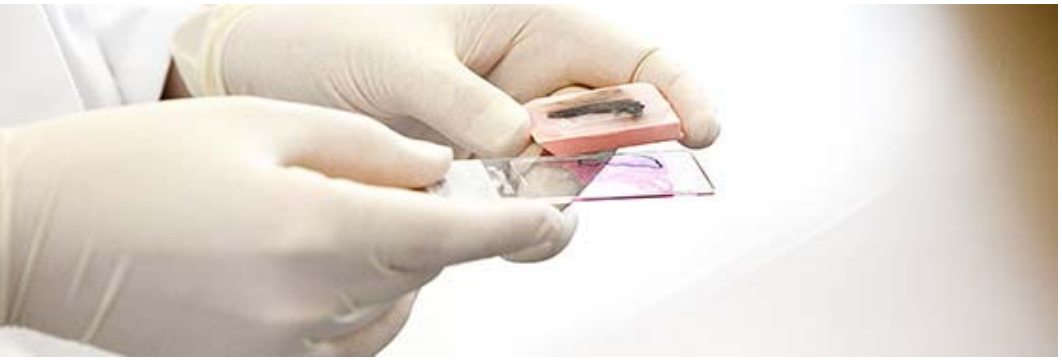
New aspects in preanalytics



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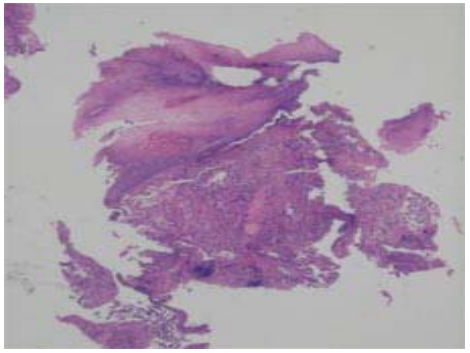
Research Topics of the TUM lab for Experimental Pathology in Munich

- Development and validation of molecular biomarkers
- Improvement of tissue quality for diagnosis and research
- Intratumoral heterogeneity of human cancers
- Quantitative (phospho)protein analysis of tissue samples

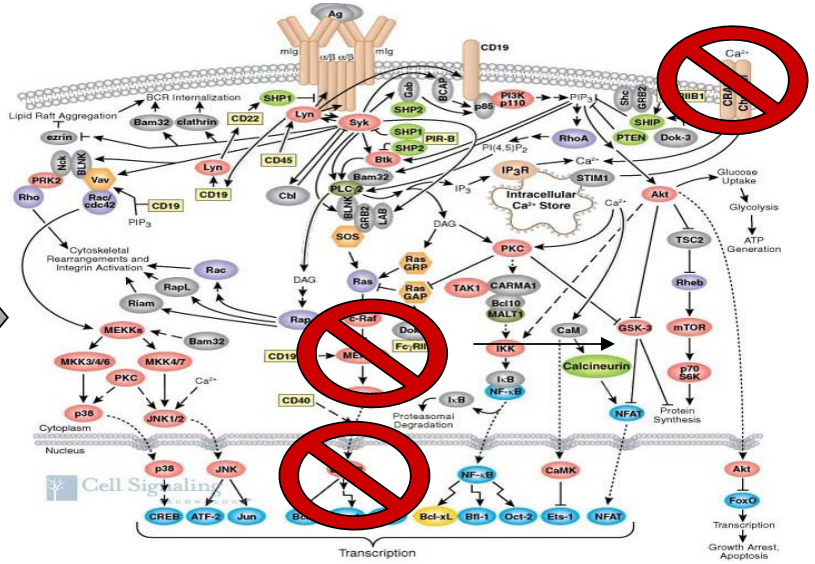
Tissue bank of the Medical School of MRI/TUM in the spotlight

- Optimal logistics with a pathologist directly in place in the operating room
- >25.000 frozen samples
- >1 million FFPE samples
- Collection of tissues fixed with an alternative fixative
- State-of-the-art molecular analysis offered

The current revolution: molecular profiling for individualized therapy

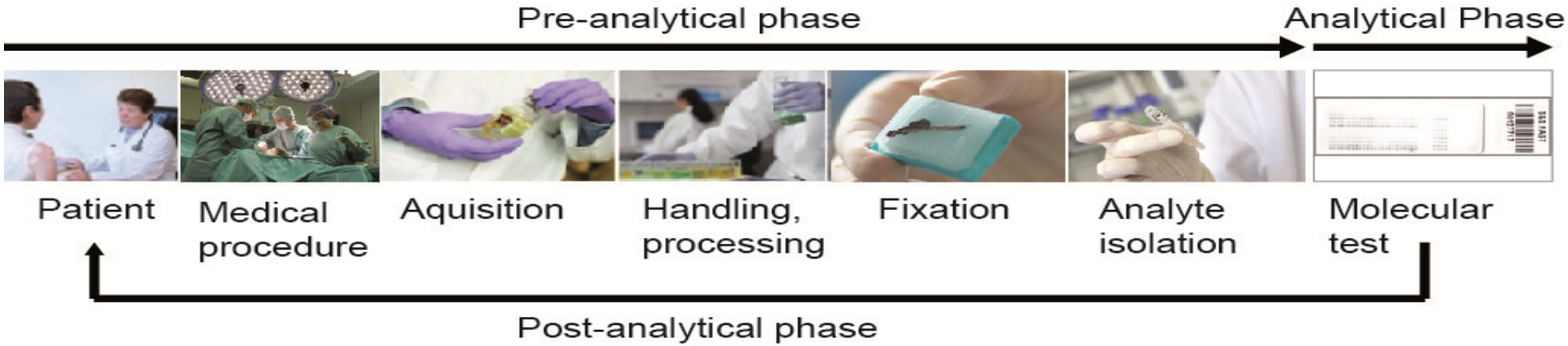


Tissue sample
(FFPE, frozen or alternative fixative)



Identify, block, and monitor deregulated protein networks

Protein analysis of clinical tissue samples - Consider the entire workflow!

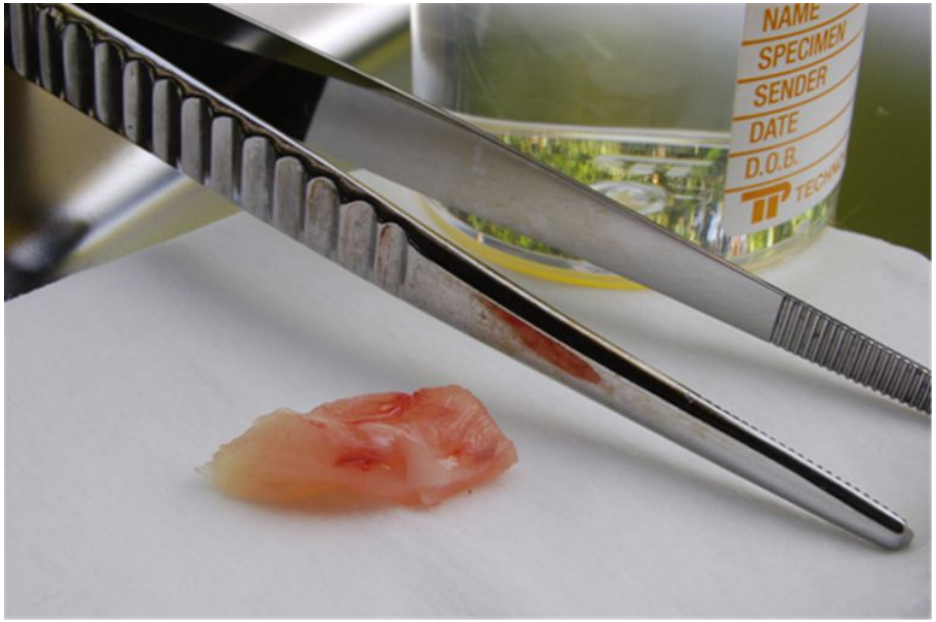


www.m4.de www.spidia.eu http://biospecimens.cancer.gov

-> Medical Treatment of the patient
-> Transport of specimen to pathology
-> Specimen reception

Specimen-types commonly received in a histopathology lab:

- Excision specimens (surgical biopsies),
- Incisional biopsy specimens
- Punch biopsies
- Shave biopsies
- Curettings
- Core biopsies
- ….



http://www.leicabiosystems.com/pathologyleaders/an-introduction-to-specimen-preparation/

Stabilisation/Fixation

Objective: to prevent decay and preserve cells and tissues in a “life-like” state.

http://www.leicabiosystems.com/pathologyleaders/an-introduction-to-specimen-preparation/

Grossing

- „Cut up“
- Careful examination and description of the specimen
- Larger specimens may require further dissection to produce representative pieces from appropriate areas
- The tissues selected for processing will be placed in cassettes

http://www.leicabiosystems.com/pathologyleaders/an-introduction-to-specimen-preparation/

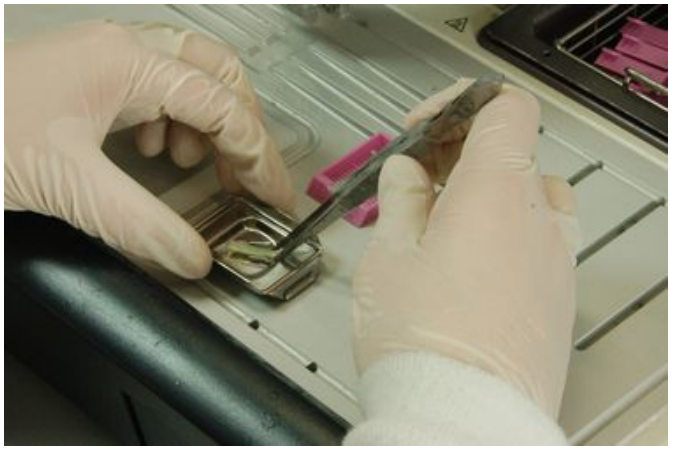
Processing

- “tissue processors”
- specimens are infiltrated with a sequence of different solvents, finishing in molten paraffin wax

http://www.leicabiosystems.com/pathologyleaders/an-introduction-to-specimen-preparation/

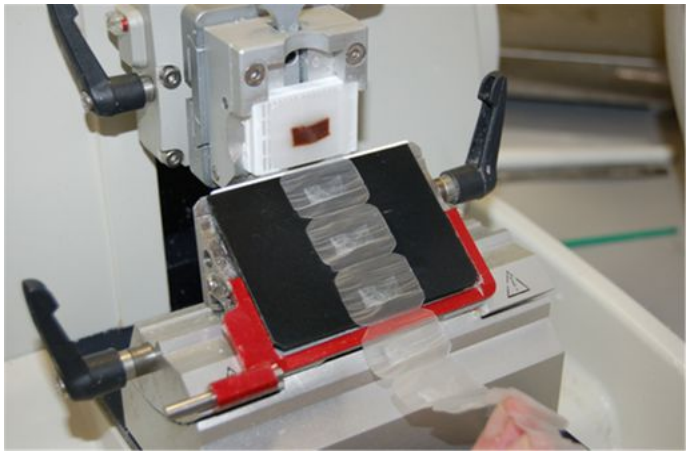
Embedding

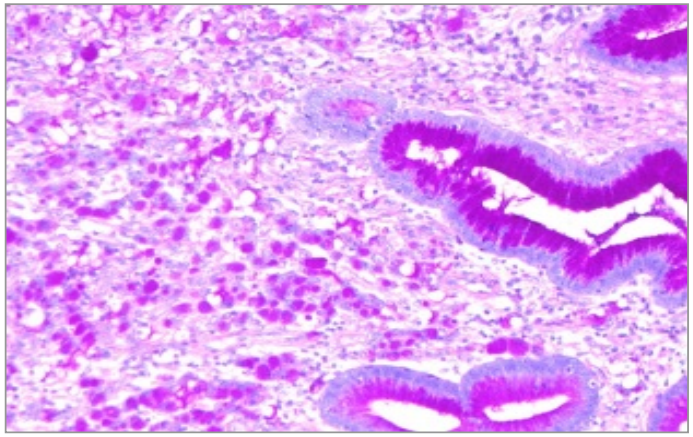
- specimens are placed in an embedding centre where they are removed from their cassettes and placed in wax-filled molds
- specimen “block” is allowed to solidify on a cold surface
- The block containing the specimen is now ready for section cutting or storing



http://www.leicabiosystems.com/pathologyleaders/an-introduction-to-specimen-preparation/

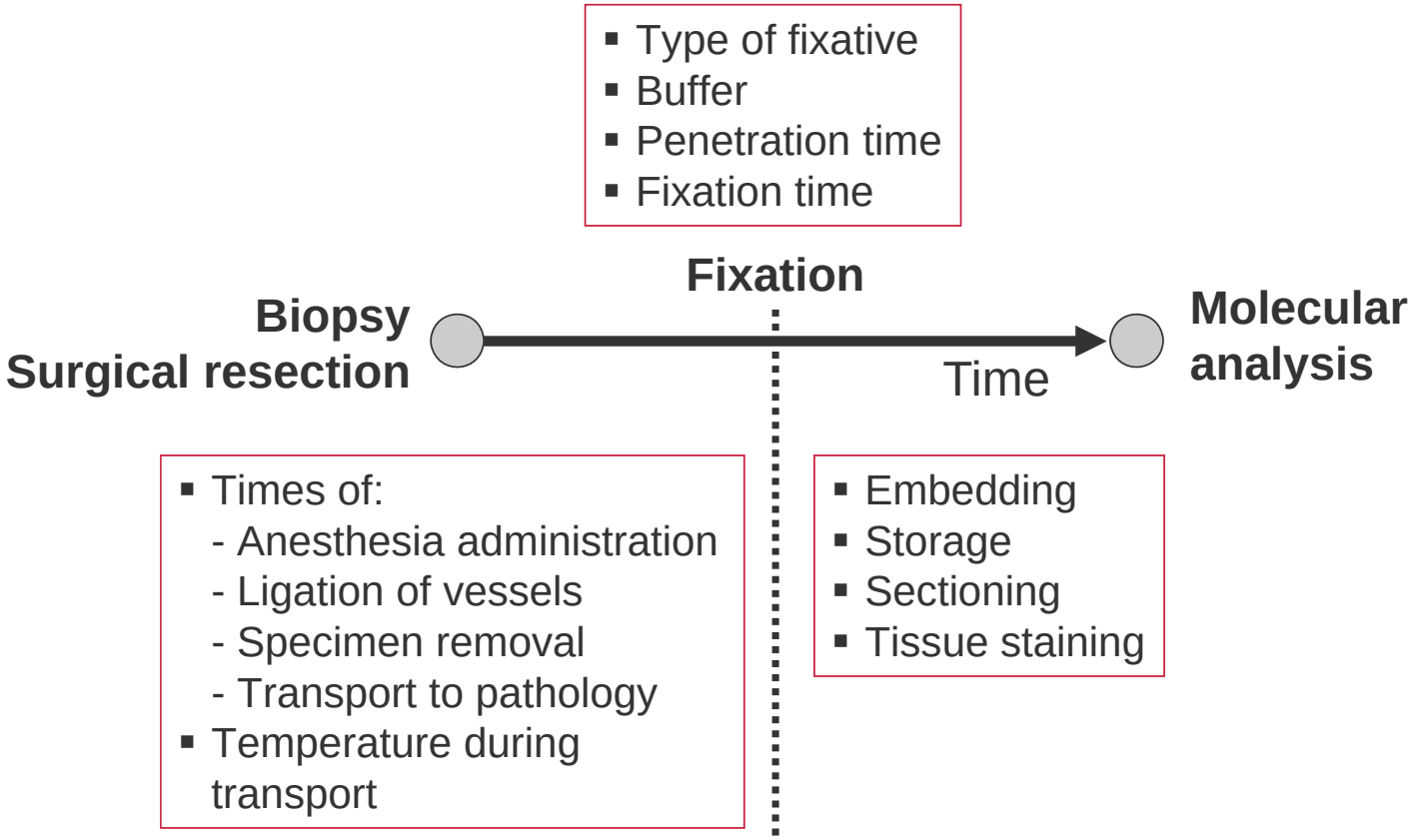
Cutting and staining





http://www.leicabiosystems.com/pathologyleaders/an-introduction-to-specimen-preparation/

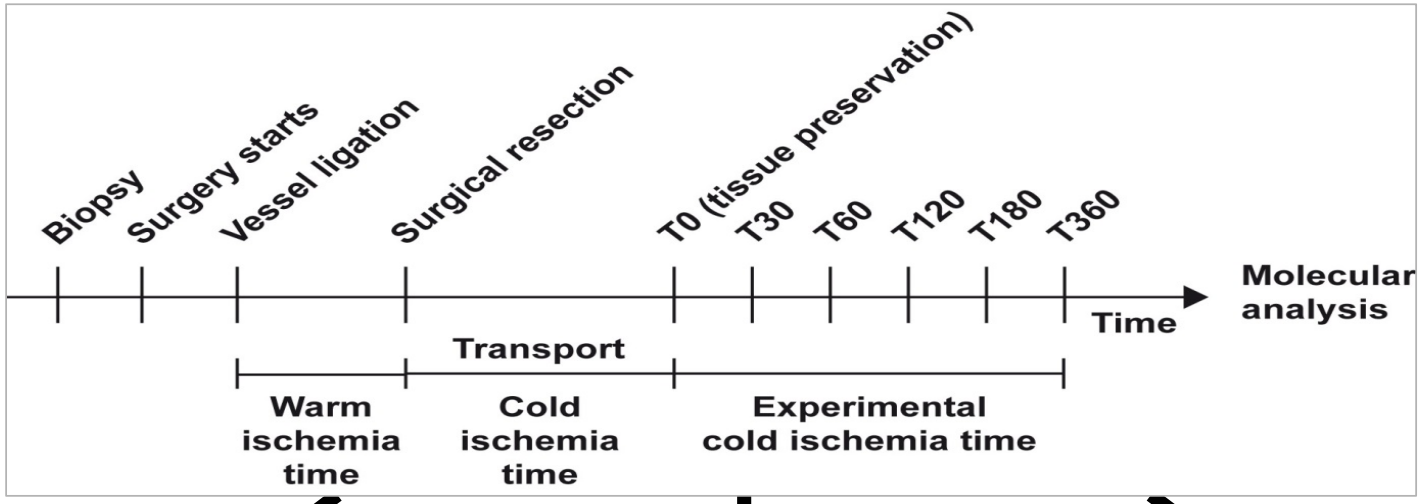
Problem for biomarker analysis: pre-analytical variables during tissue processing



Becker KF and Taylor CR. Appl Immunohistochem Mol Morphol. 2011

Standards for the pre-analytical phase

Identifying the critical steps during tissue processing



SPIDIA

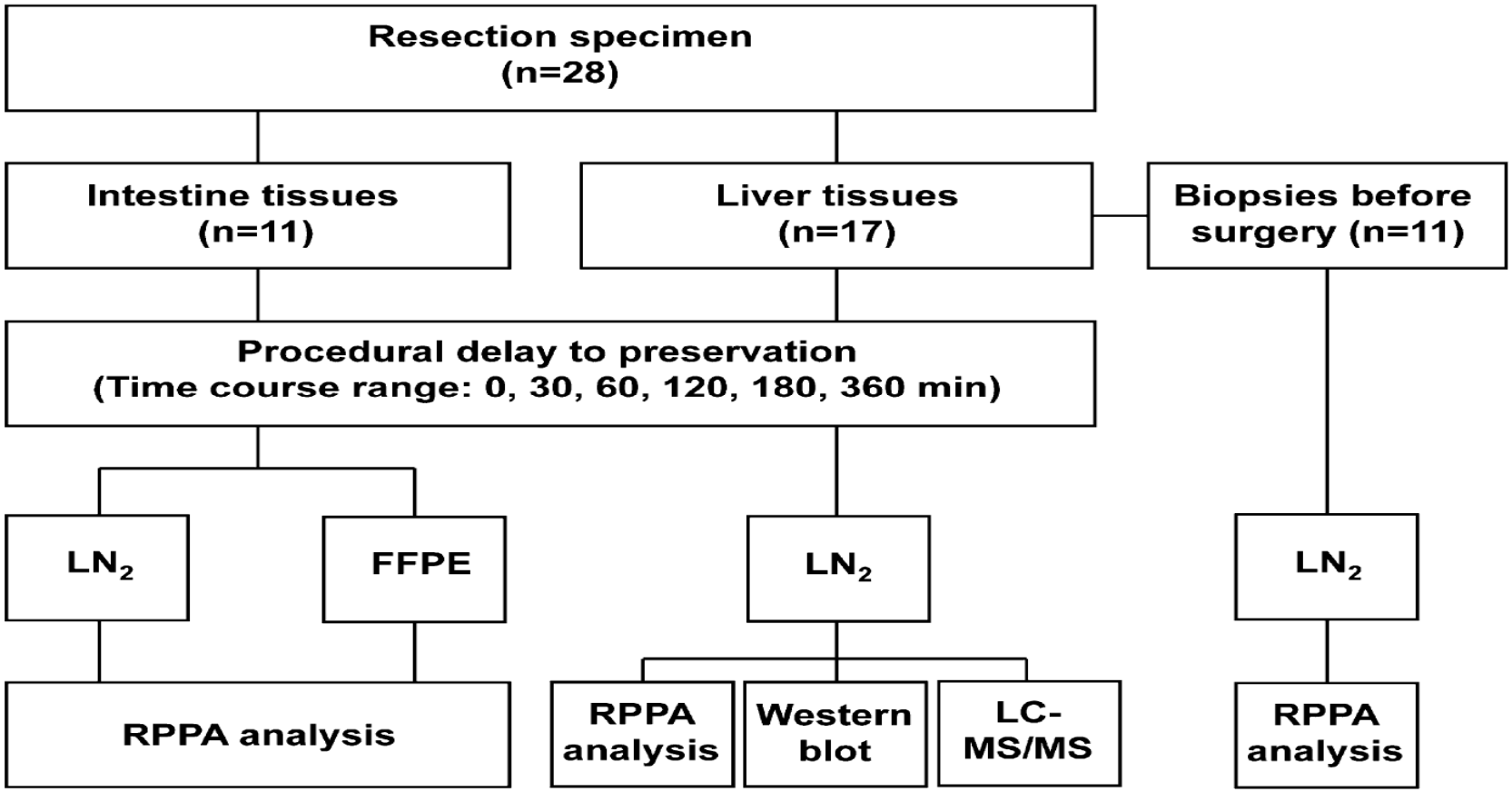
RNA analysis

Protein analysis

Metabolome analysis

European Committee for Standardization (CEN)

Examples


Resection specimen
(n=28)
Intestine tissues
(n=11)
Liver tissues
(n=17)
Biopsies before surgery (n=11)
Procedural delay to preservation
(Time course range: 0, 30, 60, 120, 180, 360 min)
LN2
FFPE
LN2
LN2
RPPA analysis
RPPA analysis
Western blot
LC-MS/MS
RPPA analysis

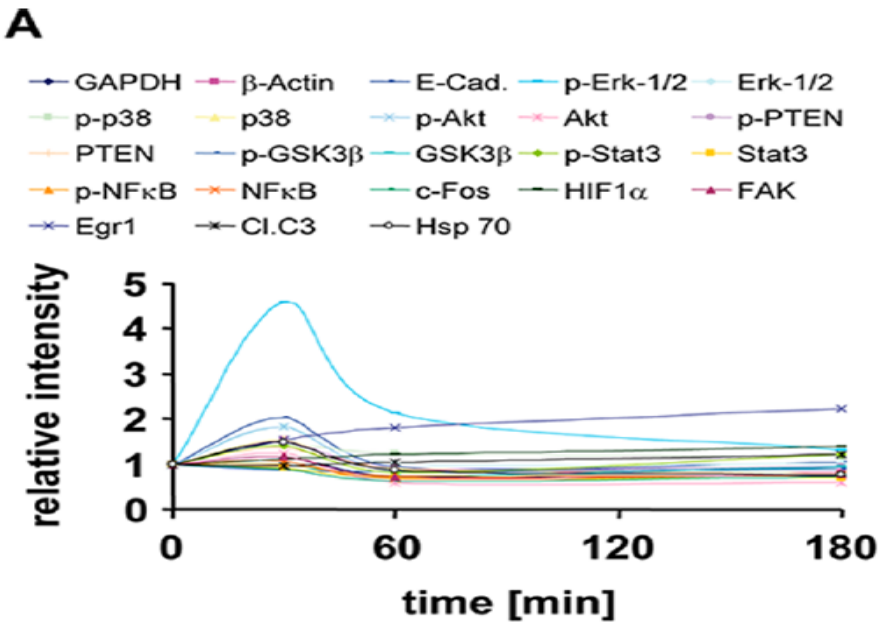
Proteins analysed by RPPA

- 33 proteins analysed
  - 20 total proteins
  - 13 phosphoproteins

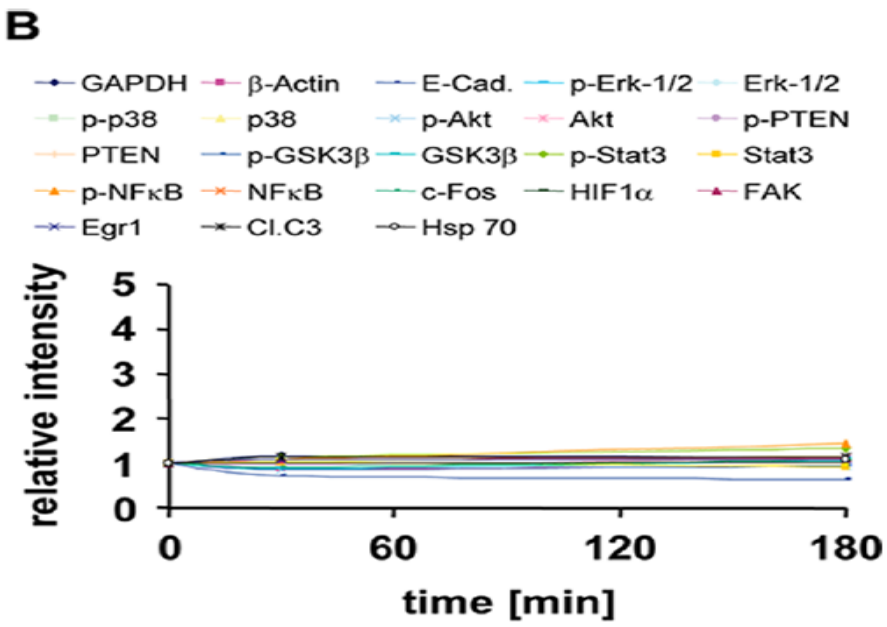
No	Protein
1	EGFR
2	P-EGFR
3	HER2
4	P-HER2
5	HER3
6	P-HER3
7	HER4
8	VEGFR
9	P-VEGFR
10	PI3K
11	P-PI3K
12	AKT
13	P-AKT
14	ERK
15	P-ERK
16	HGF
17	cMET

No	Protein
18	P-cMET
19	beta-Catenin
20	P-beta-Catenin
21	GSK3-beta
22	P-GSK3-beta
23	Axin
24	Cytokeratin 18
25	P-Cytokeratin 18
26	GAPDH
27	Beta-actin
28	p38
29	P-p38
30	PTEN
31	P-PTEN
32	HIF1-alpha
33	Cleaved caspase 3

P-ERK responds to delayed cold ischemia – but not in all patients



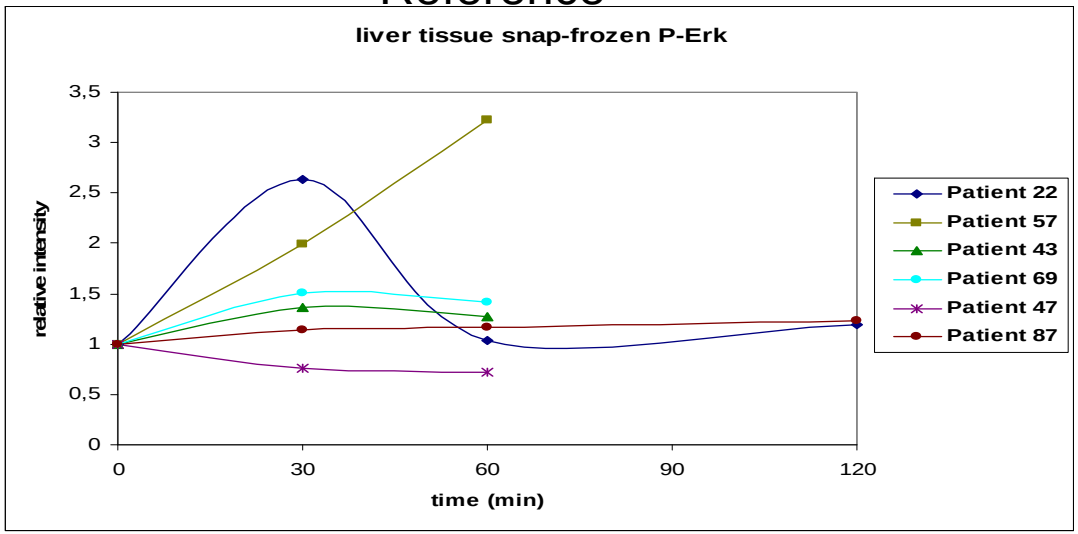
Patient 1



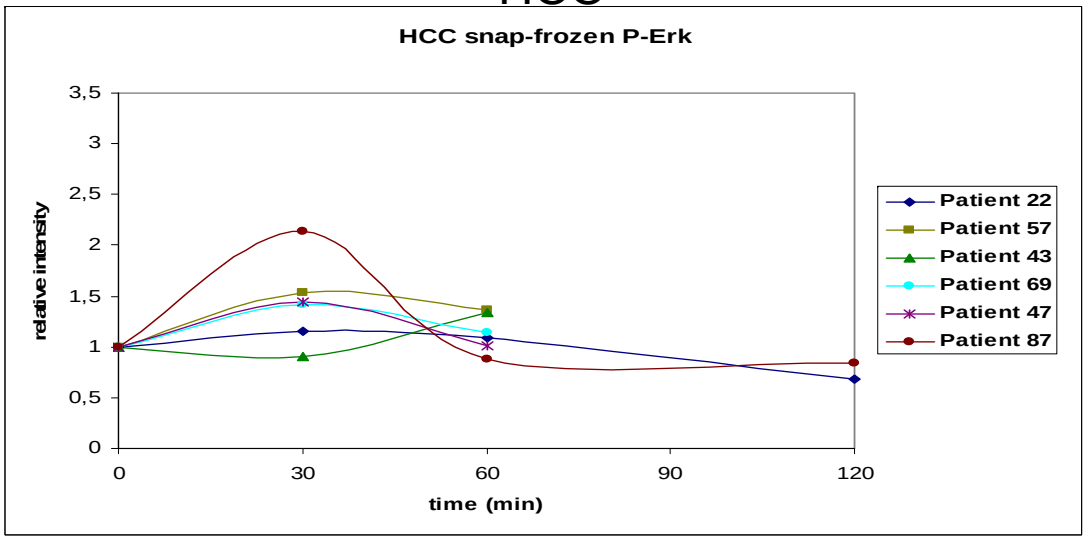
Patient 2

P-ERK responds to delayed cold ischemia – also in hepatocellular carcinoma (HCC)

Reference

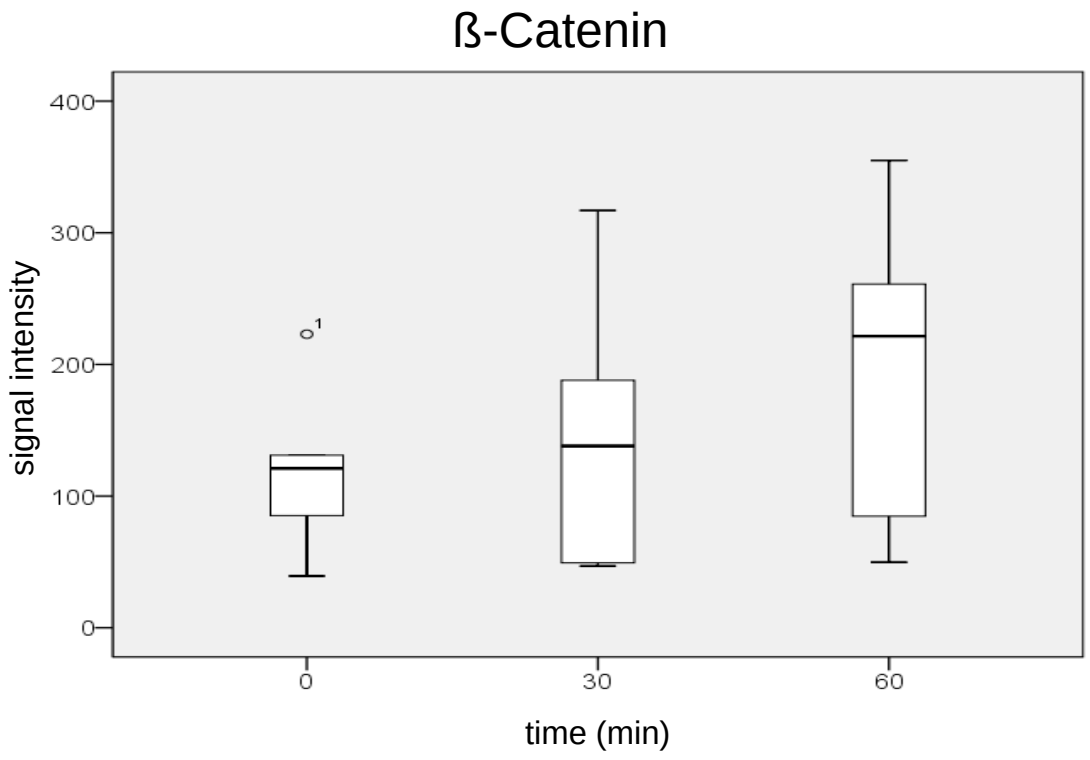


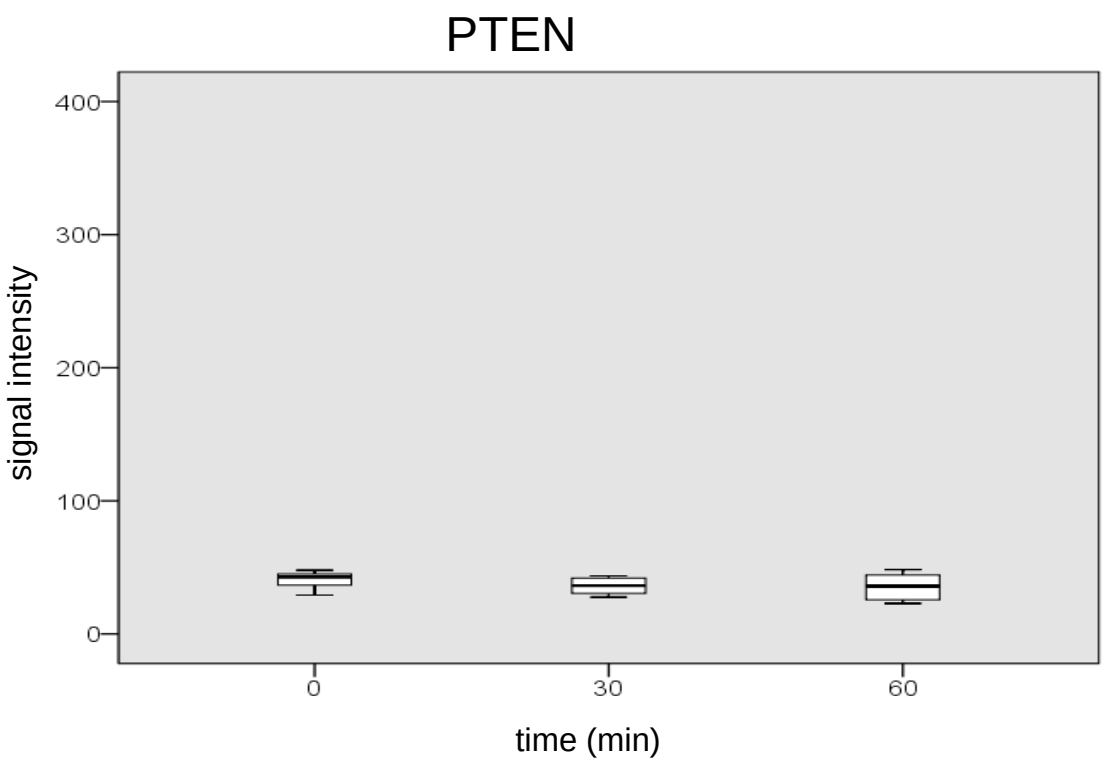
HCC



Interpatient variability

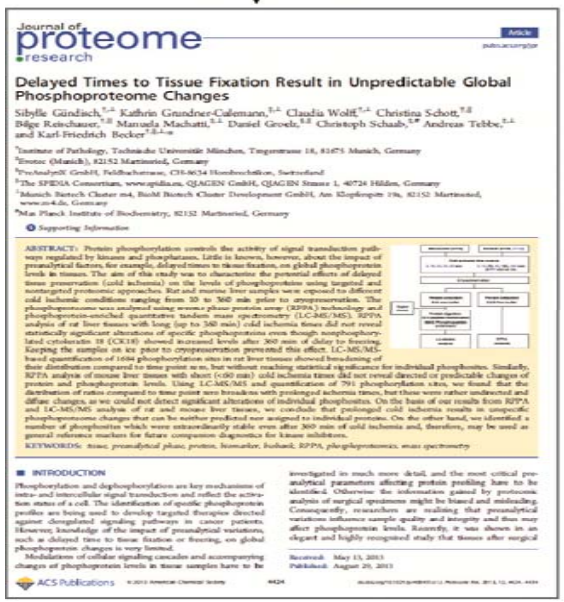
(HCC)





SPIDIA´s ways to exploit scientific results

Publication



Journal of proteome research

Article

Delayed Times to Tissue Fixation Result in Unpredictable Global Phosphoproteome Changes

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Supporting Information

ABSTRACT: Protein phosphorylation controls the activity of signal transduction pathways regulated by kinases and phosphatases. Little is known, however, about the impact of preanalytical factors, for example, delayed times to tissue fixation, on global phosphoprotein levels in tissues. The aim of this study was to characterize the potential effects of delayed tissue preservation (cold ischemia) on the levels of phosphoproteins using targeted and nontargeted proteomic approaches. Rat and murine liver samples were exposed to different cold ischemic conditions ranging from 10 to 360 min prior to cryopreservation. The phosphoproteome was analyzed using reverse phase protein array (RPPA) technology and phosphoprotein-enriched quantitative tandem mass spectrometry (LC-MS/MS). RPPA analysis of rat liver tissues with long (up to 360 min) cold ischemia times did not reveal statistically significant alterations of specific phosphoproteins even though nonphosphorylated cytokeratin 18 (CK18) showed increased levels after 360 min of delay to freezing. Keeping the samples on ice prior to cryopreservation prevented this effect. LC-MS/MS-based quantification of 1684 phosphorylation sites in rat liver tissues showed broadening of their distribution compared to time point zero, but without reaching statistical significance for individual phosphosites. Similarly, RPPA analysis of mouse liver tissues with short (<30 min) cold ischemia times did not reveal directed or predictable changes of protein and phosphoprotein levels. Using LC-MS/MS and quantification of 791 phosphorylation sites, we found that the distribution of ratios compared to time point zero broadens with prolonged ischemia times, but these were rather undirected and diffuse changes, as we could not detect significant alterations of individual phosphosites. On the basis of our results from RPPA and LC-MS/MS analysis of rat and mouse liver tissues, we conclude that prolonged cold ischemia results in unspecific phosphoproteome changes that can be neither predicted nor assigned to individual proteins. On the other hand, we identified a number of phosphosites which were extraordinarily stable even after 360 min of cold ischemia and, therefore, may be used as general reference markers for future companion diagnostics for kinase inhibitors.

KEYWORDS: tissue, preanalytical phase, protein, biomarker, biobank, RPPA, phosphoproteomics, mass spectrometry

INTRODUCTION

Phosphorylation and dephosphorylation are key mechanisms of intra- and intercellular signal transduction and reflect the activation status of a cell. The identification of specific phosphoprotein profiles are being used to develop targeted therapies directed against deregulated signaling pathways in cancer patients. However, knowledge of the impact of preanalytical variations, such as delayed time to tissue fixation or freezing, on global phosphoprotein changes is very limited.

Modulations of cellular signaling cascades and accompanying changes of phosphoprotein levels in tissue samples have to be investigated in much more detail, and the most critical preanalytical parameters affecting protein profiling have to be identified. Otherwise the information gained by proteomic analysis of surgical specimens might be biased and misleading. Consequently, researchers are realizing that preanalytical variations influence sample quality and integrity and thus may affect phosphoprotein levels. Recently, it was shown in an elegant and highly recognized study that tissues after surgical

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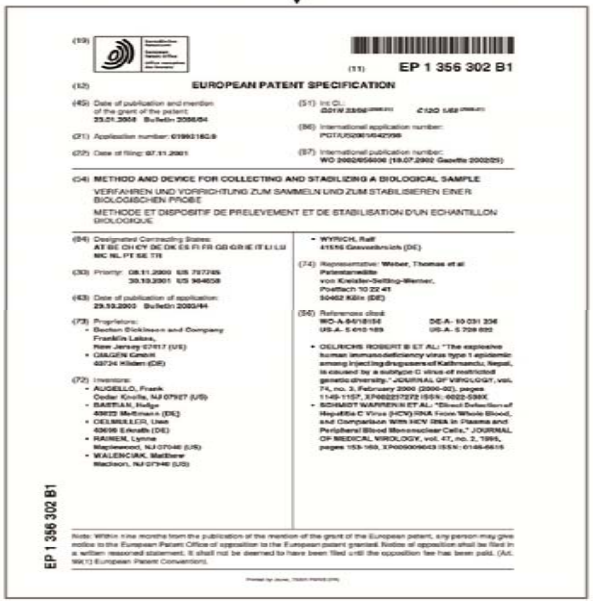
ACS Publications

IP



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(86) International application number: PCT/US2001/042099

(22) Date of filing: 07.11.2001

(87) International publication number: WO 2002/056006 (18.07.2002 Gazette 2002/29)

(54) METHOD AND DEVICE FOR COLLECTING AND STABILIZING A BIOLOGICAL SAMPLE

VERFAHREN UND VORRICHTUNG ZUM SAMMELN UND ZUM STABILISIEREN EINER BIOLOGISCHEN PROBE

METHODE ET DISPOSITIF DE PRELEVEMENT ET DE STABILISATION D'UN ECHANTILLON BIOLOGIQUE

(84) Designated Contracting States: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE TR

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US-A- 5 410 169 US-A- 5 708 830

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EP 1 356 302 B1

Standard

CEN/TC 140
Date: 2014-06
TC 140 WI 00140098
CEN/TC 140
Secretariat: DIN

Molecular *in-vitro* diagnostic examinations — Specifications for pre-examination processes for frozen tissue — Extracted proteins

Molekularanalytische in-vitro diagnostische Verfahren — Spezifikationen für präanalytische Prozesse für gefrorene Gewebeproben — Extrahierte Proteine

Élément introductif — Élément central — Élément complémentaire

ICS:

Descriptors:

Document type: Technical Specification
Document subtype:
Document stage: Publication
Document language: E

C:\Users\kh\Desktop\tissue_protein-cryo 2014-07-04fb.doc STD Version 2.5a

SPIDIA SPIDIA SPIDIA

Institut für Pathologie
Fakultät für Medizin
Technische Universität München

Published CEN Technical Specifications

Molecular in-vitro diagnostic examinations — Specifications for pre-examination processes for blood
— Part 1: cellular RNA
— Part 2: genomic DNA
— Part 3: cell free circulating DNA

Molecular in-vitro diagnostic examinations — Specifications for pre-examination processes for FFPE tissue
— Part 1: RNA
— Part 2: Proteins
— Part 3: DNA

Molecular in-vitro diagnostic examinations — Specifications for pre-examination processes for snap frozen tissue
— Part 1: RNA
— Part 2: Proteins

Molecular in-vitro diagnostic examinations — Specifications for pre-examination processes for metabolomics in urine, serum and plasma

CEN, European Committee for Standardization

Example

CEN/TC 140

Date: 2014-06

TC 140 WI 00140097

CEN/TC 140

Secretariat: DIN

Molecular *in-vitro* diagnostic examinations — Specifications for pre-examination processes for FFPE tissue — Extracted proteins

Molekularanalytische in-vitro diagnostische Verfahren — Spezifikationen für präanalytische Prozesse für FFPE Gewebeproben — Extrahierte Proteine

Élément introductif — Élément central — Élément complémentaire

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Descriptors:

Document type: Technical Specification
Document subtype:
Document stage: Publication
Document language: E

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The SPIDIA4P project builds on SPIDIA's results and is funded by the European Union's Horizon 2020 research and innovation programme.
AIM: additional International Standards

Solid Tissues / Tumours

- FFPE tissue — Part 1: Isolated RNA (ISO/IS)
- FFPE tissue — Part 2: Isolated proteins (ISO/IS)
- FFPE tissue — Part 3: Isolated DNA (ISO/IS)
- **FFPE Tissue – in situ staining including Immunohistochemistry (IHC) (ISO/IS)**
- Frozen tissue — Part 1: Isolated RNA (ISO/IS)
- Frozen tissue — Part 2: Isolated proteins (ISO/IS)
- **Frozen Tissue – Isolated DNA (CEN/TS)**
- **Fine Needle Aspirates (FNAs) – Isolated DNA (CEN/TS)**
- **Fine Needle Aspirates (FNAs) – Isolated RNA (CEN/TS)**
- **Fine Needle Aspirates (FNAs) – Isolated Proteins (CEN/TS)**

Printed in Bold: New SPIDIA4P documents

The SPIDIA4P project

Whole Blood including Liquid Biopsies

- Venous whole blood — Part 1: Isolated cellular RNA (ISO/IS)
- Venous whole blood — Part 2: Isolated genomic DNA (ISO/IS)
- Venous whole blood — Part 3: Isolated circulating cell free DNA Plasma (ISO/IS)
- **Venous whole blood — circulating tumour cells, (CTCs), isolated DNA (CEN/TS)***
- **Venous whole blood — circulating tumour cells, (CTCs), isolated RNA (CEN/TS)***
- **Venous whole blood — circulating tumour cells, (CTCs) , preparation for analytical CTC staining (CEN/TS)**
- **Venous whole blood — Isolated exosomes and isolated nucleic acids therefrom and ccfRNA (CEN/TS)***
- **Metabolomics — Urine, Whole blood plasma and Serum : International ISO Standard (ISO/IS)**

Non-invasive Body Fluids

- **Urine and other body fluids — Isolated cfDNA (CEN/TS)**
- **Saliva — Isolated human DNA (CEN/TS)** - **Saliva and stool — Isolated microbiome DNA (CEN/TS)**

Printed in Bold: New SPIDIA4P documents

ISO 20387:2018 (August)
Biotechnology -- Biobanking -- General requirements for biobanking

1 Scope

This document specifies general requirements for the competence, impartiality and consistent operation of biobanks including quality control requirements to ensure biological material and data collections of appropriate quality.

This document is applicable to all organizations performing biobanking, including biobanking of biological material from multicellular organisms (e.g. human, animal, fungus and plant) and microorganisms for research and development.

Summary

- Pre-analytical phase needs to be improved
- Variations of protein and phosphoprotein profiles
- Exploitation of research results as International Standards by SPIDIA/SPIDIA4P
- 9 CEN Technical Specifications were published in 2015
- ISO Standards are curently being drafted
- New International Standards are being developed by SPIDIA4P
- ISO International Standard for biobanking was published in August 2018

Institut für Pathologie
Fakultät für Medizin
Technische Universität München

Thanks to all the wonderful people in the different consortia or institutions

www.m4.de

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German Biobank Node
bbmri.de

Bayerisch-Tschechische Hochschulagentur
Česko-bavorská vysokoškolská agentura

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Bayerisches Staatsministerium der Finanzen, für Landesentwicklung und Heimat