further, I would like to take this opportunity to formally challenge our SOPs and information documents in QIS as needing to be updated to better reflect what the validation data shows, what is suggested in current literature and what we have learned and experienced with DNA profiles over the last few years, in the interest of continuous quality improvement. Could you please advise on the most appropriate channel for me to request a review of the LOD and how we use it?

Thanks

Kylie

From: Justin Howes <[redacted]>
Sent: Friday, 24 April 2020 4:33 PM
To: Kylie Rika <[redacted]>; Allan McNevin <[redacted]>
Subject: RE: [redacted]

Hi both
Healthy discussion is great! Firstly, yes this is not a world of fully continuous interpretations yet so we need to use what we have developed and supported as a Management Team.

We have supported LOD=16RFU since the PP21 validation in 2012, and continually supported this value through a number of reassessments since then. Our PP21 training presentations support above LOD of 16 and our case mgt SOPs support this as well, including the intuitive exclusion workflow and no. contributor guidelines.

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Independently I have assessed this profile and am satisfied with the interpretation as entered. I don't think a rework will work here; the only option would be a microcon. I think this is suitable to review as it is.

Regards
Justin

Justin Howes
Team Leader - Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Police Services Stream
Forensic & Scientific Services, Health Support Queensland, Queensland Health

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p [redacted] m [redacted]
a [redacted]
e [redacted] w www.health.qld.gov.au/healthsupport/businesses/forensic-and-scientific-services

From: Kylie Rika <[redacted]>
Sent: Friday, 24 April 2020 10:41 AM
To: Allan McNevin <[redacted]>

Cc: Justin Howes <[redacted]>
Subject: RE: [redacted]

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I also note the following from Taylor, Buckleton and Bright:

Does the use of probabilistic genotyping change the way we should view sub-threshold data?

Duncan Taylor, John Buckleton & Jo-Anne Bright

3. Conclusion

Continuous systems (at least STRmix as trialled here) can overcome the issues of missing low-level data with minimal effects on the outcome of the analysis. The effects of overestimation of the number of contributors may not be too severe as long as the system has been reliably validated for this policy. This situation should not be used to enable a reduction of valid quality practices such as replication and careful expert inspection of profiles and cannot be assumed to be conservative. However, any system, even one possessing the soundest theoretical basis, that cannot withstand the rigours of practical use, is destined to remain nothing more than a nice idea. We have discussed strategies to mitigate the effect of uncertainty in the number of trace contributors present when sub-threshold information is present in a DNA profile. We support replication and lowering the AT whenever practical. The use of sub-threshold data without lowering the AT may be useful in some cases. The effects of mis-assignment of N in either direction are relatively mild and restricted to LRs less than one when comparing known contributors and low LRs greater than one when comparing known non-contributors.

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New baseline work with VFP will be a good opportunity to re-consider the way we consider LOD and LOR (or just one AT). It doesn't matter where you put a threshold you will always have peaks under it that you need to decide what to do with.

Thanks
Kylie

From: Allan McNevin <[redacted]>
Sent: Thursday, 23 April 2020 2:43 PM
To: Kylie Rika <[redacted]>
Subject: RE: [redacted]

Hi Kylie,
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Cheers
Al

Allan McNevin
Senior Scientist – Evidence Recovery

Evidence Recovery Team, Forensic DNA Analysis
Forensic & Scientific Services, Health Support Queensland, Queensland Health

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From: Kylie Rika <[redacted]>
Sent: Wednesday, 11 March 2020 11:38 AM
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Subject: RE: [redacted]

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I:\Change Management\Verification of Equipment (post part replacement)\Review Baseline 3130xl B post laser change January 2017/Summary Report 3130xl B laser change January 2017 vfinal

Feel free to ask another scientist for their opinion.

Thanks
Kylie

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To: Kylie Rika <[redacted]>
Subject: RE: [redacted]

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Cheers
Al

Allan McNevin
Senior Scientist – Evidence Recovery

Evidence Recovery Team, Forensic DNA Analysis
Forensic & Scientific Services, Health Support Queensland, Queensland Health

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From: Kylie Rika <[redacted]>
Sent: Thursday, 6 February 2020 2:54 PM
To: Allan McNevin <[redacted]>
Subject: RE: [redacted]

D2

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Sent: Thursday, 6 February 2020 12:12 PM
To: Kylie Rika <[redacted]>
Subject: RE: [redacted]

HI,
I had another look. I'm still not seeing greater than 3P, can you point me in the direction of what you are seeing?
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Al

Allan McNevin
Senior Scientist – Evidence Recovery

Evidence Recovery Team, Forensic DNA Analysis
Forensic & Scientific Services, Health Support Queensland, Queensland Health

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Sent: Thursday, 6 February 2020 11:37 AM
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Can you please have another look

Thanks
Kylie

Kylie Rika
Senior Scientist - Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Police Services Stream
Forensic & Scientific Services, Health Support Queensland, Queensland Health

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KR-11

Kylie Rika

From: Kylie Rika
Sent: Tuesday, 5 May 2020 11:08 AM
To: John Doherty
Subject: RE: concerns

Thanks John

There isn't a flaw in the validation, rather a flaw in the management decision made at the time based on the data. The decision was based on convenience and is now outdated. I have raised this issue with Justin on a number of occasions, as have other staff, however he does not seem open to considering the alternative viewpoint.

The issue is that it doesn't matter how scientifically sound an argument of mine is – because it is coming from me and Justin doesn't like to be challenged, he won't listen/accept my opinion.

I agree that the scientific debate on a DNA profile interpretation should be documented and I would like to include the email chain as a notation in the Forensic Register if OK with you. However, until now, this approach has been discouraged.

I would like to accept your offer of help to resolve this matter with Justin as I feel I am currently stuck between doing the right thing in my work and just doing what I am told to have an easier life at work

I appreciate your time in reading my concerns.

Regards
Kylie

From: John Doherty <[redacted]>
Sent: Tuesday, 5 May 2020 8:51 AM
To: Kylie Rika <[redacted]>
Subject: RE: concerns

Good morning Kylie

On point one, I don't feel fully qualified to get into the technical aspects here, but offer up the following:
Where a method is validated, it is expected that we will then use the validation values in our interpretations. Anything outside of this goes against our scientific rigour (and kind of negates the validation). However, that can also lead to a very binary way of thinking which takes subjectivity away from the expert. Sometimes an expert will see something that causes them concern. The point of expertise, is that 2 experts can have differing opinions. This is why in such scenarios I have previously worked in, rather than trying to influence the decision of the second expert, a third expert would be brought in blind and asked to review the work without knowledge of the two differing opinions. This essentially leads to two experts having a shared opinion which can be reported. The third opinion however, needs to remain on file, with a subsequent explanation as to why the differing opinion has not been used, or why it has been considered and discounted.
On the surface of it, from what I can gather, it looks like what you want to do is outside of the agreed SOP. That would usually be grounds enough for discounting that opinion. If however, there is a serious flaw in the validation, then that is a matter of greater concern.

On the second point, my interpretation is slightly different. It looks like he's throwing ideas around and one of those, which is clearly not appropriate, is to share a password with a trusted colleague. He does seem to be preferring the option to find a way to achieve this remotely and I can tell you that he did raise it with Cathie, who raised it with me. I have emailed the Supt to find a solution. Perhaps if you wish to reply to him, you can say something like:

"Thank you Justin. I would imagine that the first option is the only way to achieve the outcome whilst maintaining appropriate security standards, so I will await your further advice."
Some of the work we're doing with Tess is around finding ways to challenge each other without being confrontational. The sentence I provided above is much gentler than "the ethics of the second suggestion is highly questionable and I won't be doing that" for example.

If you want, I can try to come up with some words to hep you challenge Justin on the first issue. This may be something Tess can also help with?

Regards

John Doherty
Executive Director

Forensic and Scientific Services
Health Support Queensland, Queensland Health

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From: Kylie Rika <[redacted]>
Sent: Sunday, 3 May 2020 6:54 PM
To: John Doherty <[redacted]>
Subject: concerns

Hello John

I am writing to bring to your attention two matters that I am deeply concerned about.

1. A direction given to me by Justin that I fear might touch on Section 127, 1b of the Criminal Code 1899 - he has directed me to review and validate a DNA result that I believe to be incorrect (see attached documentation)

2. An email sent to me by Justin suggesting that I ask one of my staff members to give me their login and password details for the Forensic Register. (see attached documentation)

I believe that Justin directing me to do these things is not only unethical (or possibly has some legal context), but I also feel bullied.

Can you please advise how I am to handle this situation?

Kind regards
Kylie

Kylie Rika
Senior Scientist - Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Police Services Stream
Forensic & Scientific Services, Health Support Queensland, Queensland Health

Please note that I may be working from a different location during the COVID-19 Pandemic. The best contact method is via email.

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KR-12

Kylie Rika

From: Kylie Rika
Sent: Wednesday, 17 June 2020 4:25 PM
To: John Doherty
Subject: Re: concern (follow up)

Yes see you then. Thanks
Kylie

Sent on the go with Vodafone

From: John Doherty <[redacted]>
Sent: Wednesday, June 17, 2020 4:13:13 PM
To: Kylie Rika <[redacted]>
Subject: Re: concern (follow up)

Ok, can we say 2:30 in my office?

sent from my iPhone

John Doherty
Executive Director
Forensic & Scientific Services
Health Support Queensland

From: Kylie Rika <[redacted]>
Sent: Wednesday, June 17, 2020 4:09:37 PM
To: John Doherty <[redacted]>
Subject: RE: concern (follow up)

Hi John

Monday after 2pm is fine. I won't be working from home that day so I can come to your office if that suits?

I am on standby for court next week so if I am a no-show it will be because they have called me last minute to give evidence.

Thanks
Kylie

From: John Doherty <[redacted]>
Sent: Wednesday, 17 June 2020 4:05 PM
To: Kylie Rika <[redacted]>
Subject: RE: concern (follow up)

Thanks Kylie

I'm happy to meet for a catch up and chat this through. Unfortunately, I can't do that until next Monday afternoon (after 2pm)... does that work for you? I know it's after the first workshop (which is on Monday morning...)... I possibly could od before that workshop if it was better for you?

John

From: Kylie Rika [redacted]>
Sent: Wednesday, 17 June 2020 3:57 PM
To: John Doherty <[redacted]>
Subject: concern (follow up)

Good afternoon John

Thank you again for your help with this matter. Since we last spoke about this on MS Teams, I moved forward as per your advice and sent Justin and Allan the below email (14 May)

Neither of them responded to me until I sent a follow up email to them on 2 June. Allan did not respond at this time but Justin did.

He said that my assistance was no longer required with this sample as he was going to get someone else to review it for Allan. The new reviewer suggested that Allan rework the sample (as did I) – which he did. The sample has subsequently been reported as a 4 person mixture, not a 3 person mixture. So Allan's original interpretation of 3 person mixture (which I was questioning) was incorrect.

With regards to me challenging the LOD – this is still in progress but so far at least 14 of the 19 reporters support a request being put forward to review the LOD. Interestingly when I looked up the Australasian Statspwg recommendations I found:

Recommendation 13: LOD

We recommend that the limit of detection be set as a convenient round number above the average baseline plus 10 standard deviations (SD). Information down to the baseline plus 3 SD may be used for information purposes, for instance to guide on the number of contributors or on non-concordances.

From our last chat on MS Teams, I recall you asked me to let you know if there was any blowback from me raising this concern with you. The answer to that is yes, particularly with Justin. Unfortunately the retribution has been subtle and insidious and if I were to call them out on it, they could easily turn the coin to say things like "that's not what I meant", "you took it the wrong way" etc…

I am very keen to move forward with positive relationships but this is difficult when it is not mutual.

I really hope the process with Tess can bring positivity and look forward to seeing what happens in the workshops. I will be bringing my best self to these, as I hope the others do too.

From our last chat and from the presentations meeting today, I have been left feeling unsure if I have done something wrong or not. If I have done something wrong, I am eager to know as I only ever want to do the right thing at work. I understand that a meeting with you might be better than this email so please let me know if you would like to see me.

Thanks for your time and your help with my concern.

Kylie

From: Kylie Rika
Sent: Tuesday, 2 June 2020 3:14 PM
To: Justin Howes <[redacted]>; Allan McNevin <[redacted]>
Subject: RE: [redacted] follow up

Hi both

I just wanted to follow up on this email I sent to you on the 14 May, as I have not yet had any replies.

Also, as you know, I have been working on the STRmix discussion paper (what to do with old versions of STRmix) and as part of my research for this, I came across the attached **STATSWG recommendations for the interpretation of DNA** (which I believe have been endorsed by BSAG).

I note Recommendation 13: LOD as follows:

Recommendation 13: LOD

We recommend that the limit of detection be set as a convenient round number above the average baseline plus 10 standard deviations (SD). Information down to the baseline plus 3 SD may be used for information purposes, for instance to guide on the number of contributors or on non-concordances.

Note: the term limit of detection is inappropriate and we are seeking an alternative. One suggestion is that the term 'limit of detection' refer to the average + 3SD and the term 'baseline' refer to average + 10SD

Thanks
Kylie

From: Kylie Rika
Sent: Thursday, 14 May 2020 12:08 PM
To: Justin Howes <[redacted]>; Allan McNevin <[redacted]>
Subject: RE: [redacted]

Hi both

I have used the relevant SOPs and guidelines to finalise the review of this sample.

There are many aspects that I consider when interpreting a DNA profile. One cannot simply "not see" other peaks that are below LOD and they do help build a picture of the profile as a whole, however I would never use this alone to make a call on a DNA profile. In fact with this DNA profile [redacted] if I was simply blind to peaks below LOD, my call on this DNA profile is that it is still complex unsuitable due to the followings reasons:

Degradation – is observed and according to the number of contributors guidelines, the sample should be reworked
Stochastic range – at least two of the contributors in this sample are within the stochastic range (below ~300 rfu) and according to the number of contributors guidelines, further investigation may be required in this scenario
Allelic Imbalance – this is observed within this DNA profile and according to the number of contributors guidelines, the sample should be reworked
Reworking – according to the number of contributors guidelines, this is the type of sample that should be reworked despite it being a P3 sample

As a reviewer it is my opinion that either this sample is reworked or called complex unsuitable.

Allan if you decide to not rework then you will have to get a third expert to give their opinion on this sample. If it turns out that the third person agrees with your interpretation, then my opinion as original reviewer will need to be

documented including the grounds for the dismissal of my viewpoint. This could be included in the FR as a notation against the sample.

As a side discussion, I note that none of our SOPs actually list LOD as being 16rfu except for 33538 which is an information document, not a SOP. If the definition of LOD is mean + 3 SD, then that is 8 rfu.

I also note the following from the number of contributors guidelines:

The aim of these guidelines is to assist in the assessment of the number of contributors for mixed DNA profiles obtained using the PowerPlex 21 system. These guidelines should be used in conjunction with the training and experience of the scientist. There may be features within the DNA profile other than those detailed in this document that may inform the number of contributors. If the scientist observes information/behaviours within the DNA profile that override these guidelines, it is acceptable for these observations to be used in the determination of the number of contributors. There are also certain reworks that are required, for example for quality reasons, before a reasonable assessment of the number of contributors can be made and these should be performed separately to the guidelines provided. Background information has also been presented in this document as this has been considered in the development of the recommendations provided. References to stochastic effects relate to peaks which may drop out or be imbalanced.

Further, I would like to take this opportunity to formally challenge our SOPs and information documents in QIS as needing to be updated to better reflect what the validation data shows, what is suggested in current literature and what we have learned and experienced with DNA profiles over the last few years, in the interest of continuous quality improvement. Could you please advise on the most appropriate channel for me to request a review of the LOD and how we use it?

Thanks
Kylie

From: Justin Howes <[redacted]>
Sent: Friday, 24 April 2020 4:33 PM
To: Kylie Rika <[redacted]>; Allan McNevin <[redacted]>
Subject: RE: [redacted]

Hi both
Healthy discussion is great! Firstly, yes this is not a world of fully continuous interpretations yet so we need to use what we have developed and supported as a Management Team.

We have supported LOD=16RFU since the PP21 validation in 2012, and continually supported this value through a number of reassessments since then. Our PP21 training presentations support above LOD of 16 and our case mgt SOPs support this as well, including the intuitive exclusion workflow and no. contributor guidelines.

We don't have any SOPs or guidelines supporting below this value. We are supported in below LOR within our documentation for assistance in determining possible number of contributors.

We use, and have used for years the same value for assessment of controls; we are not supported in our quality system below this value.

We'll keep moving with the values we have supported and we can look forward to the assessments of these values with VFP in conjunction with the latest versions of GMIDx and STRmix.

Independently I have assessed this profile and am satisfied with the interpretation as entered. I don't think a rework will work here; the only option would be a microcon. I think this is suitable to review as it is.

Regards
Justin

Justin Howes
Team Leader - Forensic Reporting and Intelligence Team
Forensic DNA Analysis, Police Services Stream
Forensic & Scientific Services, Health Support Queensland, Queensland Health
Please note that I may be working from a different location during the COVID-19 Pandemic. The best contact method is via email.
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Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.

From: Kylie Rika <[redacted]>
Sent: Friday, 24 April 2020 10:41 AM
To: Allan McNevin <[redacted]>
Cc: Justin Howes <[redacted]>
Subject: RE: [redacted]

Thanks Allan

In a world where we are not yet fully continuous, we have no choice but to assign thresholds. However, we must also take into consideration the experience and subjectivity of the analyst. We have DNA profile interpretation guidelines to assist in helping to group staff's interpretation approaches as closely together as possible, but the fact still remains, these are guidelines and interpretation is subjective.

I would be more confident explaining why I had considered the 21 peak at D2 (when it is only a few rfu less than the 25 and is clear from bassline and has good morph) than explaining why I ignored it because we have an arbitrary LOD of 16rfu (which isn't even the true LOD). 16rfu was decided on out of convenience after the laser change in 2017. I note that the # of contrib. guidelines talks about not using S/T peaks under LOD, but those guidelines were developed before the laser change. I guess what I am saying is that in the interests of continuous improvements in the way we interpret, we are probably overdue for a re-think on some things.

I also note the following from Taylor, Buckleton and Bright:

Does the use of probabilistic genotyping change the way we should view sub-threshold data?

Duncan Taylor, John Buckleton & Jo-Anne Bright

3. Conclusion

Continuous systems (at least STRmix as trialled here) can overcome the issues of missing low-level data with minimal effects on the outcome of the analysis. The effects of overestimation of the number of contributors may not be too severe as long as the system has been reliably validated for this policy. This situation should not be used to enable a reduction of valid quality practices such as replication and careful expert inspection of profiles and cannot be assumed to be conservative. However, any system, even one possessing the soundest theoretical basis, that cannot withstand the rigours of practical use, is destined to remain nothing more than a nice idea. We have discussed strategies to mitigate the effect of uncertainty in the number of trace contributors present when sub-threshold information is present in a DNA profile. We support replication and

lowering the AT whenever practical. The use of sub-threshold data without lowering the AT may be useful in some cases. The effects of mis-assignment of N in either direction are relatively mild and restricted to LRs less than one when comparing known contributors and low LRs greater than one when comparing known non-contributors.
We believe that treating the number of contributors as an unknown nuisance variable is the best long-term solution. An even better solution would be to combine the treatment of number of contributors as a nuisance variable with an expert system that utilises fluorescent signal directly and has models for different known artefacts. In such a system all data would be treated probabilistically and the tyranny of thresholds would be completely abolished. We are not aware of any system that can perform at this level and so can provide no examples of how it would perform.
Last, we suggest that some profiles are simply too complex and should not be interpreted. Ultimately it is the role of the scientist to assess each profile on its own merits and the case context in order to determine if and how analysis will proceed.

New baseline work with VFP will be a good opportunity to re-consider the way we consider LOD and LOR (or just one AT). It doesn't matter where you put a threshold you will always have peaks under it that you need to decide what to do with.

Thanks
Kylie

From: Allan McNevin <[redacted]>
Sent: Thursday, 23 April 2020 2:43 PM
To: Kylie Rika [redacted]>
Subject: RE: [redacted]

Hi Kylie,
Apologies for the delayed response. I have consulted with Justin, and his advice was that the inclusion of any possible peaks below the agreed upon 16RFU LOD limit is not a supported approach. There was a previous discussion and subsequent voting e-mail where the management team agreed to maintaining this LOD. Please discuss this with Justin if you would like more information or would like to discuss further the inclusion of sub LOD peaks.
Cheers
Al

Allan McNevin
Senior Scientist – Evidence Recovery
Evidence Recovery Team, Forensic DNA Analysis
Forensic & Scientific Services, Health Support Queensland, Queensland Health
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From: Kylie Rika <[redacted]>
Sent: Wednesday, 11 March 2020 11:38 AM
To: Allan McNevin <[redacted]>
Subject: RE: [redacted]

Hi Allan

In my opinion this profile is 4p or complex and for me I would say complex.

I can't justify calling the 25 peak at D2 sub thresh and then just ignoring the 21 peak just because it is below LOD when they both look very similar. In addition our actual calculated LOD is 8 not 16 as per:

I:\Change Management\Verification of Equipment (post part replacement)\Review Baseline 3130xl B post laser change January 2017/Summary Report 3130xl B laser change January 2017 vfinal

Feel free to ask another scientist for their opinion.

Thanks
Kylie

From: Allan McNevin <[redacted]>
Sent: Thursday, 6 February 2020 3:01 PM
To: Kylie Rika <[redacted]>
Subject: RE: [redacted]

Hi,

I've uploaded a further zoom from GMIDX on the D2 locus, there is a 16,19 "major" 18,20 above threshold in the minor, 22 & 25 subthreshold, everything else is below LOD

Cheers
Al

Allan McNevin
Senior Scientist – Evidence Recovery
Evidence Recovery Team, Forensic DNA Analysis
Forensic & Scientific Services, Health Support Queensland, Queensland Health
[redacted]
e [redacted] w www.health.qld.gov.au/healthsupport/businesses/forensic-and-scientific-services

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From: Kylie Rika <[redacted]>
Sent: Thursday, 6 February 2020 2:54 PM
To: Allan McNevin <[redacted]>
Subject: RE: [redacted]

D2

From: Allan McNevin <[redacted]>
Sent: Thursday, 6 February 2020 12:12 PM
To: Kylie Rika <[redacted]>
Subject: RE: [redacted]

HI,
I had another look. I'm still not seeing greater than 3P, can you point me in the direction of what you are seeing?
Thanks
Al

Allan McNevin
Senior Scientist – Evidence Recovery
Evidence Recovery Team, Forensic DNA Analysis
Forensic & Scientific Services, Health Support Queensland, Queensland Health
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From: Kylie Rika <[redacted]>
Sent: Thursday, 6 February 2020 11:37 AM
To: Allan McNevin <[redacted]>
Subject: [redacted]

Hi Allan

It is my opinion that this is >3p

Can you please have another look

Thanks
Kylie

Kylie Rika
Senior Scientist - Forensic Reporting and Intelligence Team
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KR-13

Kylie Rika

From: Kylie Rika
Sent: Tuesday, 23 June 2020 10:45 AM
To: John Doherty
Subject: Taylor 2017 Sub threshold data.pdf
Attachments: Taylor 2017 Sub threshold data.pdf

Good morning John

Thank you again for the chat yesterday. In light of our chat on LOD/thresholds, I thought I would send you the attached paper from Taylor, Buckleton and Bright – a paper we didn't have at the time of setting and making rules around LOD and LOR back in 2017. I have highlighted a part in yellow of particular interest.

If we do end up having an external person come in to look at things – we would probably have a list of a few DNA interpretation issues that are currently the cause of two schools of thought/contention. I think it would be wise to make the most of this persons time/expertise to help us.

Thanks again
Kylie

KR-13-1

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Does the use of probabilistic genotyping change the way we should view sub-threshold data?

Duncan Taylor, John Buckleton & Jo-Anne Bright

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Does the use of probabilistic genotyping change the way we should view sub-threshold data?

Duncan Taylor[a,b*], John Buckleton[c,d] and Jo-Anne Bright[c]

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(*Received 11 August 2015; accepted 15 November 2015*)

The sensitivity and resolution of modern DNA profiling hardware is such that forensic laboratories generate more data than they have resources to analyse. One coping mechanism is to set a threshold, above the minimum required by instrument noise, so that weak peaks are screened out. In binary interpretations of forensic profiles, the impact of this threshold (sometimes called an analytical threshold – AT) was minimal as interpretations were often limited to a clear major component. With the introduction of continuous typing systems, the interpretation of weak minor components of mixed DNA profiles is possible and consequently the consideration of peaks just above or just below the analytical threshold becomes relevant. We investigate here the occurrence of low-level DNA profile information, specifically that which falls below the analytical threshold. We investigate how it can be dealt with and the consequences of each choice in the framework of continuous DNA profile interpretation systems. Where appropriate we illustrate how these can be implemented using the probabilistic interpretation software STRmix. We demonstrate a feature of STRmix that allows the analyst to guide the software, using human observation that there is a low-level contributor present, through user-designated prior distributions for contributor mixture proportions.

Keywords: DNA profile interpretation; sub-threshold; likelihood ratios; analytical threshold

1. Introduction

The primary method for the analysis of a DNA sample is amplification by polymerase chain reaction (PCR), which incorporates a flurophore. This is followed by separation of the fragments by capillary electrophoresis. The output is a trace of fluorescence versus time that is referred to as an electropherogram (epg). Most laboratories set an analytical threshold (AT), above which peaks are labelled at analysis. The AT is often set well above the level of electronic noise. Peaks in the epg may be artefactual or allelic. Epg analysis software can recognise and filter some of the well-characterised artefacts, but many still require the judgement of a human analyst. Many of these remaining artefactual peaks can be recognised by position or morphology. In binary interpretations, the impact of these weak peaks was minimal as interpretations were often limited to the interpretation of a clear major component. With the introduction of continuous typing systems the interpretation of weak minor components of mixed DNA profiles is

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possible and consequently the consideration of peaks just above or just below analytical threshold becomes important.

There have been numerous published methods that describe how the AT could be determined. For a review the reader is referred to the work of Bregu et al.[1]. Some recognise that there are different factors that affect the AT, such as dye colour, input DNA amount or instrument[1,2]. The ideal situation is that these factors are considered on a sample by sample (and even locus by locus) basis and applied to the profile[3]. However, in order to balance the laboratory's sample processing capability with interpretation needs, the laboratories may need to apply a single AT that applies to all profiles, or an AT that is based on dye label, and is set at a level designed to screen out much low level artefactual fluorescence. Thus, it is of value to address the issue of sub-AT information from a standpoint that continues to address the balance between sample processing and interpretation. As such, the purpose of this work is to examine the effects of using a sub-AT threshold signal on interpretation rather than investigating methods to determine the AT. This work considers that no matter where the AT is set, peaks will exist below it that appear allelic and may affect interpretation.

This work evaluates some options for analysts to deal with sub-threshold information and the risks or benefits associated with each in the context of analysis within a continuous DNA interpretation system. We introduce a novel method for dealing with sub-threshold data implemented within the STRmix programme that allows the user to specify a prior belief in mixture proportions.

Much of the discussion will be dominated by the topic of choosing a number of contributors for analysis, which is where the sub-AT peaks will have their biggest impact on interpretations.

There have been various works that look at the consequences of overestimation or underestimation of the number of contributors[4,5]. In general, the consequences of underestimation are that known contributors are excluded due to the forced pairing of peaks that in reality do not pair. The consequence of overestimation is more complex; doing so can have very little effect on a major contributor to a DNA profile and a more marked effect on a minor contributor. This is only true for continuous systems that take peak heights into account. For a semicontinuous system the effect of overestimation will have an effect on all contributors to a mixture as more genotype sets are considered for all contributors to the mixture (see Benschop et al.[6]). There is also a greater number of non-contributors that are given relatively neutral likelihood ratios (*LR*s) as the analysis is accounting for more potential dropout.

The Scientific Working Group on DNA Analysis methods (SWGDAM) guidelines for the validation of probabilistic genotyping systems[7] advise a study of over and underestimation of contributor numbers (at 4.1.6.4) so that the impact of the above-mentioned issues are known for the system being validated. There are methods available that do not require a number of contributors to be assigned[8]; however, the majority of current probabilistic software programmes do require a choice of number of contributors.

This leads to the question of how, if at all, sub-threshold information should be taken into account when making the choice of number of contributors. We consider four broad categories for consideration:

(1) ignore the presence of sub-threshold peaks when interpreting DNA profiles;
(2) change the method by which data are generated (either by lower the AT or carrying out replicate PCRs);

(3) use informed priors on mixture proportion in a probabilistic system;
(4) do not interpret the DNA profile.

1.1. *Ignore the presence of sub-threshold peaks when interpreting DNA profiles*

To consider the performance and consequence of utilising sub-threshold information when carrying out an interpretation we first start by considering the scope of the issue. We do this in two ways; first via a simulation designed to give an indication of how ignoring sub-threshold information will lead to an underestimate of the number of contributors in the most high-risk situations and, secondly, a demonstration of the practical consequences of ignoring sub-threshold data.

We first start by considering the probability that by ignoring sub-threshold information, a low-level two-person mixture would be assigned as a single source profile. We do this by simulating two contributors with low levels of DNA and different levels of allele sharing and over various analytical thresholds. Twenty-one locus profiles were simulated and the peak heights and AT are intended to be realistic for an Applied Biosystems 3130 capillary electrophoresis (CE) system (Thermo Fisher Scientific, CA). Details of this simulation are given in Appendix 1.

Simulation was chosen in this part of the study because it allows for control over the experimental conditions and for a large number of experiments (for example, Table 1 gives the results of 150,000 simulated mixtures).

Table 1 gives the number of simulations (out of 1000) of two low-level contributors that when combined collectively gave a profile that looked like a single contributor. Simple allele count per locus was used to assign the number of contributors. Use of peak heights is likely to be superior but at such low-levels this is not likely make a significant difference to the count[9].

Inspection of Table 1 suggests that, under the trialled circumstances, there is a high probability of the alleles from two individuals masquerading as a low-level single source profile. The table also shows that this effect is likely to be reduced at lower AT.

This simulation informs the probability of assigning one donor if there are in fact two. It is important not to confuse this with the probability that there are two if we assign one. This latter probability is what we really want. To obtain this probability we need the prior probabilities that there are one or two contributors in a profile. We are allowed to know what type of sample it is and what analysis regime we have employed but we cannot use profile information itself. We will use equal priors for this work, accepting that this was an arbitrary choice. Making this choice will restrict the lower bound probability that a profile is single source, given that it appears as single source to 0.5. Using these priors the probabilities in Table 2 are obtained (details of the calculation appear in Appendix 2).

For the CE system that we are simulating here it is likely that peaks above 30 rfu that have passed expert inspection are all allelic. This suggests that for an AT = 100 or 50 rfu there is a possible strategy of using peaks below the threshold to help improve the assignment of the number of contributors.

These results suggest that ignoring sub-threshold peaks when interpreting low level putatively mixed DNA profiles is likely to lead to underestimation of the number of contributors and thereby has the potential to lead to incorrect interpretations. It is unlikely that a blanket rule to ignore such information would be sustainable. There may be concern that these *in silico* mixtures ignore the effect of stutters. Any stutters misassigned as allelic tends to increase the allele count and hence have no effect at all in the direction of underestimation.

Table 1. The number of simulations (out of 1000) of two low-level contributors that gave a profile that looked like a single contributor based on allele count at 21 loci.

			Average peak height of Contributor 2 (rfu)									
			20	40	60	80	100	120	140	160	180	200
Average peak height of contributor 1 (rfu)	AT = 100 rfu	20	722	734	705	642	549	436	344	230	199	179
		40	734	947	869	718	559	337	194	118	113	78
		60	705	869	746	530	302	119	52	36	17	9
		80	642	718	530	283	95	22	6	3	0	0
		100	549	559	302	95	19	4	0	0	0	0
			Average peak height of Contributor 2 (rfu)									
			10	20	30	40	50	60	70	80	90	100
	AT = 50 rfu	10	754	694	633	557	448	356	249	201	168	137
		20	694	757	520	378	239	122	71	34	33	15
		30	633	520	305	151	57	19	10	2	1	1
		40	557	378	151	70	19	4	0	0	0	1
		50	448	239	57	19	2	0	0	0	0	0
			Average peak height of Contributor 2 (rfu)									
			10	20	30	40	50	60	70	80	90	100
	AT = 30 rfu	10	709	504	315	227	117	71	57	40	30	40
		20	504	302	110	32	16	5	1	0	2	0
		30	315	110	16	1	0	0	0	0	0	0
		40	227	32	1	0	0	0	0	0	0	0
		50	117	16	0	0	0	0	0	0	0	0

Table 2. The probability that the peaks above AT are from a single source (S) given that they look like a single source on simple allele count (AS), Pr(S|AS).

Masking	0.2		0.5	
Mean peak height in range AT (rfu)	10–50 rfu	10–100 rfu	10–50 rfu	10–100 rfu
	Pr(*S*|*AS*)			
30	0.91	0.98	0.70	0.82
50	0.66	0.87	0.56	0.67
100	0.56	0.61	0.59	0.56

We do however look at a number of *in vitro* mixtures. A range of four person mixtures were amplified using GlobalFiler (Thermo Fisher Scientific, CA), as per the manufacturer's instructions. Amplification fragments were resolved using the ABI PRISM 3130xl Genetic Analyser and analysed in GeneMapper ID-X to obtain peak height information for each profile. These mixtures are samples 22 to 31 from Ref. 10, amplified in triplicate except for sample 23 where there were only two replicates, leading to a total of 29 profiles. We reproduce the relevant mixture information from Ref. 10 in Table 3.

Profiles were analysed using ATs of 30 rfu, 50 rfu and 100 rfu. While it is possible to construct simpler mixtures that could be used in this experiment, we chose four-person mixtures due to the high probability that the number of contributors can be underestimated, the higher probability that masking or dropout may occur and as an example of profiles where the use of sub-AT information could have an important impact on the interpretation. Later (in Table 4) we show how, for the data sets used, the number of contributors could be underestimated over half the time.

Profiles were analysed using STRmix V2.3 which utilises models described in Refs 11–13 (exact software settings used are available from the corresponding author on request). In all analyses the Y-indel locus and DYS391 were ignored. A uniform probability for an allelic drop-in of 0.0017 was used (up to 75 rfu) for the 30 rfu and 50 rfu AT and a drop-in probability of zero was used for the 100 rfu AT, in line with laboratory observations.

Two experiments were carried out to investigate the consequences of ignoring the sub-threshold information when determining the number of contributors.

Table 3. Mixture proportions and PCR setup.

Tubes	mixture ratios for contributor C_1:C_2:C_3:C_4	Total DNA added to PCR (pg)
22	1:1:1:1	400
23		200
24		50
25		20
26		10
27	4:3:2:1	400
28		200
29		50
30		20
31		10

Table 4. Assigned number of contributors (based on peak count) are given showing the effect that lowering AT or carrying out replicates has on the ability to determine the number of contributors.

			AT = 10 rfu		AT = 30 rfu		AT = 50 rfu		AT = 100 rfu	
Template (pg)	ratio	replicate	1 PCR	3 PCR	1 PCR	3 PCR	1 PCR	3 PCR	1 PCR	3 PCR
400	1:1:1:1			4		4		4		4
		1	4		4		4		4	
		2	4		4		4		4	
		3	4		4		4		4	
	4:3:2:1	1	4	4	4	4	4	4	4	4
		2	4		4		4		4	
		3	4		4		4		4	
200	1:1:1:1	1	4	4	4	4	4	4	4	4
		2	4		4		4		4	
	4:3:2:1	1	4	4	4	4	4	4	3	3
		2	4		4		4		3	
		3	4		4		4		3	
50	1:1:1:1	1	3	4	3	4	3	3	1	2
		2	4		3		2		1	
		3	4		3		3		2	
	4:3:2:1	1	3	4	3	4	2	3	2	2
		2	4		3		3		2	
		3	3		3		3		2	
20	1:1:1:1	1	3	4	2	2	1	1	0	1
		2	3		2		1		0	
		3	3		2		1		1	
	4:3:2:1	1	2	3	2	2	1	2	0	1
		2	3		1		1		1	
		3	3		2		2		1	
10	1:1:1:1	1	2	3	1	1	1	1	0	0
		2	2		1		1		0	
		3	2		1		1		0	
	4:3:2:1	1	2	3	1	2	1	1	0	0
		2	2		1		0		0	
		3	3		2		1		0	

Experiment 1. Utilising sub-threshold information

First, the correct number of contributors was assigned to each profile during analysis and the *LR*s were calculated using the propositions:

H_p: The person of interest (POI) and three unknown individuals are the sources of DNA.

H_d: Four unknown individuals are the sources of DNA.

The POI was varied to be each of the four known contributors and 186 randomly selected non-contributors. *LR*s were calculated using an in-house self-declared Caucasian GlobalFiler database and using the product rule. This amounts to 116 STRmix analyses compared with known donors and 5394 comparisons to non-donors.

Experiment 2. Ignoring sub-threshold information

In this experiment, the number of contributors was chosen ignoring sub-threshold information i.e. based purely on the number of detected peaks above the varying AT. Using the chosen number of contributors, *N*, *LR*s were calculated using the propositions:

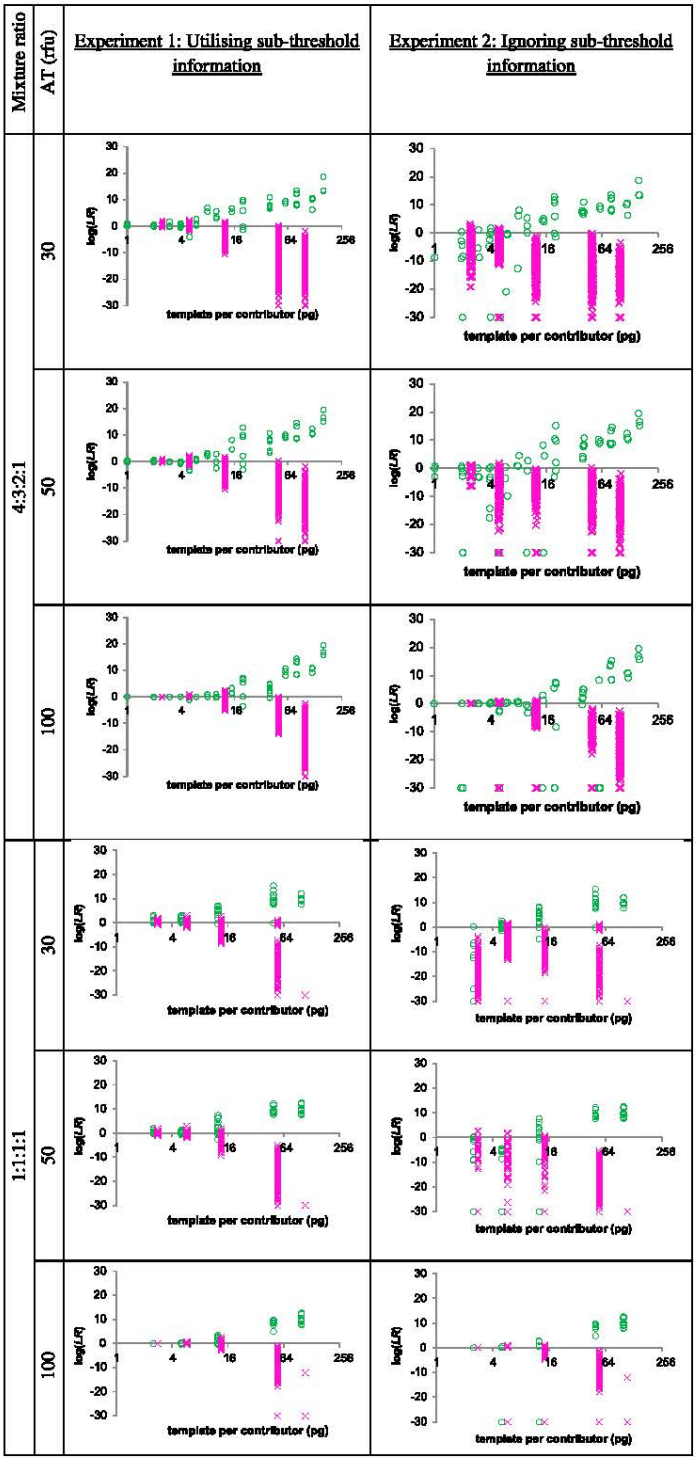

Figure 1. $\text{Log}_{10}(LR)$ versus template per contributor (pg) using sub-threshold information (experiment 1) or ignoring sub-threshold information (experiment 2) for a range of four person profiles.

H_p: The POI and (N–1) unknown individuals are the sources of DNA.

H_d: N unknown individuals are the sources of DNA.

The POI was varied to be each of the four known contributors and 186 randomly selected non-contributors. *LR*s were calculated using an in-house self-declared Caucasian GlobalFiler database and using the product rule.

Figure 1 shows the $\log_{10}(LR)$ produced for these comparisons. The *LRs* produced from comparisons to known contributors are signified by a green point and those produced from comparisons to known non-contributors are signified by a pink cross. A minimum value for $\log_{10}(LR)$ of –30 was used, and any *LR*s obtained that fell below this were given the value of –30. The amount of DNA contributed by each known contributor was known from the experimental design. When comparing to non-contributors, the choice of input DNA (for Figure 1) was not known as the non-contributor could align with any of the contributors' input DNA amounts. For known non-contributors the amount of input DNA was assigned as the total amount of DNA added to the PCR divided by the number of contributors. Due to the amount of information present in these graphs we also provide (as supplementary material) the same information but displayed by plotting the $\log_{10}(LR)$ value when considering or ignoring sub-threshold information against each other.

Figure 1 shows that underestimating the number of contributors can cause a $\log_{10}(LR)$ to become less than 0 (sometimes to minimum cap of the graphs) of a true trace contributor in some cases (note the scattered green circles at low log(*LR*) for low template). This is the expected outcome for underestimation[4,5]. We have chosen profiles that are most difficult to interpret due to complexity and high levels of dropout. In addition, a detailed examination of peak heights will be of some but limited use since the donor in dispute is trace and at the limits of the AT. In theory there should be a greater ability to exclude using fewer contributors and this is visible in the results (note the generally lower values for the crosses in the right-hand set of graphs in Figure 1).

This experiment looks at the consequences of underestimation of N and shows that utilising sub-threshold information can partially mitigate the issue. However, use of sub-threshold peaks should be tempered by the relative strength and amount of the putative additional contributor. When assigning a number of contributors based on sub-threshold information there is a risk that an overestimation can occur if any artefacts are considered allelic. It should therefore be balanced by reference to the previously published work[5,14] which showed that an increase in N beyond that required, can alter the *LR* for a true trace contributor and mildly increase the risk of low grade *LR* greater than one.

1.2. Change the method by which data are generated (either by lowering the AT or carrying out replicate PCRs)

To investigate the extent to which generating additional data can assist in interpretation we considered two possible strategies, first a lowering of the AT and second by generating additional PCR replicates. It has already been shown[10] that providing additional, relevant information into the analysis of DNA profile data increases the ability to distinguish a true from a false proposition. We also recognise that due to reasons of practicality there is going to be a limit to which laboratories are willing to lower their AT, and as stated in the introduction, no matter where this level is, there will always be data that appear just below it. We show the effect of lowering the AT as a means to

assist laboratories in their choice of AT, when they will inevitably have to weigh up throughput considerations again data generation.

We analyse the 29 mixed DNA profiles outlined in Table 3 using four different AT (10, 30, 50 and 100 rfu) and considering each of the three PCR replicates individually or in combination in order to determine the number of contributors.

Table 4 shows the effect that lowering AT, using sub-threshold information, or carrying out replicates has on the ability to determine the number of contributors for the data used in this study. For example, inspection of the 1:1:1:1 mixture results at 20 pg individual DNA from Table 4 shows that at AT=50 rfu each of the three individual profiles (1 PCR) appeared to have originated from only one contributor based on allele count. When the AT was reduced to 30 rfu the profiles appeared to have originated from two contributors with more unmasked alleles observed for each contributor. At 10 rfu, when all three replicates are analysed together (3 PCR), the correct assignment of four contributors is made.

1.2.1. Replication

Replication led to some improvement particularly at the fringes when significant portions of the data are dropping out. This can be seen in Table 4 in the 50 pg samples using an AT of 30 rfu, all six of these samples individually detected information that could be described by three individuals, but were clearly four when taking multiple replicates into account. The results in Table 4 also show that amplification can only assist so much. Sticking with an AT of 30 rfu, any samples that were amplified with 10 pg or 20 pg of DNA remained describable by fewer than four individuals even with three replicates. For these samples there is a need to consider what the correct answer is. For example, if the peaks above AT come from three of the four contributors, the 'correct' answer is probably nearer to three rather than four.

There is a resource cost associated with routine repeat amplifications that will need to be considered in forensic laboratories.

1.2.2. Lowering the AT

Comparing graphs vertically in Figure 1 shows very little noticeable improvement in the ability to discriminate true from false donors. However comparing rows horizontally in Table 4 suggests that lowering the AT or using sub-threshold information leads to improved ability to assign the number of contributors. There is a cost in expert time in using very low thresholds. Although no evidence is presented here we assume that at very low thresholds even the most skilled experts will let through artefacts occasionally.

Swaminathan et al.[15] created a continuous method for contributor number assignment (called NOC*It*) and compared this to maximum allele count and maximum likelihood methods. When carrying out the maximum allele count method they found that allowing the AT to shift to the point of baseline noise (19 to 52 rfu) performed worse at estimating the number of contributors than having it fixed at a higher level above baseline noise (50 rfu). While the text does not specifically comment on the reasons for this finding, it may be due to low level artefacts, or stutters appearing above the ratio threshold used being counted as allelic.

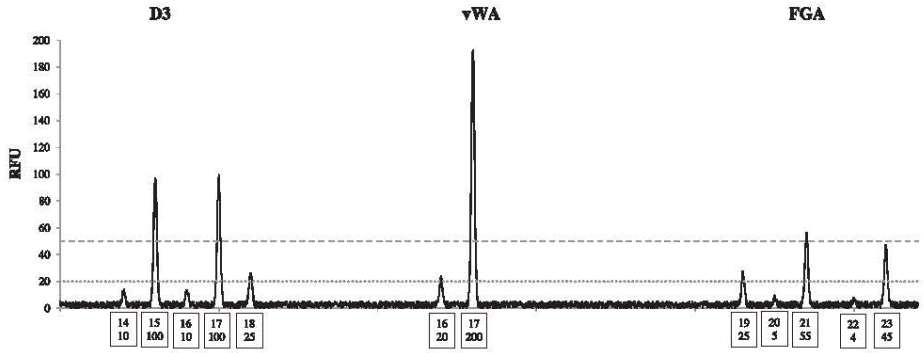

Figure 2. Three loci of a mixed DNA profile with AT shown as a dashed line for 50 rfu and dotted line for 20 rfu. Boxes show peak designation and height.

1.3. Use informed priors on mixture proportion in a probabilistic system

It is possible to provide the analytical system with information that a low level sub-threshold contributor is believed to exist. Consider the mixed DNA profile shown in Figure 2. The known sources of DNA are:

Contributor 1: D3:[15,17], vWA:[17,17], FGA:[21,23]

Contributor 2: D3:[17,18], vWA:[16,18], FGA:[19,19]

In this instance considering the AT as 50 rfu there appears to be a sub-threshold contributor present; however, the detected information present in the profile can be described by a single contributor. Peaks detected at 50 rfu are too weak to be paired with complete certainty at D3 or designated as a homozygote at vWA (using only a single replicate), although their pairing would be the most supported combination. There is therefore likely to be a mild impact of the presence of the sub-threshold peaks on the detected peaks, i.e. the presence of the sub-threshold D3:18 means we would accept a [15,18] or [17,18] pairing for the 'major' some proportion of the time with the 17 or 15 peaks (respectively) coming from a second contributor. The analyst may choose to use the presence of the sub-threshold peaks to consider the profile as originating from two individuals.

We demonstrate the power that providing information, even seemingly minor, can have on the ability of continuous systems to interpret DNA profile data. Before carrying out the experiment there are several predictions that can be made from theory. Consider two *LR*s that could be calculated from these data.

Proposition pair 1

H_{p1}: Contributor 1 and an unknown individual are the sources of DNA.

H_{d1}: Two unknown individuals are the sources of DNA.

Proposition pair 2

H_{p2}: Contributor 2 and an unknown individual are the sources of DNA.

H_{d2}: Two unknown individuals are the sources of DNA.

Table 5. LRs produced for comparison to contributors to epg shown in Figure 2.

		Uniform priors AT = 50 rfu	Using informed priors AT = 50 rfu	Uniform priors AT = 20 rfu
Contributor 1	*LR*	63	108	310
Contributor 2		0.097	0.24	6

If the profile is analysed as a two-person mixture with no guiding information from the analyst even with no significant imbalances in the observed peaks then the analysis will likely split the profile into two roughly equal contributors. Proposition pair 1 will yield an *LR* that favours H_{p1} as most of Contributor 1's peaks are detected, but it will be low as the genotype probability will be spread approximately evenly across a number of genotypes. Proposition pair 2 will yield an *LR* that will likely provide some support for H_{d2} to the profile. The reason for this is that Contributor 2's peaks are not detected and so their presence would have to be explained with multiple dropouts. If the system is supplied with some guiding information that there are two unevenly contributing individuals then we would expect that more weight would be placed on pairing the observed peaks for the major, which we would expect to translate to an *LR* that provides more support for H_{p1} in proposition pair 1. For contributor 2 to be the minor contributor, their peaks have still dropped out; however, now the system is expecting a low template contributor and will be more tolerant of dropout. We therefore would expect the *LR* obtained from proposition pair 2 to be closer to one. Finally, when reading to AT of 20 rfu then more information is given to the system. Informed priors for mixture proportion are no longer required as the information being used to interpret the profile is all being used in the analysis. We would expect a divergence of mixture proportion to be obtained naturally from the data provided and that the *LR* produced from either proposition pair will support the corresponding prosecution proposition.

We now turn to results obtained in practice. The DNA profile in Figure 2 was analysed using STRmix V2.3.06 first using an AT of 50 rfu and providing the system with no information beyond that it has originated from two individuals. Owing to the low peak heights under these circumstances the mixture proportions obtained were 47%:53%.

Secondly the same analysis was carried out in STRmix but supplying mild prior distributions for mixture proportions of $N(0.75, 0.25)$ for contributor 1 and $N(0.25, 0.25)$ for contributor 2. We use priors on the mixture proportion; however, we realise that it is in fact the template DNA amount that these priors will be acting on. Priors for mixture proportions are displayed for the ease of the user because doing so does not need them to consider how other effects within the DNA profile such as degradation and locus specific amplification efficiencies interact with the template to generate peak heights. Mixture proportions will automatically scale with peak intensity and so the user does not need to scale their priors for each similarly proportioned mixture. We also recognise that Gaussian distributions extend beyond the interval [0,1] but only apply them within this range.

The mean of the posterior for mixture proportions from the analysis were 85%:15%. The third analysis was for data using AT of 20 rfu, and not providing informed priors for mixture proportion. This time the mean of the posterior for mixture proportions from the analysis were 79%:21%. The *LR*s when comparing contributors to the three analyses can be seen in Table 5. The trend of *LR*s fits what is expected by

theory and demonstrates the point that even just supplying the information that the analyst has a prior belief in the mixture proportions based on sub-threshold data (without supplying that specific data to the analysis system) aids in the analysis and produces a result that is more intuitively aligned with the human assessment.

1.4. Do not interpret the DNA profile

At the laboratory at Forensic Science South Australia, an audit of samples received over a one-month period revealed that 54% of samples fell into what is classically called transfer or contact DNA and 34% of samples yielded a total DNA concentration of less than 10 pg/μL. There would be many more that would possess less than this level for individual contributors to mixed samples. These profiles are likely to suffer from significant allelic dropout and be within the range where sub-threshold information will be present.

A simple solution to the problems of interpreting epgs with sub-threshold peaks might be to deem all such profiles as too complex; however, given the portion of profiles that this group would represent it is unlikely this would be a sustainable practice. We do not mean this to be an excuse to interpret poor quality data, quite the contrary, instead we mean this statement to highlight the need to determine what data can be interpreted (which we hope we have started in this work).

The question must be asked whether certain profiles *should* be analysed. This is a different question to whether a profile *can* be analysed. Taking a position of theoretical purity, all data can be analysed as long as models exist to describe it. As the information content of the data decreases, or the uncertainty surrounding the interpreted profile increases, there will be an inevitable drop in the discriminating power the model will provide using the data. This is the desired behaviour and correctly represents the strength of the data. There is no limit to which this thinking can be applied. For example, the models already exist that an analyst could obtain an epg that exhibits a single weak peak of putative artefactual status and choose to analyse it, considering it may originate from anywhere between one and five individuals. After what is likely to be several hours of processing and analysis, utilising highly complex statistical, mathematical and biological theory and being provided with many pages of detailed output the interpretation system would no doubt inform the analyst of what they already knew, there is no information in the datum to discriminate true from false propositions.

Whether something should be analysed will depend on a number of factors, many of which will not directly relate to the epg in question. Ultimately it will be a decision made by the analyst that the potential discriminating power that epg could provide, in the context of the case and laboratory environment, is worth the interpretation and analysis time.

2. Interpretation of putative stutter peaks

When interpreting a DNA profile that has a major component and one or more minor components that are in the same peak height range as stutter of the major, then some assessment of the nature of small peaks in stutter positions will need to be made by the analyst.

It is worth discussing the 2006 ISFG[16] Recommendation 6, which states:

> If the crime profile is a major/minor mixture, where minor alleles are the same size (height or area) as stutters of major alleles, then stutters and minor alleles are indistinguishable.

> Under these circumstances alleles in stutter positions that do not support H_p should be included in the assessment.

It is the authors' experience that this statement is sometimes taken as meaning 'all peaks in stutter positions must be treated as allelic' as it has been used as such for interpretational attack in court. We suggest that this is not the intent of the authors of Ref. 16 when making this recommendation. In the same publication, the preceding sentence gives an example of when the recommendation would have an effect, and states that under those circumstances '...the probability of stutter must be considered...'. Probabilistic systems take into account the ambiguous nature of peaks by calculating the probability of that peak if it is purely stutter as opposed to it being partially allelic (given a number of parameters dealing with intrinsic properties of the DNA profile such as DNA amounts, degradation, genotype sets, etc.). Sometimes the choice of number of contributors will mean that the certain peaks within the profile will be considered unambiguously as entirely stutter, however this is a perfectly acceptable outcome. To consider all peaks in stutter positions as allelic would see an overestimation of the number of contributors in a large proportion of samples and would be against the ethos that each party is allowed its best explanation of the evidence.

This leaves the analyst with the task of making an assessment of the nature of peaks in stutter positions as to their status. There is a risk here of either overestimating or underestimating the number of contributors to the profile and we point the reader to Refs 4 and 5 for the outcomes of either of these eventualities when using a continuous system including examples of ambiguous stutter peaks. Our intention in this paper is not to trial or recommend methods for dealing with ambiguous peaks in stutter positions and we do not do so. All we suggest is that the method used should take into account known stutter values for alleles/loci and the profile should be considered holistically, which may include an assessment of the presence of peaks below the AT.

3. Conclusion

Continuous systems (at least STRmix as trialled here) can overcome the issues of missing low-level data with minimal effects on the outcome of the analysis. The effects of overestimation of the number of contributors may not be too severe as long as the system has been reliably validated for this policy. This situation should not be used to enable a reduction of valid quality practices such as replication and careful expert inspection of profiles and cannot be assumed to be conservative. However, any system, even one possessing the soundest theoretical basis, that cannot withstand the rigours of practical use, is destined to remain nothing more than a nice idea. We have discussed strategies to mitigate the effect of uncertainty in the number of trace contributors present when sub-threshold information is present in a DNA profile. We support replication and lowering the AT whenever practical. The use of sub-threshold data without lowering the AT may be useful in some cases. The effects of mis-assignment of N in either direction are relatively mild and restricted to *LR*s less than one when comparing known contributors and low *LR*s greater than one when comparing known non-contributors.

We believe that treating the number of contributors as an unknown nuisance variable is the best long-term solution. An even better solution would be to combine the treatment of number of contributors as a nuisance variable with an expert system that utilises fluorescent signal directly and has models for different known artefacts. In such a system all data would be treated probabilistically and the tyranny of thresholds would

be completely abolished. We are not aware of any system that can perform at this level and so can provide no examples of how it would perform.

Last, we suggest that some profiles are simply too complex and should not be interpreted. Ultimately it is the role of the scientist to assess each profile on its own merits and the case context in order to determine if and how analysis will proceed.

Acknowledgements

We thank Lisa Melia and Johanna Veth and two anonymous reviewers for their valuable comments that greatly improved this manuscript.

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Appendix 1

Peaks for each of the two contributors were simulated from a lognormal distribution with mean μ and variance $\frac{4}{\mu}$. With probability 0.2 a peak was masked. Masking can be thought of as happening because a major contributor is present or because the two traces mask each other. The number of peaks per locus was counted and any profile that had only 0–2 peaks per locus was checked to see that it did have contributions from each contributor. This is the number of profiles out of the 1000 simulations appearing in Table 1.

Appendix 2

Let

S be the event that the peaks above AT come from a single source;

T be the event that the peaks above AT come from two sources;

AS be the event that the peaks above AT appear to come from a single source by simple allele count.

Values for the mean μ were drawn from either U[10,50 rfu] and U[10,100 rfu] for each of the two contributors. $\Pr(AS|S)$ and $\Pr(AS|T)$ were calculated using the simulation described in Appendix 1 (1000 simulations were used). Masking was set at 0.2 and 0.5.

The desired probability was obtained as:

$$\Pr(S|AS) = \frac{\Pr(AS|S)\Pr(S)}{\Pr(AS|S)\Pr(S) + \Pr(AS|T)\Pr(T)}$$

and assuming $\Pr(S) = \Pr(T)$. These values appear in Table 2.

KR-14

From: Kylie Rika
Sent: Tuesday, 22 September 2020 10:16 AM
To: Justin Howes
Subject: RE: LOD

Thanks Justin

Kylie

From: Justin Howes <[redacted]
Sent: Friday, 18 September 2020 12:35 PM
To: Kylie Rika <[redacted]
Subject: RE: LOD

Hi Kylie
I thank you for sharing your feelings with me. I want to assure you that you are safe in the work environment and with your colleagues. You are safe with me and I support you in the work you undertake.

I wanted to also assure you that the change management process is a transparent process that is designed to provide staff with safety in the ability to explore ideas. It is important to raise ideas and issues for discussion, so I hope that you can continue to contribute in this space.

I have copied in a link to Benestar as a reminder that this is available to you and that I would support you if you decide to reach out to them at any time.

https://qheps.health.qld.gov.au/csd/employee-centre/workhealth-safety-wellbeing/department-employee-assistance-services-providers

Regards
Justin

Justin Howes
Team Leader - Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Police Services Stream
Forensic & Scientific Services, Health Support Queensland, Queensland Health

Please note that I may be working from a different location during the COVID-19 Pandemic. The best contact method is via email.

[redacted]

e [redacted] w www.health.qld.gov.au/healthsupport/businesses/forensic-and-scientific-services

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Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.

From: Kylie Rika <[redacted]
Sent: Wednesday, 26 August 2020 4:13 PM
To: Justin Howes <[redacted]
Subject: LOD

Hi Justin

Thank you for meeting with me last week to hear my ideas on LOD and allowing me the opportunity to ask if you thought my idea was good (a relaxing of the do not look below LOD rule for some samples).

Based on the meeting outcomes as well as information I have received from Sharon, I have decided to no longer pursue a change in the way we handle sub threshold peaks below LOD. Whilst I still feel that there is a risk to ignoring these peaks, I no longer feel safe or supported to make the change happen. I acknowledge that you support me in putting in a change request to discuss what things need to be looked at or considered for RW pathways etc, however, I also acknowledge that you were unable to provide me with direct support on the idea to look below LOD.

The way I feel I have been treated with this proposed opportunity for quality improvement, has left me feeling unsafe to raise further issues and ideas.

Hopefully with the work that Tess and John are doing, things will change for me in this regard.

Thanks
Kylie

Kylie Rika
Senior Scientist - Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Police Services Stream
Forensic & Scientific Services, Health Support Queensland, Queensland Health

Please note that I may be working from a different location during the COVID-19 Pandemic. The best contact method is via email.

[redacted]
e [redacted] w www.health.qld.gov.au/healthsupport/businesses/forensic-and-scientific-services

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