Accurately assessing the quality and quantity of cfDNA extractions

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Introduction

Liquid biopsy or cell-free DNA (cfDNA) testing is fast becoming an important method to overcome the difficulties and invasive nature of direct tumour testing. Ensuring that clinical laboratory extractions are efficient and of high quality is paramount due to the limited yield, scarcity, and variability of cfDNA within patient samples.

Due to these limitations, using artificial material is an ideal solution for delivering external quality assessment (EQA). In addition, using samples with a known starting concentration allows an accurate indication of extraction efficiency.

An exploratory pilot was set up to determine the feasibility and robustness of delivering an EQA for cfDNA extraction using artificial reference material that closely resembles real patient samples.

Methods

To accurately determine the extraction efficiency, novel plasma Seraseq® ctDNA Extraction Reference Material at 50ng/ml of cfDNA was utilised. This would allow for a direct comparison between laboratories using the same starting material and would enable the EQA to be scaled up without difficulties associated with sourcing real patient samples and the variability in cfDNA yield in patient plasma.

Reference material (2ml aliquot) was sent to four laboratories that each use a different cfDNA extraction technique. These were transported in temperature-controlled packaging which maintained the samples at -20°C. The extracted cfDNA was returned to GenQA at ambient temperature for analysis.

GenQA determined the mass using volume by weight and concentration using the *AP3B1* bio-rad ddPCR assay. The quality was assessed using Agilent's TapeStation cell-free DNA assay, which provided a percentage of cfDNA within the sample, a sizing profile and the average size of cfDNA in base pairs (bp).

Results

DNA extraction techniques

Four different extraction methods were used:

- Qiasymphony DSP Circulating DNA Kit - custom protocol
- Roche COBAS® cfDNA Sample Preparation Kit
- Nonacus Cell3™ Xtract
- Maxwell RSC / ccfDNA LV Plasma Kit

Volume of cfDNA extracted

- Laboratories were provided with 2ml of plasma and asked to extract and send back the DNA within pre-weighed extraction tubes.
- Once the DNA was returned to GenQA, the tubes were re-weighed and the volume of cfDNA was calculated.
- The balance has an uncertainty of measurement (UoM) of ±0.00052g which has been taken into consideration within the figures.
- The volume extracted ranged from 43.2 µl – 179.8 µl..
- A summary of the volume of cfDNA extracted is available in figure 1 and table 1.

Figure 1 – Summary of volume of cfDNA extracted (µl)

Label indicate the extraction method used, and error bars indicate the UoM.

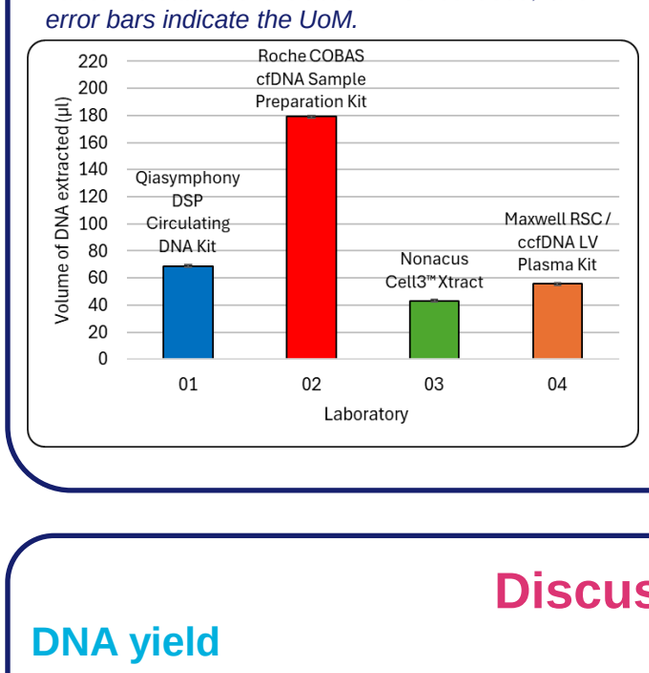


Figure 2 – Summary of concentration of cfDNA extracted (ng/µl)

Label indicate the extraction method used, and error bars indicate the UoM.

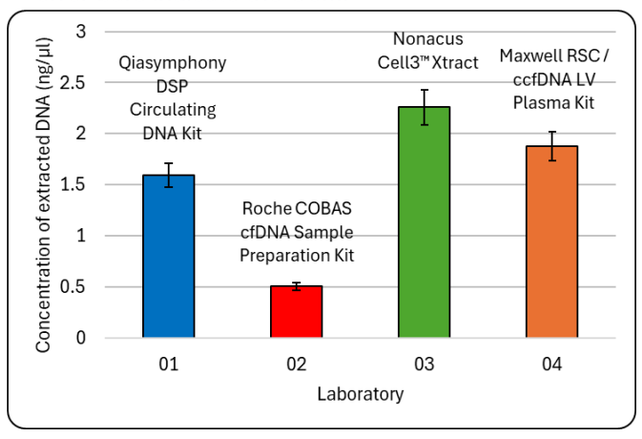
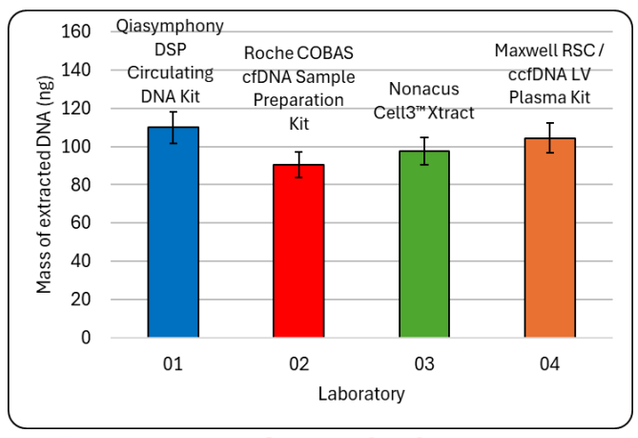


Figure 3 – Summary of mass of cfDNA extracted (ng)

Label indicate the extraction method used, and error bars indicate the UoM.



Concentration of cfDNA extracted

- The concentration was assessed using the using the *AP3B1* bio-rad ddPCR assay.
- This assay was chosen as it represents a house keeping gene with a stable copy number.
- The ddPCR equipment has been shown internally to have an UoM of ±7.46% which has been taken into consideration within the figures.
- The concentration of DNA extracted ranged from 0.51ng/µl – 2.26ng/µl.
- A summary of the concentration of cfDNA extracted by each laboratory is shown in figure 2 and table 1.

Mass of cfDNA extracted

- The mass of DNA was determined using the concentration by ddPCR and volume of cfDNA by weight.
- The resulting mass of cfDNA laboratories extracted ranged from 90.4ng – 109.9ng.
- The uncertainty of measurement associated with the balance and ddPCR is detailed within the figures and tables.
- A summary of the mass of cfDNA extracted by each laboratory is shown in figure 3 and table 1.

Quality of extracted cfDNA

- The fragment size of the extracted cfDNA was determined using Agilent's TapeStation cell-free DNA assay.
- The average peak size ranged from 186-190 base pairs (bp).
- The sizing profiles indicated 2 peaks for all samples; 1 large peak ~160bp and a second smaller peak ~305bp. See figure 4.
- The assay identified that the percentage of cfDNA within the sample was greater than 96% for all laboratories.
- A summary of the TapeStation results is displayed in figure 4 and table 1.

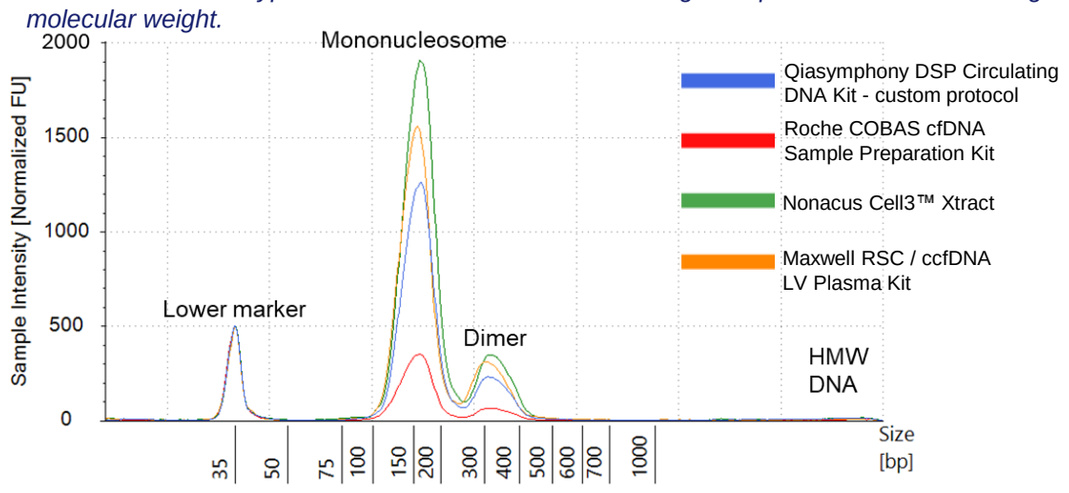
Table 1 – Summary of pilot EQA results

Table includes results from ddPCR and TapeStation analysis.

laboratory ID	Extraction method	DNA volume (µl)	Concentration (ng/µl)	Mass (ng)	%age cfDNA content	Average peak size (bp)
01	Qiasymphony DSP Circulating DNA Kit	68.9	1.60	109.94 ± 8.2	98	189
02	Roche COBAS cfDNA Sample Preparation Kit	179.1	0.51	90.40 ± 6.7	97	186
03	Nonacus Cell3™ Xtract	43.2	2.26	97.57 ± 7.3	99	190
04	Maxwell RSC / ccfDNA LV Plasma Kit	55.6	1.88	104.37 ± 7.8	99	187

Figure 4 – Overlayed Agilent TapeStation Electropherograms for all methods

Labels indicate the type of DNA associated with each fragment peak. HMW refers to high molecular weight.



Discussion

DNA yield

- The volume of cfDNA extracted was very variable with over a 4-fold difference between the lowest and highest volume samples.
- The concentration of the extracted cfDNA varied between laboratories.
- All laboratories showed >90% recovery rate based on the expected mass of 100ng cfDNA within the plasma. Although some showed extraction >100ng, this is the nominal value for the plasma stated by the manufacturer and there may be some variability in the cfDNA mass.

DNA quality

- All laboratories extracted a high percentage of cfDNA with minimal high molecular weight (HMW) DNA. As this is artificial plasma, there is not expected to be any HMW DNA present as may be present within real samples. It does indicate minimal contamination of the samples during the extraction process.
- The peak fragment sizes are consistent between the different laboratories and represents the sizing that would be expected from a real patient sample.

Conclusions

- Despite the difference in volume and concentration of cfDNA extracted between the laboratories, the resulting mass was very consistent between the different laboratories, demonstration efficient extraction of the cfDNA.
- The peak sizes indicated by Tapestation are reflective of the expected peak sizes for this material, indicating the extraction protocols are not shearing the DNA during their process.
- The reference material closely mimics real patient plasma and is suitable for a larger scale pilot EQA for laboratories worldwide.
- The current storage of samples at -20°C may prove difficult when sending the EQA to regions that have long transit times. Therefore, further studies into the stability of the samples at 2-8°C would be beneficial to be able to provide the EQA at an economic price in the future.

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Conflicts of interest: The authors have no conflicts of interest to declare.

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