Poster EP17.002 Review of the DNA quantification external quality assessment

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Introduction

GenQA is an external quality assessment (EQA) provider for end-to-end genomics testing. Covering over 100 different EQAs from 11 different disciplines. The DNA quantification EQA has been provided by GenQA for 6 years, initially set up to aid with standardization of assessing sample quantity for the NHS England 100,000 genomes project, it is now openly available to all participants. Accurate quantification is important for several applications, and when samples fall outside the required specifications for a test, this can result in a failed test or sample rejection which could ultimately lead to delays in returning patients results or re-sampling of patients.

Methods

- For the 2021 DNA quantification EQA, six DNA samples were provided (Table 1) along with an elution buffer to use as a blank if necessary. Participants were asked to quantify the DNA using their routine double-stranded DNA (dsDNA) specific method.
- Results submitted by proforma detailing the concentration of DNA measured and the method the laboratory used to quantify the DNA
- Results assessed using statistical analysis to benchmark laboratories performance

Results

Methodologies

Sixty-two laboratories participated in the EQA and a total of 18 different methodologies were used to quantify the DNA samples. The different methodologies can be split into three methodology types: Fluorometric, Spectrophotometric and qPCR. A summary of the methods used for the different sample types can be seen in Figure 1.

Figure 1 - Methods used by laboratories to determine DNA concentration

Methods used to determine the DNA concentration, grouped by type of quantification method

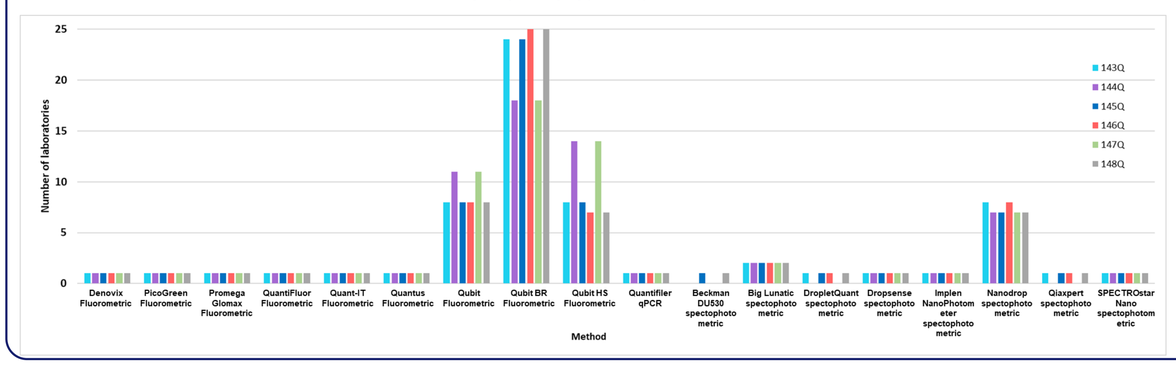


Table 1 – Summary of samples provided and EQA median values by quantification type

Note: One laboratory used qPCR to determine the concentration, for this, the single laboratory's value is displayed as a median cannot be provided

Sample name	Source of DNA	EQA consensus median concentration (ng/µl)	Fluorometric median concentration (ng/µl)	Spectrophotometric median concentration (ng/µl)	qPCR concentration (ng/µl)
2021_143Q	Somatic cell line	145.0	138.5	187.0	165.5
2021_144Q	Formalin fixed paraffin embedded tissue (FFPE)	35.6	34.4	128.2	15.8
2021_145Q	Whole Peripheral Blood	61.5	56.9	85.0	65.9
2021_146Q	Fresh tissue	75.0	72.7	92.0	78.9
2021_147Q	Formalin fixed paraffin embedded tissue (FFPE)	17.4	16.4	63.2	9.8
2021_148Q	Whole Peripheral Blood	93.4	85.1	127.3	63.5

Analysis

Results were analysed by benchmarking and comparison of results between laboratories. The statistical analysis for the scoring of the results was determined with consultation from a chartered statistician. This involved the following steps:

- Removal of outliers.
- Determining the best fit distribution model based upon Akaike's Information Criterion (AIC).
- Bootstrapping to determine a better estimate of the parameters of the distribution.
- Calculation of percentiles of the fitted distribution in an analogous way to using the mean and standard deviations for a normal distribution.

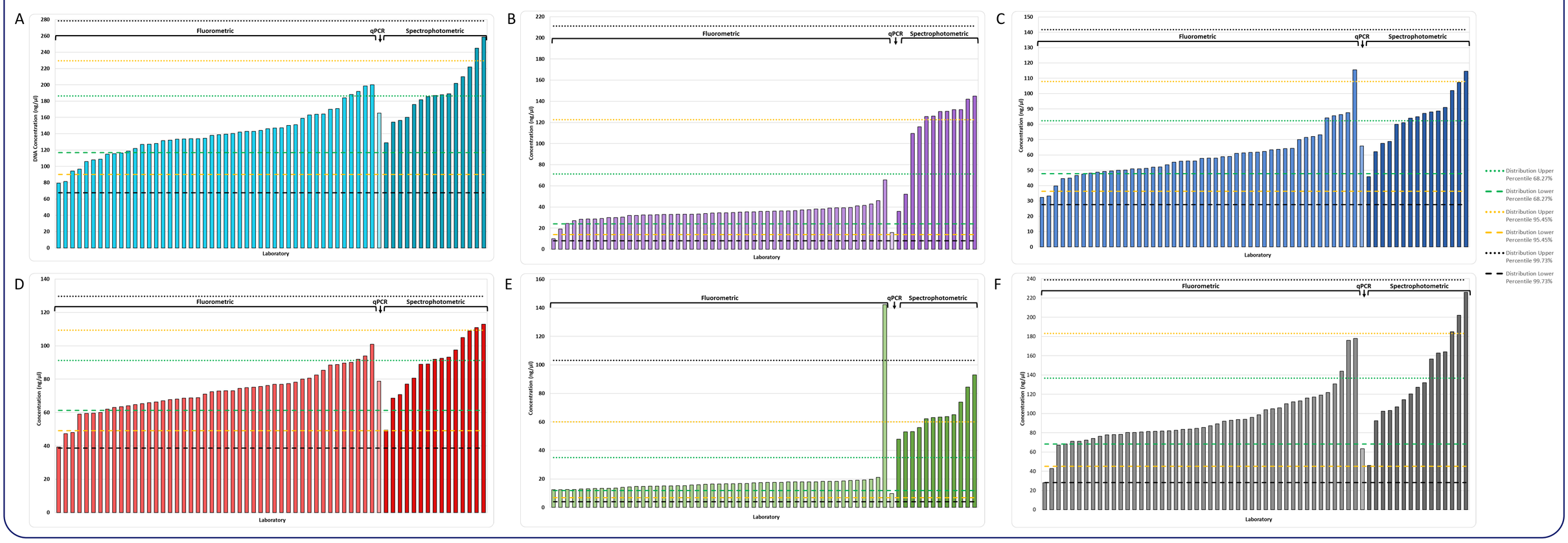
Results

Individual sample results

The results were analysed for each sample (Figure 2). Sample 143Q, 146Q and 148Q were best fitted as gamma distributions and samples 144Q, 145Q and 147Q were best fitted as log normal distributions. The type of quantification method had an impact on the concentrations measured, with spectrophotometric methods generally reporting higher concentration results (Table 1 and Figure 2). The higher concentration measurements was particularly evident for DNA extracted from FFPE (Figures 2B and 2E).

Figure 2 - DNA concentration measurements reported by laboratories with percentile thresholds

Arranged in ascending concentration by technique type identified by different shading: from left to right- fluorometric techniques, qPCR and spectrophotometric techniques. A-143Q, B- 144Q, C-145Q, D-146Q, E-147Q, F-148Q



Results

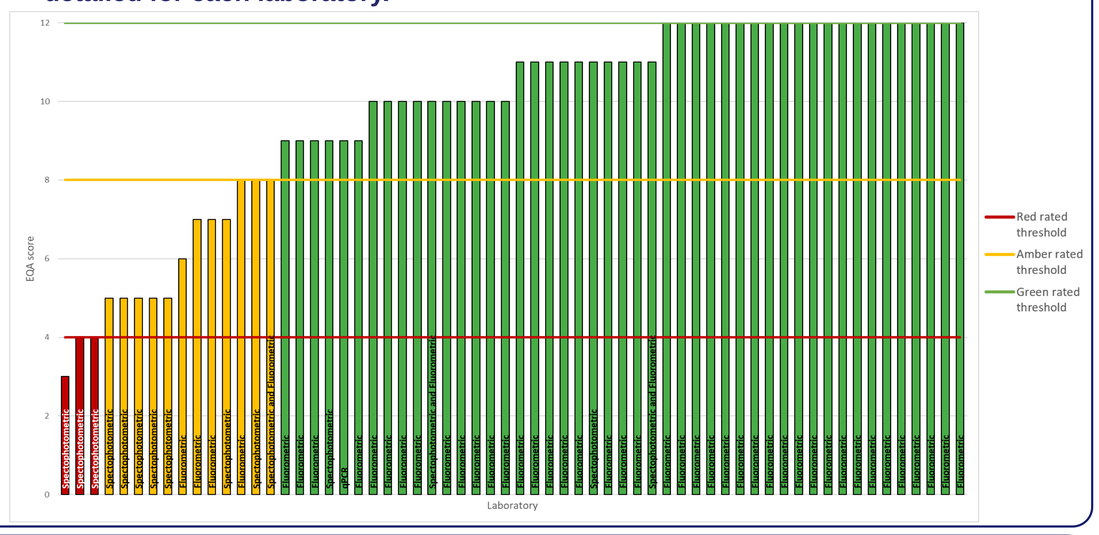
EQA scoring

For each sample, laboratories received 2 marks if their measured concentration was within 68.27 percentile of the fitted distribution, 1 mark if their measured concentration was between 68.27 and 95.45 percentile of the fitted distribution and 0 marks if their measured concentration was greater than or less than 95.45 percentile of the fitted distribution.

A total of 12 marks were available for the EQA. Scoring threshold were set, with participating laboratories scoring 9-12 marks classified as satisfactory with no recommendations (green rated), laboratories scoring 5-8 marks classified as satisfactory with a recommendation to review their DNA quantification protocol (amber rated) and laboratories scoring 0-4 marks classified as poor performers with a recommendation to urgently review their DNA quantification protocol (red rated). A summary of the scoring for the laboratories can be found in Figure 3.

Generally, laboratories scored well in the EQA, with only three laboratories receiving poor performance and the majority of laboratories (47 out of 62) receiving satisfactory performance with no recommendations. Laboratories using spectrophotometric methods generally scored lower (Figure 3).

Figure 3 - Participant EQA scores with the type of quantification method detailed for each laboratory.



Conclusion

The results from the EQA highlight the variability of DNA concentration measurements across and between methods and highlights the need for better standardisation of DNA quantification and continued need for DNA quantification EQAs to allow laboratories to bench mark and standardise their methods.

Spectrophotometric methods often detect contaminants within DNA extracted from FFPE samples that may skew results and this EQA has also identified this, and it is therefore recommended that for this sample type, a different quantification method should be considered.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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