Professor Linzi Wilson-Wilde OAM PhD

ABN 18 568 796 588

REPORT

Report to: Walter Sofronoff QC, Commissioner
Commissioner of Inquiry into Forensic DNA Testing in Queensland

Report Date: 7 August 2022

Request: Opinion as to appropriateness of process by which scientists are not performing micro-concentration where quantification is between 0.001 ng/uL and 0.0088 ng/uL.
To advise:

- Whether this process constitutes international best practice;
- Whether it would be preferable for reporting scientists to decide on a case-by-case basis before amplification whether a sample should be micro-concentrated before amplification;
- The risks posed by this process, if any, to the accuracy of results produced by the laboratory.

Information Reviewed:

Document	Date Issued
Letter – Sofronoff to Wilson-Wilde – Expert opinion about micro-concentration – 27 July 2022	27-07-2022
Email from Helen Wells received 2 August 2022	02-08-2022
QHFSS document – DNA Extraction and Quantification of Samples Using the QIAsymphony SP and AS Modules, number 34132V6	10-05-2021
QHFSS document – Concentration of DNA Extracts using Microcon® Centrifugal Filter Devices, number 34040V4	18-02-2021
QHFSS document – A Review of the automatic concentration of DNA extracts using Microcon® Centrifugal Filter Devices: Options for QPS consideration.	January 2018
Komonski, D.I., Marignani, A., Richard, M.L., Frappier, J.R.H. and Newman, J.C., 2004. Validation of the DNA IQ™ System for use in the DNA extraction of high volume forensic casework. Canadian Society of Forensic Science Journal, 37(2), pp.103-109.	2004
Ip, S.C., Lin, S.W. and Lai, K.M., 2015. An evaluation of the performance of five extraction methods: chelex® 100, QIAamp® DNA blood mini kit, QIAamp® DNA investigator kit, QIAsymphony® DNA Investigator® kit and DNA IQ™. Science & Justice, 55(3), pp.200-208.	2015
Kanokwongnuwut, P., Martin, B., Taylor, D., Kirkbride, K.P. and Linacre, A., 2021. How many cells are required for successful DNA profiling?. Forensic Science International: Genetics, 51, p.102453.	2021
Dilley, K., Pagan, F. and Chapman, B., 2021. Methods for ensuring the highest DNA concentration and yield in future and	2021

retrospective trace DNA extracts. Science & Justice, 61(2), pp.193-197.	
Doran, A.E. and Foran, D.R., 2014. Assessment and mitigation of DNA loss utilizing centrifugal filtration devices. Forensic Science International: Genetics, 13, pp.187-190.	2014
Barbaro, A., Staiti, N., Cormaci, P. and Saravo, L., 2004, April. DNA profiling by different extraction methods. In International Congress Series (Vol. 1261, pp. 562-564). Elsevier.	2004
Identity Automation™ DNA IQ™ System Protocol for the Hamilton Microlab®STAR Line of Liquid-Handling Workstations	2019
QIAsymphony® DNA Investigator® Handbook	2013
QIAamp® DNA Investigator Handbook	2020
DNA IQ™ System—Small Sample Casework Protocol	2021

Comments

- There are numerous methods and instruments available for the extraction, purification and concentration of DNA from biological material. The methodology chosen by a laboratory is dependent on various factors including:
    - scientific considerations – that the method is generally accepted and has been published in a peer-reviewed journal, the biological material (e.g. blood, hair, trace sample) and substrate type (e.g. clothing, swab, tape lift) to be extracted, compatibility with existing laboratory equipment and the compatibility of the DNA extraction process with downstream amplification methods. The method should be repeatable and reproducible, demonstrated through validation/verification prior to implementation, and
    - management considerations – the throughput required (sample numbers analysed), cost (funding received) and available resources (available staff numbers).
- There is no recognised international best practice for a specific methodology that should be applied to the extraction of DNA from biological material and methods utilised are highly laboratory dependant.
- Microcon® Centrifugal Filter devices can be used to purify and concentrate samples containing low levels of DNA or samples with any remaining inhibitors (for example sulfites found in denim).
- At each stage in the DNA collection and analysis process, it is expected that some DNA will be lost. A loss of up to 30% during the DNA extraction process has been reported, however this is highly dependent on the sample type and DNA extraction method used.
- The use of a DNA concentration step after the DNA extraction process can result in further DNA loss, with large net losses reported in research. Although this was reported with devices which had a molecular weight cut-off rating of 30 Kilodaltons (K), whereas the Microcon® Centrifugal Filter devices can filter down to 10K. However, even at 10K, some loss would be expected, and the benefits of concentration would not likely be achieved unless and 5-fold reduce in elute volume could be achieved (i.e. 100uL reduction down to 20uL).
- Microcon® Centrifugal Filter devices can be costly, with limited ability to automate and add an additional time-consuming step in the DNA analysis process. This may be important for forensic laboratories whose clients require shortened turnaround times.
- Current extraction kits, such as the QIAamp® DNA investigator kit, QIAsymphony® DNA Investigator® kit and DNA IQ™ can produce purified DNA of sufficient quality to proceed

directly to amplification without undergoing any further purification or concentration. DNA recovery of up to 95% has been reported using the QIAamp® DNA investigator kit, using an optimized methodology.

- The QIAsymphony DNA Investigator Kit can be configured to control the DNA extract elution volume from 400uL down to 30uL (depending on the sample type and protocol used), negating the need for a specific concentration step.
- Depending on the extraction methodology used, a concentration step may improve the chance of obtaining a DNA profile in samples containing low levels of DNA.
- The laboratory operational policy regarding procedures for when to concentrate a sample, is a balance between results (probability of obtaining a DNA profile and the completeness of the DNA profile obtained), sample throughput and resource management.
- It is feasible for a high throughput laboratory to optimise and validate its extraction protocols without the need for a routine DNA concentration step. I note I have not reviewed any of the QHFSS DNA extraction validation documents.
- If after the extraction step, the eluted DNA undergoes quantification and the concentration of the DNA is found to be low, a concentration step could be used to potentially increase the chance of obtaining an informative DNA profile. However, the decision to do so should be dependent on the sample type (blood, semen, trace), case type (volume or serious offence) and quantitation result. This could be implemented at the reporting scientist's discretion.

Opinion

- There is no specific international best practice for the extraction, purification, and concentration of DNA from forensic samples.
- Laboratory policy regarding the use of Microcon® Centrifugal Filter devices should consider the balance between scientific and management considerations and could be determined on a sample-to-sample basis, considering sample type, case type and quantitation result.
- A concentration step after DNA extraction will not affect the accuracy of a DNA result (it should not result in an incorrect profile) but may affect the success rate (the proportion of informative DNA profiles obtained). The effect (negative or positive) on the success rate will be determined by the optimization of the extraction methodology and sample type to be extracted. It is therefore important that the entire extraction process is appropriately validated.