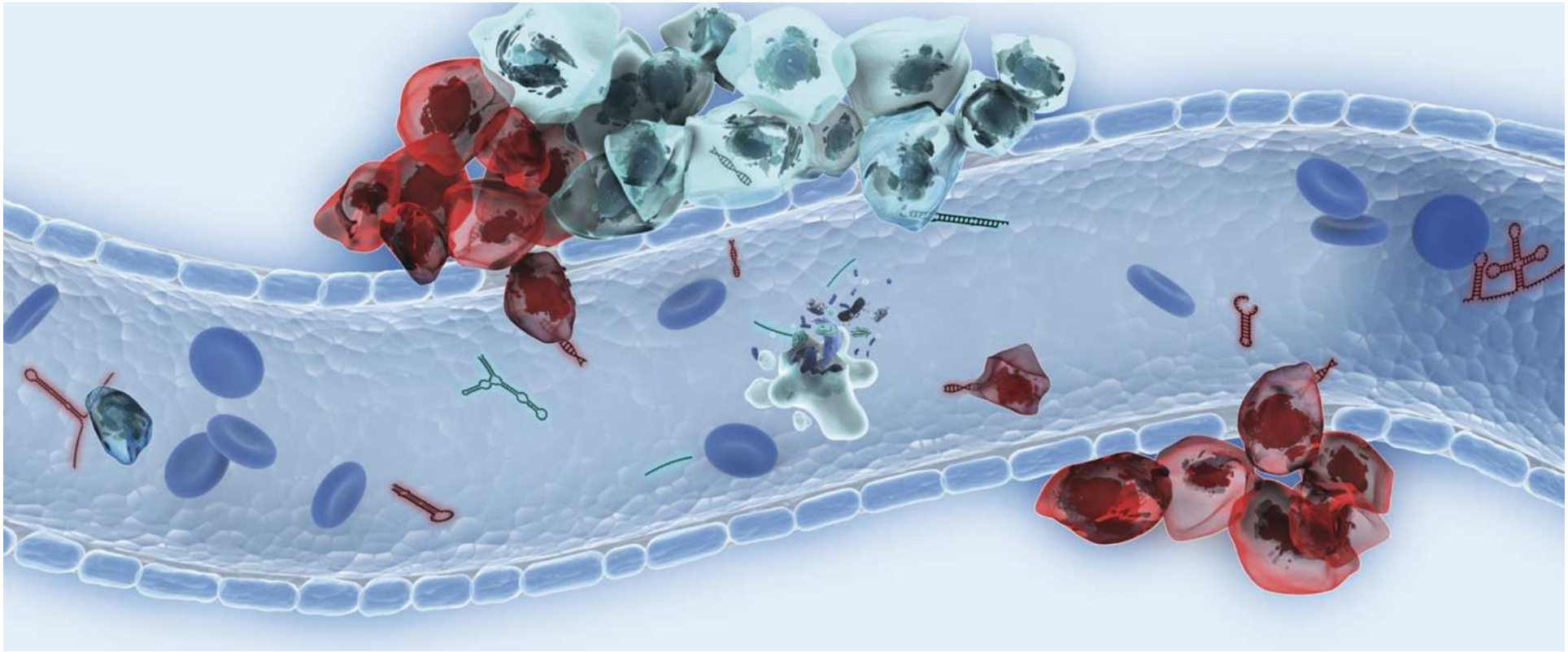


Circulating Cell-Free DNA Pre-analytics: The Importance of Standardized Workflows for Liquid Biopsy Applications

Dr. Andrea Ullius, Scientist, PAXgene Product Development, PreAnalytiX (QIAGEN)

Deficiencies in Routine Healthcare Call for Workflow Standardization

- Diagnostic errors cause about 10% of all patient deaths and about 17% of adverse events

  Institute of Medicine (IOM) Report Sept. 2015

- The pre-analytical phase accounts for 46% to 68% of such errors observed during the total testing process

  Medical Laboratory Observer, May 2014

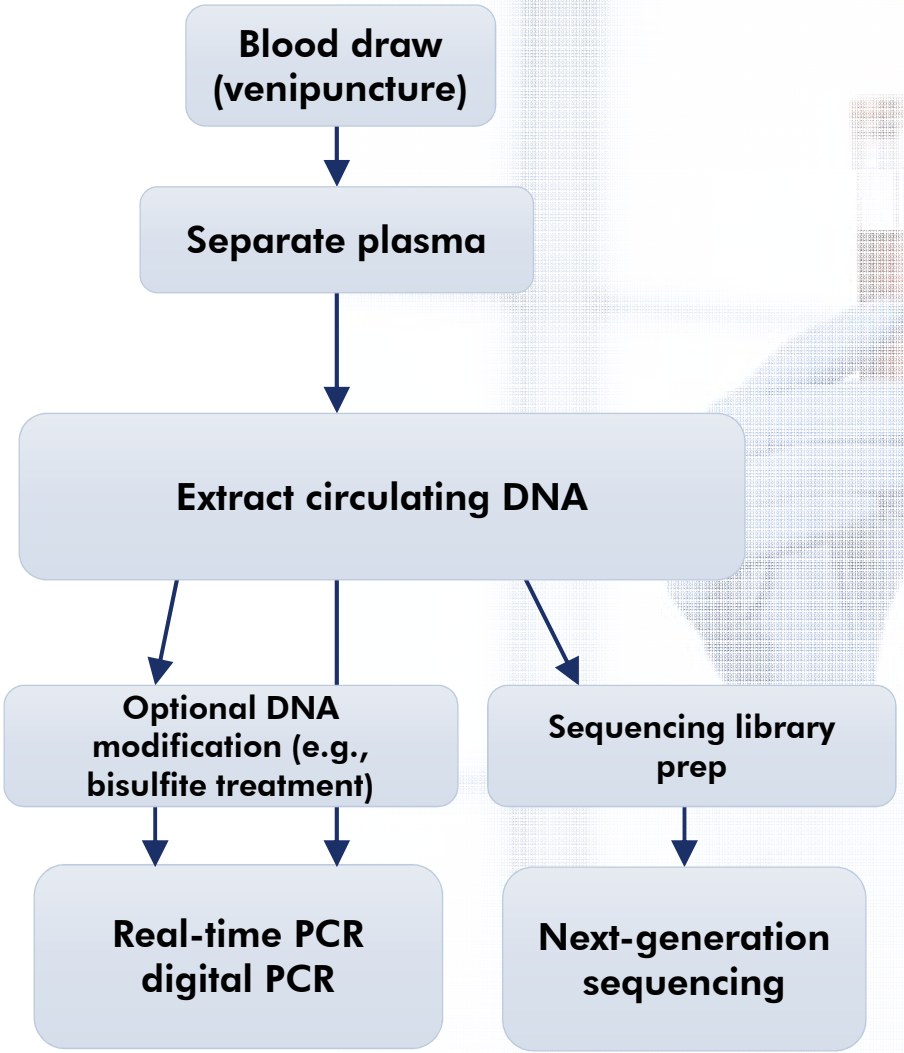
SPIDIA & SPIDIA4P: Standardization and Improvement of Pre-analytical Procedures for *in vitro* Diagnostics *EU FP7-HEALTH (GA. no. 222916) & EU HORIZON 2020 (GA. no. 733112)*

SPIDIA

SPIDIA4P

- QIAGEN coordinating both initiatives (16 & 19 Partners)
- 22 CEN Technical Specifications and ISO Standards planned or developed (highly consensus-driven international and European processes)
- E.g., Specifications for pre-examination processes for venous whole blood in Molecular in vitro diagnostic examinations
  - Part 3: Isolated circulating cell-free DNA from plasma CEN/TS 16835-3: 2015

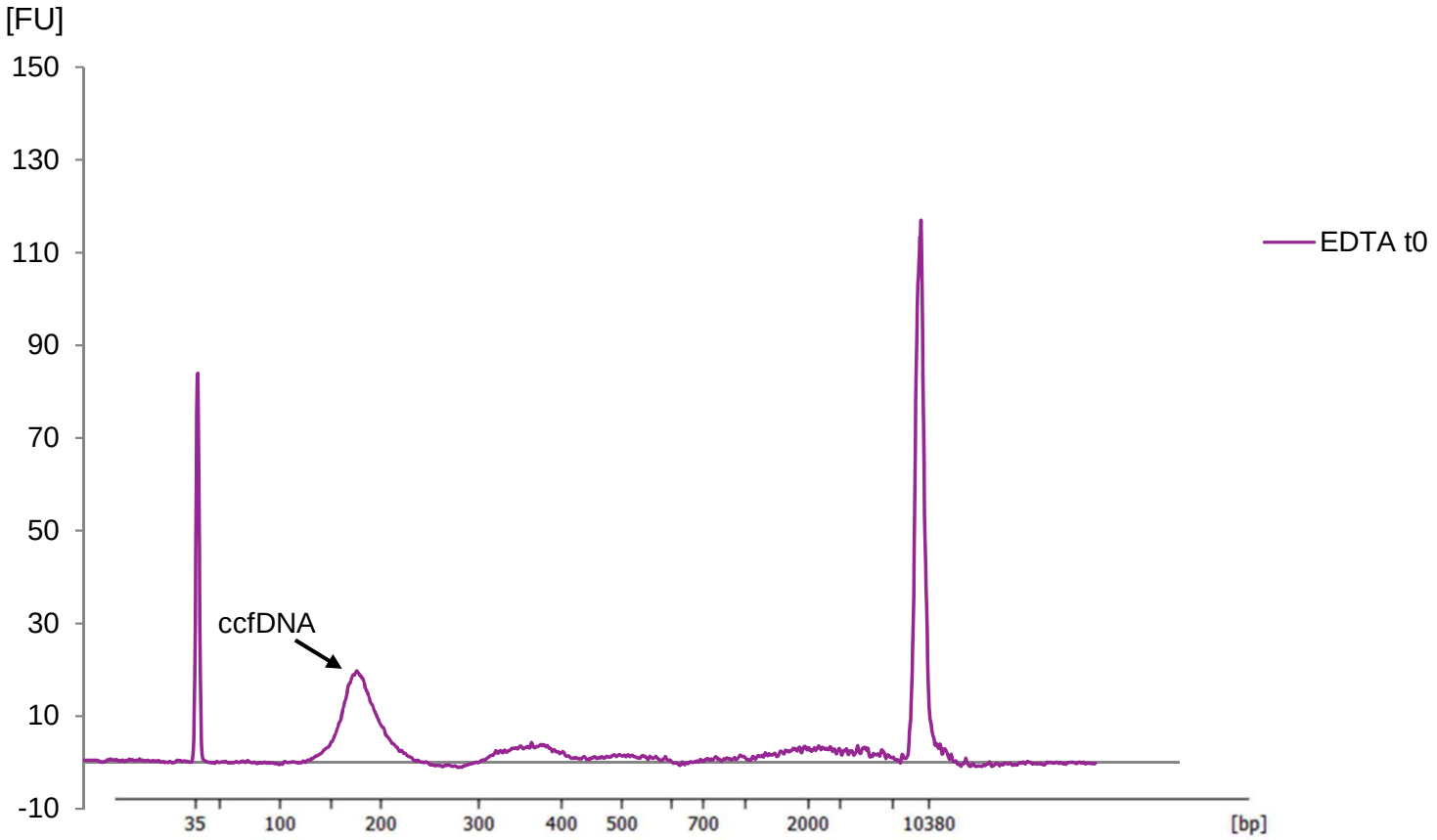
Critical Points Along the Workflow



- Avoid release of cellular nucleic acids (gDNA)
- Enable highly efficient large-volume nucleic acid extraction
- Avoid fragment size bias to improve sensitivity
- Work with low input amount demands sensitive pre-amplification and library prep

Problem: Need for Stabilization of ccfDNA

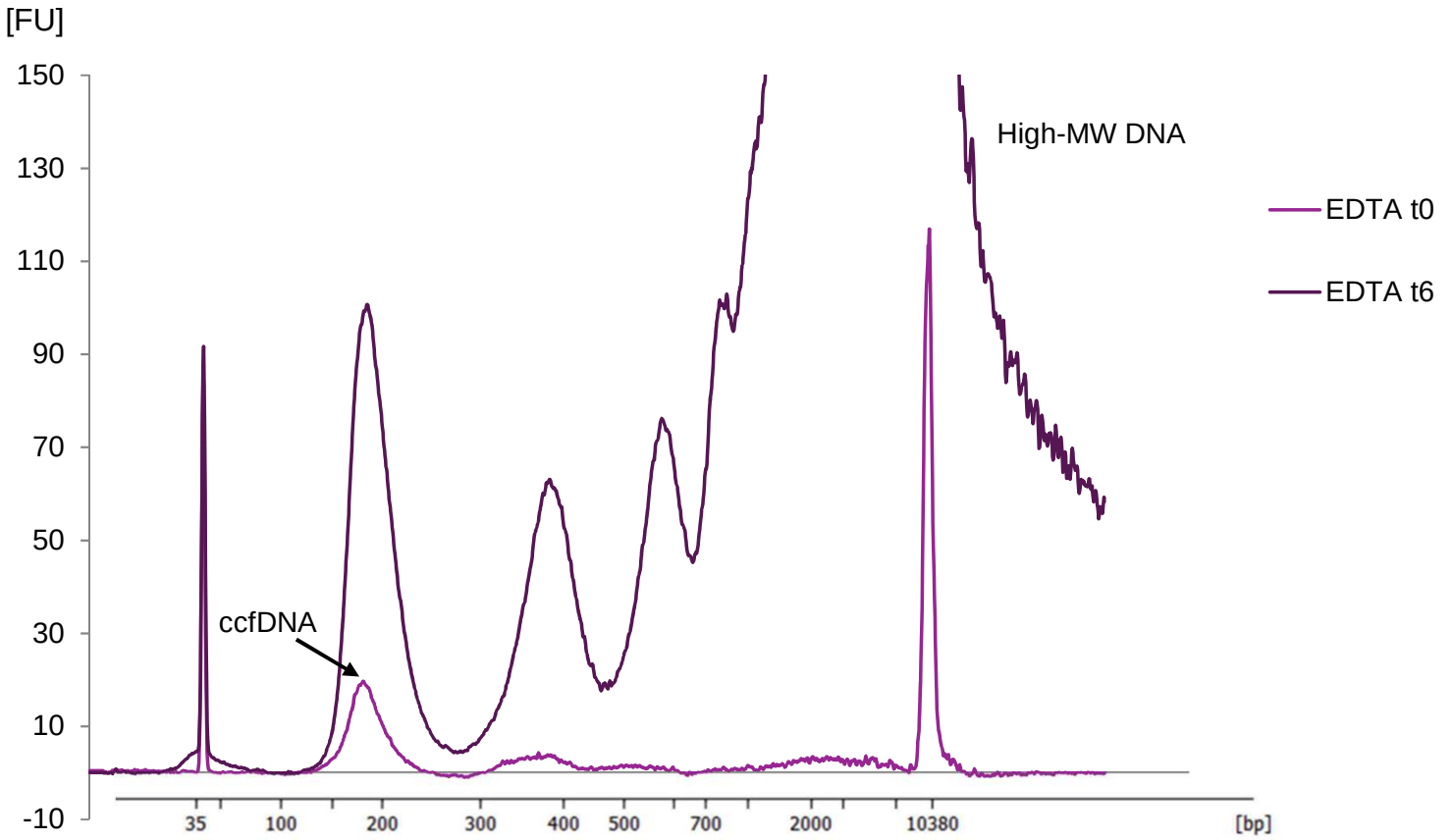
- ccfDNA fragment size profile from healthy donor



ccfDNA was extracted from EDTA plasma of 1 subject directly after blood draw (t0). 1 µl eluate was analyzed using the Agilent® High Sensitivity DNA Kit.

Problem: Need for Stabilization of ccfDNA

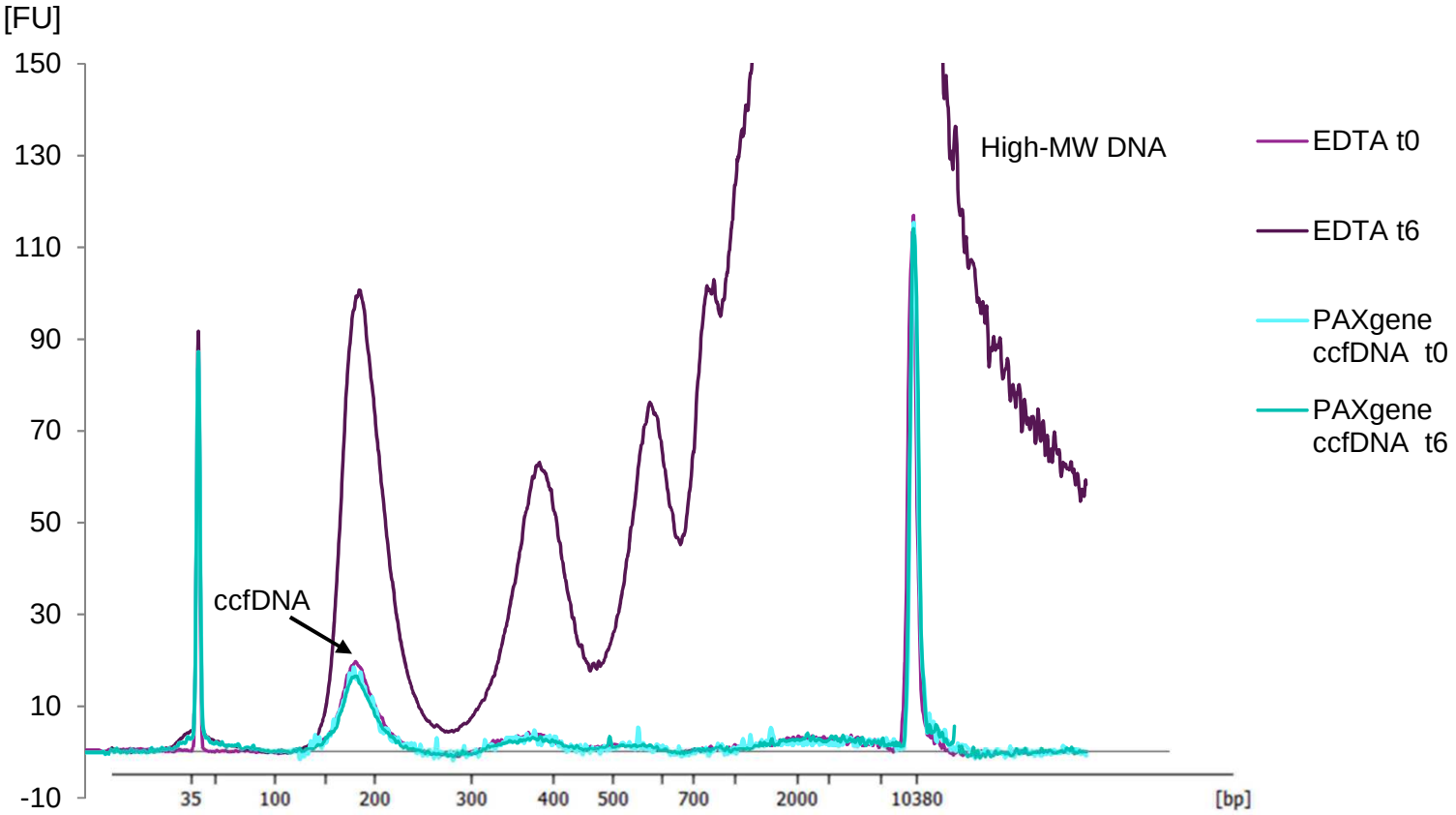
- Apoptosis of white blood cells leads to dilution of naturally occuring ccfDNA



ccfDNA was extracted from EDTA plasma of 1 subject directly after blood draw (t0) and after 6 days at room temperature (t6). 1 µl eluate was analyzed using the Agilent® High Sensitivity DNA Kit.

Solution: Prevent Blood Cell Lysis and Release of Genomic DNA

- PAXgene® Blood ccfDNA Tubes (RUO)* help prevent release of gDNA into plasma



ccfDNA was extracted from EDTA and PAXgene plasma of 1 subject directly after blood draw (t0) and after 6 days at room temperature (t6). 1 µl eluate was analyzed using the Agilent® High Sensitivity DNA Kit.

* For Research Use Only. Not for use in diagnostic procedures.

PAXgene Blood ccfDNA Tube (RUO)* Features

Unique stabilization of extracellular levels of ccfDNA

- Effective stabilization at RT minimizes background gDNA and maximizes ccfDNA yield from plasma
  - White blood cells – helps prevent release of gDNA
  - Red blood cells – helps minimize hemolysis
- Non-crosslinking NA preservation – no DNA modification

BD Vacutainer® plastic tube with BD Hemogard™ safety closure

- Helps minimize risk of tube breakage
- Enhanced safety for healthcare and lab personnel
- Helps minimize contamination between samples
- Provides consistent blood draw volume

Integrated pre-analytical workflow

- Seamless integration into manual or automated prep with QIAamp® and QIAsymphony® circulating DNA extraction technology

* For Research Use Only. Not for use in diagnostic procedures.

System Approach: QIAsymphony PAXgene Blood ccfDNA Kit (RUO)*

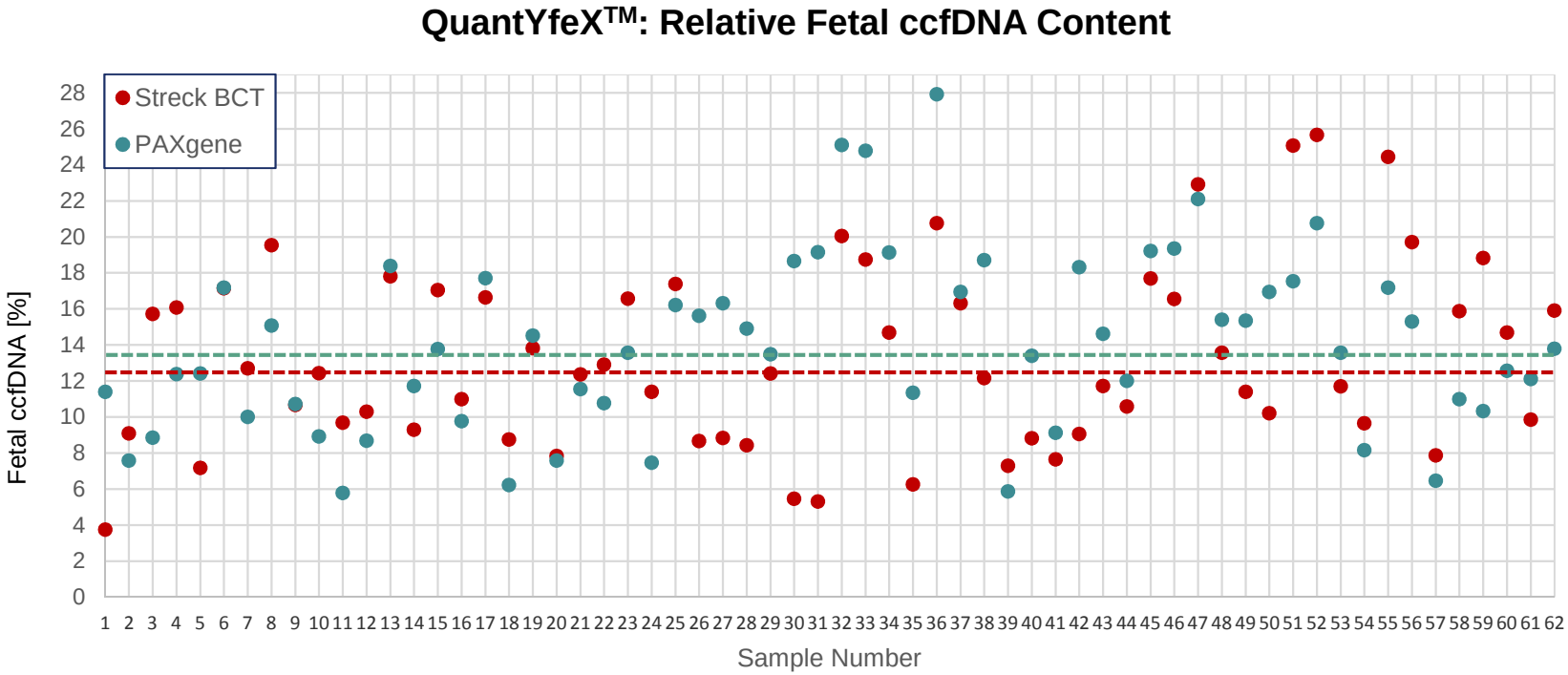
PAXgene Blood ccfDNA Tubes (RUO)	**2.4 or 4.8 ml Plasma**	**QIAsymphony PAXgene Blood ccfDNA (RUO)**

- Dedicated isolation technology for use with PAXgene Blood ccfDNA Tube (RUO)* — Complete system: ccfDNA stabilization + extraction
  - Binding chemistry optimized for use with PAXgene ccfDNA Tube reagent
  - Optimized input volumes to accomodate higher volume plasma
  - Optional custom protocols for primary tube handling

- Two protocol lines
  - Standard protocol similar to QIAsymphony DSP Circulating DNA Kit† protocols (≤500 bp)
  - Large fragment protocols enable co-isolation of large fragments (>500 bp) with flexible elution volume (60, 100, 150 µL)

* For Research Use Only. Not for use in diagnostic procedures.
† Intended for in vitro diagnostic use.

Equivalence between PAXgene Blood ccfDNA Tubes (RUO)* and current method

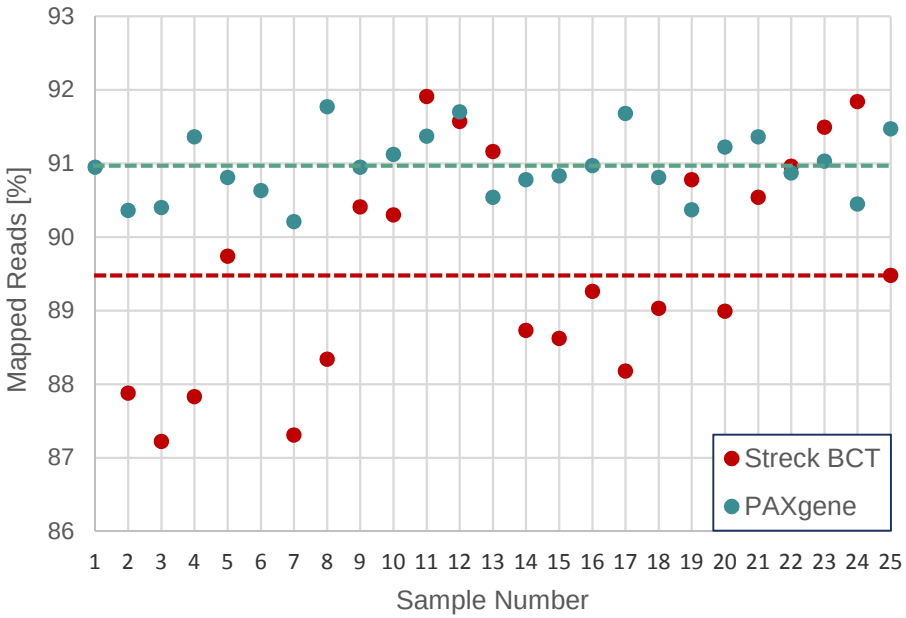


Relative fetal fraction of paired plasma samples collected in PAXgene Blood ccfDNA Tubes or Streck Cell-Free DNA BCT tubes. Relative amount of fetal ccfDNA in maternal background was quantified with methylation sensitive QuantYfeX QC Assay. Relative fetal ccfDNA fractions in maternal blood were not significantly different between the two different tubes (p-value: 0.20 paired T-Test, two-tailed distribution). PAXgene median: 13.7% (samples 1-30 with 2 centr. 12.0%; samples 31-62 with 1 centr. 15.4%), Streck BCT: median 12.4% (median indicated as dotted lines).
Data courtesy of LifeCodexx.

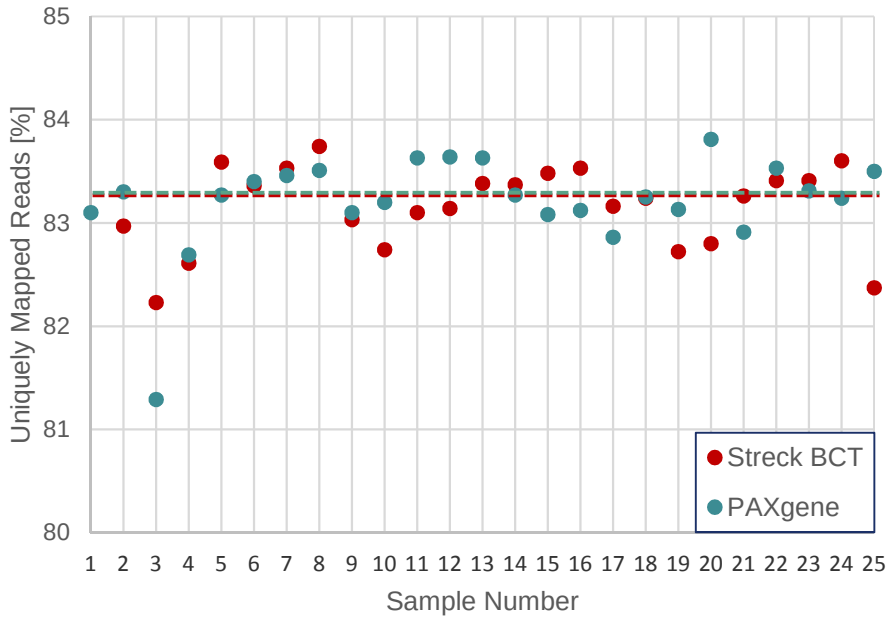
* For Research Use Only. Not for use in diagnostic procedures.

Equivalence between PAXgene Blood ccfDNA Tubes (RUO)* and current method

A)



B)

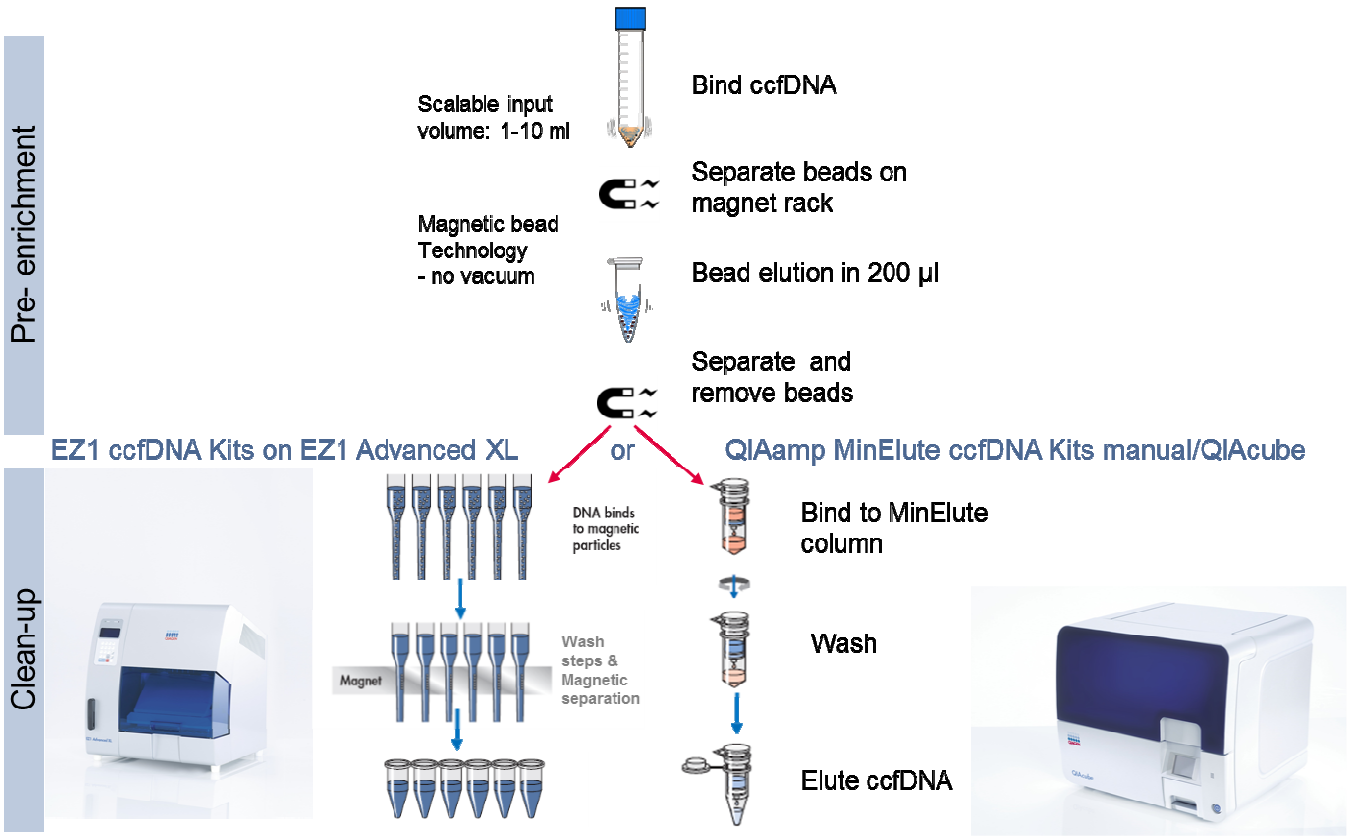


NGS read out quality for paired plasma samples collected in PAXgene Blood ccfDNA Tubes or Streck Cell-Free DNA BCT tubes. ccfDNA from Streck BCTs and PAXgene tubes sequenced on Illumina HiSeq™ with NEB library preparation. There were significantly more mapped reads with ccfDNA from PAXgene tubes (p-value 1.78E-04, two-tailed paired T-test). PAXgene median: 90.95% (samples 1-13 with 2 centr. 90.95%; samples 14-25 with 1 centr. 90.92%), Streck BCT: median 89.37% (A). There was no significant difference in unique mapped reads. PAXgene median: 83.27% (samples 1-13 with 2 centr. 83.30%; samples 14-25 with 1 centr. 83.25%), Streck BCT: median 83.25% (B);
median indicated as dotted lines. **Data courtesy of LifeCodexx.**

* For Research Use Only. Not for use in diagnostic procedures.

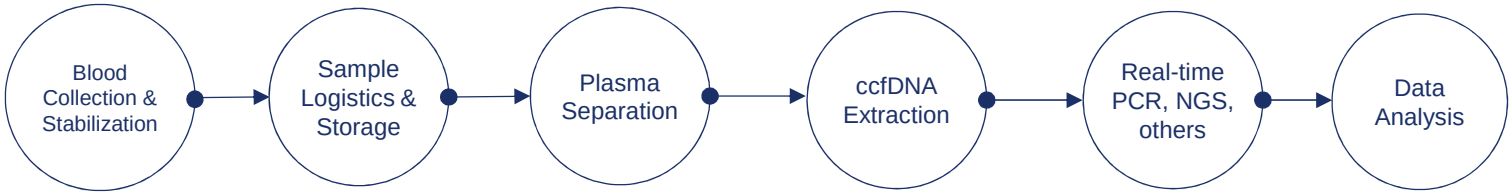
Manual ccfDNA Extraction: QIAamp MinElute® ccfDNA Kit

PAXgene Blood ccfDNA Tubes (RUO)*	1–10 ml Plasma Input	QIAamp MinElute ccfDNA Mini & Midi Kit (MBA)† NEW



* For Research Use Only. Not for use in diagnostic procedures.
† For molecular biology applications. Not intended for the diagnosis, prevention or treatment of a disease.

ccfDNA from Plasma — Sample-to-Insight Workflows



Manual / Semi-Automated	PAXgene Blood ccfDNA Tubes (RUO)*	1–10 ml Plasma Input	QIAamp MinElute ccfDNA Mini & Midi Kit (MBA)† NEW
Manual / Semi-Automated	PAXgene Blood ccfDNA Tubes (RUO)	1–10 ml Plasma	EZ1® ccfDNA Mini & Midi Kit (MBA) NEW
Automated	PAXgene Blood ccfDNA Tubes (RUO)	2.4 or 4.8 ml Plasma	QIAsymphony PAXgene Blood ccfDNA (RUO)
Automated	Any Stabilization Tube (incl. PAXgene Blood ccfDNA Tube)	2 or 4 ml Plasma	QIAsymphony DSP Circulating DNA Kit (IVD)‡

Integrated Workflow
No protocol modifications or pretreatments. Optimized results.

„Generic“
Flexibility to process different BCTs.

Complete preanalytical workflow solutions from QIAGEN & PreAnalytiX A QIAGEN / BD Company

* For Research Use Only. Not for use in diagnostic procedures.
† For molecular biology applications. Not intended for the diagnosis, prevention or treatment of a disease.
‡ Intended for in vitro diagnostic use.

Thank You!