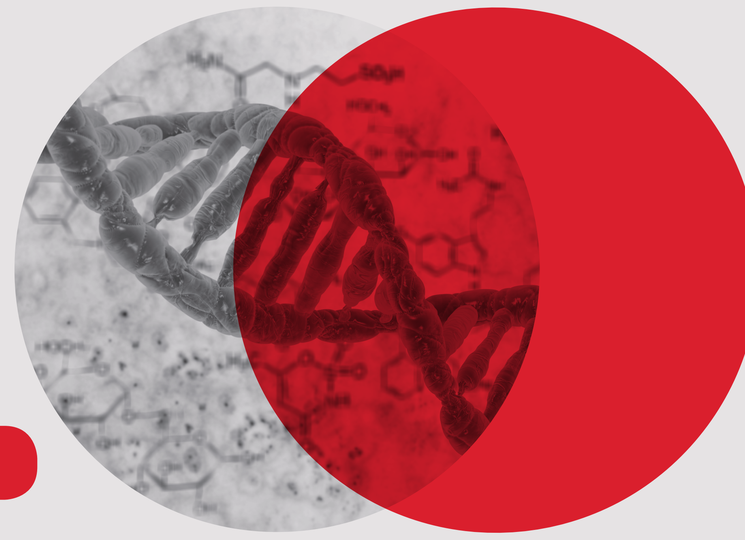
Evaluation of an external quality assurance pilot program for the quality assessment of DNA extracts



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Introduction

The accuracy and quality of any genetic test directly depends on the quality of the initial DNA extract. The DNA extraction process should recover high amounts of non-contaminated intact DNA from the source tissue. Current external quality assurance (EQA) programs for DNA extraction require participating laboratories to perform DNA extraction on an EQA provided fresh blood sample or specific tissue type. However, this strategy is restrictive for laboratories who regularly perform DNA extraction on other tissue types or for biobanks who have vast stores of archived specimens that are unrelated to the EQA material. To address this, a pilot EQA program was developed to evaluate the pre-diagnostic quality of DNA extracted from multiple tissue types. For this EQA program, participants were required to submit to the RCPAQAP their own extracted DNA from any tissue type. Here we report the results of the pilot survey. Quality of DNA extracts submitted were assessed using standard quality assuring statistical measures for population concordance testing.

Methods

A total of fourteen laboratories enrolled in the 2017 pilot survey with eleven laboratories participating. Participating laboratories were requested to submit five DNA extracts at 50ng/uL from any tissue type except formalin-fixed paraffin-embedded (FFPE) tissue. DNA extracts from fifteen different tissue types were analysed in the 2017 pilot survey. The majority (64%) of samples received were DNA extracts isolated from blood. Fifty-one DNA extracts (93%) were isolated using commercial kits or reagents; four (7%) were isolated using in-house or salting out methods. Table 1 lists the manufacturers of commercial DNA extraction kits used by participating laboratories.

Table 1. Manufacturers of commercial DNA extraction kits used.

Manufacturer	Number of Samples / %
Qiagen	28 (51%)
PerkinElmer	7 (13%)
Macherey-Nagel	5 (9%)
Sigma-Aldrich (Merck)	5 (9%)
GE Healthcare Life Sciences	5 (9%)
Applied Biosystems (Thermo Fisher Scientific)	1 (2%)

All DNA extracts were evaluated for integrity and PCR amplifiability using three testing strategies:

(i) Assessment of DNA integrity by 4200 TapeStation (Agilent Technologies)

Duplicate analyses of each DNA extract were performed on the 4200 TapeStation, and the average DNA Integrity Number (DIN) were used for Z-score calculations [*(DIN value – population mean DIN)/standard deviation of population DIN*] to determine population concordance. Z-scores within two standard deviations from the mean were regarded as good quality DNA and considered to be concordant. Z-scores greater than two standard deviations below the mean were deemed as highly fragmented DNA and considered discordant.

(ii) Assessment of PCR amplifiability by multiplex PCR

Following analysis of DNA integrity, each DNA extract were characterised by multiplex PCR across five gene loci (*AF4, AFF1, ZBTB16, RAG1* and *TBXAS1*) (1). Agarose gel electrophoresis was performed to visualise all PCR products. Amplification of all five PCR products were considered concordant. DNA extracts that failed to amplify across the five gene loci were considered discordant.

(iii) Relative quantification (RQ) by real-time PCR

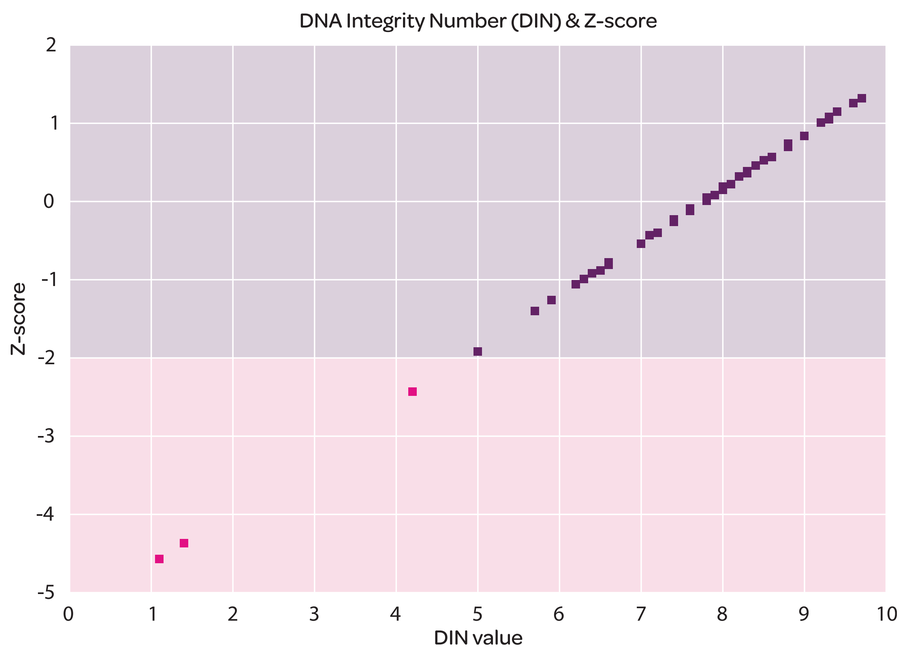
DNA extracts were further analysed across eleven additional gene loci (*TERT, LRP1B, RBFOX1, ROBO2, DMD, PDE4D, EYS, CNTNAP2, ASTN2, PRKG1, CNTN5*) using real-time PCR. Cycle threshold values generated for each gene were used to determine the relative quantity fold change using the *TERT* gene as the internal endogenous reference. RQ values of all genes were averaged for each DNA extract to derive a final RQ value. The closer the RQ value is to one, the closer the relative DNA copy number is to the reference control blood sample (i.e., no duplications or deletions). The generation of an RQ value was therefore considered concordant for each DNA extract. In contrast, DNA extracts that failed to amplify across the eleven gene loci were considered poor quality DNA and therefore discordant.

Results

DNA integrity assessment

Fifty-two DNA extracts (95%) achieved a Z-score within two standard deviations of the population mean and were considered concordant (Figure 1). The remaining three (5%) DNA extracts had low DIN values (indicative of highly fragmented DNA) and Z-scores greater than -2 were considered discordant.

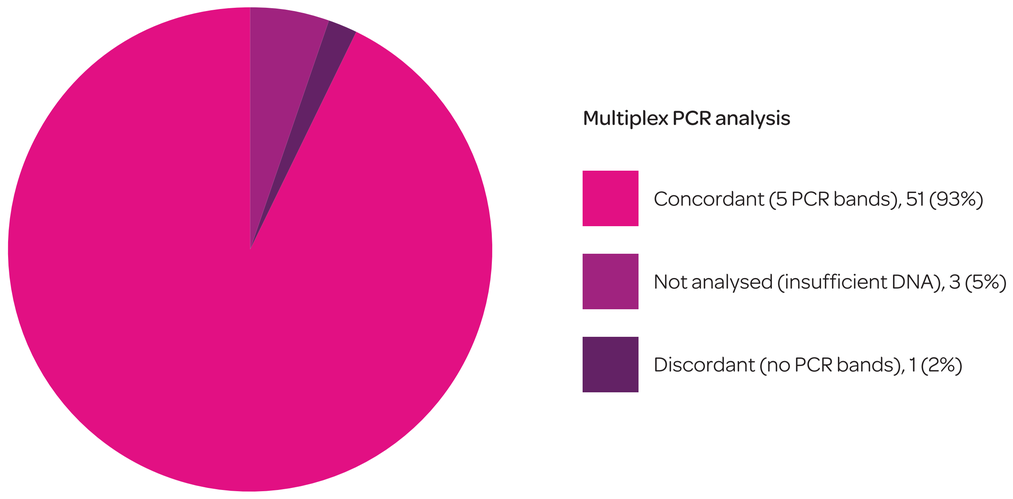
Figure 1. DNA Integrity Number (DIN) and Z-score for each DNA extract. DNA extracts with Z-scores between -2 and 2 (purple squares; top shaded area) were considered concordant and those greater than -2 were discordant (pink squares; bottom shaded area).



PCR efficiency assessment by multiplex PCR

Fifty-two (95%) of DNA extracts submitted were further assessed by multiplex PCR. The remaining three (5%) were not analysed due to the insufficient supply of DNA. Amplification of all five PCR products were observed in fifty-one (93%) of the DNA extracts (Figure 2). Two extracts that were discordant for DNA integrity were successfully amplified and considered concordant.

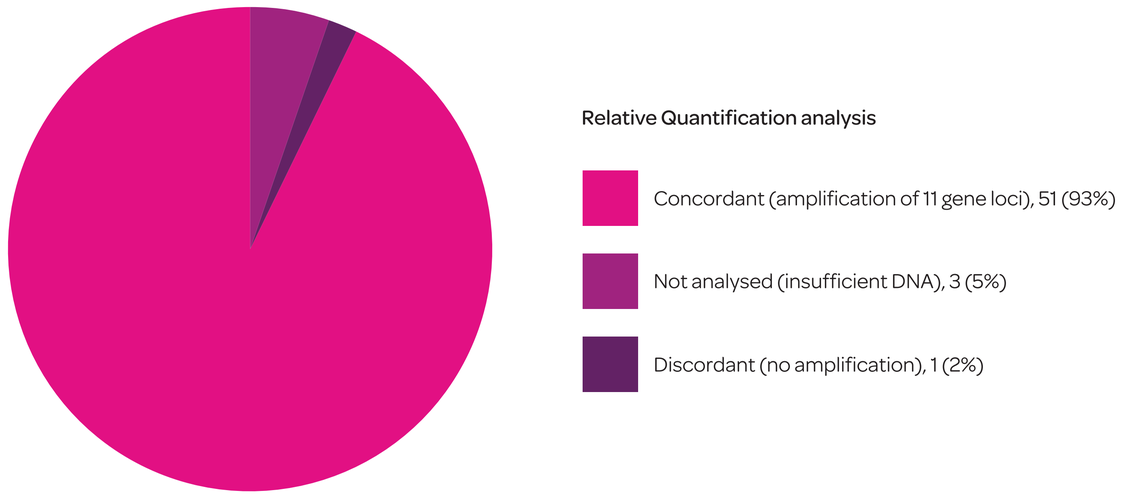
Figure 2. Concordance of DNA extracts using multiplex PCR analysis.



Relative quantification by real-time PCR

Relative quantification of eleven gene loci using real-time PCR was performed on fifty-two (95%) DNA extracts submitted; three (5%) of DNA extracts were not analysed due to insufficient DNA. Only one (2%) sample failed to amplify and was considered discordant (Figure 3).

Figure 3. Concordance of DNA extracts using relative quantification analysis.



Conclusion

- The overall quality of DNA extracts submitted by participating laboratories were good and were considered applicable for downstream diagnostic analyses.
- 98% of DNA extracts submitted were considered concordant (i.e., had high DNA integrity and were suitable for downstream genetic analyses).
- Only one sample failed to amplify using both multiplex and real-time PCR and was considered to be of poor DNA quality and not suitable for genetic diagnostic analyses.
- The data from this pilot survey suggests that DNA extracts with low DNA integrity may still be fit for purpose and used for diagnostic characterisation.
- This novel EQA is therefore applicable for total DNA extraction quality testing for multiple tissue types.
- NEW PILOT PROGRAMS: The RCPAQAP have now introduced a pilot program to assess FFPE DNA extracts for 2018 and are developing a pilot program to assess the quality of extracted circulating free DNA.

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Reference

1. van Dongen JJM, Langerak AW, Bruggemann M, *et al.*, (2003). Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations

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