Assessing the performance of cfDNA extraction from plasma

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Member of UK NEQAS consortium

Introduction

The use of specific and sensitive methodologies for the detection of somatic variants in circulating tumour DNA (ctDNA) requires efficient extraction of high-quality cell free DNA (cfDNA) from plasma samples. Many samples fail to provide reportable results which may be due to sub-optimal DNA extraction.

The global external quality assessment (EQA) provider, GenQA has extensive experience in delivering the assessment of the quality and quantity of DNA extracted from different sample types.

GenQA delivered a pilot EQA for cfDNA extracted from 50 laboratories from the same plasma samples to provide an external measurement of the performance of the extraction processes.

Material & methods

- Each laboratory received two Seraseq® ctDNA contrived plasma samples which contained cfDNA at a concentration of 50ng/ml (201C) and 80ng/ml (202C).
- Laboratories were not informed of the sample concentration and were instructed to extract cfDNA and return to GenQA within tubes provided.
- The returned DNA was assessed by GenQA by determining the volume by weight, and concentration by droplet digital PCR (ddPCR) to determine the overall mass of DNA and therefore the extraction efficiency.
- Tapestation cell free DNA assay was also run to determine the quality of the extracted cfDNA and identify any contamination of the samples.
- Laboratories were scored based on the quality and quantity of DNA extracted and provided with an overall Summary Report detailing all participants results and an individual laboratory report with scoring and feedback on their extraction.

Discussion

- The recovery of cfDNA was lower than expected for sample 202C. This is potentially due to extraction method being at capacity with the higher concentration sample and therefore laboratories were not able to extract all the DNA present.
- Some laboratories extracted greater than the nominal values provided by the manufacturer, however it should be noted that for this batch the average concentration after extraction from the manufacturer was 57ng/ml for 201C and 87ng/ml for 202C, therefore the concentration may be higher than the nominal value.
- For two laboratories, the proportion of DNA within each peak did not match the expected 4:1 ratio, which indicates that the extraction method is not as efficient at extracting DNA either at lower molecular weight (~160bp) or higher molecular weight (~320bp).

Conclusion

The **extraction efficiency varied greatly** between laboratories which may impact on the ability of a laboratory to obtain a reportable result for ctDNA testing.

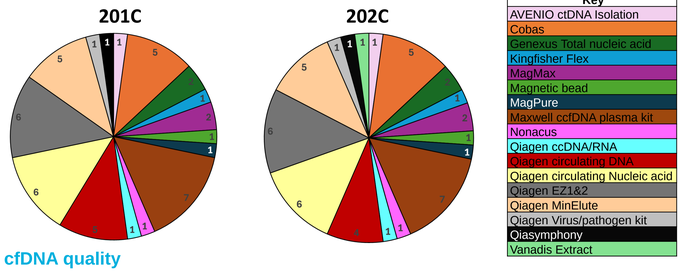
The sizing profiles for most laboratories were consistent and closely resembled the results of real patient samples demonstrating the ability to use contrived reference samples to measure the effectiveness of cfDNA extraction processes.

Results

DNA extraction techniques

- There were 17 different extraction methods used, as detailed in Figure 1.

Figure 1– cfDNA extraction methods used by participating laboratories

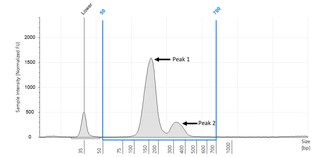


cfDNA quality

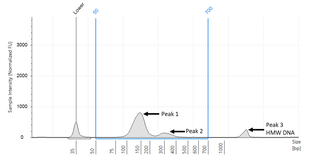
- The quality of cfDNA extracted was assessed using Agilent's TapeStation cell-free DNA assay.
- The majority of laboratories produced good quality extracts. An example electropherogram of a high quality sample is displayed in Figure 2a.
- For 96% of participating laboratories, the electropherograms indicated 2 peaks; 1 large peak (peak 1) between 137-171bp which contained ~80% of the DNA and a second smaller peak, which ranged from 268-338bp (peak 2) which contained ~20% of the DNA.
- One laboratory's electropherogram indicated a third small peak >800bp (peak 3), indicating contamination of high molecular weight (HMW) DNA (Figure 2b).
- For one laboratory, no defined peaks were produced, indicating an issue during the extraction process (Figure 2c).

Figure 2 – Electropherograms produced during cfDNA analysis

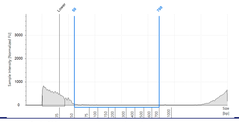
a) Example of electropherogram of a high quality sample extracted by a participating laboratory.



b) Electropherogram produced indicating contamination with HWM DNA



c) Electropherogram indicating an issue during extraction process



cfDNA mass

- The mass of DNA was determined by using volume by weight and concentration using the *AP3B1* Bio-rad ddPCR assay (Table 1 and Figures 3 and 4).
- For 4 laboratories, the mass could not be determined due to issues with distinguishing between positive and negative droplets during ddPCR.
- For 1 laboratory no droplets were produced during the droplet generation process, this was the same laboratory which did not produce any peaks for the TapeStation.

Table 1 – Summary of mass of cfDNA extracted by participating laboratories

Sample	Nominal mass provided by manufacturer (ng)	Mean mass of extracted DNA by participants (ng)	Lowest mass extracted by participant (ng)	Highest mass extracted by participant (ng)
201C	100	102	24.5	173.9
202C	160	135	52.7	203.0

Figure 3 – Mass of cfDNA extracted by participating laboratories for plasma sample 201C

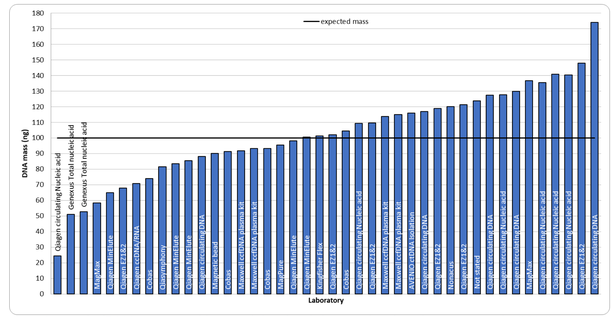
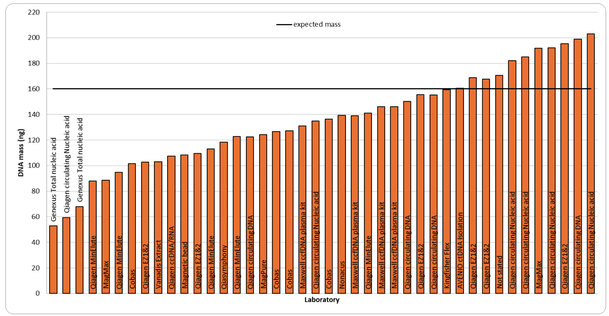


Figure 4 – Mass of cfDNA extracted by participating laboratories for plasma sample 202C



Acknowledgements The authors would like to thank the GenQA Sample Handling Specialist Advisory Group, the representatives within the GenomeMET project (www.genomemet.org), for the support in developing the EQA and the EQA participants.

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