Getting the most from your sample: Inter-laboratory variability in DNA extracted from blood and formalin-fixed paraffin-embedded (FFPE) tissue

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Introduction

With an increase in testing of higher molecular weight DNA, the demand for high quality and quantity of DNA is becoming more inportant. GenQAs DNA extraction external quality assessment (EQAs) allows laboratories from a variety of countries, using an array of methods to benchmark their DNA extraction methods against other laboratories that have received the same sample.

GenQAs DNA extraction EQAs were originally set up to aid laboratories for the 100,000 Genomes Project to ensure their methods produced sufficient quality and quantity of DNA for Next Generation Sequencing (NGS). The DNA extraction from blood EQA was first run in 2014 and DNA extraction from FFPE tissue in 2015. Following this success, the EQA was extended and is now available to all GenQA participant, along with the DNA extraction from fresh frozen tissue and DNA extraction from saliva EQAs.

Methods

DNA extraction from blood

- Three samples of volume 1ml (157B), 3ml (158B) and 4.5ml (159B) were sent out. Participants were asked to extract the DNA using their routine extraction method and return the DNA using the tubes provided.
- Returned DNA was tested to determine the quantity of DNA by ddPCR and quality of DNA by TapeStation.

DNA extraction from FFPE

- Three samples from 3 different tissue sources: Gastrointestinal stromal tumour (GIST) (154T), lung tissue (155T) and colorectal tissue (156T) were provided. Two 5µm slide mounted or rolled sections were provided for each sample. Participants were asked to extract the DNA using their routine extraction method and return the DNA using the tubes provided.
- Returned DNA was tested to determine the quantity of DNA by ddPCR and quality of DNA by preparing library pools that were then run on TapeStation.

Results - DNA extraction from blood

Methodology

Fifty-seven laboratories participated in the EQA and a total of 15 different methodologies were used to extract the DNA. Chemagen and Qiagen were the extraction methods used by the majority of laboratories.

Mass of DNA extracted

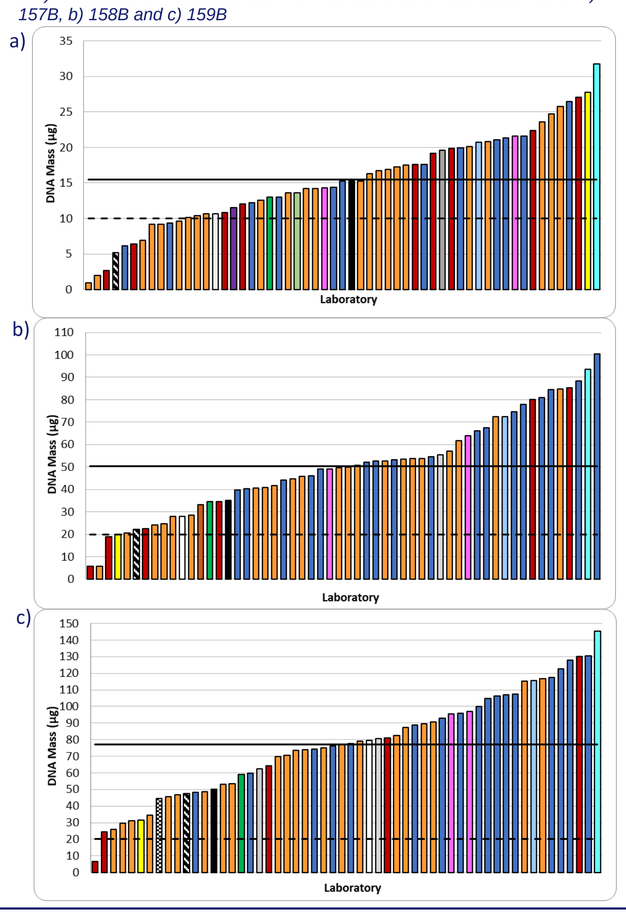
The mass of DNA extracted by the laboratories was determined based on the weight of DNA and the concentration determined using ddPCR. A measurement uncertainty of 12.6% was added to the concentration values to account for UoM for the ddPCR. The mass of DNA extracted from a 1ml blood sample ranged from 0.95µg to 31.75µg, 3ml sample ranged from 5.85µg to 100.27µg and 4.5ml sample ranged from 6.63µg to 145.23µg (Figure 1). Scoring thresholds were set based on the standard mass of DNA required per sample volume for NGS. The mass of DNA extracted was highly variable across different laboratories and within different method groupings.

Methodology key for Figures 1 and 2

- Autogen
- Chemagen
- Illustra
- InnuPREP
- Invitrogen
- Kurabo
- Macherey Nagel
- Manual and Qiagen
- Maxwell
- Not stated
- Omega Mag-bind
- Phenol
- Promega
- Qiagen
- Roche
- Tiangen

Figure 1 – Summary of mass of DNA extracted.

mass of DNA extracted from each blood sample in ascending order with mean mass extracted (solid black line) and scoring threshold (dashed black line). Colour of the bar indicates the method of DNA extraction used. a) 157B, b) 158B and c) 159B



DNA Quality

The quality of the DNA was analysed using the TapeStation, using the genomic screentape, which produces a DNA integrity Number (DIN). The DIN ranges from 0-10, with 0 being the most degraded and 10 being highly intact. The DIN varied as follows:

- 1ml sample: 5.8 – 8.3
- 3ml sample: 6 - 8.9
- 4.5ml sample: 6 – 8.3

Laboratories were scored based on the DIN, as follows (Figure 2):

- DIN of >6.8: 2 marks – good quality
- DIN of 6 -6.7 : 1 mark – average quality
- DIN <6 : 0 marks – low quality

The majority of laboratories received a score of 1, indicating average quality DNA extracted.

Scoring

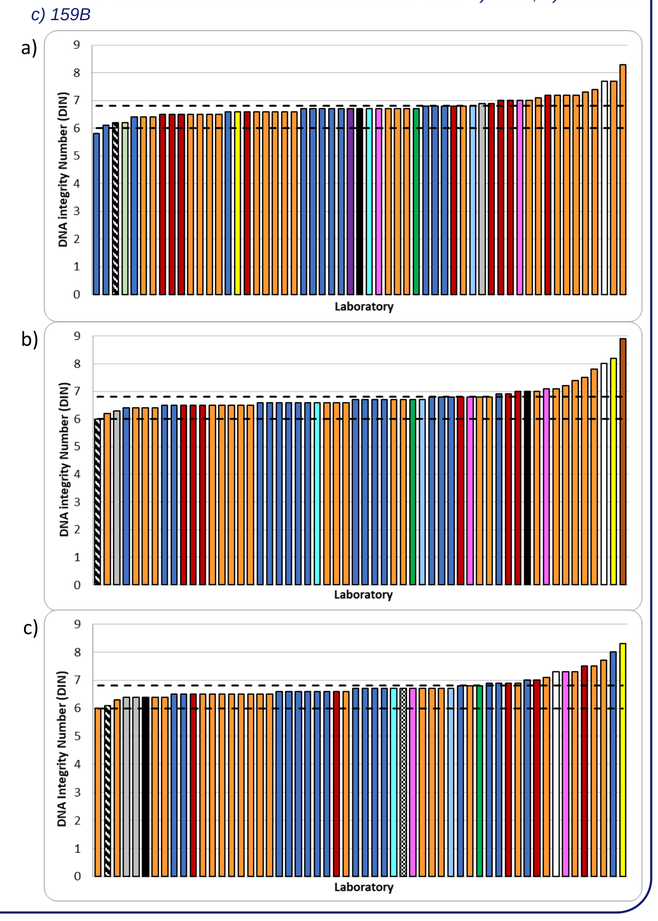
Laboratories were scored out of a total of 12 marks and performance criteria was applied as follows:

- 0-4 marks – Red rated – Poor performance
- 5-8 marks – Amber rated – Satisfactory
- 9-12 marks – Green rated – Satisfactory

No laboratories were red rated, ten laboratories were amber rated and 47 were green rated

Figure 2 – Summary of DIN of the extracted DNA

DNA Integrity Number (DIN) of DNA extracted from each blood sample in ascending order with scoring thresholds indicated (dashed black lines). Colour of the bar indicates the method of DNA extraction used. a) 157B, b) 158B and c) 159B



Results – DNA extraction from FFPE

Methodology

Fifty-one laboratories participated in the EQA and a total of 7 different methodologies were used to extract the DNA. Qiagen and Maxwell were the extraction methods used by the majority of laboratories.

Mass of DNA extracted

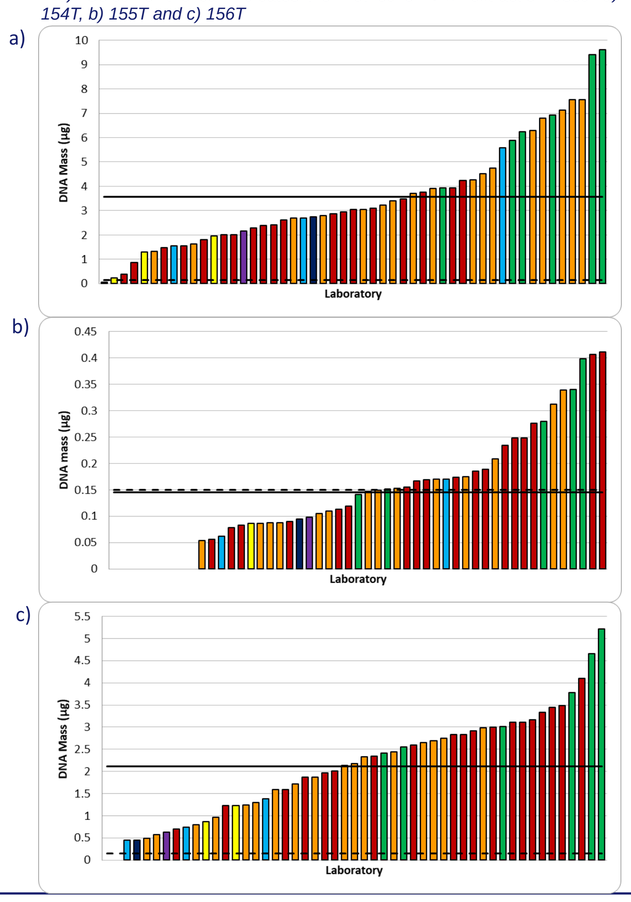
The mass of DNA extracted by the laboratories was determined based on the weight of DNA and the concentration determined using ddPCR. A measurement uncertainty of 12.6% was added to the concentration values to account for UoM for the ddPCR. The mass of DNA extracted from the GIST sample ranged from 0.065µg to 9.61µg, the lung tissue sample ranged from undetectable to 0.411µg and colorectal tissue sample ranged from undetectable to 5.219µg (Figure 3). The mass of DNA extracted was variable for the different samples.

Methodology key for Figures 3 and 4

- Manual-Qiagen
- Maxwell
- Qiagen
- RecoverAll
- Roche
- Thermofisher
- Zytovision

Figure 3 – Summary of mass of DNA extracted.

Mass of DNA extracted from each FFPE sample in ascending order with mean mass extracted (solid black line) and scoring threshold (dashed black line). Colour of the bar indicates the method of DNA extraction used. a) 154T, b) 155T and c) 156T



DNA Quality

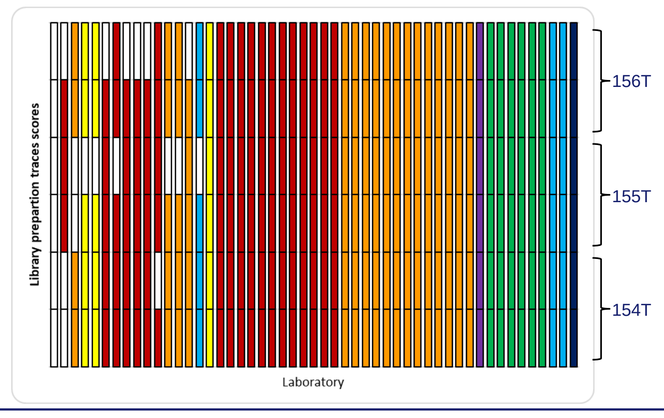
To assess the quality of DNA extracted, library pools were prepared for each sample and run on TapeStation with the High Sensitivity D1000 ScreenTape. The traces produced were independently reviewed and scored by two clinical scientists with extensive experience in NGS. Traces were scored as follows (Figure 4):

- Good quality trace: 2 marks
- Average quality trace: 1 mark
- Poor quality trace: 0 marks.

The majority of laboratories had good quality library preparations for the samples.

Figure 4 – Summary of library preparation trace scores.

Each section of the chart represents 1 mark. Coloured sections represent the number of marks achieved by a laboratory for that sample. Colour of each section indicates the method of DNA extraction used.



Scoring

Laboratories were scored out of a total of 6 marks.

Sample 156T was provided as an educational sample as it was from an aged tissue block. This sample was not scored for the EQA.

The EQA demonstrated that laboratories were still able to extract good quality and quantity of DNA from a 10-year-old FFPE tissue block .

In addition, the mass of DNA for sample 155T was not scored as the scoring threshold was greater than the mean mass of DNA extracted for this sample.

Performance criteria was applied as follows:

- 0-2 marks – Red rated – Poor performance
- 3-4 marks – Amber rated – Satisfactory
- 5-6 marks – Green rated – Satisfactory

One laboratory was red rated, two laboratories were Amber rated and 48 were green rated.

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

Conclusion

The variability in mass of DNA extracted is clear in both EQAs and demonstrates that many laboratories are not maximising the yield of DNA for the sample provided. Based on these results, as variety is seen by laboratories using the same methods, it demonstrates that there are many other factors that are effecting the quantity of DNA laboratories can extract. The results highlight the importance of EQA for laboratories to determine how their extraction methods compare and where there is room for improvement in their extraction processes.

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