European Society of Pathology

Tissue banks: Pitfalls and good practice in diagnostic accuracy and molecular analysis.

"Standards for optimal pre-analytical phase in surgical pathology"

Peter Riegman

Head Erasmus MC Tissue Bank
Collection point of the Central Biobank

SPIDIA

SPIDIA4P

13:00-14:00 CET Monday, March 25, 2024

No conflict of interest to report

*The research here presented has received funding from the European Union Seventh Framework Programme FP7 under **grant agreement n°222916**, Project acronym: **SPIDIA** and Project full title: "Standardisation and improvement of generic Pre-analytical tools and procedures for In vitro DIAgnostics" and **n°73112 SPIDIA4P** Standardisation of generic pre-analytical procedures for in-vitro diagnostics for Personalized Medicine*

Erasmus MC
University Medical Center Rotterdam

Deficiencies in Routine Healthcare and Research

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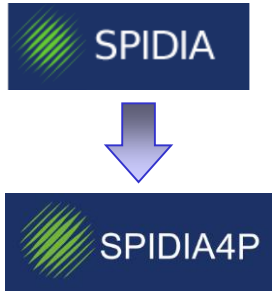
- *Diagnostic errors cause about 10% of patient deaths and about 17% adverse events*
  Institute of Medicine (IOM) Report Sept 2015

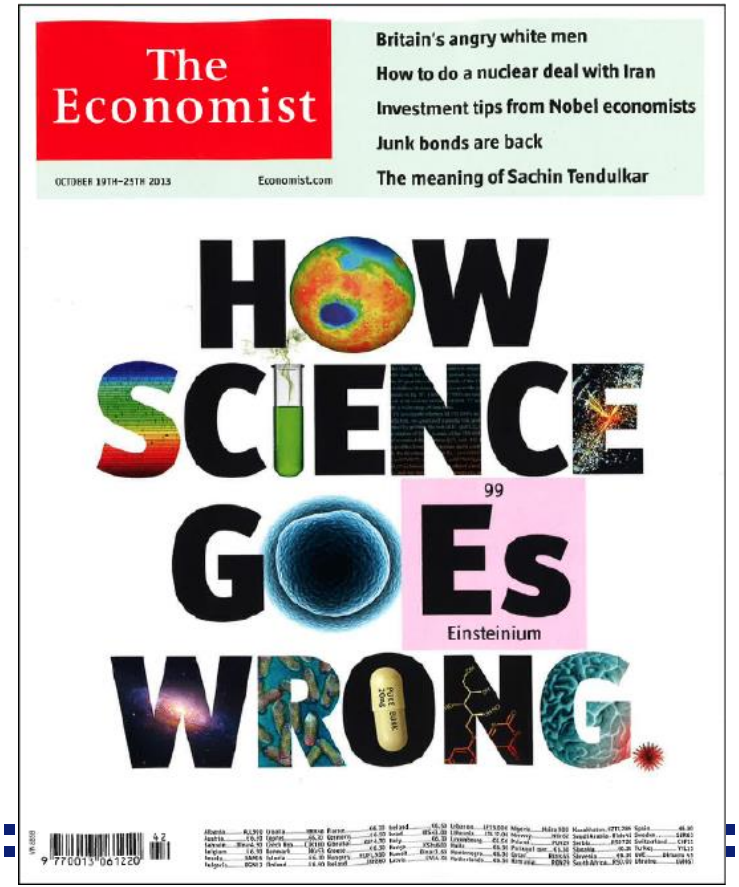
- *Pre-analytical phase accounts for 46% to 68% of clinical laboratory errors*
  Medical Laboratory Observer, May 2014

- *SPIDIA comparison experiments => pre-analyitical contributions to sample variations and differences between institutes*
  Blood and tissues using RNA expression arrays, proteomics and metabolomics

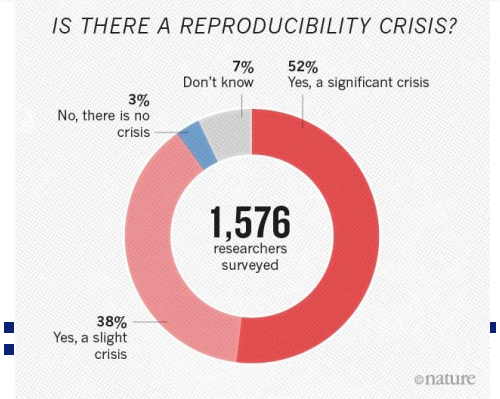
- *Irreproducible preclinical research exceeds 50% => $28B/year wasted in the US*
  PLoS Biol., June 2015



Reproducibility crisis:



Too many of the findings that fill the academic ether are the result of shoddy experiments or poor analysis (see pages 21-24). A rule of thumb among biotechnology venture-capitalists is that half of published research cannot be replicated. Even that may be optimistic. Last year researchers at one biotech firm, Amgen, found they could reproduce just six of 53 "landmark" studies in cancer research. Earlier, a group at Bayer, a drug company, managed to repeat just a quarter of 67 similarly important papers. A leading computer scientist frets that three-quarters of papers in his subfield are bunk. In 2000-10 roughly 80,000 patients took part in clinical trials based on research that was later retracted because of mistakes or improprieties.



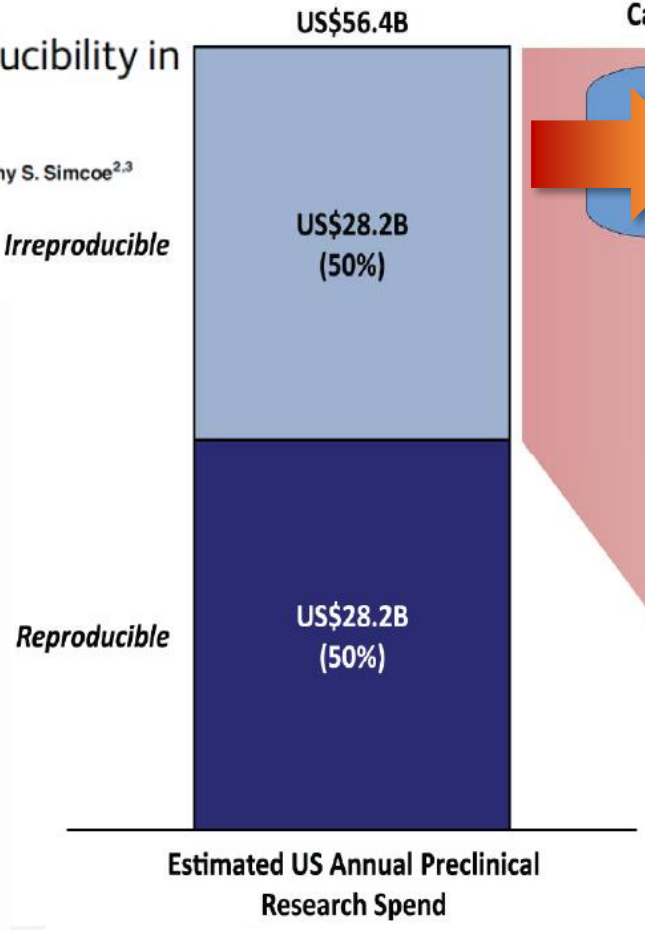
Reproducibilty crisis

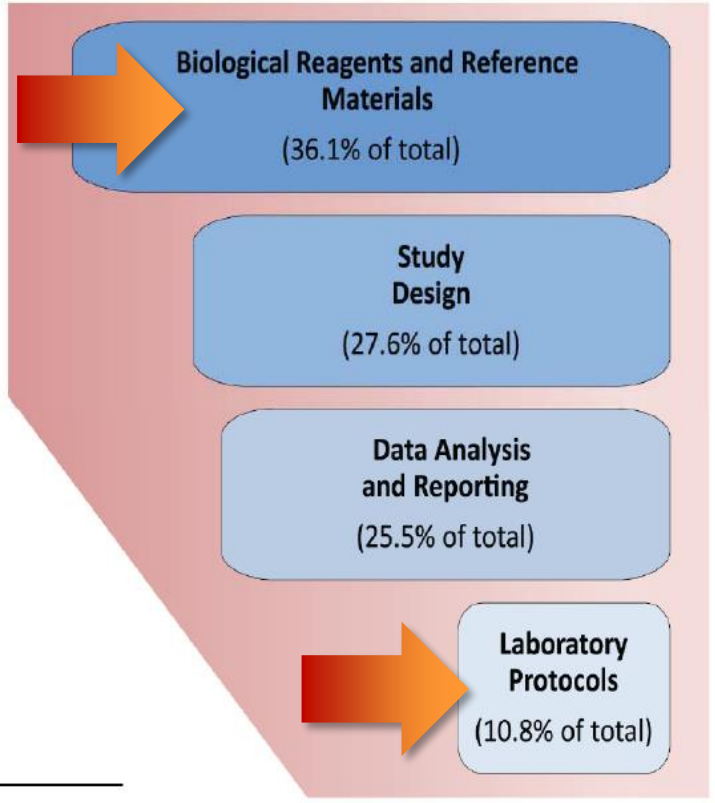
PLOS BIOLOGY

PERSPECTIVE

The Economics of Reproducibility in Preclinical Research

Leonard P. Freedman[1]*, Iain M. Cockburn[2], Timothy S. Simcoe[2,3]





Reproducibility

Survey (Nature):

Biomaterials

Different institutes

Different sample collection methods

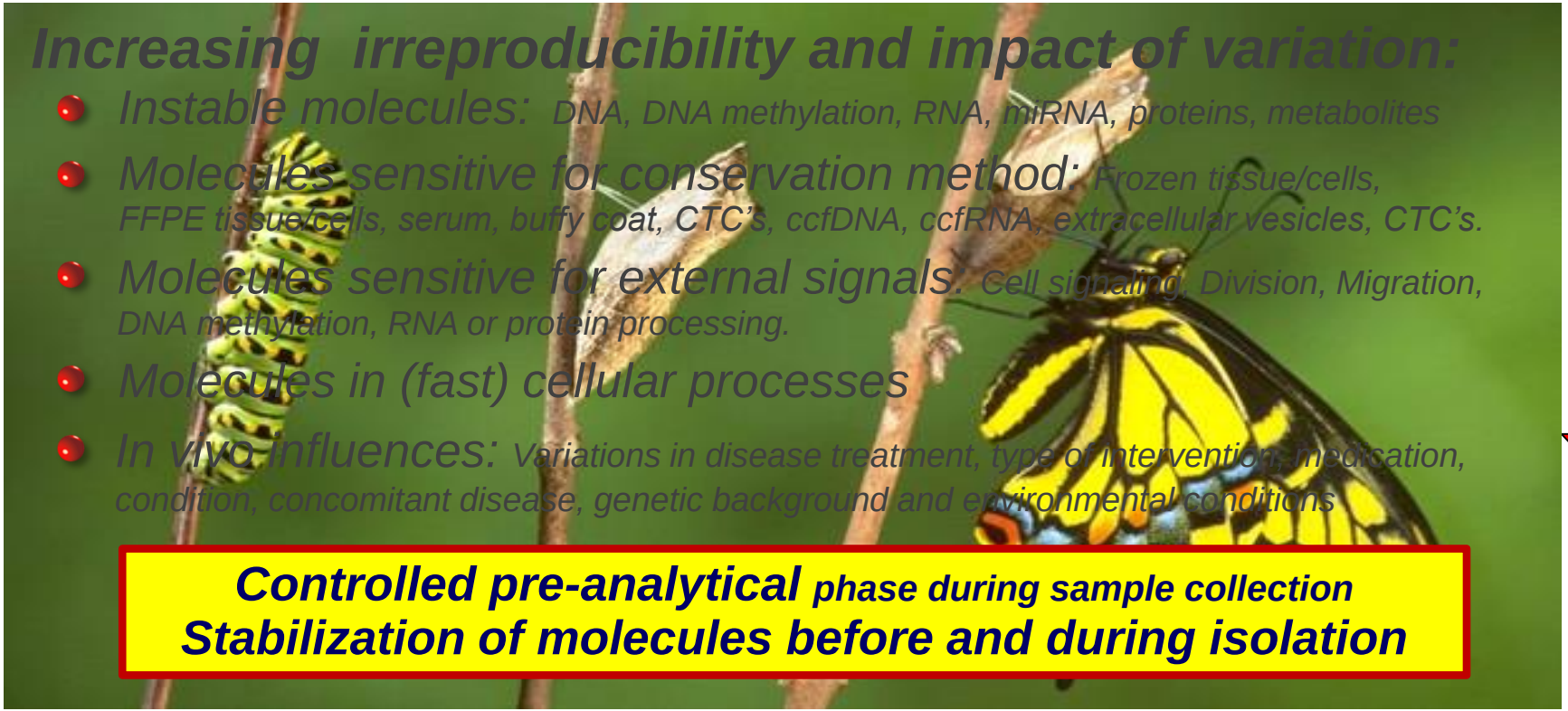
Different sample variations

Different results / Batch effects

Reproducibility crisis

Best reproducible sample types:

DNA isolated from blood – Especially when validated in big studies with collections from different institutes.



Diagnosis is Result of Entire Workflow

Improvements:

- ***Pre-analytical Technologies***
- ***International ISO & CEN Standards***
- ***External Quality Assessment (EQA) Schemes***
- ***Implementation*** *- Healthcare, Biobanking, Research*

ISO 15189: Specification, Verification and Validation of pre-analytical workflows is an essential part of analytical test development

+ = Entire workflow

= Pre-analytical phase or pre-analytical workflow

Critical sources frozen & FFPE tissue

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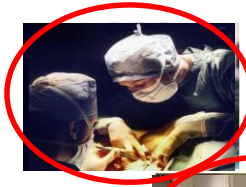

Medication
Surgical procedure
Warm ischemia

Transport
Temperature
Cold ischemia

Sample processing
Mech. alteration
Selection+annotation

Sampling

Freezing
Freezing rate
Temperature

Cryostorage
Temperature
Temp. shifts

Fixation
Fixative
Time

Embedding
Temperature

Diagnosis
Disease codes

Storage
Time
temperature

Sample preparation

Internal & Institute variety
Institute variety
Controllable variety

Robust tests ONLY

Modified slide made available by Kurt Zatloukal for SPIDIA4P members

Controlling sample variation:

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University Medical Center Rotterdam

Medical Treatment

Hospital integrated Biobanking

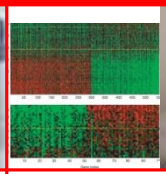
Medical Research










Donor Consent | Medical/ Surgical Procedure | Transport | Receipt | Processing | Storage | Annotation | Quality | Release, Distribution Transport | Scientific Analysis | Restocking/ Destruction

Pre-acquisition

- Antibiotics
- Type of intervention
- Type of anesthesia
- Total treatment
- Arterial clamp time
  - Warm Ischemia
  - Cold Ischemia
- Patient Condition
- Genetic Background
- Environment

Acquisition

- Processing Conditions/Time
- Sample D… (…)
  - Sa…ple/Patient
- Temperature
  - Cold Ischemia
- Stabilization / Fixation Agent
  - Pre-cooled Isopenta…
  - Formalin
- SOPs / Workinstructions

Post Acquisition

- Storage Media
- Storage Method
- Storage Temperature
- Quality Controls
- Disease Site
- Data Standards
- SOPs / Workinstructions

QA & QC
SOP's & Workinstructions
Guidelines
ISO 15189:2012
ISO 20387

ISO

Release

- Request
- Study Design
- Multidisciplinary
- Ethics
- Statistics
- Environment
- Definition
- / Workinstructions
- Transfer Agreement

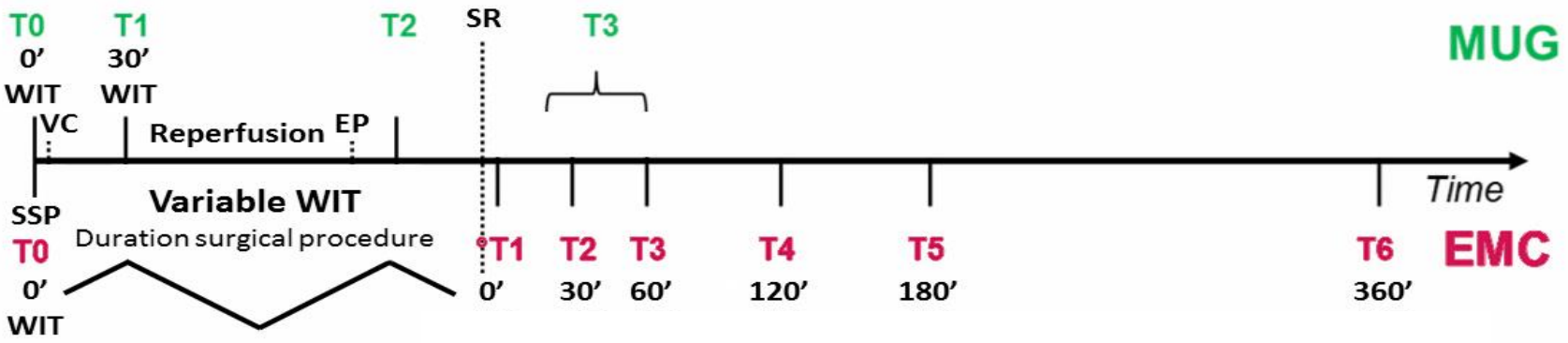
Pre-analytical phase

Simulation of Specimen Collection and Transport

Liver samples - adjacent to colon metastasis during surgery
9 patients - 3 samples per time point - Outside tumor area

Two institutes EMC and MUG with different surgical procedures

- *MUG Pringle Maneuver*
- *EMC without*



Simulation of Specimen Collection and Transport

SPIDIA

Methods:

Expression array and comparison of genes of interest with Δct

Results:

- *Some (5%) up and down regulated genes – Warm ischemia only*
- *Some stable genes – Household genes*
- *Most genes - High variation in gene expression*

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Typical upregulated RNA expression example

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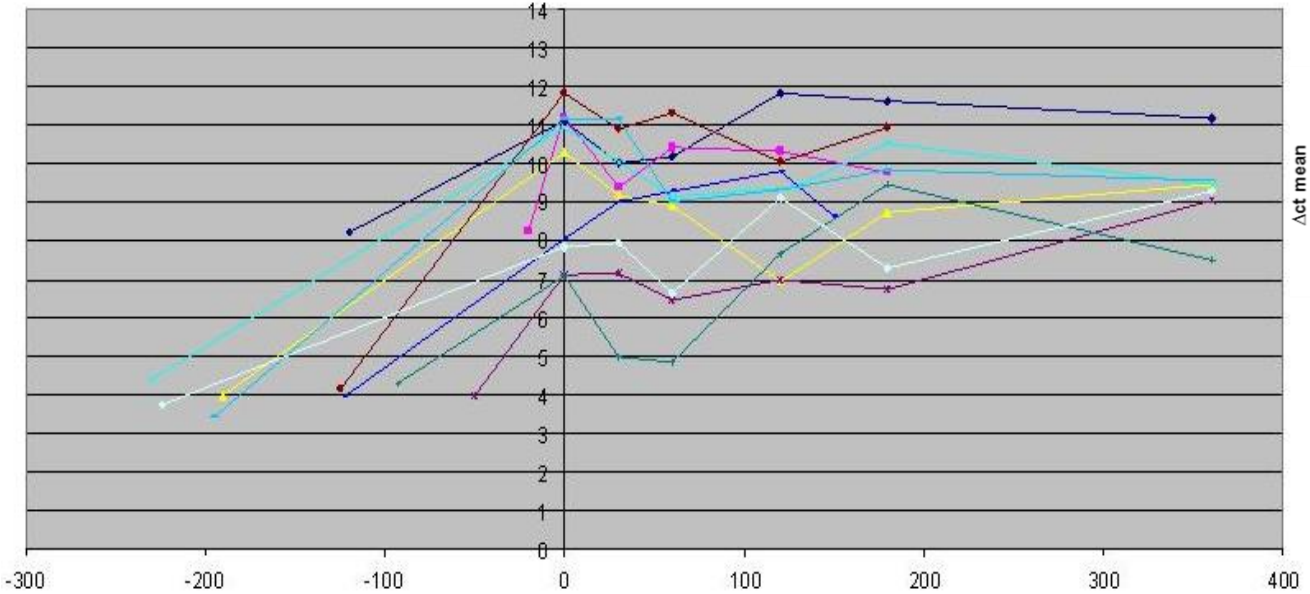
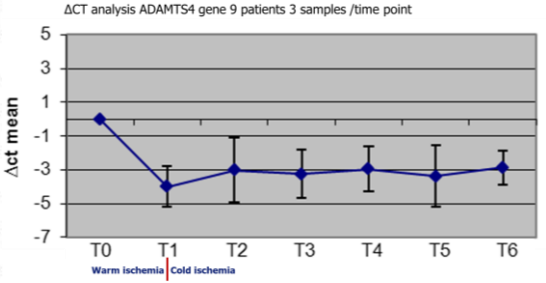
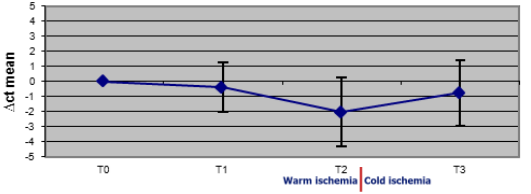
Time course expression analysis ADAMTS4 gene 9 patients 3 samples /time point



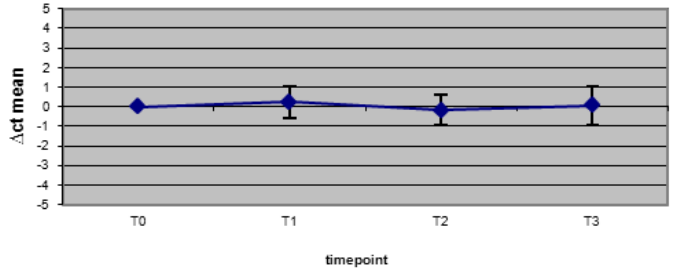
Warm ischemia | *Cold ischemia*

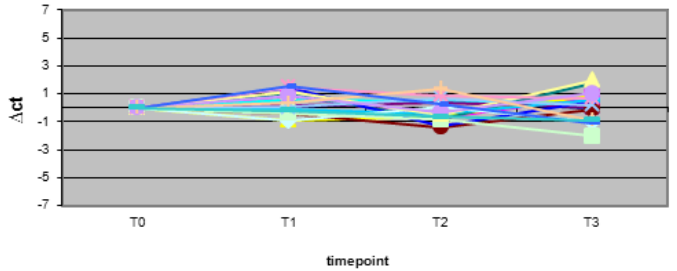




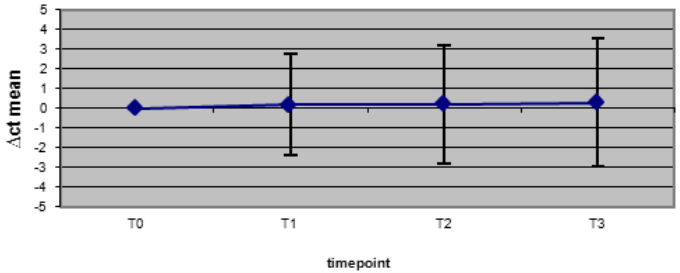
Typical Stable and Unstable Genes (Δct)

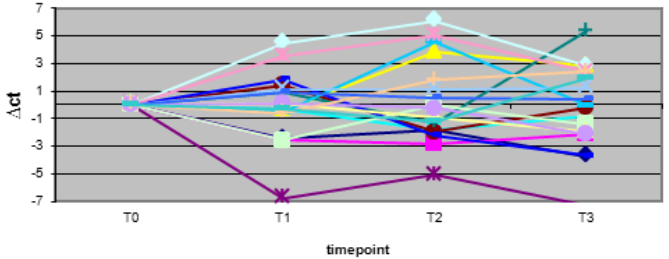
Time course expression analysis NONO_S01 gene 9 patients 3 samples /time point



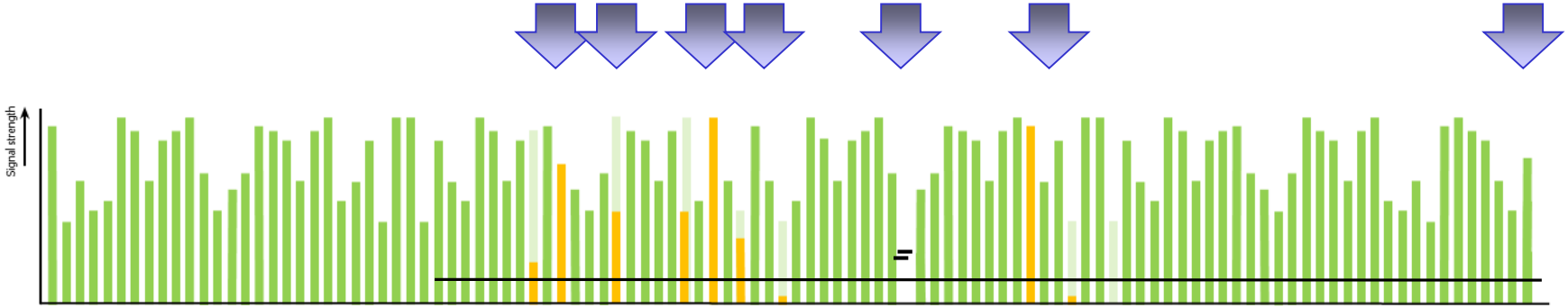


Time course expression analysis FOSB_5pM02 gene 9 patients 3 samples /time point





FFPE Tissue Processing: Theoretical Simulation of Pre-analyitical Influences



- *In vivo - no influence*
- *Medication/anesthesia/intervention/pain relief/treatment of infection*
- *Genetic background/condition*
- *Warm ischemia*
  - *-short*
  - *-long*
- *Cold ischemia*
- *FFPE Fixation*
  - *-Over fixation*
- *Embedding*
  - *-other embedding method*
- *Isolation*

Frozen biopsy/Blood
-Isolation

Frozen sample chirugic resection
-Isolation

Controlling sample variation:

Sample metadata:
Tool for identification and control of sample variation

Use sample metadata in practice:
To evaluate if a sample is fit for purpose and exchangeable

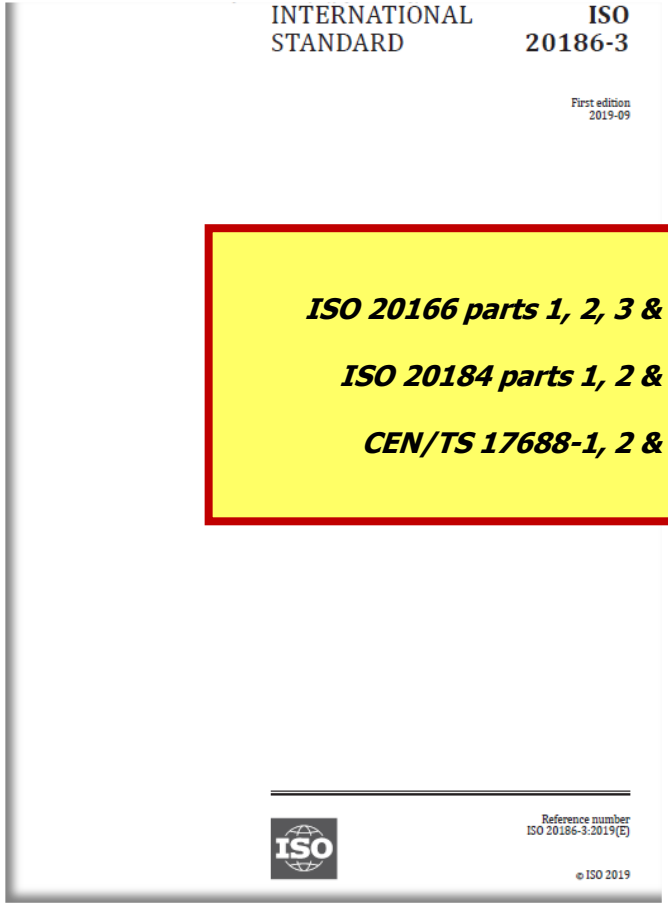
- **Equal for all samples** - No sample variations
- **Fit for Purpose** - Margins

Control pre-analytical variations

1. **Standardize** *method equal for all samples*
2. **Avoid** *– Risk Assessment……..…Alternative method*
3. **Document variation** *…….....Sample metadata*

22 CEN & ISO Standard Documents

SPIDIA4P


INTERNATIONAL STANDARD

ISO 20186-3

First edition
2019-09

Reference number
ISO 20186-3:2019(E)

© ISO 2019

ISO 20166 parts 1, 2, 3 & 4

ISO 20184 parts 1, 2 & 3

CEN/TS 17688-1, 2 & 3

- 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) — RNA, Proteins, DNA, in situ staining
  - **Tissue** (Frozen) – RNA, Proteins, DNA
  - **Fine Needle Aspirates** – RNA, Proteins, DNA
  - **Saliva** – DNA
  - **Urine & Body Fluids** – cfDNA
  - **Metabolomics** – Urine, Serum, Plasma
  - **Microbiome** – Stool, Saliva etc.

published CEN *published ISO* *final stage*

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22 CEN & ISO Standard Documents

SPIDIA4P

ISO 15189:2022

Informative referall to the pre-analytical technical standards

Published two European technical standards CEN

FprCENTS 17981-1
*In vitro diagnostic Next Generation Sequencing (NGS) workflows – Part 1: Human **DNA** examination*

FprCENTS 17981-2
*In vitro diagnostic Next Generation Sequencing (NGS) workflows – Part 2: Human **RNA** examination*

On track to develop general ISO standards for NGS

Co-funded by the European Union
Grant agreement 874719

- Molecular in-vitro diagnostic examinations - Specifications for **pre-examination processes** for:
  - **Blood** — Cellular RNA, gDNA, ccfDNA, ccfRNA
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  - **Metabolomics** – Urine, Serum, Plasma
  - **Microbiome** – Stool, Saliva etc.

published CEN *published ISO* *final stage*

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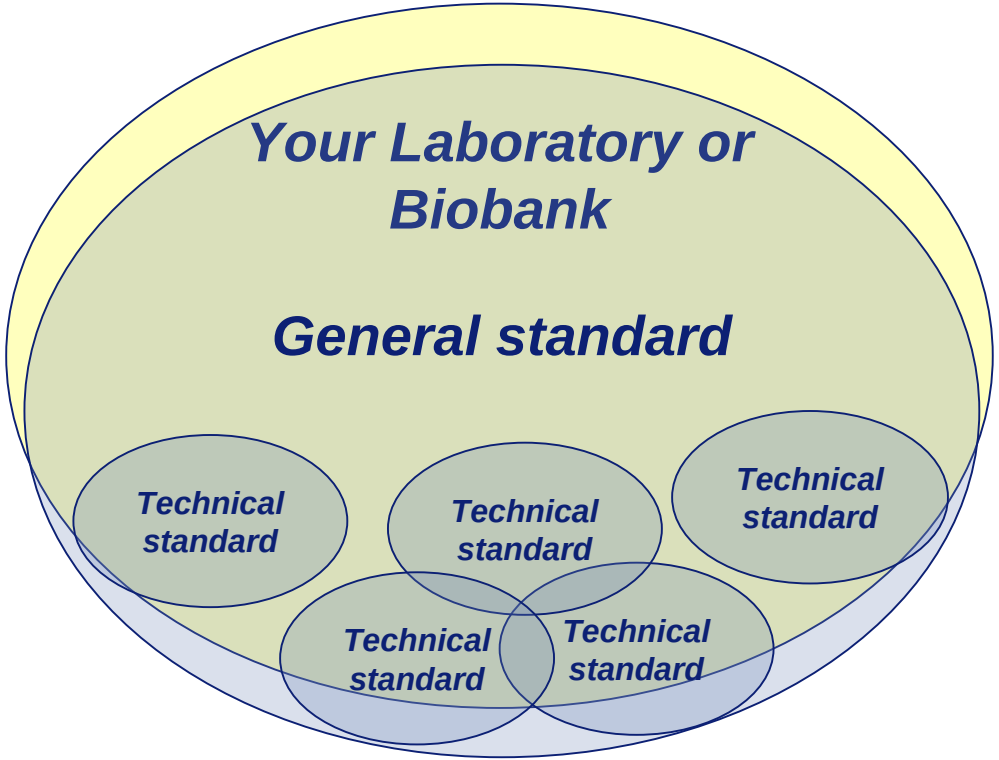
General Standards and Technical standards

General Standards *(ISO 15189 or ISO 20387)*

- **Not** normative for specific situations
- Refers to pre-analytical technical standards

Technical standards *(SPIDIA4P standards)*

- Normative for specific situations
- Can be used in general standard in case the specific situation is used



Difference Diagnostic - Scientific Workflow

= Entire diagnostic workflow

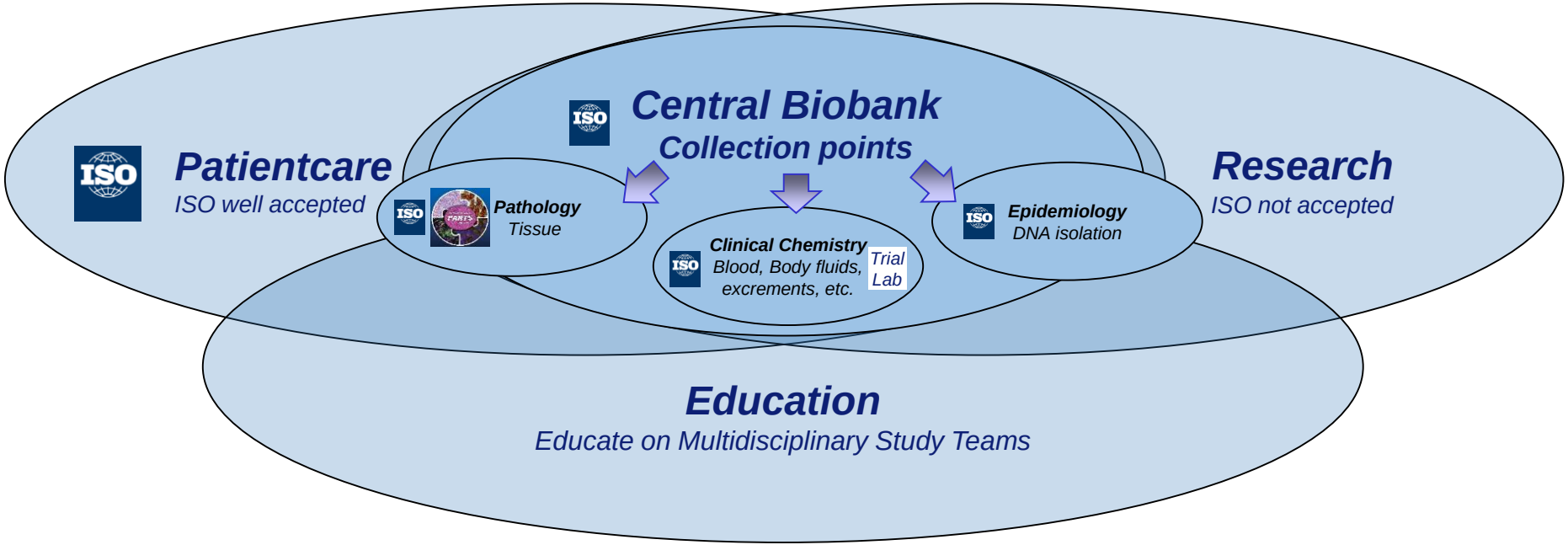
= Entire scientific workflow

= Pre-analytical phase = Biobank

Increase reproducibility by Implementation of Standards

- *Same for diagnostics, research and product development*
- *Healthcare, Biobanking for Research, Diagnostic and Pharma Industries*

Academic Medical Center biobank infrastructure



General standards: ISO 15189 or ISO 20387 AND
Specific (SPIDIA4P) pre-analytical ISO technical standards

=> High quality and standardized samples

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

The research here presented has received funding from the European Union Seventh Framework Programme FP7 under grant agreement n°222916, Project acronym: SPIDIA and Project full title: "Standardisation and improvement of generic Pre-analytical tools and procedures for In vitro DIAgnostics" and n°73112 SPIDIA4P Standardisation of generic pre-analytical procedures for in-vitro diagnostics for Personalized Medicine