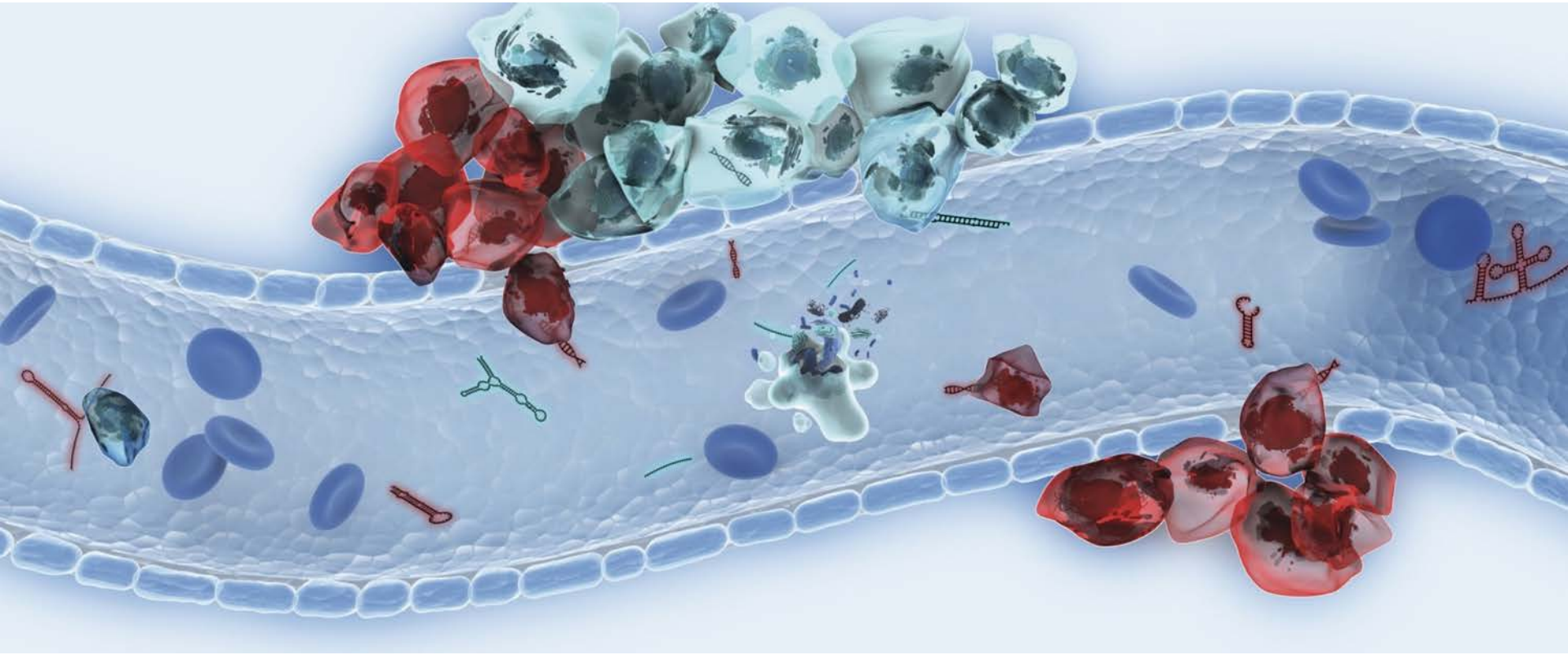

Circulating Free DNA Pre-Analytics: Importance of ccfDNA Stabilization and Extraction for Cancer Detection and Profiling

Dr. Martin Horlitz, Manager MDx Development, QIAGEN GmbH

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Agenda

- Importance of Pre-Analytics in Sample-to-Insight Workflows
- Sample Stabilization: PAXgene Blood ccfDNA Tube
- ccfDNA Purification: Manual and Automated Solutions
- Pre-Analytical ccfDNA Solution: PAXgene Blood ccfDNA System

Deficiencies in Routine Healthcare Call for Improvements

- It is estimated that more than 70% of clinical decisions are based on information derived from laboratory test results

  ADVANCE for Administrators of the Laboratory. July 2005

- 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

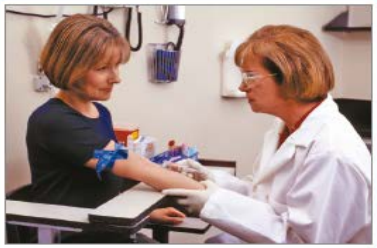
- This unnecessary expenditure caused by pre-analytical errors can be extrapolated to a typical U.S. hospital with approximately 650 beds to $1.2 million per year.

  Green SF. Clin Biochem. 2013

New Integrated System Covering the ccfDNA Pre-analytical Workflow

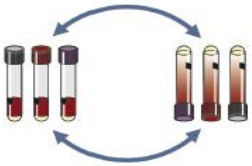
Doctor's Office Hospital → **Laboratory**

Blood Collection → **Storage Transport** → **Plasma Separation** → **ccfDNA isolation** → **Analytical Test** → **Data Analysis**

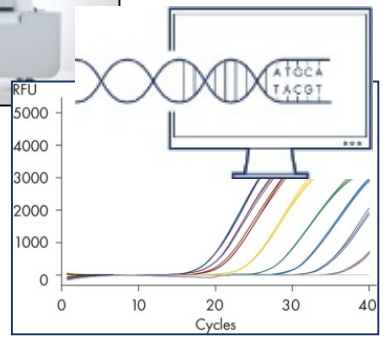


QIAsymphony

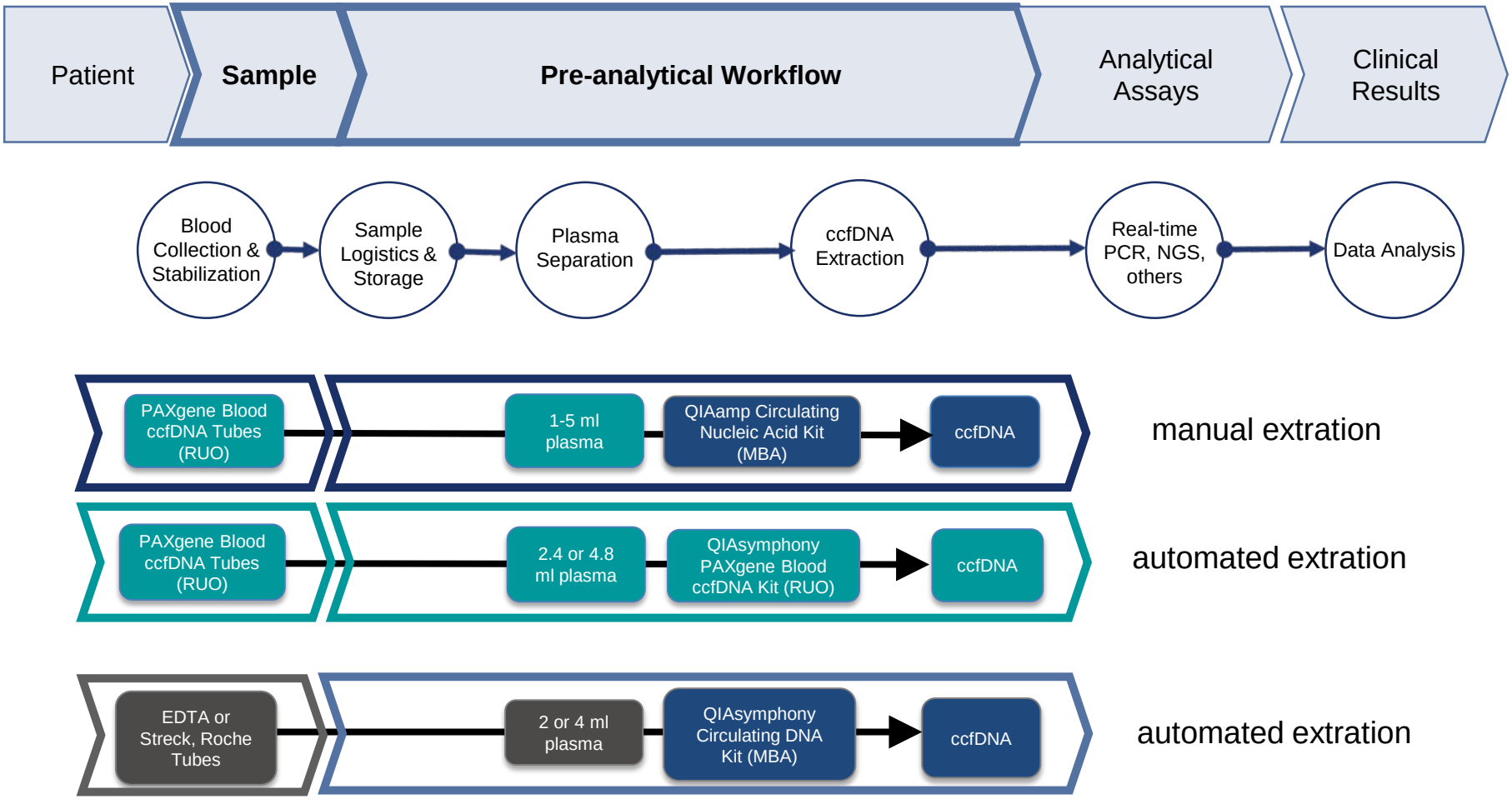
Tube



Kit



ccfDNA from Plasma — Sample-to-Insight Workflow



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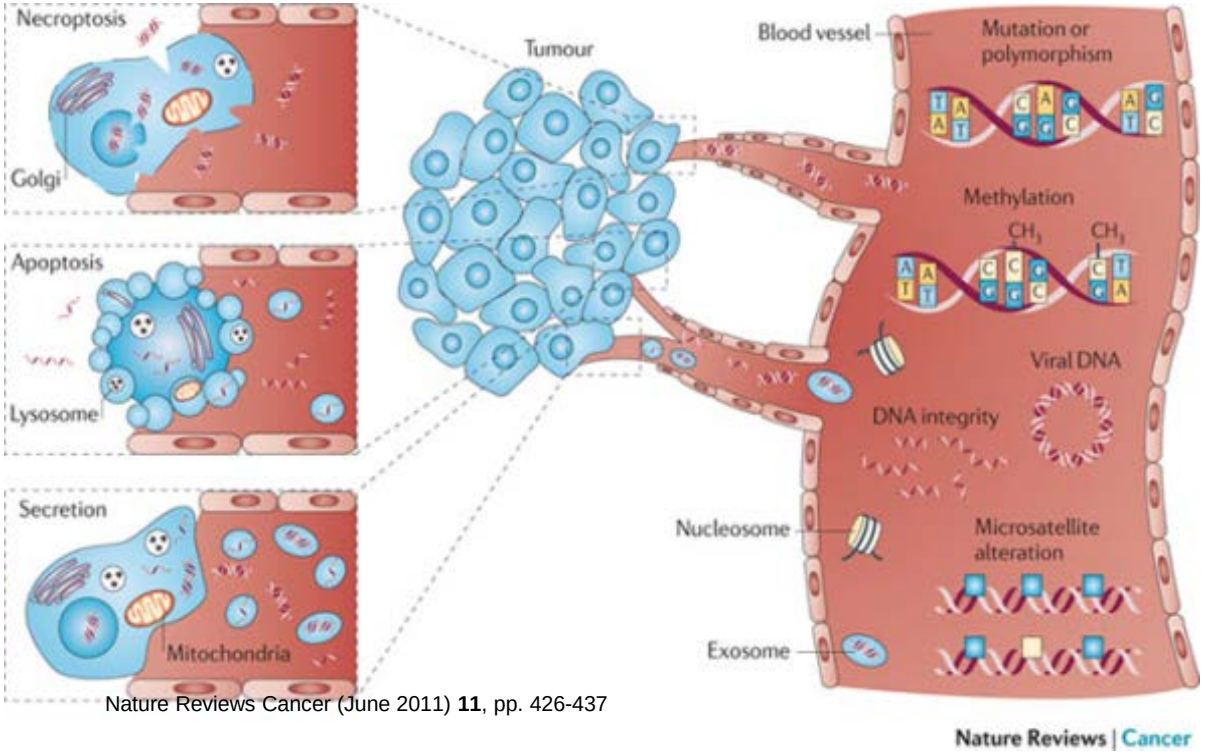
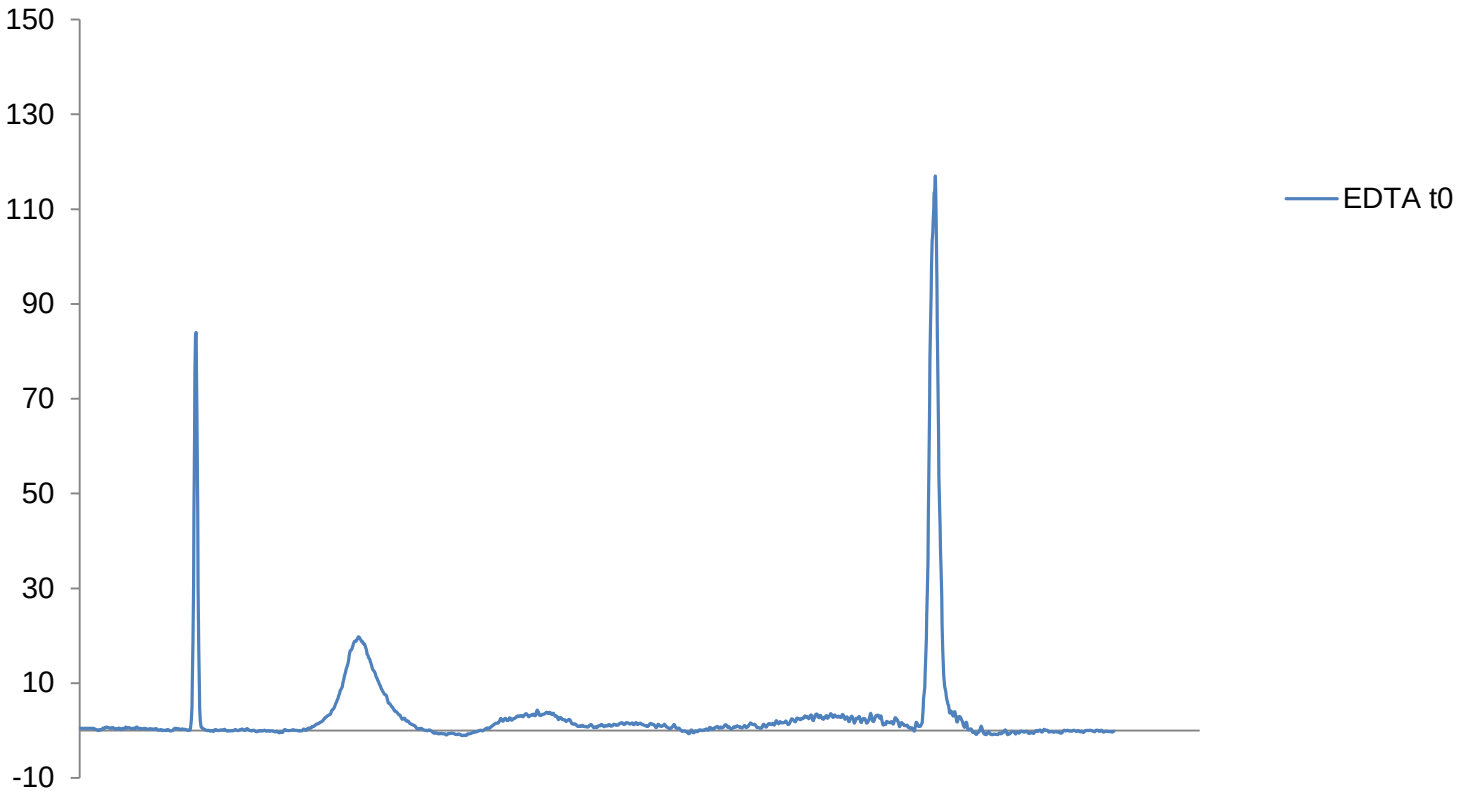
Circulating Cell-free Nucleic Acids — Challenges

- Usually low concentrations in plasma, serum, urine and other body fluids (1-50 ng DNA/ ml plasma)
- Highly fragmented (<500bp)
- Longer fragments (≥ 500bp) – possible from necrotic processes
- Background DNA can be caused through hemolysis
- NIPT: important to maintain fetal fraction without dilution of maternal DNA
- Cancer: Target DNA may only be a few molecules per sample / must not be obscured by cellular gDNA



Nature Reviews Cancer (June 2011) **11**, pp. 426-437

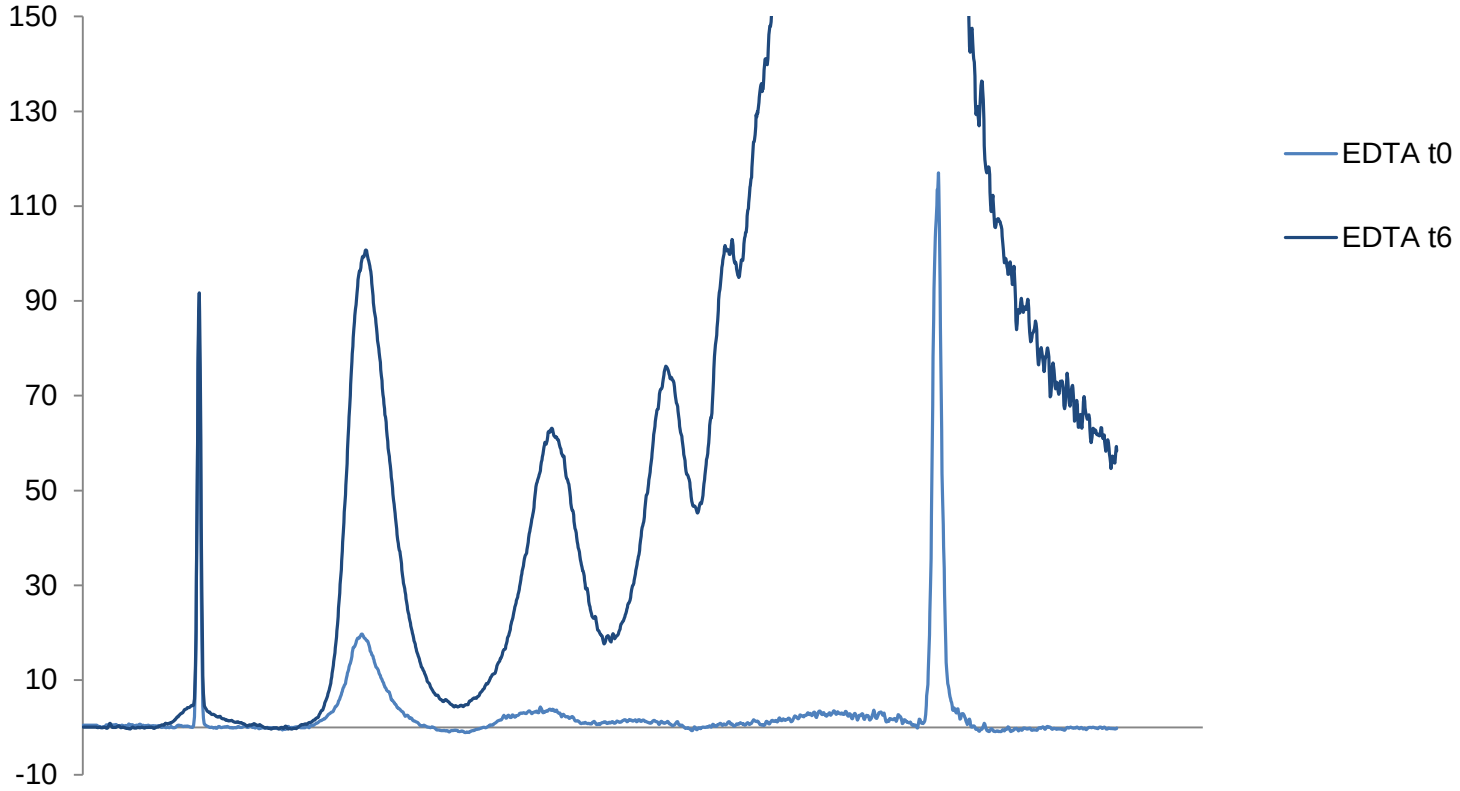
Need for Stabilization of ccfDNA — The Problem



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.

Need for Stabilization of ccfDNA — The Problem

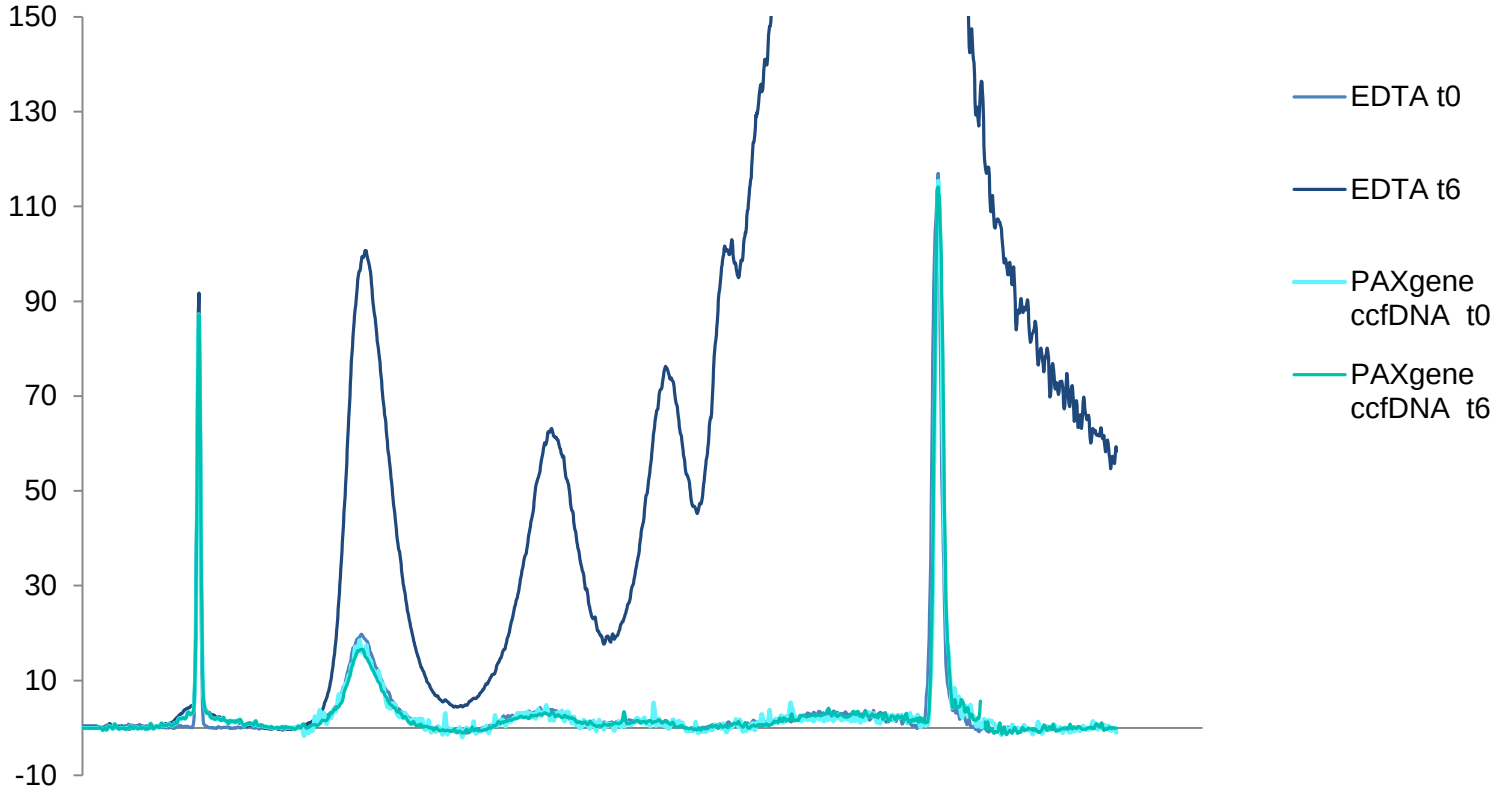
- Apotosis of white blood cells leads to dilution of naturally occuring ccfDNA



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.

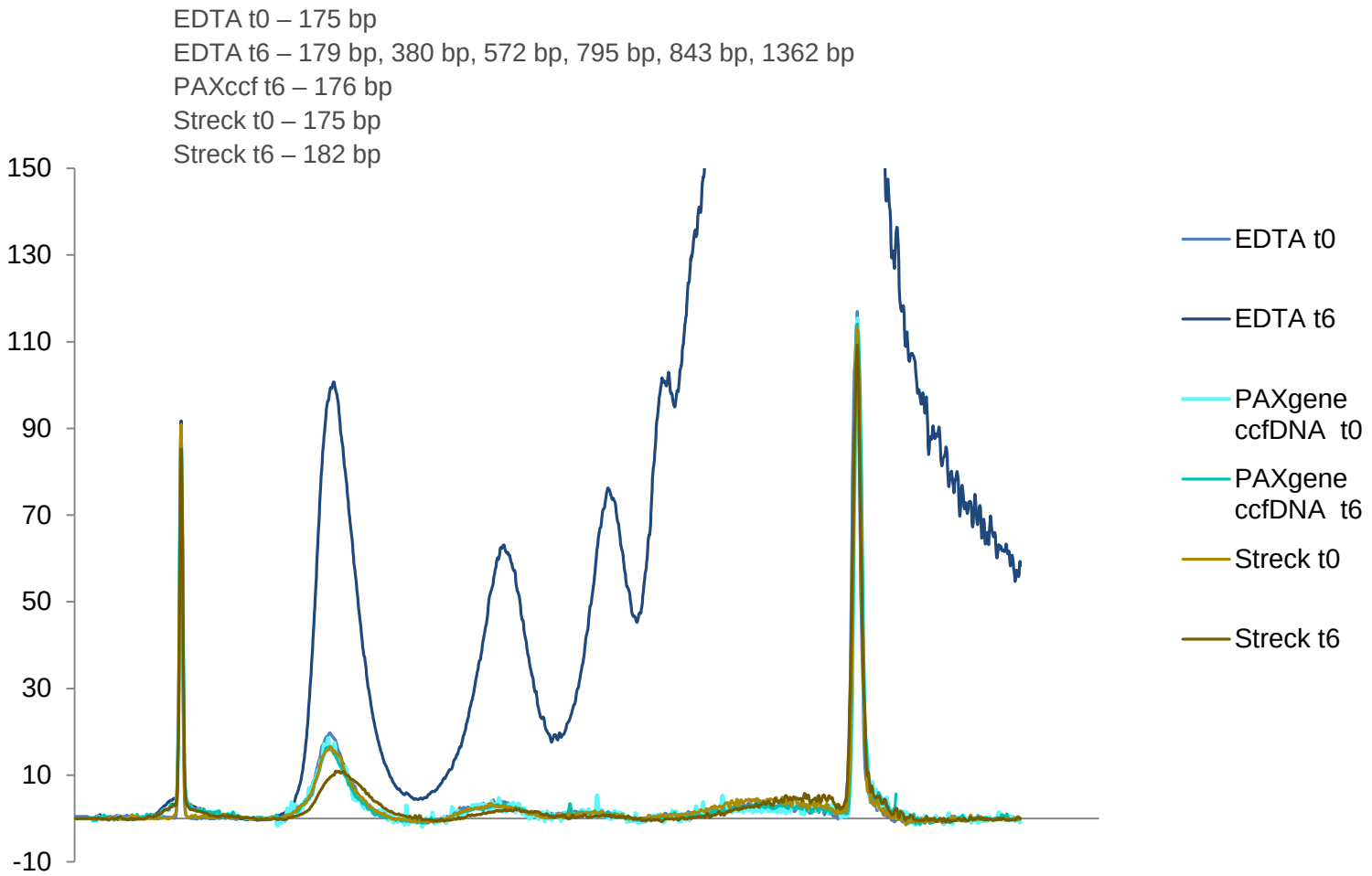
Prevention of Blood Cell Lysis and Release of Genomic DNA

- PAXgene Blood ccfDNA stabilization helps prevent release of gDNA into the 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.

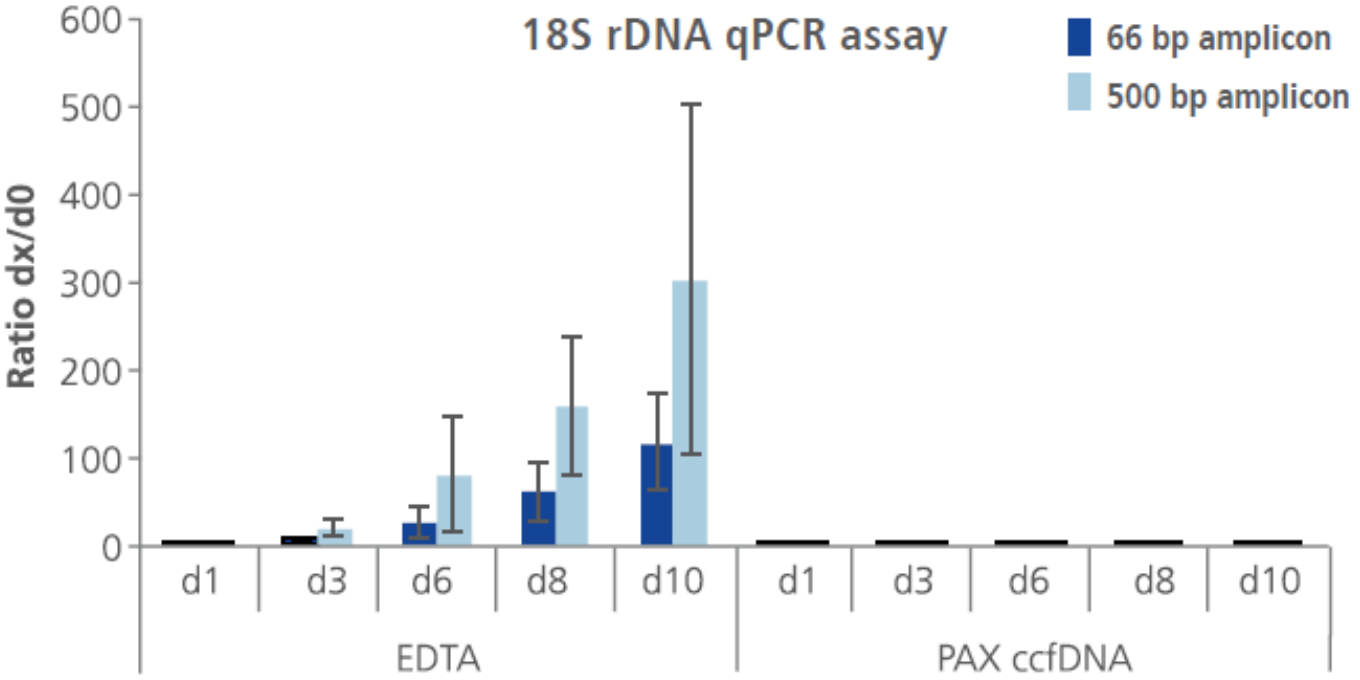
Effects of Cross-Linking & Lack of Stabilization



PAXgene Blood ccfDNA stabilization helps prevent release of gDNA into the plasma.
ccfDNA was extracted from EDTA, Streck 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.

Stabilization of ccfDNA in Whole Blood

Stabilization of Whole Blood at Room Temperature with No Increase in ccfDNA Levels



PAXgene Blood ccfDNA stabilization helps prevent release of genomic DNA from white blood cells. Plasma was extracted from whole blood of 6 subjects; ccfDNA was isolated and yield was quantified by real-time PCR (18S rDNA gene, 66 bp/500 bp amplicon).

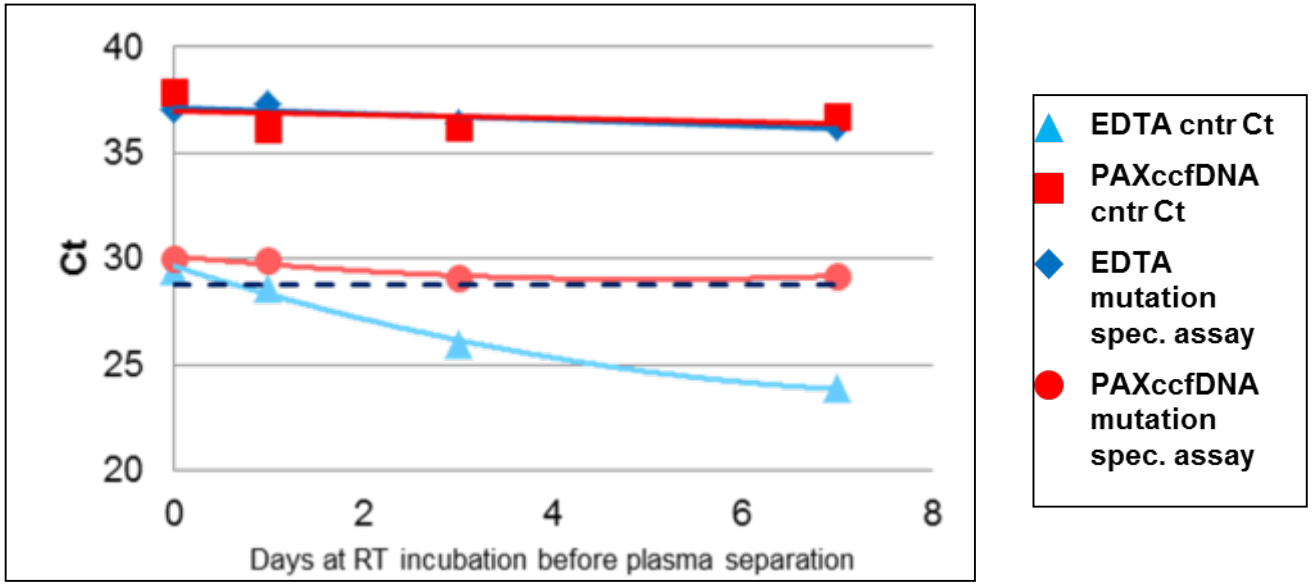
qPCR Analysis of Spiked-in Mutated DNA

PAXgene Blood ccfDNA Samples:

- Constant Ct values, valid mutation detection over storage time

K2-EDTA Samples:

- Critical cut-off value passed after 1 days storage, resulting in false negative calls



Reliable mutation detection in PAXgene Blood ccfDNA Samples. Reference DNA (digested) equivalent to 500 copies of cancer biomarker spiked into blood from 12 donors collected into PAXgene Blood ccfDNA or K2-EDTA Tubes. ccfDNA extraction with QIAamp cNA kit at day 0 and after storage at room temperature for 1, 3 and 7 days. Mutation detection by qPCR (Arms/Scorpions technology). Valid results with delta Ct [Ct mutation – Ct control assay] ≤ 8

PAXgene Blood ccfDNA Tube (RUO) Features

- **Unique Stabilization of extracellular levels of ccfDNA**
- Effective Stabilization
  - White blood cells – Prevent release of gDNA
  - Red blood cells - Minimal hemolysis
  - → maximum ccfDNA yield from plasma, minimized background gDNA
- Non-crosslinking NA preservation – no DNA modification

- **BD Vacutainer® Plastic Tube with Hemogard™ Safety Closure**
- Minimized risk of tube breakage
- Enhanced safety for healthcare and lab personnel
- Minimal contamination between samples
- Consistent blood draw volume

- **Integrated Preanalytical Workflow**
- Seamless integration into manual or automated prep with QIAamp and QIAsymphony Circulating DNA extraction technology

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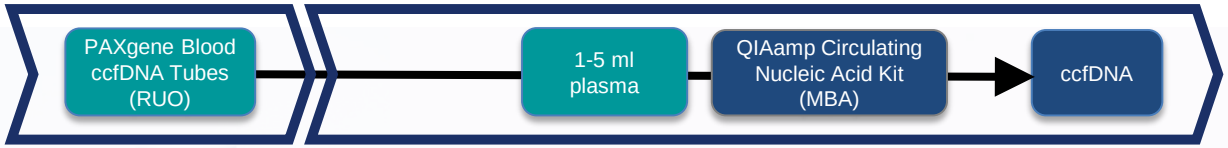
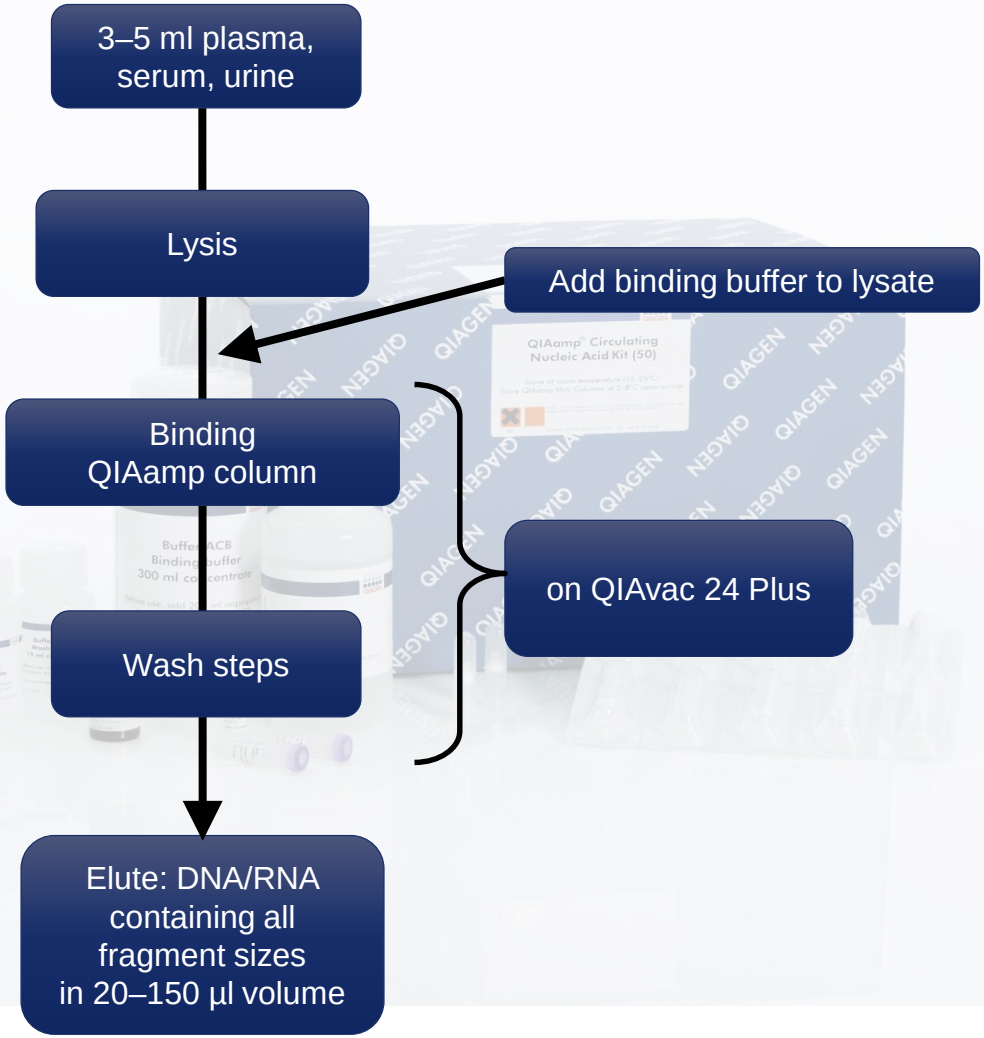
Manual ccfDNA Extraction: QIAamp® Circulating Nucleic Acid Kit



Vacuum-based manual processing of 24 samples in 2–3 hours

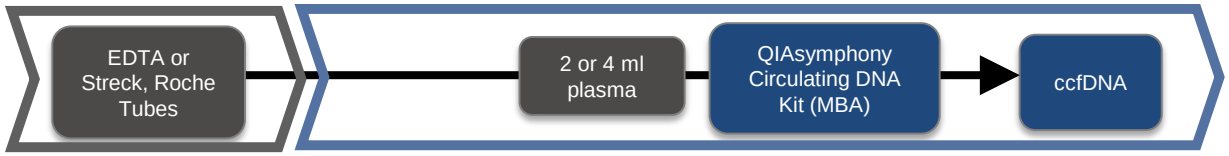
Supported blood collection tubes:

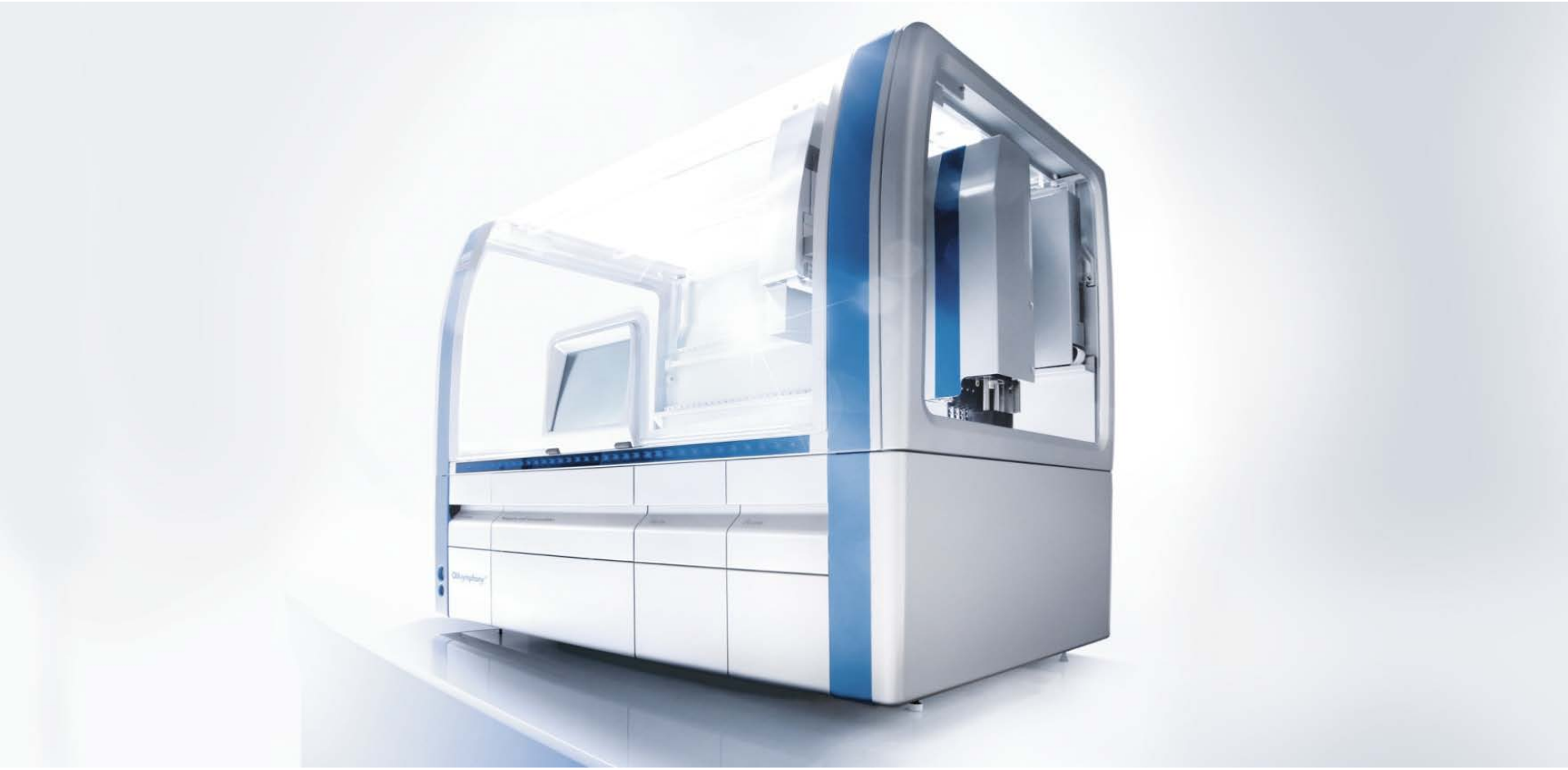
PAXgene Blood ccfDNA
EDTA / Citrate / Serum BCT
Streck Cell-free DNA BCT *



* with extended lysis step

Automated Solution: QIAsymphony ccfDNA Extraction





QIAsymphony: ccfDNA extraction Using New Chemistry

Novel extraction chemistry allows for processing large-volume samples (plasma or serum) on the QIAsymphony SP instrument — up to 96 samples per run:

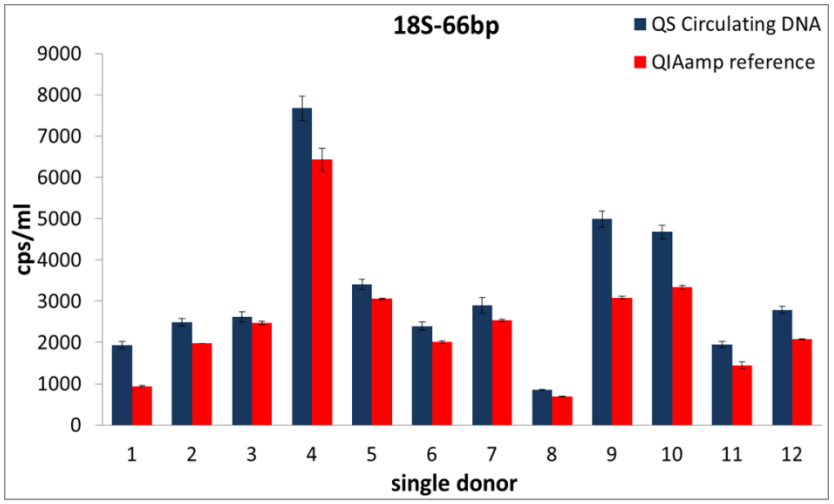
Extracted ccfDNA is compatible with different assay applications (qPCR, next-gen sequencing, arrays)

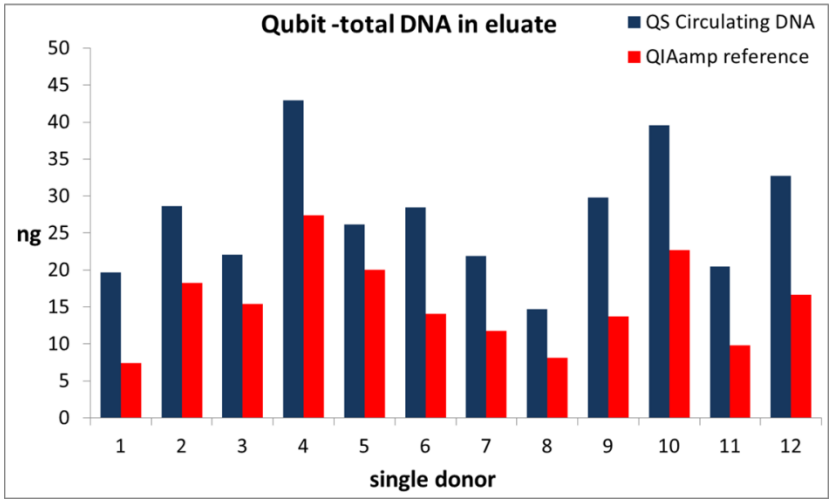
New QIAsymphony ccfDNA Kit: DNA Yield Compared to Manual Kit

QIAsymphony ccfDNA extraction compared against QIAamp Circulating Nucleic Acid Kit

Set-up of experiment:

- Blood samples from 12 healthy donors
- Blood collection in Cell-Free DNA BCT (Streck)
- Plasma was separated from whole blood by double centrifugation
- 4 ml plasma volume as sample input
  - QIAamp® Circulating NA Kit (elution in 60 µl) (**red**)
  - QIAsymphony® Circulating DNA Kit (**blue**)
- Circulating DNA yield determined by real-time PCR; results were calculated as target copies per ml plasma
- Circulating DNA yield determined by Qubit™ dsDNA HS Assay Kit; results were calculated as total DNA yield from 4 ml plasma

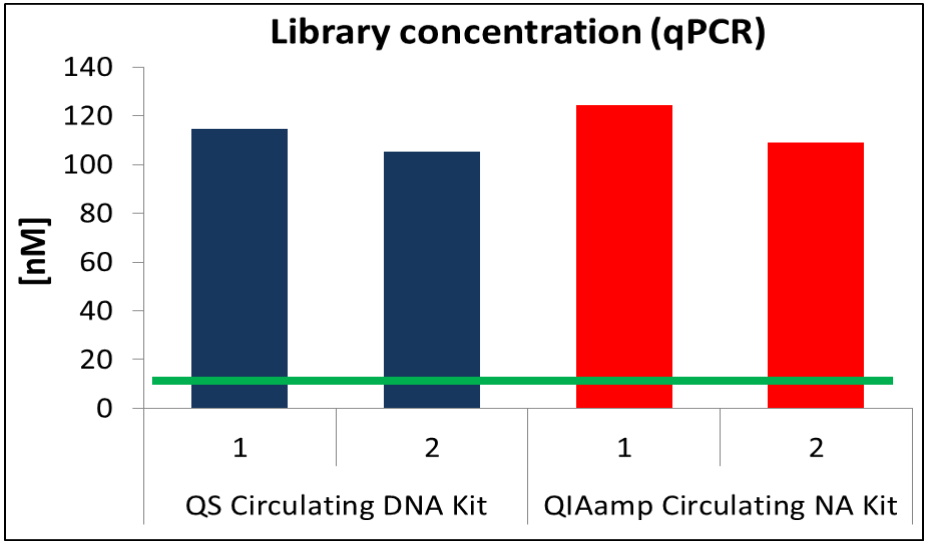


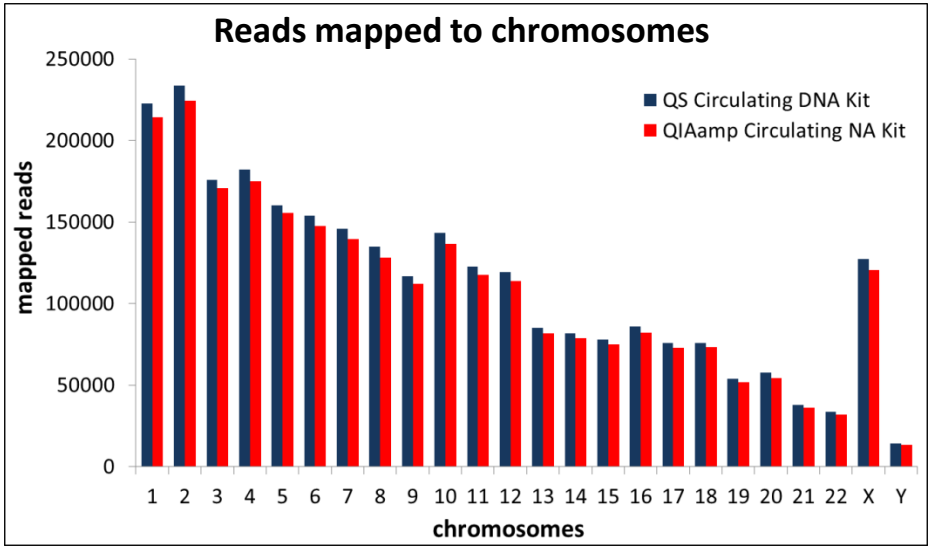


Compatibility of Extracted ccfDNA in Library Prep and NGS

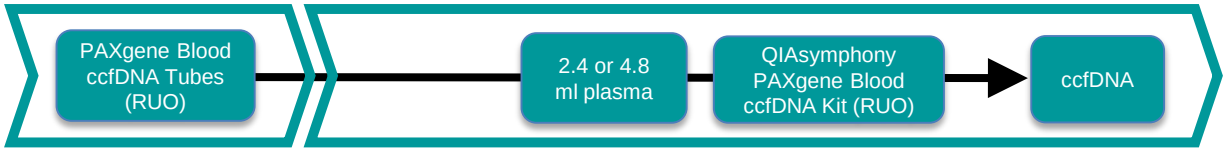
Set-up of experiment:

- Plasma pool from Cell-Free DNA BCT (Streck)
- Plasma ccfDNA extraction:
  - QIAsymphony® Circulating DNA Kit (blue)
  - QIAamp® Circulating NA Kit (red)
- Library prep: GeneRead Library Prep I Core Kit: Input 10 µl (2.3–5.6 ng)
- 10 nM required for Illumina Sequencing applications (green)
- MiSeq NGS run using 2 nM (10 µl) Calculation of mapped reads distributed on chromosomes (lower figure)





QIAsymphony PAXgene Blood ccfDNA Kit

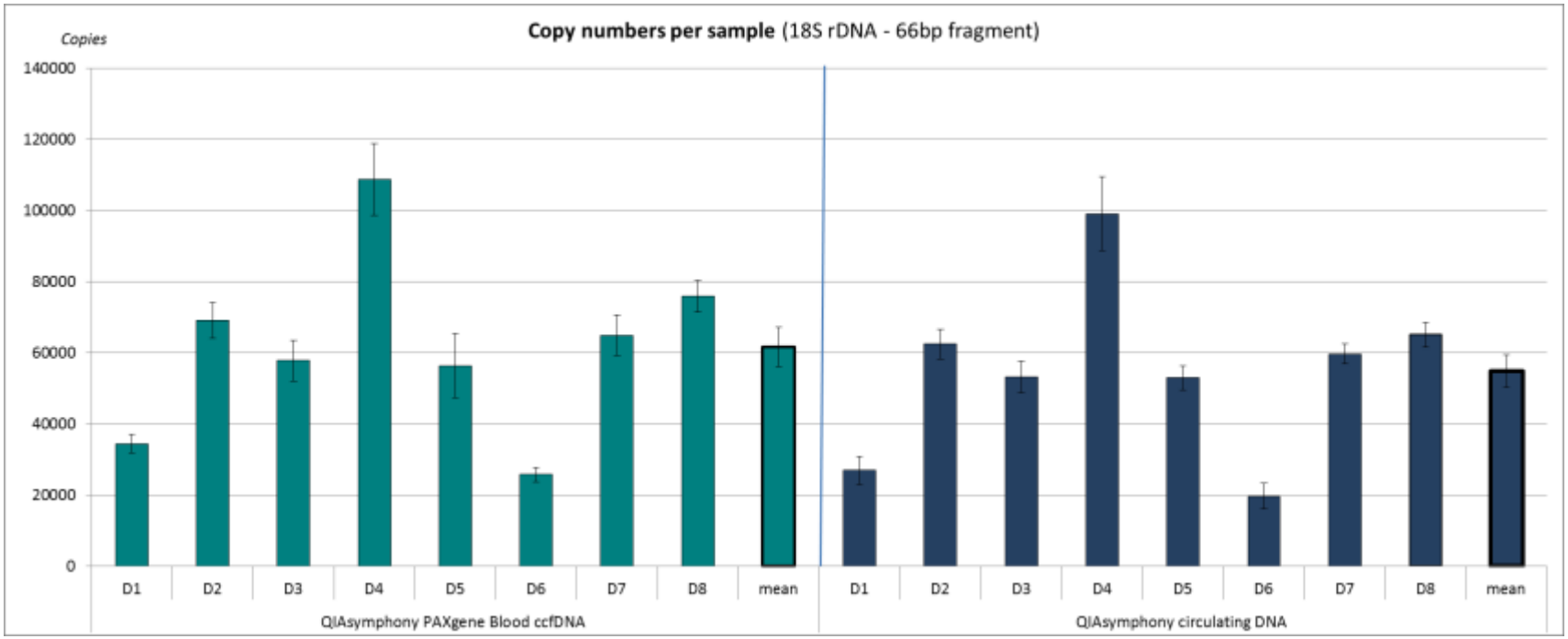


- Dedicated isolation technology for use with PAXgene Blood ccfDNA Tube (RUO)
  - Binding chemistry optimized for use with PAXgene ccfDNA tube reagent
  - Optimized input volumes to accomodate higher volume plasma

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

QIAsymphony ccfDNA Extraction — System Approach vs Generic

- Slight advantages with PAXgene System approach compared with multi-purpose QIAsymphony circulating DNA kit used in conjunction with the PAXgene ccfDNA Tube



Plasma was extracted from whole blood of 8 subjects; ccfDNA was isolated with the QIAsymphony Circulating Nucleic Acid protocol (4ml) or the QIAsymphony PAXgene Blood ccfDNA LAF protocol (4.8ml) and the corresponding kits. Yield was quantified by real-time PCR (18S rDNA gene, 66 bp amplicon).

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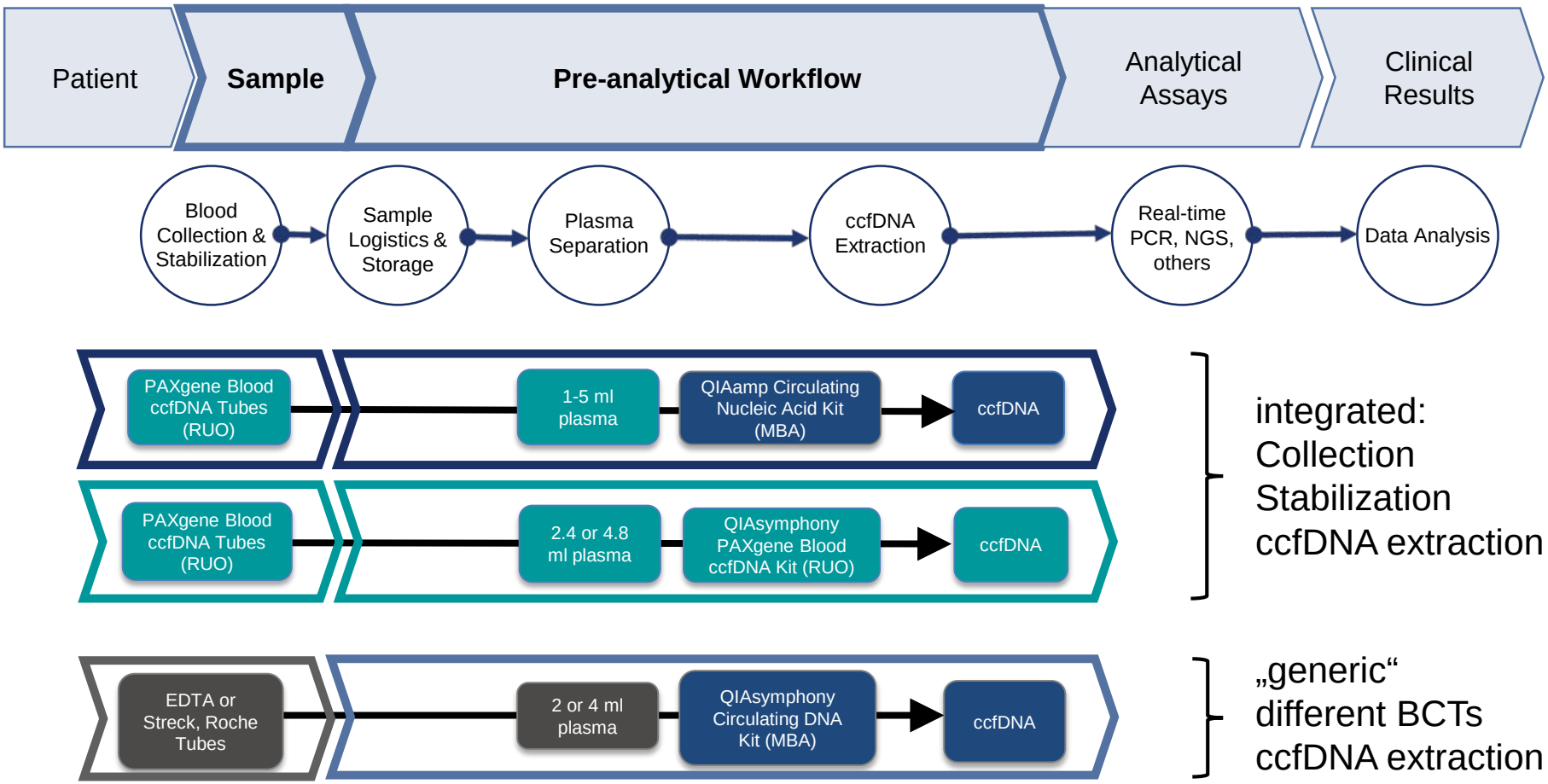
ccfDNA from plasma — Sample-to-Insight orkflow



Complete preanalytical workflow from QIAGEN & PreAnalytiX

Questions?