New Standards for Liquid Biopsies Pre-analytical Workflows: A Key for Reliable Diagnostics, Research and Biobanking

2nd ISBER Biospecimen Research Symposium
Berlin, February 6th 2019

Dr. Uwe Oelmüller, SPIDIA4P Coordinator, QIAGEN GmbH

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

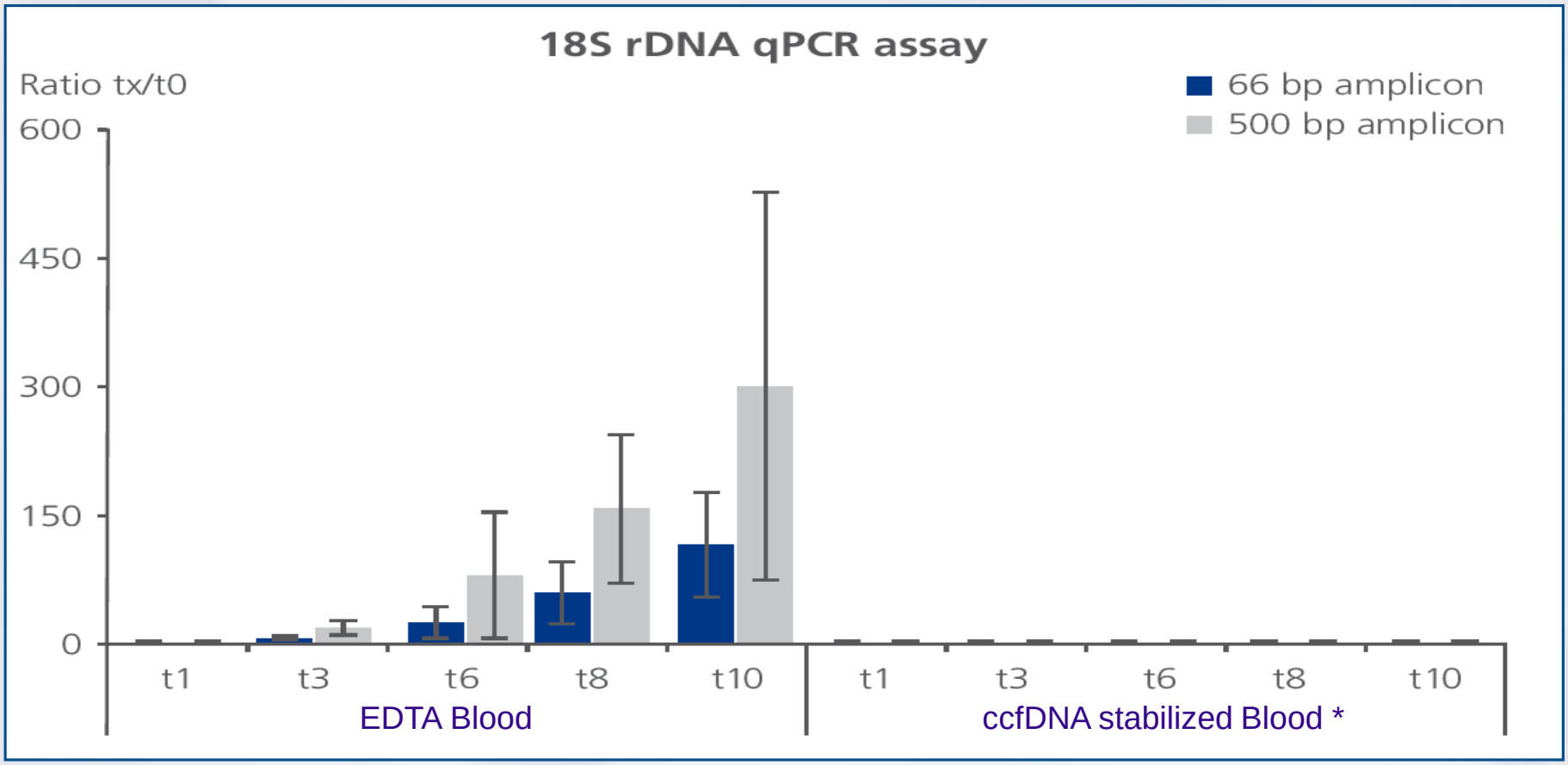
- Irreproducible preclinical research exceeds 50%, US $28B / year spent on preclinical research that is not reproducible - in the US

  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

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

Pre-analytical Impacts on Blood ccfDNA Profiles

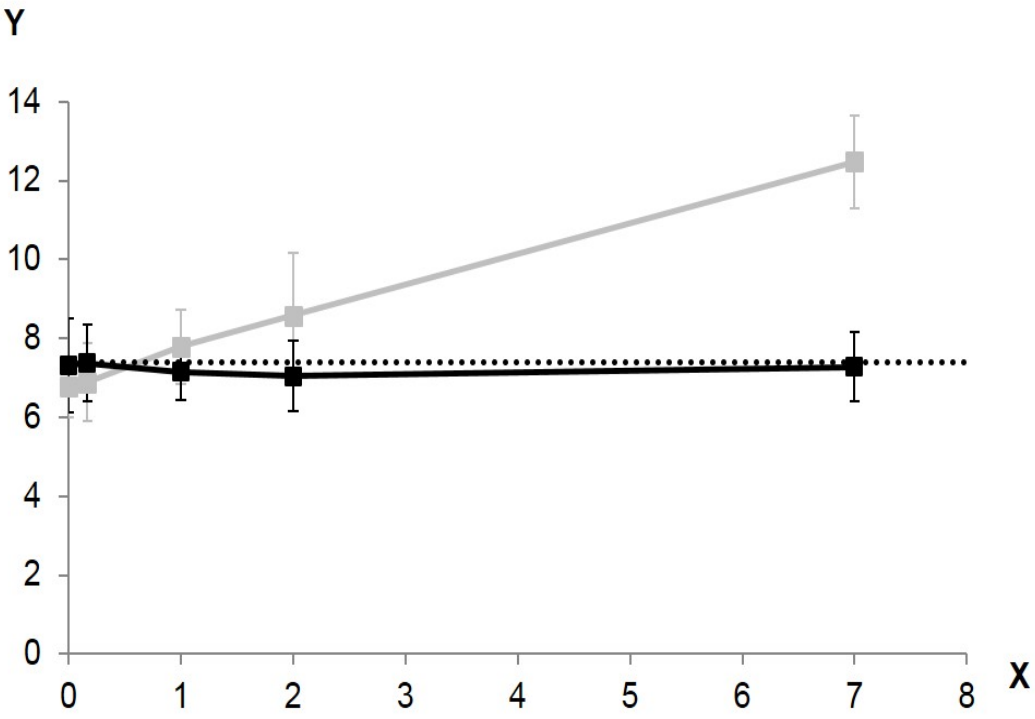


* PAXgene Blood ccfDNA Tube

Andrea Ullius[1,2], Joachim Bonnet[3], Wera Hofmann[3], Markus Stumm[4], Nadine Dettmann[1,2], Katharina Pfaff[1,2], Franziska Heese[1,2] and Daniel Grölz[1,2]. [1]QIAGEN GmbH, Hilden, Germany; [2] PreAnalytiX GmbH, Hombrechtikon, Switzerland; [3]LifeCodexx AG, Konstanz, Germany; [4] Centre for Prenatal Diagnostics and Human Genetics, Berlin, Germany

ESBB Congress London, 2015

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)

SPIDIA4P, unpublished data

Three Major Efforts for Improvements

- **Technologies** for securing high quality samples
- **International Standards** for pre-analytical workflows
- **Implementation** in healthcare, biobanking, research etc.

New Technologies and Standards for Pre-analytical Workflows

SPIDIA – FP7 (2008 – 2013)

- ⇨ 16 Partners and additional collaborators incl. US NCI
- New technologies for sample collection, stabilization, processing, transport, storage (Blood, Tissues)
- 9 EU CEN/TS Standard Documents

SPIDIA4P – H2020 (2017 – 2020)

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

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

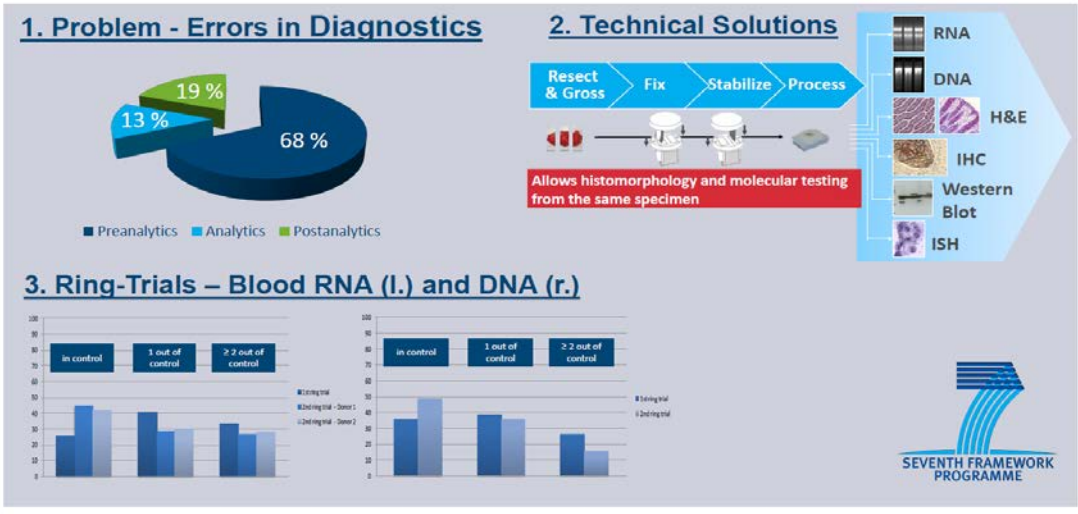
The SPIDIA project has received funding under the Seventh Research Framework Program of the European Union, FP7-HEALTH-2007-1.2.5, under grant agreement no. 222916. The SPIDIA4P project receives funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no.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

SPIDIA's Road to Standardization

Vienna Agreement 1991

- 2018: Progressing to ISO/FDIS
- 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



SPIDIA

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

CEN CENELEC

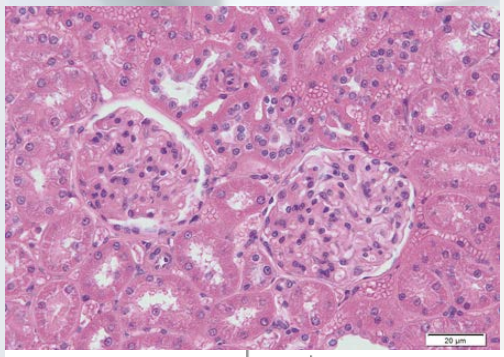
European Commission

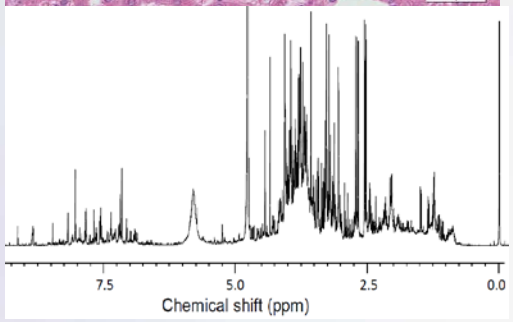
Highly Consensus Driven Process for Developing Standards

- **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 European 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** (Alliance of European medical technology industry associations, founded by **EDMA**), **EPBS** (European Association for Professions in Biomedical Science), **CANCER-ID**, **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

10 CEN Technical Specifications
5 progressed to ISO/IS

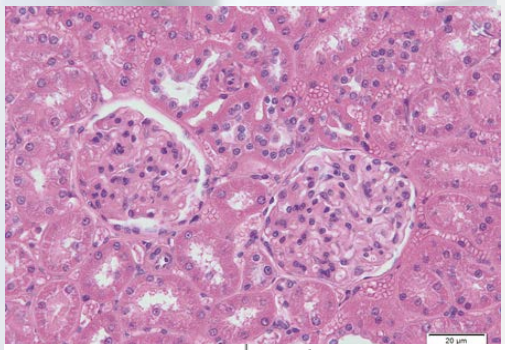


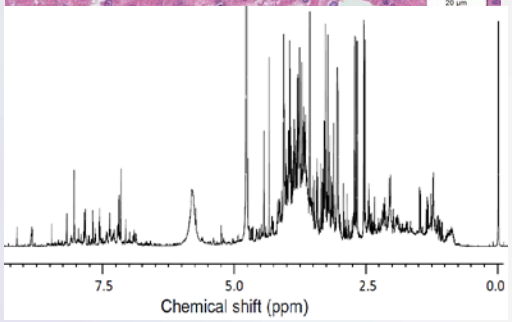


- Molecular in-vitro diagnostic examinations - Specifications for pre-examination processes for
  - blood — Cellular RNA
  - blood — Genomic DNA
  - **blood — Circulating cell free DNA**
  - FFPE tissue — DNA ⇨ ISO/IS 12/2018
  - FFPE tissue — RNA ⇨ ISO/IS 12/2018
  - FFPE tissue — Proteins ⇨ ISO/IS 12/2018
  - frozen tissue — RNA ⇨ ISO/IS 12/2018
  - frozen tissue — Proteins ⇨ ISO/IS 12/18
  - frozen tissue — DNA (new 2018)
  - metabolomics in urine, serum and plasma

⇨ Professional societies and organizations play a central role in implementation (e.g. BBMRI-ERIC, ESP, OECI, EFLM, MedTech etc.)

SPIDIA4P - More Standards, EQAs and Support Tools to come





. . . . pre-examination processes for

- **Venous whole blood — CTCs: DNA, RNA, stains & proteins**
- **Venous whole blood – Exosomes: nucleic acids; ccfRNA**
- **Urine & other body fluids – cfDNA**
- Saliva – Human DNA
- Saliva and stool – Microbiome DNA
- Fine Needle Aspirates (FNAs) – DNA, RNA, proteins
- FFPE Tissue – in situ stainings incl. IHC
- Metabolomics of body fluids: International ISO Standard

- **. . . plus control tools (EQA schemes)**
- **. . . plus implementation tools**
- **. . . plus proof of commercial success (SMEs, e.g. Inivata Ltd.)**

CEN/TS 16835-3 - Pre-examination Process for Blood ccfDNA

TECHNICAL SPECIFICATION

SPÉCIFICATION TECHNIQUE

TECHNISCHE SPEZIFIKATION

CEN/TS 16835-3

October 2015

ICS 11.100.30

English Version

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

Tests de diagnostic moléculaire in vitro - Spécifications relatives aux processus pré-analytiques pour le sang total veineux - Partie 3: ADN libre circulant extrait du plasma

Molekularanalytische in-vitro-diagnostische Verfahren - Spezifikationen für präanalytische Prozesse für venöse Vollblutproben - Teil 3: Aus Plasma isolierte zirkulierende zellfreie DNS

This Technical Specification (CEN/TS) was approved by CEN on 31 August 2015 for provisional application.

The period of validity of this CEN/TS is limited initially to three years. After two years the members of CEN will be requested to submit their comments, particularly on the question whether the CEN/TS can be converted into a European Standard.

CEN members are required to announce the existence of this CEN/TS in the same way as for an EN and to make the CEN/TS available promptly at national level in an appropriate form. It is permissible to keep conflicting national standards in force (in parallel to the CEN/TS) until the final decision about the possible conversion of the CEN/TS into an EN is reached.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.

EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

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Ref. No. CEN/TS 16835-3:2015 E

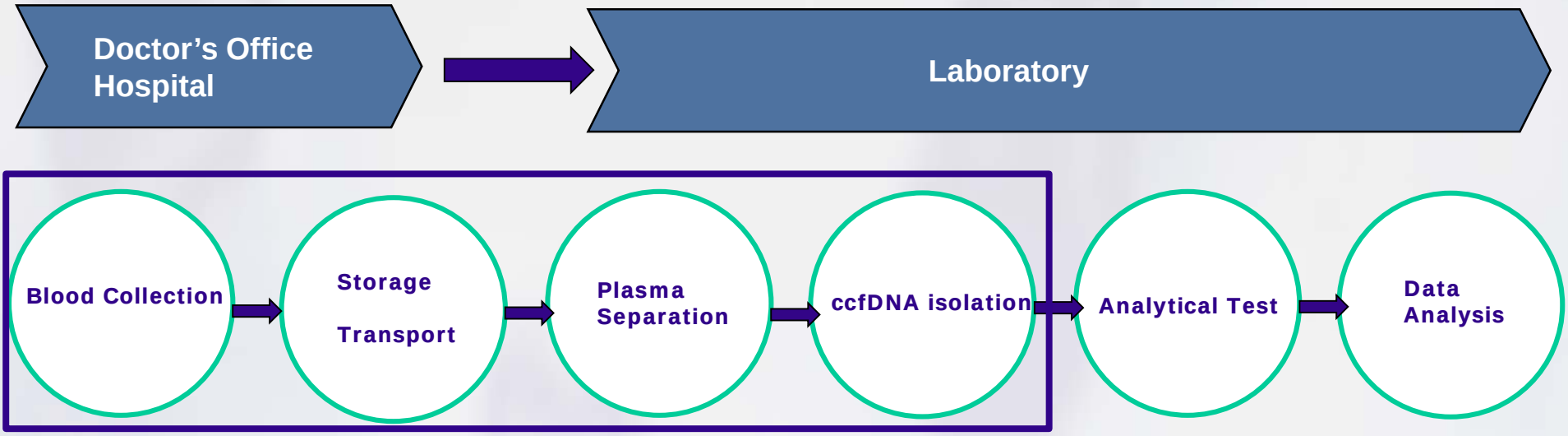
Ask-Shop 14.09.2016

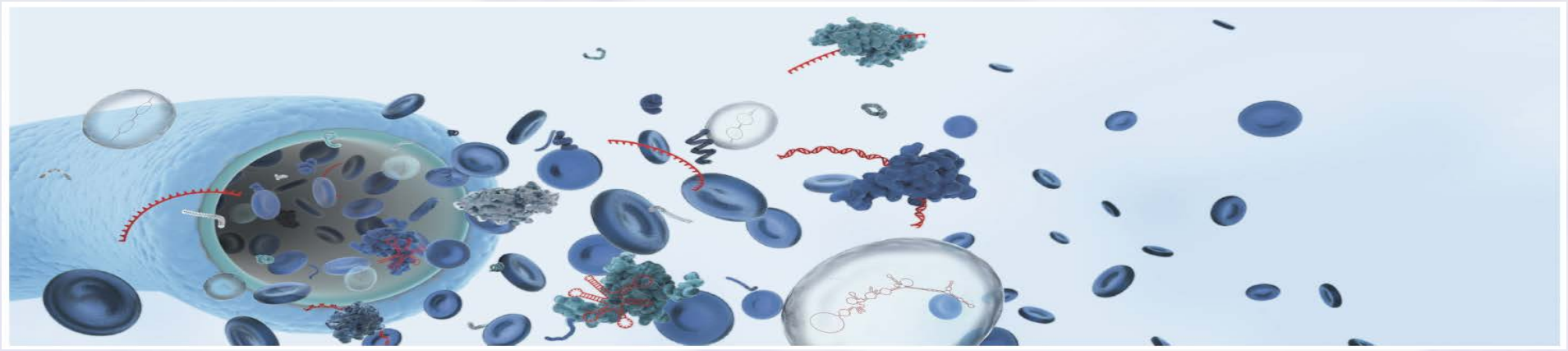
ISO/IS Standard expected for 2019

CEN/TS 16835-3 - Pre-examination Process for Blood ccfDNA

ccfDNA Diagnostic Workflow – Starting with the Patient (CEN/TS 16835-3)

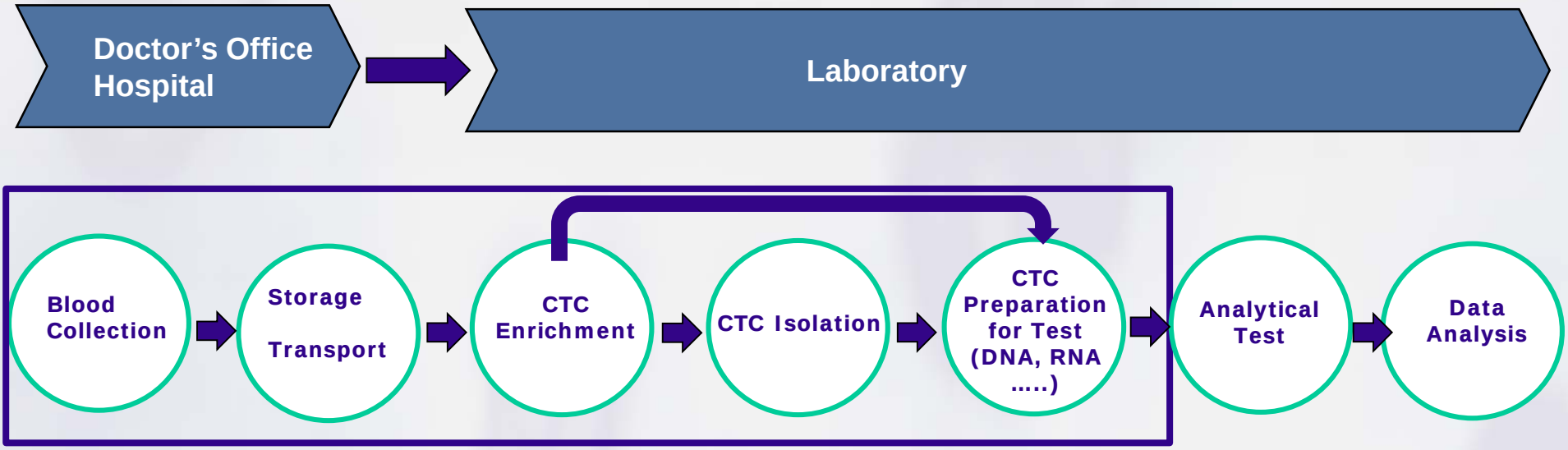
ISO/International Standard development is in final FDIS ballot preparation





CTC / Blood Rare Cells Diagnostic Workflow – Starting with the Patient

CEN/TS development is ready for approval ballot including commenting



- Low CTC abundance and high WBC background (1-10 CTCs in 10^6–10^8 WBCs) (~0.0001%-0.00001%)

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

New European Medical Device and In Vitro Diagnostic Regulations 2017

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

- Also pre-analytical workflow parameters become mandatory (IVDR)
  - 6. PRODUCT VERIFICATION AND VALIDATION (Annex II)
  - 6.1. Information on analytical performance of the device
  - 6.1.1. Specimen type

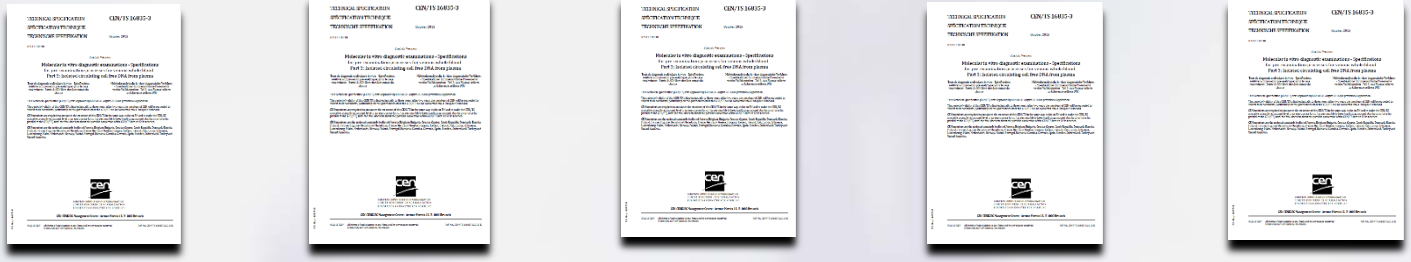
    This Section shall describe the different specimen types that can be analysed, including their stability such as storage, where applicable specimen transport conditions and, with a view to time-critical analysis methods, information on the timeframe between taking the specimen and its analysis and storage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

SPIDIA4P

Role of Standards and Technologies

EU IVDR – In-vitro-Diagnostic Device Regulation

Pre-analytical workflow parameters



EN ISO & CEN Standards

SOPs

Technologies & Products

Pre-analytical Workflow - Same Standards for all Segments

- **Biobanks**
  - Source for high quality samples
  - ⇨ BBMRI-ERIC plays a central role
- **Biomedical & Translational Research**
  - Academia
  - Pharma industry
  - Diagnostic Industry
- **Diagnostics**
  - High sample quality is the safe way
  - Analytical assay might tolerate lower quality or not ⇨ Validation studies

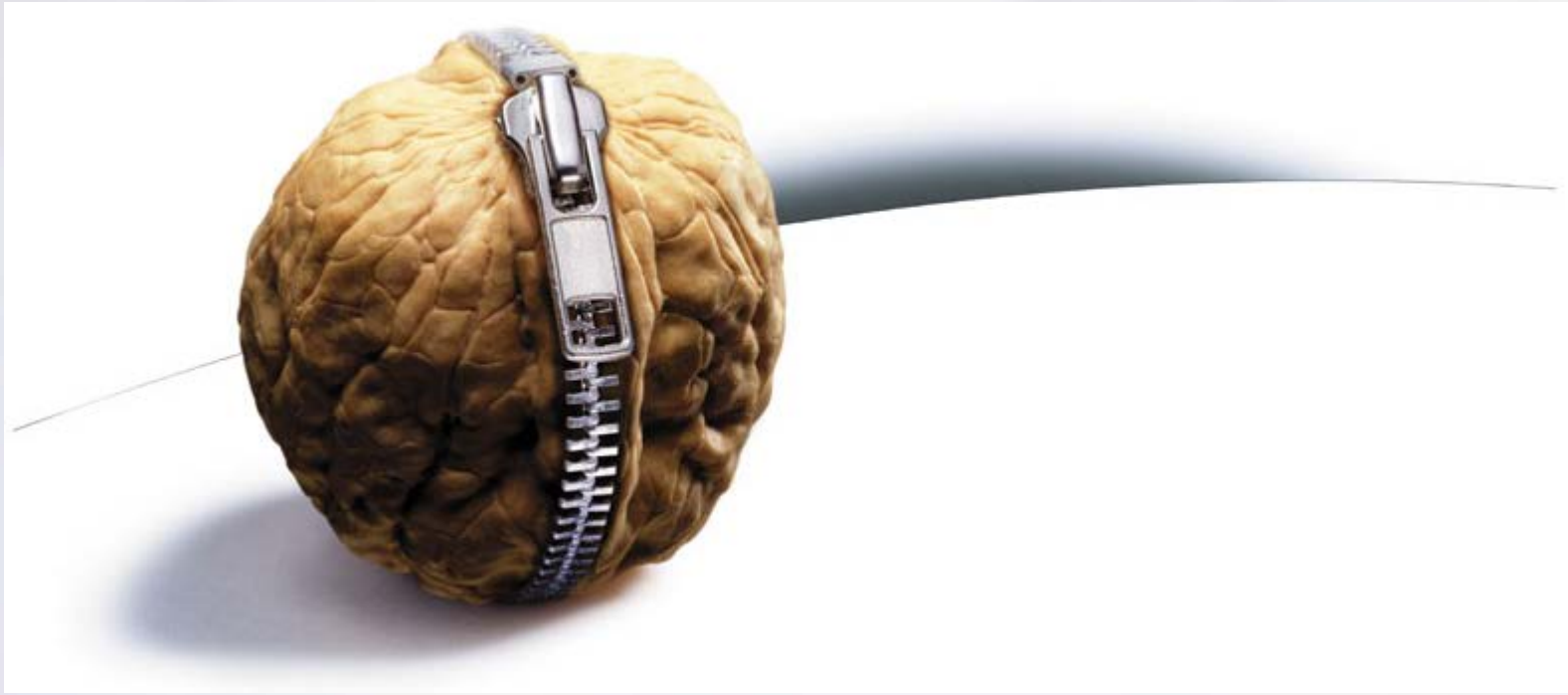
SPIDIA4P

A big Thank You goes to . . .

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

Thank you!

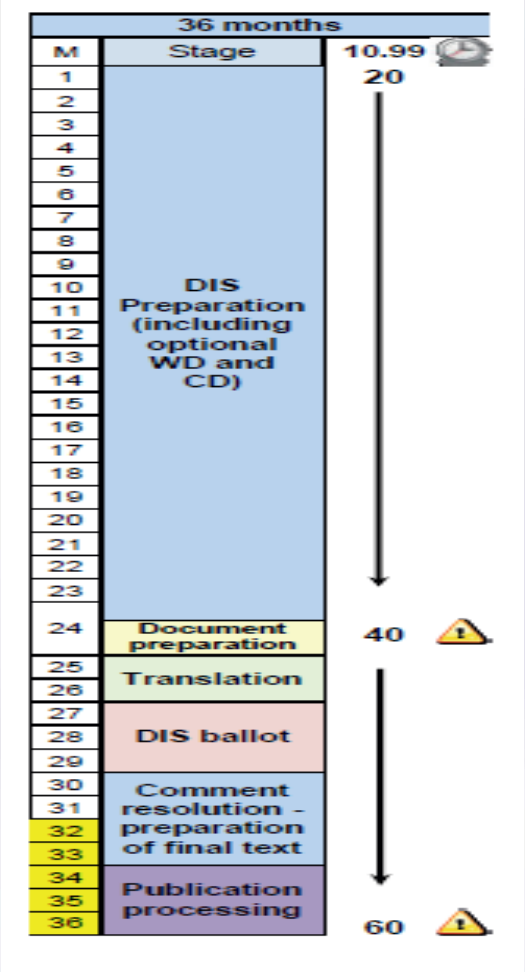
Questions ?

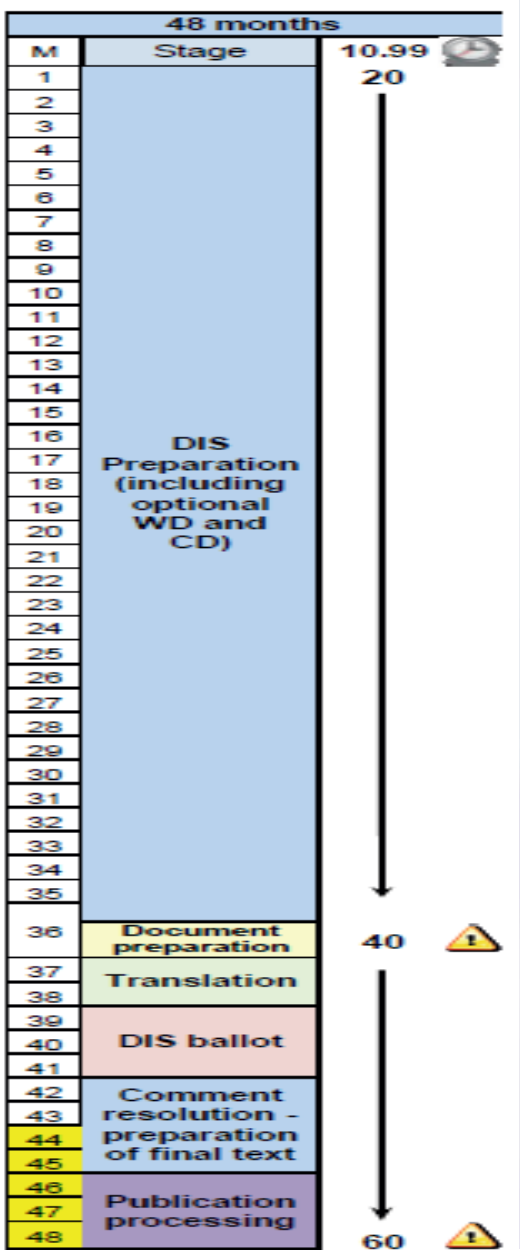


ISO/IS Development – Usually a 36 to 48 Months Period

ISO/TC 212

- Technical Committee for Clinical laboratory testing and in vitro diagnostic test systems
- 41 member countries, 22 observing members

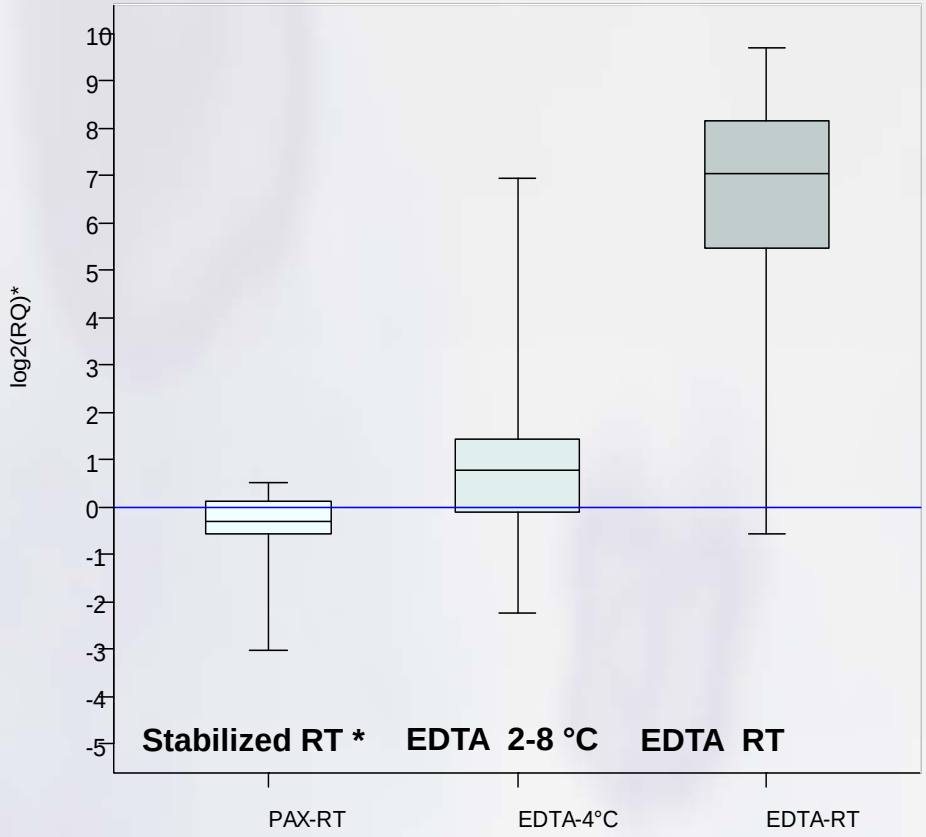




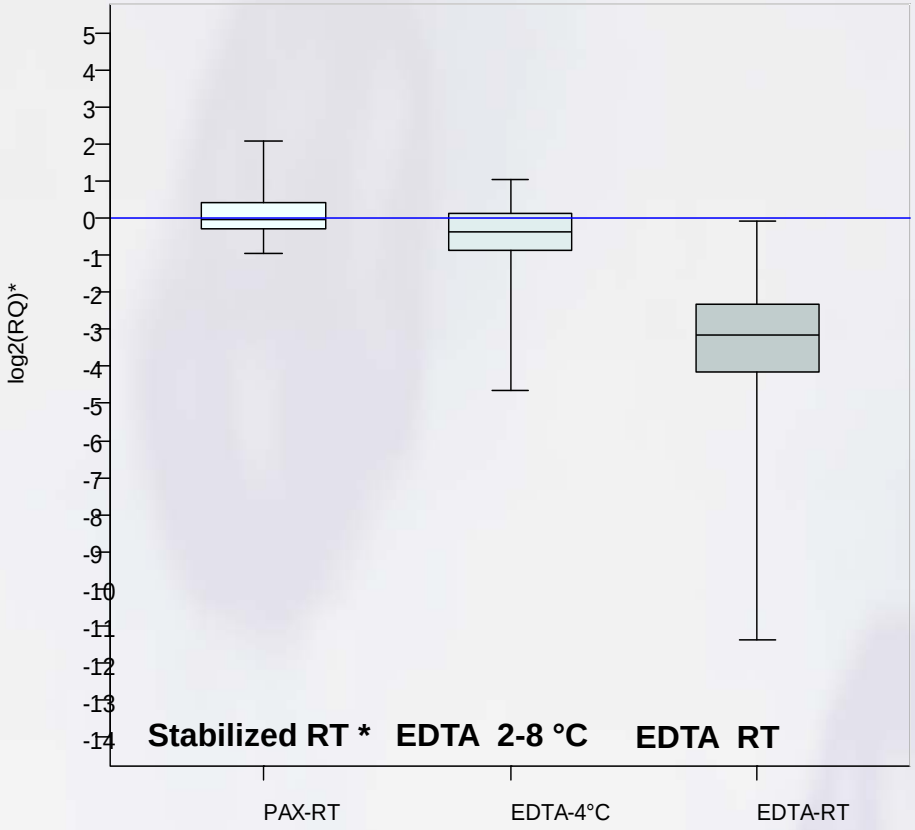
Source: https://www.iso.org/files/live/sites/isoorg/files/developing_standards/docs/en/Target_date_planner_4_ISO_standards_development_tracks_2017.pdf

Changes of Blood Cellular RNA Profile: 48 Hours After Collection

Up-regulated FOSB mRNA level



Down-regulated TNFRS mRNA level



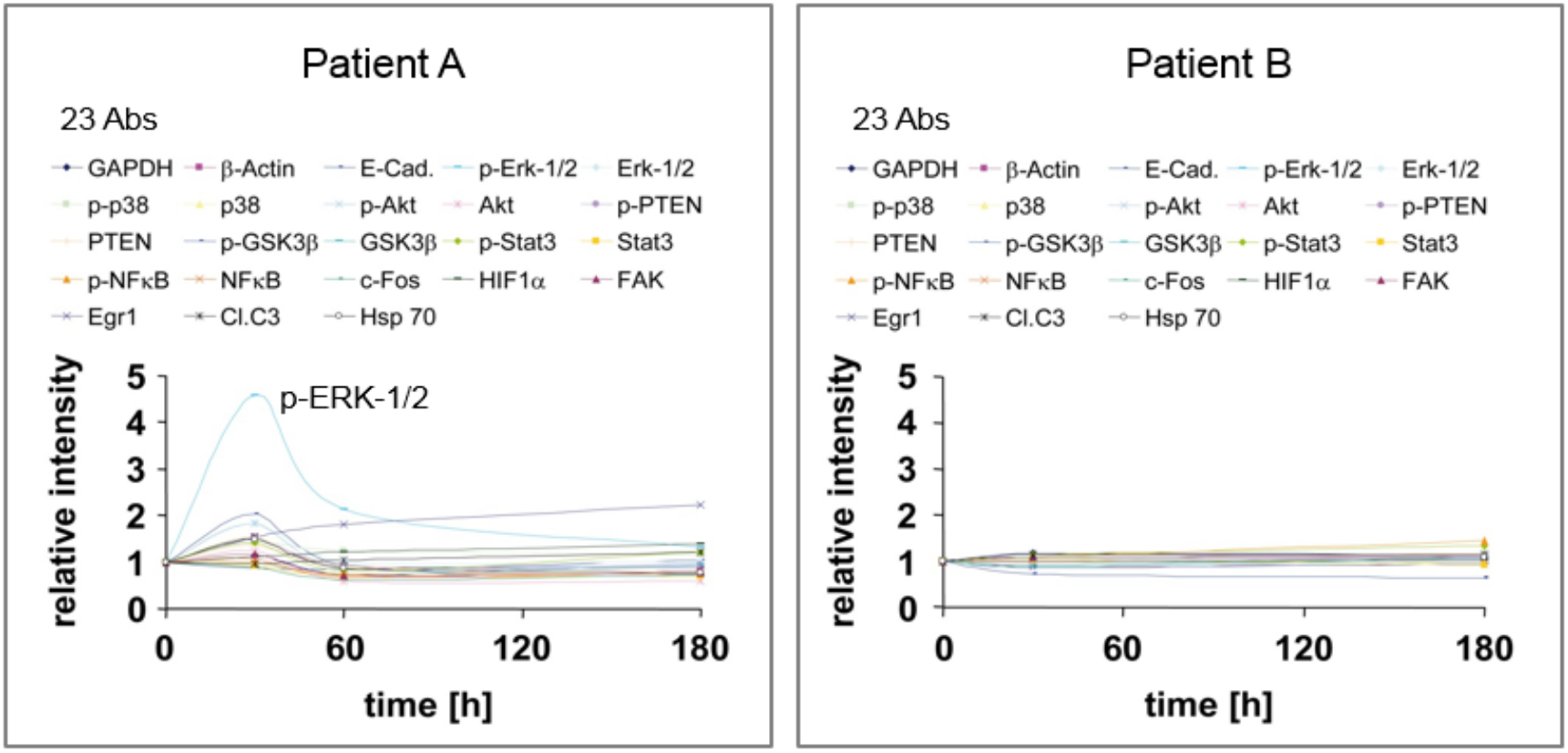
* PAXgene Blood RNA

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.

Inter-Patient Samples Variability

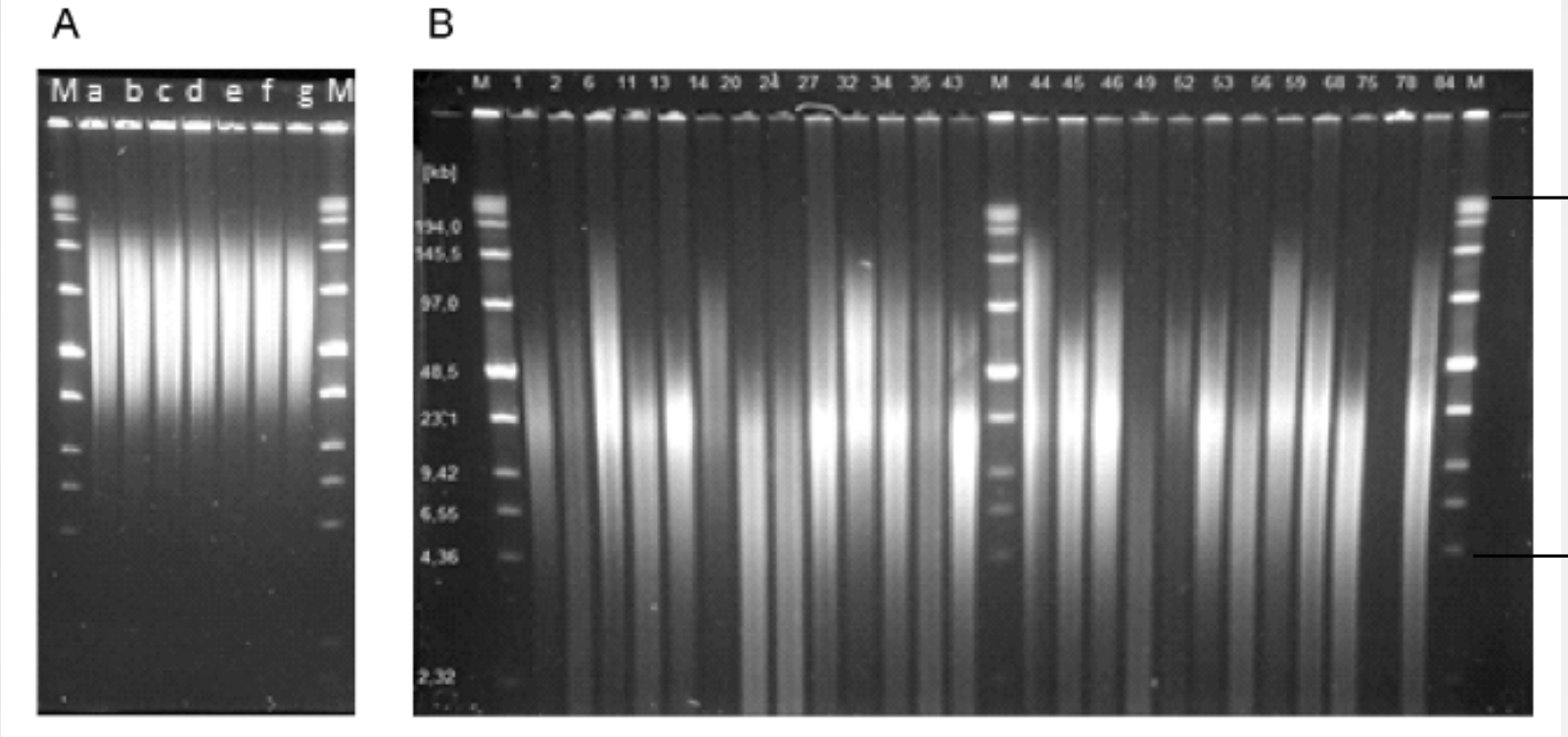
Impact of ischemia time on protein expression of intestine



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

Gündisch et al., J. Proteome Res. 2012

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



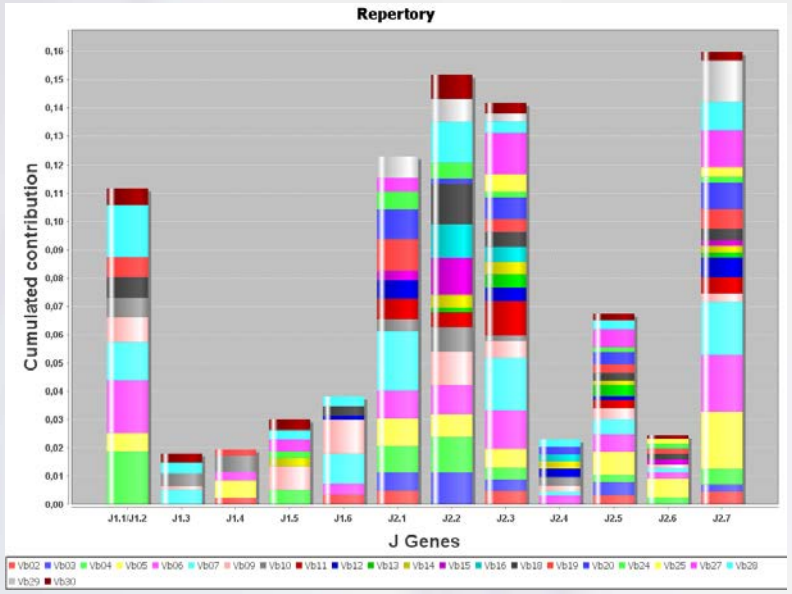
A: gDNA isolated immediately after blood collection at SPIDIA Laboratory

B: gDNA isolated by ring trial participating laboratories

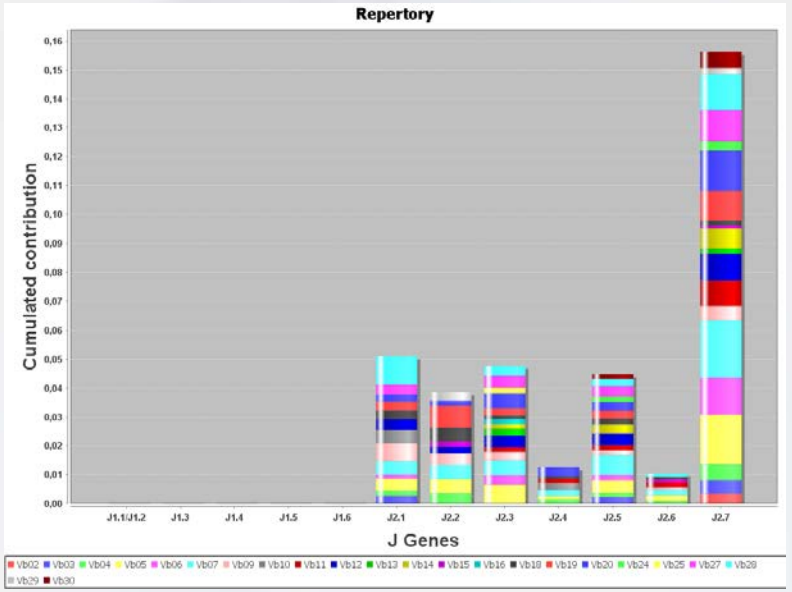
Malentacchi, F., Ciniselli, CM., Pazzagli, M. et al. (2015) Influence of pre-analytical procedures on genomic DNA integrity in blood samples: the SPIDIA experience. Clin Chim Acta. 440:205-10.

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.

International Grant Consortia Network on Diagnostic Workflow Technologies & Standardization