Pre-Analytical Sample Processing in Biobanking

Practical Laboratory Course

February 8 - 10, 2017

Institute of Pathology, Medical University of Graz, Austria

Organizers:

BBMRI.at
Biobanking and BioMolecular resources Research Infrastructure Austria

Medizinische Universität Graz

Christian Doppler Forschungsgesellschaft

Sponsors:

PreAnalytiX

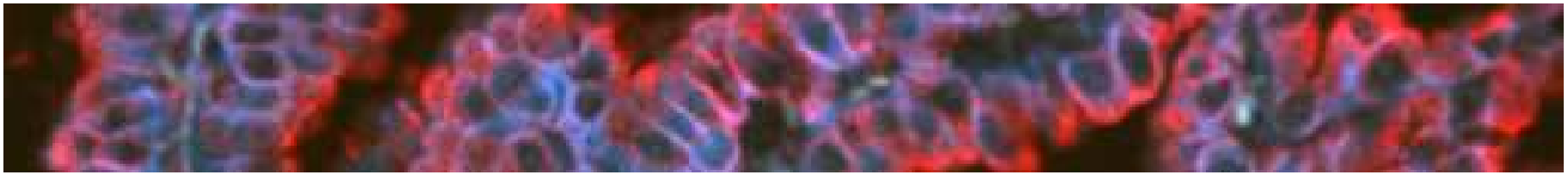
QIAGEN

AlphaMetrix Biotech

Roche

HVD LIFE SCIENCES

MICRONIC
INNOVATION IN SAMPLE STORAGE



Pre-analytical Sample Processing in Biobanking

Graz, February 8th- 10th, 2017

Introduction &
the case for sample pre-analytics

Kurt Zatloukal M.D.
Institute of Pathology
Medical University of Graz
Austria

Relations with Industry

- **Co-founder:** Gobal Action 4 Health Institute, Dublin
- **Shareholder:** Alacris Theranostics, Berlin
- **Consultancy:** AstraZeneca
  GlaxoSmithKline
  Daiichi Sankyo
  Kapsch BusinessCom AG
- **Research grants:** Qiagen
  Siemens

Rationale for the Course

- Standardization of pre-analyticial processes is key to guarantee reliability of analytical results
- Same requirements for diagnostics and biobanks
- Increasing demand in the context of personalized medicine and companion diagnostics
- Information on new and upcoming CEN/ISO standards
- Practical advice for implementation of CEN TS

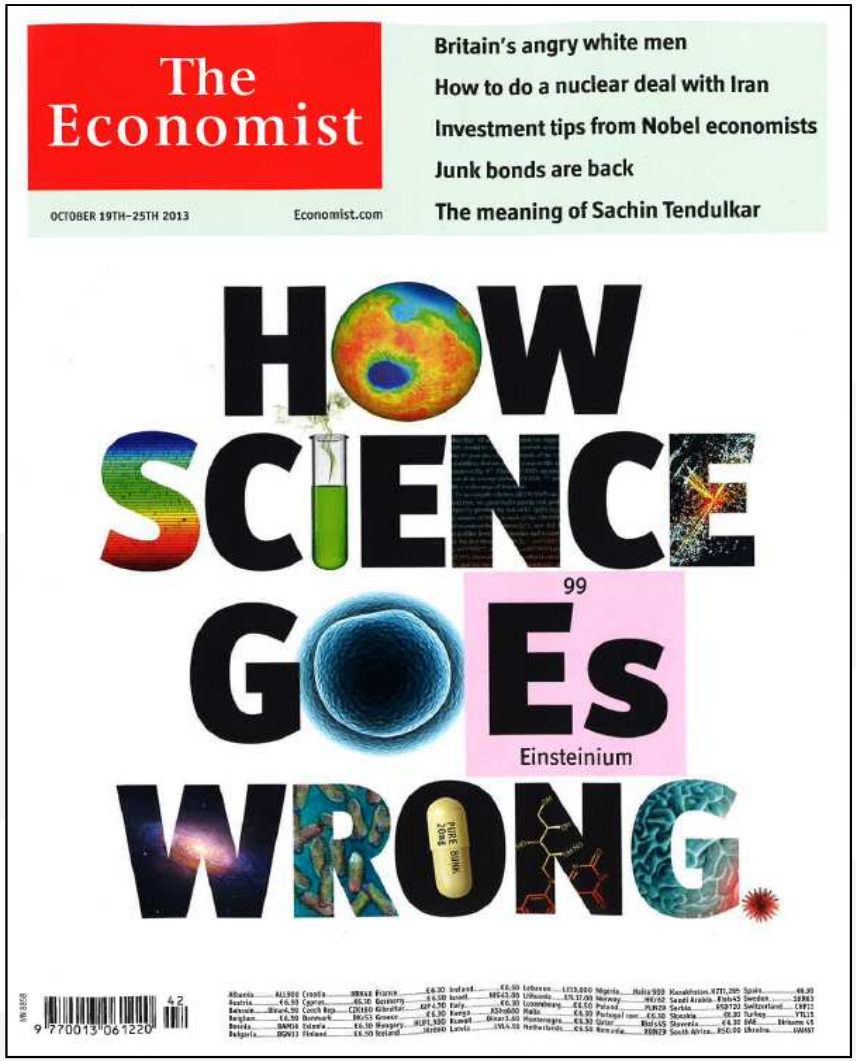
Programme and Content

- Introduction to the topic
- Practical demonstration and „hands on“
  - Collection of samples
  - Sample stabilization
  - Sample selection
  - Isolation of analytes
  - Quality control

Guest lectures:

- Giorgio Stanta, Trieste: Analysis of RNA from FFPE tissue
- Karl F. Becker, Munich: Analysis of proteins
- Claudio Luchinat, Florence: Analysis of metabolites

The Problem of Not Reproducible Studies



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.

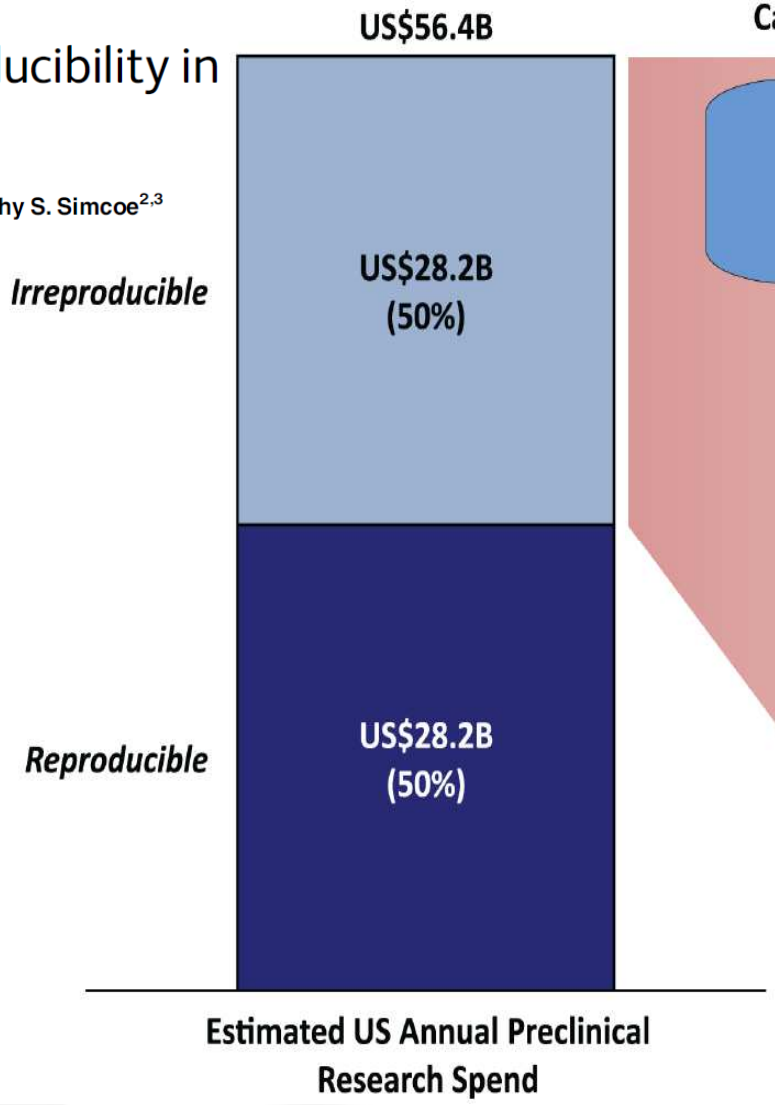
Economic Impact of Biosample Quality in R&D

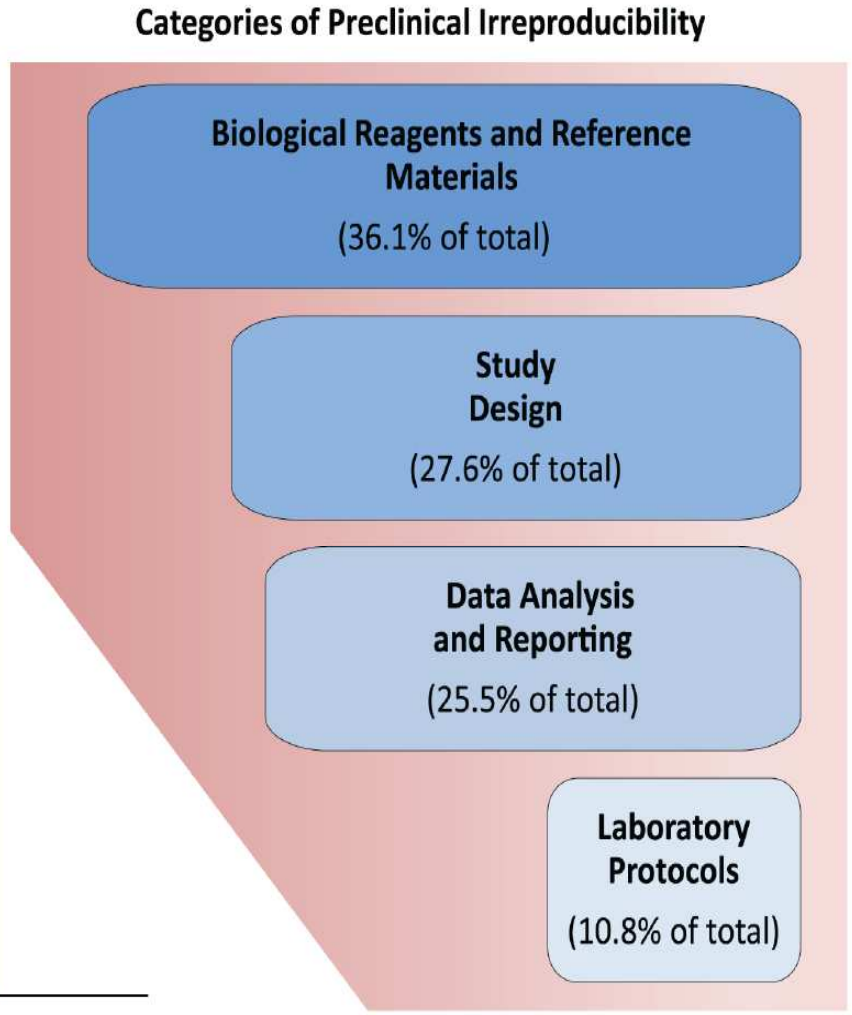
PLOS | BIOLOGY

PERSPECTIVE

The Economics of Reproducibility in Preclinical Research

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





Pre-analytical Errors in Medical Diagnostics

- 46% - 68% of diagnostic testing process errors are in the pre-analytical phase

Plebani M, Clin Chem Lab Med. 2006

Impact of Errors in Medical Diagnostics

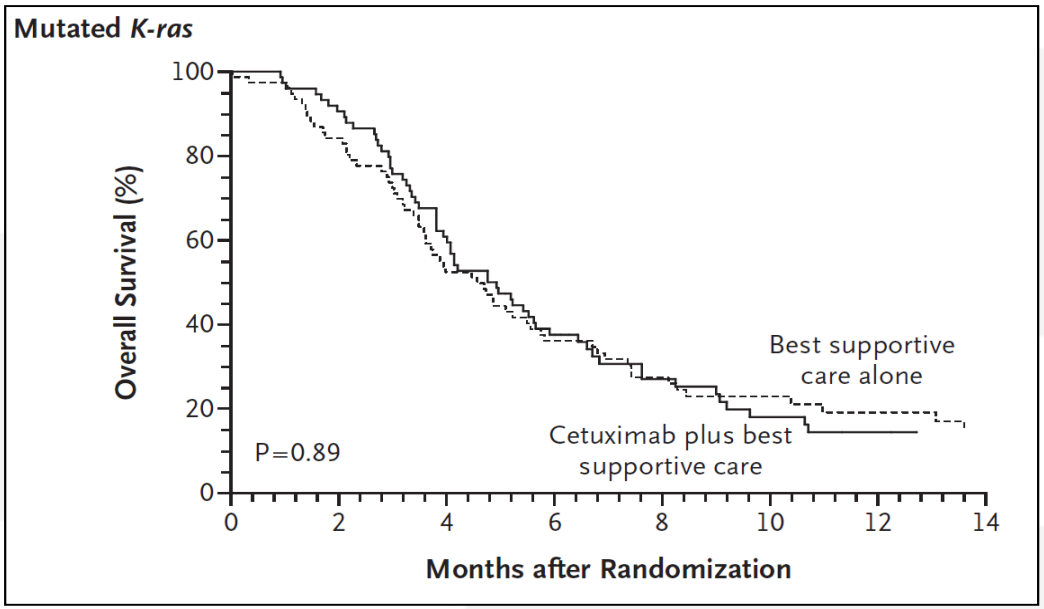
- 5 percent of U.S. adults experience a diagnostic error
- 10 percent of patient deaths can be attributed to diagnostic errors
- 6 to 17 percent of adverse events in hospitals are related to diagnostic errors

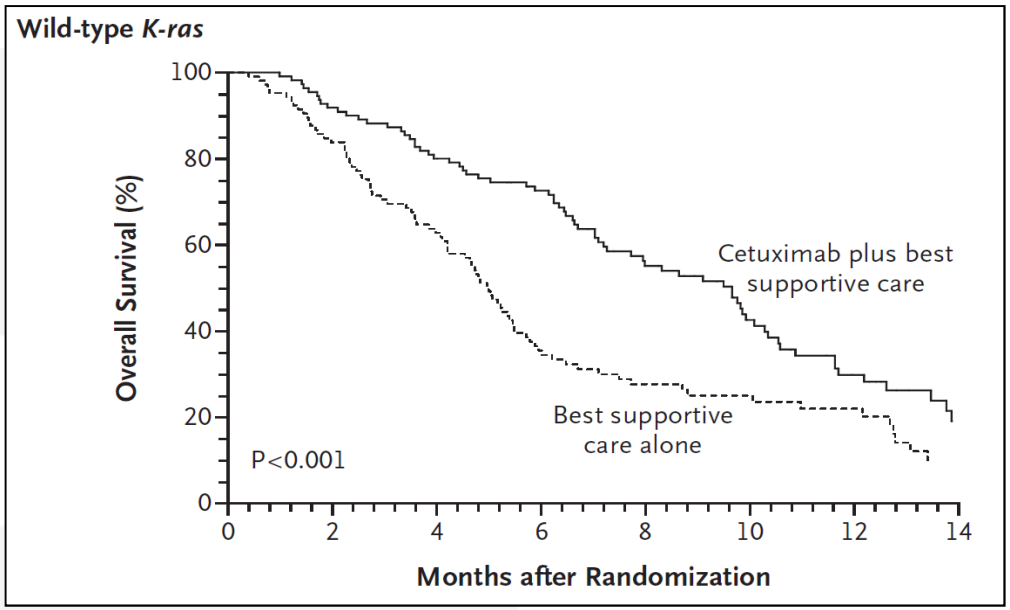
Institute of Medicine
SEPTEMBER 2015
Improving Diagnosis in Health Care

The National Academy of Sciences.

Efficacy of Drugs Depend on a Companion Diagnostic to Assess Mutational Status in Patients

The Example of *K-RAS* testing for Cetuximab therapy





Karapetis et al., NEJM 2008

Companion Diagnostics for Cancer Therapy (FDA)

DRUG	DISEASE	TARGET	ASSAY	TECNOLOGY
Afatinib	NSCLC	EGFR	RT-PCR	Rotor-Gene
Brentuximab Vedotin	Hodgin Lymph., sALCL	CD30	IHC	
Cetuximab (1)	CRC	EGFR	IHC	
Cetuximab (2)	mCRC	KRAS	RT-PCR	Rotor-Gene
Crizotinib	NSCLC	ALK	FISH	
Dabrafenib	Melanoma	BRAF	PCR	ABI 7500
Denileukin Diftitox	cut TCL	CD25	IHC	
Erlotinib	NSCLC	EGFR	RT-PCR	Cobas
Everolimus	mRCC, NEC	mTOR	LC-MS/MS	
Exemestane	Breast Ca	Aromatase (ER/PR)	IHC	
Fulvestrant	Breast Ca	ER	IHC	
Gefitinib	NSCLC	EGFR	RT-PCR	Cobas
Imatinib (1)	CML	Ph+	RT-PCR, FISH	
Imatinib (2)	GIST	c-Kit	IHC	
Imatinib (3)	MDS	EGFR	FISH	
Imatinib (4)	HES	FIP1L1-PDGFRα	RT-PCR	
Lapatinib	Breast Ca	HER2/NEU	IHC, FISH	
Olaparib	Breast Ca	BRCA1/2	PCR,	Sanger seq.
Panitumumab (1)	CRC	EGFR	IHC	
Panitumumab (2)	mCRC	KRAS	RT-PCR	Rotor-Gene
Pertuzumab	Breast Ca	HER2/NEU	IHC FISH	
Tamoxifen	Breast Ca	ER	IHC	
Tositumomab	(f)NHL	CD20 antigen	IHC	
Trastuzumab	Breast , Gastric Ca	HER2/NEU	IHC, FISH, CISH	
Vemurafenib	Melanoma	BRAF	RT-PCR	Cobas

Examples of Drugs in Personalized Medicine

Drug	Action	Company	Cancer	Therapy costs US$
Bosutinib	Src Inh	Pfizer	CML	82000.-
Cetuximab	EGFR Inh.	ImClone BMS/Merck	Colon Ca	61000.-
Axitinib	Tyr K Inh.	Pfizer	Renal Ca	59000.-
Pomalidomid	Angiog Inh.	Celgene	Myeloma	52000.-
Lenalidomid	Angiog Inh.	Celgene	Myeloma	95000.-
Erlotinib	EGFR Inh.	Roche	Lung/Panc Ca	55000.-
Lapatinib	Her2 Inh.	GSK	Breast Ca	34000.-
Crizotinib	ALK Inh.	Pfizer	Lung Ca	67000.-
Vemurafenib	B-Raf Inh.	Roche/ Daiichi Sankyo	Melanoma	54000.-

Source: ISI Group, Economist

Personalized Medicine Relies on Biomarkers to Select the Right Patients to Treat

case	cost reduction	patients screened	patients saved failure under treatment
trastuzumab Her2 IHC+ v. none	62,3%	3350	714
trastuzumab Her2 IHC+ v. none (a)	58,2%	1379	308
trastuzumab Her2 IHC+ PTEN+ v. Her2 IHC+	14,5%	991	56
trastuzumab Her2 FISH+ PTEN+ v. Her2 IHC+	29,5%	1002	111
erlotinib EGFR+ v. none	37,8%	994	158

D. Wine, Thesis MIT 1985

Diversification of Companion Diagnostics

FDA approved companion diagnostics for use in formalin fixed, paraffin embedded (FFPE) tumour tissue samples include:

A) EGFR Companion Diagnostics:

Cetuximab (Erbitux®) / gefitinib (Iressa®): DAKO EGFR PharmaDx kit (IHC).
Afatinib (Gilotrif®): Qiagen, therascreen EGFR RGQ PCR kit (PCR).
Erlotinib (Tarceva®): Roche Molecular Systems Inc, Cobas® EGFR mutation kit, (PCR test for exon 19 deletions and exon 21 (L858R) substitution mutations of the EGFR gene

B) HER2 Companion Diagnostics:

Trastuzumab (Herceptin®):Ventana Medical Systems, INFORM HER-2/NEU (FISH).
Trastuzumab (Herceptin®): Abbott Molecular Inc, PATHVYSION HER-2 DNA Probe Kit (FISH).
Trastuzumab (Herceptin®): Biogenex Laboratories Inc, INSITE HER-2/NEU KIT (m McAb for IHC).

Only Quality Defined Biological Samples Will Lead to Reliable Data

The garbage in – garbage out problem

Samples

Pre-analytics

Basic research

Biomarker

Targets for therapy

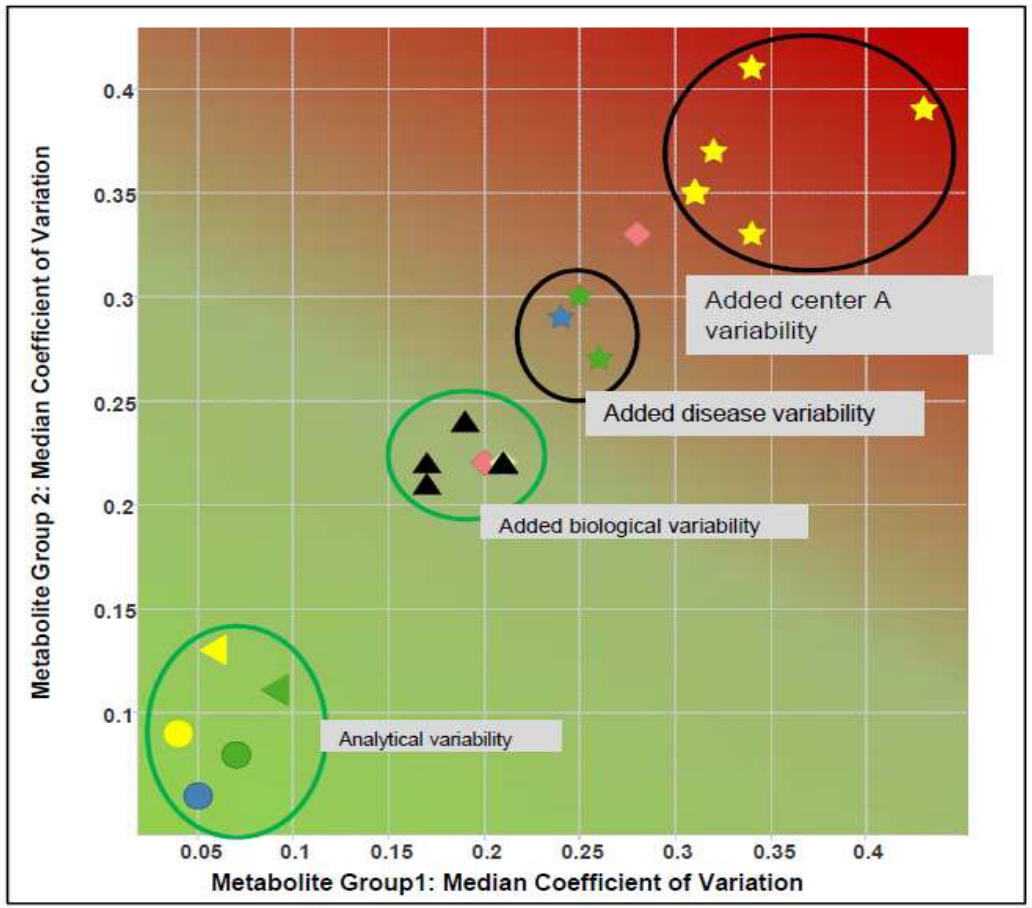
Patient

Biobanks

Data

Knowledge

Poor Standardization of Different Centres



- Metanomics Health's proprietary MxP™ Broad Profiling of >760 human plasma samples
- Variability increases from analytical < biological < disease < center A
- Centers B and C are best performers, center A is in need of process optimization

Color by
Center anonymous
- Center A
- Center A blood donation department
- Center B
- Center B blood donation department
- Center C
- Reference center 1
- Reference center 2
- Reference center 3
- Reference center 4
- Reference center 5

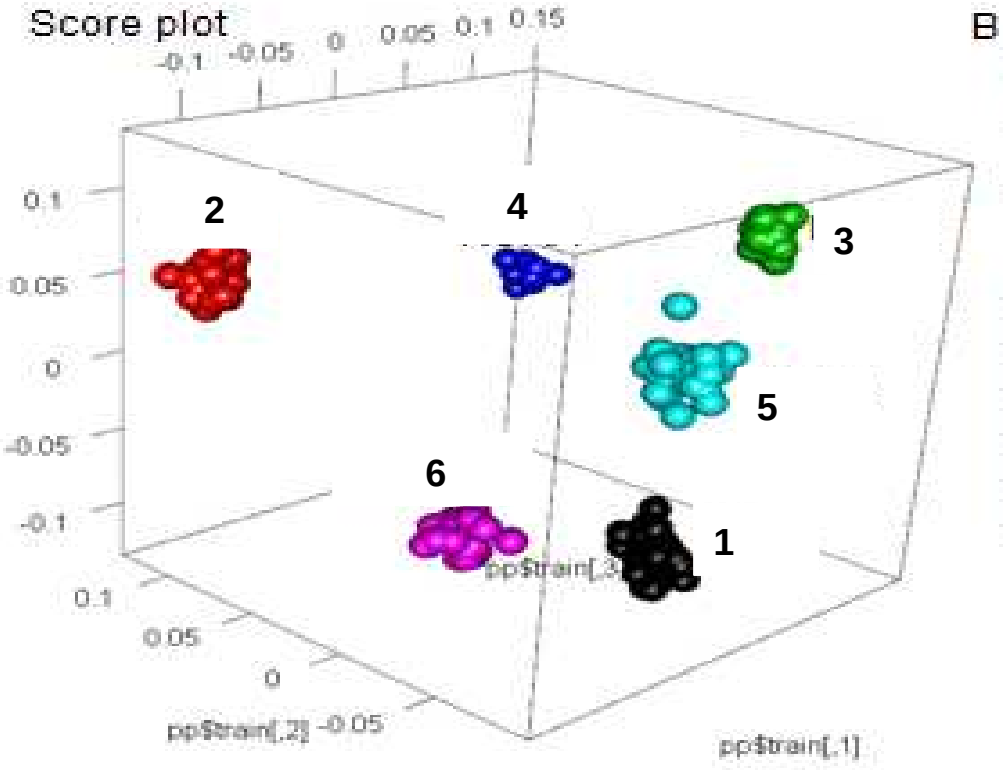
Shape by
Variability group
- Analytical variability
- Disease
- Healthy control
- MTXH Reference
- MxPool™

metanomics health

The Source of Samples Determins the Metabolome Signature

PCA-CA on samples of plasma containing EDTA:
In this case the EDTA peaks were eliminated to avoid spectral differences that could be introduced by different concentrations of the anticoagulant agent

A



B

Cross-validation
Discrimination accuracy = 89%

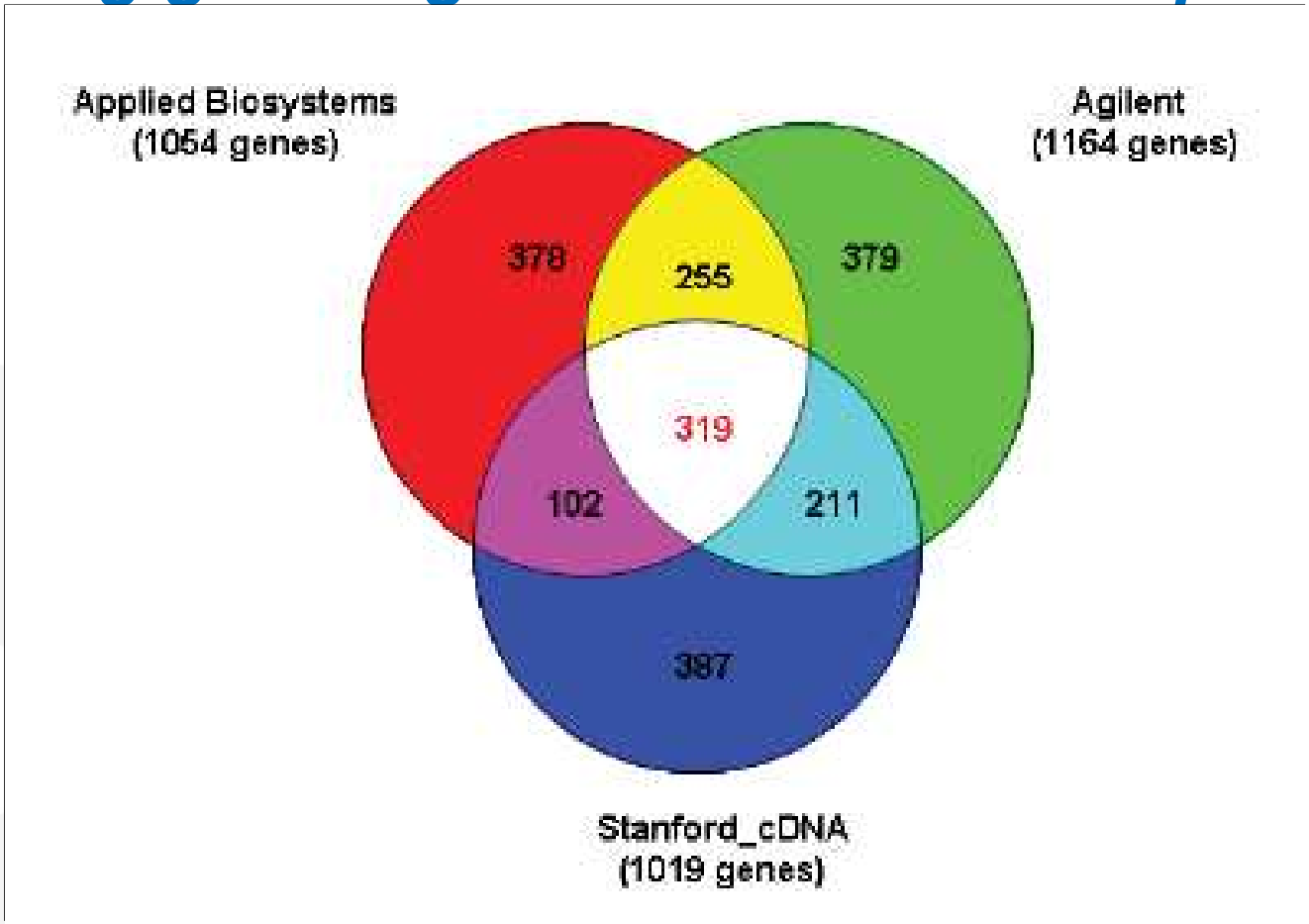
Confusion matrix

	[,1]	[,2]	[,3]	[,4]	[,5]	[,6]
[1,]	**94.8**	2.2	0.0	2.5	0.5	0.0
[2,]	3.1	**93.1**	0.0	0.9	2.3	0.5
[3,]	11.0	2.1	**71.2**	0.0	14.4	1.3
[4,]	5.7	6.2	0.0	**86.2**	0.9	1.0
[5,]	1.3	0.9	1.3	1.0	**95.2**	0.2
[6,]	2.5	0.0	0.0	0.0	0.7	**96.8**

CERM Univ. Florence

Impact of DNA –Array Platform on Gene Signatures

Overlapping gene signatures of different platfroms



Sorlie et al., BMC Genomics 2006

Sample Management

Reproducibility Depends on Quality

OBBR Office of Biorepositories and Biospecimen Research

GARBAGE IN ⇒ GARBAGE OUT

Many SOPs Around the World: Which are the Best?

OBBR Office of Biorepositories and Biospecimen Research

- Impossible to call any one "best" (even NCI's)
  - All have strengths and weaknesses
  - No single set of SOPs are applicable to all clinical and research analytical platforms
  - Very few SOPs are based on scientific evidence

Where we need to go

from C. Compton

USA


National Cancer Institute
U.S. National Institutes of Health | www.cancer.gov
OBBR
Office of Biorepositories and Biospecimen Research
Launch NCI Best Practices
Launch caHUB
Sign Up For Updates
Search
About OBBR
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Biospecimen Research Network
caHUB
News and Events
Public Resources
Biospecimen Research Network

Europe


SPIDIA
Standardisation and improvement of generic pre-analytical tools and procedures for in-vitro diagnostics
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ABOUT SPIDIA
SPIDIA is a 4.5-year project, funded by the European Union FP7 programme to the value of 9 million Euros, which brings together a consortium of 16 leading academic institutions, international organisations and life sciences companies.

Tissue Sample Quality: Critical Issues

Medication
Surgical procedure
Warm ischemia

Fixation
Fixative
Time

Transport
Temperature
Cold ischemia

Embedding
Temperature

Sample processing
Mech. alteration
Selection+annotation

Diagnosis
Disease codes

Aliquotting

Storage
Time
temperature

Freezing
Freezing rate
Temperature

Sample preparation

Cryostorage
Temperature
Temp. shifts

Analysis

SPIDIA

Sources of Diversity

Medication
Surgical procedure
Warm ischemia

Transport
Temperature
Cold ischemia

Sample processing
Mech. alteration
Selection+annotation

Aliquotting

Freezing
Freezing rate
Temperature

Cryostorage
Temperature
Temp. shifts

Fixation
Fixative
Time

Embedding
Temperature

Diagnosis
Disease codes

Storage
Time
temperature

Sample preparation

Analysis

can be avoided
can be reduced
not avoidable

SPIDIA

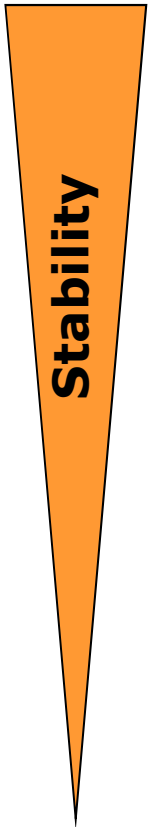
Parameters for Tissue-Based Analysis

Sample variables

- Tissue type (organ)
- Diseased/normal
- Sample type (biopsy/surgery)
- Peri-operative effects
- Ischemia
- Processing
- Fixation
- Storage
- Analysis

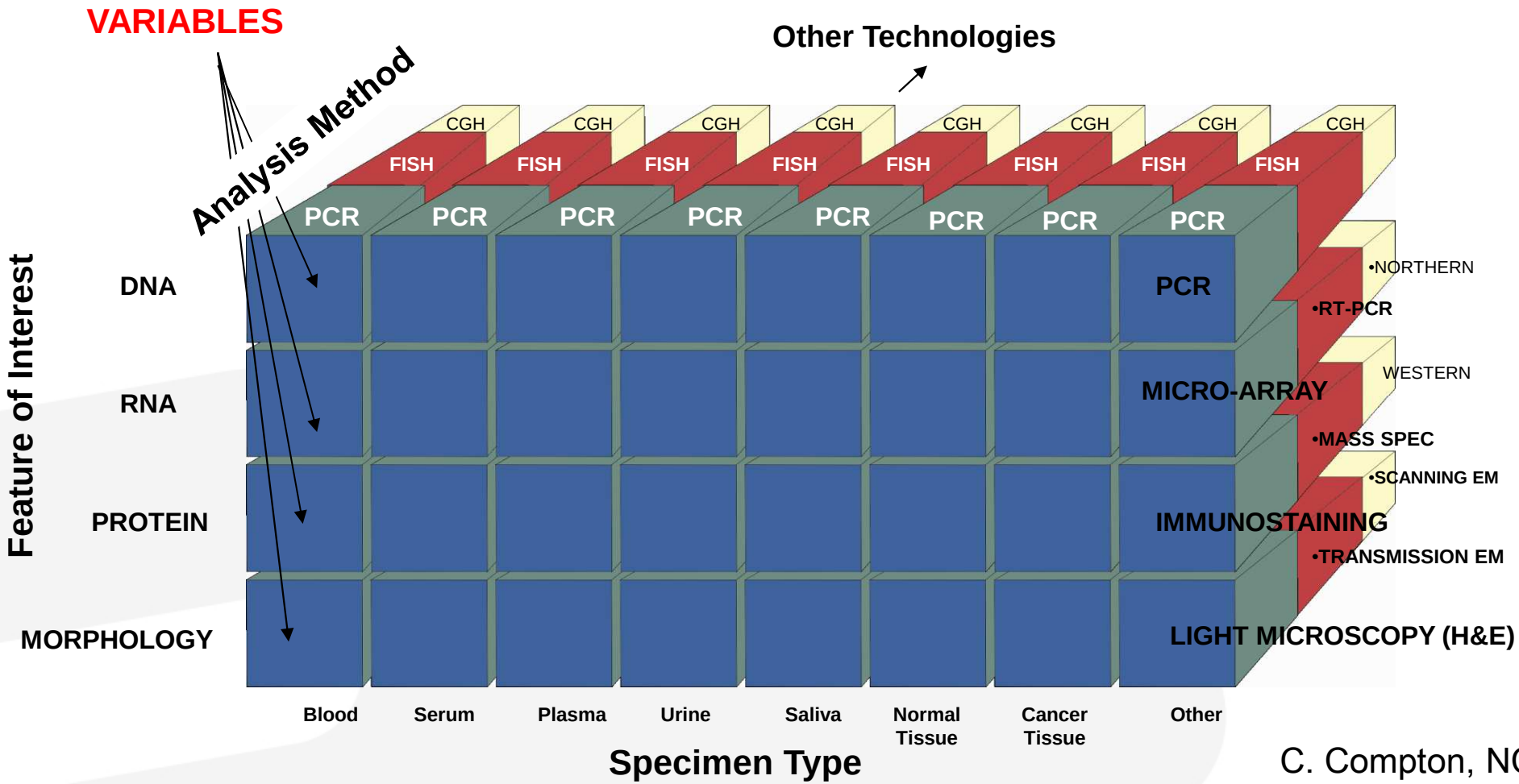
Readout

- Morphology
- Antigenicity
- Mol.structure
- Biomolecules
  - DNA
  - Protein
  - Protein mod.
  - RNA
  - Metabolites
- Interactomes



SPIDIA

NCI „Ice cube“ Framework for Generation of Evidence-based Standards



C. Compton, NCI

An Emerging International Framework for Biobanking-related Standards and Regulations

EUROPEAN COMMISSION

Brussels, 26.9.2012
COM(2012) 541 final

2012/0267 (COD)

Proposal for a

REGULATION OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

on *in vitro* diagnostic medical devices

6. PRODUCT VERIFICATION AND VALIDATION

The documentation shall contain the results of verification and validation testing and/or studies undertaken to demonstrate conformity of the device with the requirements of this Regulation and in particular the applicable general safety and performance requirements.

This includes:

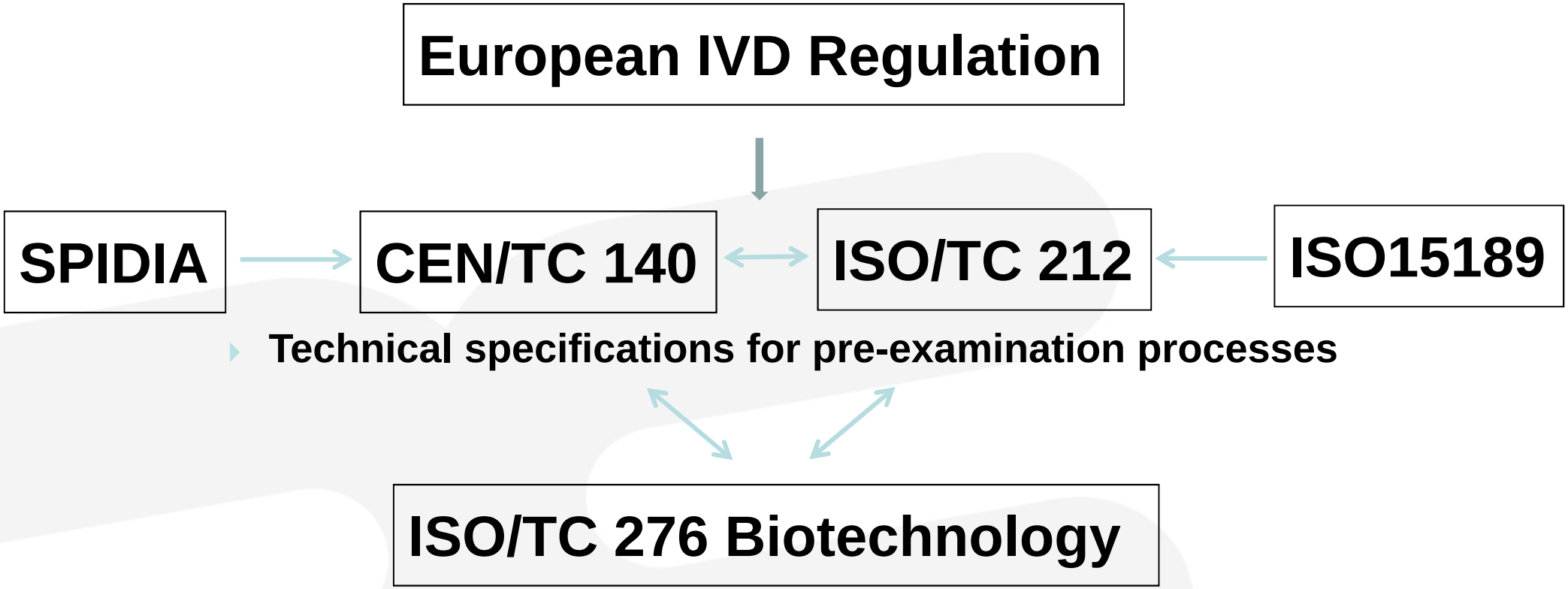
6.1 Information on analytical performance

6.1.1 Specimen type

This section shall describe the different specimen types that can be used, including their stability (e.g. storage and where applicable transport conditions) and storage conditions (e.g. duration, temperature limits and freeze/thaw cycles).

6.1.2 Analytical performance characteristics

An Emerging International Framework for Sample-related Standards



CEN Technical Specifications for Pre-examination Processes

- Frozen tissue — Part 1: Isolated RNA (CEN/TS 16826-1:2015)
- Frozen tissue — Part 2: Isolated proteins (CEN/TS 16826-2:2015)
- FFPE tissue — Part 1: Isolated DNA (CEN/TS 16827-3:2015)
- FFPE tissue — Part 2: Isolated RNA (CEN/TS 16827-1:2015)
- FFPE tissue — Part 3: Isolated proteins (CEN/TS 16827-2:2015)

- Venous whole blood — Part 1: Isolated cellular RNA (CEN/TS 16835-1:2015)
- Venous whole blood — Part 2: Isolated genomic DNA (CEN/TS 16835-2:2015)
- Venous whole blood — Part 3: Isolated circulating cell free DNA from plasma (CEN/TS 16835-3:2015)

- Metabolomics in urine, venous blood serum and plasma (CEN/TS16945:2016)

More to Come ……

- **Venous whole blood — isolated circulating tumour cells, (CTCs) and circulating organ cells, (COCs), isolated DNA, RNA, proteins**
- **Venous whole blood – Isolated exosomes isolated nucleic acids**
- **Urine and other body fluids – isolated cfDNA**
- **Saliva – isolated human DNA**
- **Saliva and stool – isolated microbiome DNA**
- **Frozen Tissue – isolated DNA**
- **Fine Needle Aspirates (FNAs) – isolated DNA, RNA, proteins**
- **Metabolomics of body fluids: International ISO Standard: ISO/TC 212**
- **FFPE Tissue – in situ stainings including immunohistochemistry (IHC): ISO/TC 212**

Molecular in-vitro diagnostic examinations — Specifications for pre-examination processes for frozen tissue — RNA (draft content)

Outside the laboratory

- Primary tissue collection manual
- Information about the primary sample donor
- Information on the primary tissue sample
- Information on the primary tissue sample processing
- Transport requirements

Inside the laboratory

- Information on the primary tissue sample receipt
- Pathological evaluation of the specimen
- Cryo-preservation of the specimen
- Storage requirements
- Isolation of the total RNA
- General information for RNA isolation procedures
- Using commercial kits
- Using the laboratories own protocols
- Quality assessment of isolated RNA
- Storage of isolated RNA

Scope of the Technical Specifications

Applicable to molecular *in-vitro* diagnostic examinations (e.g., *in-vitro* diagnostic laboratories, laboratory customers, *in-vitro* diagnostics developers and manufacturers, institutions and commercial organizations performing biomedical research, biobanks, and regulation authorities.

Key Pillars

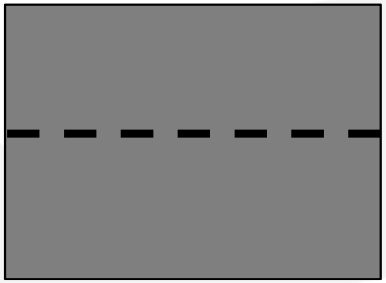
- Validation: reliability
- Standardization: reproducibility
- Documentation: accountability

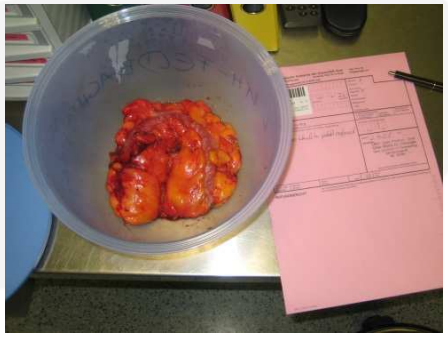
Bringing Light into a Black Box

outside the laboratory

inside the laboratory

?





Patient ***Pre- Analytics*** ***Analytics***

Molecular in-vitro diagnostic examinations — Specifications for pre-examination processes: Principles

- Documentation, documentation, documentation ……
- Few concrete procedures

  Standard buffered formalin solution

  10 % formalin solution containing 3.7 % by mass (corresponding to 4% by volume) formaldehyde, buffered to pH 6.8 to pH 7.2

  no TE-buffer for RNA
- Definitions
- Not included:
  - Biosafety, biosecurity
  - Informed consent, counselling

Thank you
and my Team