Review and Assessment of the Use of "No DNA Detected" by Queensland Health Forensic and Scientific Services (QHFSS)

Requested by Commission of Inquiry
into Forensic DNA Testing in Queensland

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Introduction

1. This Report addresses an inquiry by Commission of Inquiry into Forensic DNA Testing in Queensland regarding the validity and/or accuracy of reporting of “No DNA Detected” by Queensland Health Forensic and Scientific Services (QHFSS) based on the laboratory’s protocol on a DNA quantification assay known as the Quantifiler™ Trio DNA Quantification Kit (manufactured by Thermo Fisher Scientific). The use of an analytical procedure in a forensic laboratory (or for that matter any diagnostics service laboratory) should be validated in order to understand its capabilities and limitations, so that proper protocols and interpretation guidelines can be implemented. The goal is to guarantee that the method is “fit for purpose” in the manner it is being employed. The reporting of “No DNA Detected” can have significant impact on decision processes by the various clients and stakeholders; therefore, it is imperative that a finding is supported by well-constructed internal validation studies and that it is conveyed in a manner that is understood. Indeed, QHFSS in its own definitions document states “no DNA was detected above the limit of detection at the quantitation stage” (see page 39 of QHFSS’ document titled “Explanations of Exhibit Results for Forensic Register” valid from July 12, 2021) which conveys that the reported finding is based on the limits of the test procedure. This statement of “No DNA Detected” in itself is appropriate; many forensic laboratories also issue a similar statement. Indeed, the qualifying language by QHFSS would seem appropriate as it describes that what is meant regarding “No DNA detected” in the context with methodological limitations.

> **No DNA detected**
> This item/sample was submitted for DNA analysis; however no DNA was detected above the limit of detection at the quantitation stage. No further processing was conducted on this item.
> Mnemonic = NDNAD
> (PP21 or P+)

2. However, the statement is not based on data from a properly designed validation study. Because there are problems with the validation study performed by QHFSS the statement may not convey well, the true limit of detection of DNA in a sample within the context of the methodology and as simply stated. The opinion in this Report is based on:

    a. QHFSS summary report titled “Validation of Quantifiler® Trio” authored by Pierre Acedo, Megan Mathieson, Luke Ryan and Cathie Allen (September 2015)

    QHFSS standard operating protocol titled “Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit” (valid from 03/05/2022 and approved by Cathie Allen)

    b. Communications between and among QHFSS administration and staff

c. The manufacturer's Quantifiler™ HP and Trio DNA Quantification Kits User Guide (October 2018)

d. The manufacturer's product bulletin on Quantifiler HP and Quantifiler Trio DNA Quantification Kits

e. Three "Statement of Witness" documents produced by QHFSS reporting scientists and corresponding casefile information

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

g. Personal experience

3. The primary focus of this Report is on the inadequate process by QHFSS to determine the limit of detection (LoD) for its DNA quantitation assay which in turn impacts the validity of the statement "No DNA Detected."

Validation

4. The purpose of determining the quantity and quality of DNA is multifold: 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. Thus, a DNA quantitation assay is a critical part of the DNA analytical process.

5. The Quantifiler™ Trio DNA Quantification Kit is used by QHFSS to determine the quantity and quality of DNA that has been extracted from items of evidence and reference samples. The fundamental technology (i.e., Real-Time PCR) and this specific kit are well validated for the purpose, and there is no concern raised about the kit's general use. However, all methods have limitations, and it is incumbent upon users to determine those limitations (characterized through validation studies) to ensure interpretation of results is commensurate with underlying supporting data. In addition, the manufacturer recommends that "Each laboratory using the Quantifiler HP and Trio kits should perform its own internal validation studies" (Product Bulletin and similar language in User Guide).

6. QHFSS did perform a validation study on the kit. Assuming, however, that the QHFSS Validation Report on the Quantifiler™ Trio DNA Quantification Kit adequately reflects the design and studies performed, then there are serious concerns with the experimental design and statistical analyses that were applied.

7. It is not necessary to describe the scientific underpinnings of a Real-Time PCR quantitation assay; simply the quantity of DNA is determined over the duration of an assay (measured

by number of cycles with each cycle accumulating signal if DNA is present) by the amount of fluorescent signal generated typically <40 cycles of Real-Time PCR. The greater the signal is the greater the concentration of DNA in the sample. For the signal intensity to indicate detectable DNA it must meet a predetermined threshold value set in the software. If the signal is not sufficient to raise above this threshold, the recorded result(s) is "undetermined." This outcome can have a few root causes: there is no DNA in the tested sample; DNA may be present, but below the LoD for the assay; and/or there is inhibition of the assay due to co-purified compounds that have a negative impact on the assay. While a number of indicators can be used to assess a possible root cause of an undetermined result, an important aspect (for this Report) for stating "No DNA Detected" is the LoD.

8. The LoD indicates the smallest concentration of DNA that can be detected or distinguished from a sample containing no DNA within a stated confidence level. A precise and accurate definition of the LOD, determined by proper experimental design, is essential for the common understanding of its meaning among scientists, clients, and stakeholders. To determine the LoD of the DNA quantitation test system, one needs first to understand the limit of a blank sample or for herein the limit of a negative control (LNC). The LNC is the highest fluorescent signal that is expected to be found when replicates of a sample that contain no DNA (known as negative controls and reagent blanks in forensic DNA typing) are assayed. Even with no DNA in a sample a signal may be generated due to analytical noise. Validation studies determine if a negative sample(s) may yield an analytical signal that may be consistent with a signal generated by a (low level) DNA sample. Such a study helps distinguish the level of analytical noise from the level of signal of DNA in a sample. The quantitation assay is not capable of measuring DNA concentrations down to or almost zero. Thus, the LoD is some signal higher than the LNC. The concentration of DNA that can be distinguished reliably from noise should be determined empirically. The linear range of the assay (based on the manufacturer user guide) is between 0.005 ng/µl and 100 ng/µl. Samples with DNA concentrations below a value of 0.005 ng/µl cannot be accurately and precisely quantified. Also, it should be noted that preparing samples at such low-level concentrations is not precise nor accurate so DNA concentrations tested may not be the true values. The LoD of this quantitation assay is at a concentration below the linear range of the assay. It should not be misconstrued that inaccurate calibration of DNA concentration below 0.005 ng/µl is an indication of the LoD. The LoD is used solely for determining the presence of DNA within the limits of the assay. While the predicted quantities may vary substantially from the true concentration of DNA, the LoD, if properly defined, is a value that can support that DNA is present within the detection limits of the assay.

9. There are different approaches to determine the LoD, none appear to have been used by QHFSS in its validation study. One approach to determine the DNA concentration that can be distinguished from the LNC is to measure the signal in replicate samplings of negative samples, determine the mean (i.e., average) and standard deviation (i.e., the amount of variation from the mean value) of those replicates, and then calculate the LoD as that mean plus some number of standard deviations (which typically can range from 2 to 10). The weakness of this approach for the quantitation assay is two-fold: it is not based on any detection of DNA, and the negative samples that are described in the QHFSS validation study did not yield a measurable signal; the assay returned "undetermined" for the reagent

blanks (see QHFSS Validation Report). A better approach is to analyze a number of replicate samples at low DNA concentrations to determine the signal that is consistent with the presence of DNA. There are several ways to design replicate testing and statistical calculations (similar to above for the LCN and with a reasonable degree of certainty) that can be performed to calculate the LoD, which are not described further herein.

10. It appears that the LoD of 0.001 ng/µl was made by edict by QHFSS and not derived by some intended experimental design and proper statistical analyses. QHFSS tested known quantity samples down to 0.001 ng/µl concentrations and then took some of those samples through the DNA typing process. A few known samples (NIST standard samples) with an intended target amount of less than 0.001 ng/µl were tested but as stated by QHFSS "The percentage inaccuracies at the lowest concentration (0.0001ng/µL) were excluded from the results as high levels of inaccuracy and variation was observed from all standard sets" (Validation Report). This justification for not including the data is not supported as calibration below 0.005 ng/µl is inaccurate and varies substantially (which QHFSS acknowledges in its validation report as well as did testing below 0.005 ng/µl (down to 0.001 ng/µl). Moreover, it ignores the prime issue of detection of DNA. Additionally, there is no mention in the validation report whether any of these samples at less than 0.001 ng/µl were subjected to DNA typing. None the less, QHFSS did derive results for some samples that yielded values less than 0.001 ng/µl. Quantitative values less than the QHFSS 0.001 ng/µl LoD threshold were obtained for unknown quantity samples (operationally unknown because the samples were intentionally damaged and/or degraded). For example, see Table 25 (included below) of the QHFSS validation study in which samples irradiated with UV light for 4 hours yielded an average quantity of 0.0001 ng/µl and an average of 4 alleles were observed after DNA typing. Also, QHFSS obtained detectable DNA typing results for undetermined results – 1.67 alleles on average (albeit unlikely to be informative DNA data).

Table 25: Average Quantifiler Trio quantification results.

Sample	UV Exposure	Average IPCCT	Average Ct Value (SAT)	Average Quant Value (SAT)	Average Ct Value (LAT)	Average Quant Value (LAT)	Degradation Index	Average #Allele (Total 42)
1	Nil	28.24	26.6378	2.0580	24.1924	2.5834	0.7966	42.00
2	5 min	27.49	28.5263	0.4871	28.0807	0.1619	3.0153	42.00
3	10 min	26.62	29.3653	0.2609	30.7718	0.0240	10.8882	36.33
4	20 min	27.16	29.8703	0.1743	32.1978	0.0086	20.3921	35.33
5	30 min	27.35	31.0887	0.0687	35.3250	0.0009	75.3547	24.33
6	40 min	27.35	31.3946	0.0544	35.1298	0.0011	53.0365	26.00
7	50 min	27.25	31.7351	0.0420	37.6957	0.0002	250.4552	23.00
8	1 hour	27.23	32.2540	0.0282	39.0460	0.0001	444.4416	21.33
9	2 hours	27.26	33.8743	0.0084	39.6577	0.0000	194.4811	18.33
10	4 hours	27.09	39.3915	0.0001	undetermined	undetermined	n/a	4.00
11	8 hours	27.12	undetermined	undetermined	undetermined	undetermined	n/a	1.67
12	24 hours	26.75	undetermined	undetermined	undetermined	undetermined	n/a	0.00

Thus, DNA was detected with a quantified amount of less than 0.001 ng/µl (an order of magnitude less than the LoD stated by QHFSS). These results are similar to those presented in the manufacturer's guide (see the table below).

Report on "No DNA Detected"

Table 13 Dynamic range of male samples using the Quantifiler™ Trio DNA Quantification Kit and the GlobalFiler™ kit

Sample number	Expected quantity (ng/µL)	Quantifiler™ Trio DNA Quantification Kit			GlobalFiler™ kit
		Avg measured quantity of SA target (ng/µL)	Avg measured quantity of Y target (ng/µL)	Avg measured quantity of LA target (ng/µL)	Avg% of alleles recovered (15 µL DNA input)
1	120	123 ± 24	111 ± 13	128 ± 15	100
2	100	99 ± 16	87 ± 13	103 ± 15	100
3	80	84 ± 15	74 ± 8	88 ± 9	100
4	60	64 ± 11	55 ± 8	67 ± 8	100
5	40	46 ± 7	39 ± 4	46 ± 7	100
6	20	22 ± 2	18 ± 1	22 ± 3	100
7	10	9.9 ± 1.5	9 ± 0.7	10 ± 0.9	100
8	5	4.6 ± 0.96	4.3 ± 0.6	5.2 ± 0.9	100
9	1	0.69 ± 0.26	0.8 ± 0.16	1 ± 0.19	100
10	0.5	0.39 ± 0.044	0.39 ± 0.04	0.52 ± 0.05	100
11	0.10	0.08 ± 0.007	0.07 ± 0.004	0.1 ± 0.01	100
12	0.05	0.04 ± 0.005	0.04 ± 0.005	0.05 ± 0.007	100
13	0.03	0.03 ± 0.005	0.02 ± 0.007	0.02 ± 0.006	100
14	0.01	0.009 ± 0.003	0.01 ± 0.002	0.01 ± 0.001	100
15	0.01	0.005 ± 0.001	0.004 ± 0.001	0.006 ± 0.002	88
16	0.003	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.0004	82
17	0.0016	0.0008 ± 0.001	0.001 ± 0.00032	0.001 ± 0.001	20
18	0.0008	0.0006 ± 0.001	0.00002 ± 0.00004	0.001 ± 0.0003	4
19	0.0004	0.0002 ± 0.001	0.0002 ± 0.0002	0.00009 ± 0.0002	2
20	0.0002	0.0002 ± 0.001	0.0001 ± 0.0002	0.00013 ± 0.0002	0
21	0.0001	0.0001 ± 0.001	0.001 ± 0.0003	0.001 ± 0.0003	0
22	0.00005	0.0002 ± 0.001	0.00009 ± 0.0002	0.00009 ± 0.0002	0
NTC	0	–	–	–	–

11. Additionally, the manufacturer's product bulletin makes it clear that the kit has "improved sensitivity at sub-picogram levels of DNA" which are values below 0.001 ng/ul. Figure 1 (see the Figure below from the bulletin) of the product bulletin shows detectable results below 0.001 ng/µl to support the claim of lower sensitivity of detection. Also see the manufacturer's website (https://www.thermofisher.com/order/catalog/product/4482910) on the Quant Trio DNA Quantitation Kit in which it indicates that the limit of detection is <0.001 ng/µl.

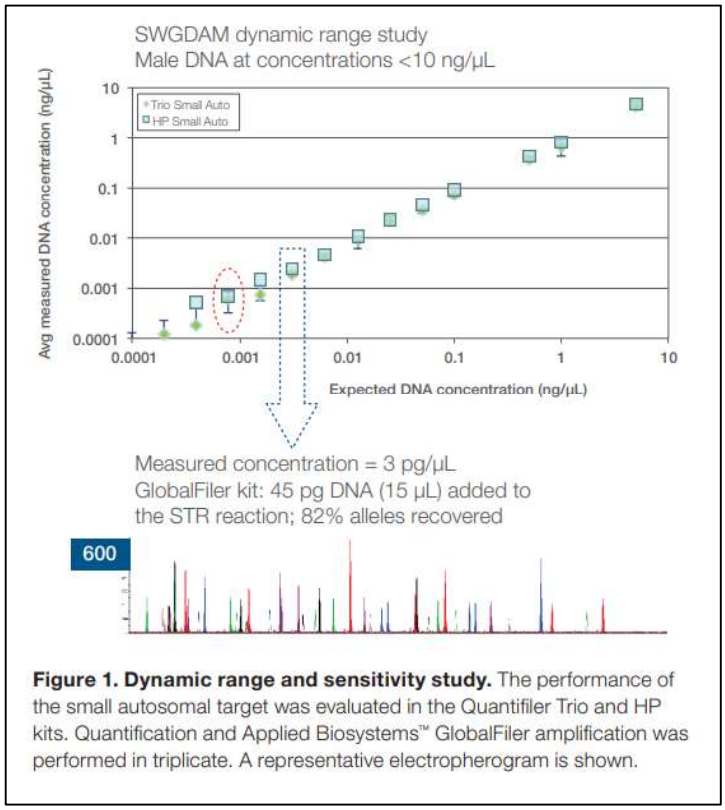

Figure 1. Dynamic range and sensitivity study. The performance of the small autosomal target was evaluated in the Quantifiler Trio and HP kits. Quantification and Applied Biosystems™ GlobalFiler amplification was performed in triplicate. A representative electropherogram is shown.

12. Thus, there is good scientific support within QHFSS and externally that the presence of DNA may be determined below 0.001 ng/ul. QHFSS should have tested replicate samples (a suitable number of replicates) with concentrations less than 0.001 ng/µl (to include negative samples) to determine if signal could be obtained above "undetermined" and then analyzed some set of these samples for potential DNA typing results. QHFSS has not defined the LoD of its quantitation assay.

Statements and correspondence Case Notes

13. A few examples of corresponding Case Notes were provided that displayed a "No DNA Detected" finding. The specific cases are not included for privacy reasons. A couple of examples are shown below.

Testing Detail

Police Report					Linked No.	
NDNAD - No DNA Detected						
T.SA (Qty)	T.IPC (Cr)	T.LA (Qty)	T.Y (Qty)	T.SA (DI)	IPCCT	LOWQT
0.000859	27.835672	0.001268	0.000448	0.677521	N	Y

27/10/2021 11:52	Result	NDNAD - No DNA Detected T.SA (Qty): 0.00063	440186	440201

14. The quantity values listed in these examples are within the range of values that QHFSS and the manufacturer have found detectable DNA as well as some samples have yielded some DNA typing signals. QHFSS, as do many other users of the quantitation assay, rely on what is known as the small or short autosomal target (T.SA in the above examples) for determining the concentration of DNA in a sample. The long or large autosomal target is also informative about quantity but tends to be used more so for assessing the degree of DNA degradation (in comparison with the short autosomal target) in a sample. In the first example above the concentration derived for the long autosomal target is above target threshold 0.001 ng/µl invoked by QHFSS and yet "No DNA Detected" still was reported. QHFSS does not describe in its standard operating protocol how to interpret this result.

15. It should be noted again that at levels below 0.005 ng/µl the determined DNA concentration is not accurate. While inaccurate, a signal at or above a well-defined LoD is an indication that DNA is present in the sample. However, because of this inaccuracy it is not always predictable for a sample(s) with concentrations at or near the LoD whether typable DNA results can be obtained. Nevertheless, it is reasonable to establish a practical or operational threshold based on a likelihood of obtaining typable or better yet informative results. Based on the total quantity (or portion of the extract used for subsequent analysis) contained with an extract, an initial decision may be: there is sufficient DNA to proceed with consuming some or all of the low-level sample for DNA testing; to concentrate the sample; to subject the sample to alternate testing; or to maintain the sample for some future technology that may be more sensitive than the methodologies currently within the laboratory.

16. Another factor to consider is that only a portion of the DNA extract can be placed into the PCR tube (or well) for DNA typing. Thus, the total recoverable DNA is not used in a single assay. There are methods to concentrate the sample so that more DNA template can be placed into the PCR. Because sample concentration does result in loss of DNA (supported by data in QHFSS Validation study), sample concentration should be assessed on the impact of sample loss and potential of increasing analytical success on a sample initially deemed "insufficient". On a case-by-case basis samples can be concentrated which should be determined by relevant parties (a system already in place between the Police and QHFSS) or established as a routine practice. QHFSS understands that not all sample can be placed into a PCR and that concentration is an option to increase typing success. Indeed, procedures in this regard have been implemented at QHFSS to attempt to improve typing success.

Ability to obtain DNA results when the quantitation is below 0.001 ng/µl

17. In my own experience, samples with low concentrations or deemed "undetermined" have at times (not routinely though) yielded DNA typing results (indeed the QHFSS validation demonstrated that some DNA typing signal can be obtained; also see product bulletin Figure above). Laboratories do report "no DNA was detected in a sample" (or something similar) but typically it is based on a well-defined LoD and other criteria such as all three targets in the quantitation assay yield an "undetermined" result and an internal positive control (known as the IPC) yields a positive result within acceptable ranges. There is no

such direction in the QHFSS quantitation standard operating protocol. Any signal above "undetermined" typically is considered an indication of the presence of DNA; however, if during validation studies analytical noise is detected in negative samples, then the determination of the presence of DNA would be based on some signal above that noise. Interpretation regarding "undetermined" values is only briefly mentioned (three times) in the QHFSS quantitation protocol and does not appear to be part of the decision process for interpretation of "No DNA Detected." Perhaps to ensure proper communication to all stakeholders, going forward, a finding of "No DNA Detected" should be accompanied with qualifiers (in the case Report and/or other communications) such as "within the limits of the assay" or "although not routine but because of limitations of the assay, a sample containing a low-level DNA concentration or undetermined result may yield typable DNA results" or "there are methods to concentrate the DNA that may enable obtaining a typable result or alternate procedures that can be considered," to name a few possible qualifiers. Low quantities of DNA that more likely would not yield a typable result with the standard STR protocol of the laboratory may yield results with other methods, such as massively parallel sequencing of mitochondrial DNA. So, the concept of no typable results or low success of obtaining typable results also is dependent on the sensitivity of the particular analytical testing applied.

18. To summarize, the reporting of "No DNA Detected" is acceptable and appropriate language. This statement signifies that the quantitation system yielded a value (i.e., DNA concentration) below the LoD. It does not convey (at least to scientists) that a sample was devoid of DNA. Simply the statement conveys, given the limitations of the quantitation system, that DNA could not be detected with any confidence. The manner that QHFSS undertook to validate the Quantifiler™ Trio DNA Quantification Kit and accompanying Real-Time PCR instrument did not define the LoD. Therefore, it is unknown at what threshold value the presence of DNA can be distinguished from background noise. However, data from the QHFSS validation study and manufacturer data indicate that the LoD likely is lower than 0.001 ng/µl (the value that QHFSS invoked as its LoD). The corresponding Case Note files that were provided show quantitation values between 0 and 0.001 ng/µl (and one target above 0.001 ng/µl) which indicate that the Real-Time PCR instrument detected some fluorescence. However, it is not known with confidence whether DNA was detected or that signal was attributable to analytical noise. QHFSS should with all due speed perform a proper LoD study so that the finding of "No DNA Detected" is reliably based on the sensitivity and limitations of the quantitation system.

Response to questions asked by the Commission

19. The Commission of Inquiry into Forensic DNA Testing in Queensland posed a series of questions or instructions regarding the issue of reporting "No DNA Detected" by QHFSS. These questions and the responses are:

    a. The statement quoted in paragraph 1 above is true for every sample that returns a quantitation value of less than 0.001ng/µL?

    Paragraph 1 in the Commission's inquiry is:
"In the case of samples that return a quant value below 0.001ng/µL the practice at QHFSS is to report this result to the Queensland Police Service by means of a shared

data interface, the "Forensic Register", by stating, as required by the laboratory's manual of *Explanations of Exhibit Results for Forensic Register*:"

"*No DNA detected*", and that,
"*This sample was submitted for DNA analysis; however no DNA was detected above the limit of detection at the quantitation stage. No further processing was conducted on this item*."

Response: Clearly, the statement is not correct for some samples yielding values below 0.001 ng/µl. However, without a valid study to determine the LoD, it is not possible to discern at what threshold value the statement "No DNA Detected" would be supportable.

b. If the statement is untrue or if it is misleading in any respect, please explain why that is so.

Response: First, the statement can be incorrect for samples that yield values above a well-defined LoD and less than 0.001 ng/µl. QHFSS did not perform studies to determine a proper LoD; instead QHFSS invoked a LoD of 0.001 ng/µl. Second, if no other processing were done to the sample to increase the amount of DNA per unit volume of an extract, from a scientific or practical perspective that even if incorrect the majority of samples would not yield usable DNA results. Third, however, also from a scientific point of view, the statement would be misleading and be uninformative to other scientists within and without the QHFSS and could lead to nonproductive decision making on how to proceed after the quantitation has been determined. For example, a value near 0.001 ng/µl, although not accurate, could translate into a total quantity of DNA in an extract approaching 100 pg (a potentially typable amount of DNA). However, only about ~1/6th of that sample can be placed into a PCR, which translates into a low probability of obtaining a usable DNA result with the current methodology employed by QHFSS. Given the sensitivity of detection of current STR kits and other novel technologies, an effective concentration methodology could yield sufficient DNA to generate usable DNA results for some samples. For example, improvements in the extraction process such as reducing the final volume of recovered extract would effectively concentrate the DNA prior to quantitation, which in turn could effect an overall more successful typing process. If the QHFSS scientists are uninformed, the motivation for process improvement is hampered. Fourth, the findings may mean different things to the various clients and stakeholders, who likely do not have strong science backgrounds. Particularly in the English-based adversarial system, the opposing sides need to be properly informed to make judicious decisions on how to proceed with their cases. How that plays out depends on case circumstances and is beyond the scope of this Report; the only point here is that valid statements should be provided by the scientists.

c. The statement quoted in paragraph 5 above is true for every sample that returns a quantitation value of less than 0.001ng/µL;

Paragraph 5 of the Commission's inquiry is:
"In formal witness statements for use in court that are signed by scientists, the usual description given uses words to the effect of "DNA was not detected in this sample and therefore they were not tested further" without further explanation. You will be briefed with examples of statements using similar words."

Response: As stated above the statement may be incorrect regarding "DNA was not detected in the sample." Values less than 0.001 ng/µl may indeed indicate detectable DNA and for those samples stating "No DNA Detected" would be technically incorrect. Without a properly defined LoD one cannot say which samples yielding values less than 0.001 ng/µl contain detectable DNA and which samples would be properly deemed "No DNA Detected." However, "not tested further" would be a correct statement because QHFSS typically does not proceed with testing DNA samples that yield a quantitation value below 0.001 ng/µl. The court is unlikely to appreciate the subtlety or nuances of "No DNA Detected" and qualifying language would be helpful to better inform. A standard lexicon document could be provided that defines what this and other terminology and jargon mean to bridge that gap.

d. If the statement is untrue or if it is misleading in any respect, please explain why that is so.

Response: See responses to b and c.

e. What words could be used to accurately describe the situation in the Forensic Register or in formal witness statements.

Response: Qualifying statements should be developed by the laboratory in collaboration with its clients and stakeholders. Together they can determine language that conveys what the finding means. As already described in this Report qualifying language could be "within the limits of the assay" or "although not routine but because of limitations of the assay, a sample containing a low-level DNA concentration or undetermined result may yield typable DNA results" or "there are methods to concentrate the DNA that may enable obtaining a typable result or alternate procedures that can be considered," or a combination of these and other possible wording. All relevant parties should work together to create language that is well-understood and concomitant with the scientific underpinnings.

f. The Commission presented the following language stated by QHFSS (found on page 39 of QHFSS' document titled "Explanations of Exhibit Results for Forensic Register" valid from July 12, 2021) and asked for an opinion:

"For Powerplex 21: This comment is used when the quantitation value is less than the limit of detection (LOD) for amplification. QPS can request processing of the sample to restart should they require it.

> For Profiler Plus: This comment is used for Volume Crime Priority 3 samples only when the quantitation value is undetermined, and there is no indication of inhibition.”
>
> Response: These quotes appear to be inconsistent regarding the LoD and using the statement of “No DNA Detected.” The first statement bases the decision process on a quantitation of less than the LoD, and the second statement bases the decision process on a quantitation result of undetermined. The quantitation of DNA should be independent of the STR typing kit. There is no explanation, at this time, for these different scenarios.

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 05 September 2022 and describes the opinions and conclusions of the undersigned.

Bruce Budowle