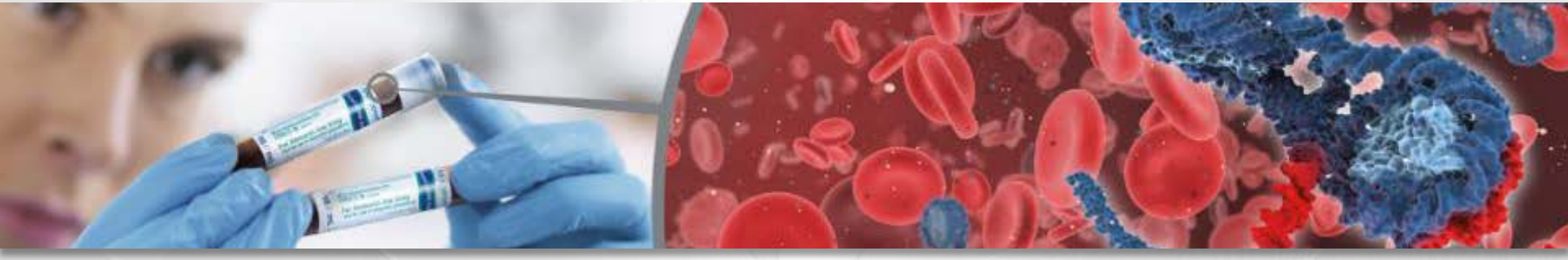
Preanalytical Considerations and Workflow Solutions for Liquid Biopsies

Specimen, Standards & Technologies



European Biobank Week – Industry Workshop - Live in Your Living Room

18 November 2020

Dr. Tomasz Krenz

Who we are

PreAnalytiX®, a joint venture between **BD** and **QIAGEN**, develops, manufactures and sells integrated and standardized systems for sample collection, stabilization and purification of high-quality RNA, microRNA, ccfDNA, and DNA from human blood, bone marrow, or tissue specimens.

Our mission

To enable and improve the diagnosis, treatment and monitoring of disease by providing clinicians and researchers with preanalytical systems that yield superior quality samples for molecular diagnostic testing.

Legal Disclaimer

- The PAXgene® Blood RNA System is available for IVD use only when the PAXgene Blood RNA Tube (762165) is used in combination with the PAXgene Blood RNA Kit (762164 or 762174).
- The PAXgene® Blood ccfDNA Tube (RUO) is for Research Use Only in the U.S. The PAXgene Blood ccfDNA Tube (CE-IVD) is available in some parts of the world outside the U.S.
- The CE-marked PAXgene Blood ccfDNA Tube for in vitro diagnostic use is available in the following countries: AT, AU, BE, BG, CA, HR, CY, CZ, DK, EE, FI, FR, DE, GR, HU, IS, IN, ID, IE, IT, JP, KR, LV, LI, LT, LU, MT, NL, NZ, NO, PL, PT, RO, RU, SG, SK, SI, ES, SE, CH, TH, UK, VN
- For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN or PreAnalytiX kit handbook or user manual. QIAGEN or PreAnalytiX kit handbooks and user manuals are available at **www.qiagen.com** or **www.preanalytix.com** or can be requested from QIAGEN Technical Services, PreAnalytiX Technical Services or your local distributor.

Agenda

- Need for pre-analytical workflow standardization to reduce diagnostic errors
- Pre-analytical factors that influence the outcome of ccfDNA analysis
- International initiatives and requirements to standardize pre-analytical workflows
- Liquid biopsy preservation and workflow solutions

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Demand for Improvements and Workflow Standardization

- 150,000 papers documenting thousands of claimed biomarkers, but fewer than 100 have been validated for routine clinical practice

  Bring on the biomarkers, George Poste, Nature 2011

- 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 errors observed during the total testing process

  Medical Laboratory Observer, May 2014

- Unnecessary expenditure caused by pre-analytical errors in a typical U.S. hospital (~ 650 beds) of ~ $1.2 million per year

  Green SF. Clin Biochem. 2013

- Irreproducible preclinical research exceeds 50%, US $28B / year spent on preclinical research that is not reproducible - in the US alone

  Freedman LP, Cockburn IM, Simcoe TS (2015) PLoS Biol 13(6): e1002165.doi:10.1371/journal.pbio.1002165

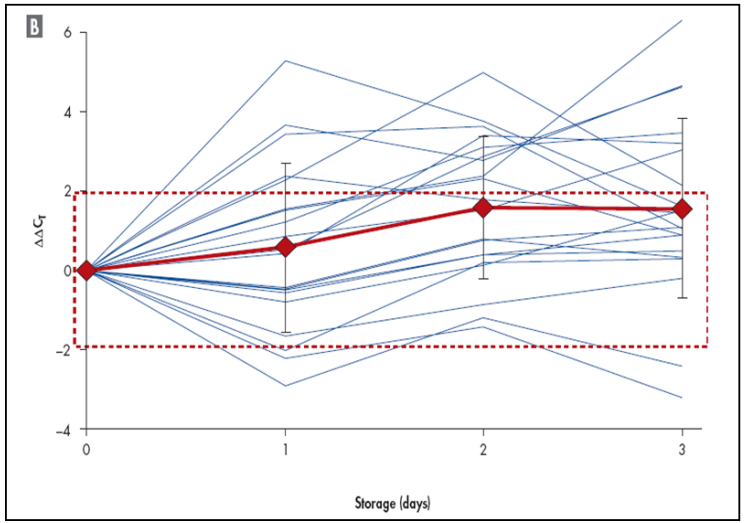
An Analytical Test Result is the Result of an Entire Workflow

Specifying, developing and verifying preanalytical workflows has to be part of the analytical test development

European Conference. Standards: Your Innovation Bridge. Brussels (2014). SPIDIA Booth.

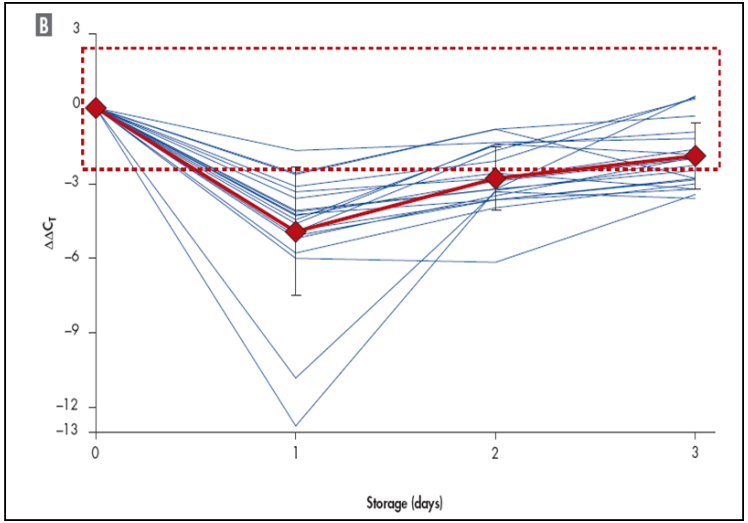
Blood RNA Profiling - Challenges with Individual Sample Kinetics

Transcription degradation and induction in human EDTA blood stored at room temperature



IL-1b mRNA

Guenther K. et al. AMP Poster (2005)



c-fos mRNA

Guenther K. et al. CLI 5, 26-28 (2008)

Deficiencies in Routine Healthcare and Research

Agenda

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Pre-analytical Factors that Influence the Outcome of ccfDNA Analysis

Preanalytical step affecting ccfDNA	Challenge	Recommendation
Blood collection tube	Release of genomic DNA from leukocytes; PCR inhibition	Use of a dedicated ccfDNA stabilization tube Use of EDTA tube, in case no dedicated ccfDNA stabilization tube is available

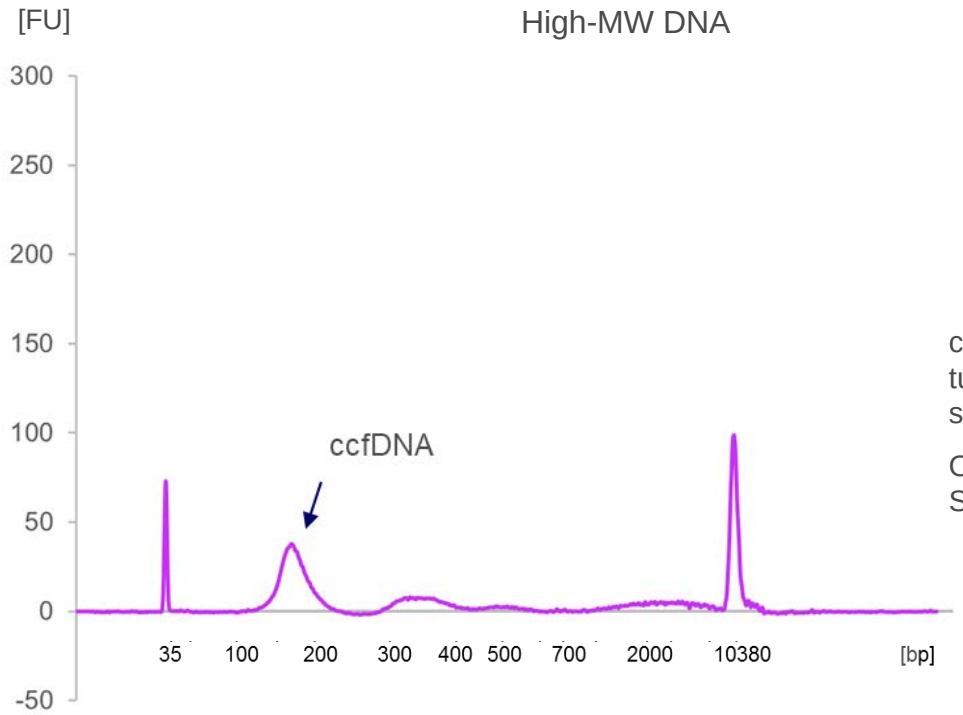
„the entire workflow, including specimen/sample storage and transport conditions, and its impact on the stability of biomolecules intended to be examined shall be verified and validated“

ISO 20186-3:2019
Molecular in vitro diagnostic examinations — Specifications for pre- examination processes for venous whole blood — Part 3: Isolated circulating cell free DNA from plasma.

Grölz et al. Curr Pathobiol Rep. 2018;6(4):275-286

Pre-analytical Factor - Blood Collection Type Impact on Size Distribution

Apoptosis of white blood cells leads to release of high molecular weight DNA



ccfDNA extracted from plasma collected using EDTA tubes from a subject directly after blood draw (t0) and after seven days at room temperature (T7d, 25°C)

One-microliter eluates analyzed using Agilent© High Sensitivity DNA Kit

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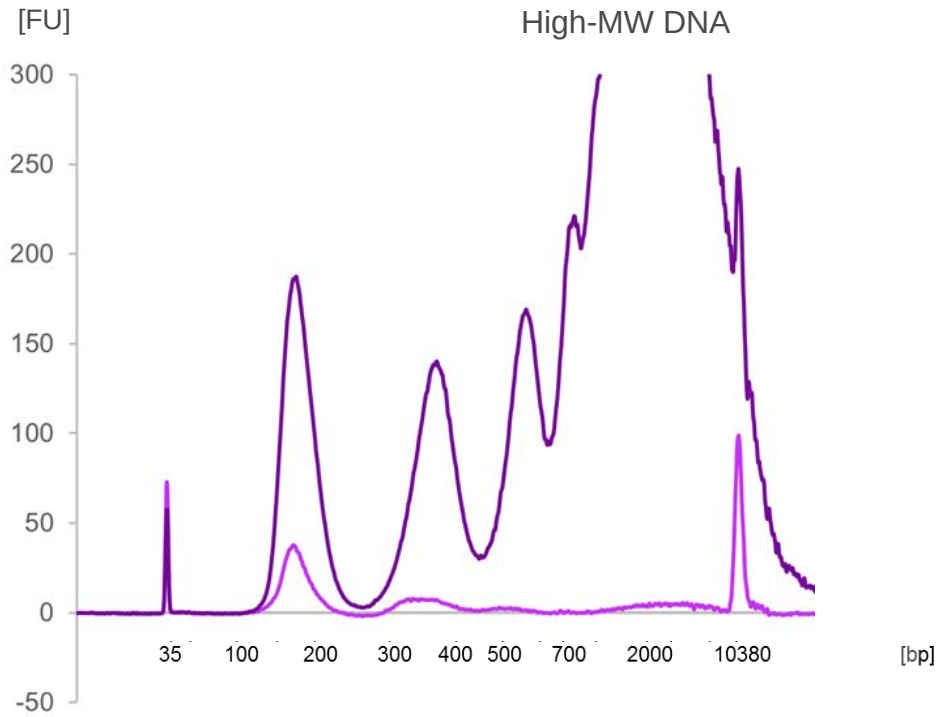
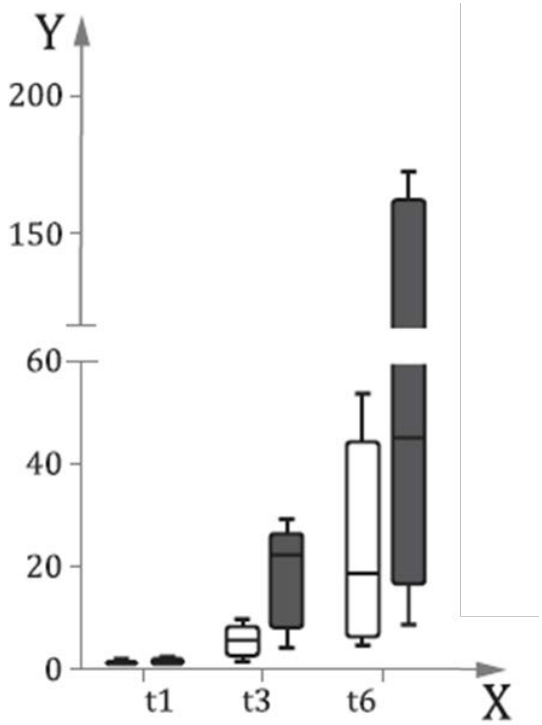


ccfDNA extracted from plasma collected using EDTA tubes from a subject directly after blood draw (t0) and after seven days at room temperature (T7d, 25^{o}C)

One-microliter eluates analyzed using Agilent High Sensitivity DNA Kit

Pre-analytical Factor – Blood Collection Impact on Yield

Apoptosis of white blood cells leads to increased yield and dilution of the target ccfDNA



18S rDNA gene, 66 bp amplicon

18S rDNA gene, 500 bp amplicon

X whole blood storage duration at room temperature before plasma generation (t1,3,6: 1, 3, and 6 days)

Y ratio of 18S rDNA copy numbers determined in plasma after indicated blood storage durations versus immediately after blood collection (t0)

ISO 20186-3:2019
Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for venous whole blood
— Part 3: Isolated circulating cell free DNA from plasma. Annex A.

Pre-analytical Factor – Plasma Processing Protocol

Follow manufacturer's centrifugation protocols to avoid gDNA carryover or reduced volume of separated plasma

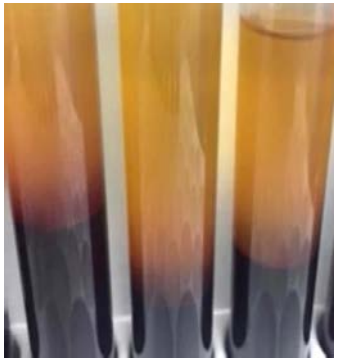
Manufacturer recommendation for PAXgene Blood ccfDNA Tube*

- 1st Spin 15 min at 1600-3000 x g
- 2nd Spin 10 min at 1600-3000 x g
- Medium brake

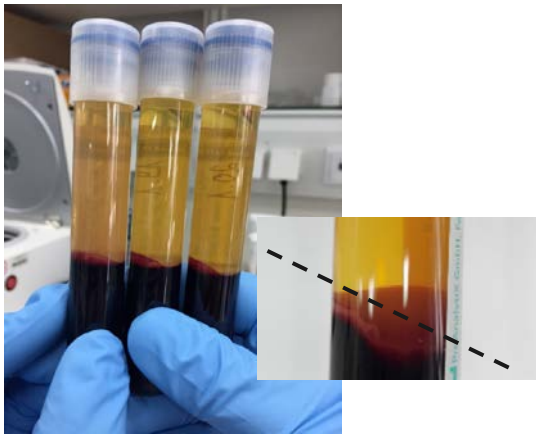
⇒ Best outcome: Maximum recovery of clear plasma with compact level interface

Reduced centrifugation time:

5 min at 1600 x g

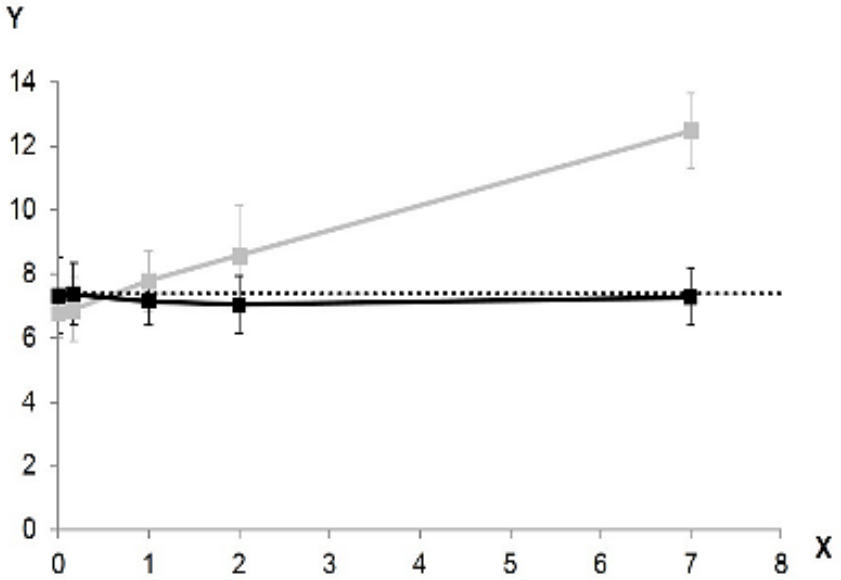


Maximum brake:



*For Research Use Only in the U.S. The PAXgene Blood ccfDNA Tube (CE-IVD) is available in some parts of the world outside the U.S.

Post Blood Collection ccfDNA Profile Changes - Impact on EGFR Test



X venous whole blood storage duration (in days) before plasma preparation

Y ΔCT = CT (mutant) - CT (wildtype control)

EDTA Blood

Stabilized Blood

Threshold (given by the examination provider)

The average of 7 donors is shown

ISO 20186-3:2019
Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for venous whole blood
— Part 3: Isolated circulating cell free DNA from plasma. Annex A.

Blood collected in EDTA and PAXgene Blood ccfDNA Tubes*
Spiked with restriction enzyme treated EGFR DNA with mutation T790M, equivalent to 200 copies ccfDNA tested with the commercially available EGFR Plasma PCR Kit (RUO)

*For Research Use Only in the U.S. The PAXgene Blood ccfDNA Tube (CE-IVD) is available in some parts of the world outside the U.S.

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SPIDIA: New Technologies and Standards for Pre-analytical Workflows

SPIDIA – FP7 (2008 – 2013)

- ⇨ 16 Partners
- New technologies for sample collection, stabilization, processing, transport, storage (Blood, Tissues)
- 9 EU CEN Standards

SPIDIA4P – H2020 (2017 – 2020)

- ⇨ 19 Partners
- ⇨ 14 associated consortia & stakeholder organizations
- 13 additional new CEN & ISO Standards
- EQAs
- European implementation

www.spidia.eu ⇨ **New Website. Subscribe to the newsletter!**

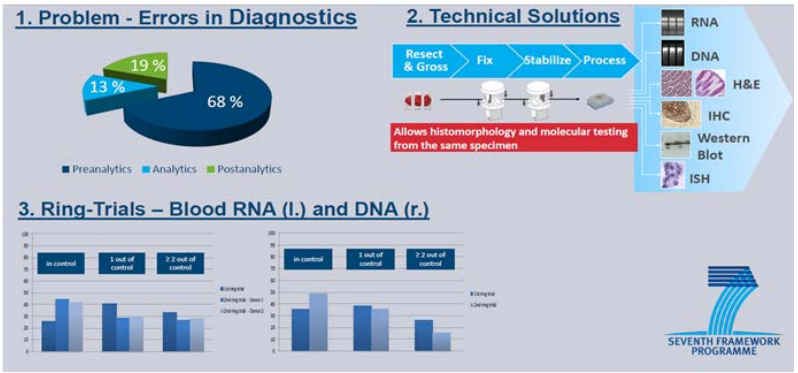
The SPIDIA project has received funding under the Seventh Research Framework Program of the European Union, FP7-HEALTH-2007-1.2.5, under grant agreement no. 222916. The SPIDIA4P project receives funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no. 733112.

SPIDIA's Road to Standardization

Vienna Agreement 1991

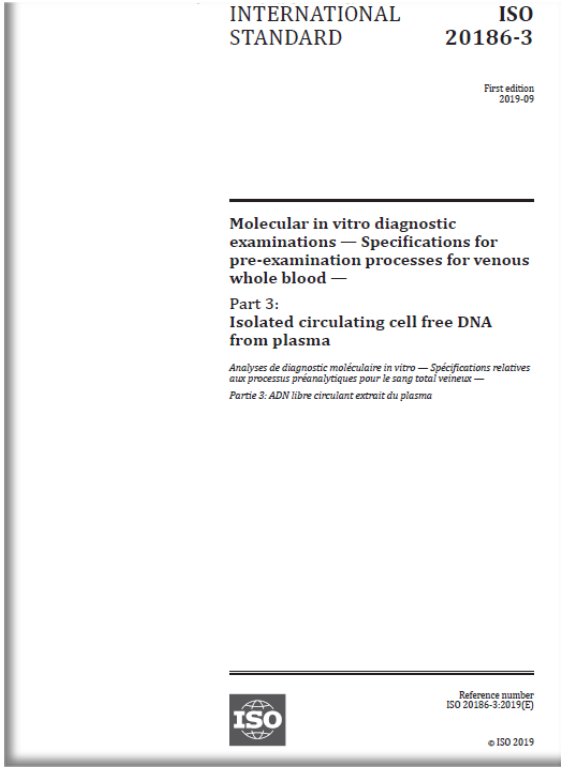
- 2019: 8 ISO/International Standards
- 2014: 8 new projects for ISO Standards approved in ISO/TC 212 „*Clinical laboratory testing and in vitro diagnostic test systems*"

- 2015: 9 CEN Technical Specifications published
- 2013: 9 new projects approved in CEN/TC 140 „*In vitro diagnostic medical devices*"
- 2010: Start of standardization work



European Conference. Standards: Your Innovation Bridge. Brussels (2014). SPIDIA Booth.

22 CEN & ISO Standard Documents and EQAs by 2021



INTERNATIONAL STANDARD **ISO 20186-3**

First edition 2019-09

Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for venous whole blood —

Part 3:
Isolated circulating cell free DNA from plasma

Analyses de diagnostic moléculaire in vitro — Spécifications relatives aux processus préanalytiques pour le sang total veineux —
Partie 3: ADN libre circulant extrait du plasma

Reference number ISO 20186-3:2019(E)

© ISO 2019

Molecular in-vitro diagnostic examinations - Specifications for pre-examination processes for

- Blood — Cellular RNA, gDNA, ccfDNA, *ccfRNA*
- Blood – *Exosomes, ccfRNA*
- Circulating Tumor Cells – **DNA, RNA, staining**
- Tissue (FFPE) — DNA, RNA, Proteins
- Tissue (Frozen) – RNA, Proteins, **DNA**
- Tissue (FFPE) - *staining*
- Fine Needle Aspirates – *DNA, RNA, Proteins*
- Saliva – **DNA**
- Urine & Body Fluids – *cfDNA*
- Microbiome – *Stool, Saliva etc.*

published CEN

published ISO

in development

https://www.spidia.eu/

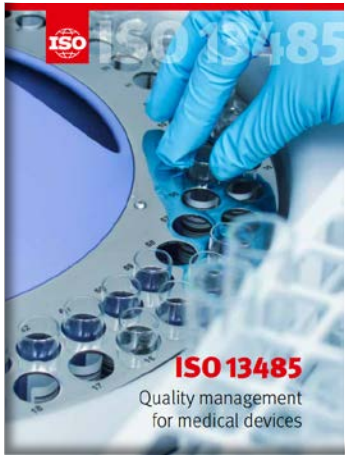
New In Vitro Diagnostic Regulations 2017 (IVDR)

- New European In Vitro Diagnostic Regulation in force since May 2017
- Pre-analytical workflow parameters in several sections
  - 6. PRODUCT VERIFICATION AND VALIDATION (Annex II)
  - 6.1. Information on analytical performance of the device
  - 6.1.1. Specimen type

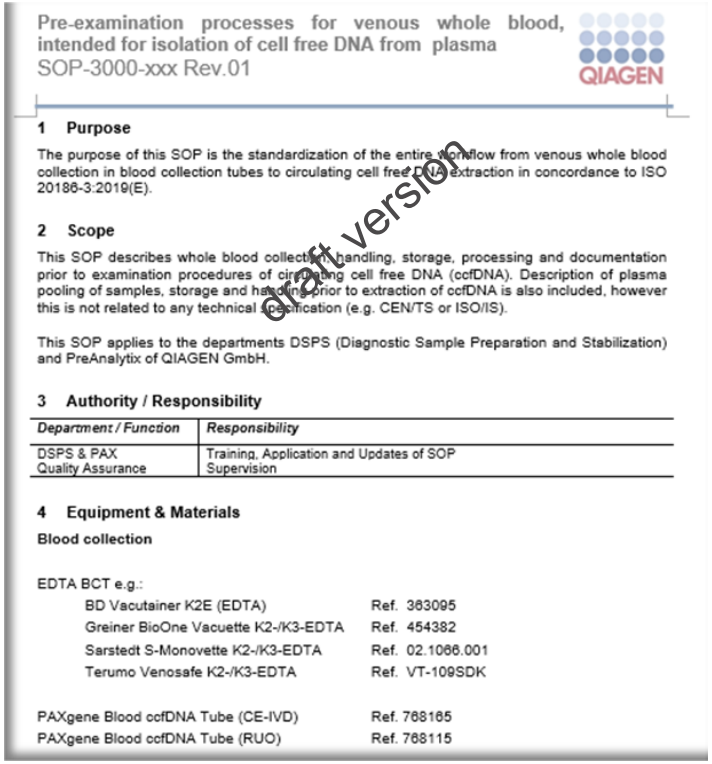
    This Section shall describe the different specimen types that can be analysed, including their stability such as storage, where applicable specimen transport conditions and, with a view to time-critical analysis methods, information on the timeframe between taking the specimen and its analysis and storage conditions such as duration, temperature limits and freeze/thaw cycles

Implementation of Preanalytical Standards

PreAnalytiX, a QIAGEN/BD Company, and SPIDIA4P partner



Product Development Process


Pre-examination processes for venous whole blood, intended for isolation of cell free DNA from plasma
SOP-3000-xxx Rev.01

QIAGEN

1 Purpose

The purpose of this SOP is the standardization of the entire workflow from venous whole blood collection in blood collection tubes to circulating cell free DNA extraction in concordance to ISO 20186-3:2019(E).

2 Scope

This SOP describes whole blood collection, handling, storage, processing and documentation prior to examination procedures of circulating cell free DNA (ccfDNA). Description of plasma pooling of samples, storage and handling prior to extraction of ccfDNA is also included, however this is not related to any technical specification (e.g. CEN/TS or ISO/IS).

This SOP applies to the departments DSPS (Diagnostic Sample Preparation and Stabilization) and PreAnalytix of QIAGEN GmbH.

3 Authority / Responsibility

Department / Function	*Responsibility*
DSPS & PAX Quality Assurance	Training, Application and Updates of SOP Supervision

4 Equipment & Materials

Blood collection

EDTA BCT e.g.:

BD Vacutainer K2E (EDTA)	Ref. 363095
Greiner BioOne Vacuette K2-/K3-EDTA	Ref. 454382
Sarstedt S-Monovette K2-/K3-EDTA	Ref. 02.1066.001
Terumo Venosafe K2-/K3-EDTA	Ref. VT-109SDK

PAXgene Blood ccfDNA Tube (CE-IVD)	Ref. 768165
PAXgene Blood ccfDNA Tube (RUO)	Ref. 768115

draft version

Certification according to ISO 13485

Company Quality Manual: Process Landscape

Global Process SOPs incl. legal requirements

Technical SOPs for pre-analytical workflows based on ISO & CEN standards

ISO 20186-3 – Pre-examination Processes for Blood ccfDNA

Contents

ISO 20186-3:2019 - Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for venous whole blood — Part 3: Isolated circulating cell free DNA from plasma

Product Development According to ISO Standards

Blood donation date	07.01.2020
Experiment	MRa0053 Feasibility Precision02
Standard	According to ISO 20186-3 Isolated circulating cell free DNA from plasma

Technical SOP: pre-examination process for ccfDNA based on ISO standard

Worksheet: **Outside the laboratory**

Donor Information, Blood Collection and Transport into the Laboratory

Donor ID	Type and number (#) of blood tubes collected	Blood sample ID (labeling)	Time Blood collection [hh:mm]	Gender	Health status and relevant lifestyle factors of the blood donor	Venipuncture technique	Phlebotomist - Name	Tampering with and/or additions to primary sample	Blood collection tubes sample storage conditions until transport into the lab	Blood sample storage time until transport into the lab. [min]	Blood collection tubes transport conditions
MRa0053 D1	10ml BD-EDTA spray dried (1) 10ml PAXgene Blood ccfDNA Tube (9)	MRa0053 D1-E1 D1- P1-9	8:50	unknown	healthy	BD blood collection set, 23G	BAD - N. Mustermann	Underfilled 50%	horizontal, room temp.	< 60min	horizontal in bags
MRa0053 D2	10ml BD-EDTA spray dried (1) 10ml PAXgene Blood ccfDNA Tube (9)	MRa0053 D1-E1 D1- P1-9	9:00	male	healthy	Sartstedt blood collection set, 21 G	BAD - N. Mustermann	Incomplete mixing 2x	standing upright in tray, room temp.	< 60min	standing upright in tray
MRa0053 D3	10ml Streck cfDNA (10)	MRa0053 D3-T1 bis T10	9:10	female.	healthy	Sartstedt blood collection set, 23 G	BAD - N. Mustermann	According IFU	….	….	….
…											
…											
…											

example

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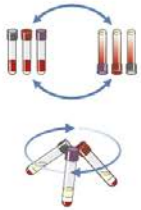
Pre-analytical Steps: Part of a Whole Diagnostic Test Workflow

PreAnalytiX
A QIAGEN / BD Company

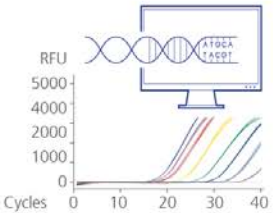
Sample Collection	Preanalytical Workflow	Analytical Assays	Insight

QIAsymphony

PAXgene Blood ccfDNA Tube

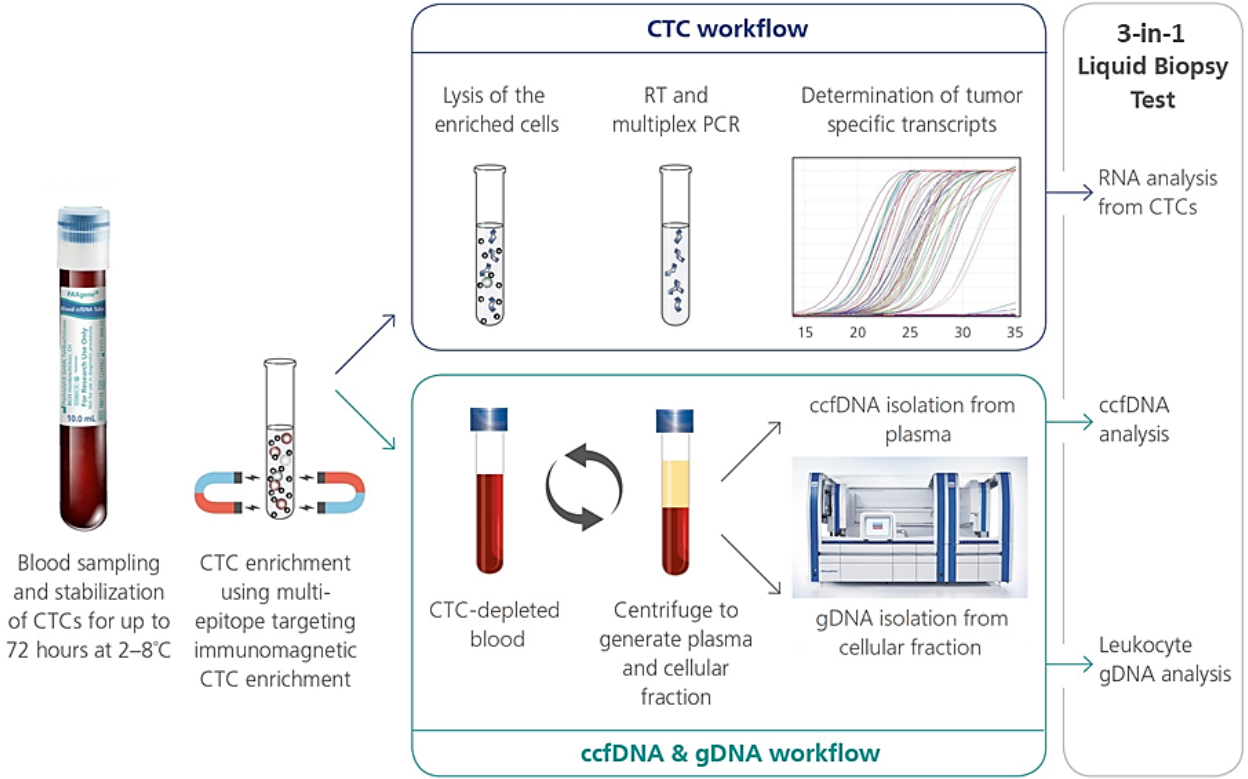


QIAsymphony
PAXgene Blood ccfDNA Kit



Blood collection and stabilization → Transport, storage, plasma separation → ccfDNA extraction → Real-time PCR, NGS, other → Data analysis

Multimodal Analysis: Extraction and Analysis of CTC RNA, ccfDNA and gDNA from a Single Blood Sample*†



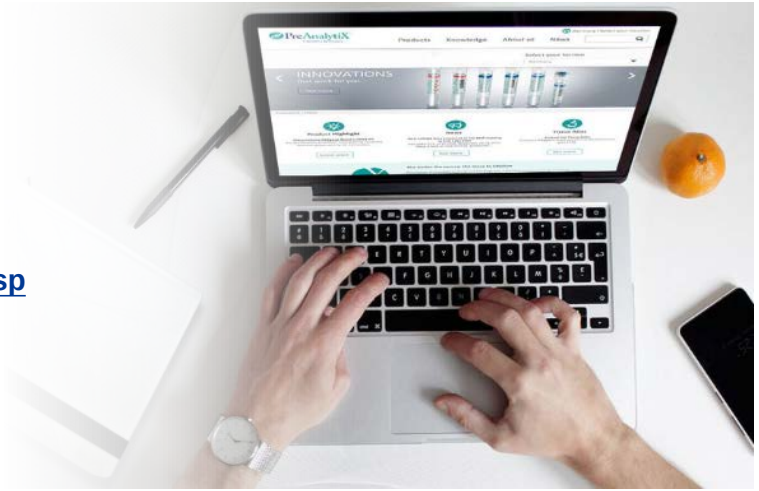
*The workflow presented is For Research Use Only. Not for use in diagnostic procedures.
†This research was conducted using the PAXgene Blood ccfDNA Tube (RUO) which is available in the United States and other parts of the world outside of Europe.

Babayan et al. [abstract]. Proceedings: AACR Advances in Liquid Biopsies 2020; January 13-16, 2020; Miami, FL.

Thank you for your attention

Questions?

Q&A session



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