Standardized Pre-analytics: The Key for Reliable and Sensitive Molecular Cancer Diagnostics & Research

AACR Annual Meeting 2023, April 17th

Dr. Uwe Oelmueller, SPIDIA4P Coordinator, QIAGEN - Germany & PreAnalytiX - Switzerland

www.spidia.eu

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Potential Conflicts of Interest

- Employee of QIAGEN GmbH, Germany
- Management Committee Co-Chair at PreAnalytiX (QIAGEN/BD Company)
- Participation in QIAGEN's regular long-term incentive program (LTIs)
- Co-inventor on several patents and patent applications on pre-analytical workflow technologies

New Technologies and Standards for Pre-analytical Workflows

SPIDIA (2008 – 2013) – EU 7th Research Framework Program

- ⇨ 16 Partners
- ⇨ co-work with US NCI's BRN & GTEx programs and CLSI
- New technologies for sample collection, stabilization, transport, storage, processing (Blood, Tissues)
- 9 EU CEN Standards

SPIDIA4P (2017 -2021) – EU Horizon 2020 Research & Innovation Program

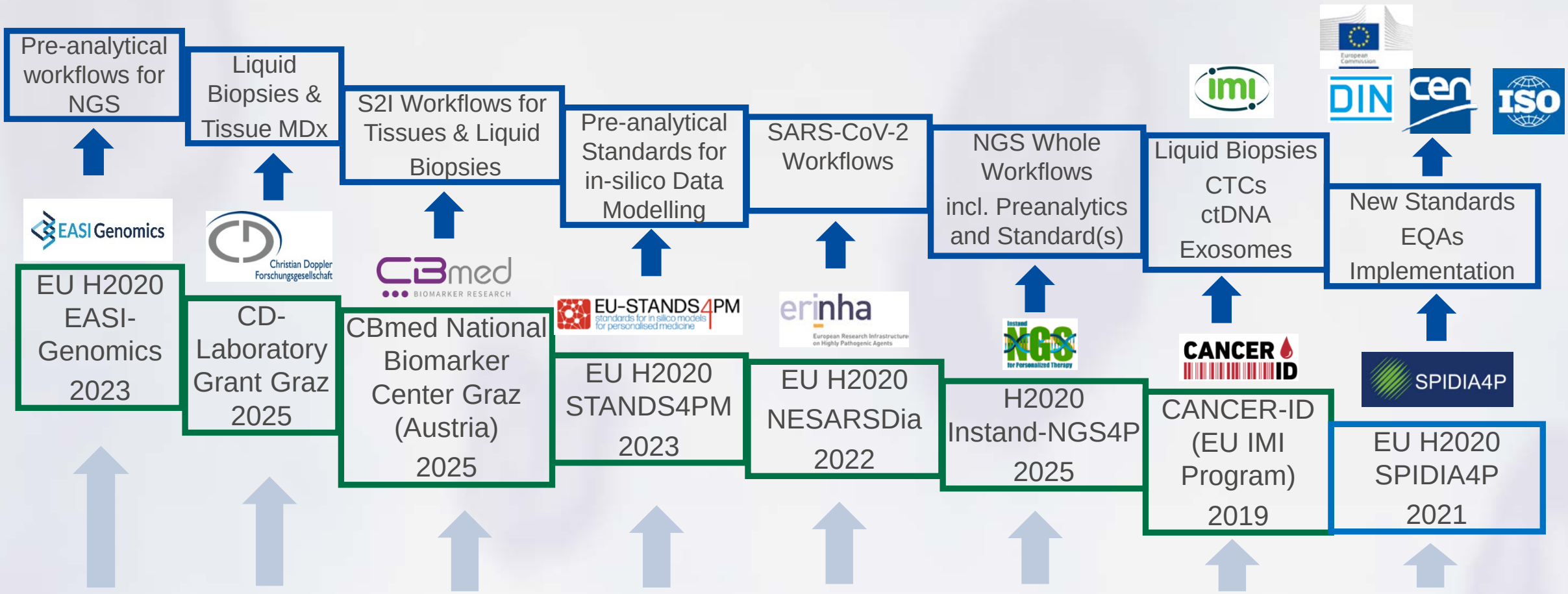
- ⇨ 19 Partners
- ⇨ 14 associated consortia & stakeholder organizations
- 13 additional new CEN & ISO Standards
- EQAs
- European and International implementation

www.spidia.eu ⇨ **Subscribe the Newsletter!**

The SPIDIA project 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 has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement no. 733112.

Largest Consortia Network for Pre-analytics in Community

⇨ Tech Developments, Standards, EQAs, Implentation, Consulting, Education



Deficiencies in Routine Healthcare and Research demand for Improvements

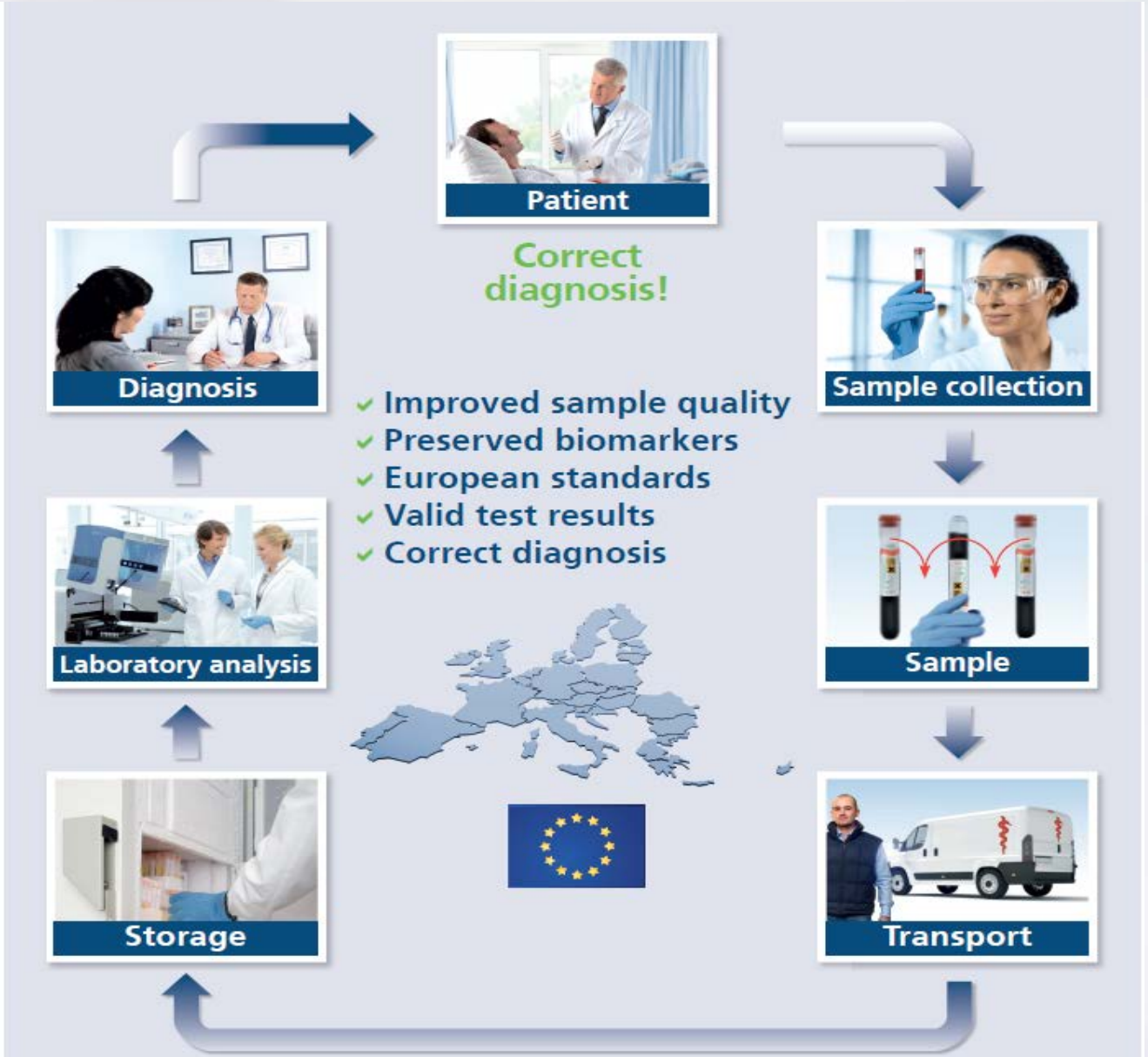
- Pre-analytical phase accounts for 46% to 68% of clinical laboratory errors

  Medical Laboratory Observer, May 2014

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

  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

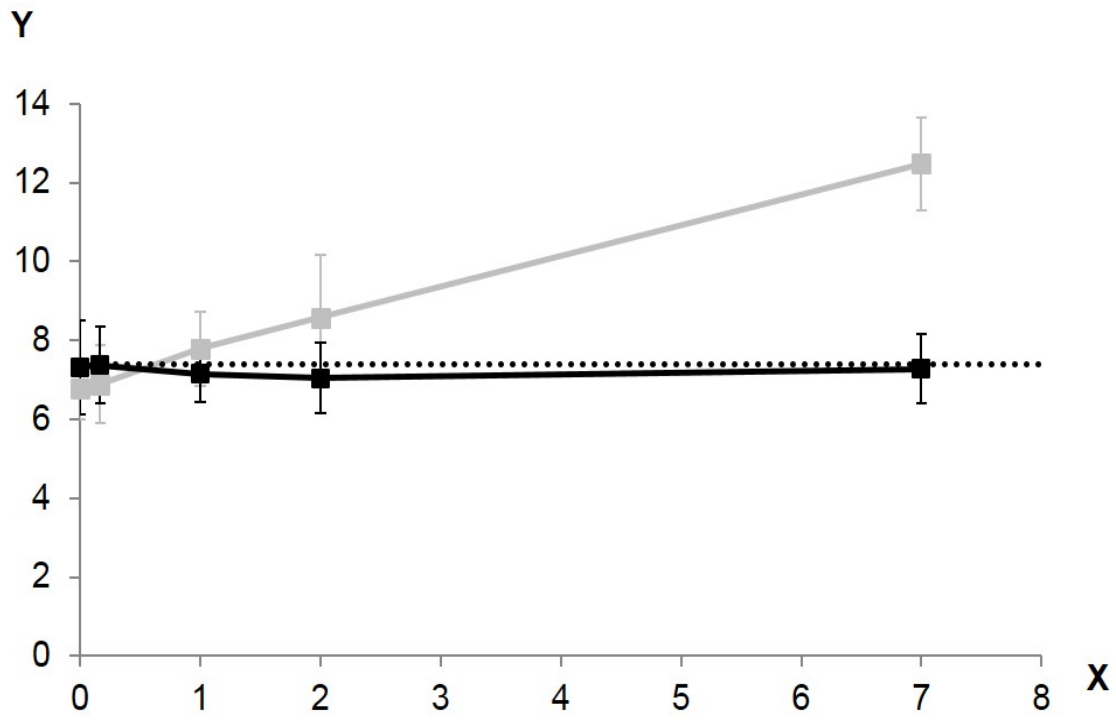


Specifying, developing, verifying and validating preanalytical workflows is an essential part of analytical test development

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

Post Blood Collection ccfDNA Profile Changes

Research Study: Impact on EGFR Test Results



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)

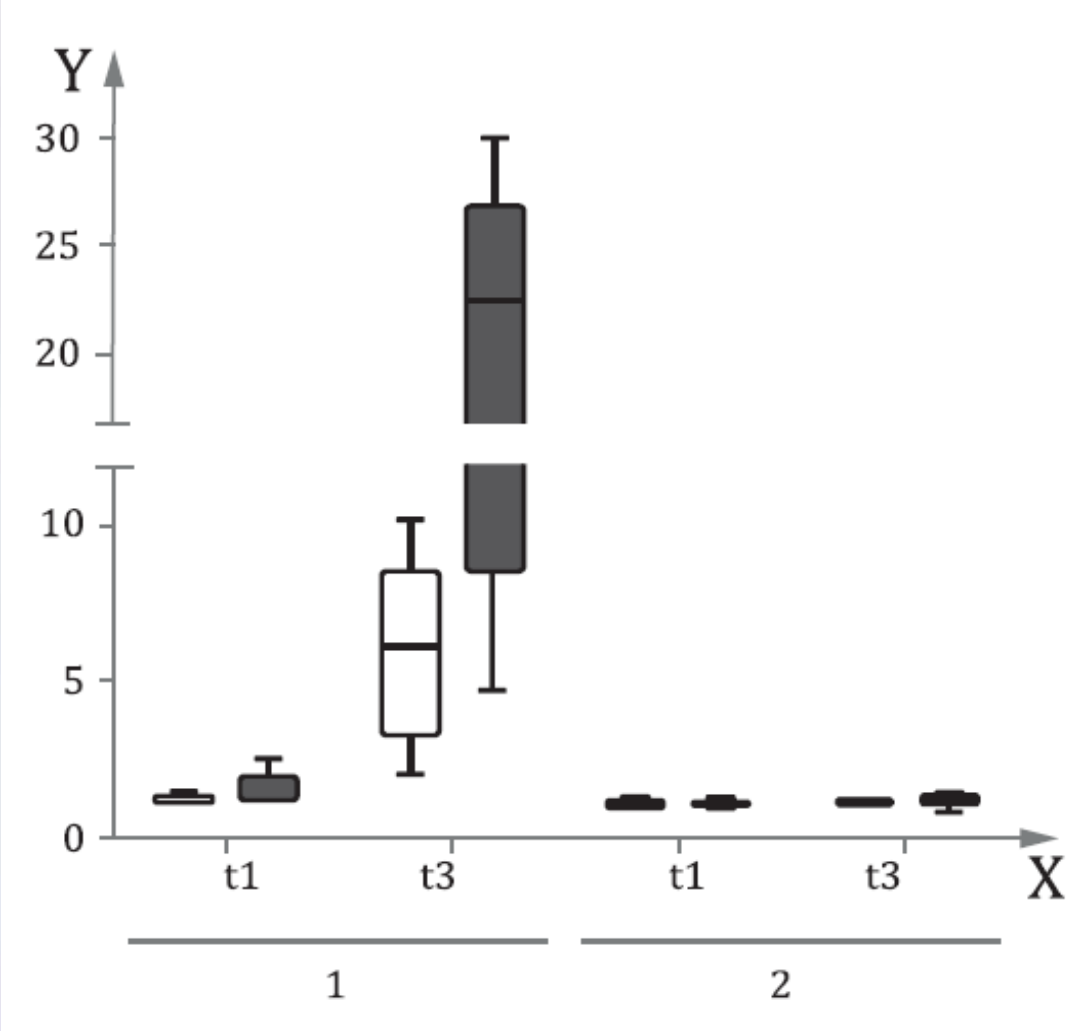
The average of 8 donors is shown

- Spiked restriction enzyme treated EGFR DNA with mutation T790M in all samples, equivalent to 200 copies
- ccfDNA tested with the commercially available EGFR Plasma PCR Kit (RUO)
- Mutation detected when result is below dotted line ⇨ mutation not detected in stored EDTA blood but in stabilized blood

Source:

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 Specimen Storage – Post Collection Release of DNA Dilutes Original ccfDNA Profile



⇨ Apoptosis of white blood cells leads to increased DNA 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)

1: EDTA Blood

2: stabilized Blood: with ccfDNA profile stabilizer

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.

Major Efforts for Improvements

- Pre-analytical Technologies
- International ISO & CEN Standards
- External Quality Assessment (EQA) Schemes

SPIDIA's initial Road to Standardization via CEN and ISO

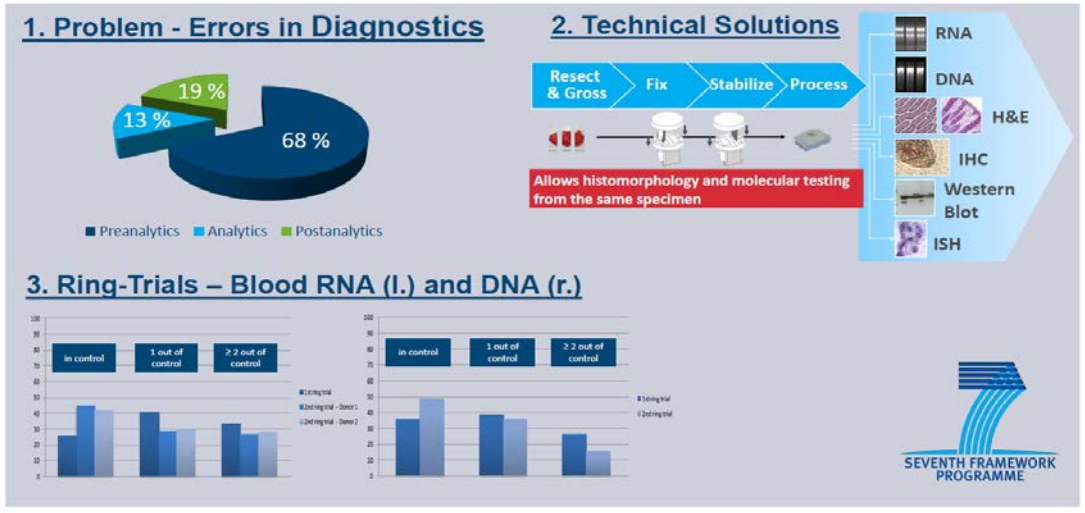
under Vienna Agreement (1991)

ISO

- 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*"

cen

- 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



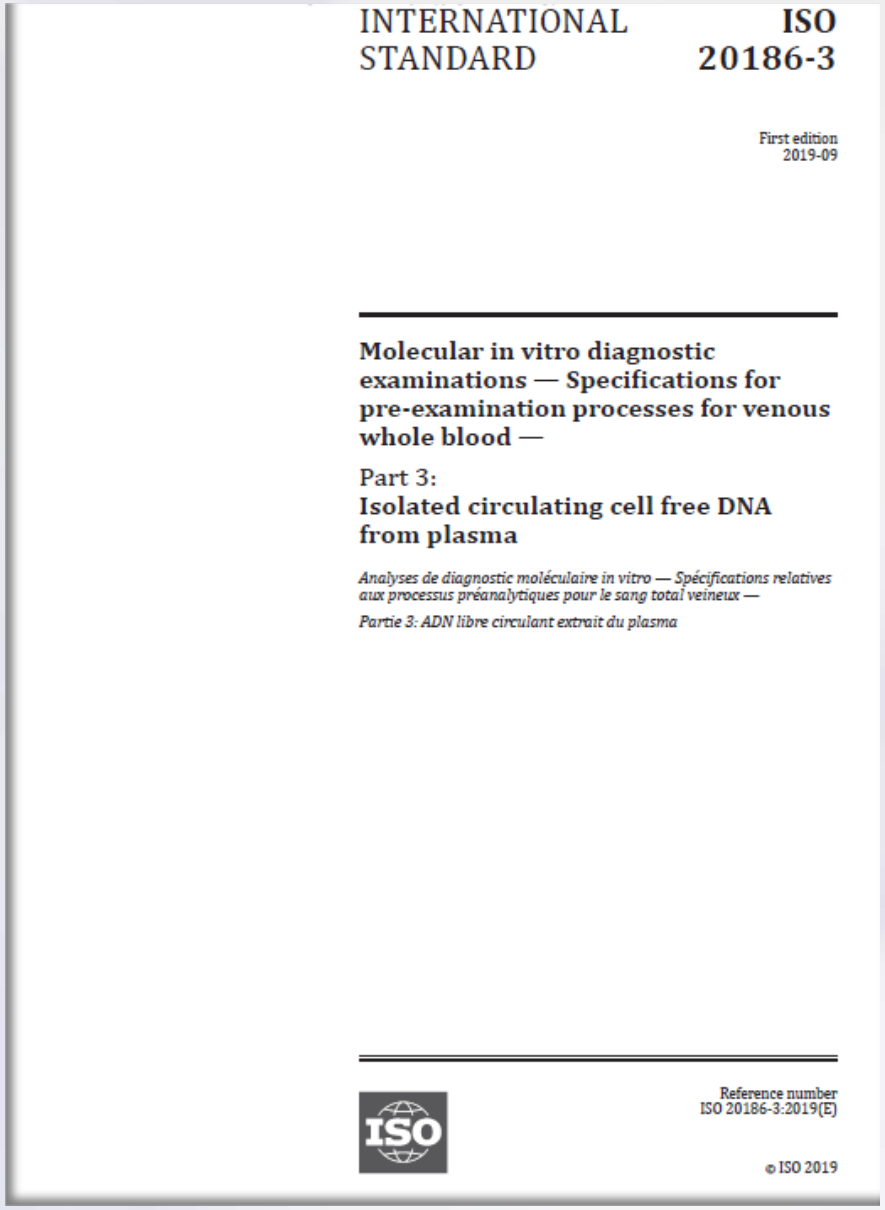
SPIDIA

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

CEN CENELEC

European Commission

22 CEN & ISO Standard Documents and EQAs (2012 – 2022)



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 / EVs
  - **Blood Tumor Cells** – DNA, RNA, staining
  - **Tissue** (FFPE) — DNA, RNA, Proteins
  - **Tissue** (Frozen) – DNA, RNA, Proteins
  - **Tissue** (FFPE) – in situ staining
  - **Fine Needle Aspirates** – DNA, RNA, Proteins
  - **Saliva** – DNA
  - **Urine & Body Fluids** – cfDNA
  - **Metabolomics** – Urine, Serum, Plasma
  - **Microbiome** – Stool, Saliva etc.

published CEN ⇨ progressing at ISO published ISO

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

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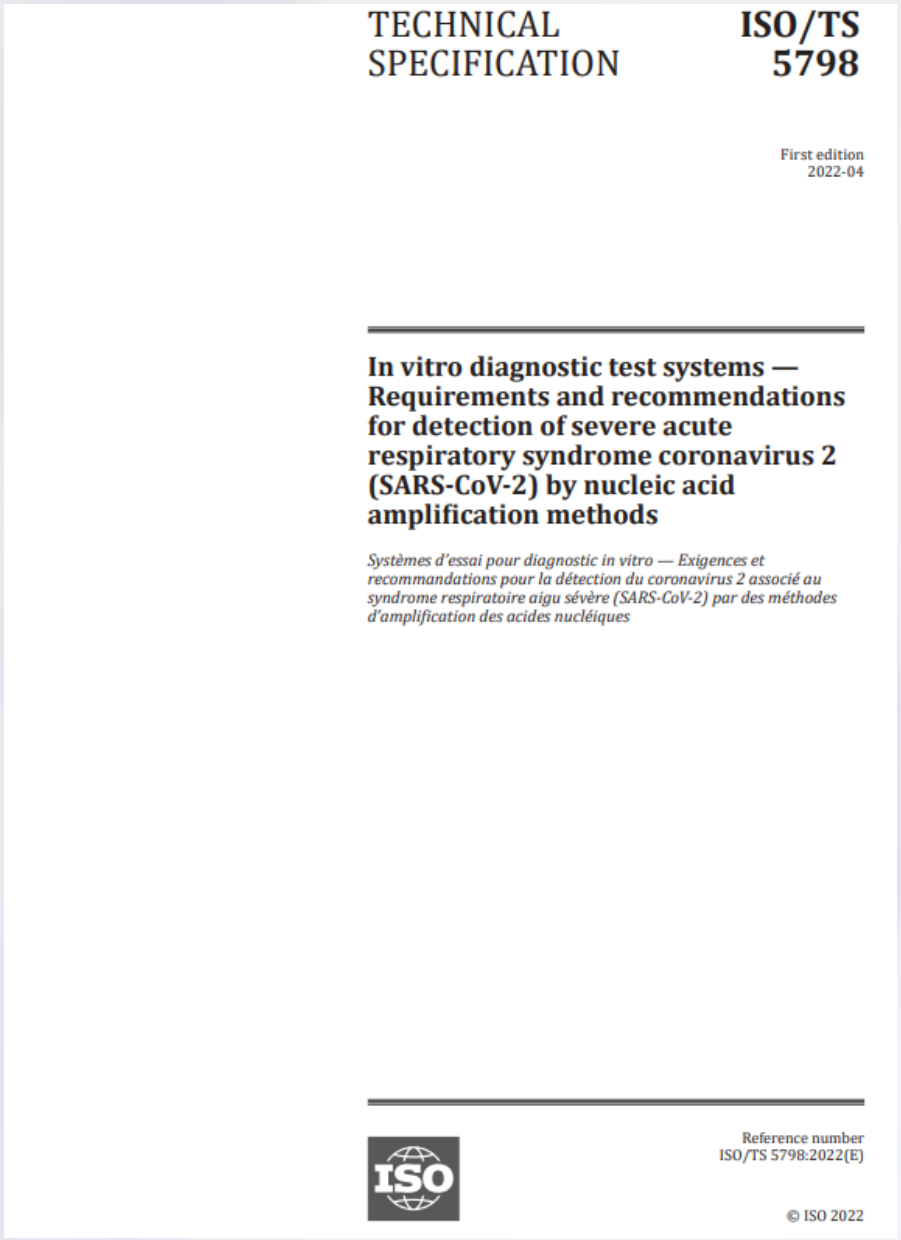
Example:

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

Pre-analytical Workflow - Same Standards for all Segments

- **Biobanks**
  - Source for good quality samples ⇨ required for biomarker & analytical test development
- **Biomedical & Translational Research**
  - Academia
  - Pharma industry
  - Diagnostic Industry
- **Diagnostics**
  - High sample quality is the safe way
  - Analytical assay might tolerate lower quality or not ⇨ Verification studies

Pre-analytical Workflow Parameters also in other new Standards



TECHNICAL SPECIFICATION

ISO/TS 5798

First edition
2022-04

In vitro diagnostic test systems — Requirements and recommendations for detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by nucleic acid amplification methods

Systèmes d'essai pour diagnostic in vitro — Exigences et recommandations pour la détection du coronavirus 2 associé au syndrome respiratoire aigu sévère (SARS-CoV-2) par des méthodes d'amplification des acides nucléiques

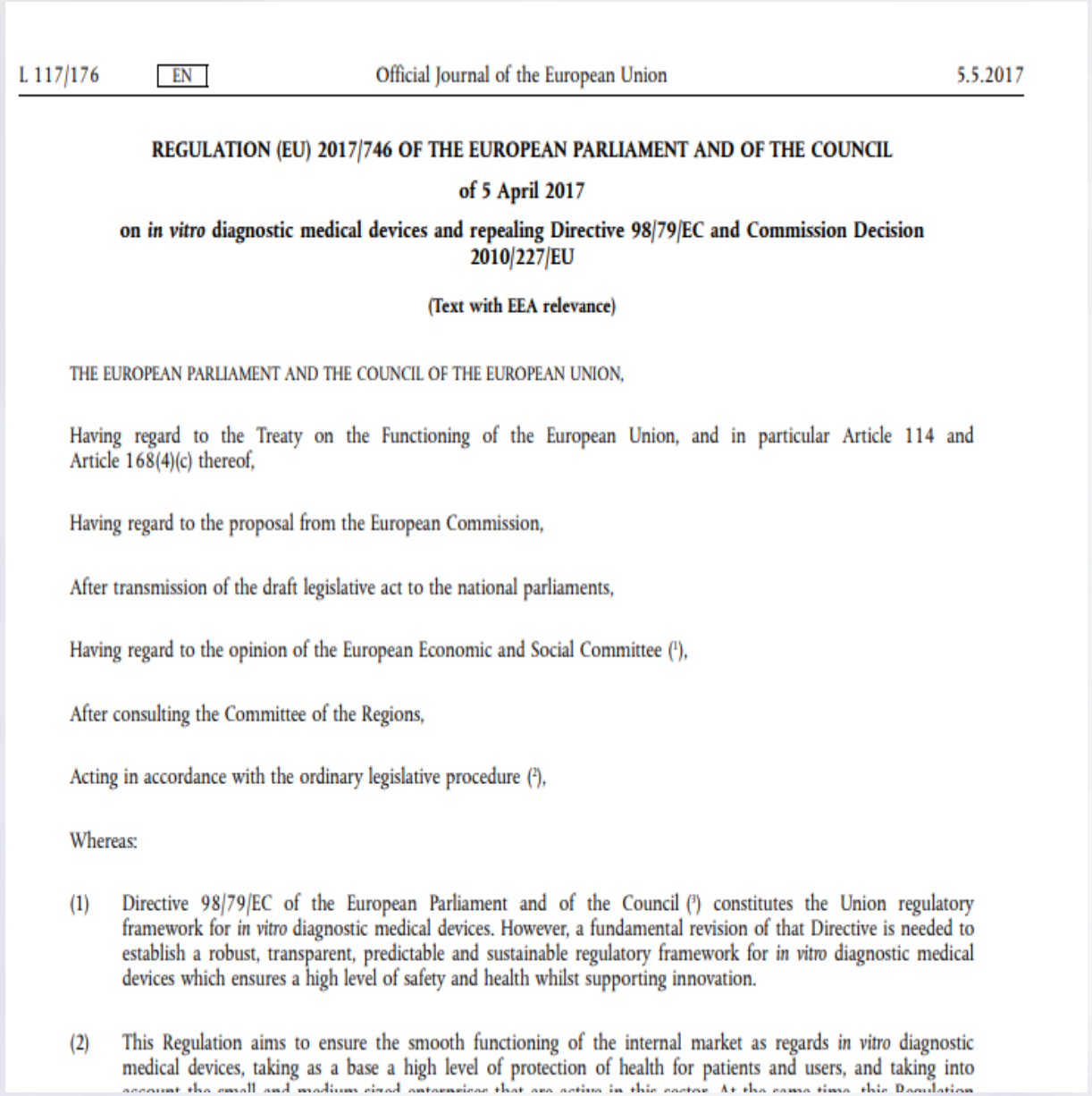
ISO

Reference number
ISO/TS 5798:2022(E)

- Example:

  ISO/TS 5798:2022 on SARS-CoV-2 detection

New EU In Vitro Diagnostic Medical Device Regulation (IVDR)



L 117/176 EN Official Journal of the European Union 5.5.2017

REGULATION (EU) 2017/746 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 5 April 2017

on *in vitro* diagnostic medical devices and repealing Directive 98/79/EC and Commission Decision 2010/227/EU

(Text with EEA relevance)

THE EUROPEAN PARLIAMENT AND THE COUNCIL OF THE EUROPEAN UNION,

Having regard to the Treaty on the Functioning of the European Union, and in particular Article 114 and Article 168(4)(c) thereof,

Having regard to the proposal from the European Commission,

After transmission of the draft legislative act to the national parliaments,

Having regard to the opinion of the European Economic and Social Committee ([1]),

After consulting the Committee of the Regions,

Acting in accordance with the ordinary legislative procedure ([2]),

Whereas:

(1) Directive 98/79/EC of the European Parliament and of the Council ([3]) constitutes the Union regulatory framework for *in vitro* diagnostic medical devices. However, a fundamental revision of that Directive is needed to establish a robust, transparent, predictable and sustainable regulatory framework for *in vitro* diagnostic medical devices which ensures a high level of safety and health whilst supporting innovation.

(2) This Regulation aims to ensure the smooth functioning of the internal market as regards *in vitro* diagnostic medical devices, taking as a base a high level of protection of health for patients and users, and taking into

- entered into force on 26 May 2017
- has replaced the EU's Directive on in vitro diagnostic medical devices (IVDD 98/79/EC)
- transition period from IVDD to IVDR ended on 26 May 2022
- amendment in Article 10 paragraphs 2, 3, 4 (Dec. 2021): devices CE-marked according to IVDD with valid certificates prior to transition end can still be sold for defined limited periods (classes D, C, B, and class A sterile)

EU IVDR requires Specification, Verification and Validation of Pre-analytical Workflow Parameters

- **Pre-analytical workflow parameters** in several sections & clauses, e.g. Annex II:

  - 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

- **State-of-the-Art** required for device developments in various law articles and annexes

QIAGEN *therascreen* PIK3CA RGQ PCR Assay – Preanalytical Workflow Parameters

May 2019

therascreen® PIK3CA RGQ PCR Kit Instructions for Use (Handbook)

Σ 24

Version 1

IVD

For in vitro diagnostic use

Rx only (For prescription use only)

For use with Rotor-Gene® Q MDx (US) instrument

For use with QIAamp® DSP DNA FFPE Tissue Kit

For use with QIAamp® Circulating Nucleic Acid Kit

REF 873121

QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, Germany

R1 MAT 1115877EN

Sample to Insight

QIAGEN

- FDA approved in 2019: CDx test

  Presence of PIK3CA mutations in cancer tissue or plasma from patients with breast cancer is linked with response to treatment with PIQRAY® (alpelisib) / Novartis

➢ Preanalytical workflow parameters are specified as part of the approved test

⇨ Example: Collection and storage duration:

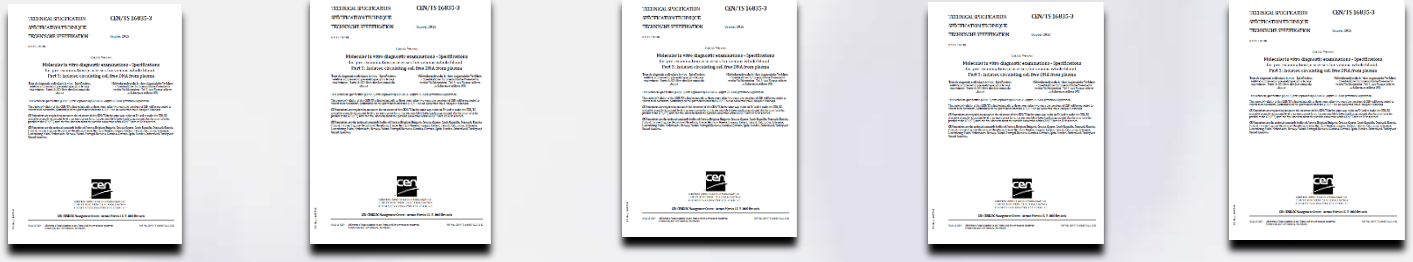
Whole peripheral venous blood collected in K_2EDTA blood collection tubes must be processed to obtain plasma within four hours of blood collection. Failure to do so may result in genomic DNA contamination of the sample. For further information on the isolation of plasma from whole blood, refer to Appendix A of the *QIAamp DSP Circulating Nucleic Acid Kit Handbook*.

Product claims may differ from country to country based on regulations and approvals. Contact your country representative for further details.

Role of Legislation, Standards and Technologies

New EU IVDR – in-vitro Diagnostic Device Regulation 2017

Pre-analytical workflow parameters



EN ISO & CEN Standards

SOPs

Technologies & Products

Major Efforts for Improvements

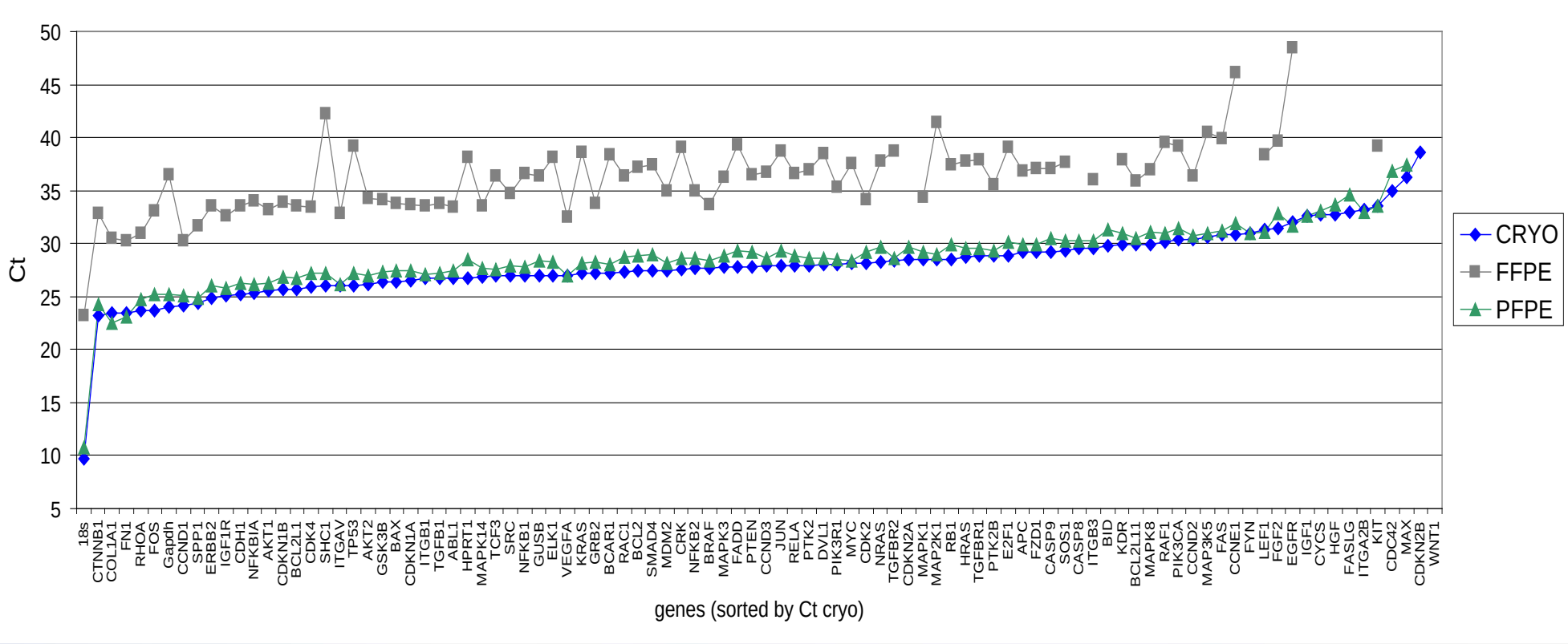
- **Pre-analytical Technologies**
- **International ISO & CEN Standards**
- **External Quality Assessment (EQA) Schemes**

New Technology Developments without Cross-Linking Methods

Research Study by Med.Univ.Graz (Austria)

RNA Profiling – Cry vs FFPE vs PFPE (PAXgene Tissue Fixed Paraffin Embedded)

Mammacarcinoma Case: TaqMan® Array Gene Signature 96-Well Plate



Viertler *et al*.. A New Technology for Stabilization of Biomolecules in Tissues for Combined Histological and Molecular Analysis. J Mol Diagn 2012, 14:458-466.

TaqMan

Consortium Member PreAnalytiX developed Products based on new Crosslinking-free Stabilization Technologies

- PAXgene Tissue System & Workflows *
  - Molecular and Morphology Stabilization
  - Recent academic partner studies demonstrated also Microbiome Stabilization

    Radani *et al.*. Gastro Help Advances 2022, 1 (5): 755-766

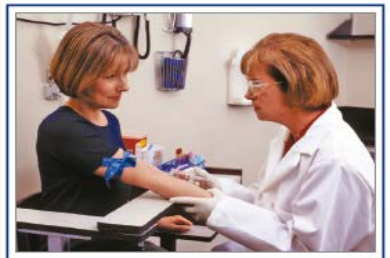
- PAXgene Blood ccfDNA Tube & Workflows *
  - Circulating Nucleic Acids Stabilization
  - Liquid Biopsies Multimodality options
  - Crosslinking-free technology can enable sensitivity advantages

    Schmidt *et al.*. Clin Chim Acta 2022, 469: 94-98

    Voss *et al.*. PLoS ONE 16(7): e0253401

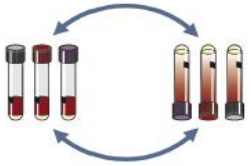
* PAXgene Tissue products and PAXgene Blood ccfDNA products are **For Research Use Only**. Not for use in diagnostics procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

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

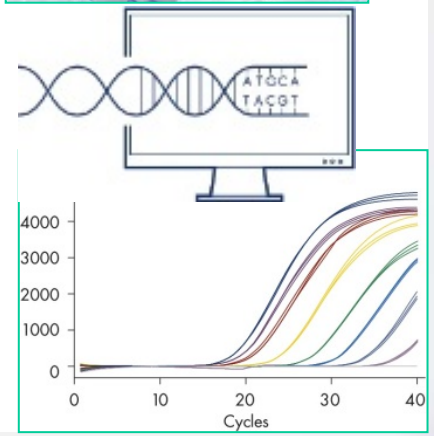


QIAsymphony

Tube

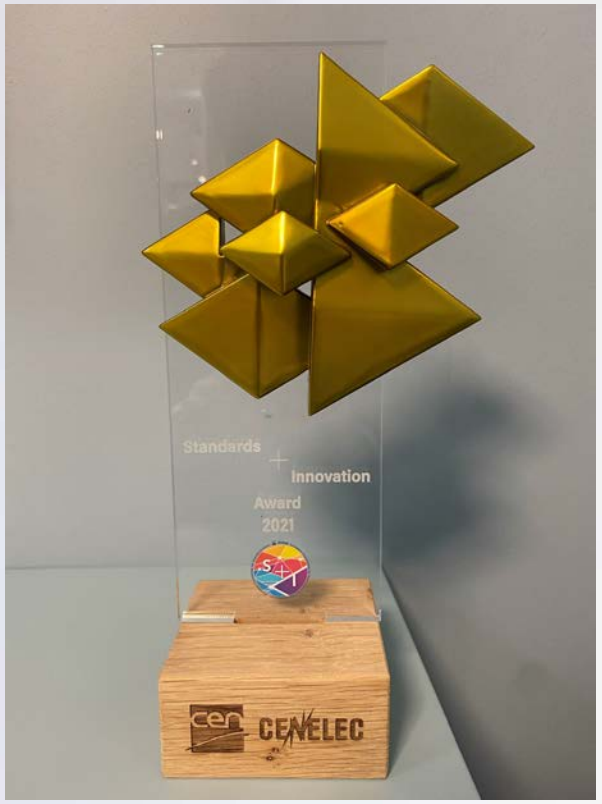


Kit



Specimen Collection & Stabilization → Storage Transport → Sample Processing → Analyte Isolation → Analytical Test → Data Analysis

EU CEN-CENELEC Standards+Innovation Project Award 2021



CEN president Vincent LAFLÈCHE announced SPIDIA4P as winner of the "Project Award 2021"

A big Thank You goes to . . .

. . . to the SPIDIA & SPIDIA4P Consortium Members, CEN/TC 140, ISO/TC 212 and all European and International Partners!

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Thank you for attending!

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A QIAGEN / BD Company

Questions?

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Faraia Shah - faraia.shah@bd.com

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