OECI Oncology Days
Brno, 21st - 23rd June 2017
Pathology Day

SPIDIA4P: PREANALYTICAL CONDITIONS OF TISSUES

Serena Bonin
Department of Medical Sciences
Università degli Studi di Trieste
What is pre-analytics?

Pre-analytical phase: covers all steps from the clinicians requests to the beginning of the analytical examination, included nucleic acid or protein extractions

Clin Chem. 2015 Jul;61(7):914-34
Why extractions into pre-analytics?

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Why pre-analytics?

- Standardization of pre-analytical 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

Sample source determines the metabolome signature
Why pre-analytics?

- Physicians rely on accurate laboratory test results for diagnosis and guiding therapy: more than 70% of clinical decisions are based from information derived from laboratory results (MLO Med Lab Obs. 2014 May;46(5):22, 24, 26)

- $10^7$ € of funding may be lost each year in clinical trials in the EU due to pre-analytical and analytical problems (Ann Transl Med. 2016 May;4(9):181)

Clin Biochem. 2016 Dec;49(18):1313-1314
Why pre-analytics?

- Medical research irreproducibility, which slows down the translation into medical practice

Sources of variability related to clinical research irreproducibility

- Tissue and macromolecule pre-analytical preservation (pre- and fixation procedures)
- Selection and standardization of analytical procedures (standardization of procedures, controls, interpretation of results)
- Heterogeneity on morphological and molecular level

The Economist. 2013 Oct How Science goes wrong
Why pre-analytics?
Why in FFPE?

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

Original design made available by Kurt Zatloukal

Formalin fixation and paraffin embedding are part of a globally applied method of tissue preservation; however, they also represent a multistage process that is far from standardized. A recent review article published by our office identified 15 preanalytical factors associated with formalin fixation and paraffin embedding tissue processing that have documented effects on immunohistochemistry (IHC) efficacy and many more that were unaddressed or under-addressed in the scientific literature. While technological
Medication
Surgical procedure
Warm ischemia

Transport
*Temperature*
*Cold ischemia*

Sample processing
*Mech. alteration*
*Selection+annotation*

Fixation
*Fixative*
*Time*

Embedding
*Temperature*

Diagnosis
*Disease codes*

Aliquotting

Freezing
*Freezing rate*
*Temperature*

Cryostorage
*Temperature*
*Temp. shifts*

Sample preparation

Original design made available by Kurt Zatloukal
PREANALYTICAL CONDITIONS

Vacuum system

Cold fixation and defined time of fixation

can be avoided

can be reduced

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

- **blood**: cellular RNA – CEN/TS 1865-1
- **blood**: genomic DNA - CEN/TS 1865-2
- **blood**: cell free circulating DNA - CEN/TS 1865-3
- **FFPE tissue**: RNA - CEN/TS 16827-1
- **FFPE tissue**: Proteins- CEN/TS 16827-2
- **FFPE tissue**: DNA- CEN/TS 16827-3
- **snap frozen tissue**: RNA CEN/TS 16826-1
- **snap frozen tissue**: Proteins CEN/TS 16826-1
- **metabolomics** in urine, serum and plasma CEN/TS 16945

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CEN/TS- European Technical Specification

Target groups
✓ *in-vitro* diagnostic laboratories
✓ *in-vitro* diagnostics developers and manufacturers
✓ Institutions and commercial organizations performing biomedical and clinical research
✓ Biobanks
✓ Regulation authorities

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BBMRI-ERIC Self assessment Survey

[BBMRI-ERIC website](#)
Representatives of the BBMRI-ERIC Quality Expert Working Groups from 18 different countries
BBMRI-ERIC Self assessment Survey

Self-Assessment Survey

* Please type in your e-mail address

Please please provide us with some information by answering the following questions:

Is your organisation located in a BBMRI-ERIC Member/Observer state? See http://www.bbmri-eric.eu/national-nodes/
- Yes
- No

Are you in contact with the coordinating office from the National Node in your country? See http://www.bbmri-eric.eu/national-nodes/
- Yes
- No

Have you purchased the required CEN Technical Specifications as a basis for your sample handling procedure? See http://www.bbmri-eric.eu/services/standardisation/
- Yes
- No

Please select the required BBMRI-ERIC Self-Assessment Surveys from the list below:

- Specifications for Pre-examination processes for snap frozen tissue – Part 1: Isolated RNA; CEN/TS 16826-1:2015
- Specifications for Pre-examination processes for snap frozen tissue – Part 2: Isolated proteins; CEN/TS 16826-2:2015
- Specifications for Pre-examination processes for FFPE tissue – Part 3: Isolated DNA; CEN/TS 16827-3:2015

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<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
<th>Next</th>
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<tbody>
<tr>
<td>12) Donor/patient ID documented should</td>
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<tr>
<td>13) Health status of donor/patient documented should</td>
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<td>14) Medical treatment documented should</td>
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<td>15) Start of warm ischemia documented</td>
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<tr>
<td>16) Date of vessel ligation/arterial clamping time should</td>
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<tr>
<td>17) Time of vessel ligation/arterial clamping time should</td>
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<tr>
<td>Information on the primary tissue sample</td>
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<tr>
<td>18) Date of tissue removal from the body should</td>
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<tr>
<td>19) Time of tissue removal from the body should</td>
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<tr>
<td>20) Tissue type and condition documented</td>
<td></td>
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<tr>
<td>21) Organ of organ and location within should</td>
<td>Yes</td>
<td>No</td>
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<td>22) Type and start of fixation documented</td>
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<tr>
<td>23) Date of start should</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>24) Time of start should</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>25) Fixative type should</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>26) Fixative condition should</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>Information on the primary tissue sample processing</td>
<td></td>
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<tr>
<td>27) Modifications after removal from body documented</td>
<td>Yes</td>
<td>No</td>
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<td>28) Selection/use of transport containers performed should</td>
<td>Yes</td>
<td>No</td>
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<td>29) Selection/use of stabilisation procedures for transport of unfixed primary tissue performed should</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>30) Labelling of the transport container performed should</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>31) Documented, when several aliquots of a single sample with different features are in one container should</td>
<td>Yes</td>
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SPIDIA for personalised medicine: Standardisation and improvement of generic pre-analytical tools and procedures for in-vitro diagnostics

- 48-month project
- Key experts of 19 stakeholder organisations
- Aims: pre-analytical procedures, European and international standardisation organisations’ processes (CEN and ISO), external quality assurance, quality management, ethics and regulatory demands
- [www.spidia.eu](http://www.spidia.eu)

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CEN

- RNA - CEN/TS 16827-1
- Proteins- CEN/TS 16827-2
- DNA- CEN/TS 16827-3

ISO/TC 212

- ISO/DIS 20166-1, -2,-3: Molecular in vitro diagnostic examinations: Specifications for pre-examination processes for FFPE tissues (1-RNA, 2-protein, 3-DNA)

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- RNA CEN/TS 16826-1
- Proteins CEN/TS 16826-1

ISO/TC 212

- ISO/DIS 20184-1, -2,: Molecular in vitro diagnostic examinations: Specifications for pre-examination processes for frozen tissues (1-RNA, 2-protein)

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• cellular RNA – CEN/TS 1865-1
• genomic DNA - CEN/TS 1865-2
• cell free circulating DNA - CEN/TS 1865-3

ISO/TC 212

• ISO/DIS 20184-1, -2,: Molecular in vitro diagnostic examinations: Specifications for pre-examination processes for venous whole blood(1-RNA, 2-gDNA, 3-cfDNA from plasma)

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## CEN Technical Specifications for Pre-examination Processes

**Development of 12 new CEN/TS and 2 ISO standards & Raising awareness for and implementation of standards**

<table>
<thead>
<tr>
<th>CEN/TS</th>
<th>ISO</th>
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<tbody>
<tr>
<td>4 Venous whole blood circul. tumor cells — RNA, DNA, protein &amp; staining procedures</td>
<td>1 FFPE tissue – in-situ staining</td>
</tr>
<tr>
<td>1 Venous whole blood exosomes — cfc RNA</td>
<td>1 Metabolomics – urine, plasma, serum</td>
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<td>1 Frozen tissue — DNA</td>
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<tr>
<td>1 Urine/other body fluids - cfcDNA</td>
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<td>3 fine needle aspirates – RNA, DNA, protein</td>
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<tr>
<td>1 Saliva &amp; stool microbiomes– DNA</td>
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<tr>
<td>1 Saliva — DNA</td>
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13 new External Quality Assurance Schemes corresponding to the pre-analytical standards portfolio

- Venous Whole Blood: Genomic DNA and cellular RNA, viable PBMC, Cell Free Circulating DNA (ccfDNA), Cell Free Circulating RNA (ccfRNA), Circulating Tumour Cells (CTCs)
- FFPE tissue: DNA, RNA, protein
- Frozen tissue: Genomic DNA, RNA, protein
- Saliva: DNA
- Stool: DNA

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Acknowledgements:
SPIDIA4P EIPC
BBMRI-BBMRI-ERIC WG for FFPE tissue processing
And Andrea Wutte

QUESTIONS?

Thank you for your attention