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Möglichkeiten der Ergebnisverwertung aus Forschungsprojekten

Publikation

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 fixation. Keeping the samples on ice prior to cryopreservation prevented this effect. LC-MS/MS-based quantification of 1686 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 (<60 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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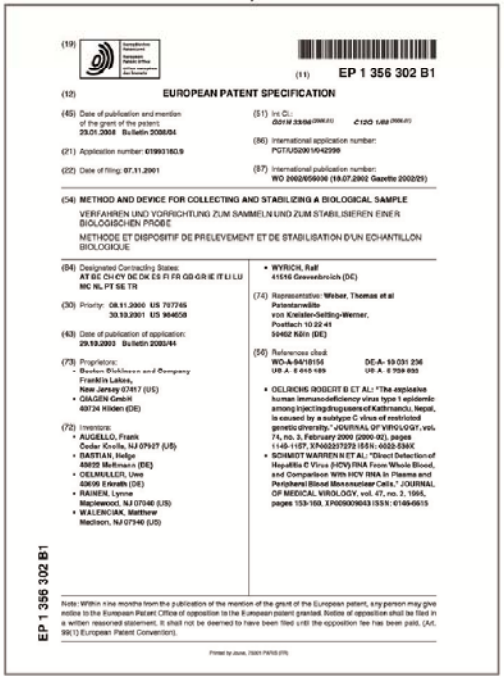
Patent



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(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

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EP 1 356 302 B1

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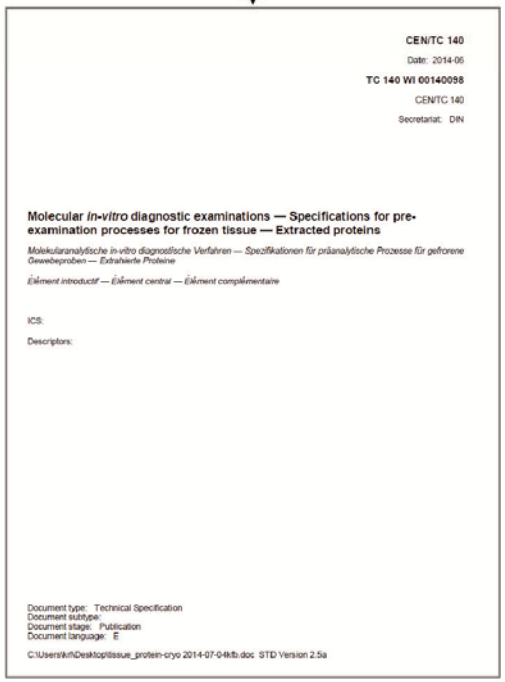
Standard



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

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Normen und Spezifikationen als Beitrag zur Innovationsförderung

Hightech-Strategie 2020 für Deutschland

- „**Normung und Standardisierung werden in Deutschland zunehmend integraler Bestandteil des Forschungs- und Innovationsprozesses,** denn frühzeitig eingeleitet fördern sie den Transfer von Forschungsergebnissen in marktfähige Produkte und Dienstleistungen und den schnellen Marktzugang von Innovationen“

Bundesministerium für Bildung und Forschung

IDEEN INNOVATION WACHSTUM
Die Hightech-Strategie für Deutschland

Ideen. Innovation. Wachstum
Hightech-Strategie 2020 für Deutschland

- „Eine **aktive Beteiligung an Normungs- und Standardisierungsaktivitäten** verschafft der deutschen Wirtschaft zudem globale Wettbewerbsvorteile.“

HIGHTECH-STRATEGIE Ideen zünden!

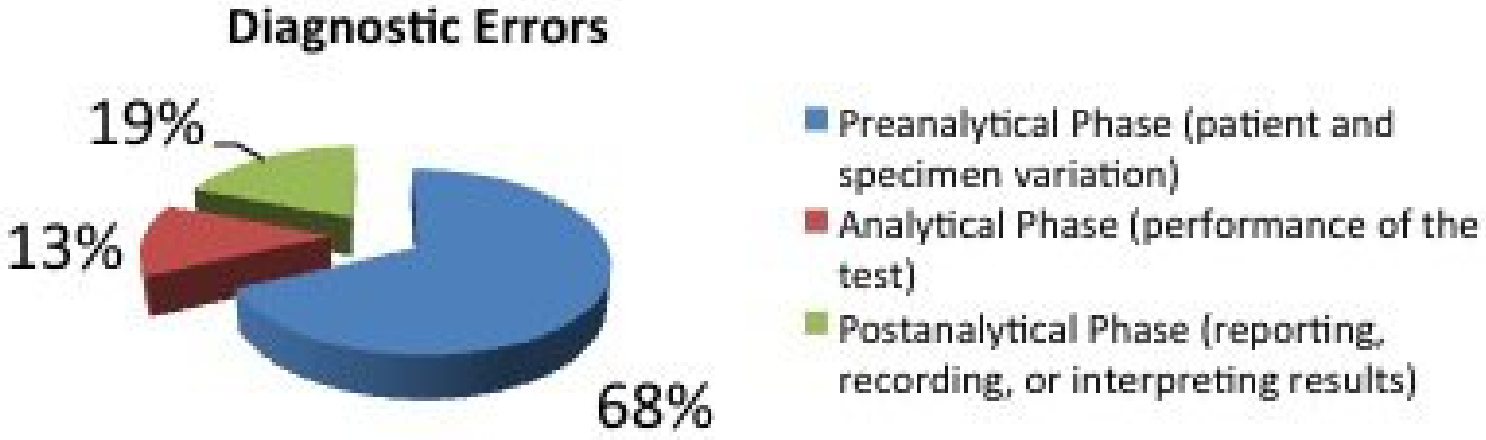
Quelle: Hightech-Strategie 2020, BMBF 2010 (Hrsg.), S. 10

Entwicklung von Standards für die Bioprobensammlung und *in vitro* Diagnostik

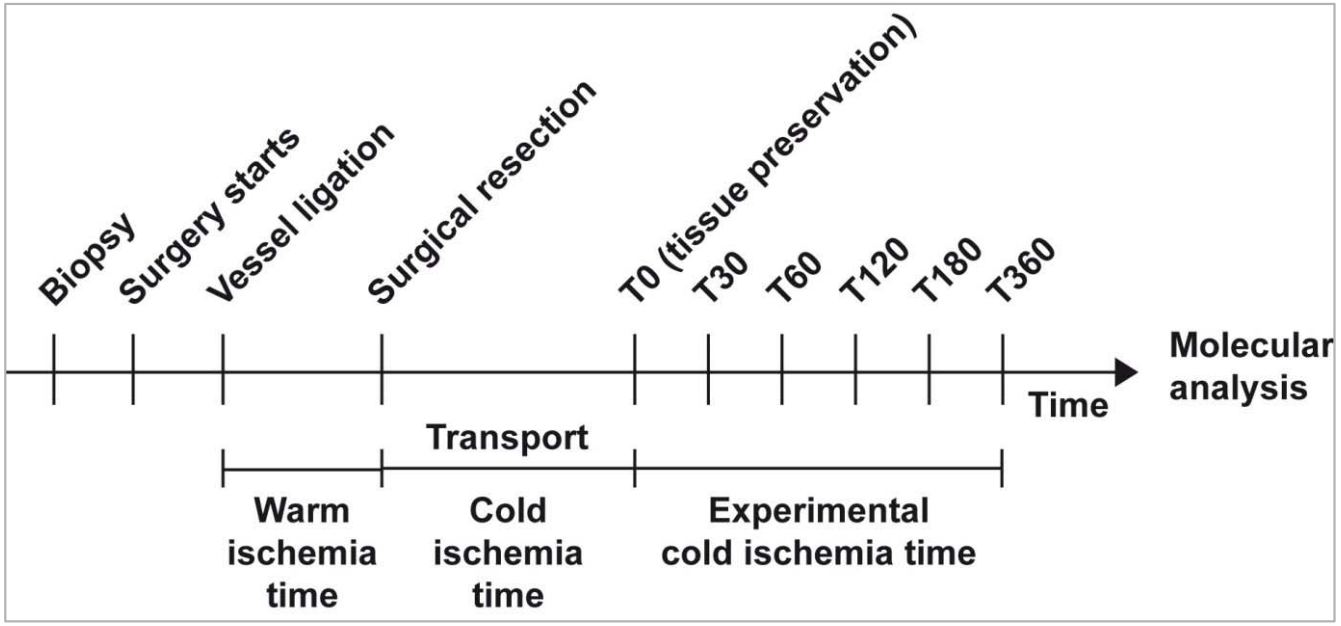
Pre-analytical phase

Analytical phase

Patient | Medical procedure | Acquisition | Handling, processing | Fixation, storage | Analyte extraction | Assay



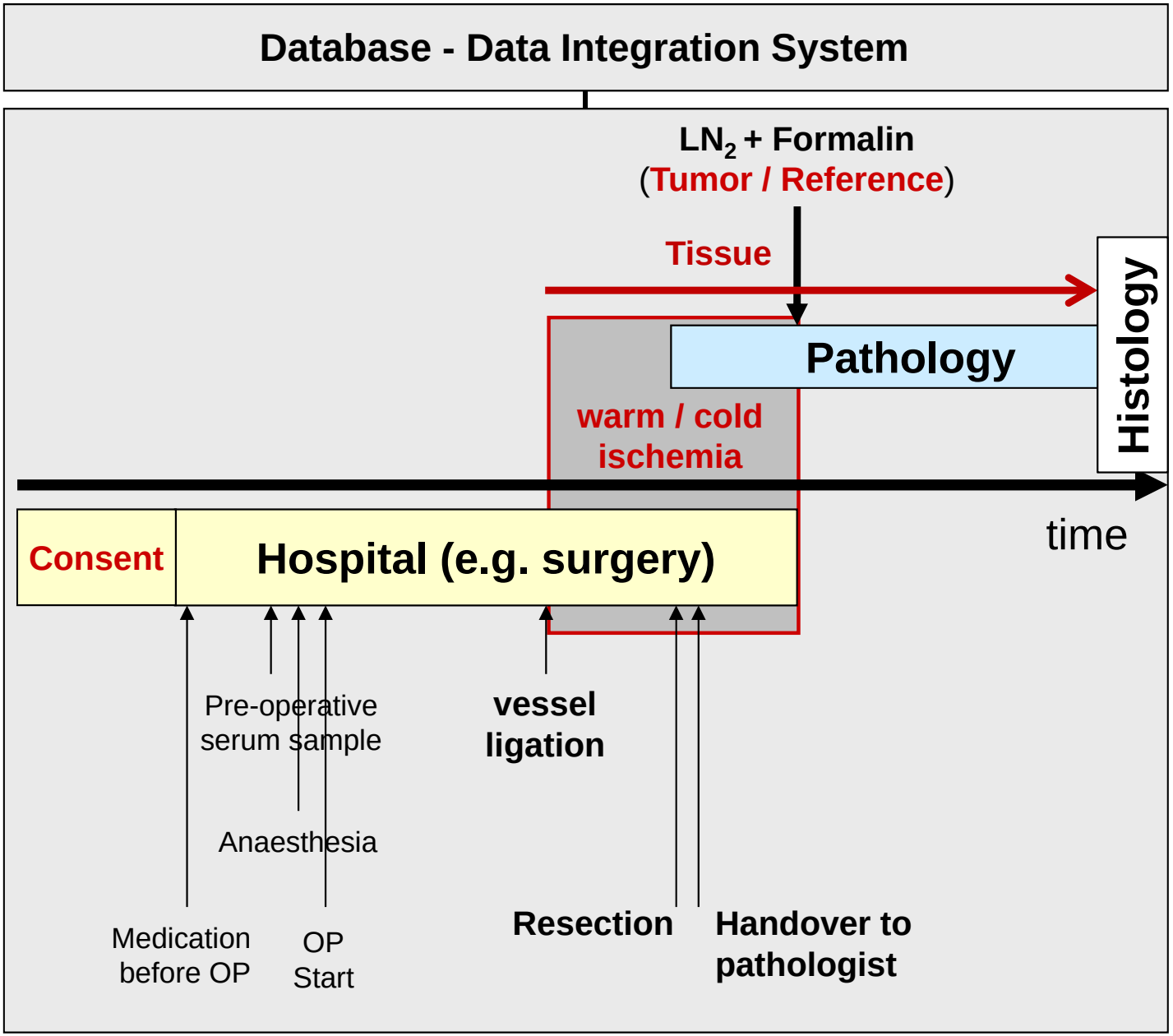
Entwicklung von Standards für die Bioprobensammlung und *in vitro* Diagnostik



RNA analysis | **Protein analysis** | **Metabolome analysis**

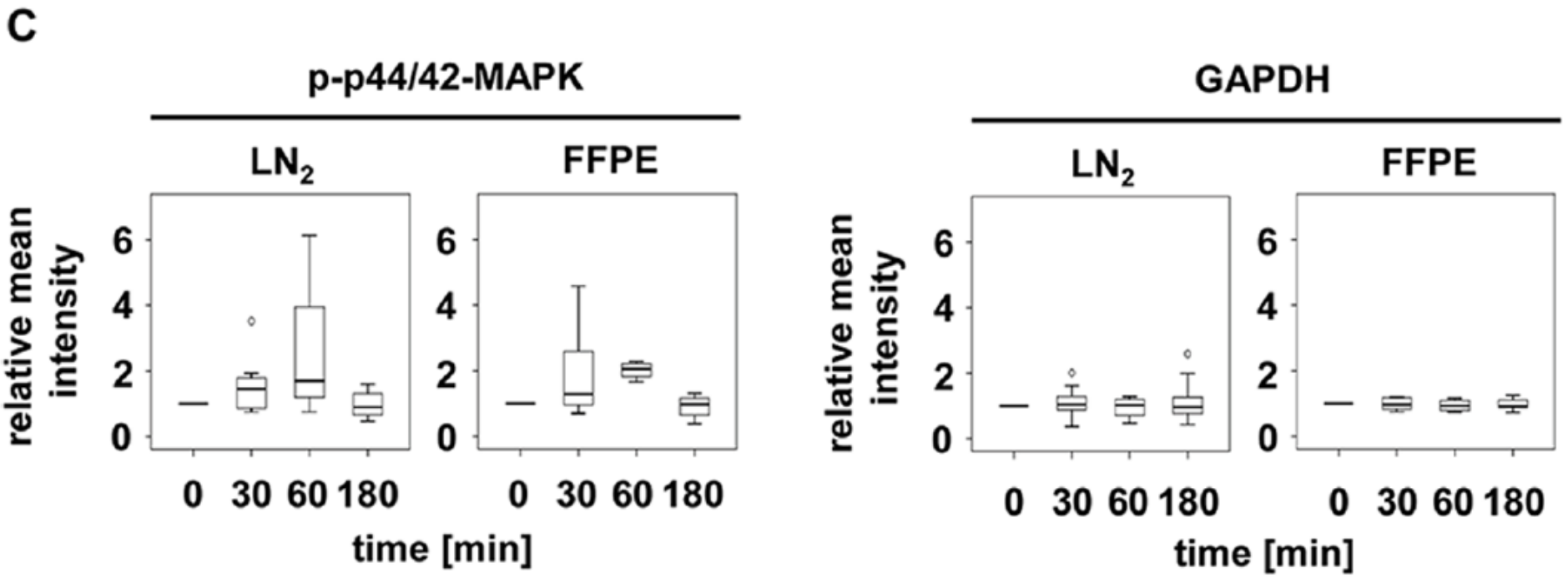
European Committee for Standardization (CEN)

Collection of tumor tissue samples exposed to different ischemic conditions



1411 samples from 128 patients

Impact of cold ischemia duration on protein levels



Gündisch et al. 2012, J Proteome Res
Gündisch et al. 2013, J Proteome Res

Example of a European Standard for the preanalytical phase

TECHNICAL SPECIFICATION
SPÉCIFICATION TECHNIQUE
TECHNISCHE SPEZIFIKATION

FINAL DRAFT
FprCEN/TS 16827-1

March 2015

ICS 11.100.10

English Version

Molecular in vitro diagnostic examinations - Specifications for pre-examination processes for FFPE tissue - Part 1: Isolated RNA

Tests de diagnostic moléculaire in vitro - Spécifications relatives aux processus préanalytiques pour les tissus FFPE - Partie 1: ARN extrait

Molekularanalytische in-vitro-diagnostische Verfahren - Spezifikationen für präanalytische Prozesse für FFPE-Gewebe - Teil 1: Isolierte RNS

This draft Technical Specification is submitted to CEN members for formal vote. It has been drawn up by the Technical Committee CEN/TC 140.

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.

Recipients of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

Warning : This document is not a Technical Specification. It is distributed for review and comments. It is subject to change without notice and shall not be referred to as a Technical Specification.

EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

Ref. No. FprCEN/TS 16827-1:2015 E

Contents

6/9 Technischen Spezifikationen sind Ende 2015 von CEN publiziert worden*

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

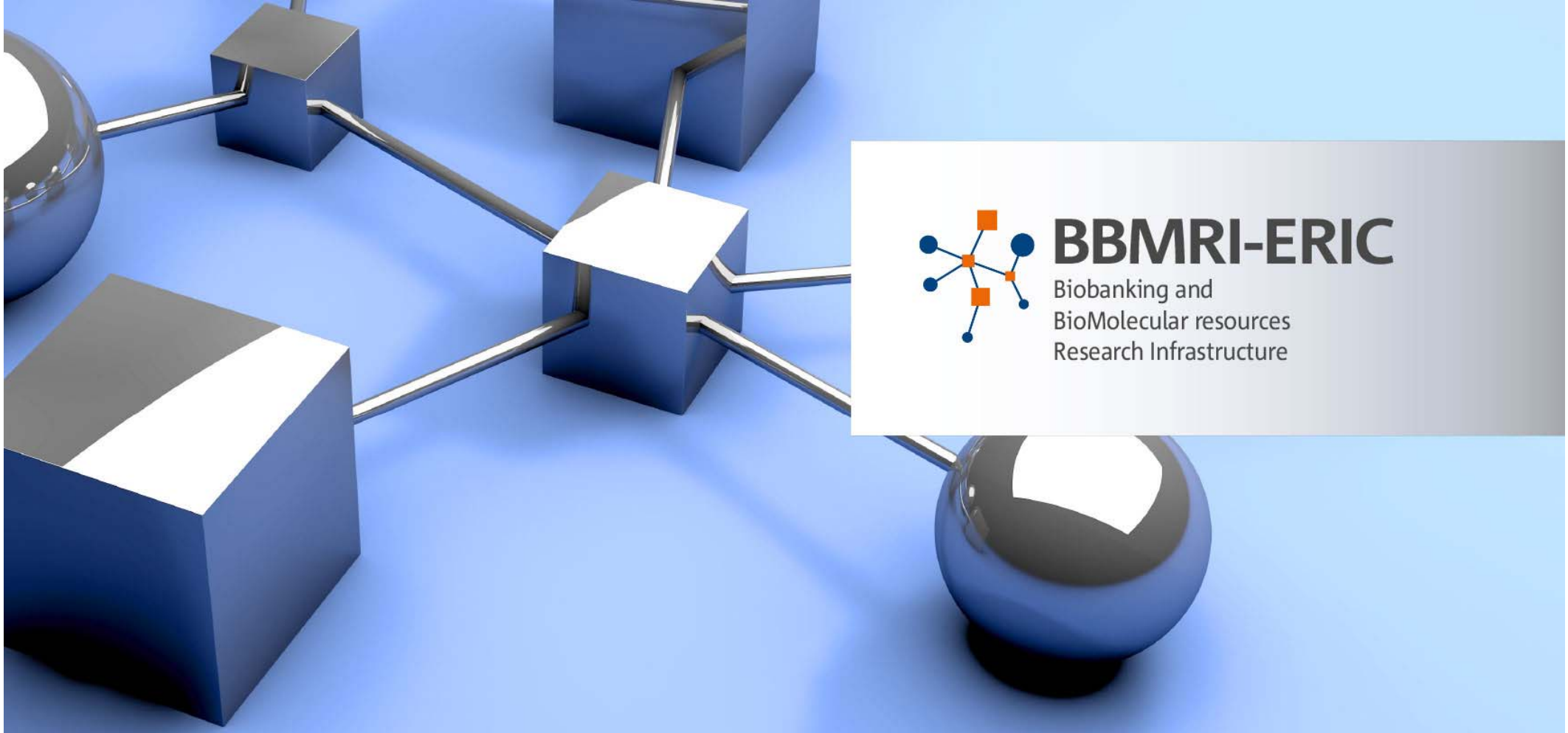
Molecular in-vitro diagnostic examinations — Specifications for pre-examination processes for snap frozen tissue
— Part 1: RNA
— Part 2: Proteins

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

Relevant für *in vitro* Diagnostiklabore und deren Kunden, Hersteller von *in vitro* Diagnostika, Molekulare Pathologie, Biobanken, Akkreditierungsorganisationen, akademische und industrielle medizinische Forschung, …

CEN, European Committee for Standardization

- Beziehbar über Beuth Verlag: www.beuth.de



BBMRI-ERIC
Biobanking and
BioMolecular resources
Research Infrastructure

slides provided to Prof. Karl-Friedrich Becker

Andrea Wutte

26 November 2016 (for presentation purpose – call for technical experts BBMRI-ERIC)

BBMRI-ERIC **Work Programme 2016 Quality**

Work Stream 2.1 CEN/TC 140 / ISO 212 Quality of the sample

- Set up Expert-WG for Evaluation of Pre-examination processes
- **Evaluation of the pre-examination processes of the 9 published CEN/TC 140 Standards by the BBMRI-ERIC experts of the field**
- Definition of Evaluation Tasks (Criteria, Documentation, Outcome and Timeline)
- Nomination of Experts for specific Evaluation task
- Evaluation process
- WG meetings (TC, Webinars, meetings) to discuss and evaluate developments
- Documentation for Self-assessment (deliver main criteria for Self-Assessment-Tool and Documentation for community use)

Der Weg zu einem ISO-Standard

- 2017: Publication of ISO Standards
- 2014: 8 new projects for ISO Standards approved in ISO/TC 212 „Clinical laboratory testing and *in vitro* diagnostic test systems”

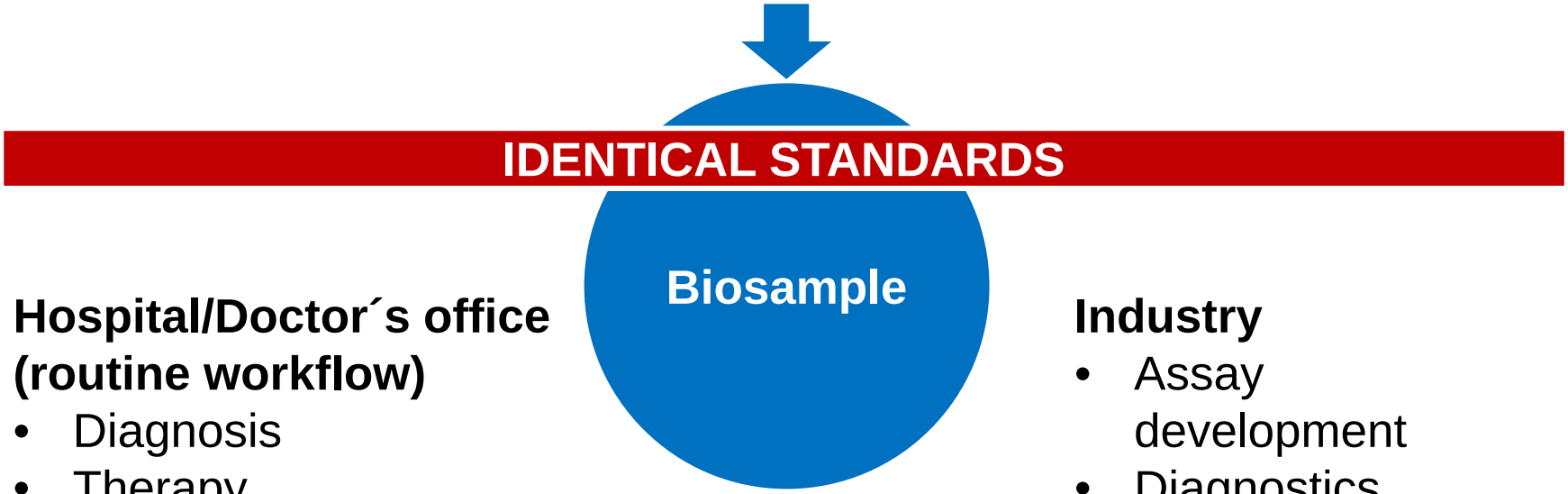
FDA **CLIA** European Society of Pathology DAkkS Deutsche Akkreditierungsstelle

- 2015: 9 CEN Technical Specifications are being published
- 2013: 9 new projects approved in CEN/TC 140 „In vitro diagnostic medical devices“
- 2010: Start of standardization work

CEN, European Committee for Standardization
ISO, International Organization for Standardization

Aim: One Standard for the same workflow

Sample donor/Patient



Hospital/Doctor´s office (routine workflow)
- Diagnosis
- Therapy

Biobanks
- Research
- Biomarkers
- Clinical data

Industry
- Assay development
- Diagnostics
- Drugs

- ISO/TC 140 (In vitro Diagnostics)
- ISO/TC 276 (Biotechnology -> Biobanks)
- FDA, CLIA, EDMA, CANCER ID, ESP, …

Zusammenfassung

- Verwertung von Forschungsergebnissen als Standard
- Prä-analytische Phase zur Probensammlung muss verbessert werden
- 6/9 CEN Standards zur Prä-Analytik wurden 2015 publiziert
- ISO Standards zur Prä-Analytik werden derzeit erstellt
- Publikation der ISO Standards geplant für 2017
- Relevant für
  - *in vitro* Diagnostik Labore und Kunden dieser Labore
  - Hersteller von Diagnostika
  - Biobanken
  - Industrielle und akademische medizinische Forschung
  - Akkreditierungsorganisationen, Zulassungsbehörden
  - …

- International gleiche Standards für die Bioprobengewinnung im Krankenhaus und Arztpraxen, für Biobanken und Industrie

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IMPACTS

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