Liquid Biopsy Assays – The entire Workflow counts

esib – European Summit of Industrial Biotechnology

Graz, November 11th

Dr. Uwe Oelmueller, QIAGEN GmbH, PreAnalytiX GmbH

SPIDIA4P funded by the EU (H2020 grant agreement no. 733112, 2017-2021).

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

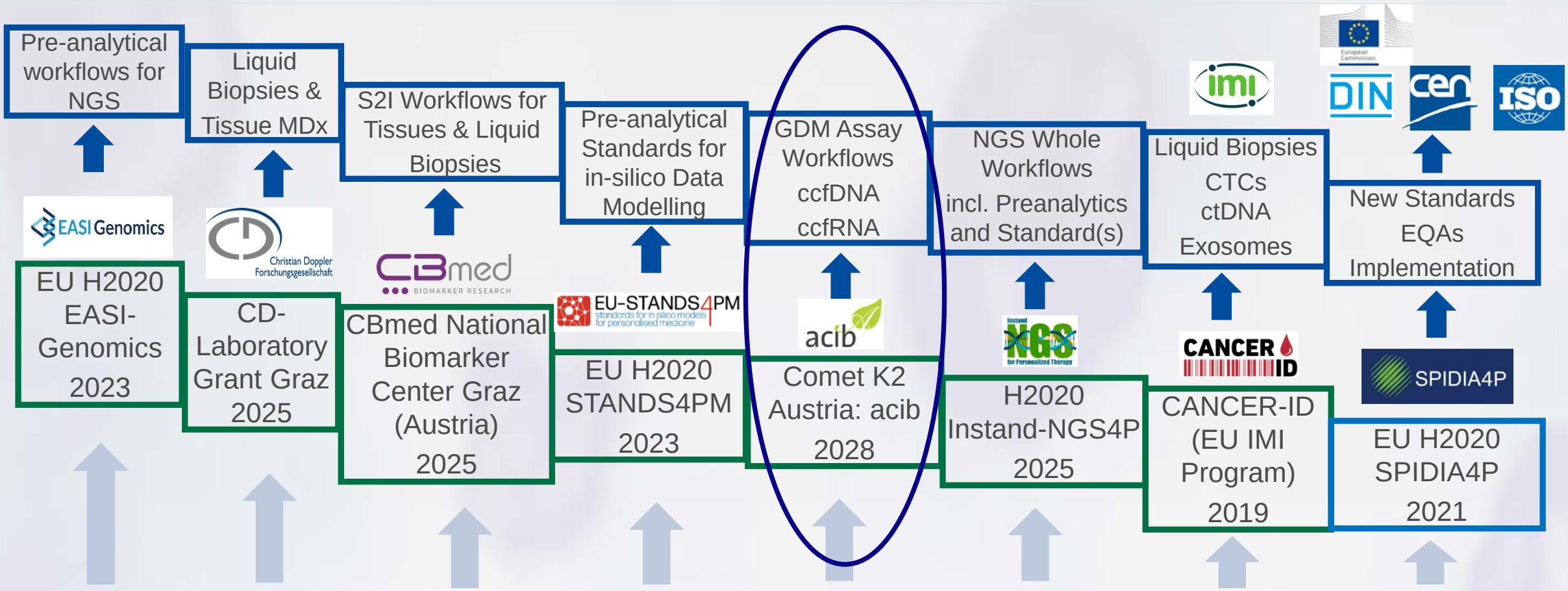
- ⇨ 19 Partners
- ⇨ 14 associated consortia & stakeholder organizations
- 13 additional new CEN & ISO Standards
- EQAs
- European and International implementation

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, Implementation, Consulting, Education



Deficiencies in Routine Healthcare and Research demand for Improvements





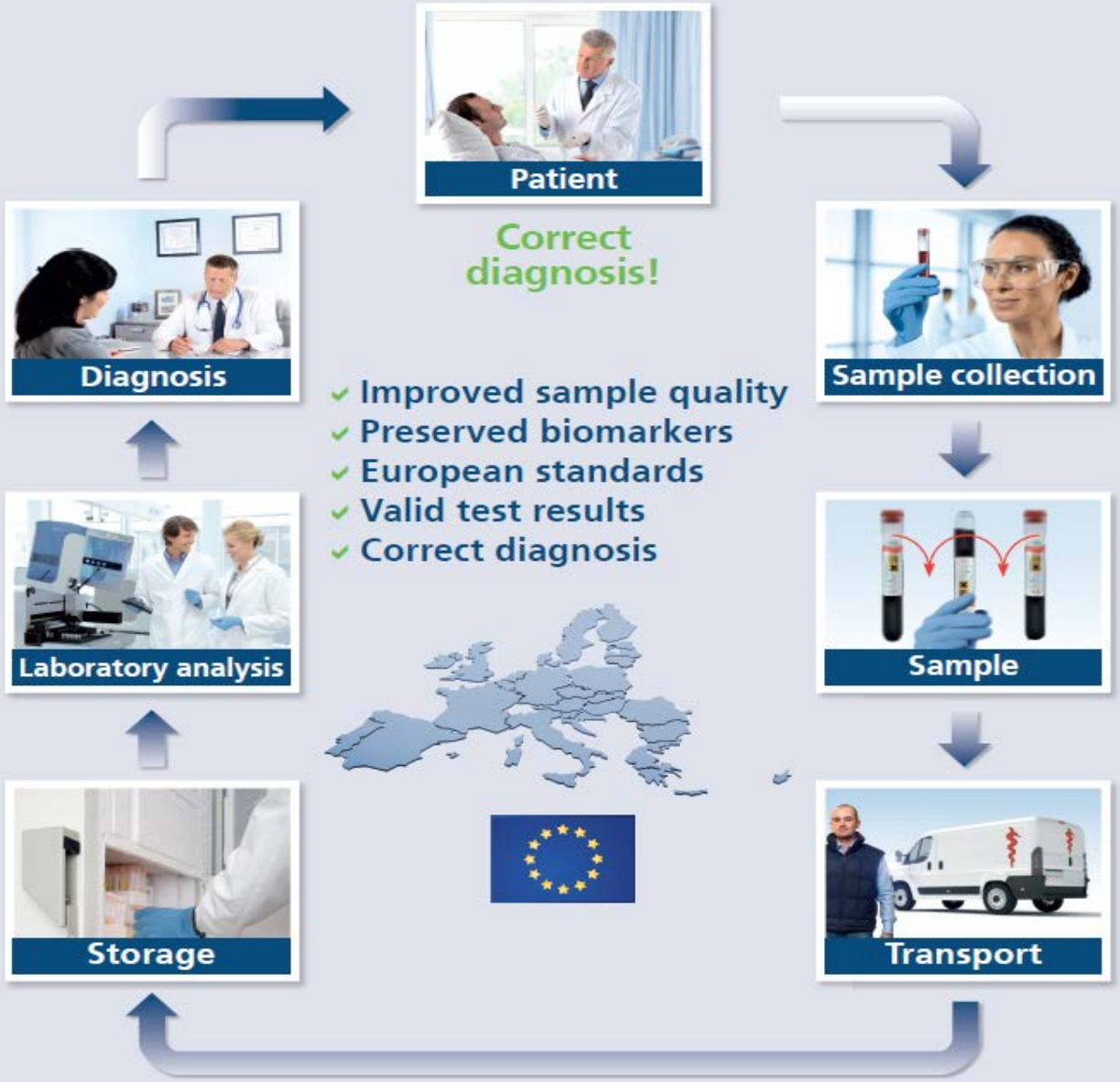
- Pre-analytical phase accounts for up to 70% of clinical laboratory errors

  Cornes M.. Annals of Clinical Biochemistry, 57/1, 2020

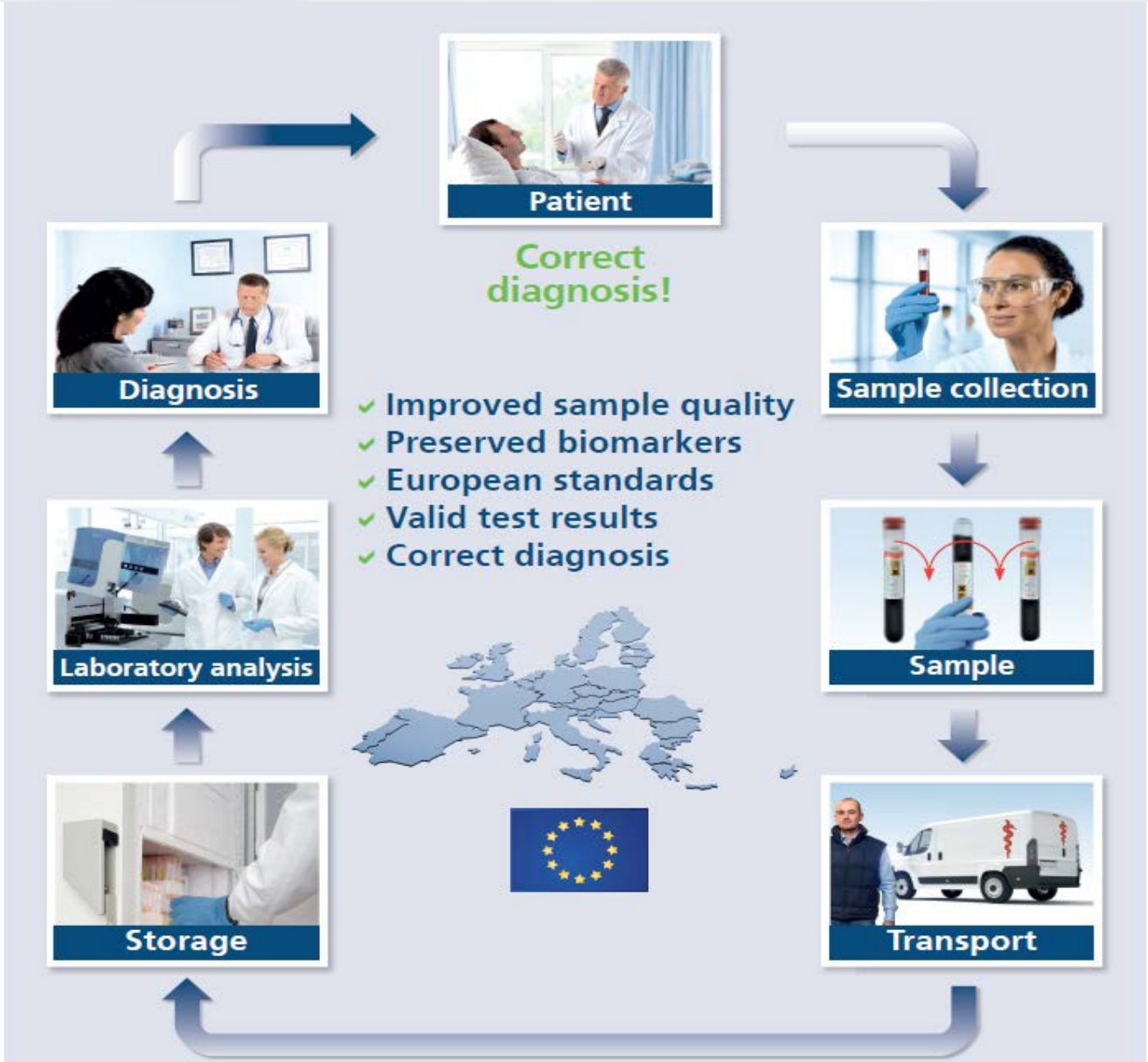
- Irreproducible preclinical research exceeds 50%, US $28B / year spent on preclinical research that is not reproducible - in the US

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

Good Quality Analytical Diagnostic Test Results are Crucial



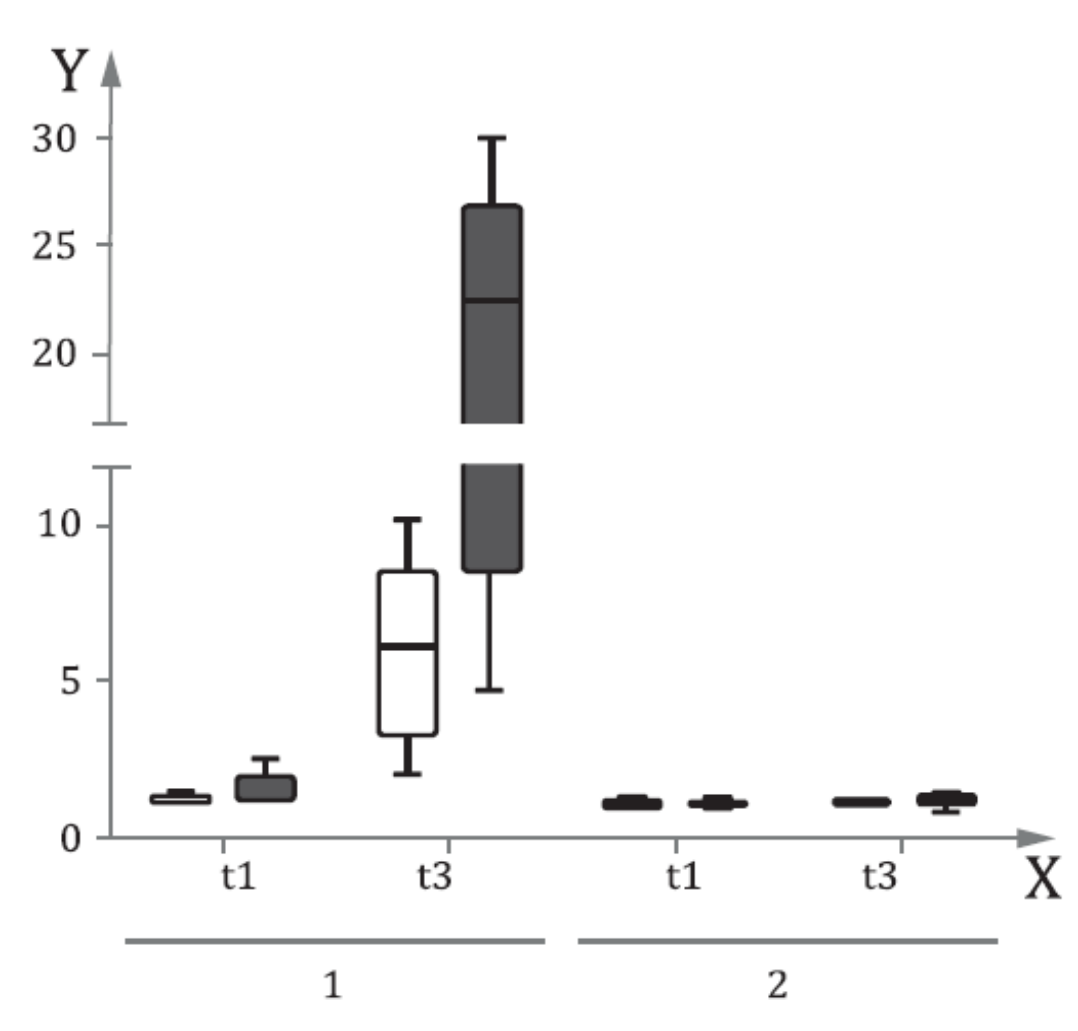
Good Quality Analytical Diagnostic Test Results are Crucial



⇨ An analytical test result is the result of an entire workflow starting and ending with the patient

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

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



⇨ Lysis 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)

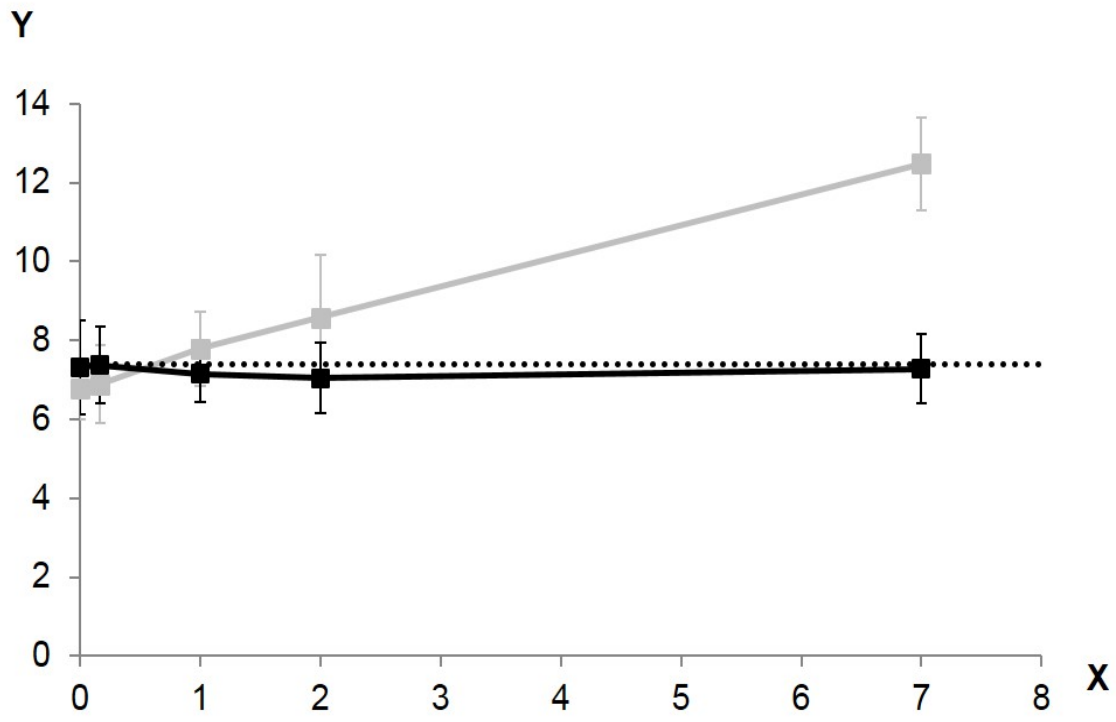
1: EDTA Blood

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.

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.

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.

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Efforts for Improvements

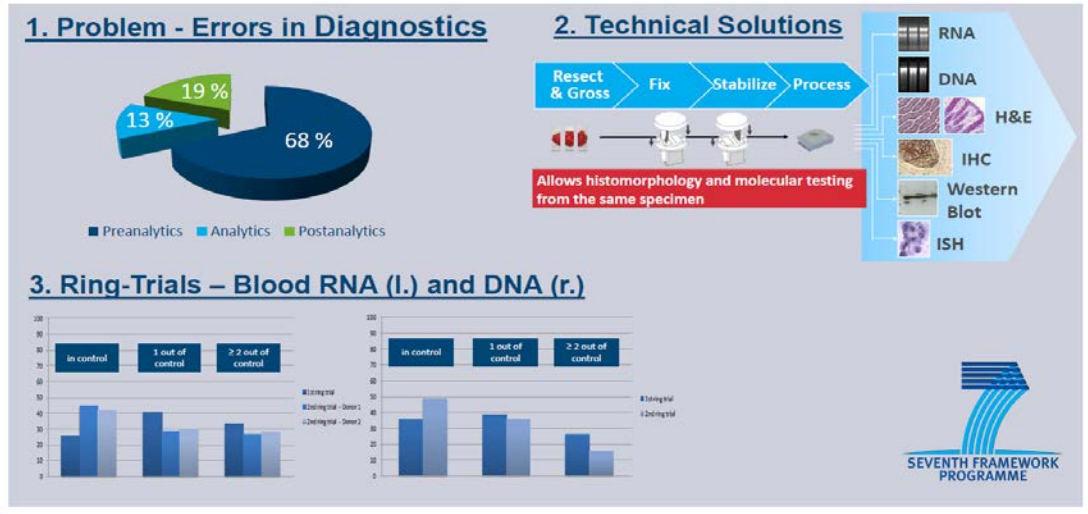
- Pre-analytical Technologies
- International ISO & CEN Standards
- External Quality Assessment (EQA) Schemes

The Road to Pre-examination Standardization via CEN & ISO

under Vienna Agreement (1991)

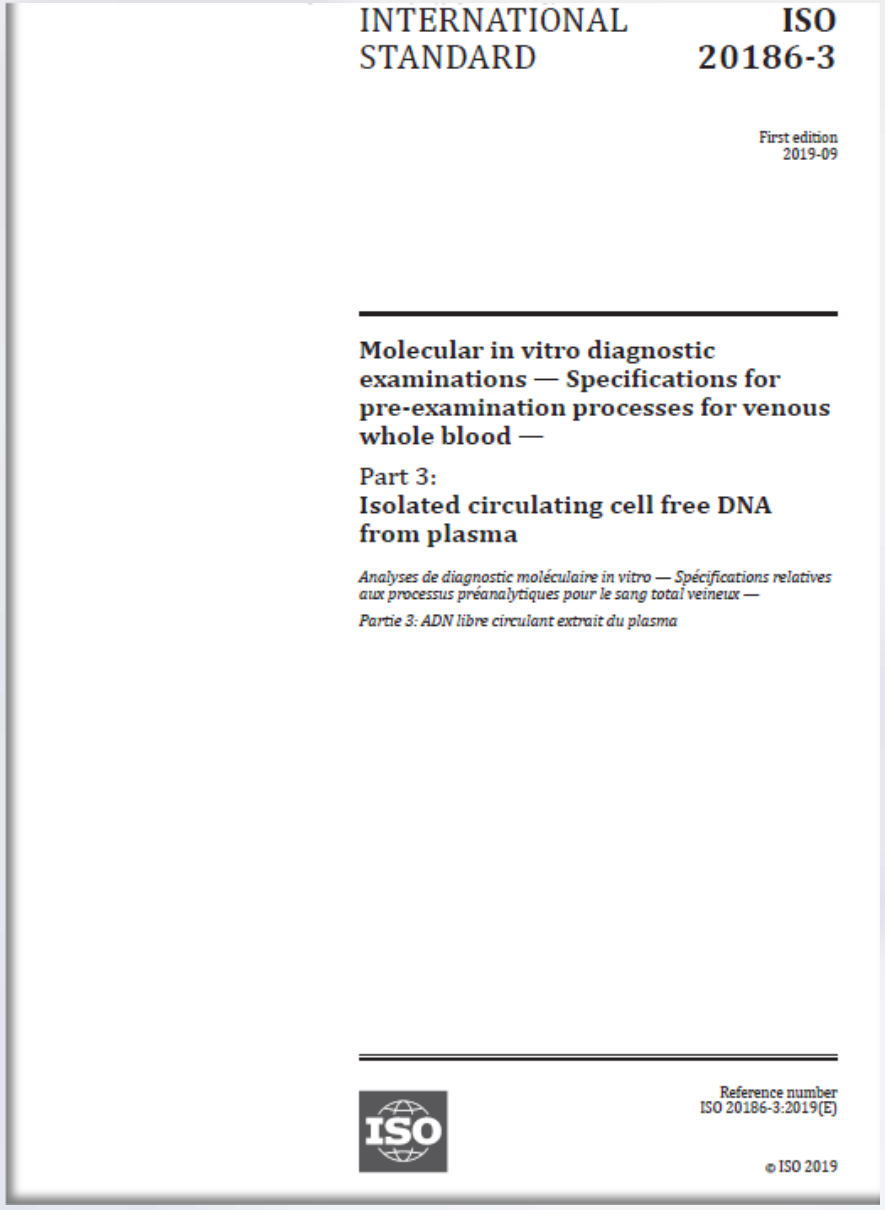
- 2026 : 16 ISO International Standards
- 2014: 8 new projects for ISO Standards approved in ISO/TC 212 „*Clinical laboratory testing and in vitro diagnostic test systems*"

- 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 Pre-Examination Documents (2012 – 2026)



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

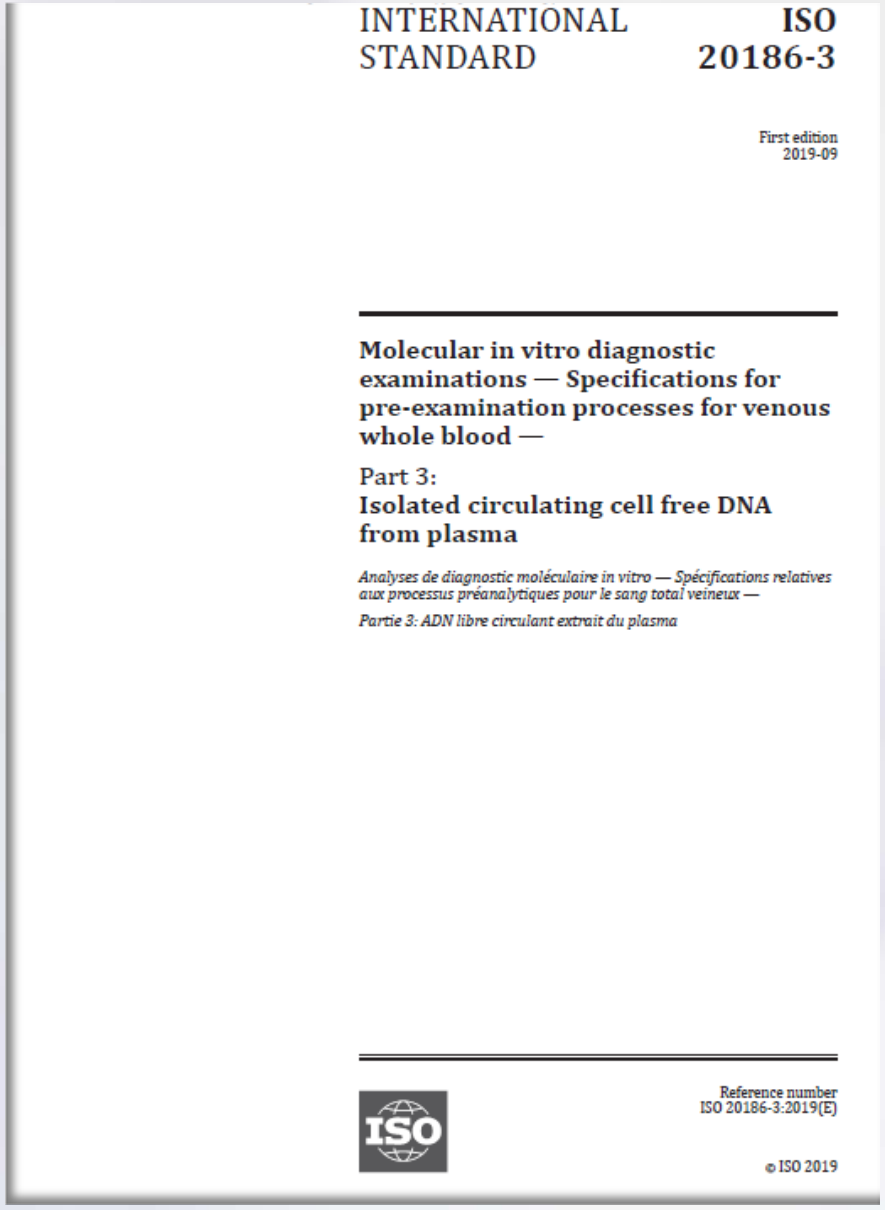
Reference number
ISO 20186-3:2019(E)

© 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

22 CEN & ISO Standard Documents and EQAs (2012 – 2026)



INTERNATIONAL STANDARD

ISO 20186-3

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

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  - **Saliva** – DNA
  - **Urine & Body Fluids** – cfDNA *New: 2026*
  - **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

US FDA and EU IVDR Workflow Requirements for Assay Developments

Sampling

Follow standardized protocols for sample collection, storage, processing and handling (showcased by liquid biopsy ctDNA)

Assays

Assay validation encompasses entire system from sample collection to output of assays (showcased by liquid biopsy ctDNA)

US FDA *presentation at AACR Annucal Congress 2023, Orlando US*

EU IVDR

…. specimen types that can be analysed, including their stability such as storage, …. 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

EU IVDR *Annex II , 6. PRODUCT VERIFICATION AND VALIDATION, 6.1.1*

US FDA: New Guidance on Developing Blood Collection Tubes for Cell-Free Nucleic Acids (2026)

This content is from the eCFR and is authoritative but unofficial.

Title 21—Food and Drugs
Chapter I—Food and Drug Administration, Department of Health and Human Services
Subchapter H—Medical Devices
Part 862—Clinical Chemistry and Clinical Toxicology Devices
Subpart B—Clinical Chemistry Test Systems

Authority: 21 U.S.C. 351, 360, 360c, 360e, 360j, 360l, 371.
Source: 52 FR 16122, May 1, 1987, unless otherwise noted.

Editorial Note: Nomenclature changes to part 862 appear at 73 FR 35341, June 23, 2008.

§ 862.1676 Blood collection device for cell-free nucleic acids.

(a) *Identification.* A blood collection device for cell-free nucleic acids is a device intended for medical purposes to collect, store, transport, and handle blood specimens and to stabilize and isolate cell-free nucleic acid components prior to further testing.

(b) *Classification.* Class II (special controls). The special controls for this device are:

(1) Design verification and validation documentation must include appropriate design inputs and design outputs that are essential for the proper functioning of the device for its intended use, including all of its indications for use, and must include the following:

(i) Documentation demonstrating that appropriate, as determined by FDA, measures are in place (*e.g.*, validated device design features and specifications) to ensure that users of blood collection device for cell-free nucleic acids devices are not exposed to undue risk of bloodborne pathogen exposure and operator injury during use of the device, including blood collection, transportation, and centrifugation processes.

(ii) Documentation demonstrating that appropriate, as determined by FDA, measures are in place (*e.g.*, validated device design features and specifications) to ensure that the device reproducibly and reliably collects, transports, stabilizes, and isolates cell-free nucleic acids of sufficient yield and quality suitable for downstream applications as appropriate for its intended use. At a minimum, these measures must include:

(A) Data demonstrating that blood samples collected in the device have reproducible cell-free nucleic acid yields that are suitable, as determined by FDA, for downstream testing as appropriate for the intended use, including estimates of within-lot, within-device, and lot-to-lot variability;

(B) Data demonstrating that cell-free nucleic acid yields isolated from blood specimens collected into the device do not add clinically significant bias to test results obtained using the downstream application(s) described in the intended use. For devices indicated for use with multiple downstream applications, data demonstrating acceptable performance for each type of claimed use or, alternatively, an appropriate, as determined by FDA, clinical justification for why such data are not needed;

(C) Data demonstrating that the device appropriately stabilizes cell-free nucleic acids after sample collection, during storage, and during transport over the claimed shelf life of the device;

(D) Data demonstrating that samples collected in the device have minimal levels of contamination with other types of nucleic acids present in cells or cellular components, and that these levels of contamination do not interfere with downstream testing;

(E) Data from analytical or clinical studies that demonstrate that, when used as intended, the device consistently draws a blood sample volume that is within the indicated fill range;

(F) Data from analytical or clinical studies that demonstrate that, when used as intended, cell-free nucleic acid yield, stability, and quality are not significantly impacted by interference due to other parts of the device (such as reduced or excess active ingredient) or specimen collection and processing procedures (such as hemolysis, centrifugation, or mixing of blood with anticoagulant or additives); and

(G) Data from analytical studies that demonstrate that the device is suitable for its intended use across all storage and sample handling conditions described in the device labeling, including device shelf life and shipping conditions (*e.g.*, temperature, humidity, duration).

(iii) A protocol, reviewed and determined acceptable by FDA, that specifies the verification and validation activities that will be performed for anticipated device modifications to reevaluate performance claims or performance specifications. This protocol must include a process for assessing whether a modification to technology, engineering, performance, materials, specifications, or indications for use, or any combination thereof, could significantly affect the safety or effectiveness of the device. The protocol must include assessment metrics, acceptance criteria, and analytical methods for the performance testing of changes.

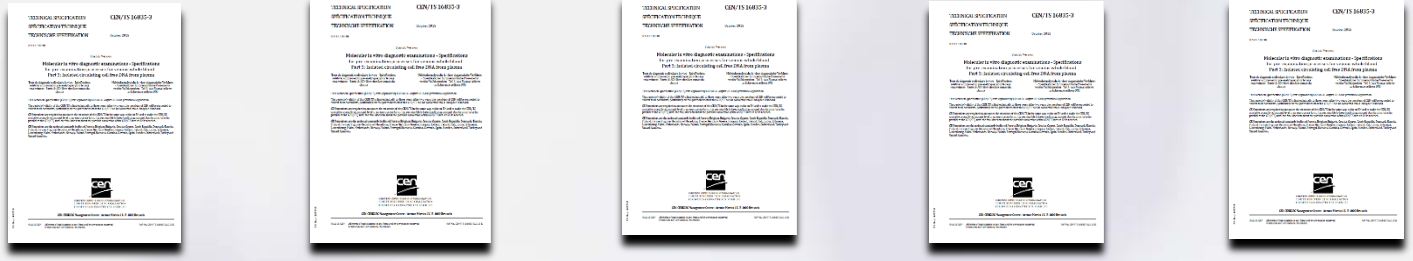
https://www.ecfr.gov/current/title-21/chapter-I/subchapter-H/part-862/subpart-B/section-862.1676

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Role of Legislation, Standards and Technologies

EU IVDR – in-vitro Diagnostic Device Regulation 2017

Diagnostic Workflow Parameters



EN ISO & CEN Standards

SOPs

Technologies & Products

Development & Implementation of a Diagnostic Test Workflow

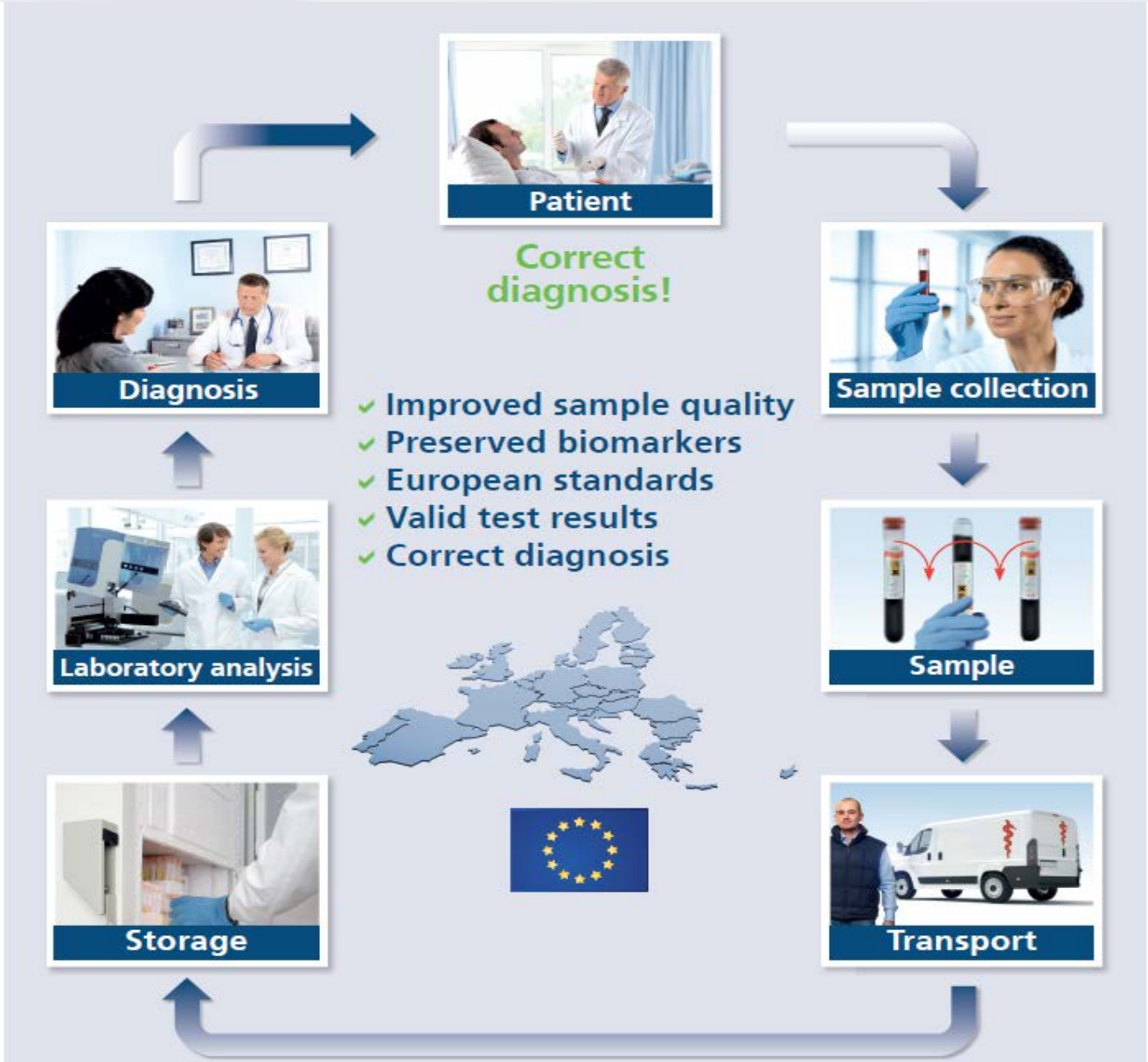




Specimen Collection & Stabilization → Storage Transport → Sample Processing → Analyte Isolation → Analytical Test → Data Analysis

Company trademarks shown for illustration purpose only under copyright

Good Quality Analytical Diagnostic Test Results are Crucial



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

Development & Implementation of a Diagnostic Test Workflow





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

Company trademarks shown for illustration purpose only under copyright

Workflow Implementation in the Laboratory
ISO 20186-3 – Documentation for ensuring correct Test Results

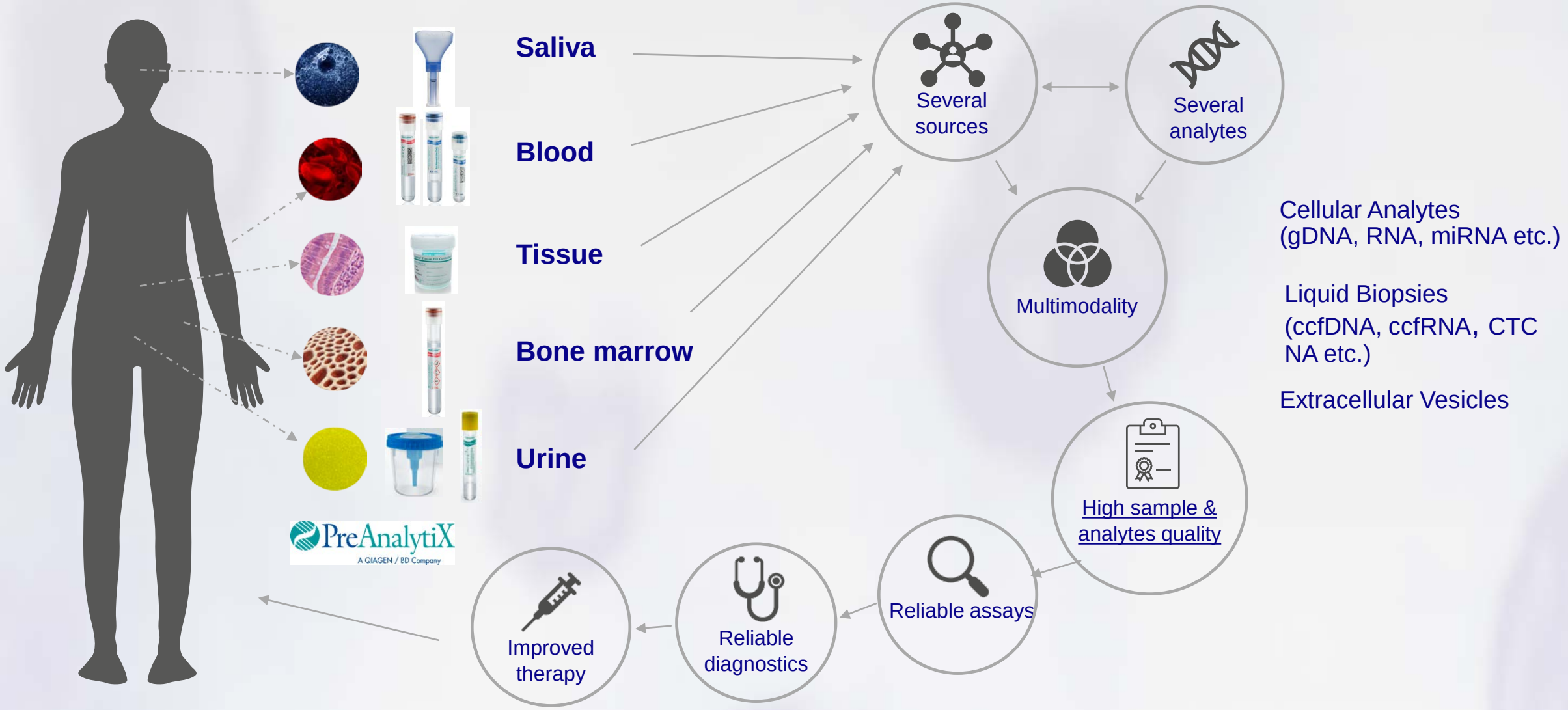
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Documentation requirements intend to monitor that the verified and validated workflows are correctly executed for ensuring specified test performance

Multi Specimens and Multianalyte Analysis - Liquid Biopsies



Cellular Analytes
(gDNA, RNA, miRNA etc.)

Liquid Biopsies
(ccfDNA, ccfRNA, CTC NA etc.)

Extracellular Vesicles

EU Parliament Event in 2019

TACKLING ISSUES ON IN VITRO DIAGNOSTICS FOR PERSONALISED MEDICINE, SPIDIA4P







Long Journey Started in 2008 - A big Thank You goes to . . .

. . . to the SPIDIA & SPIDIA4P & Instand-NGS4P & EASI-Genomics EU Consortia, CD Laboratories Graz, CEN/TC 140, ISO/TC 212 and all other many European and International Partners!



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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

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SPIDIA4P

Thank you!

Questions ?



Twofold Role of Standardization





Traditional Role of Standards

- Source of technical know-how
- Trade facilitation and opening of markets
- Providing a scientific basis for legislation in the health, safety and environment sectors

Valued-added role for research and innovation

- Speeding up innovation by providing the requisite knowledge base (technology transfer)

➢ New ideas, technologies and products benefit from standardization to get into the marketplace and to be successful

Source: Gindele 2013
http://www.iso.org/iso/home/about/conformity-assessment.htm

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