Digital PCR-based EGFR T790M Mutation Detection From Human Blood Samples Collected in PAXgene® Blood ccfDNA Tubes

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Introduction

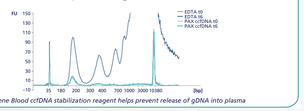
Circulating cell-free DNA (ccfDNA) is used as an analyte in liquid biopsy-based cancer research with promising applications in the fields of cancer screening, therapeutic decision making and monitoring and minimal residual disease control. However, despite the multitude of future applications, challenges regarding the sensitivity of analytical tests remain, thereby limiting the routine use of ccfDNA within the above mentioned fields.

In this study, we investigated the compatibility of the PAXgene Blood ccfDNA Tube* (PreAnalytiX) and the QIAcuity® Digital PCR System as an optimal "sample to insight" workflow with regard to the detection of EGFR T790M mutations spiked into apparently healthy human donor blood specimens.

PAXgene Blood ccfDNA Tube

The PAXgene Blood ccfDNA Tubes (RUO)* enable:

- Stabilization of ccfDNA **for 10 days up to 25°C, 7 days up to 30°C, and 3 days up to 37°C**
- Single tube collection, stabilization, transport and storage
- Standardized preanalytical sample processing



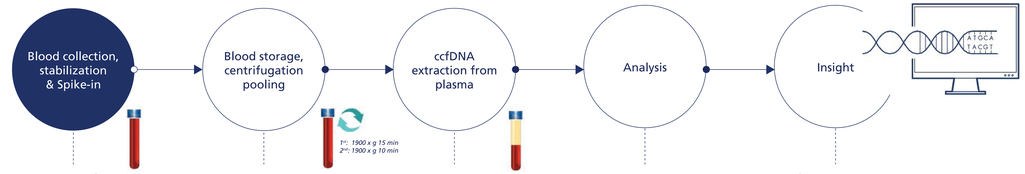
PAXgene Blood ccfDNA stabilization reagent helps prevent release of gDNA into plasma

Data from www.preanalytix.com

Methods

Blood from 20 apparently healthy consented donors was collected into PAXgene Blood ccfDNA Tubes. Within 2 h of blood draw, blood samples were either spiked with an EGFR-Multiplex 5% AF cfDNA standard (SensID) containing an EGFR T790M mutation or left unspiked as a control. Tubes were stored on the bench for 3 d at ambient temperature to simulate transport over a weekend and plasma was generated by double centrifugation. Plasma pools were generated from spiked and unspiked samples and ccfDNA was isolated with the QIAamp® Circulating Nucleic Acid Kit (QIAGEN). CcfDNA quantity was measured by Qubit® dsDNA HS Assay (Thermo Fisher Scientific) and fragment size distribution was determined by TapeStation® Cell-free DNA ScreenTape® (Agilent Technologies). EGFR T790M mutation detection was performed using the dPCR Mutation Assay EGFR 6240 Human (QIAGEN) with a 26K 24-well Nanoplate (QIAGEN) on the QIAcuity Digital PCR System (QIAGEN). Data analysis was conducted using the QIAcuity Software Suite (QIAGEN).

(Pre)-Analytical workflow for ccfDNA-based EGFR T790M mutation detection



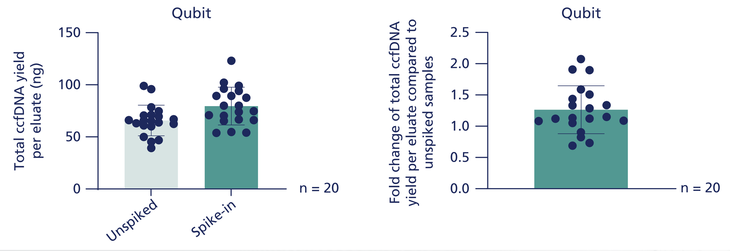
- PAXgene Blood ccfDNA Tube*
- Spike-in: EGFR-Multiplex 5% AF cfDNA standard
- Whole blood storage for 3 days at room temperature
- 1st centrifugation: 15 min, 1900 x g
- 2nd centrifugation: 10 min, 1900 x g
- QIAGEN QIAamp Circulating Nucleic Acid Kit (5ml plasma protocol)
- Qubit dsDNA HS
- TapeStation Cell-free DNA ScreenTape
- QIAcuity dPCR Mutation Assay EGFR 6240 Human
- ccfDNA based mutation detection

Results

ccfDNA quantification and fragment size distribution of unspiked and EGFR T790M mutation spiked human plasma after whole blood storage in PAXgene Blood ccfDNA tubes

- Qubit dsDNA HS assay revealed slightly increased total ccfDNA yields in EGFR T790M mutation spiked blood samples.
- TapeStation analysis (cell-free DNA ScreenTape) confirmed slightly increased total ccfDNA yield in EGFR T790M mutation spiked samples and showed distinct mono- and dinucleosomal ccfDNA peaks with minimal high molecular weight DNA content, highlighting the ccfDNA profile stabilizing performance of the PAXgene Blood ccfDNA Tube during extended blood collection tube storage.

(A) ccfDNA quantification of unspiked and EGFR T790M mutation spiked human blood samples



Total ccfDNA yield per eluate of unspiked samples and samples spiked with an EGFR-Multiplex 5% AF cfDNA standard (40ng) containing an EGFR T790M mutation. Whole blood samples were incubated for 3 d at room temperature to simulate transport over a weekend prior to plasma pool generation. After 3 d, ccfDNA was extracted and purified using QIAamp Circulating Nucleic Acid kit and isolated ccfDNA was quantified by Qubit dsDNA HS assay.

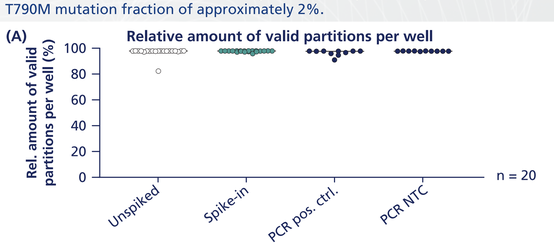
(B) Fragment size distribution profiles of unspiked and EGFR T790M mutation spiked human blood samples

Fragment size profiles of unspiked samples and samples spiked with an EGFR-Multiplex 5% AF cfDNA standard (40ng) containing an EGFR T790M mutation. Isolated ccfDNA was fragment size profiled using Agilent TapeStation Cell-free DNA ScreenTape.

Digital PCR-based EGFR T790M mutation detection from plasma extracted ccfDNA

- EGFR T790M mutation was successfully detected in spiked samples with an EGFR T790M mutation fraction of approximately 2%.

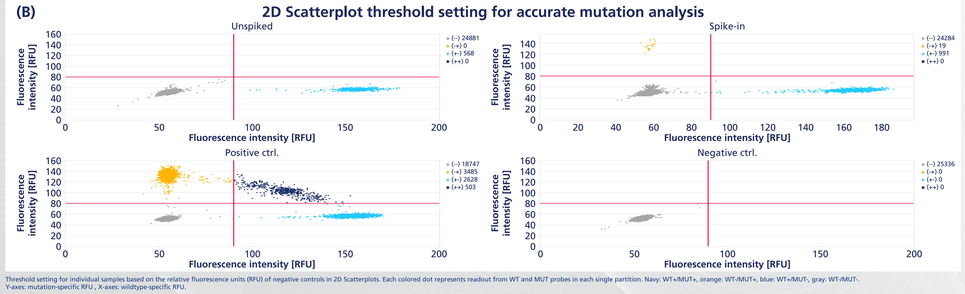
(A) Relative amount of valid partitions per well



Rel. amount of valid partitions per well for the QIAcuity 26k 24-well Nanoplate.

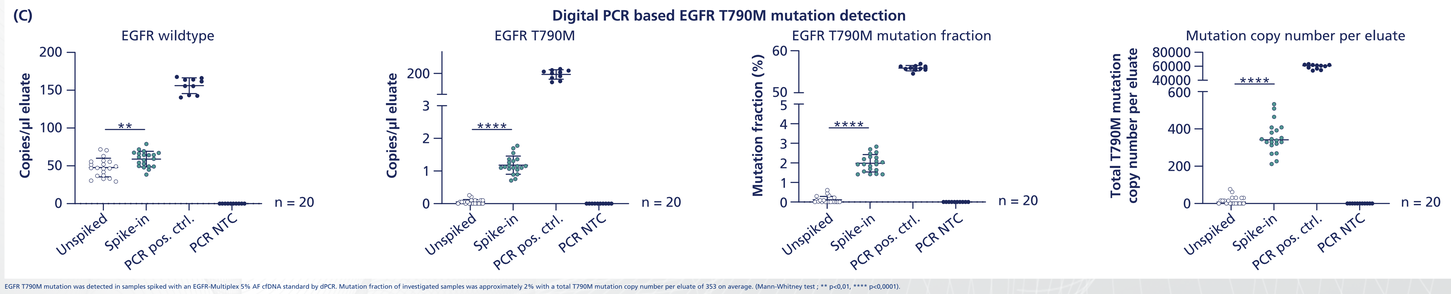
Digital PCR-based EGFR T790M mutation detection from plasma extracted ccfDNA (cont'd)

(B) 2D Scatterplot threshold setting for accurate mutation analysis



Threshold setting for individual samples based on the relative fluorescence units (RFU) of negative controls in 2D Scatterplots. Each colored dot represents readout from WT and MUT probes in each single partition. Navy: WT+/MUT+, orange: WT-/MUT+, blue: WT+/MUT-, gray: WT-/MUT-.
Y-axes: mutation-specific RFU , X-axes: wildtype-specific RFU.

(C) Digital PCR based EGFR T790M mutation detection



EGFR T790M mutation was detected in samples spiked with an EGFR-Multiplex 5% AF cfDNA standard by dPCR. Mutation fraction of investigated samples was approximately 2% with a total T790M mutation copy number per eluate of 353 on average. (Mann-Whitney test ; ** p<0,01, **** p<0,0001).

Conclusion

PAXgene Blood ccfDNA Tubes enabled high-quality ccfDNA isolation by stabilizing nucleated cells during the simulated transport condition. Spiked EGFR T790M mutations were successfully detected by dPCR following sample storage. This proof of principle study shows the combined usability of both systems for mutation detection in human blood specimens, highlighting the potential of both technologies for a broader application in the liquid biopsy field to detect oncogenic driver as well as tumor suppressor gene mutations in a multitude of tumor types.

Disclaimer

*The PAXgene Blood ccfDNA Tube is For Research Use Only in the US. Not for use in diagnostic procedures.
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