Review and Assessment of the Appropriateness of Not Concentrating Low Quantity DNA Samples by Queensland Health Forensic and Scientific Services (QHFSS)

Requested by Commission of Inquiry
into Forensic DNA Testing in Queensland

Prepared by Bruce Budowle, Ph.D.

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Introduction

1. This Report addresses an inquiry by Commission of Inquiry into Forensic DNA Testing in Queensland regarding the appropriateness of the process by which scientists at Queensland Health Forensic and Scientific Services (QHFSS) are not concentrating samples that have been determined to have concentrations between 0.001 ng/µL and 0.0088 ng/µL and alternatively practices that may be considered appropriate to address or process such low quantity DNA samples.

2. The opinion in this Report is based on:

    a. QHFSS Project #70 Phase 1 Report on Verification of Promega DNA IQ™ for the Maxwell® 16 (prepared by Megan Mathieson, Belinda Andersen, Cecilia Iannzzi, and Allan McNevin)

    b. QHFSS Report on the Validation of a Manual Method for Extracting DNA using the DNA IQ™ System (prepared by Nurthen T, Hlinka V, Muharam I, Gallagher B, Lundie G, Iannuzzi C, and Ientile V), August 2008

    c. QHFSS protocol for DNA IQ™ Method of Extracting DNA from Casework and Reference Samples, 9 November 2010

    d. QHFSS Project #163 Report on Assessment of Results Obtained from 'Automatic-Microcon' Samples (prepared by Josie Entwistle, Allison Lloyd, Kylie Rika, Thomas Nurthen, and Cathie Allen), August 2015

    e. QHFSS Project #184 on A Review of the Automatic Concentration of DNA using Microcon® Centrifugal Filter Devices: Options for QPS Consideration (prepared by Justin Howes and Cathie Allen), January 2018

    f. QHFSS Proposal #115 on Verification of a new Microcon® DNA Fast Flow Centrifugal Filter Membrane (prepared by Amy Cheng, Cecilia Iannuzzi and Allan McNevin)

    g. QHFSS protocol for Concentration of DNA Extracts using Microcon® Centrifugal Filter Devices, 18 February 2021

    h. QHFSS Assessment of Low Quantification Value DNA Samples (prepared by Cathie Allen, Justin Howes and Paula Brisotto), 21 June 2022

    i. Communications between and among QHFSS administration and staff

    j. Promega's Technical Bulletin TB296 DNA IQ™ System—Small Sample Casework Protocol (November 2021)

    k. Promega's Technical Bulletin TB297 DNA IQ™ System—Database Protocol

l. Forensic Biology Procedures Manual Extract of DNA, Virginia Department of Forensic Science, VDFS 210-D2004 FB PM Extraction of DNA, 28 May 2021.

m. Scientific literature (not specifically cited herein but generally relied upon)

n. Personal experience

4. There are various steps involved in processing DNA samples. After crime scene collection and initial serological screening, the first step of the analytical process is to extract DNA from candidate samples. Once extracted, the quantity and quality of recovered DNA in an extract is assessed. It is important to determine the quantity and quality of DNA: to enable placing optimum amounts of DNA into downstream assays (e.g., the polymerase chain reaction (PCR) for short tandem repeat (STR) typing), if sufficient DNA is available to use optimum amounts; to determine the maximum amount of DNA that can be placed into a PCR when there is less than optimum amount of DNA; to not proceed with an assay if the amount of usable DNA for an assay may not yield typable results (and that if desired alternate more sensitive methods may be pursued); to determine if inhibitor compounds may have co-purified with the DNA during extraction which may be remedied, for example, by further purification or dilution of the DNA sample; to determine if the DNA may be notably degraded; and to reduce undue consumption of biological evidence. This part of the DNA analysis process informs a scientist on what steps may be feasible to ensue regarding the potential success with extant DNA typing methodologies.

5. Scientists assess the quantity and quality of DNA contained within sample extracts to make decisions on whether to proceed with processing the samples based on the capabilities of the particular laboratory. Many laboratories set an initial input amount typically ranging from 0.1 to 0.25 ng of DNA. QHFSS uses 0.132 ng as its lowest amount of DNA for proceeding with routine analysis. This concept of a minimum amount of input DNA is an historical threshold based on studies on single source samples in which stochastic effects (i.e., variation within the DNA profile) are exacerbated. As the amount of input DNA decreases the intensity of signal associated with the components (i.e., alleles) in a DNA profile tends to become more variable, and alleles that should be comparable in signal may not be comparable. For single source samples these stochastic effects are often manageable (at long as there is sufficient signal to detect and interpret a DNA profile). However, with mixture samples interpretation can at times be quite challenging particularly for samples with three or more contributors and/or if the samples are of low quantity and quality. Historically, the threshold input quantity for samples was applied because interpretation of DNA profiles was manually performed (often termed a binary process). Manual interpretation could not handle well the vagaries of exacerbated stochastic effects (e.g., missing genetic data in a DNA profile among other issues) and thus there was a need to analyze DNA above some threshold so interpretation of the data potentially would be less likely to be challenging. Over the past decade manual interpretation approaches have been replaced with computational approaches known collectively as probabilistic genotyping (PG). PG accommodates better the stochastic effects encountered with simple to

complex forensic DNA samples allowing for analyses of lower quantity samples, lower quality samples, and more complex mixture (such as three and four person mixtures) compared with manual interpretation approaches. Thus, the initial input DNA likely can be lowered from what has been operational historically. There is a DNA input limit with PG assisted analyses, however, because at some point there is too little DNA to yield a useable (or informative) result. QHFSS employs PG for interpretation of DNA profiles for what appears to have been at least a decade. From the materials provided, it is not clear that QHFSS has performed a study to determine the minimum amount of input DNA concomitant with this capability.

6. Another consideration regarding an input DNA threshold is that it does not address the contribution of each individual in a mixture. As stated above the threshold used by QHFSS likely was based on single source samples. In a mixture of, for example, two persons at an input of 0.132 ng, each contributor would necessarily be at a lower input amount and thus any mixture interpreted with less than this threshold would fall into the region that QHFSS expresses concern with stochastic effects impacting analysis, For example, in Assessment of Low Quantification Value DNA Samples dated 21 June 2022 – similar statements can be found in other documents). QHFSS states:

> The value of 0.0088ng/µL is based on assessment of the data (and equates to 132 picograms). Validation studies conducted within the laboratory has shown that stochastic effects become apparent from DNA templates below 0.132 ng (132 picograms) making interpretation of the resultant DNA profile more complex.

7. In Project #184 QHFSS states:

> <0.132ng were found to exhibit marked stochastic effects after amplification [2].

8. Further, consider a sample with an input amount of, for example, 0.20 ng (above the input amount threshold employed by QHFSS) and is a two-person mixture. If each person equally contributed to the mixture, then each would have only contributed approximately 0.1 ng which is below the QHFSS threshold. If one person contributed 80% of the mixture and the other 20% of the mixture, then the larger amount contributor would fall above the threshold and the lower one would fall well below the threshold. Some mixtures with a low quantity minor contributor(s) may not be interpretable for the minor component and some mixtures may be interpretable. For example, a single minor contributor who has alleles that are distinct from the major contributor's alleles (and stutter peaks which are artifacts of the DNA typing process) may be interpretable even if they are just above the detection limits (or analytical threshold) of the QHFSS instrumentation. In studies performed by QHFSS (discussed further below) there does not seem to be an assessment of interpretable data from input above the threshold but with contributors that may below the input threshold. Regardless, the decision on what amount of DNA is a complex one; but with today's technologies likely can be lower than the 0.132 ng input threshold.

9. With low level sample extracts there is another limitation to consider. At QHFSS a maximum of 15μl of sample extract can be placed into a PCR tube (or well) which is the subsequent step of the DNA process known as amplification. Indeed, the 0.132 ng threshold is derived from the upper bound of the 0.001 ng/μl to 0.0088 ng/μl and this 15μl maximum volume for PCR (i.e., 0.0088 ng x 15 = 0.132 ng). Thus, without any additional processing, the input amount for samples that have a quantitation value within this range would be equal to or less than the 0.132 ng threshold. To be able to place more input DNA into the amplification process, an extract must be concentrated. Concentration increases the amount of DNA per unit volume of a sample. QHFSS uses a Microcon filtration process – a process used by other laboratories. There is no criticism of using this process per se. However, there is some loss of DNA during the concentration phase. QHFSS demonstrated in Project #184 that loss of DNA occurs. If a concentration were 100% efficient, then a 35 μl volume concentrate should be approximately three-fold greater per unit volume at least theoretically (assuming no loss of DNA during sample manipulation). The data (Figure 4 of Project #184, see graph below) show that a large portion of samples are less than three-fold. These data only consider samples yielding success (i.e., usable data). It would have been informative to determine the concentration effect and DNA loss for samples deemed "failure" (i.e., not usable data). Such information could indicate that a failure to achieve efficient concentration may impact achieving successful outcomes. One cannot determine factors impacting successful outcomes without compiling and assessing the data more completely. The overall concentration process as performed by QHFSS may be inefficient and other modifications of the methodology may be necessary (see below Responses to Issues Raised by Commission).

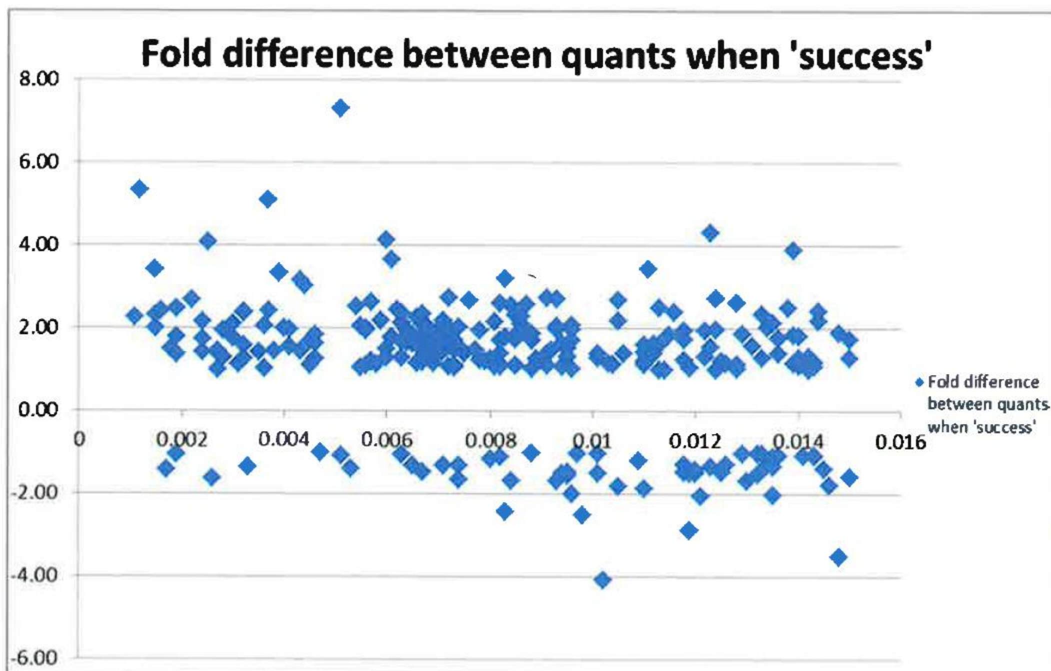

Figure 4: Quantification differences pre and post concentration

10. QHFSS concentrates a starting volume of 100 µl extract to either 35 µl (called half volume) or 15 µl (called full volume). Note that in the protocol titled "Concentration of DNA Extracts using Microcon® Centrifugal Filter Devices" dated 18 February 2021 the full volume is listed at 5 µl to 15 µl; thus, it is uncertain what volume is sought with the full volume concentration. It is not clear from the documentation provided, what the decision process and what circumstances would dictate selecting a full or half volume target. There are risks and benefits to consider with each targeted volume. The Overall benefit with concentration is being able to place more DNA into an amplification reaction so that samples that initially fall within the less successful range of 0.001 ng/µl and 0.0088 ng/µl may move to greater amounts of DNA per unit volume and fall within a more successful typing range. The limitations with concentration are potential loss of DNA; consumption of evidence; and additional labor, time and costs. The latter are not addressed further herein as quality and obtaining useful data should be the primary criteria; but costs associated with work are a necessary consideration. It is unknown from the documents provided how much loss of DNA is associated overall by targeting 35 µl or 15 µl volumes with QHFSS. If, for example, loss is greater with a 15 µl than a 35 µl concentration volume, then the decision may favor the 35 µl target. If there is similar loss with either target volume, then a balance should be considered between the chance of obtaining a usable result versus maintaining some sample for analysis with other potentially resolving technologies not within the QHFSS toolbox or for re-testing by the defense or other interested parties. Based on the documents provided, currently it is not possible to make such decisions. QHFSS should perform the necessary studies or compile the data if the studies were performed, so informed decisions can be made based on empirical results.

11. If the target is 15 µl, then the entire sample will be consumed for DNA typing. If the target is 35 µl, then approximately half the sample would remain for further analyses. Again, a balance between these two outcomes should be weighed. That balance should consider obtaining a usable result versus testing with other technologies if the first test failed to generate a usable DNA profile.

12. It should be noted that the general method of concentrating a DNA sample performed by QHFSS is an acceptable approach (although there are modifications that could improve efficiency – see below Responses to Issues Raised by Commission). The main point is there are insufficient data to make sound decisions on how to proceed, and the laboratory should address this gap as soon as possible. Currently, it is unknown if the decisions made by QHFSS impact undue consumption of evidence. However, there is one area that needs to be urgently revisited by QHFSS, that is the volume targeted for the extraction of DNA. QHFSS employs the DNA IQ™ System (Promega Corporation) to isolate and purify DNA from evidence samples. The DNA IQ System is a well-developed and validated method and there is no criticism about the technology. QHFSS differs compared with most, possibly all, laboratories that use this extraction technology or similar ones. The final extraction volume is 100 µl, while in other laboratories final extraction volumes tend to range from 35 µl to at most 50 µl. A

smaller extraction volume would in effect concentrate the sample of the extract more so than a larger volume such that initially some samples that fall into the 0.001 ng/µl to 0.0088 ng/µl would have greater concentrations. Indeed, the Promega Corporation (i.e., the manufacturer of the DNA IQ system) recommends in Technical Bulletin 296:

> 11. Add 25–100µl of Elution Buffer, depending on how much biological material was used. A lower elution volume ensures a higher final concentration of DNA.

13. With this technology too small of a volume results in diminishing returns (i.e., inefficient recovery of DNA); after a certain point of volume increase there is no gain in DNA recovery and volumes above that point will result in the same amount of DNA recovered but with a reduction of DNA concentration. As an example, the DNA IQ System protocols by the Virginia Department of Forensic Science elute DNA in volumes less than 50 µl.

14. QHFSS performed a validation study to stand up the DNA IQ System. In that study their initial recovery of DNA from blood samples in a 50 µl volume showed low yield; however, the buccal cells did not show a similar loss. Instead of evaluating potential causes such as the samples may not have been prepared properly (something that has been considered by QHFSS for other studies), the laboratory changed the methodology in two aspects – adding a chemical DTT to the extraction phase and increasing the target volume from 50 µl to 100 µl. The laboratory performed the experiment again and obtained more desirable results for the blood samples (but not for the buccal cells). When changing more than one aspect at a time in an experiment one cannot determine which one or if both improved the process (or if in this study it was a sample preparation issue). Thus, QHFSS cannot at this time attribute the enhanced DNA yield for blood in its in-house study is at all attributable to the increased target volume. While each laboratory should validate internally the methods that will be implemented and some variation is expected between laboratories, laboratories should not work within a bubble. If other laboratories are not having to increase the target value to ~100 µl, then QHFSS should consider the possibility that there is something wrong with how their studies were undertaken, at least repeat them under the initial conditions and at a minimum reach out to other laboratories and/or the manufacturer for possible guidance. The buccal cells did not show any substantial difference in total DNA yield with the change of procedure, which may suggest that there is something compromising the blood samples as opposed to the elution volume being the solution to achieve desired yields. What the actual cause and solution are unknown regarding the finding of low performance of the initial blood extraction study. The outcome was that QHFSS extracts to a larger volume than most laboratories which in turn likely reduces the concentration of the sample extract. Thus, many samples that fall within the 0.001 ng/µl to 0.0088 ng/µl range and likely samples above this range would have greater concentrations and may not have to be subjected to a concentration step at a subsequent stage of the analysis if a lower final extract volume was achieved. Most importantly, more samples could be successfully typed without additional manipulations.

15. As a side note validation studies include repeatability and reproducibility studies which are running the method by the same person and instrumentation or by different persons and instrumentation, respectively. During the validation of the DNA IQ System, QHFSS changed parts of the protocol which seemed to improve the system. While it is important to optimize a protocol, repeatability and reproducibility studies should be performed using consistent protocols. Thus, QHFSS did not accurately perform these studies.

16. The Commission indicated that:

    Previously, if a sample falls within the quantitation range of 0.001 ng/µl and 0.0088 ng/µl QHFSS has instituted different policies regarding on how to proceed. According to the Commission as of 6 June 2022 QHFSS adopted the following policy:

    - For a quantitation less than 0.001 ng/µl, the scientist reports "No DNA detected".
    - For a quantitation between 0.001 ng/µl and 0.0088 ng/µl, the sample is to be amplified without first concentrating it. If at the point of interpreting a profile a reporting scientist believes that the sample should be concentrated, it may be concentrated and amplified again.
    - For a quantitation of 0.0088 ng/µl or higher the sample is amplified and further processed.

    Prior to this policy, QHFSS implemented the following policy (from 2018 onward):

    - For all Priority 2 and Priority 3 crime scene samples with a quantitation value less than 0.0088ng/µl are reported as:
    - 0<Quant<0.001ng/µl: No DNA Detected
    - 0.001 ng/µl – 0.0088 ng/µl: DNA insufficient for further processing
    - Priority 1 samples with quantitation values in this range were to undergo concentration and proceed to amplification.

17. This approved workflow enabled extracts with quantitation values less than 0.0088ng/µl to remain available for further processing e.g., concentration) upon request by the Police. While the impact of these decisions has changed from 2018 (and prior) until recently, the following issues raised by the Commission are addressed based on the 6 June 2022 policy.

18. Most recently, the Commission indicated that the policy has been changed as of 19 August 2022 to:
    - For a quantitation between 0.001 ng/µl and 0.0088 ng/µl, the sample is to be concentrated to 35 µl before amplification. This was the process in place prior to the Options Paper decision in February 2018.

19. The Commission understands that, apart from the above procedures, two other procedures might be appropriate or more appropriate in order to ensure the best chance of obtaining a useable profile:
    - All samples in the range should be concentrated, but there should be reporting scientist discretion as to whether they are concentrated to full (15 µl) or standard (35 µl).
    - A reporting scientist should exercise a discretion as to whether a particular sample should be concentrated before amplification, and if so, to what degree.

20. The issues raised are:
    1. Whether the process adopted from 6 June 2022, or the process adopted from 19 August 2022, constitutes international best practice;
    2. Whether the laboratory has adequate experimental data or analysis about concentration to justify the process adopted from 6 June 2022 or the process adopted from 19 August 2022;
    3. Whether another procedure concerning concentration before or after amplification would be preferable and why;
    4. The risks posed by the current process, if any, to the accuracy of results produced by the laboratory.

Issue 1: Whether the process adopted from 6 June 2022, or the process adopted from 19 August 2022, constitutes international best practice.

21. Response: The issue of reporting “No DNA Detected” for quantitation values less than 0.001ng/µl was addressed previously. See “Review and Assessment of the Use of “No DNA Detected” by Queensland Health Forensic and Scientific Services (QHFSS) dated 5 September 2022.

22. The policy of samples falling within the 0.001 ng/µl and 0.0088 ng/µl will be amplified without first concentrating should be reconsidered and appears to have been changed in the 19 August policy. Without having good data on the samples that failed to yield usable data and the quality of the usable data (i.e., low signal, partial profile, etc.), it is difficult to support the approach of amplification without first concentrating. Sample will be consumed by this approach that at least the laboratory indicated had a low success rate. Whether the success rate initially determined by the laboratory or subsequent findings are accurate still requires further investigation. Although data still need to be generated and/or collated regarding the efficacy of the concentration process, it, for the moment, would be a better policy to concentrate samples to 35 µl before amplification. Additionally, QHFSS, in collaboration with other stakeholders and clients, need to determine what constitutes a low success rate. A cost-benefit analysis considering the overall Queensland system (i.e., victims, families, communities, investigators, judicial system, and government budgets) should be undertaken. It simply is

unknown what is a poor or good success rate. For example, a 10% success rate or less (dependent on the type of crime) could still be considered quite effective compared with other investigative mechanisms, may reduce the number of victims (such as stopping a serial rapist), may save medical, investigative and adjudication costs, and may exculpate individuals wrongly associated with the crime scene evidence. The bottom line is if this quantitation range is considered a low success rate for obtaining usable results, then proceeding to amplification without concentration would consume sample unnecessarily. If obtaining the most robust data possible is the primary goal (and it should be from a scientific point of view), it would seem better to concentrate the sample first (even with loss of some DNA) and then proceed to amplification. Thus, the 19 August policy is preferred to that of the 6 June policy. Criteria for discretion by a scientist to select 35 µl or 15 µl as the final volume should be defined. From the documents provided, there was no documentation detailing the criteria to opt for one volume or the other. The consequence of concentrating to the full target volume is that the sample will be consumed entirely; thus, no additional testing would be possible. Additionally, no criteria were provided on what additional discretionary considerations would be entertained by a reporting scientist, or whoever makes that decision in the current QHFSS workflow, to decide on whether a sample should proceed to amplification without concentration. The 19 August policy would seem to indicate all samples with a quantitation value falling between 0.001 ng/µl and 0.0088 ng/µl would be concentrated – so it is unclear what would be the need for discretionary choice to proceed. In addition to performing additional validation studies or analyses on the concentration methodology employed, QHFSS should develop criteria for deciding the two discretionary practices indicated.

Issue 2: Whether the laboratory has adequate experimental data or analysis about concentration to justify the process adopted from 6 June 2022 or the process adopted from 19 August 2022.

23. Response: As indicated above, and assuming adequate documentation has been provided, QHFSS does not have adequate experimental data about its concentration methodology to make informed decisions on how to proceed once a quantitation value is obtained. It is unknown if the concentration method for the "failed" designation in the study is similar to that of the "success" designation in its assessment of usable data from samples within the 0.001 ng/µl and 0.0088 ng/µl range that were concentrated. If lower performing (i.e., more DNA is lost), there may be indicators with those samples that could help assist the analyst in deciding to concentrate or not. Additionally, there may be modifications to the concentration methodology that could improve the procedure (see Response 4). Also, there is no indication on the depth of the review by QHFSS of what constitutes "usable" data. For example, while a mixture is complex, some parts of the mixture may be interpretable (such as a major contributor or a minor contributor with alleles that are distinct from another contributor(s)) while other parts may be uninterpretable. QHFSS needs to clearly define and possibly refine its criteria for what it deemed usable. Further, there may be other considerations that could impact the success

rate associated with concentration. It appears that QHFSS focused substantially on uploading profiles to the National Database for its assessment of success with concentration of samples. Profiles uploaded to the National Database are important as they provide investigative leads to help solve future crimes and link crimes that may be perpetrated by the same individual. But not all cases need to be assisted through a database search. The percentage of cases in which the police already have a person of interest, and the DNA supports or refutes that this individual is the donor of probative crime scene evidence also is part of the assessment of usable DNA data. QHFSS provided some data in this regard (Project #184) but its focus seems to be on uploads to the National Database (10.6% v the 1.45% success rate provided to the police). It also would be informative to know from police what portion of cases fall into this latter category to determine what are usable data.

Issue 3: Whether another procedure concerning concentration before amplification would be preferable and why.

24. Response: The general procedure used by QHFSS for concentration is acceptable. However, there is loss of DNA during concentration, and it is unknown what the degree of loss actually is over the entire set of samples that have been subjected to concentration. There are modifications to the concentration methodology used by QHFSS that could reduce this loss, such as using carrier RNA, reducing washing, and reducing centrifugation force and spin time. Additionally, the volume of the DNA extract recovered with the DNA IQ system is too large. By targeting 100 µl rather than a volume less than 50 µl, QHFSS likely has reduced the chance of obtaining typable results for some compromised forensic samples. It is recommended that QHFSS revisit the DNA IQ System validation, set up a rigorous study, and reach out to other parties to attempt to put online a targeted volume of less than 50 µl.

Issue 4: The risks posed by the current process, if any, to the accuracy of results produced by the laboratory

25. If one proceeds with typing without concentration for samples that fall within the 0.001 ng/µl and 0.0088 ng/µl the quality of the data likely will be lower than if more input DNA is used per analysis. Some results may be obtained in this range. But the policy does not address what quality these results should meet or if they warrant further processing. Usable results will span a wide range of nominal to full profiles. The minor contributors of mixtures could be better represented permitting a more robust analysis of the DNA profile data. The goal scientifically is to obtain the best results possible to obtain the most robust interpretation of the data possible.

The findings contained in this report are based on the information available to Bruce Budowle as of the date of the report. If additional information becomes available these findings may be subject to revision.

This report was completed on 15September 2022 and describes the opinions and conclusions of the undersigned.

Bruce Budowle