- EU SPIDIA Project Update -

Standardization and Improvement of Generic Preanalytical Tools and Procedures for In Vitro Diagnostics

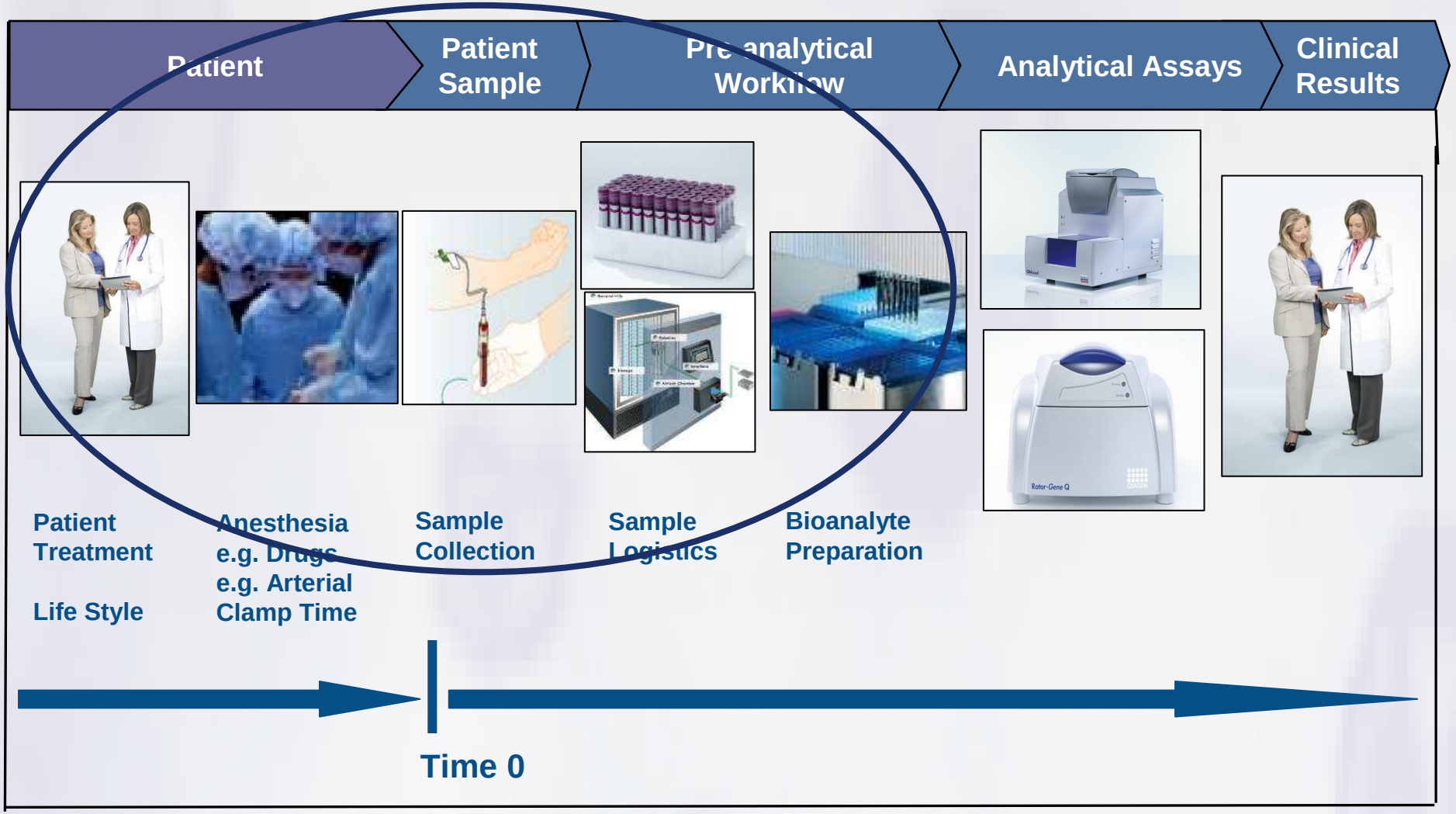
5th Annual BRN Symposium
Bethesda, February 22nd 2012

Dr. Uwe Oelmueller
SPIDIA Coordinator (QIAGEN)

Agenda

- **SPIDIA Project History and Goals**
- Results & Status
  - New Technologies & Tools
  - Pan-European Guidelines
  - Biospecimen Quality Markers

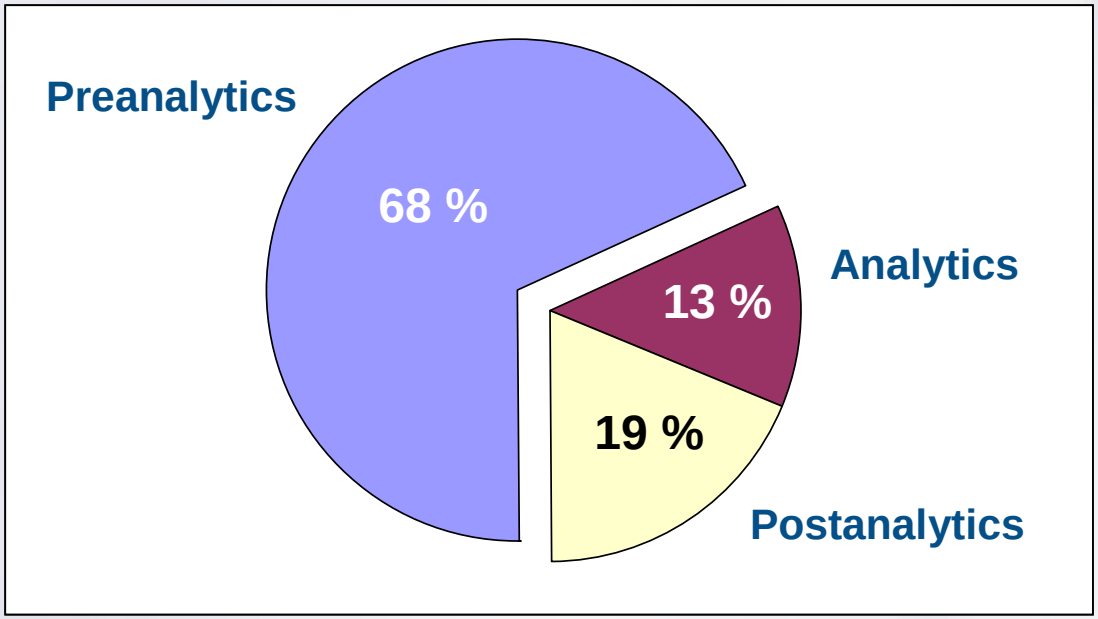
Diagnostic Workflow
From Patients to Clinical Results



It is Real Problem

"Preanalytical errors still account for nearly 60%-70% of all problems occurring in laboratory diagnostics, most of them attributable to mishandling procedures during collection, handling, preparing or storing the specimens".

Lippi G. *et al.*. Preanalytical quality improvement: from dream to reality. Clin Chem Lab Med. 2011 Jul; 49(7):1113-26. Epub 2011 Apr 25.



Costs of ~ 460,000 $ / year in an average German hospital caused by pre-analytical errors

Frost & Sullivan 2011 on behalf of BD

Project Main Goals

- Pan-European guidelines for preanalytics (Molecular – Blood, Tissue)
- New pre-analytical tools & technologies (Blood, Plasma, Tissue, Swabs)
- Sample quality markers (Blood, Tissue)
- Training and dissemination

Project Facts

Program	European Commission FP7-HEALTH
Consortium	7 public research organizations 8 companies 1 standards organization (CEN)
Coordinator	QIAGEN GmbH
Run Time	October 2008 – September 2012 (prolongation request intended)
Budget	13 Mio € (9 Mio € EC contribution)
Co-operations	NCI / OBBR, CLSI, EFCC, BBMRI and other international initiatives and organizations
Web page	www.spidia.eu
Newsletter	

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New Tissue Fixation & Stabilization
Histomorphology, IHC, RNA & DNA, Proteins

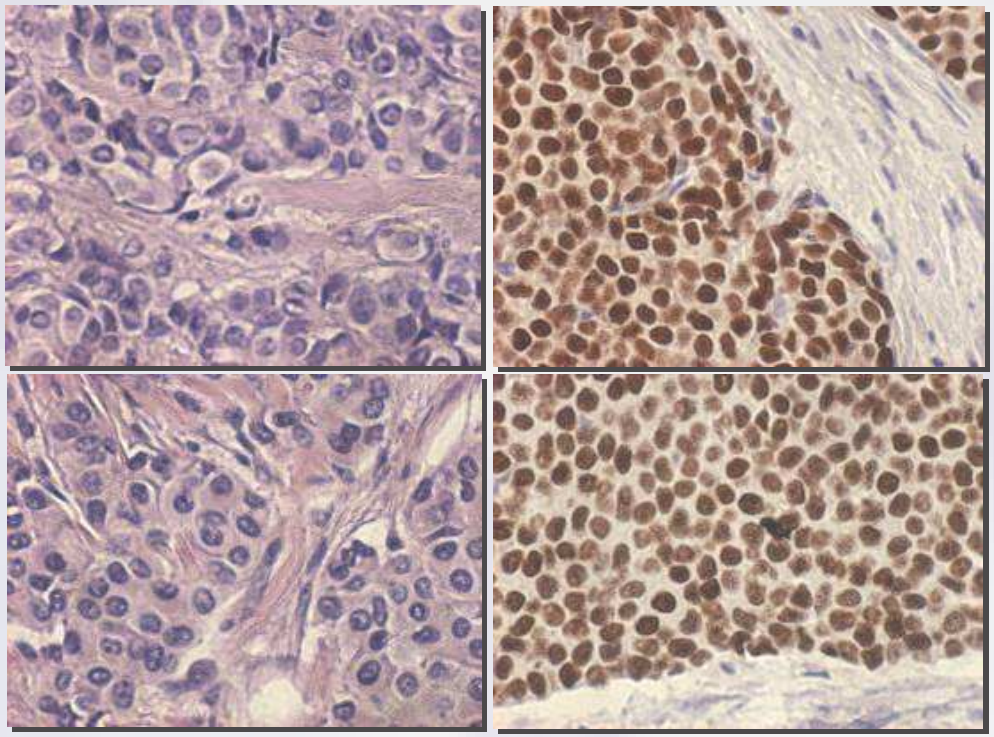
H&E Staining
IDC of Breast

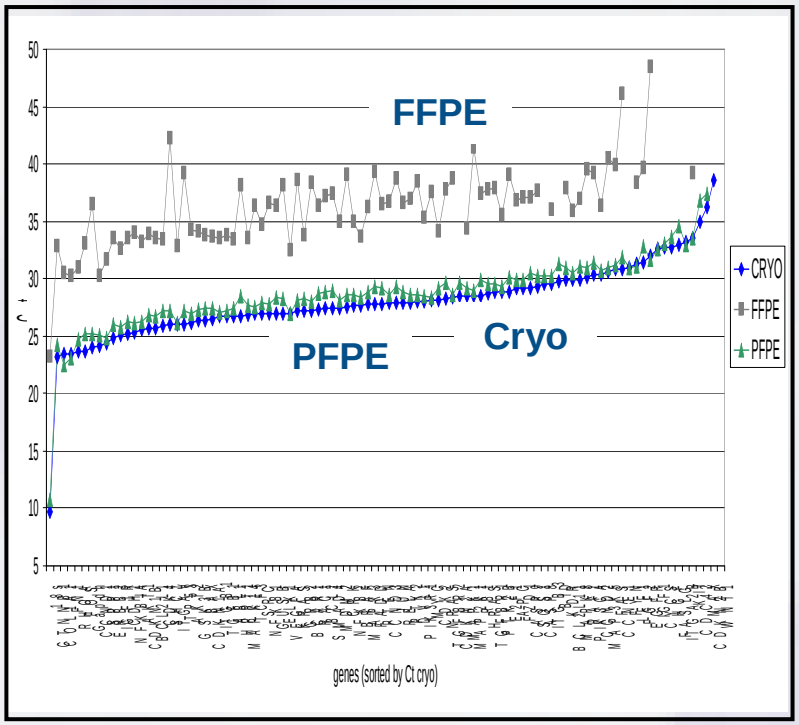
Estrogen Receptor a
(clone 1D5) IDC of Breast

Mammacarcinoma
TaqMan Array Gene Signature

Formalin

PAXgene





PFPE revealed preservation of morphology and antigenicity comparable to FFPE

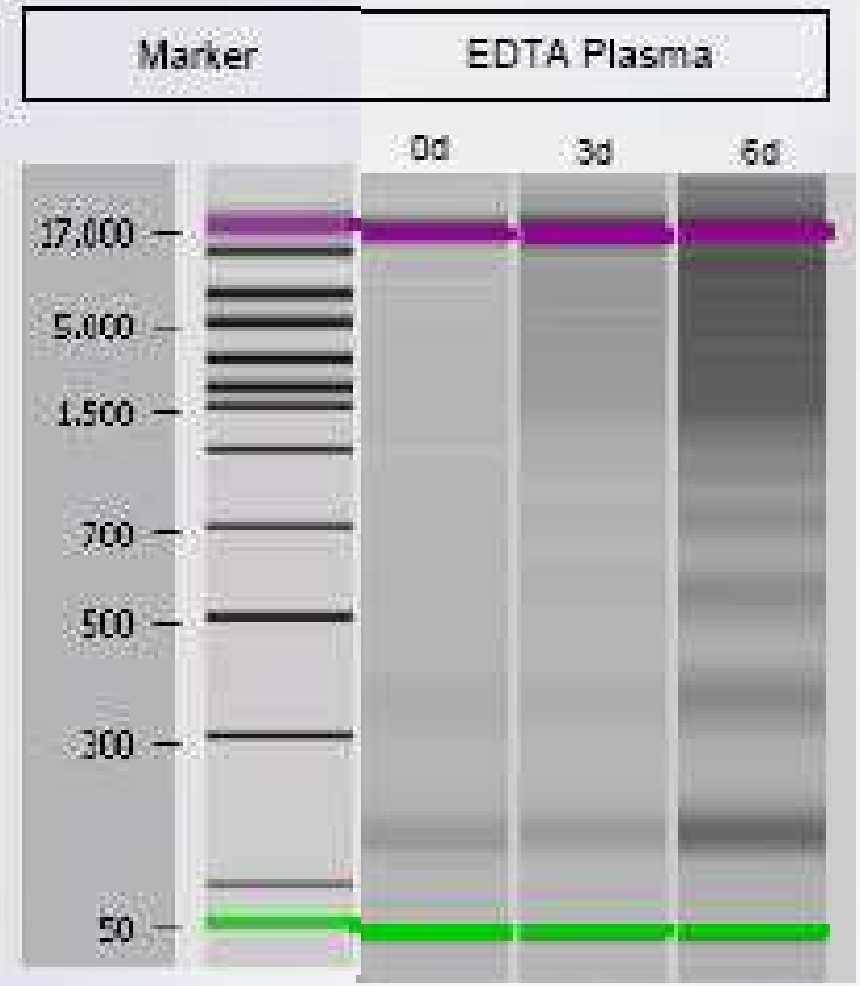
Nucleic acid analysis superior to FFPE

Kap M. *et al.,* PLoS ONE 6(11): e27704 (2011)

Viertler C. *et al.*, submitted for publication

Groelz D. et al., unpublished data.

fcNA Profiles in Whole Blood / Plasma
What is missing?



- Studies for understanding fcDNA and fcRNA profile stability / changes in whole blood and in plasma
- Development of fcDNA and fcRNA profile preservation technologies

EDTA blood was incubated for up to 6 days at room temperature. Blood fcDNA pattern stability was determined by separating the purified plasma DNA on a 2100 Agilent Bioanalyzer

Horlitz M. *et al.,* unpublished data

Ongoing Technology & Tools Developments for Other Sample Types

- **Fine Needle Aspirates**
  - Stabilization of morphology, antigenicity, DNA, RNA, proteome
- **Whole Blood**
  - Stabilization of cell morphology and biomolecule profiles
- **Swabs**
  - Stabilization and improved processing of respiratory and samples for molecular analysis
- **Stabilized Whole Blood**
  - Integrated automated sample-to-result workflows (cellular RNA, ncRNAs incl. miRNAs)

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Evidence Based Guidelines

Examples Blood DNA & RNA, Plasma fcDNA

- Phase 1 Trials - Laboratories used their workflows & tools
- Let by Prof. Pazzagli (Univ. Florence), supported by the EFCC
- Guidelines / Standards Concepts - CEN
- Phase 2 Trials - Laboratories will use SPIDIA's optimized workflows
- Guidelines / Standards Developments - CEN

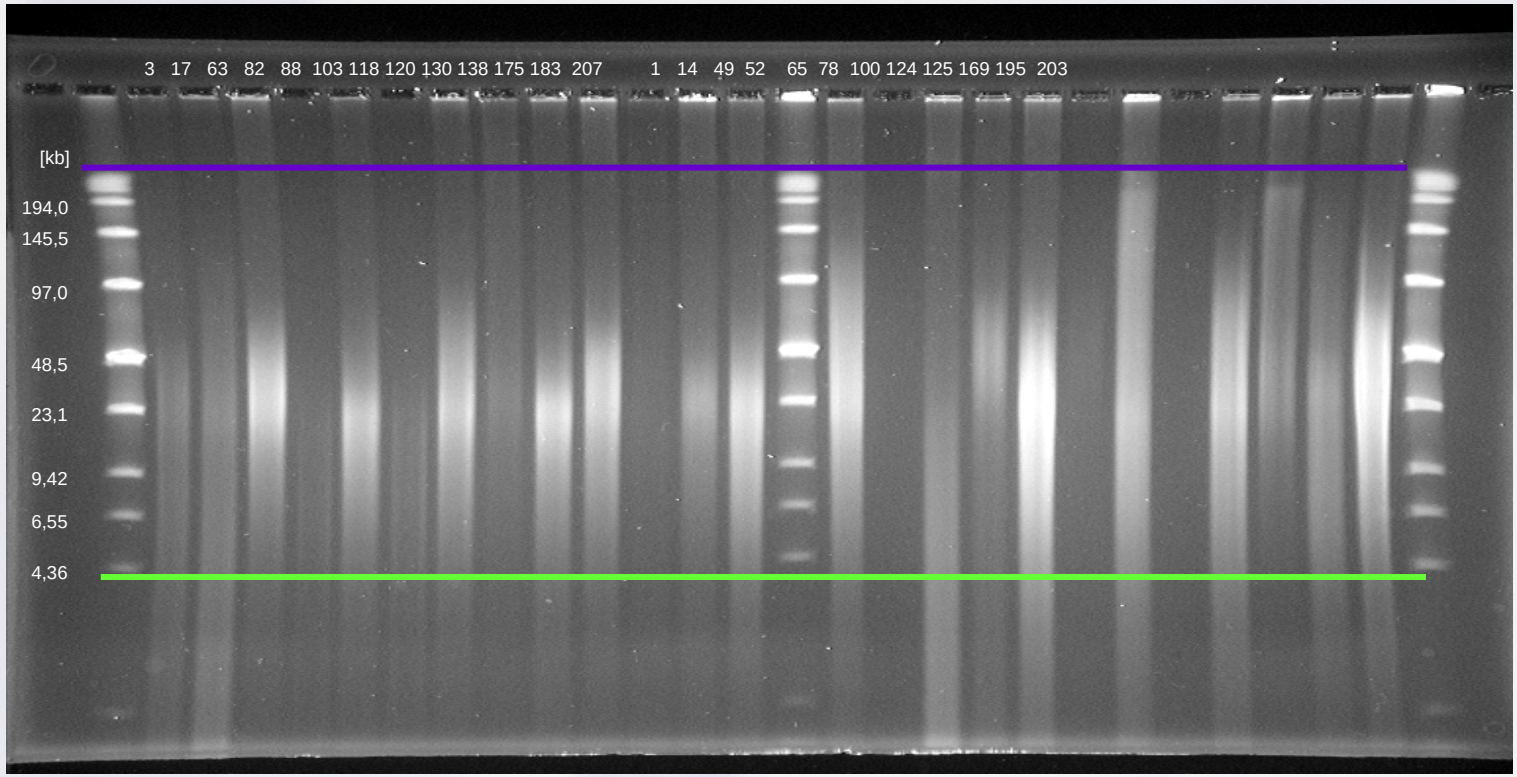
SPIDIA Trials	No. of Participants (29 countries)	Participants who sent NA samples back	Percentage of NA samples sent back
Blood RNA	102	93	91 %
Blood DNA	130	121	93 %
Plasma DNA	67	62	93 %
Total	299	276	92 %

Blood DNA Trial 1 - Examples for Pre-analytical Workflow Variations

- **Blood storage time before DNA extraction**
  - 39 labs: ≤ 6 days
  - 60 labs: 6 – 10 days
  - 53 labs: ≥ 10 days
- **Blood storage temperature before DNA extraction**
  - 18 labs: -20 ˚C
  - 129 labs: +4 ˚C
  - 9 labs: ambient temp.
- **Isolated DNA storage before analysis**
  - 20 labs: -20 ˚C
  - 111 labs: +4 ˚C
  - 27 labs: ambient temp.

Pazzagli M. *et al.,* manuscript in preparation

DNA Length Variation – Pulse Field Gel Electrophoresis



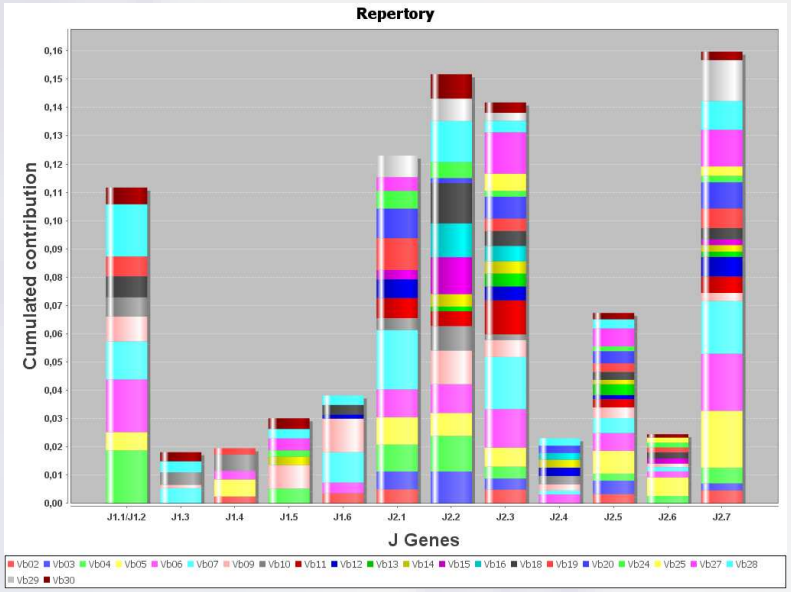
195 kb
4.36 kb

- High molecular weight DNA integrity: degradation, fragmentation
- High variability among samples

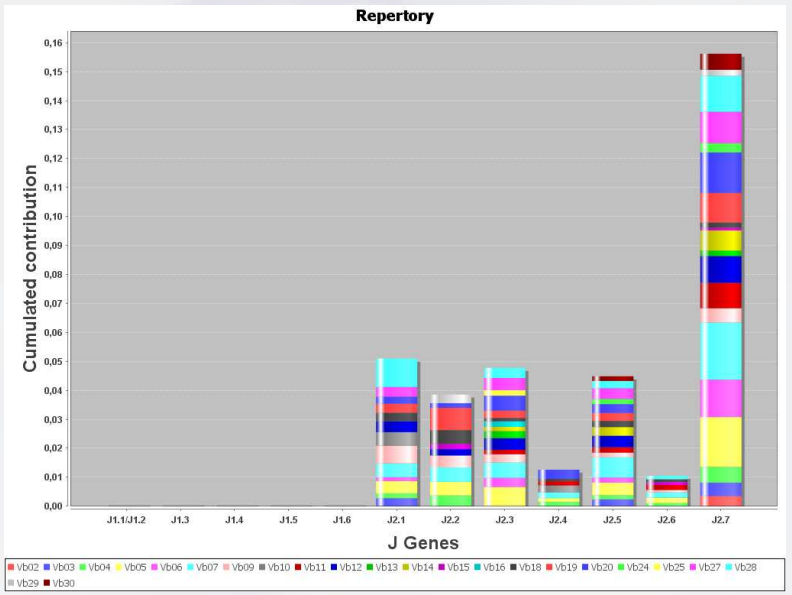
Hartmann C. *et al.*, unpublished results
Pazzagli M. et al., manuscript in preparation

Impact of DNA quality on Immune T cell Repertoire Analysis (ImmunID Technologies)

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



Ref. DNA from UNFI (DIV 54%)



Sample 38 (Poor quality) (DIV 32%)

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

L. Barraud et al. Unpublished data
Pazzagli M. et al., manuscript in preparation

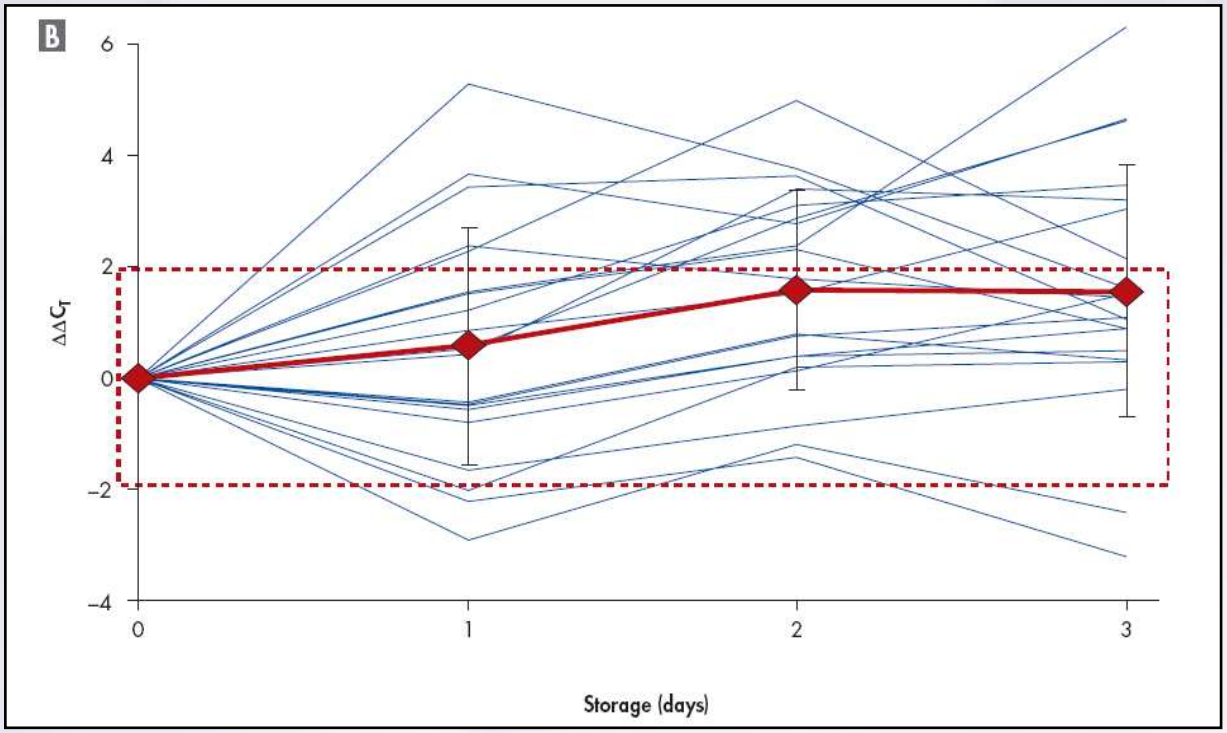
Blood RNA Ring Trial Parameters

- Purity
- Interference substances (RT-qPCR)
- Yield
- Integrity
- RNA Profile Stability / Changes

Changes of Transcripts Profiles in Blood

Individual Samples React Differently

Human EDTA Blood stored at Room Temperature over 3 days



IL-1β mRNA

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

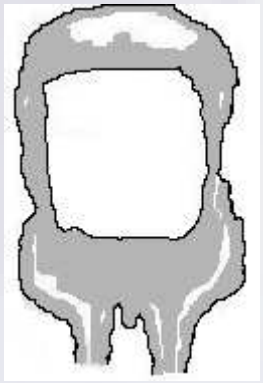
Learning from Blood RNA Ring Trial 1

- No pooling of different donors' blood
  - Accept that only sub-groups of ring trial participating laboratories get the same blood samples
- No usual blood collection bags
  - Use dedicated EDTA bags
- Immediate cooling of blood bags
  - Artificial gene induction and down regulation to be avoided
- Use of intracellular RNA markers
  - External markers will behave differently

Blood RNA Second Ring Trial

Preparation of Blood Samples

1 bag - 1 donor



Empty bag

- Filled with 39 ml of EDTA solution under sterile condition
- Filled with 461 ml blood from phlebotomy

PAXgene Blood RNA Tubes

BD EST Plastic Tubes

Proficiency Testing for Preanalytical Workflows used for Blood RNA Analysis

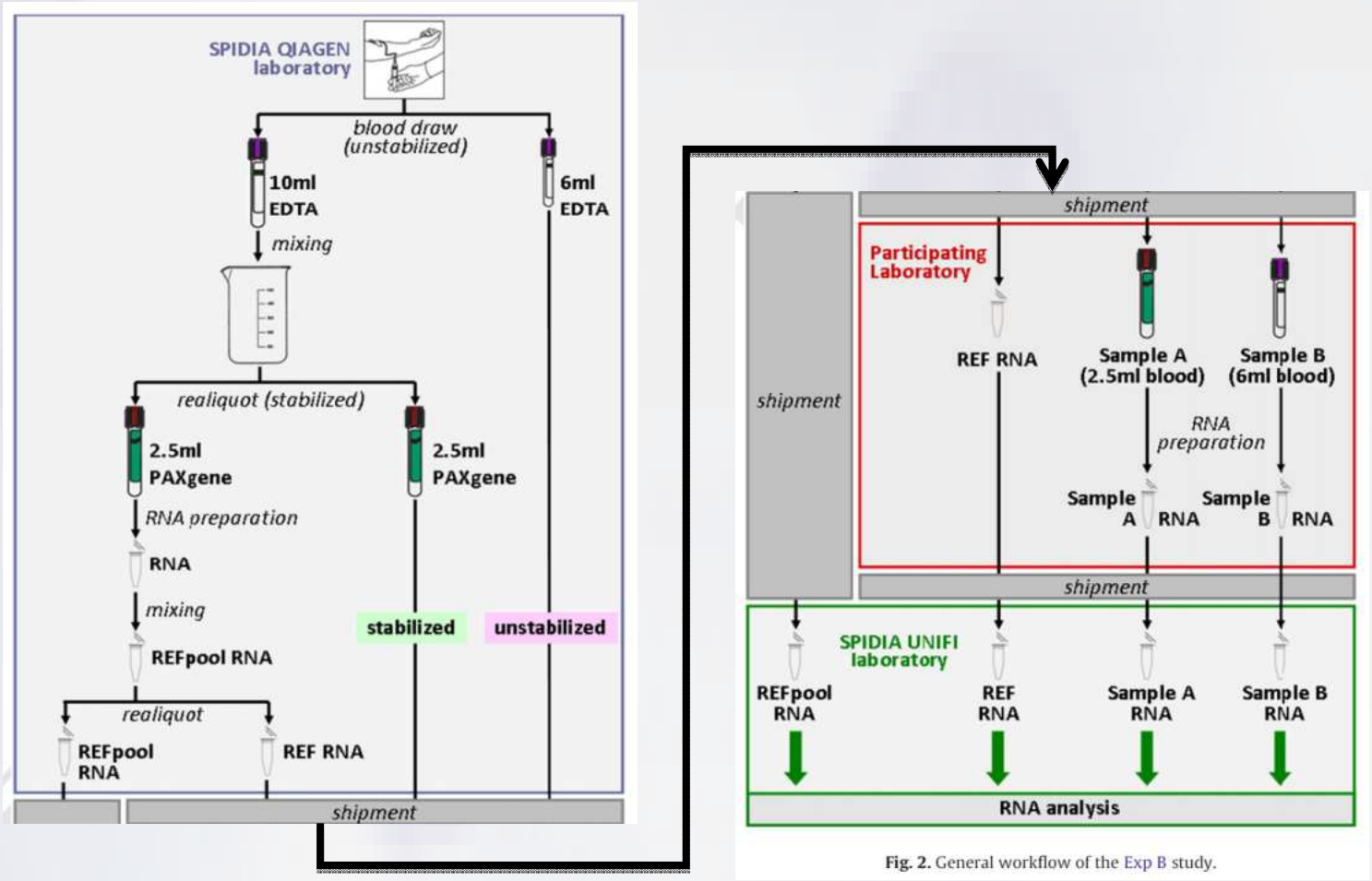
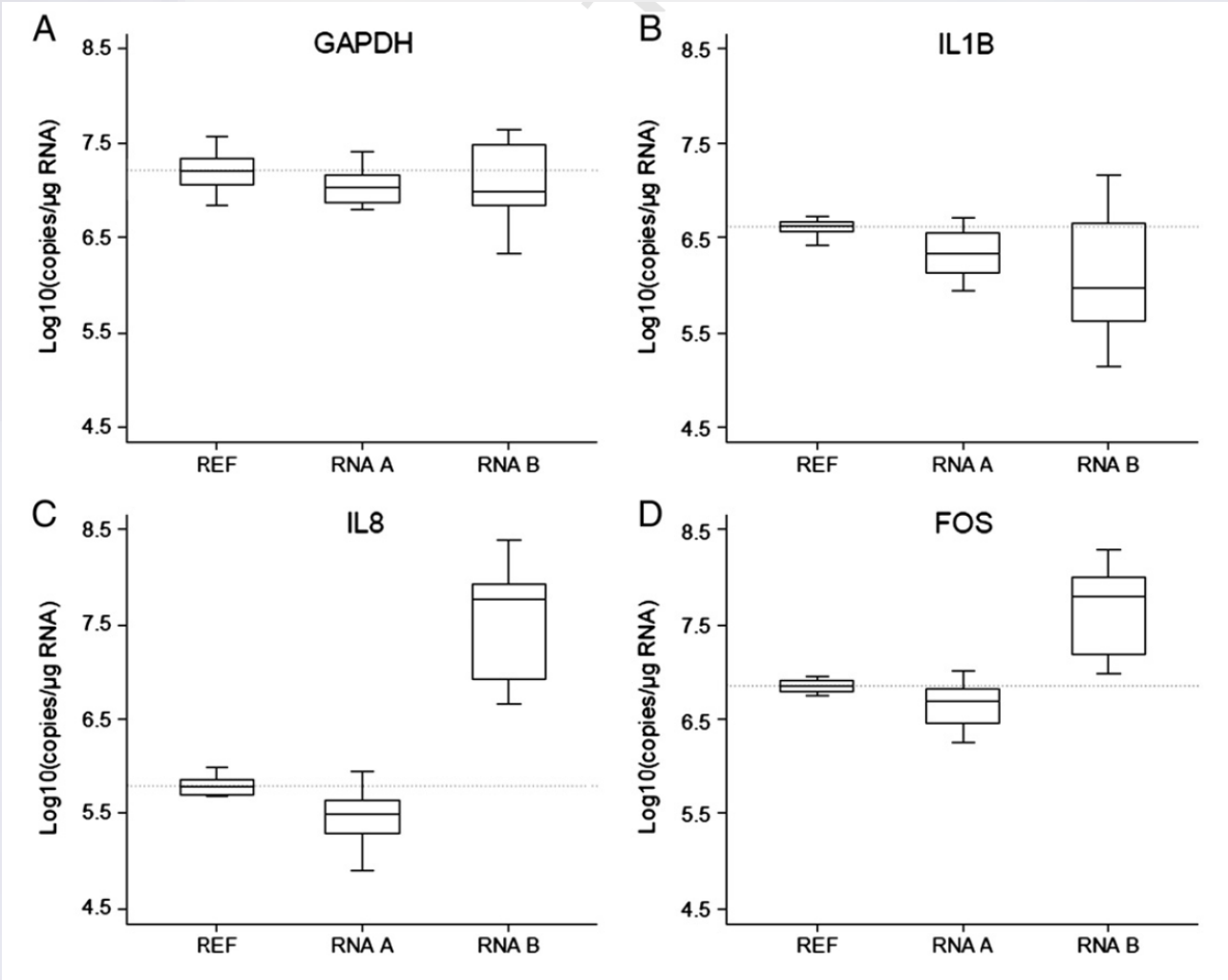


Fig. 2. General workflow of the Exp B study.

K. Günther, F. Malentacchi, P. Verderio, S. Pizzamiglio, C. M. Ciniselli, A. Tichopad, M. Kubista, R. Wyrich, M. Pazzagli, S. Gelmini. Implementation of a proficiency testing for the assessment of the preanalytical phase of blood samples used for RNA based analysis. Clin Chim Acta (2012) – in press.

Blood Sample Shipment - RNA Profile Changes

Stabilized vs. EDTA Blood



Box plots reflecting the mRNA expression of GAPDH (Panel A), IL1B (Panel B), IL8 (Panel C), and FOS (Panel D) measured in the three sample types REF, RNA A (PAXgene Blood RNA) and RNA B (EDTA). Each box indicates the 25th and 75th percentiles. The horizontal line inside the box indicates the median, and the whiskers indicate the extreme measured values. The dotted horizontal line indicates the median value of the REF samples (prior shipment) and serves for comparison.

Guenther K. et al. Clin Chim Acta. 2012, in press.

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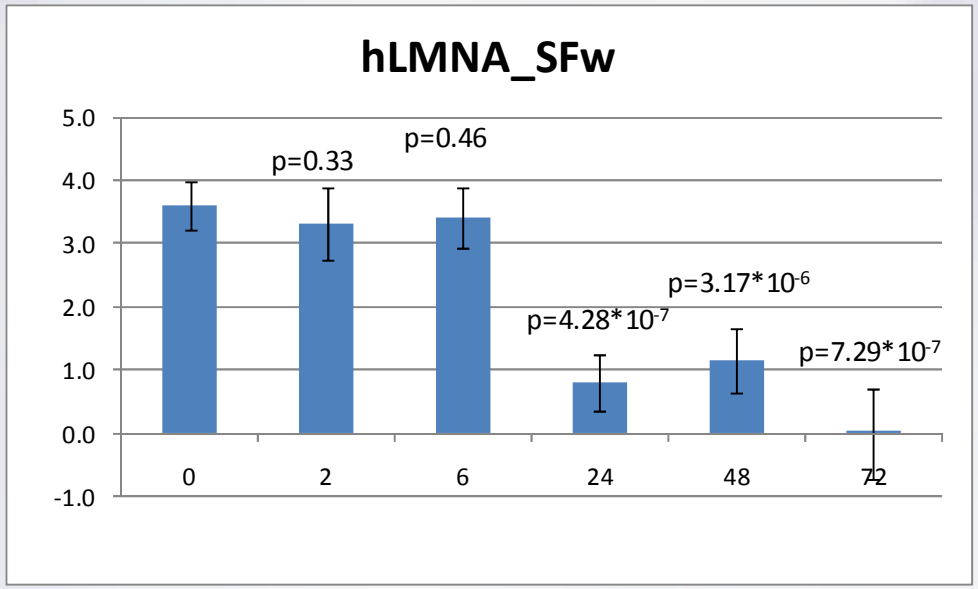
Blood RNA Quality Marker Discovery

- Quality markers measuring RNA up- & down-regulation
  - >180 micro arrays (time course experiments)
  - 11 marker candidates (specific RNA degradation or gene down regulation, specific RNA gene induction, random degradation)
  - Technical assay validation
  - Next step: Performance validation within larger donor cohorts



Rian E. *et al.*, unpublished data

Acknowledgement
SPIDIA Consortium Members

- QIAGEN GmbH - Coordinator
- Medical University of Graz *(Prof. K. Zatloukal)*
- University of Florence *(Prof. M. Pazzagli)*
- CIRMMP Florence, CERM *(Prof. I. Bertini)*
- TATAA Biocenter
- PreAnalytiX GmbH
- DIAGENIC ASA
- Aros Applied Biotechnology
- Dako Denmark
- ACIES
- Biotechnology Inst. of Czech Academy of Science *(Prof. M. Kubista)*
- European Committee for Standardization (CEN)
- ImmunID Technologies
- Erasmus Medical Center Rotterdam *(Prof. P. Riegman)*
- Technical University Munich *(Prof. H. Hoefler, Prof. K. Becker)*
- Fondazione IRCCS Istituto Nazionale dei Tumori *(Dr. P. Verderio)*

Scientific Advisory Board

- *Prof. François Rousseau* (Univ. Laval, Quebec. CanGeneTest Network)
- *Dr. Roberta M. Madej* (CLSI)

Project Ethics Committee

- *Dr. Anne Cambon-Thomsen* (CNRS, INSERM, Tolouse, France)
- *Dr. Ruth Chadwick* (ESRC Centre, Cardiff University, UK)

SPIDIA Consortium
Bi-Annual Meeting Berlin November 2011

SPIDIA

Thank you!

Questions ?