High Quality Clinical Samples:

The Key for Reliable Diagnostics and Research

Brussels, June 20th 2017

Dr. Uwe Oelmüller, SPIDIA4P Coordinator, QIAGEN GmbH

Deficiencies in Routine Healthcare demand for Improvements

- More than 70% of clinical decisions are based laboratory test results

  ADVANCE for Administrators of the Laboratory. July 2005

- 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

Improvements also needed for Research

- Researchers from 80% of more than 700 laboratories struggled to obtain standardized specimens for biomarker research

  Post G.. Bring on the Biomarkers. Nature 469, 156-157, Jan. 2011

- . . . irreproducible preclinical research exceeds 50%, resulting in approx. US$28,000,000,000 / year spent on preclinical research that is not reproducible - in the United States alone.

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

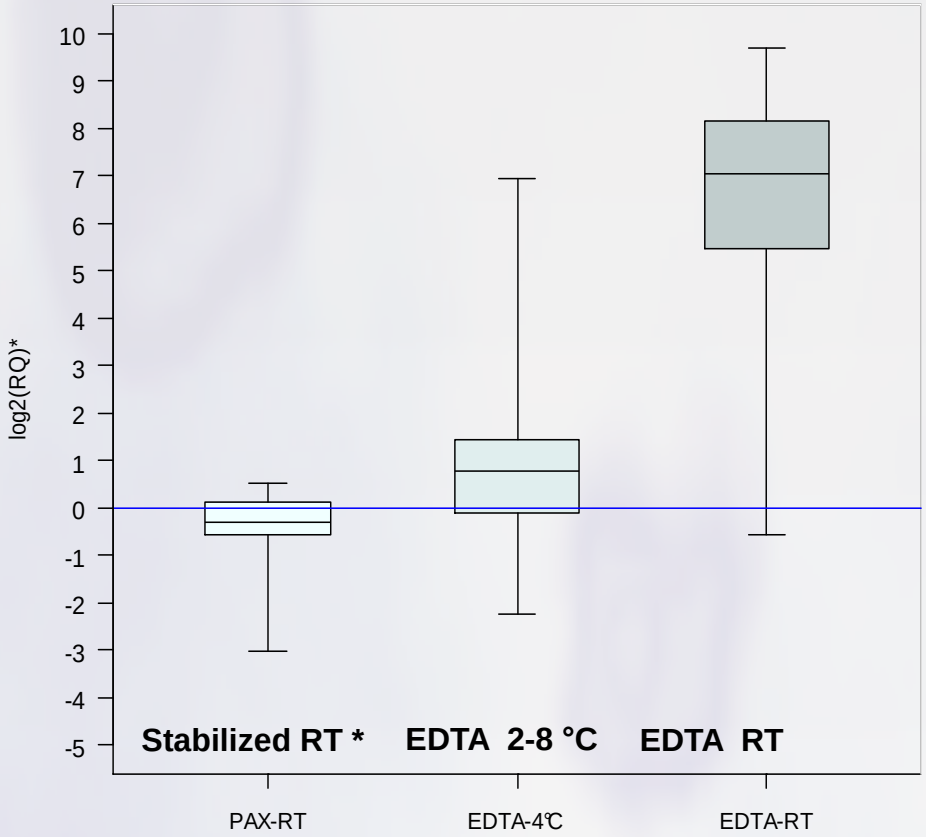
  ⇨ partly caused by pre-analytical errors

Sample-to-Insight Diagnostic Workflows

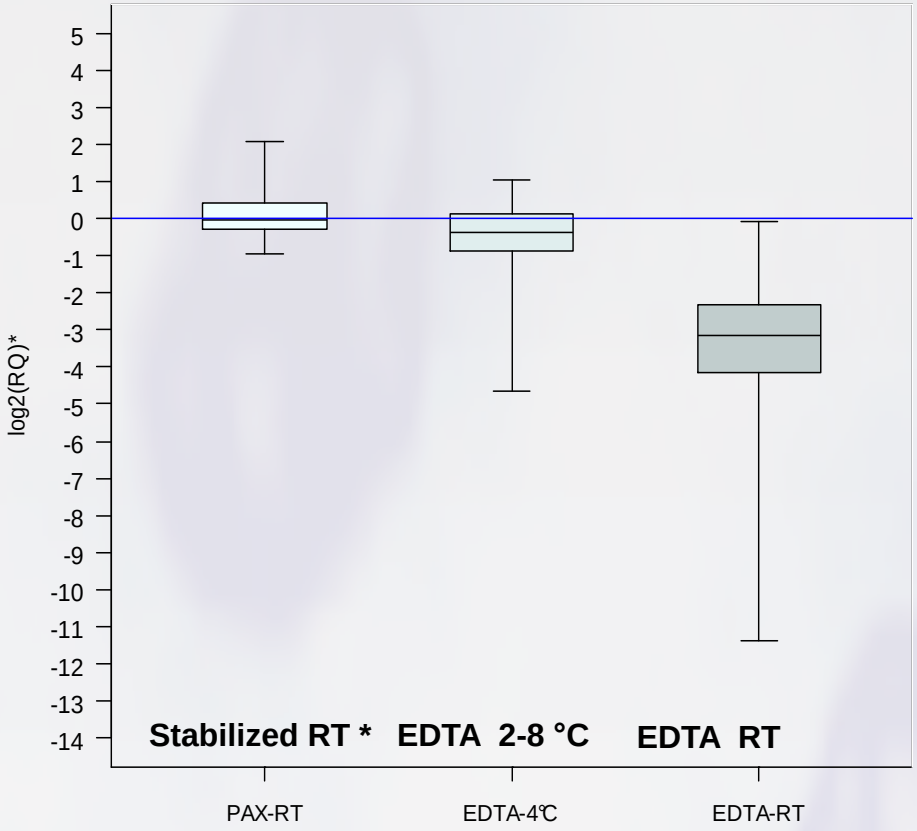
European Conference. Standards: Your Innovation Bridge. Brussels (2014). SPIDIA Booth.

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.

Two Major Efforts for Improvements

- **Technologies** for securing high quality samples
- **International Standards** for pre-analytical workflows

New Technologies and Standards for Pre-analytical Workflows

SPIDIA – FP7 (2008 – 2013)

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

SPIDIA4P – H2020 (2017 – 2020)

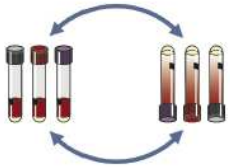
- ⇨ 19 Partners including BBMRI-ERIC
- ⇨ 14 associated consortia & stakeholders
- 13 additional new CEN & ISO Standards
- EQAs
- European implementation

www.spidia.eu

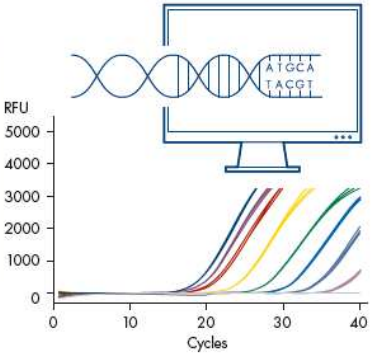
Technical Solution: Standardized Integrated Sample-to-Insight Workflows

QIAsymphony

Tube



Kit



Collection & Stabilization → **Storage Transport** → **Plasma Separation** → **ccfDNA Isolation** → **Analytical Test** → **Data Analysis**

Pre-analytical Workflow - Standards for all Segments

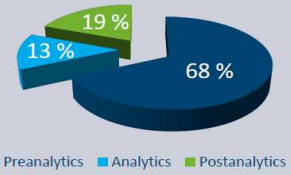
- **Biobanks**
  - Source for high quality samples
  - ⇨ BBMRI-ERIC plays a central role
- **Biomedical & Translational Research**
  - Academia
  - Pharma industry
  - Diagnostic Industry
- **Diagnostics**
  - High sample quality is the safe way
  - Analytical assay might tolerate lower quality or not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

SPIDIA's Road to Standardization

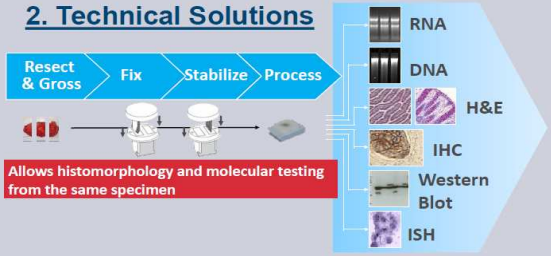
- 2017: Progressing to ISO/FDIS
- 2014: 8 new projects for ISO Standards approved in ISO/TC 212 „*Clinical laboratory testing and in vitro diagnostic test systems*"

- 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

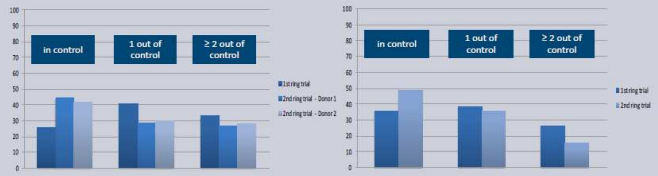
1. Problem - Errors in Diagnostics



2. Technical Solutions



3. Ring-Trials – Blood RNA (l.) and DNA (r.)

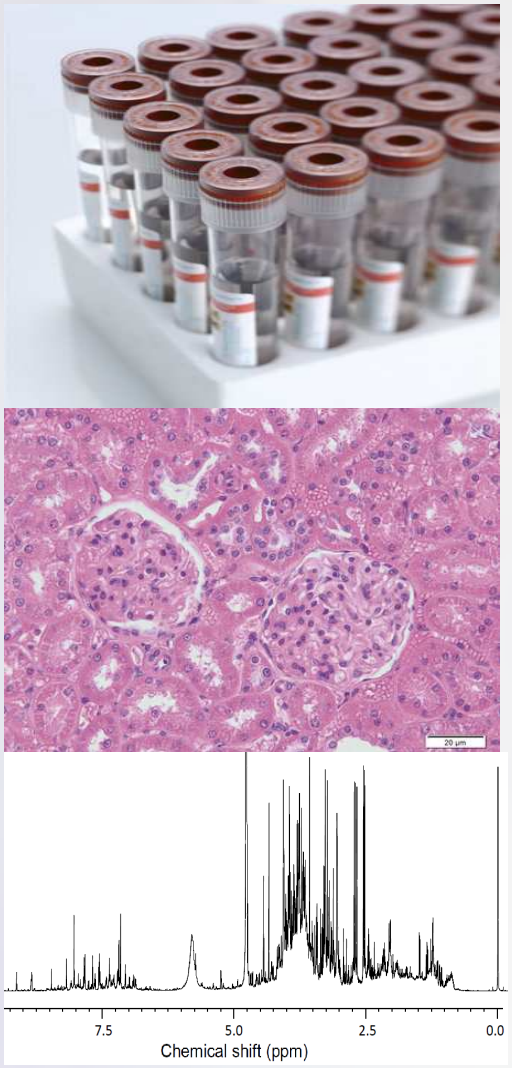


SEVENTH FRAMEWORK PROGRAMME

SPIDIA

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

9 CEN Technical Specifications released in Europe in 2015 / 16



- Pre-analytical phase: all steps from the clinicians requests to the beginning of the analytical examination

- 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
  - FFPE tissue — RNA
  - FFPE tissue — Proteins
  - frozen tissue — RNA
  - frozen tissue — Proteins
  - metabolomics in urine, serum and plasma

⇨ BBMRI-ERIC plays central role for implementation

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

ISO/IS expected for 2018

TECHNICAL SPECIFICATION **CEN/TS 16835-3**

SPÉCIFICATION TECHNIQUE

TECHNISCHE SPEZIFIKATION 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

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

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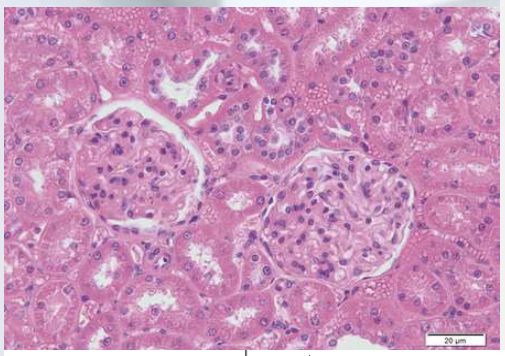
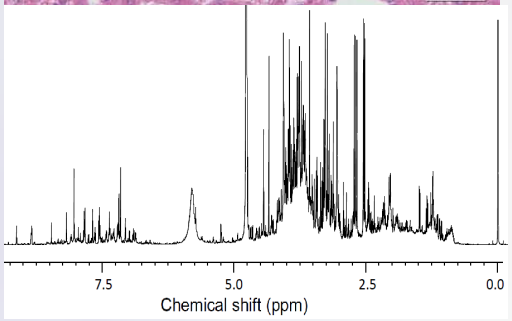
. . . more Standards to come





- 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
- Frozen Tissue – DNA
- Fine Needle Aspirates (FNAs) – DNA, RNA, proteins
- Metabolomics of body fluids: International ISO Standard
- FFPE Tissue – in situ stainings incl. IHC

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

A big Thank You goes to . . .

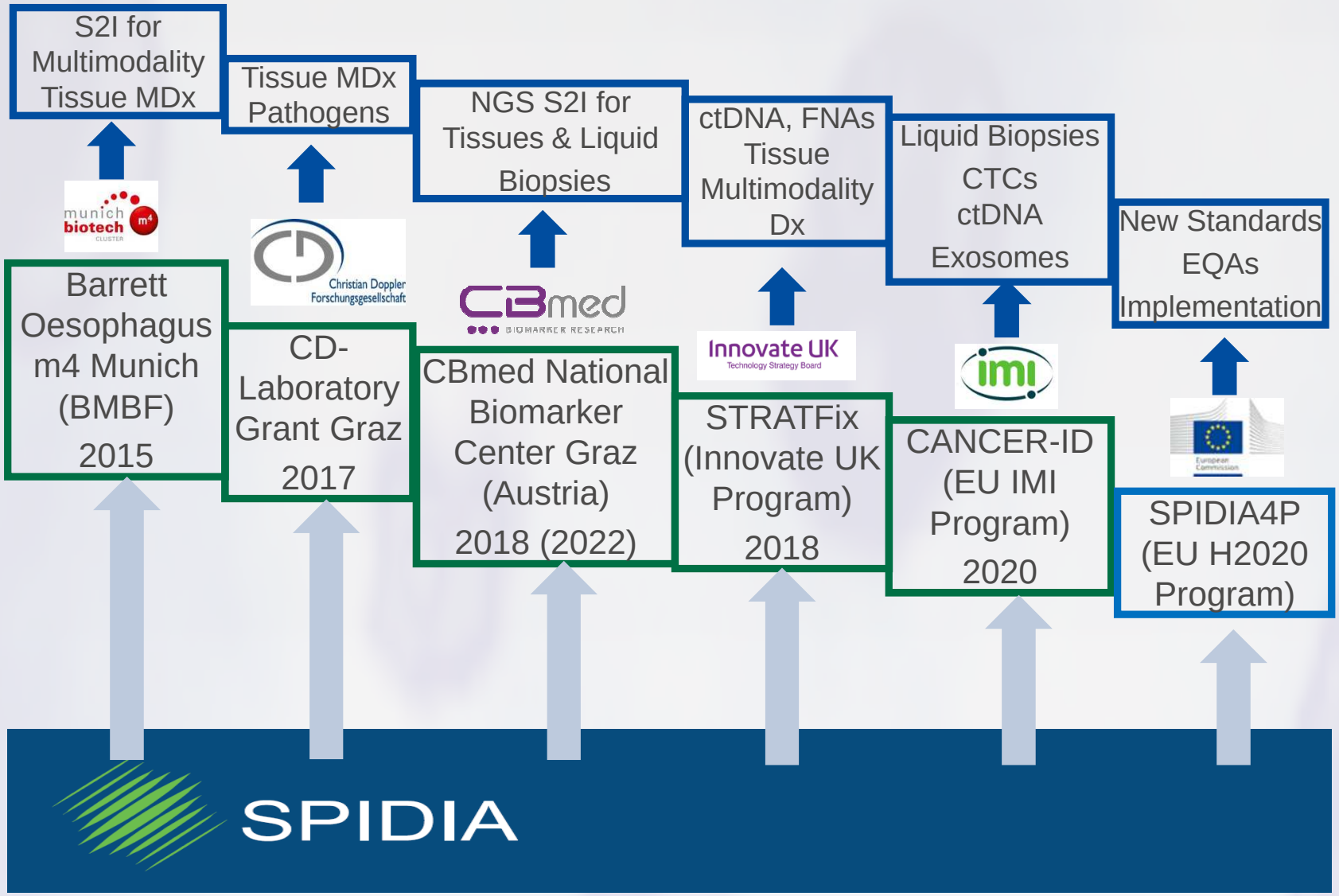
. . . to the SPIDIA & SPIDIA4P Consortium Members and all European and International Partners!

SPIDIA4P

Thank you!

Questions ?

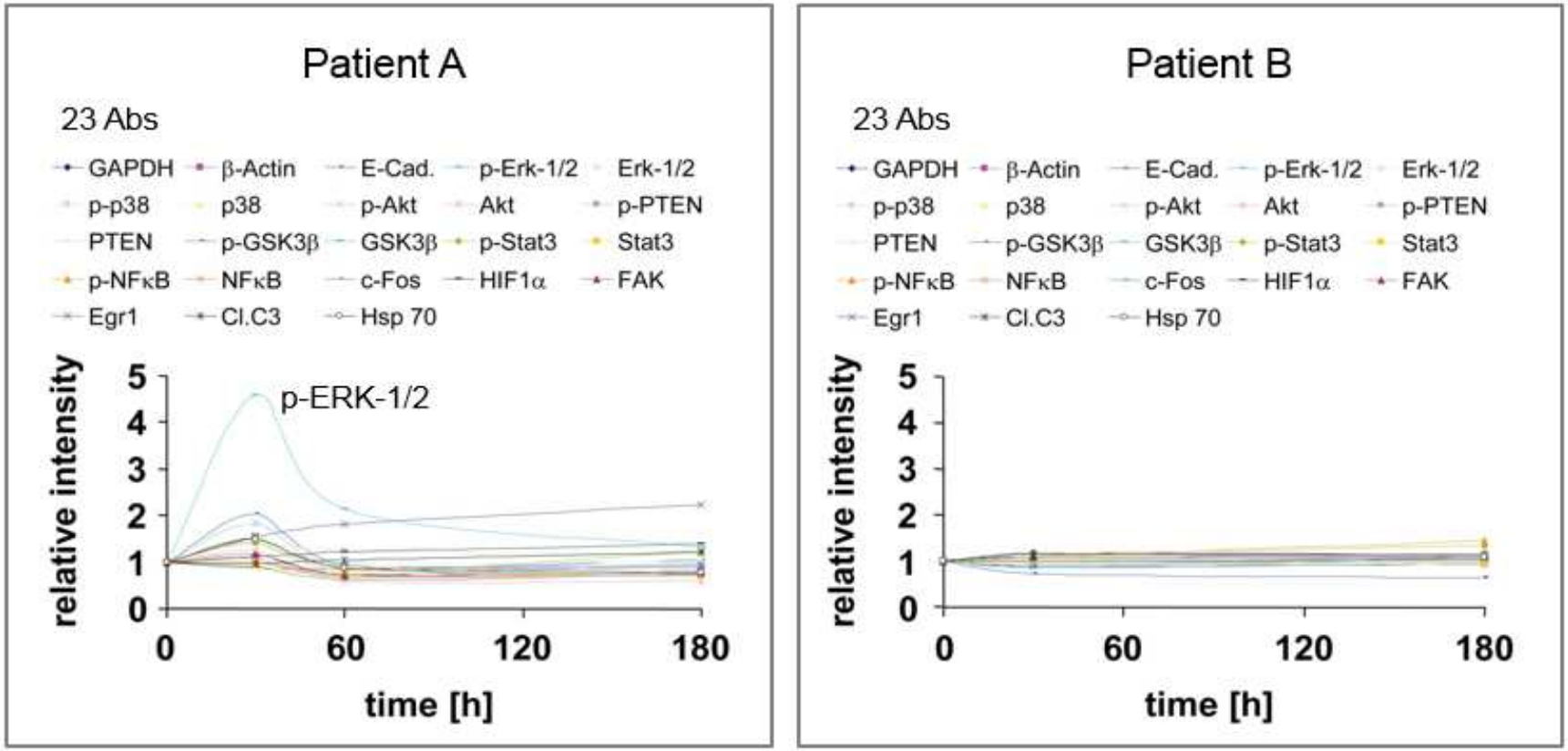
European Grant Consortia Network on Diagnostic Workflow Technologies & Standardization



SPIDIA4P

Inter-Patient Samples Variability

Impact of ischemia time on protein expression of intestine



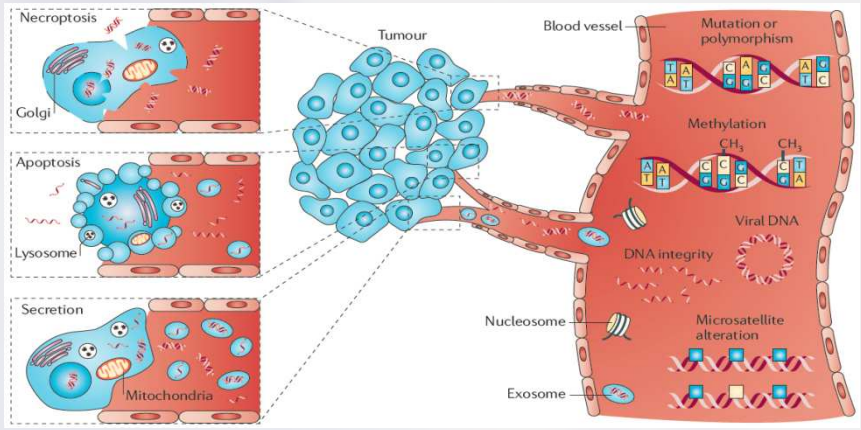
Impact of ischemia time on protein expression of non-malignant human intestine samples

Gündisch et al., J. Proteome Res. 2012

Technische Universität München

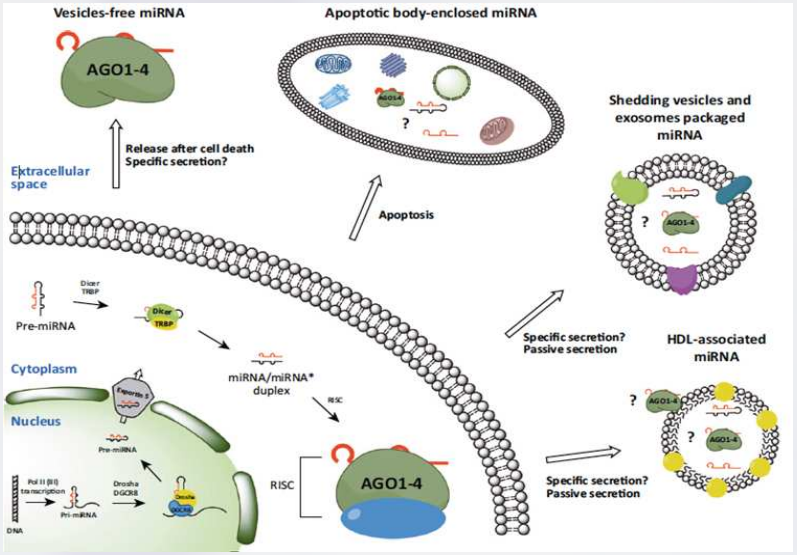
The Value of Liquid Biopsies as Blood-Based Biomarkers

ccfDNA



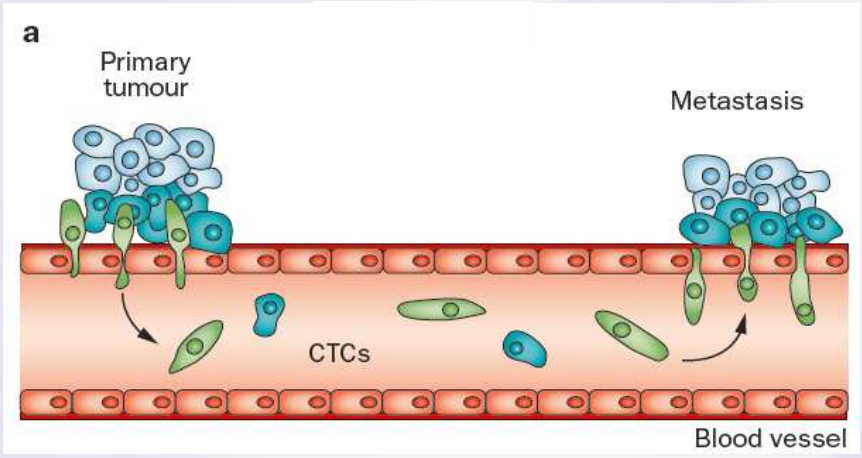
Schwarzenbach et al. (2011) Nat Rev Cancer 11:426-437

Exosomes (RNA, miRNA)



Turchinovich et al. (2013) Trends Biochem Sci 37:460-464

CTCs (multi-modality)

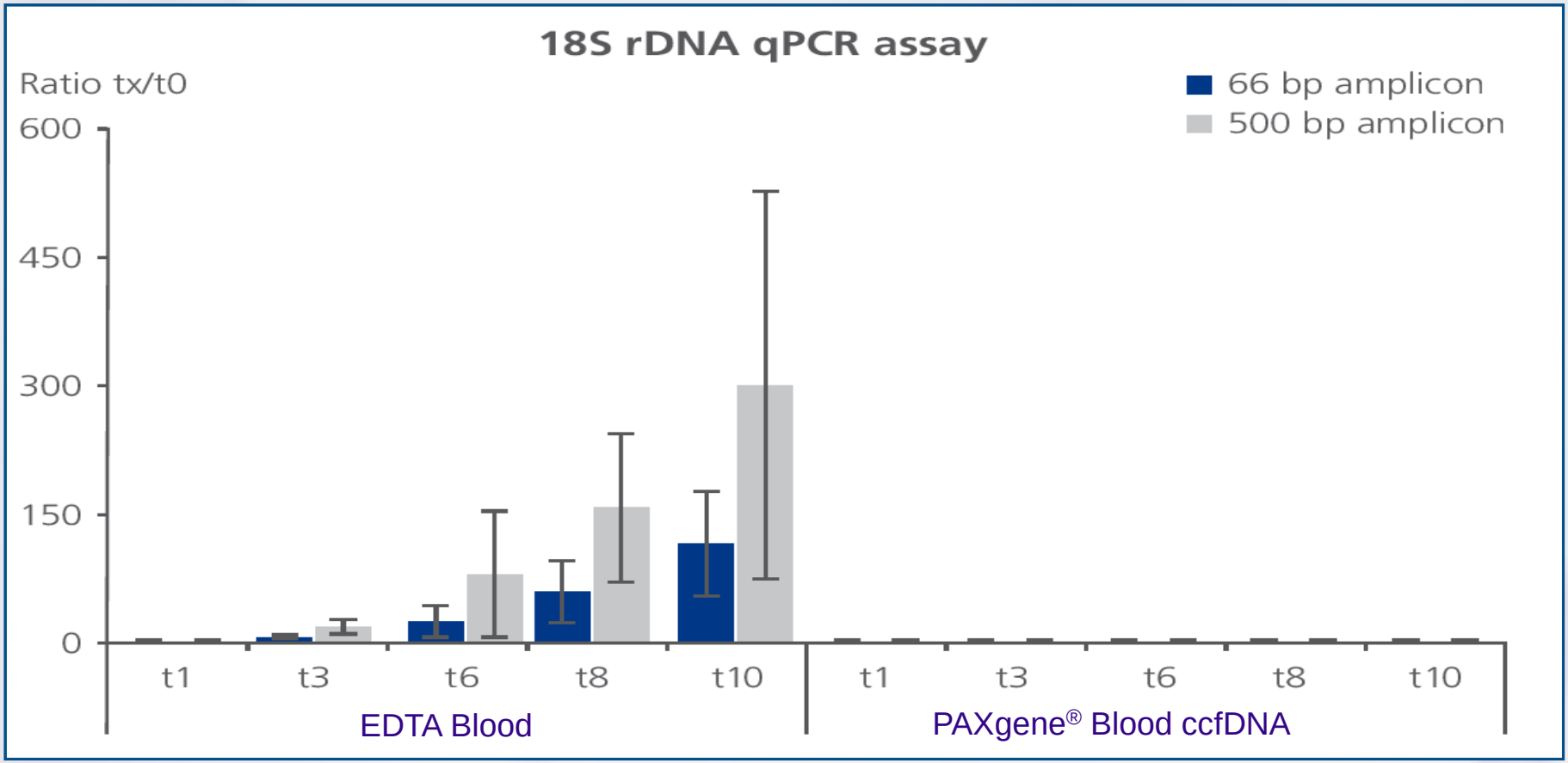


Krebs et al. (2014) Nat Rev Clin Oncol 11:129-144

. . .and ccfRNA

CANCER-ID IMI Consortium -- modified

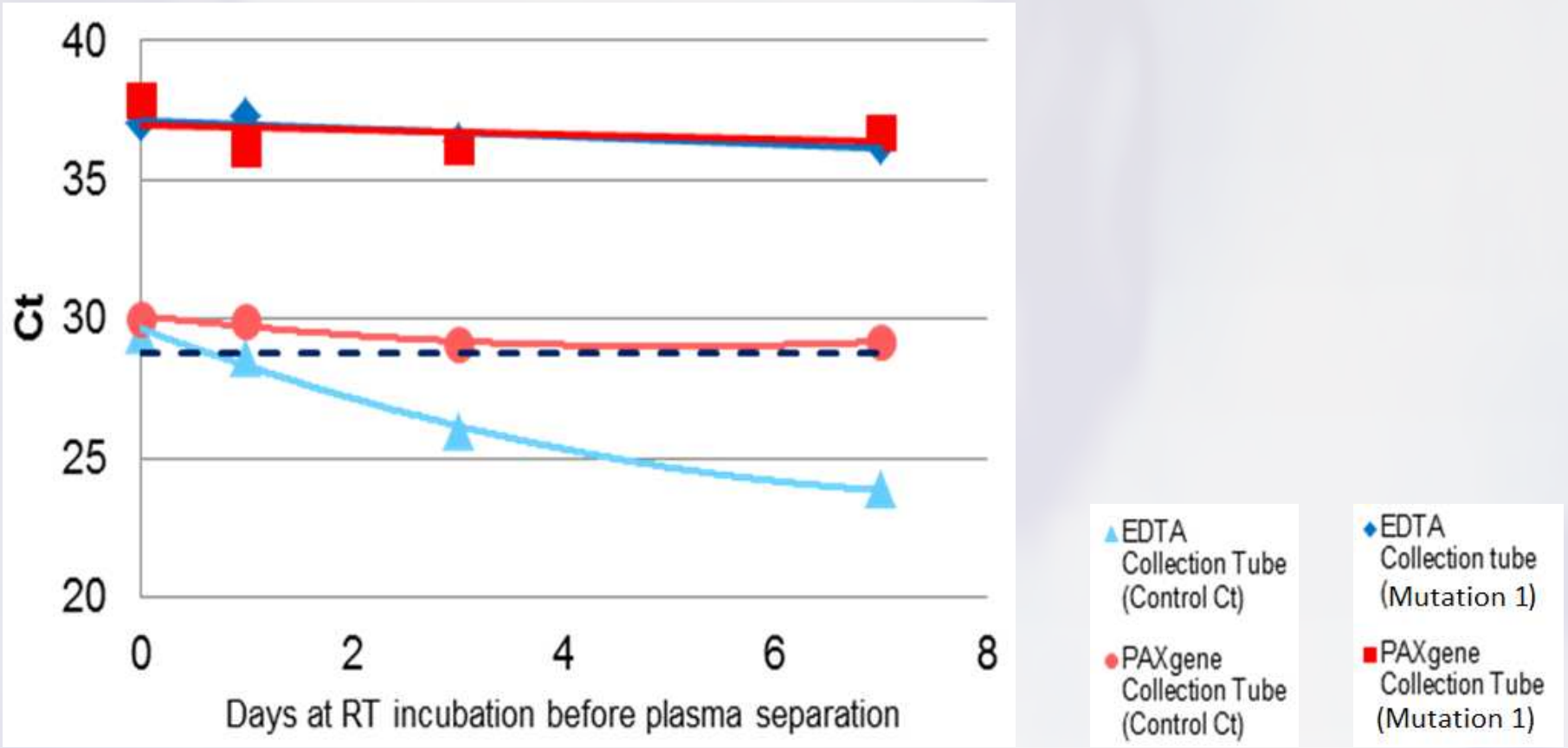
Pre-analytical Impacts on ccfDNA Analysis



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

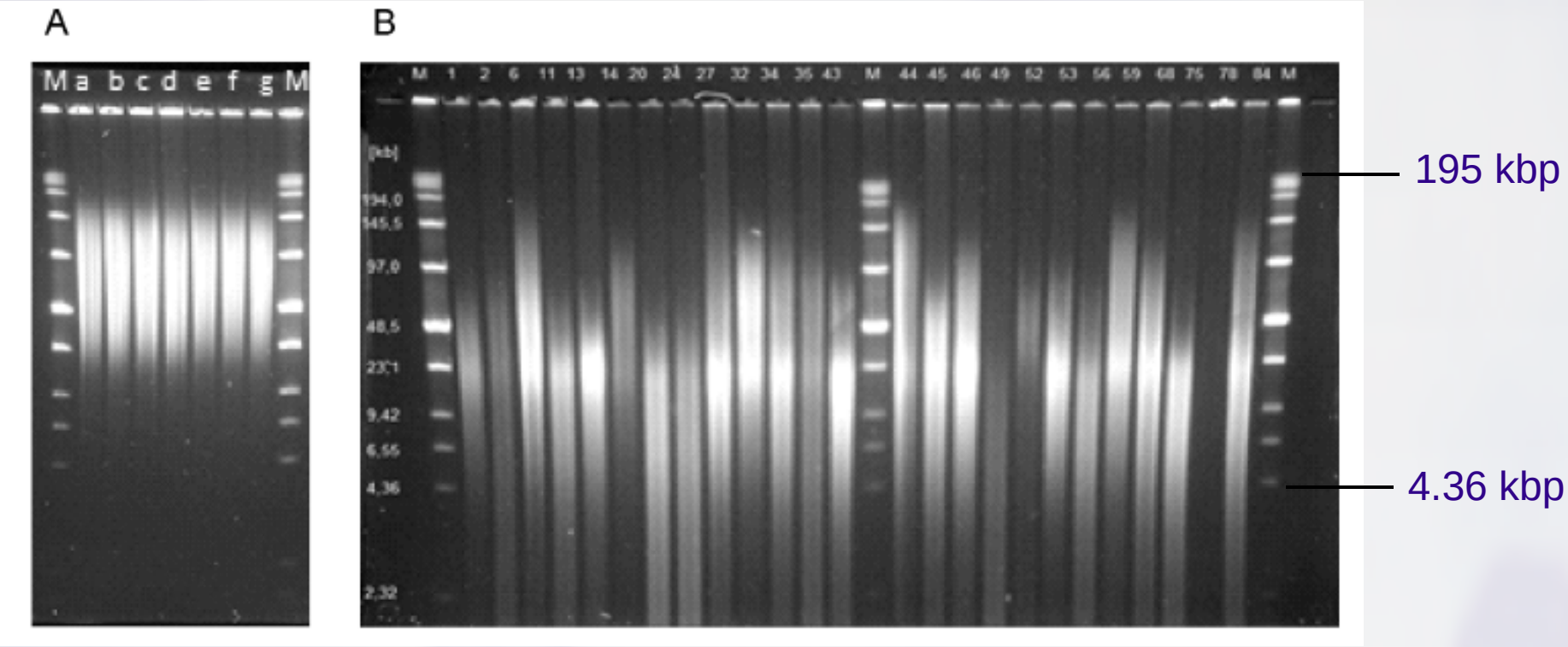
ESBB Congress London, 2015

Pre-analytical Impacts on ccfDNA based Test Development



Unpublished data provided by QIAGEN

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



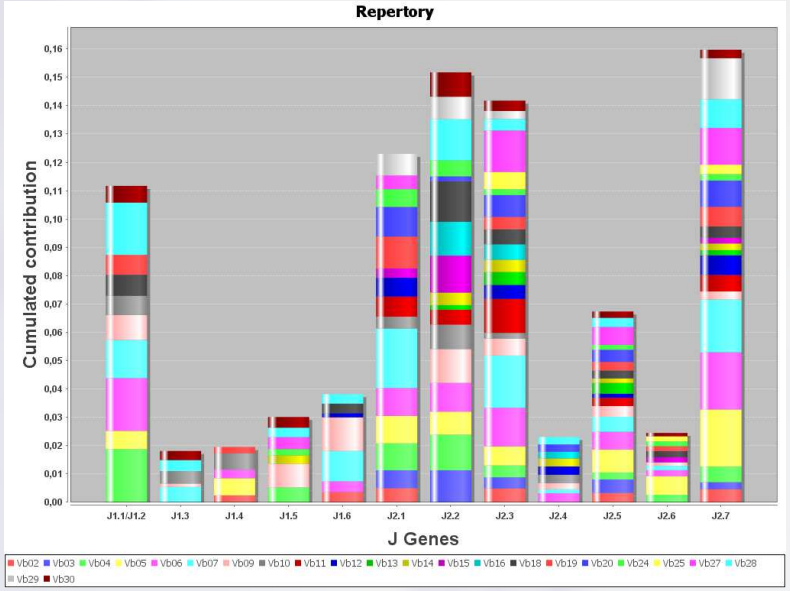
A: gDNA isolated immediately after blood collection at SPIDIA Laboratory

B: gDNA isolated by ring trial participating laboratories

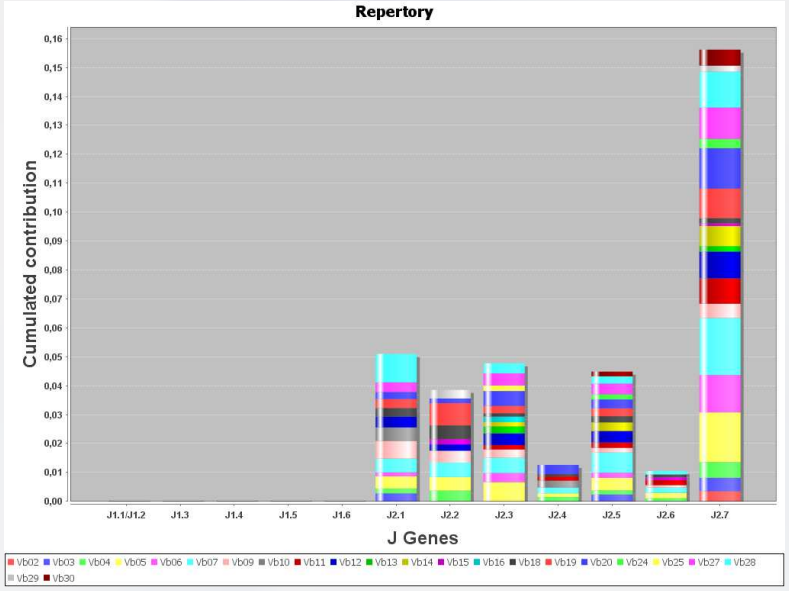
Malentacchi, F., Ciniselli, CM., Pazzagli, M. et al. (2015) Influence of pre-analytical procedures on genomic DNA integrity in blood samples: the SPIDIA experience. Clin Chim Acta. 440:205-10.

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%)



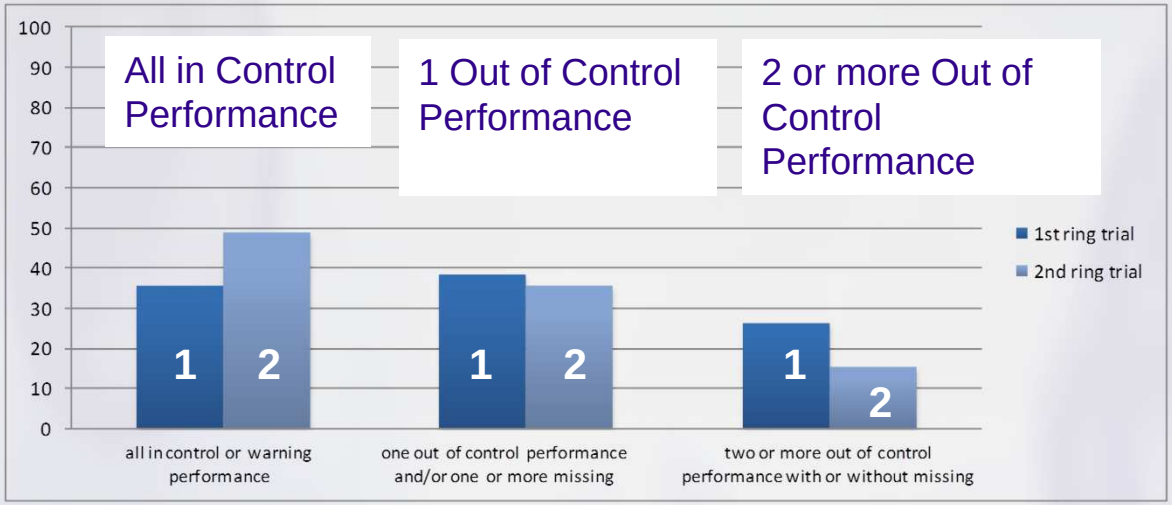
Sample 38 (Poor quality) (DIV 32%)

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

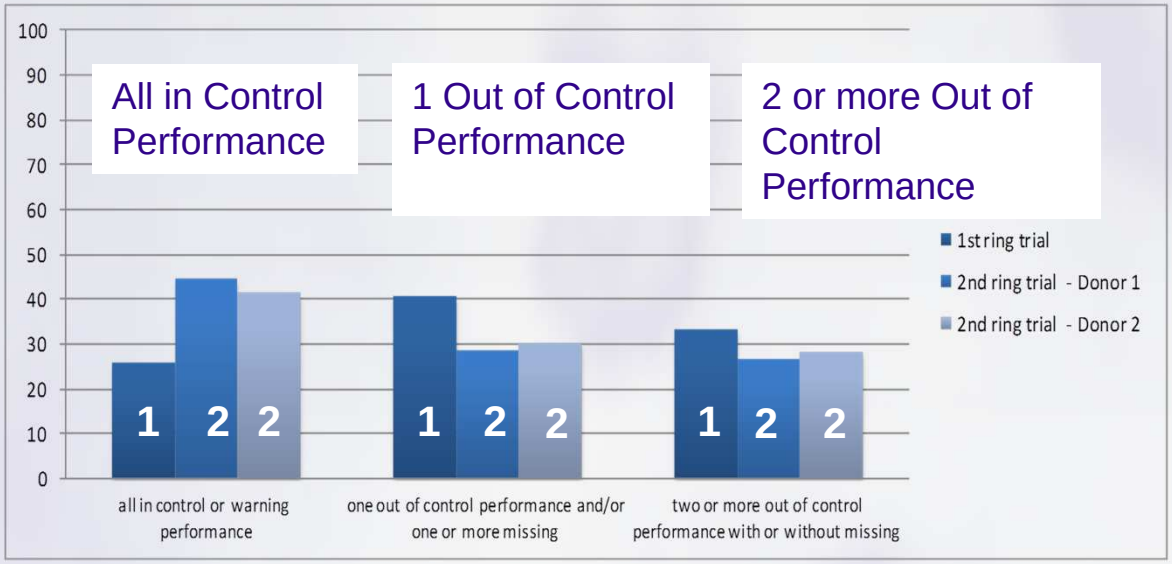
Malentacchi, F., Ciniselli, CM., Pazzagli, M. et al. (2015) Influence of pre-analytical procedures on genomic DNA integrity in blood samples: the SPIDIA experience. Clin Chim Acta. 440:205-10.

Optimized Workflows can Improve Test Results

1st vs 2nd SPIDIA European Blood Ring Trials



Blood DNA Ring Trials 1 & 2



Blood RNA Ring Trials 1 & 2

Data from SPIDIA partners IRCCS & University of Florence

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

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 need standardization to get into the marketplace and to be successful

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

CEN/TS Documents: Target Audience

Applicable to molecular in-vitro diagnostic examinations

- In-vitro diagnostic laboratories
- Laboratory customers
- In-vitro diagnostics developers and manufacturers
- Institutions & commercial organizations performing biomedical research
- Biobanks
- Regulation authorities