Rationale for defining standardized pre-analytical workflows in light of the requirements of the EU IVDR

Biomedical Research Training Workshop Week

Online, May 13th 2020

Dr. Uwe Oelmüller, SPIDIA4P Coordinator, QIAGEN GmbH

www.spidia.eu

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 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.

Deficiencies in Routine Healthcare and Research demand for Improvements

- Diagnostic errors cause about 10% of all patient deaths and about 17% of adverse events

  Institute of Medicine (IOM) Report Sept. 2015

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

  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

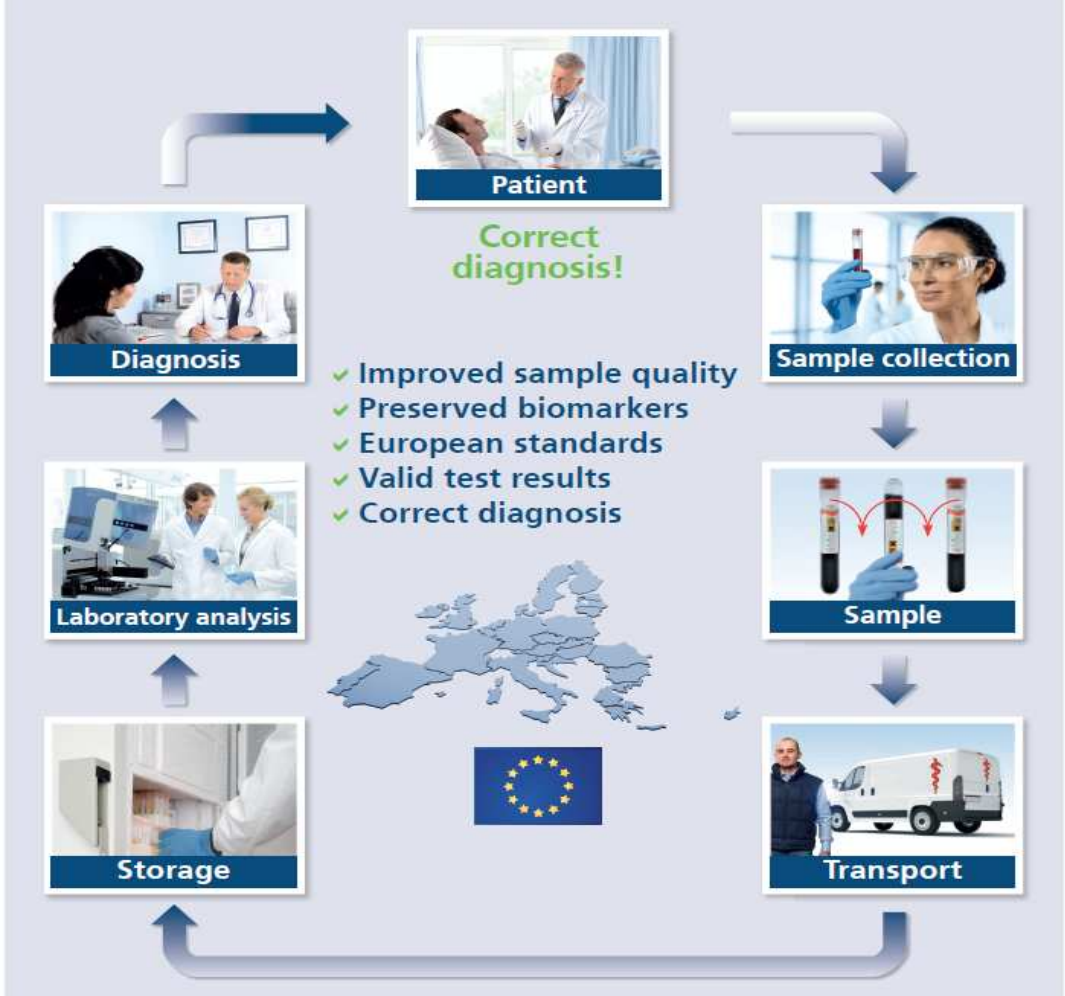
  Green SF. Clin Biochem. 2013

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

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

SPIDIA4P

An Analytical Test Result is the Result of an Entire Workflow

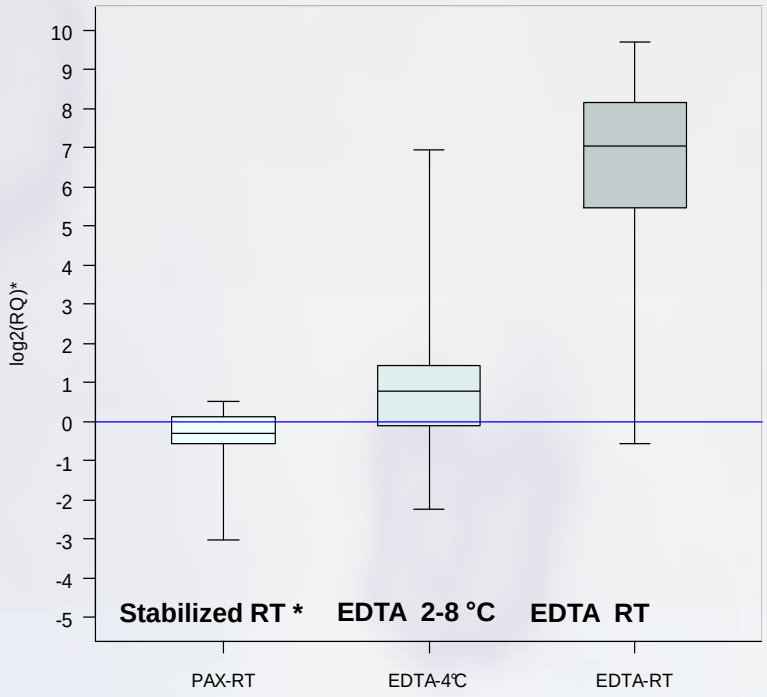


Specifying, developing and verifying preanalytical workflows has to be part of the analytical test development

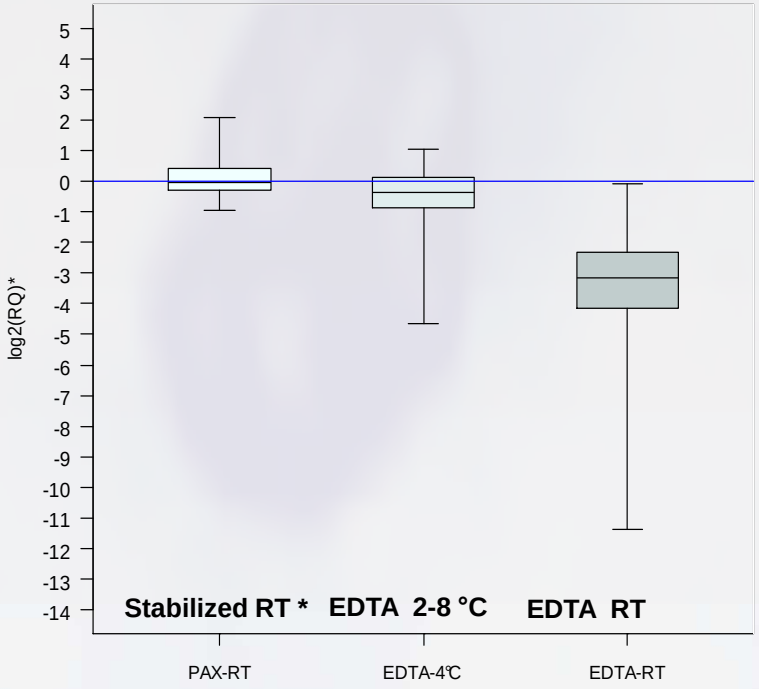
European Conference. Standards: Your Innovation Bridge. Brussels (2014). SPIDIA Booth.

Changes of Blood Cellular RNA Profile: 48 Hours After Collection

Up-regulated FOSB mRNA level



Down-regulated TNFRS mRNA level

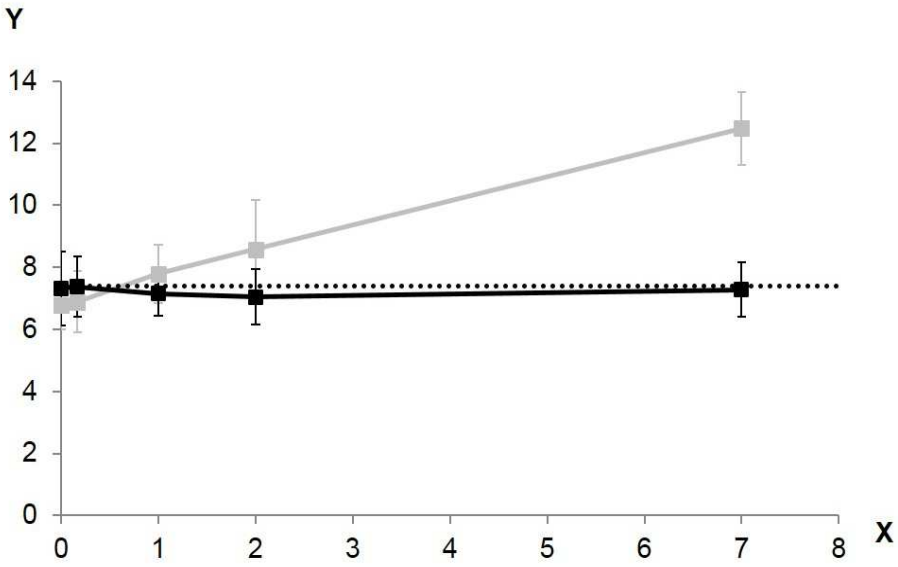


* PAXgene Blood RNA

Malentacchi F et al. (2014). SPIDIA-RNA: Second External Quality Assessment for the Pre-Analytical Phase of Blood Samples Used for RNA Based Analyses. PLoS ONE 9(11): e112293.

Zhan H et al. (2014). Biomarkers for Monitoring Pre-Analytical Quality Variation of mRNA in Blood Samples. . PLoS ONE 9(11): e111644.

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 8 donors is shown

- Spiked restriction enzyme treated EGFR DNA with mutation T790M, equivalent to 200 copies
- ccfDNA tested with the commercially available EGFR Plasma PCR Kit (RUO)

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.

Major Efforts for Improvements

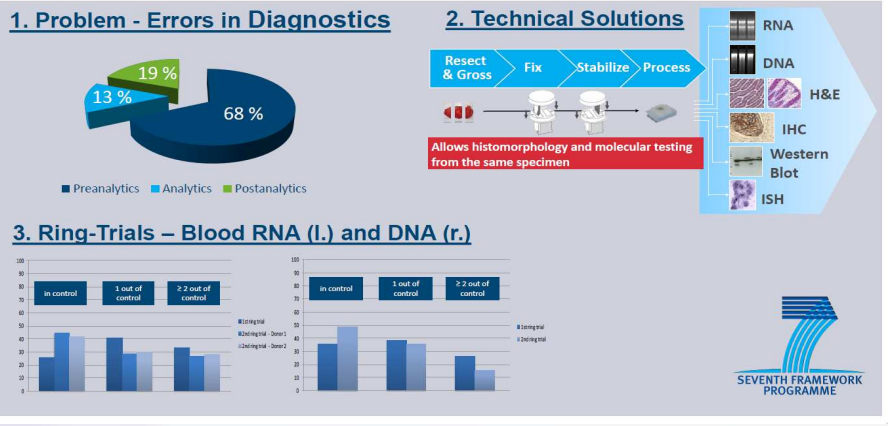
- Technologies
- International ISO & CEN Standards
- External Quality Assessment (EQA) Schemes

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.

Highly Consensus Driven Process for Developing Standards

CEN

- Recognized by the EU and the European Free Trade Association (EFTA) as being **responsible for developing standards at European level**
- Development of a European Standard (EN) or International Standard (ISO) is governed by the principles of **consensus, openness, transparency, national commitment** and **technical coherence**
- One European Standard replaces 34 national standards

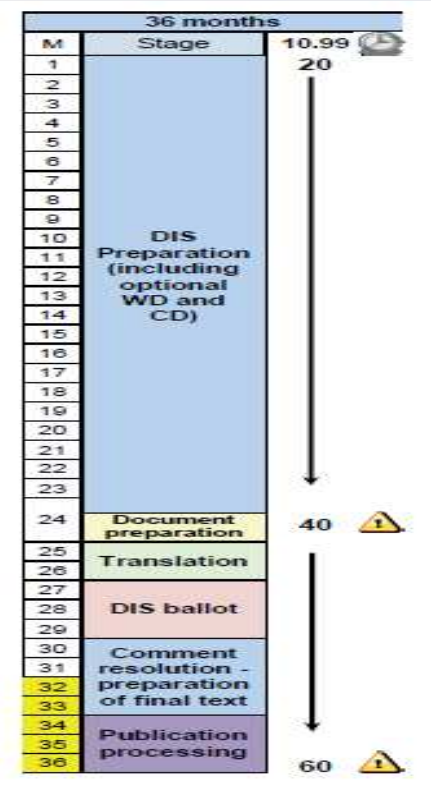
CEN/TC 140 (Committee for in vitro diagnostic medical devices)

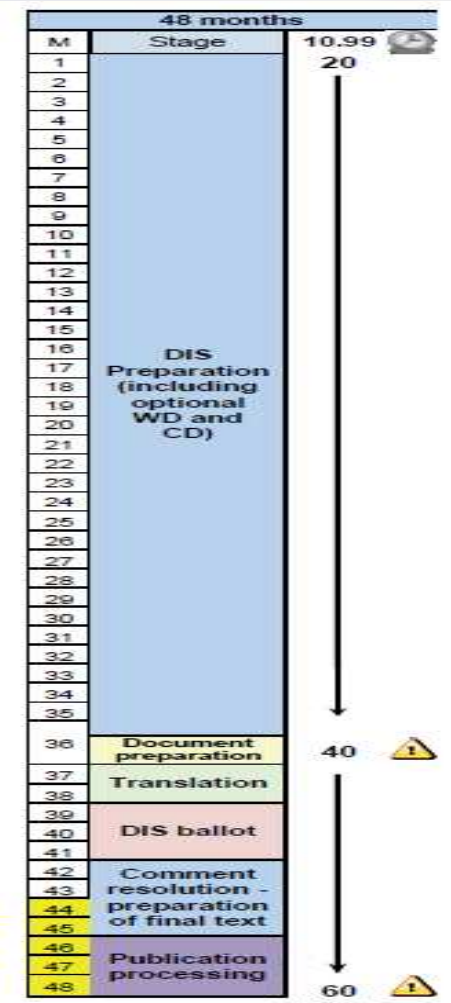
- **34 EU countries National Standards Bodies (NSB)**
- **Stakeholders in liaison & cooperations**
  - **European Commission** (EC), **ESP** (European Society of Pathology), **EFLM** (European Federation of Laboratory Medicine), **IFCC** (Int. Federation of Clinical Chemistry and Laboratory Medicine), **JISC** (Japanese Industrial Standards Committee), **MedTech Europe** (Alliance of European medical technology industry associations), **EPBS** (European Association for Professions in Biomedical Science), **BBMRI-ERIC** (Biobanking and BioMolecular resources Research Infrastructure - European Research Infrastructure Consortium), **ISO/TC 212** (Clinical laboratory testing and in vitro diagnostic test systems), **ISO/TC 276** Biotechnology

ISO/IS Development – Usually a 36 to 48 Months Period

ISO/TC 212

- Technical Committee for Clinical Laboratory Testing and in vitro Diagnostic Test Systems
- 41 member countries, 22 observing members





Source: https://www.iso.org/files/live/sites/isoorg/files/developing_standards/docs/en/Target_date_planner_4_ISO_standards_development_tracks_2017.pdf

CEN - 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

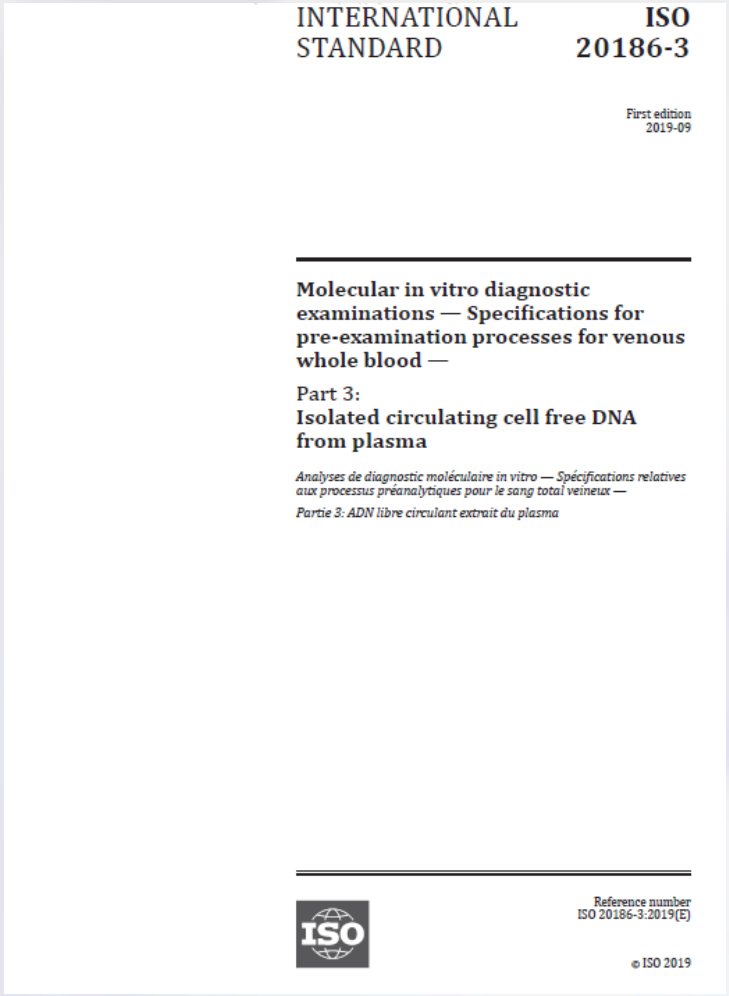
Valued-added role for research and innovation

- 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

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
  - **Blood 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
  - **Metabolomics** – Urine, Serum, Plasma
  - **Microbiome** – Stool, Saliva etc.

published CEN published ISO in development

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

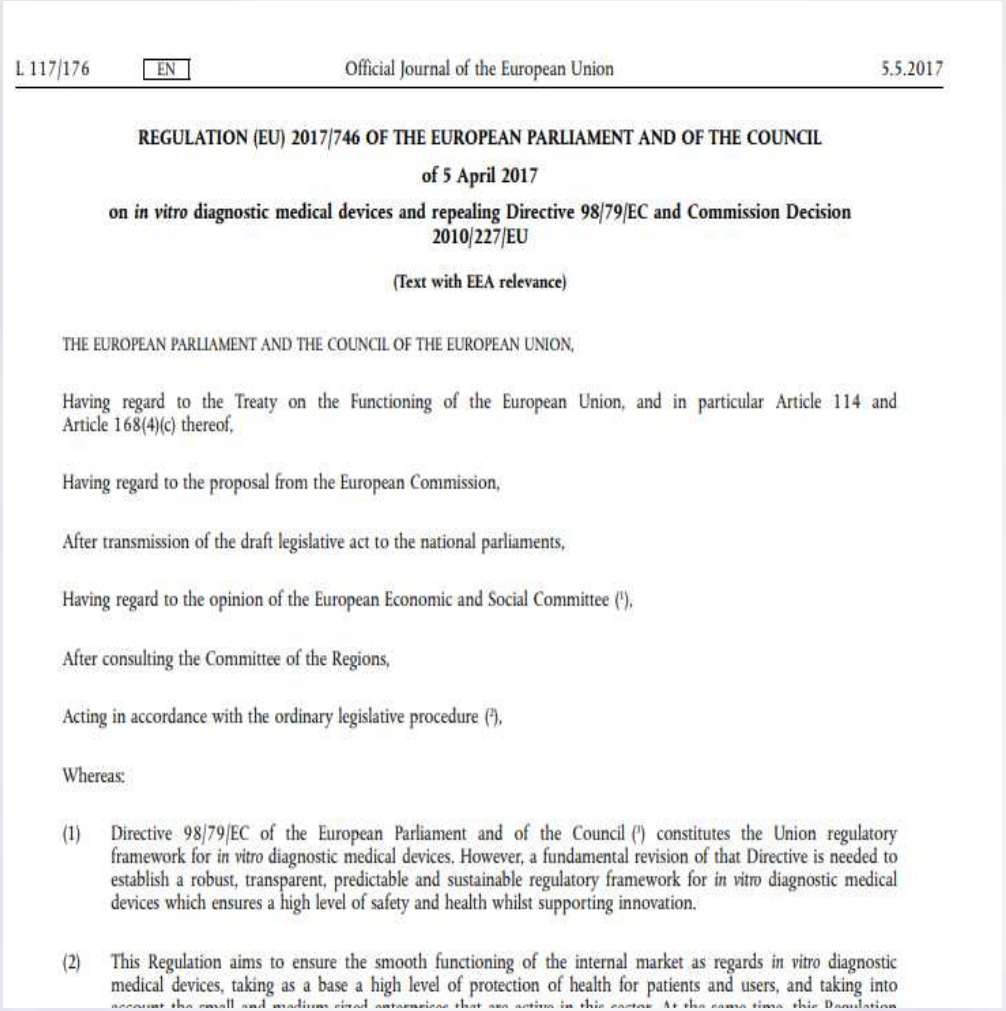
Contents

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

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

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.

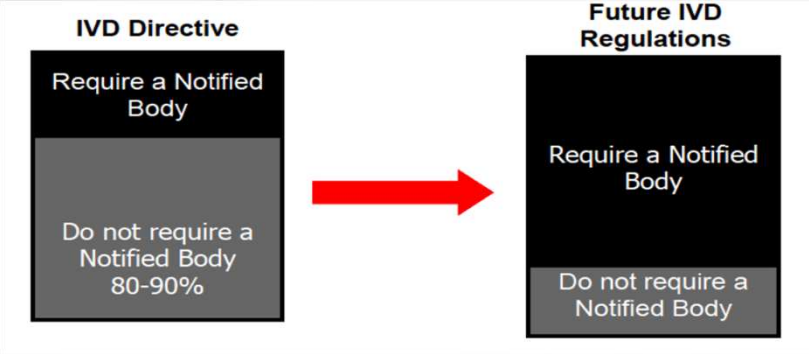
(2) This Regulation aims to ensure the smooth functioning of the internal market as regards *in vitro* diagnostic medical devices, taking as a base a high level of protection of health for patients and users, and taking into account the small and medium sized enterprises that are active in this sector. At the same time, this Regulation

- entered into force on 26 May 2017
- will replace the EU's current Directive on in vitro diagnostic medical devices (98/79/EC)
- transition period until 26 May 2022

New IVDR – Key Changes

Risk Classes

- *from list-based approach to risk-based approach*
- *four risk categories: A (low risk) to D (high risk)*



Performance Evaluation

- *process of performance evaluation defined*
- *required throughout the lifetime of the device*

Clinical Evidence

- *scientific validity, analytical performance, and clinical performance*

Post Market

- *post market performance follow-up*
- *incident reporting and trending*

Conformity Assessment Routes

- *reflect the new classification rules*
- *introduction of pre-examination process parameters*
- *more need to use a Notified Body*

Scrutiny and Traceability

- *new requirements in technical documentation*
- *unique Device Identifier (UDI)*

Source: https://www.bsigroup.com

New In Vitro Diagnostic Regulations 2017 (IVDR)

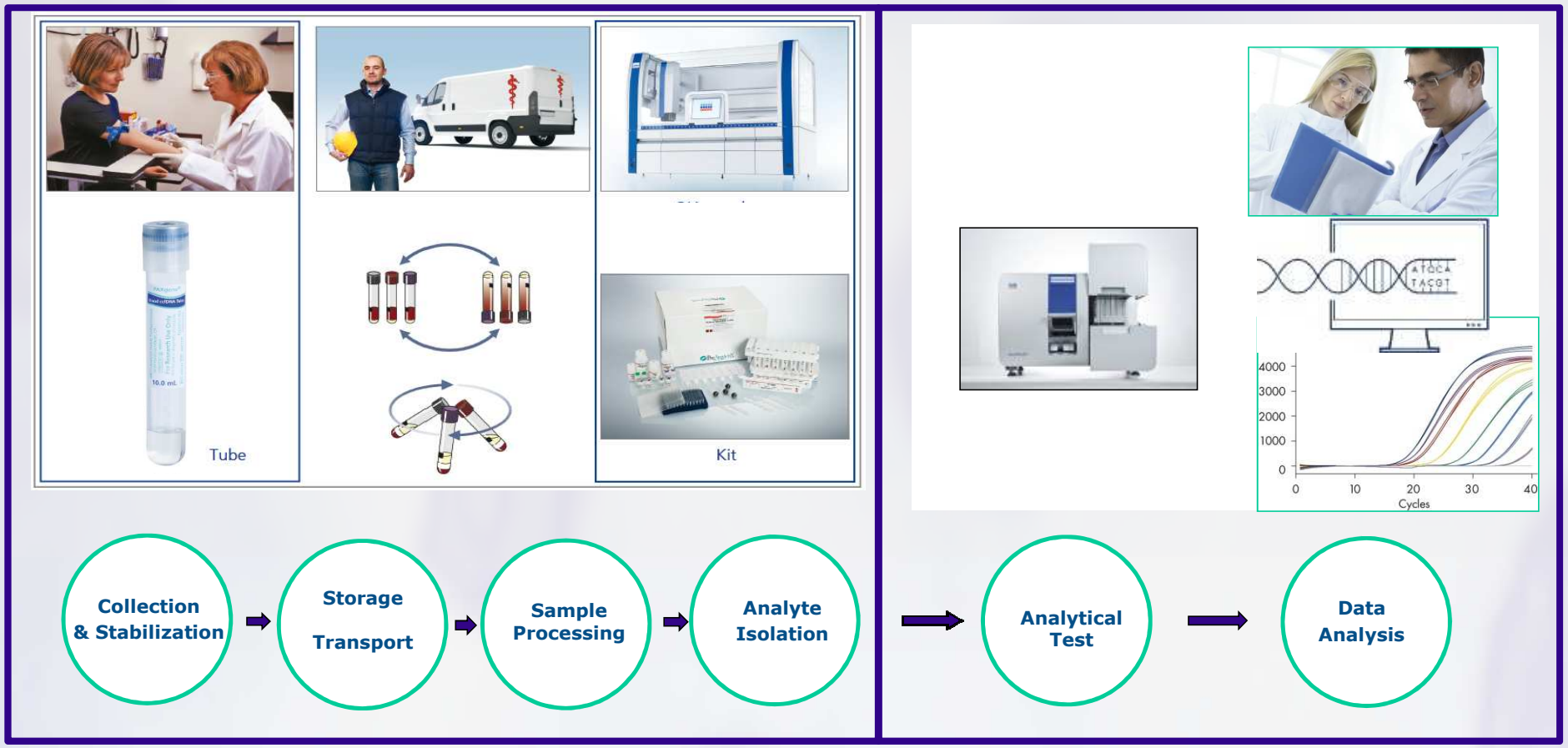
- New European In Vitro Diagnostic Regulation in force since May 2017

- 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

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



Laboratory Developed Tests in the IVDR

Main Text

(29) Health institutions should have the possibility of manufacturing, modifying and using devices in-house and thereby addressing, on a non-industrial scale, the specific needs of target patient groups which cannot be met at the appropriate level of performance by an equivalent device available on the market.

Article 5

1. A device may be placed on the market or put into service only if it complies with this Regulation when duly supplied and properly installed, maintained and used in accordance with its intended purpose.
2. . . . .
3. . . . .
4. Devices that are manufactured and used within health institutions, with the exception of devices for performance studies, shall be considered as having been put into service.
5. With the exception of the relevant general safety and performance requirements set out in **Annex I (GENERAL SAFETY AND PERFORMANCE REQUIREMENTS)**, the requirements of this Regulation shall not apply to devices manufactured and used only within health institutions established in the Union, provided that all of the following conditions are met:
    - various conditions . . . incl. ISO 15189 accreditation or national provisions where applicable . . . .

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Annex I

Chapter II

9. Performance characteristics

9.1. Devices shall be designed and manufactured in such a way that they are suitable for the purposes referred to in point (2) of Article 2, as specified by the manufacturer, and suitable with regard to the performance they are intended to achieve, taking account of the generally acknowledged state of the art. They shall achieve the performances, as stated by the manufacturer and in particular, where applicable:

(a) the analytical performance, such as, analytical sensitivity, analytical specificity, trueness (bias), precision (repeatability and reproducibility), accuracy (resulting from trueness and precision), limits of detection and quantitation, measuring range, linearity, cut-off, including determination of appropriate criteria for specimen collection and handling and control of known relevant endogenous and exogenous interference, cross- reactions; and . . .

(b) the clinical performance . . . . . .

Chapter III

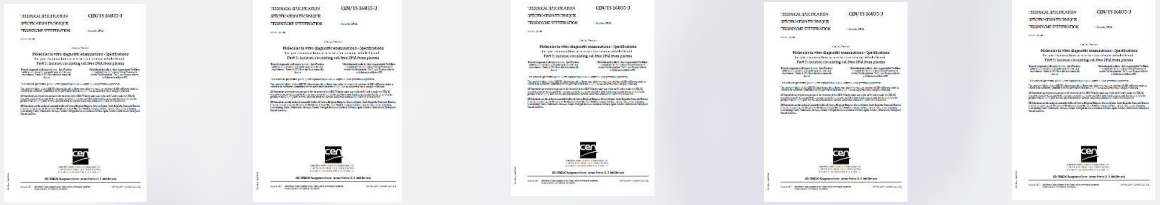
20.4.1. The instructions for use shall contain all of the following particulars:

(q) conditions for collection, handling, and preparation of the specimen;

Role of Standards and Technologies

New EU IVDR – in-vitro Diagnostic Device Regulation 2017

Pre-analytical workflow parameters



EN ISO & CEN Standards

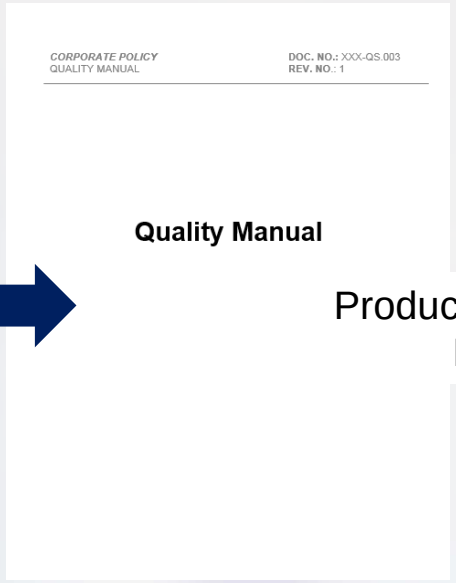
SOPs

Technologies & Products

Implementation of Preanalytical Standards

Example: SPIDIA4P partner PreAnalytiX (QIAGEN/BD Company)

Certification according to ISO 13485

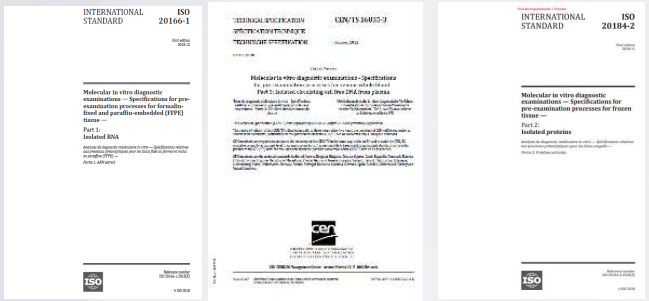

CORPORATE POLICY
QUALITY MANUAL
DOC. NO.: XXX-QS.003
REV. NO.: 1

Quality Manual

Company Quality Manual: Process Landscape

Product Development Process

Global Process SOPs incl. legal requirements


INTERNATIONAL STANDARD ISO 20166-1

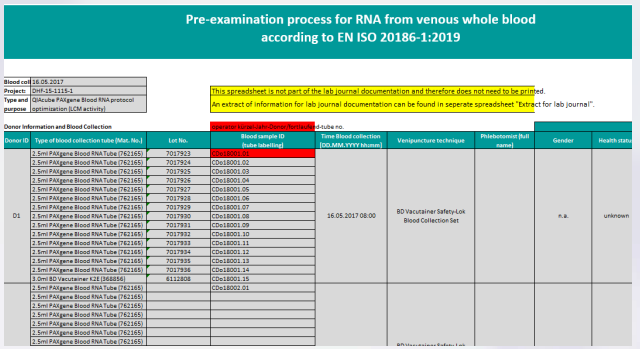
Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for formalin-fixed and paraffin-embedded (FFPE) tissue —

Part 1: Isolated RNA

INTERNATIONAL STANDARD ISO 20184-2

Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for frozen tissue —

Part 2: Isolated proteins


Pre-examination process for RNA from venous whole blood according to EN ISO 20186-1:2019

This spreadsheet is not part of the lab journal documentation and therefore does not need to be printed.
An extract of information for lab journal documentation can be found in seperate spreadsheet "Extract for lab journal".

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

ISO and CEN Standards can always be used

- New ideas, technologies and products benefit from standardization to get into the marketplace and to be successful
  - Build customer confidence that your products are safe and reliable
  - Meet regulation requirements, at a lower cost
  - Reduce costs across all aspects of your business
  - Gain market access across the world

- International Standards help businesses of any size and sector reduce costs, increase productivity and access new markets

- “Standards make market access easier, in particular for SMEs. They can enhance brand recognition and give customers the guarantee that the technology is tested and reliable”

  Jens Albens CEO, Nanotron Technologies Ltd, Germany

German SWR TV – Substantially Varying Test Results between Laboratories

https://www.swr.de/wissen/odysso/Blut-Untersuchung-Missstand-bei-Bluttests,aexavarticle-swr-77780.html

SWR - Juni 2019

EU Parliament Event on March 5th 2019

TACKLING ISSUES ON IN VITRO DIAGNOSTICS FOR PERSONALISED MEDICINE, SPIDIA4P

A big Thank You goes to . . .

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

www.spidia.eu **- New Website**

SPIDIA4P

Thank you!

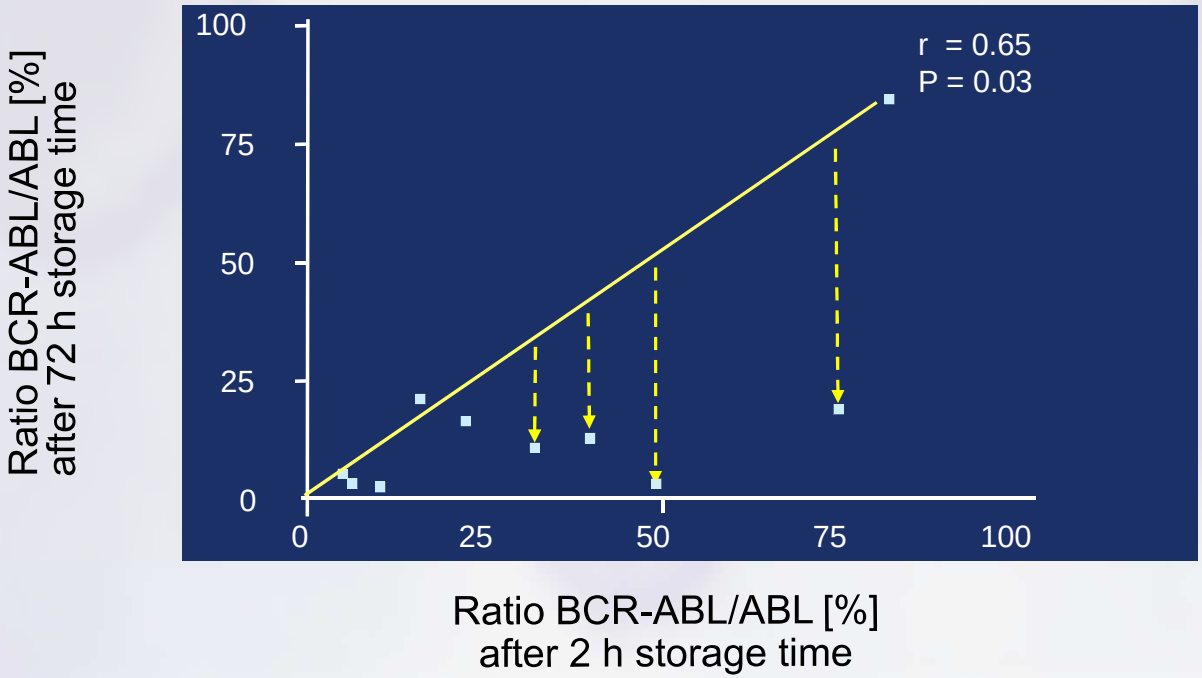
Questions ?

SPIDIA4P

Back Up Slides

Leukemia Therapy Monitoring Research Study
Blood Transcripts BCR-ABL / ABL Ratio in EDTA Tubes

Unpreserved Blood



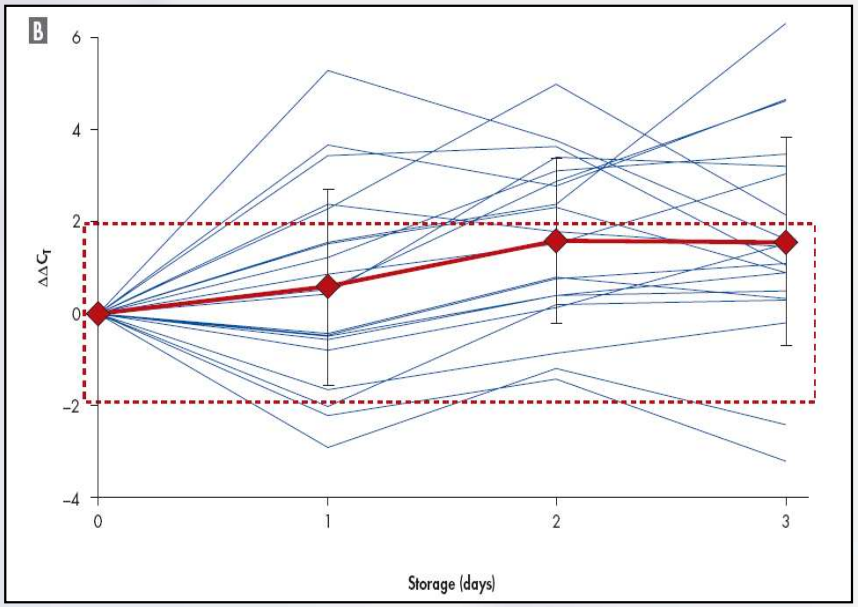
Transcripts Ratio
BCR-ABL / ABL
significantly changed after 70 h of
room temperature shipment / storage

Source: Mueller et al. (2002). Leukemia 16 (12), pp. 2395-9.

Blood RNA Quality Marker Discovery

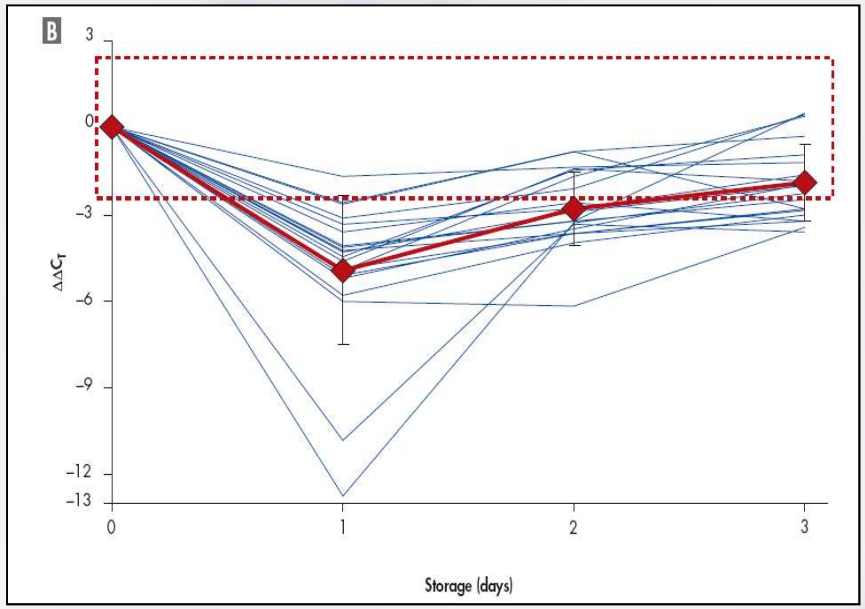
Challenge are Individual Sample Kinetics

Human EDTA Blood stored at Room Temperature over 3 days



IL-1β mRNA

Guenther K. et al.. AMP Poster (2005)



c-fos mRNA

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

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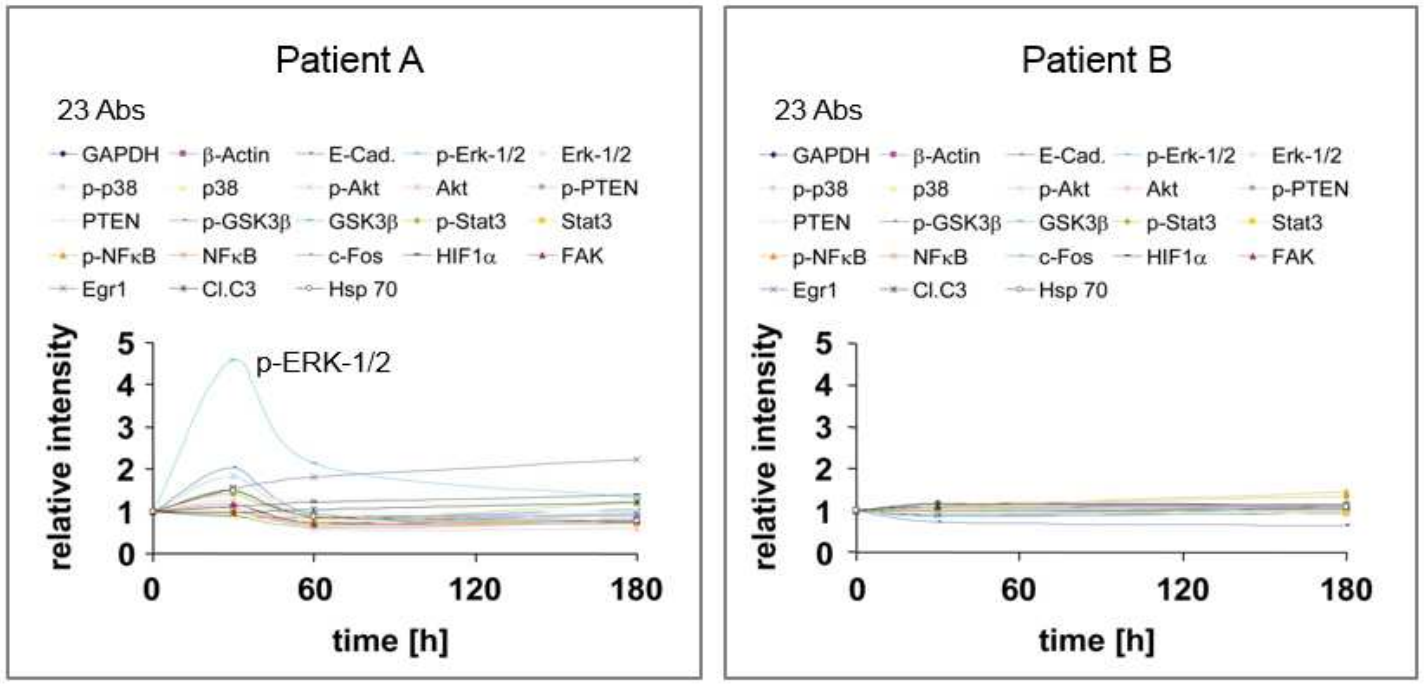
Inter-Patient Samples Variability

Impact of ischemia time on protein expression of intestine

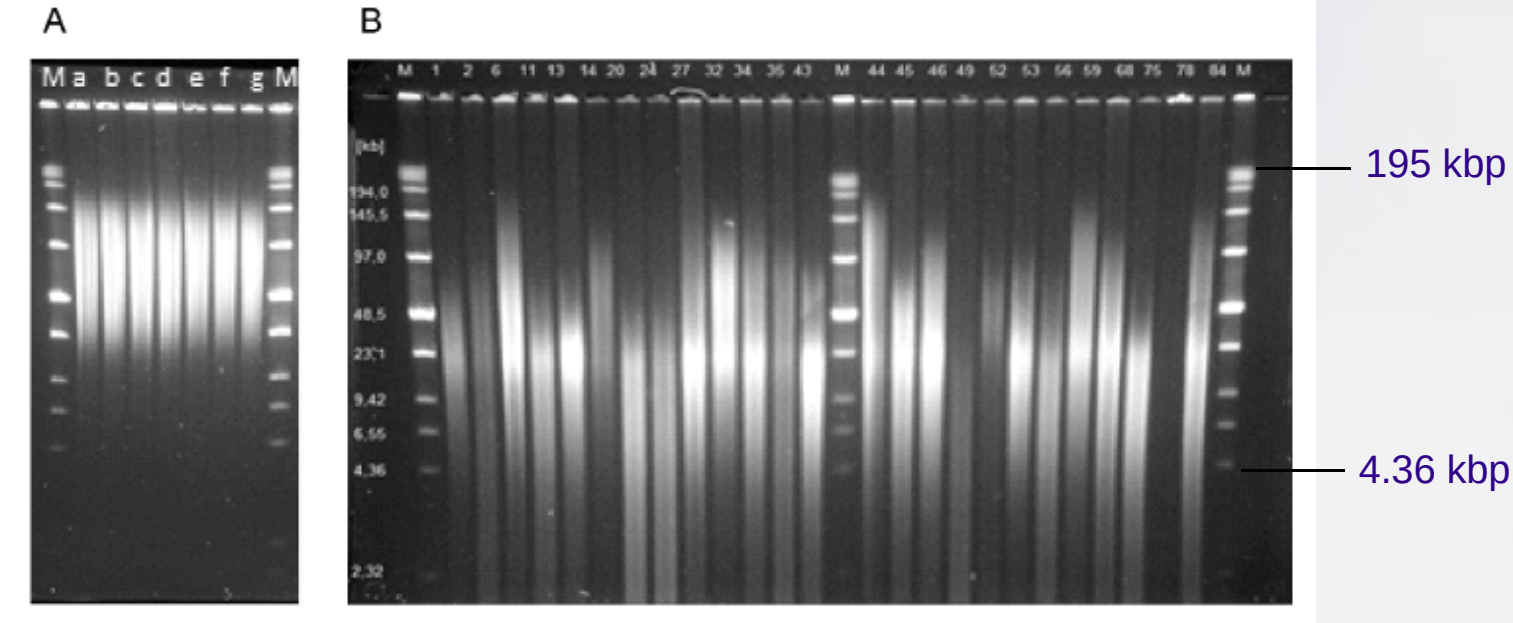


Impact of ischemia time on protein expression of non-malignant human intestine samples

Gündisch et al., J. Proteome Res. 2012

Technische Universität München

DNA Length Variation – Pulse Field Gel Electrophoresis (European Ring Trial)



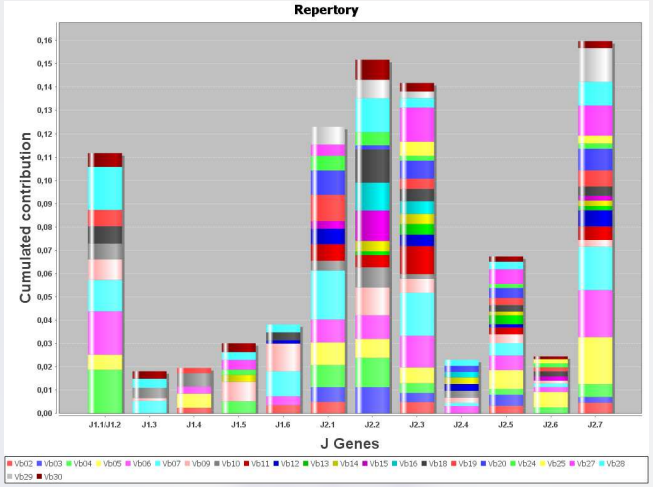
A: gDNA isolated immediately after blood collection at SPIDIA Laboratory

B: gDNA isolated by ring trial participating laboratories

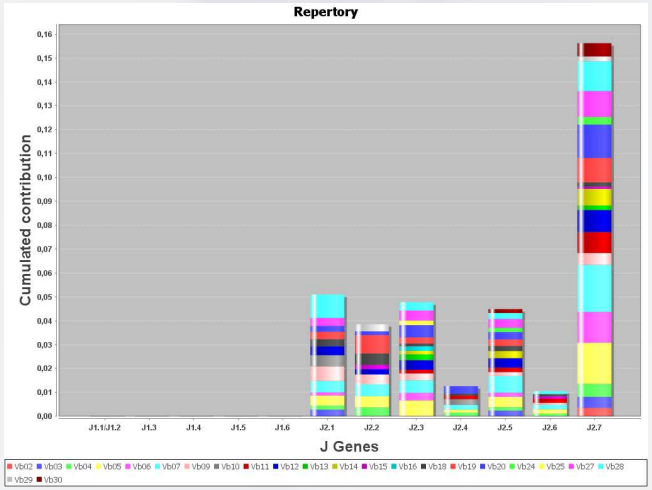
Malentacchi, F., Ciniselli, CM., Pazzagli, M. et al. (2015) Influence of pre-analytical procedures on genomic DNA integrity in blood samples: the SPIDIA experience. Clin Chim Acta. 440:205-10.

Impact of DNA quality on Immune T cell Repertoire Analysis (Ring Trial)

V contribution for each J gene – Research Trial (ImmunID Technologies, France)



Ref. DNA (DIV 54%)

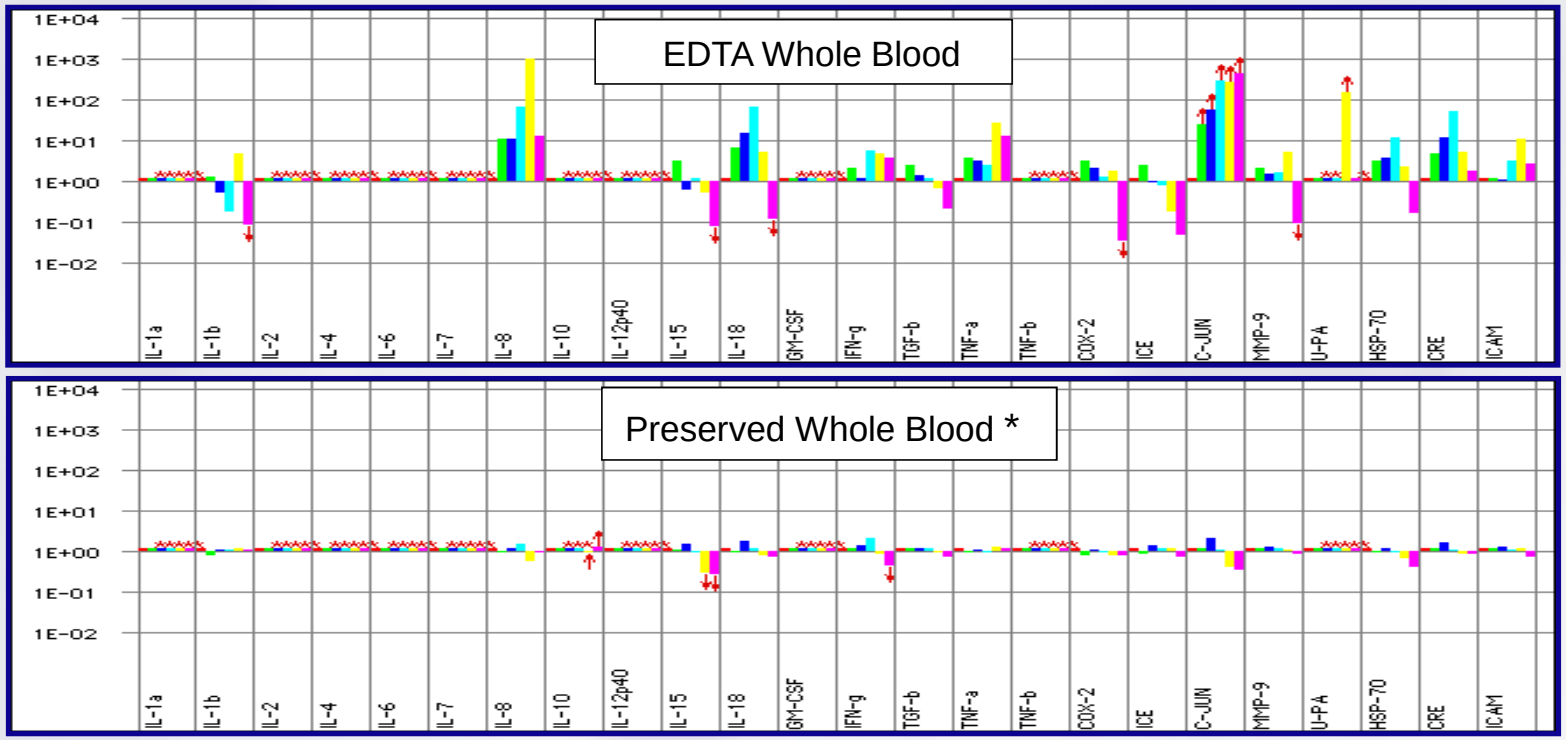


Sample 38 (Poor quality) (DIV 32%)

- Loss of all long V–J rearrangements
- Loss of part of intermediate length rearrangements

Malentacchi, F., Ciniselli, CM., Pazzagli, M. et al. (2015) Influence of pre-analytical procedures on genomic DNA integrity in blood samples: the SPIDIA experience. Clin Chim Acta. 440:205-10.

Ex Vivo Changes in Whole Blood RNA Profil



Individual samples react differently

* PAXgene Blood RNA System

Rainen et al.. Clin.Chem. 2002, 48(11):1883-90

Products of European Standardization

European Standard – EN

Goal: Development of normative specifications reflecting the current state of technology

European Technical Specification – CEN/TS

Goal: Specifications which aid market development and growth

European Technical Report – CEN/TR

Goal: Specifications of a recommendatory and explanatory nature

CEN Workshop Agreement – CWA

Goal: Special specifications developed with the rapid consensus of expert stakeholders

Largest Consortia Network for Pre-analytics in Community

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