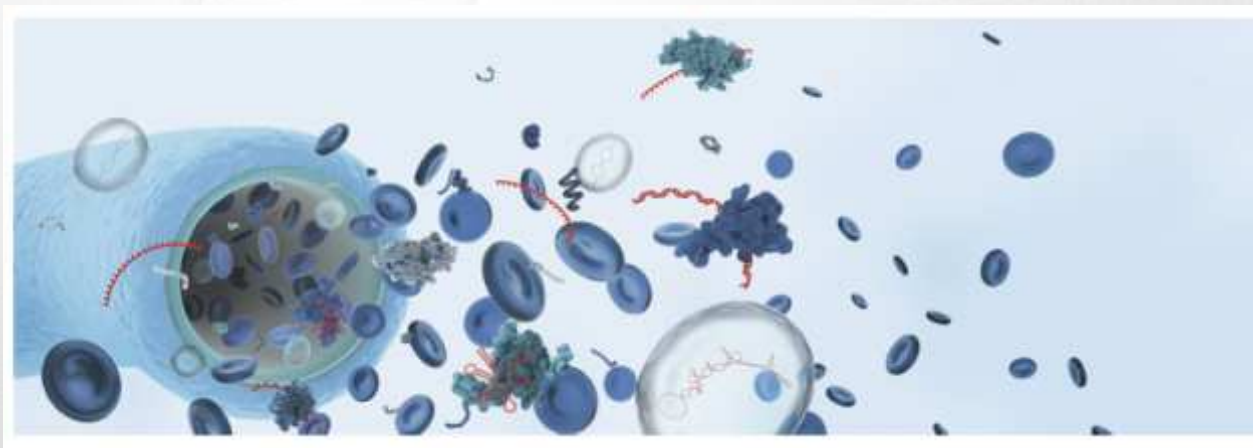


## New Technology and Workflow for Integrated Collection, Stabilization and Purification of Circulating Cell-free DNA

Dr. Thorsten Voss

Senior Scientist R&D PreAnalytiX

# New Technology and Workflow for Integrated Collection, Stabilization and Purification of Circulating Cell-free DNA



**Dr. Thorsten Voss, Senior Scientist R&D PreAnalytiX**

# PAXgene Blood ccfDNA System (RUO)

For Research Use Only. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease. The performance characteristics of this product have not been fully established.

# Content

- ❖ Introduction
- ❖ PAXgene Blood ccfDNA Tube
- ❖ QIAasymphony PAXgene Blood ccfDNA procedure
- ❖ Summary: ccfDNA from plasma

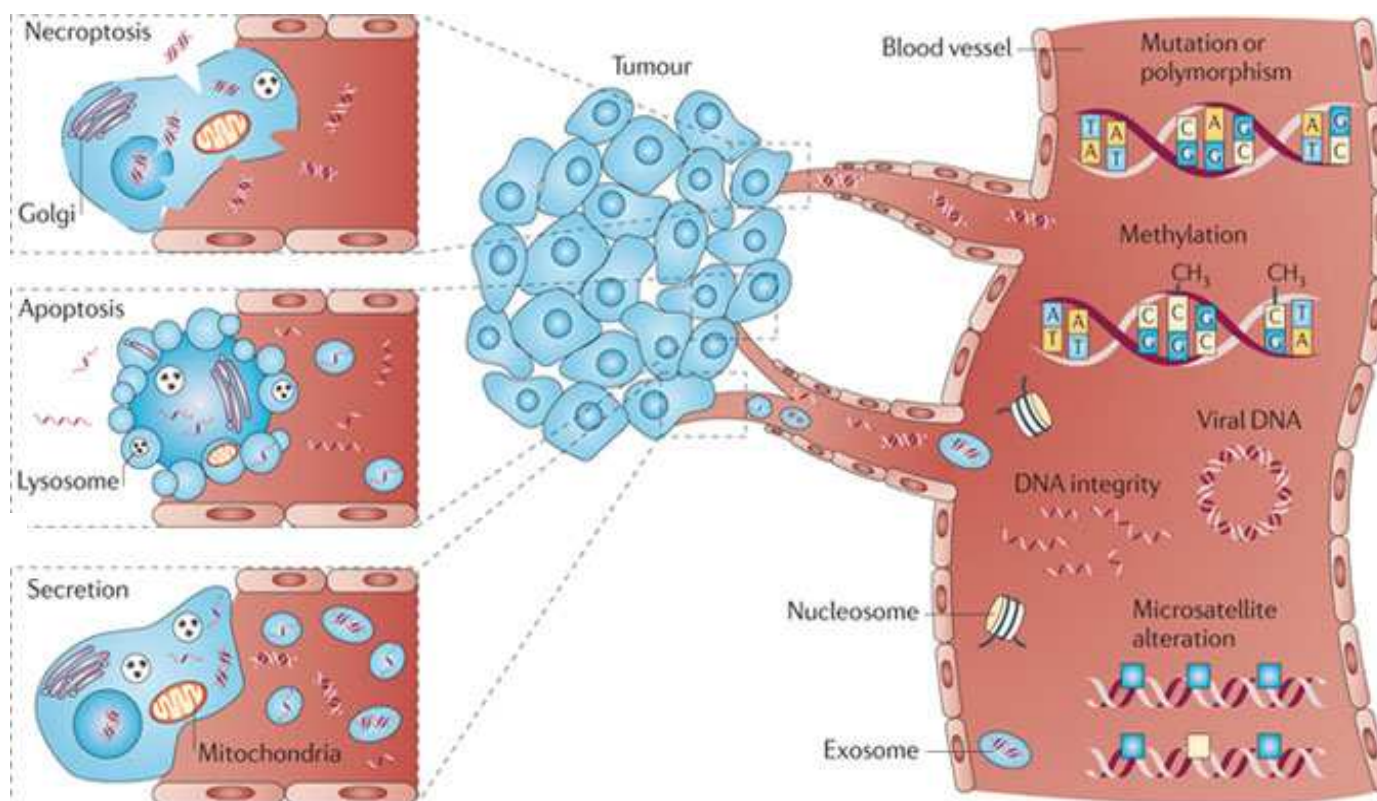




# Circulating cell-free nucleic acids

## Very short introduction

- Strongly fragmented (<500bp),  
→ but long fragments ( $\geq 500$ bp) from necrotic processes as well
- Very low concentrations in plasma, serum, urine and other body fluids (<<100ng/ml)



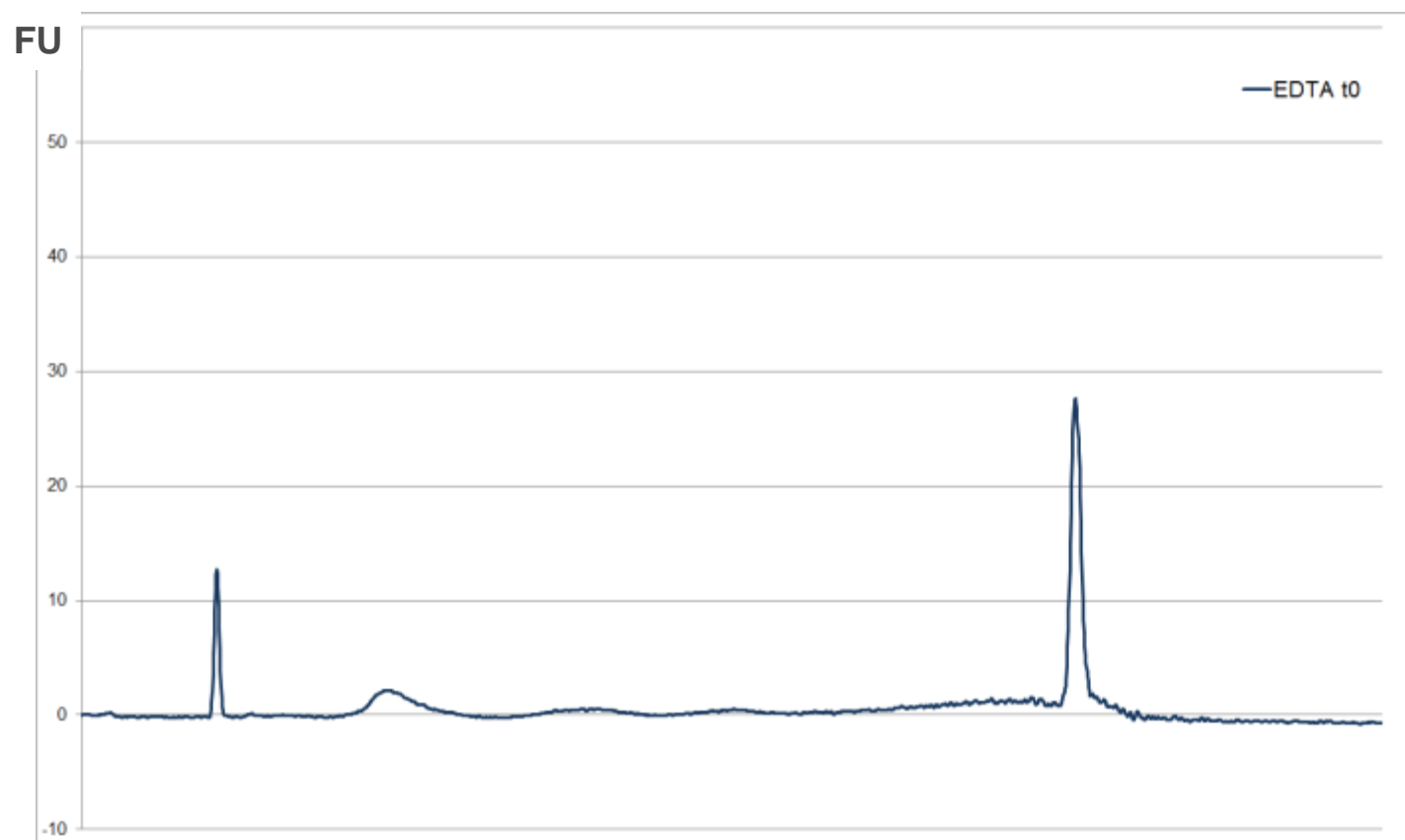
Nature Reviews Cancer (June 2011) 11, pp. 426-437

Nature Reviews | **Cancer**

# Need for stabilization of ccfDNA

## The problem

- Apoptosis of white blood cells leads to dilution of natural occurring ccfDNA



ccfDNA was extracted from EDTA plasma of 1 subject directly after blood draw (t0) and after 10 days at room temperature (t10). 1  $\mu$ l eluate was analyzed using the Agilent® High Sensitivity DNA Kit.

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# Workflow Options

## Tube formats



(Workflow) Feature	K <sub>2</sub> EDTA blood (unstab.)	Streck Cell-Free DNA BCT	PAXgene ccfDNA tube*
Blood draw volume / plasma yield per tube	10ml / ≥ 4 ml	10ml / ≥ 4 ml	~10 ml / ≥ 4ml
Tube material	Plastic	Glass	Plastic
Centrifugation @ blood collection site needed	Yes (or cooling chain)	No	No
Transport and/or storage @ room temperature possible for up to 7 days	No	Yes	Yes
Crosslinking stabilization solution	n.a.	Yes**	No
Standardized complete workflow - Tube and dedicated ccfDNA prep solution - One supplier	No	No	Yes
Optimized automation solutions	No	No	Yes

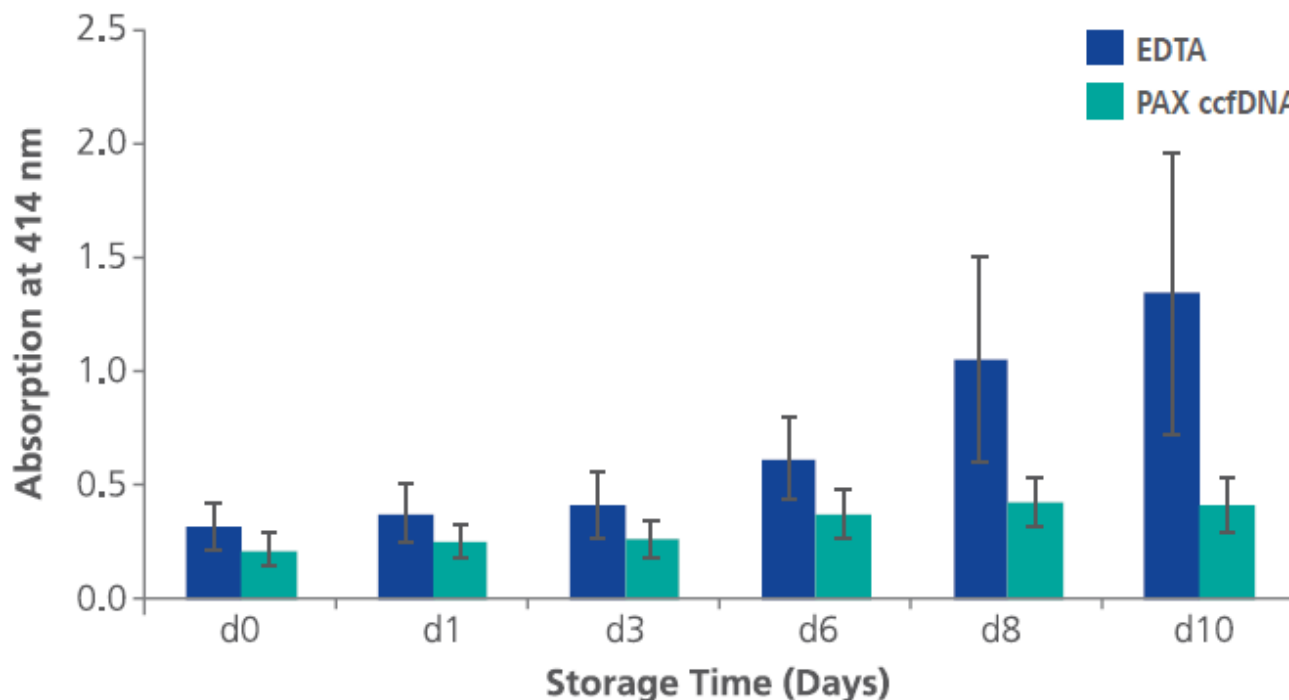
\* Designed to meet the relevant technical specifications, and designed, manufactured and distributed using systems which meet the appropriate recognised international quality standards including ISO13485 and FDA QSR's CGMP's.

\*\* According for example to WO2013123030A2, US2011111410A1, US5460797A, US5459073A.



# Prevention of red blood cell lysis during transport and storage

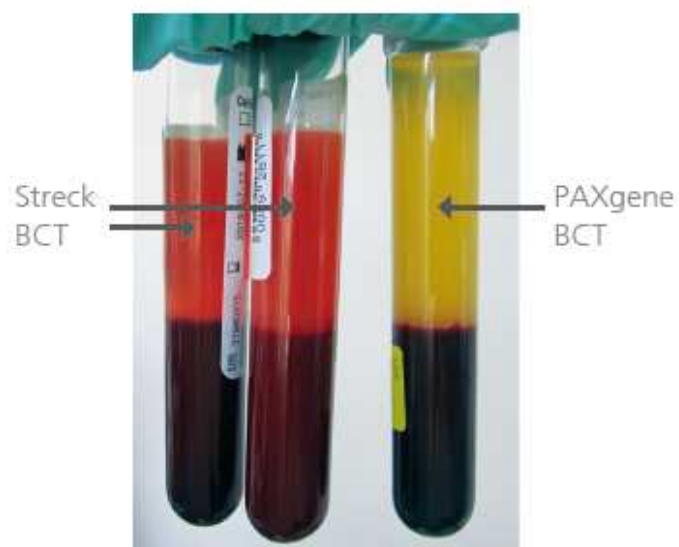
## Prevention of Hemolysis During Transport and Storage



PAXgene Blood ccfDNA stabilization helps prevent hemolysis, thus allowing distinct visual separation of plasma. Hemolysis was determined by measuring the absorption of free hemoglobin in plasma from 6 subjects at 414 nm. Mean absorption and standard deviation are shown.

# Hemolysis

## Impact on plasma separation

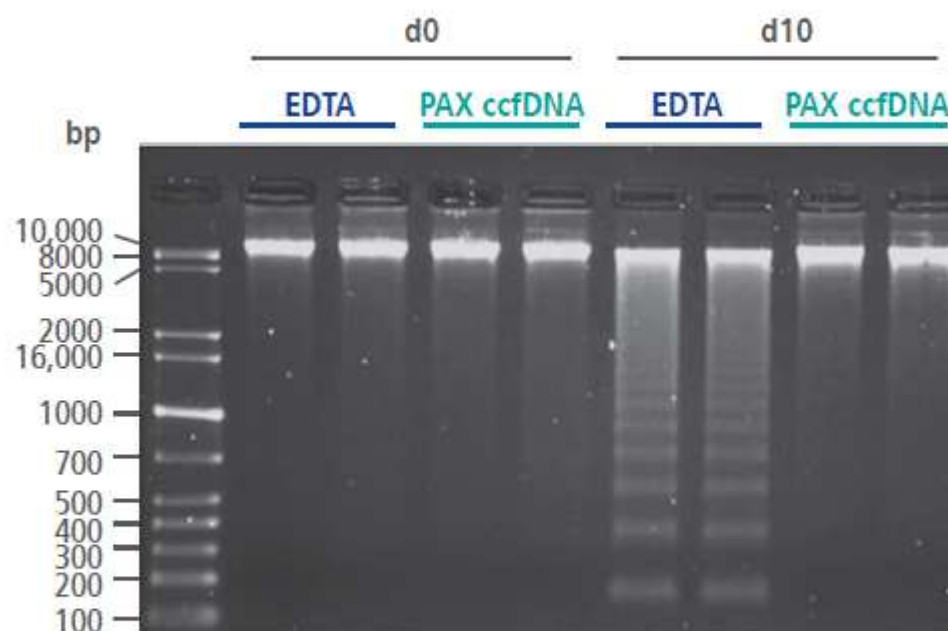


**BCTs with blood from pregnant women 3 days after collection.** Blood was transported from physician to testing laboratory and centrifuged once.

Picture kindly provided by LifeCodexx

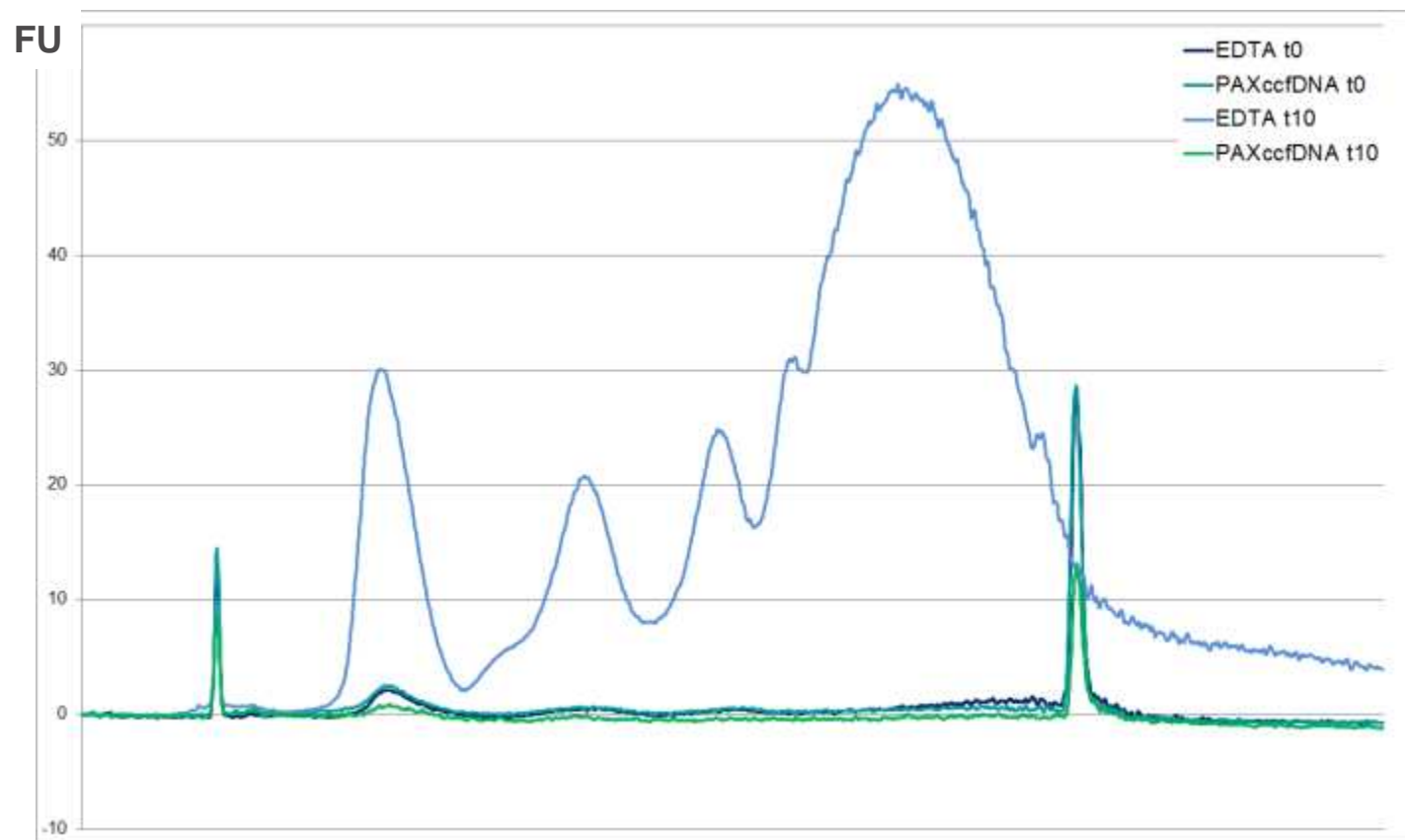
# Blood cell stabilization prevents apoptosis of white blood cells

## Stabilization of Blood Cells and Prevention of Apoptosis



PAXgene Blood ccfDNA stabilization helps prevent apoptosis of blood cells and fragmentation of genomic DNA. Genomic DNA was extracted from whole blood of 2 subjects using the QIAamp Blood Mini Kit. 400 ng DNA were separated by agarose gel electrophoresis.

# Prevention of blood cell lysis and release of genomic DNA in plasma

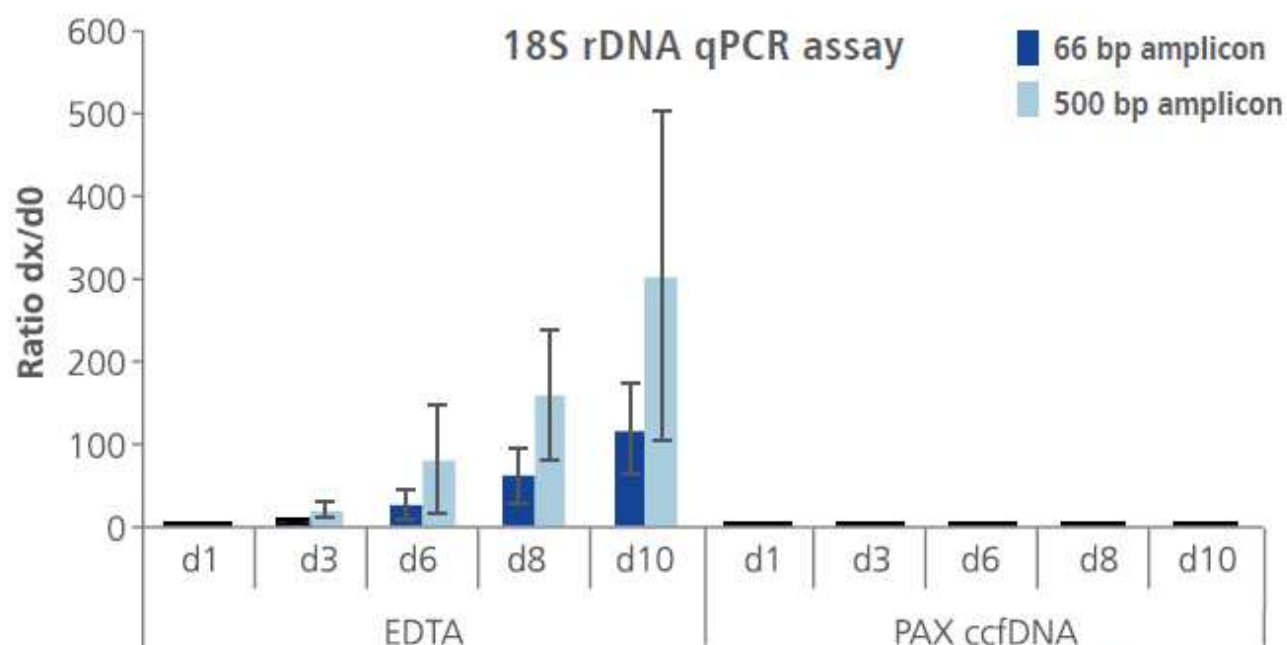


**PAXgene Blood ccfDNA stabilization helps prevent release of gDNA into the plasma.**

ccfDNA was extracted from EDTA and PAXgene plasma of 1 subject directly after blood draw (t0) and after 10 days at room temperature (t10). 1 µl eluate was analyzed using the Agilent® High Sensitivity DNA Kit.

# Stabilization of whole blood and ccfDNA during transport and storage

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

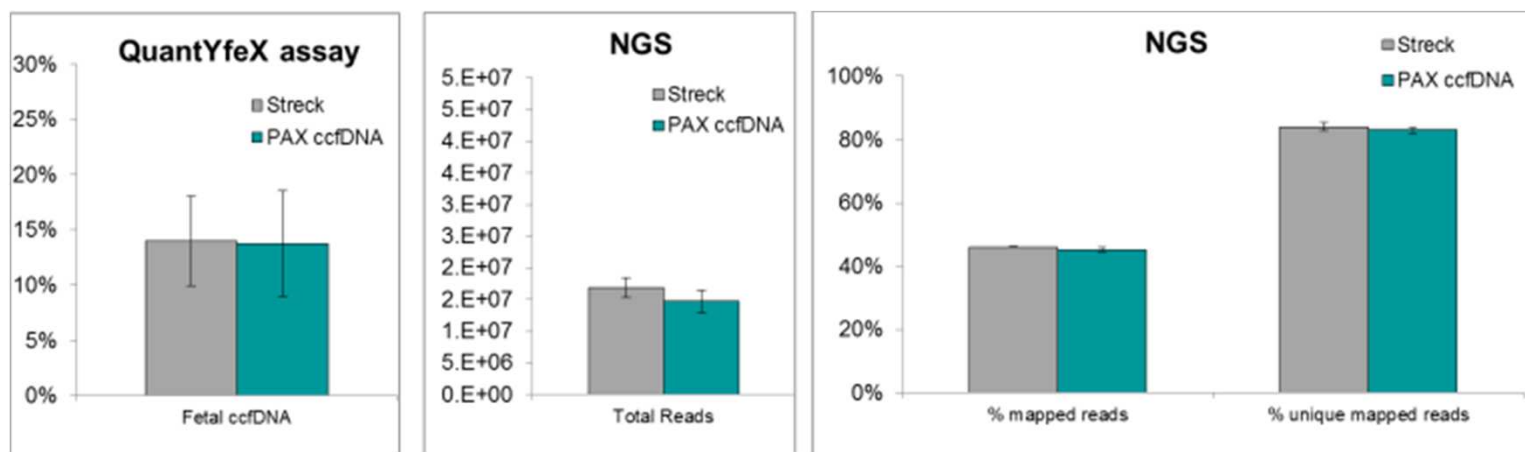


**PAXgene Blood ccfDNA stabilization helps prevent release of genomic DNA from white blood cells.** Plasma was extracted from whole blood of 6 subjects; ccfDNA was isolated and yield was quantified by real-time PCR (18S rDNA gene, 66 bp/500 bp amplicon).



# Analysis ccfDNA from stabilized maternal blood in a research study

- All specimens collected in PAXgene Blood ccfDNA tubes fulfilled the quality criterion “fetal fraction > 4%”
- Absolute fetal ccfDNA yield and fetal-to-maternal DNA ratio were comparable with the current method
- Fetal gender was concordant between collection tubes for all specimens
- All 18 specimens stored in PAXgene Blood ccfDNA tubes, then analyzed with NGS and the PrenaTest DAP.plus software, were concordant with the current method and were negative for trisomy 13, 18 and 21



# PAXgene Blood ccfDNA Tube (RUO) Safety Features

- Plastic material → *Helps to avoid tube breakage*
- Safety BD Hemogard® closure → *Helps to avoid contamination with blood*
- Prevention of hemolysis → *Plasma separation*
- No crosslinking agent in the tube → *ccfDNA preparation & assay performance*



# Content

- Introduction

- PAXgene Blood ccfDNA Tube *New in 2016 !*

- QIAasymphony PAXgene Blood ccfDNA procedure

*New in 2016 !*

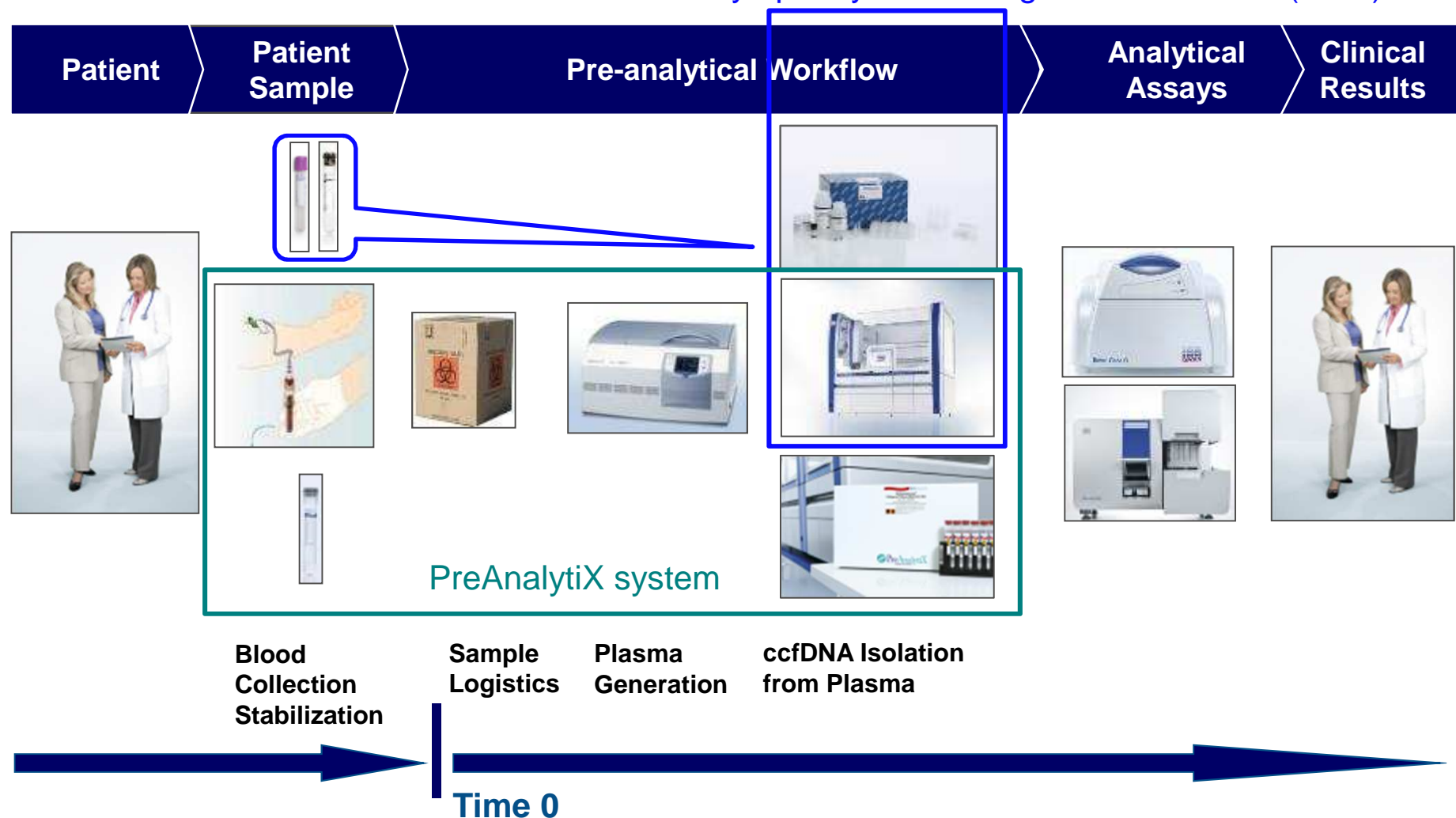
- Summary: ccfDNA from plasma



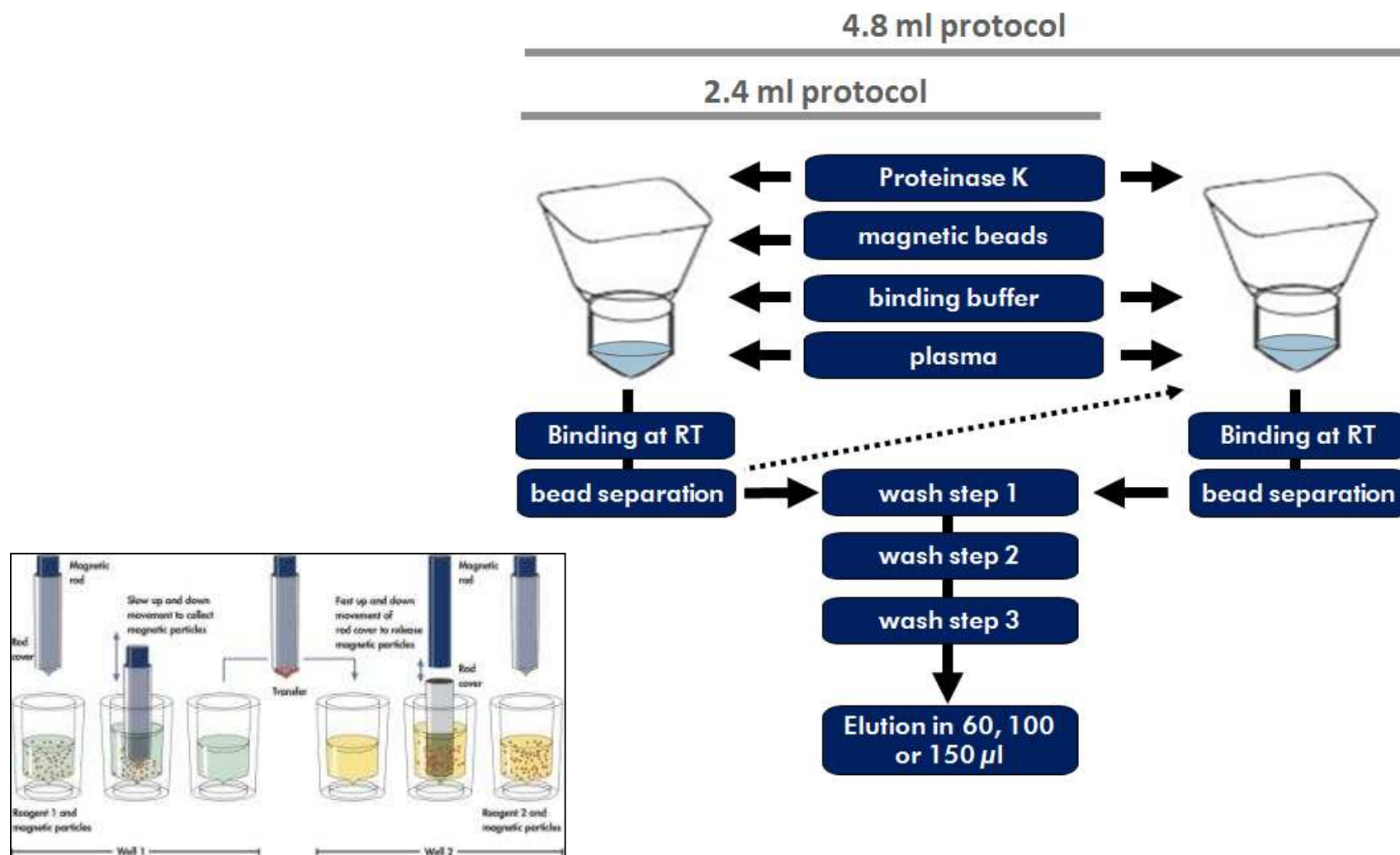
# Standardized Blood / Plasma ccfDNA Workflow



## QIASymphony Circulating Nucleic Acid Kit (MBA)



# QIAasymphony PAXgene Blood ccfDNA Protocol Workflow





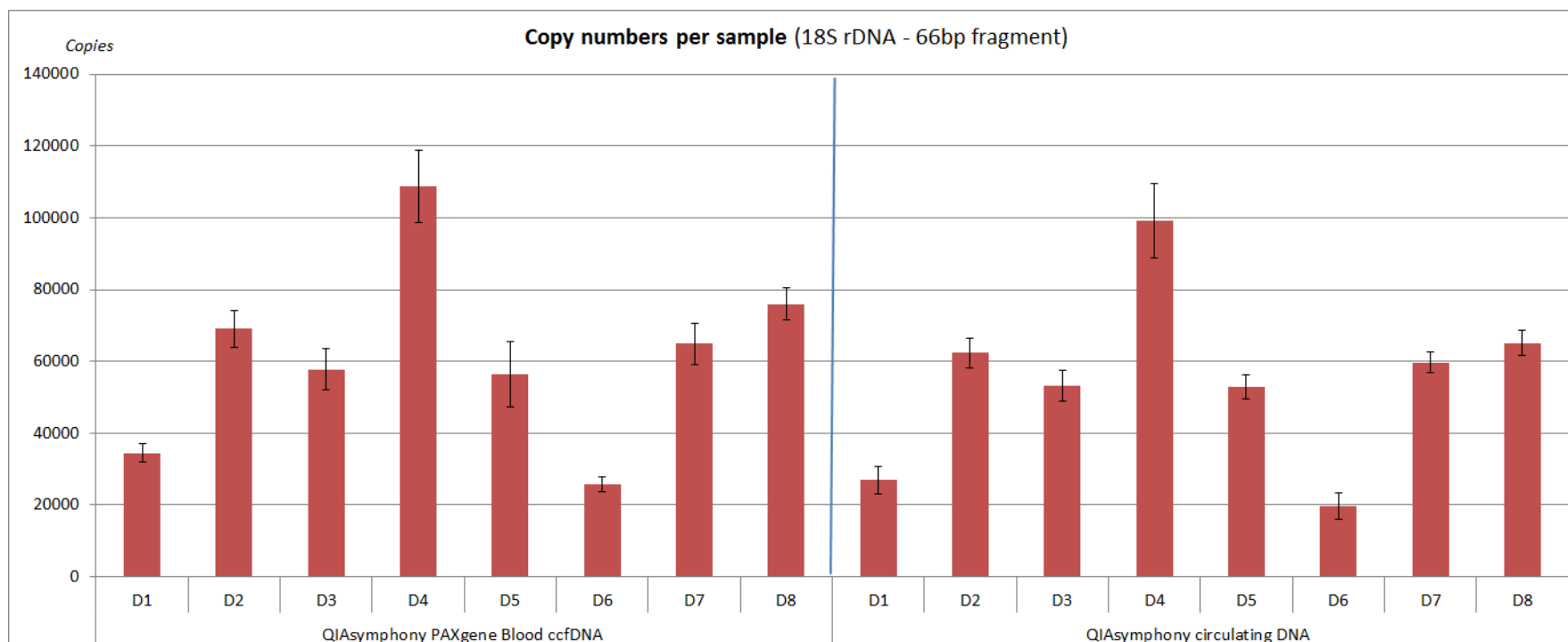
## QIAasymphony PAXgene Blood ccfDNA Kit (RUO) vs QIAasymphony Circulating Nucleic Acid Kit (MBA)



- Dedicated isolation technology for use with PAXgene Blood ccfDNA Tube (RUO)
  - Binding chemistry optimized for use with the new tube
  - Optimized protocol steps
- Two protocol lines
  - Standard protocols similar to QIAasymphony Circulating Nucleic Acid Kit protocols
  - Large fragment protocols enables additional very efficient co-isolation of large fragments
- Three different elution volumes (60, 100, 150 µl)

# Performance on the QIAAsymphony SP

- Slight advantages of dedicated compared to generic protocol when used in conjunction with the PAXgene Blood ccfDNA Tube



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

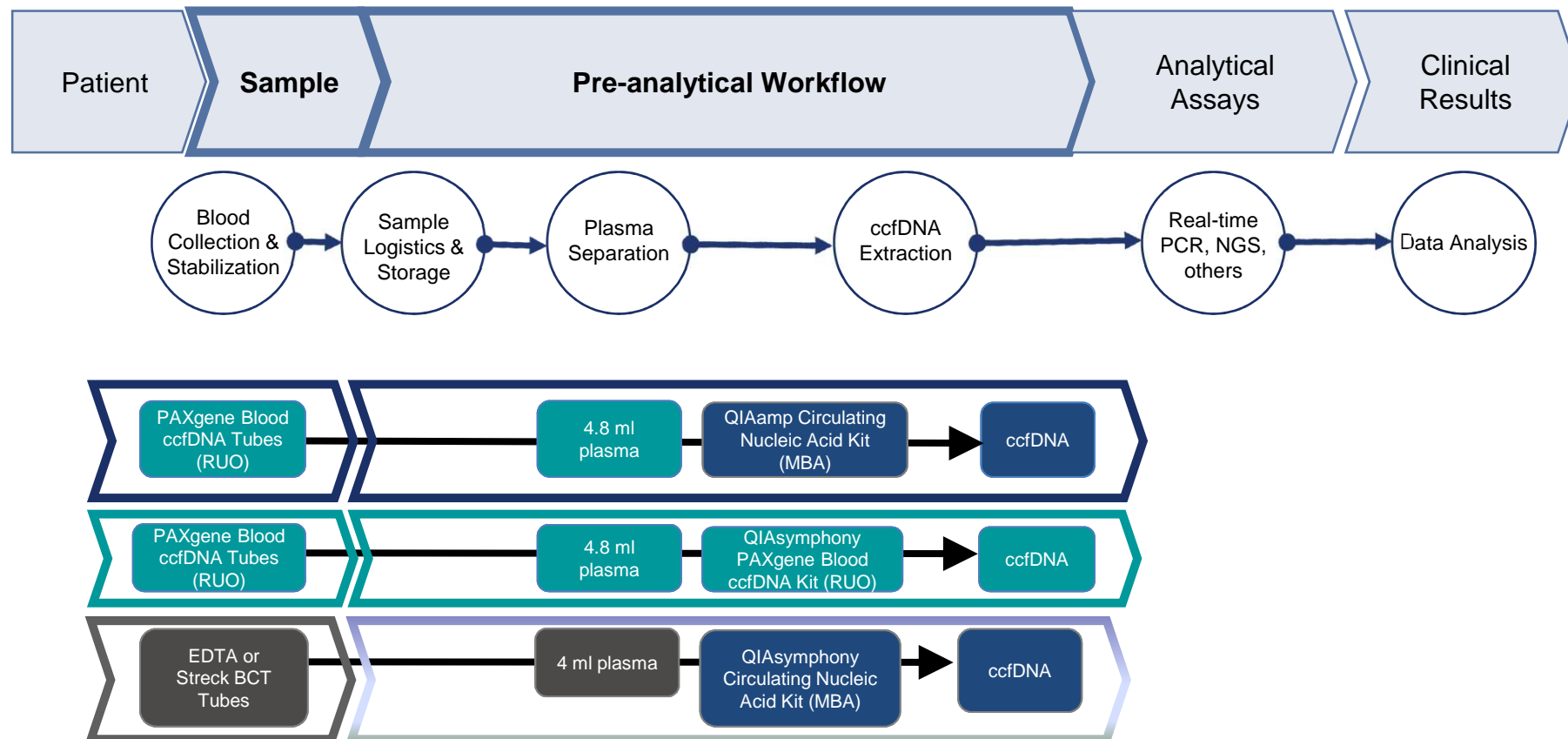
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# ccfDNA from plasma

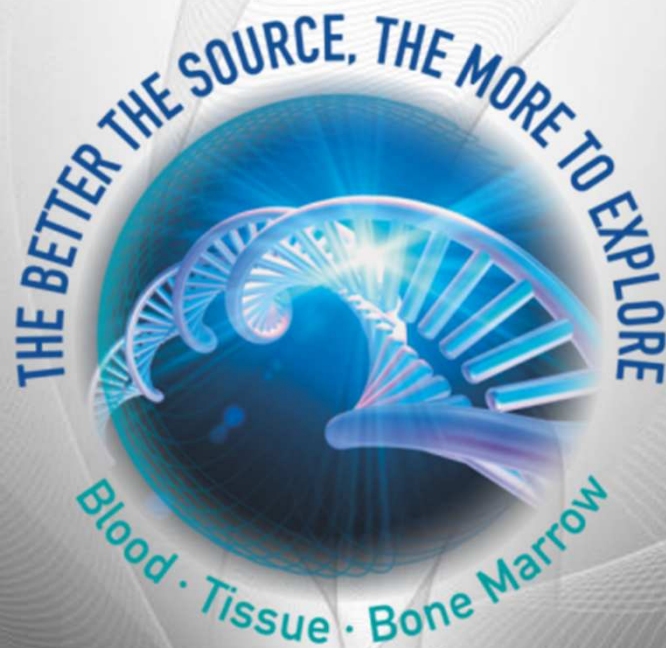
## Sample-to-insight workflow



**Complete preanalytical workflow from QIAGEN & PreAnalytiX**



# Thank you!







Thank you for attending

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## Questions?

Thank you for attending today's webinar!



**Contact QIAGEN**

Call: 1-800-426-8157

Email: [QIAwebinars@QIAGEN.com](mailto:QIAwebinars@QIAGEN.com)