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Learnings from a Pilot External Quality Assessment (EQA) for cfDNA Extraction from Plasma

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Introduction

Efficient DNA extraction is critical to ensure sensitive and accurate detection of variants in cell-free DNA (cfDNA). EMQN has established a global pre-analytical EQA for extraction of cfDNA from plasma.

The objective is to evaluate the quantity and quality of eluted cfDNA and provide an external benchmark of participants' DNA extraction processes.

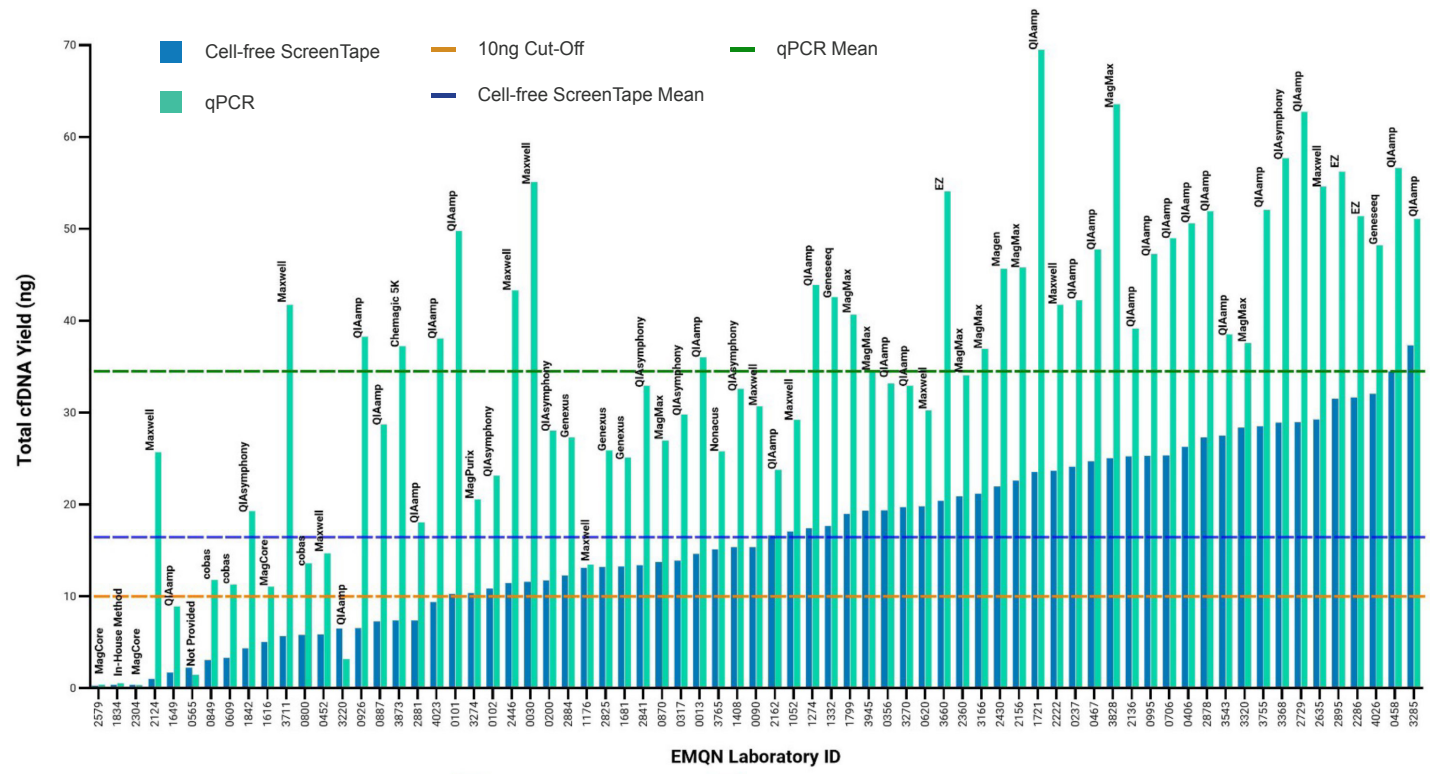
Methods

71 laboratories were provided with three 4mL artificial plasma samples containing a defined amount of cfDNA (60ng, 140ng, 360ng), manufactured by SensID GmbH,[1] shipped at room temperature.

Participants were instructed to extract the cfDNA using their routine methodology, return all eluted cfDNA and complete a form with details of the method used.

The volume of each returned sample was recorded and the cfDNA was analysed using a custom qPCR assay (88bp amplicon), the Agilent TapeStation 4150 Cell-free DNA ScreenTape assay and the Qubit™ High-Sensitivity (HS) assay. The ScreenTape assay measured cfDNA fragments between 50-700bp.

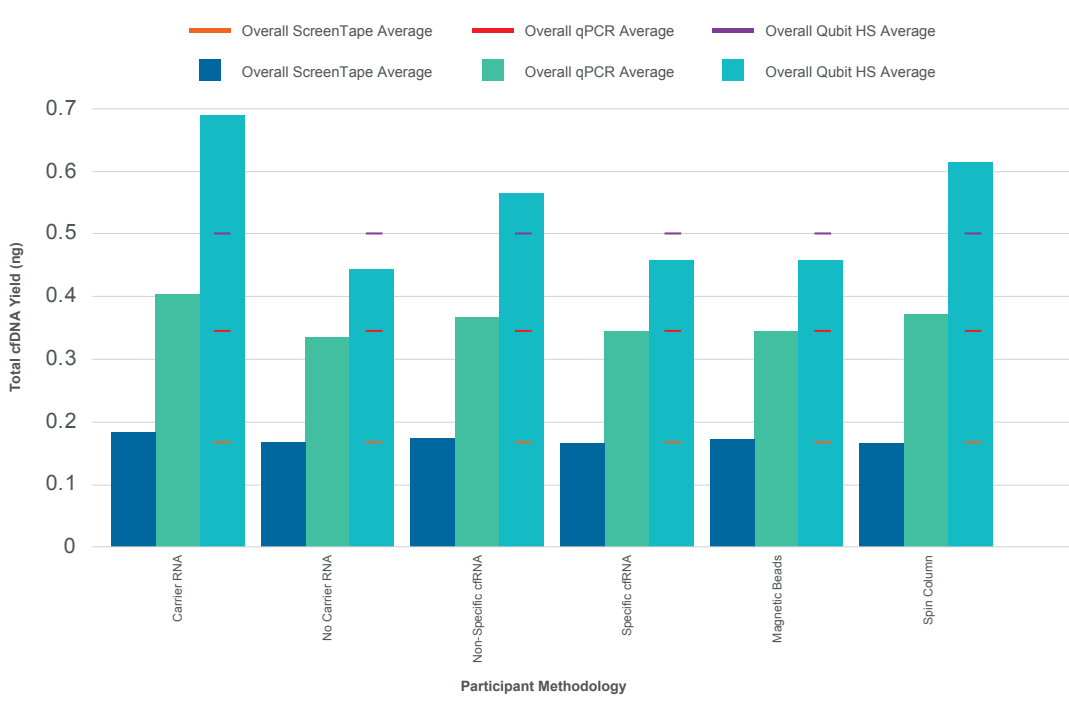
Total Extraction Yield from 60ng Sample (qPCR/ScreenTape)



Results

The % DNA retrieved was calculated by dividing the total yield by the pre-extraction yield measurement provided by SensID GmbH (59.2ng). The % DNA retrieved was 28% for the cfDNA ScreenTape (range 1-63%) and 58% for the qPCR assay (range 1-117%). The % DNA retrieved varied considerably across participants. There was variability across participants which followed the same protocol e.g. QIAamp Circulating NA kit (11-58%, n=17), Maxwell RSC ccfDNA (2-49%, n=10), MagMax Cell Free Total NA kit (23-48%, n=8). Participants were assessed based on the cfDNA ScreenTape and qPCR total yield measurements; <10ng for both assays was assigned as a 'fail' based on downstream Next Generation Sequencing (NGS) testing requirements.[2,3,4] The MagCore kit failed to achieve the minimum 10ng total yield in seven out of nine samples across all three laboratories using the method. Out of 213 samples, one was deducted for poor quality of cfDNA as the fragment size was outside mean ±2 standard deviation.

Total Extraction Yield from 60ng Sample Based on Participant Methodology



The qPCR results were higher than the Cell-free ScreenTape as the measurements will include fragments ≥88bp. An assay have been developed to evaluate the presence of high molecular weight contamination in following EQA rounds.

The % DNA retrieved from this sample using the Qubit HS kit averaged 85% of the expected quantity (range 0-308%), compared to the cfDNA ScreenTape (28%, range 1-63%) and the qPCR assay (58%, range 1-117%). Some extraction methodologies may inflate this reading due to the use of carrier RNA. Laboratories and DNA extraction kit providers frequently state quality control metrics based on the Qubit HS assay values. This value did not seem representative of the cfDNA quantity in this EQA.

Total Yield from Cases 1-3 (Cell-Free ScreenTape)

Category	Case 1 (60ng)	Case 2 (140ng)	Case 3 (350ng)
Mean	16.63	42.26	123.70
Median	15.40	40.38	119.07
Min	0.25	0.25	0.92
Max	182.60	114.45	300.96

Conclusions

The results of this pilot EQA demonstrate the majority of cfDNA extractions fit for purpose, but there was high variability in the % DNA retrieved by laboratories using the same kit suggesting there is potential to improve the efficiency of extraction for some laboratories. The peak cfDNA fragment sizes were consistent across all participants, and all samples. Only 5.6% (11/213) of samples (returned from 7 laboratories) recorded a total yield below the 10ng benchmark, we have contacted MagCore which failed to meet this metric consistently. From previous survey results, we are aware that the Qubit HS assay is commonly used for cfDNA quantification by laboratories. However, the data from this EQA suggests this technique can inflate true cfDNA yields and it may not be suitable with some methodologies. Participation in this cfDNA extraction EQA enables benchmarking against other laboratories to identify potential for improvement in cfDNA extraction procedures.

1. SensID GmbH, D-18057 Rostock, Germany. www.sens-id.com. 2. Rickles-Young, M. et al. Assay Validation of Cell-Free DNA Shallow Whole-Genome Sequencing to Determine Tumor Fraction in Advanced Cancers. J Mol Diagn. 2024;26(5):413-422. 3. NGS Basics for Cancer Research: Thermo Fisher Scientific Available at: https://www.thermofisher.com/uk/en/home/life-science/sequencing/sequencing-learning-center/next-generation-sequencing-information/cancer-research/basics.html (Accessed: 12 August 2025). 4. Lockwood, C.M. et al. Recommendations for cell free DNA assay validations. J Mol Diagn. 2023;25(12):876-897.