

Use of Agilent 4200 TapeStation system for external quality assurance of genomic DNA and cell-free DNA

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Background

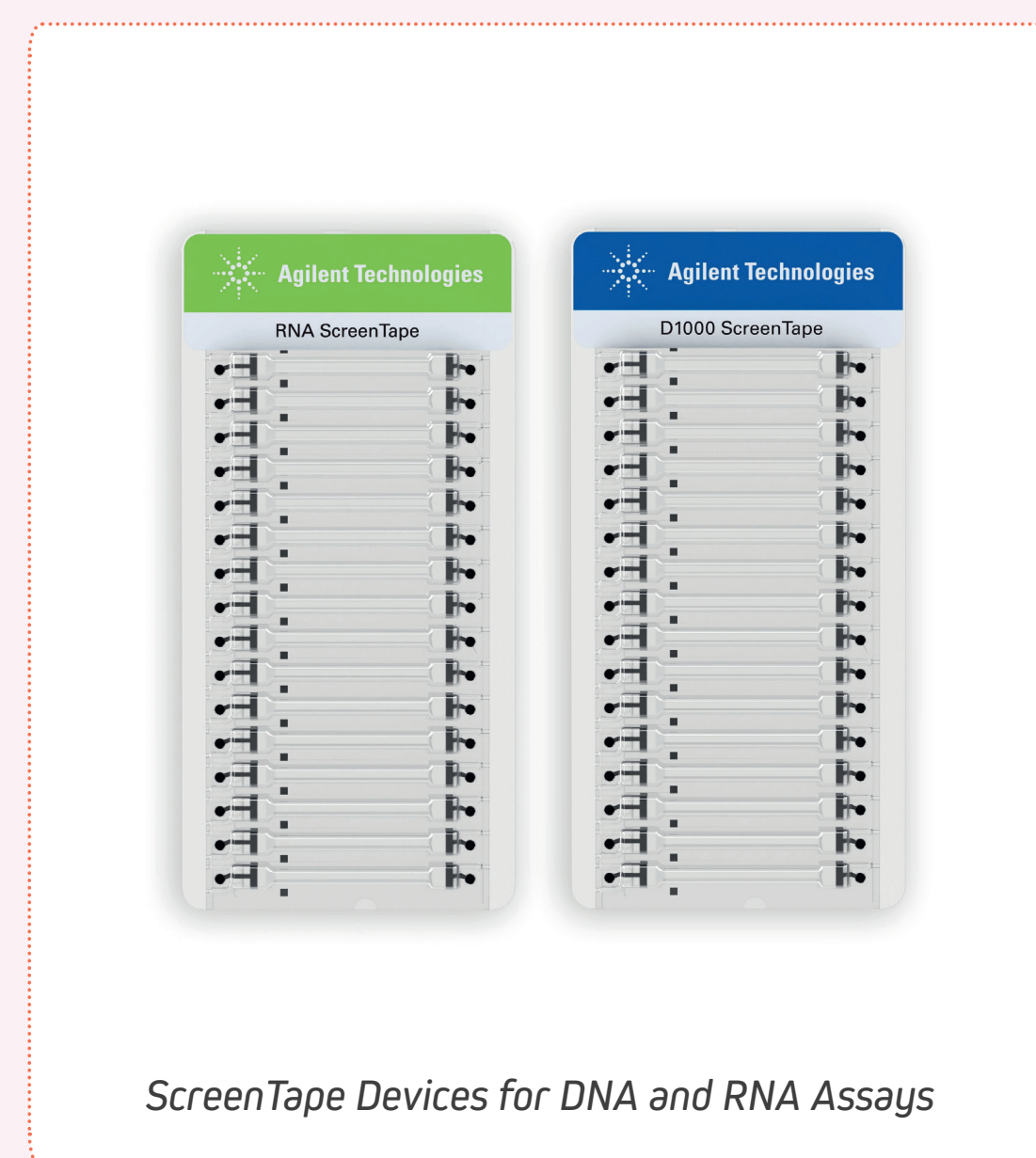
EQAs is mandatory for quality assurance of the wide variety of DNA extraction methods. DNA quality assessment by integrity and molecular size estimation can be achieved using conventional gel or automatic microfluidic electrophoresis systems (e.g Agilent 2100 Bioanalyzer system)

Here we evaluated the Agilent 4200TapeStation system, in the context of the ISBER-endorsed "Proficiency Testing (PT)" program organised by IBBL, for :

- Genomic DNA (gDNA) extraction methods from
 - Whole blood (BD Paxgene Blood DNA tubes);
 - Snap frozen tissue;
 - Formalin-Fixed Paraffin-Embedded (FFPE) cell pellet.
- Cell-free DNA (cfDNA) extraction methods from plasma isolated from whole blood collected in PaxGene cfDNA tubes, spiked-in with double stranded DNA ultramers of 200bp covering several standard cancer mutations.

4200 TapeStation System

- Automated microfluidic electrophoresis system;
- 96 well plate or 2x8 well strip sample loading format;
- Individual ScreenTape devices consisting of 16 lanes, i.e. 15 samples and 1 ladder;
- Multiple ScreenTape devices loading for maximum 96 samples run in walk away modus;
- 1 µl sample volume needed per run in concentration of 10-100 ng/µl for genomic DNA and 10-1000 pg/µl for High Sensitivity D5000 ScreenTape assays.



Methods

For gDNA:

- We evaluated the extracted DNA quality from each participant, by determining a DIN (DNA Integrity Number score for each sample run with the Genomic DNA ScreenTape assay;
- We measured DIN scores, ranging from 1 (DNA completely degraded) to 10 (DNA completely intact).

For cfDNA:

- We ran cfDNA with the 4200 TapeStation High Sensitivity D1000 and High Sensitivity D5000 kit as well as the 2100 Bioanalyzer DNA 1000 kit to determine the best detection system;
- We evaluated the extracted cfDNA quality from each participant based on the % integrated area around the 200 bp peak, corresponding to the size of the spiked-in dsDNA ultramers.

Results

For gDNA, average DIN score from all participants in our external Proficiency Testing programme:

- Stabilised blood DIN 6.7 (STDEV 0.75) (Fig. 1, 5);
- Snap frozen tissue DIN 5.67 (STDEV 1.57) (Fig.2, 6);
- FFPE cell pellets DIN 4.89 (STDEV 1.05) (Fig.3, 7).

For cfDNA, the following results were obtained from all participants (Fig.4, 8) :

- 81-89% integrated area corresponding to the 200bp peak of the spike-in;
- Contamination by cell gDNA was evident in samples from some participants;
- TapeStation HS D5000 kit outperformed the Bioanalyzer DNA1000 kit and the TapeStation HS D1000 kit.

The 4200 TapeStation system is well suited for the purpose of quality control of DNA samples extracted from clinical material; it is high throughput and user-friendly. Particularly in

the case of cfDNA, the 4200 TapeStation system allows simultaneous assessment of the size of the extracted cfDNA and of any potential contamination by white blood cell DNA.

Conclusions

The 4200 TapeStation system is more informative than the 2100 Bioanalyzer system, and is well suited for the purpose of quality control of DNA samples extracted from clinical material; it is high throughput and user-friendly and introduces DIN values, a quality label for DNA samples. Particularly in the case of cfDNA, the 4200 TapeStation system allows simultaneous assessment of the size of the extracted cfDNA and of any potential contamination by white blood cell DNA.

TapeStation Gel Images

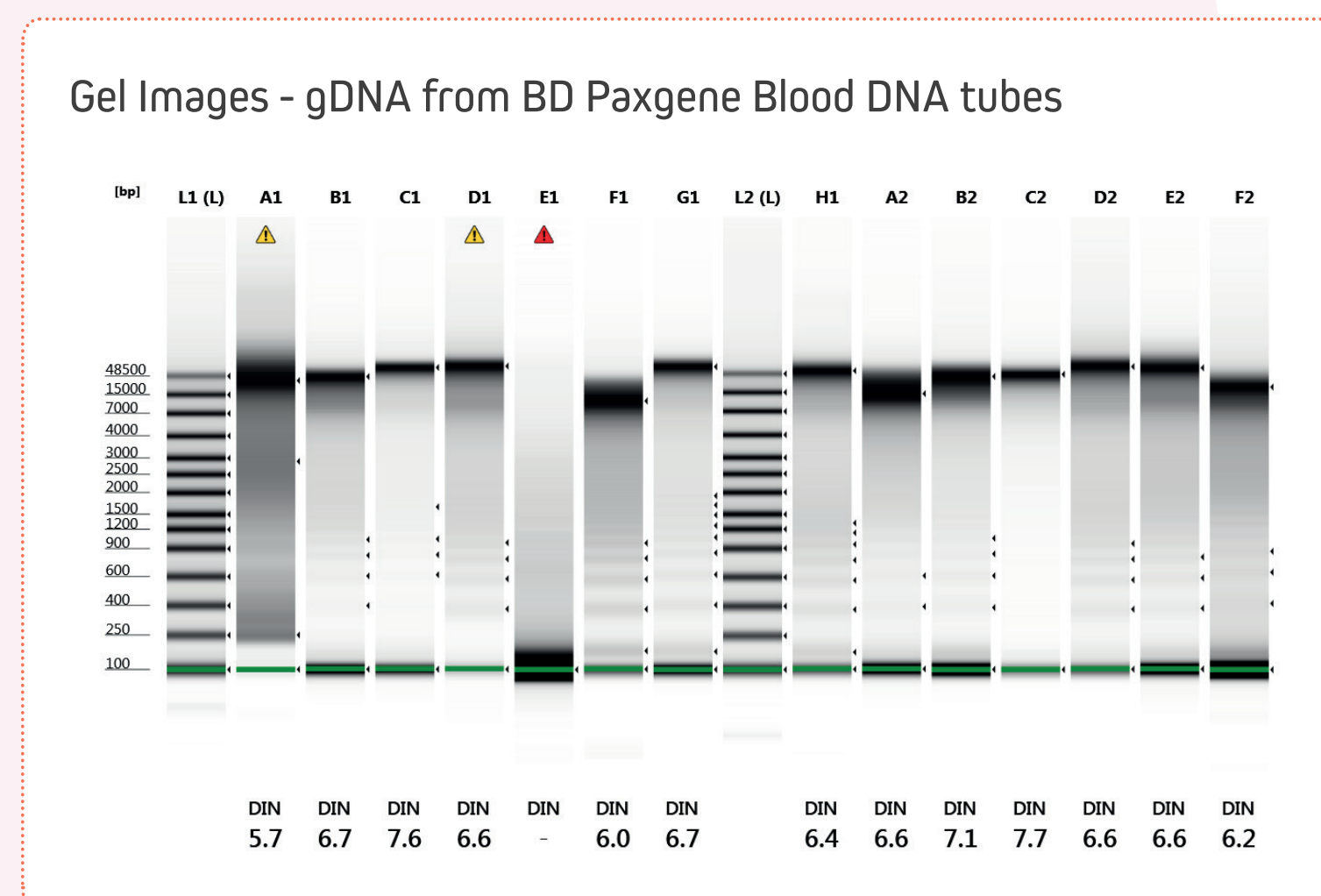


Fig.1 DNA extracted from blood collected in Paxgene DNA tubes analysed with the Agilent genomic DNA ScreenTape assay. Lanes L1 and L2 are DNA ladders. Note the DNA degradation pattern in most samples.

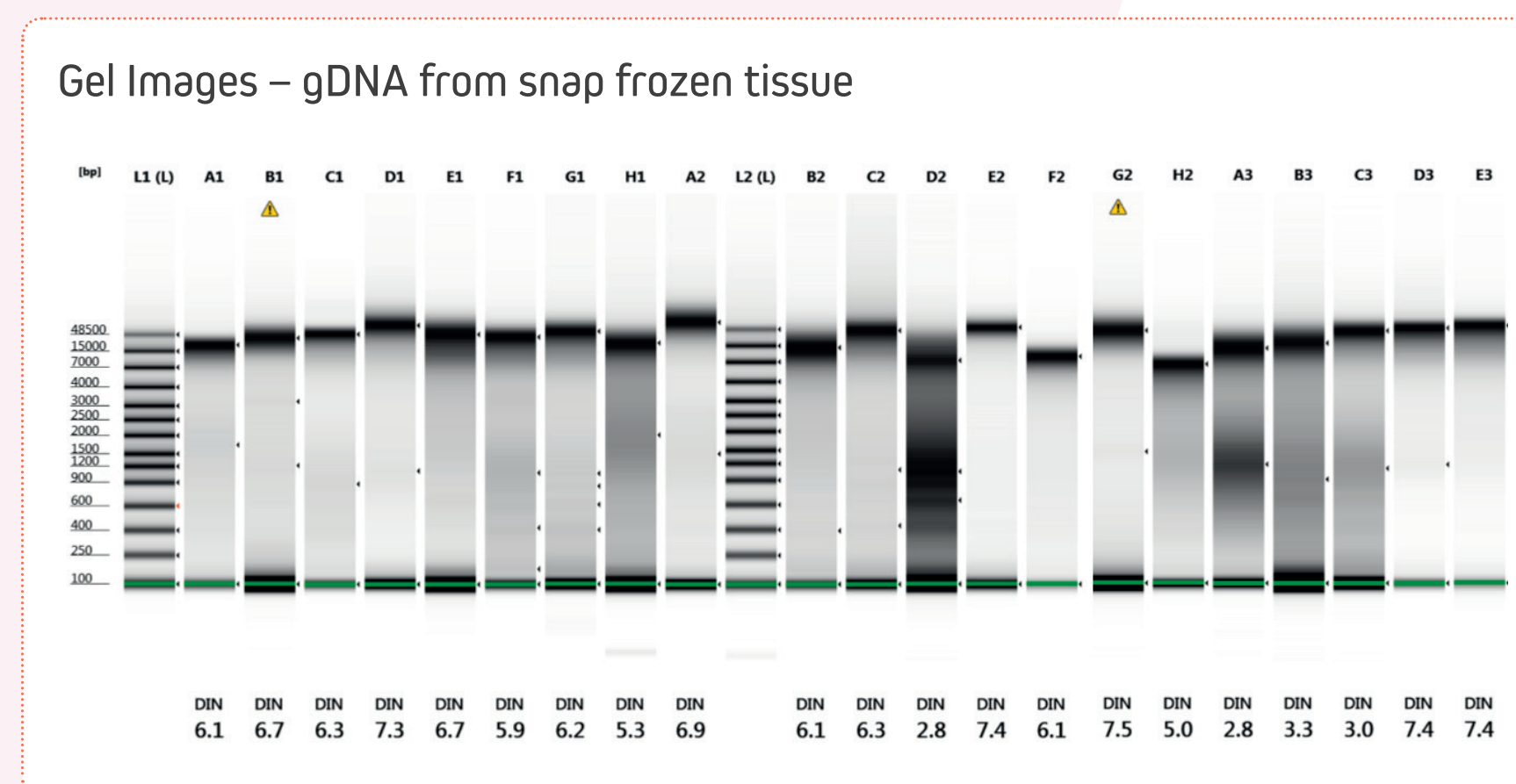


Fig.2 DNA extracted from fresh frozen tissue analysed with the Agilent genomic DNA ScreenTape assay. Lanes L1 and L2 are DNA ladders.

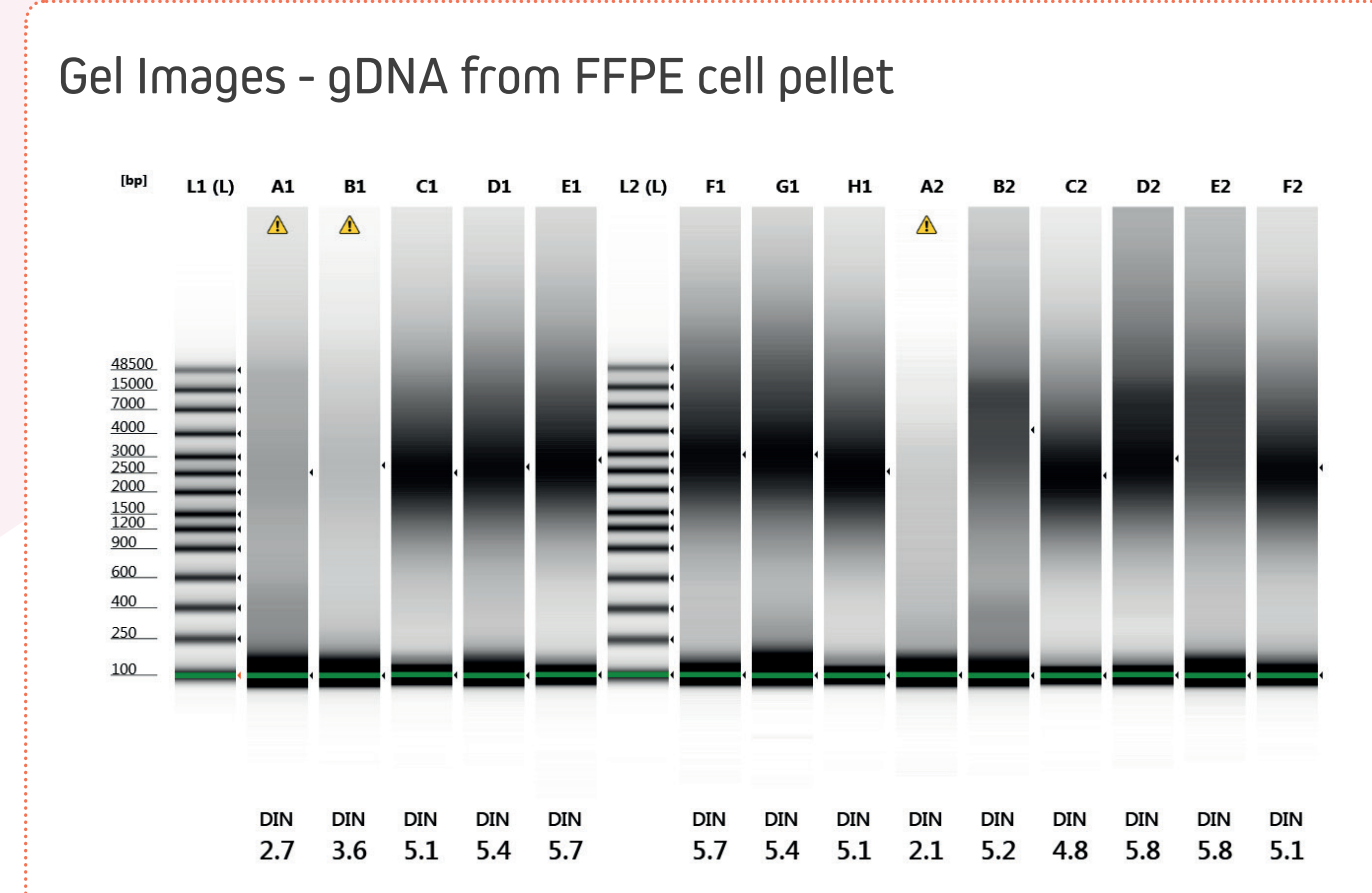


Fig.3 DNA extracted from fresh frozen tissue analysed with the Agilent genomic DNA ScreenTape assay. Lanes L1 and L2 are DNA ladders. Note the DNA smear in most samples and the lower DIN score compared to the DNA from blood.

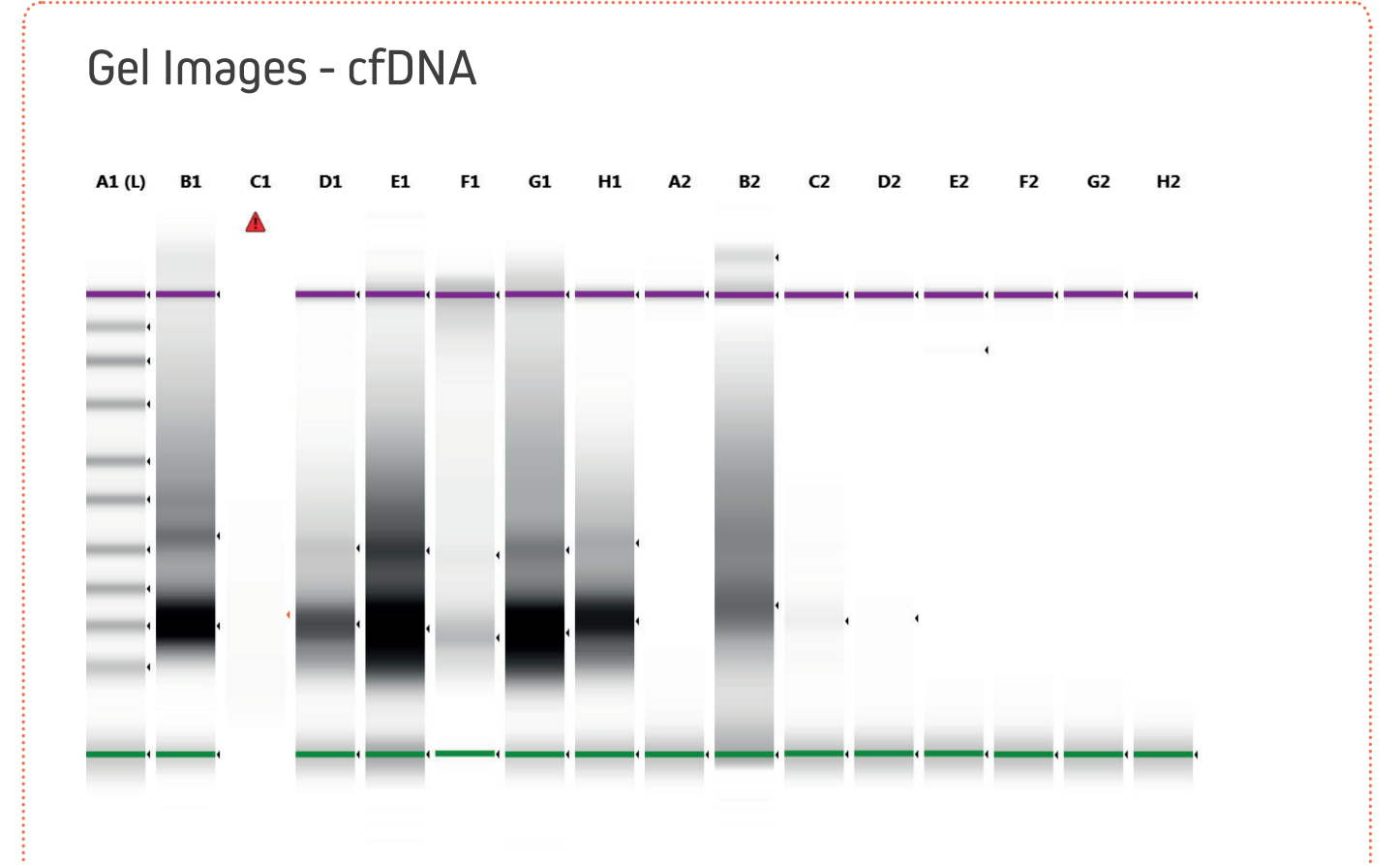


Fig.4 cfDNA extracted from plasma analysed with the Agilent HS DNA5000 ScreenTape assay. Lane L1 is DNA ladder. Note the absence of DIN score cannot be applied to cfDNA.

PT Results

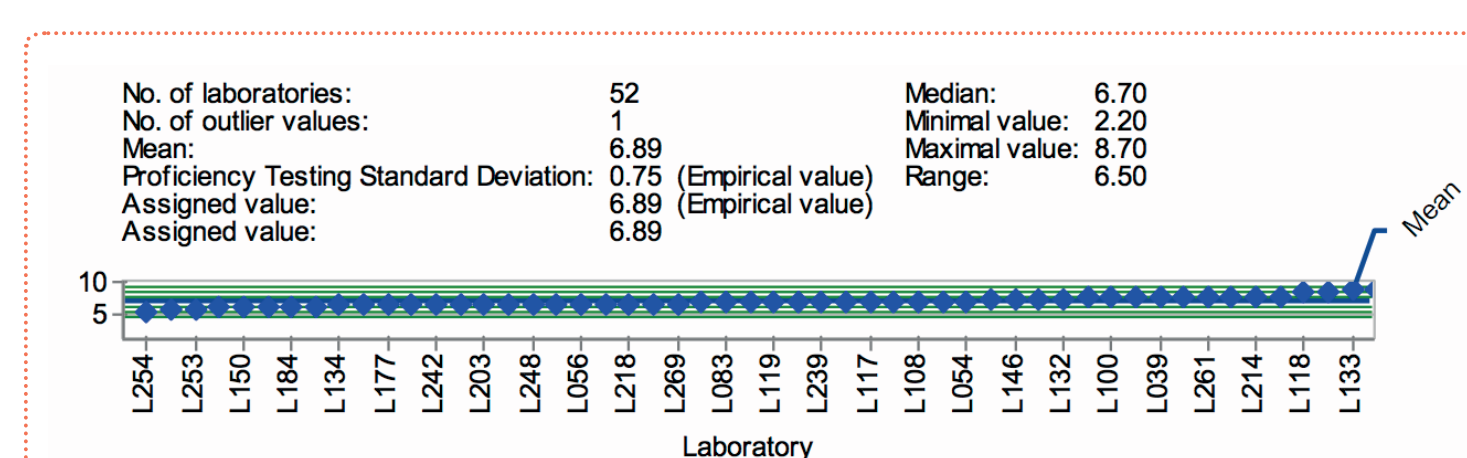


Fig.5 All Results - gDNA from BD Paxgene Blood DNA tubes

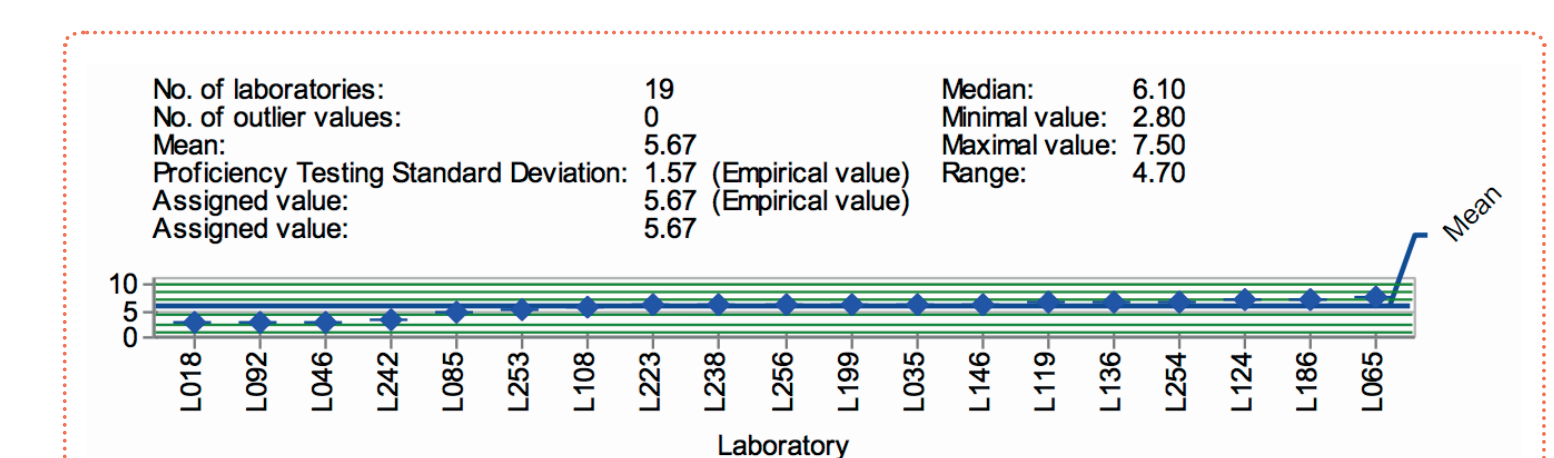


Fig.6 All Results - gDNA from snap frozen tissue

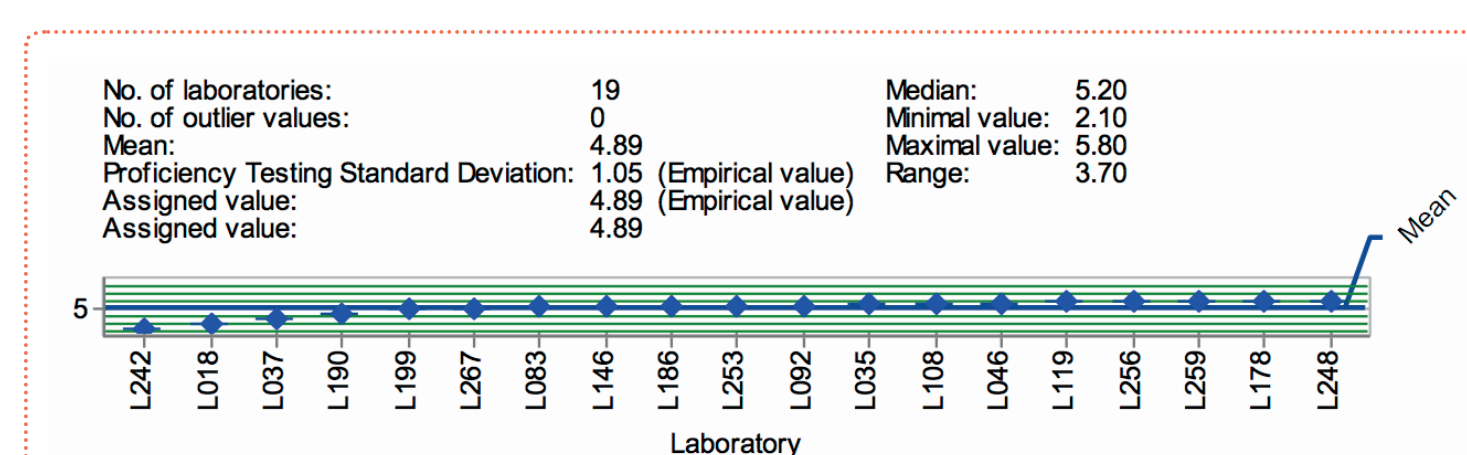


Fig.7 All Results - gDNA from FFPE cell pellets

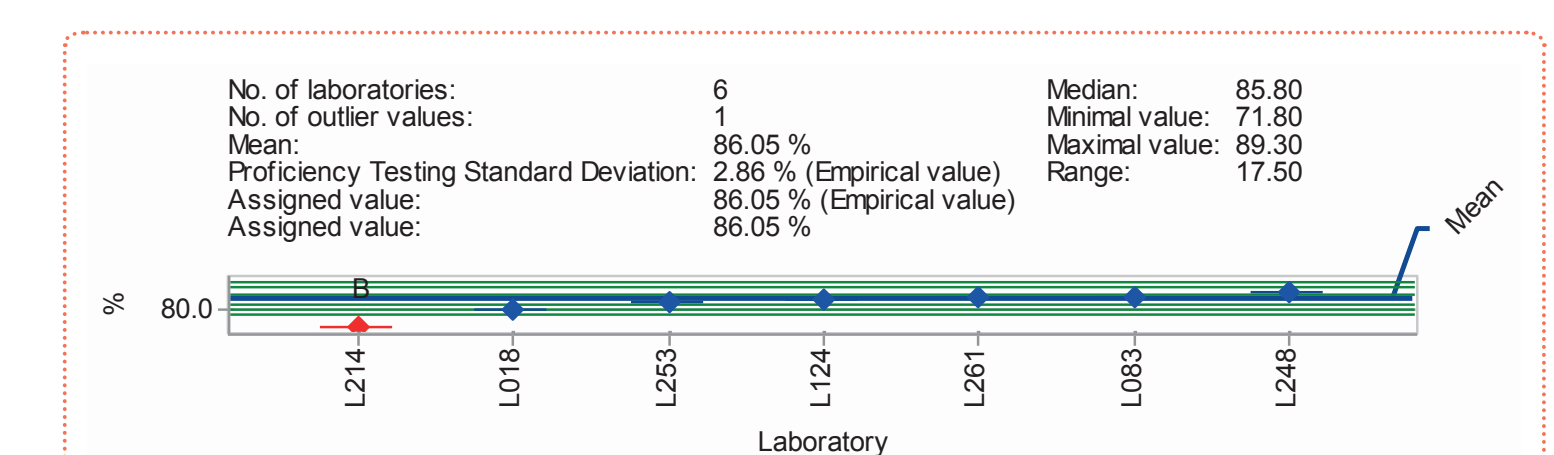


Fig.8 All Results - cfDNA