SLOVENSKI STANDARD
SIST-TS CEN/TS 16827-2:2015

01-oktober-2015

Molekularne diagnostične preiskave in vitro - Specifikacije za predpreiskovalne procese za FFPE tkiva - 2. del: Izolirani proteini

Molecular in vitro diagnostic examinations - Specifications for pre-examination processes for FFPE tissue - Part 2: Isolated proteins

Molekularanalytische in-vitro-diagnostische Verfahren - Spezifikationen für präanalytische Prozesse für FFPE-Gewebe - Teil 2: Isolierte Proteine

Tests de diagnostic moléculaire in vitro - Spécifications pour les processus préanalytiques pour tissu FFPE - Partie 2: Protéines extraites

Ta slovenski standard je istoveten z: **CEN/TS 16827-2:2015**

ICS:

11.100.10	Diagnostični preskusni sistemi in vitro	In vitro diagnostic test systems

SIST-TS CEN/TS 16827-2:2015 **en,fr,de**

TECHNICAL SPECIFICATION

SPÉCIFICATION TECHNIQUE

TECHNISCHE SPEZIFIKATION

CEN/TS 16827-2

August 2015

ICS 11.100.10

English Version

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EUROPEAN COMMITTEE FOR STANDARDIZATION
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Ref. No. CEN/TS 16827-2:2015 E

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European foreword

This document (CEN/TS 16827-2:2015) has been prepared by Technical Committee CEN/TC 140 "*In vitro* diagnostic medical devices", the secretariat of which is held by DIN.

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Introduction

Molecular in vitro diagnostics has enabled a significant progress in medicine. Further progress is expected by new technologies analysing signatures of nucleic acids, proteins, and metabolites in human tissues and body fluids. However, the profiles and/or integrity of these molecules can change drastically during primary sample collection, transport, storage, and processing thus making the outcome from diagnostics or research unreliable or even impossible because the subsequent analytical assay will not determine the situation in the patient but an artificial molecular pattern generated during the pre-examination process.

Although originally thought as being impossible due to the crosslinking activities of formaldehyde, protein extraction techniques from formalin formalin fixed and paraffin embedded (FFPE) tissues have been much improved in recent years. Heat-induced reversal of formaldehyde-induced crosslinks has been demonstrated as an essential step in the protein extraction procedures [1], [2]. Currently, most investigators accept that proteins extracted from FFPE tissue are suitable for downstream proteomic analysis [3].

However, a standardization of the entire process from primary sample collection to protein analysis is needed. Studies have been undertaken to determine the important influencing factors. This Technical Specification draws upon such work to codify and standardise the steps for FFPE tissue with regard to protein analysis in what is referred to as the preanalytical phase.

1 Scope

This Technical Specification gives recommendations for the handling, documentation and processing of FFPE tissue specimens intended for the analysis of extracted proteins during the preanalytical phase before a molecular assay is performed. This Technical Specification is applicable to molecular *in vitro* diagnostic examinations (e.g., *in vitro* diagnostic laboratories, laboratory customers, developers and manufacturers of *in vitro* diagnostics, institutions and commercial organizations performing biomedical research, biobanks, and regulatory authorities).

Protein profiles and protein-protein interactions in tissues can change drastically before and after collection (due to e.g., gene induction, gene down regulation, protein degradation). Protein species amounts can change differently in tissues from different donors / patients. The expression of genes can be influenced by the given treatment or intervention (surgery, biopsy), or drugs administered for anaesthesia or even treatment of concomitant disease as well as by the different environment conditions after the tissue removal from the body.

Furthermore, the formalin fixation and paraffin embedding process leads to modifications of the protein molecules, which can impact the validity and reliability of the analytical test results.

Therefore, it is essential to take special measures to minimize the described profile changes and modifications within the tissue for subsequent protein analysis.

This document is not applicable for protein analysis by immunohistochemistry.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN ISO 15189:2012, *Medical laboratories — Requirements for quality and competence (ISO 15189:2012, Corrected version 2014-08-15)*

ISO 15190, *Medical laboratories — Requirements for safety*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN ISO 15189:2012 and the following apply.

3.1
ambient temperature
unregulated temperature of the surrounding air

3.2
analytical phase
processes that start with the isolated analyte and include all kinds of parameter testing or chemical manipulation for quantitative or qualitative analysis

3.3
cold ischemia
condition after removal of the tissue from the body until its stabilization or fixation

3.4
FFPE
formalin fixation and paraffin embedding

3.5
FFPE tissues
formalin fixed and paraffin embedded tissues

3.6
formalin
saturated formaldehyde solution containing a mas fraction of 37 % (corresponding to a volume fraction of 40 %) formaldehyde, termed 100 % formalin

3.7
formalin fixation
treatment of a sample with standard buffered formalin solution for stabilization

3.8
pre-examination processes
preanalytical phase
preanalytical workflow
processes that start, in chronological order, from the clinician's request and include the examination request, preparation and identification of the patient, surgical procedure, collection of the primary sample(s), temporary storage, transportation to and within the analytical laboratory, aliquoting, retrieval, isolation of analytes, and end when the analytical examination begins

[SOURCE: EN ISO 15189:2012, definition 3.15, modified — An additional term was added and more details were included.]

Note 1 to entry: The preanalytical phase may include preparative processes that may influence the outcome of the intended examination.

3.9
primary sample
specimen
discrete portion of a body fluid, breath, hair or tissue taken for examination, study or analysis of one or more quantities or properties assumed to apply for the whole

[SOURCE: EN ISO 15189:2012, 3.16, modified — The term and definition is used here without the original notes.]

3.10
protein
type of biological macromolecules composed of one or more chains with a defined sequence of amino acids connected through peptide bonds

3.11
protein profile
amounts of the individual protein molecules that are present in a sample and that can be measured in the absence of any losses, inhibition and interference

3.12
protein species
amounts of a chemically clearly-defined protein corresponding to one spot on a high-performance 2-dimensional gel electrophoresis pattern

[SOURCE: Jungblut *et. al.*1996]

3.13
PTM
post translational modifications
chemical alterations to a primary protein structure, often crucial for conferring biological activity on a protein

[SOURCE: Encyclopedia of Psychopharmacology, 2010]

3.14
room temperature
temperature which is defined as 18 °C to 25 °C for the purposes of this document

3.15
sample
one or more parts taken from a primary sample

[SOURCE: EN ISO 15189:2012, 3.24, modified — The example was not taken over.]

3.16
stability
ability of a sample material, when stored under specified conditions, to maintain a stated property value within specified limits for a specified period of time

[SOURCE: ISO Guide 30:1992, 2.7]

Note 1 to entry: The measured constituent for the purpose of this document is RNA.

3.17
standard buffered formalin solution
10 % formalin solution containing a mass fraction of 3,7 % (corresponding to a volume fraction of 4 %) formaldehyde buffered to pH 6,8 to pH 7,2

Note 1 to entry: Standard buffered formalin solutions often contain methanol to inhibit oxidation and polymerization of formaldehyde.

3.18
warm ischemia

warm Ischemia is the condition where the tissue is deprived of its normal blood supply containing oxygen and nutrients while the tissue is at body temperature

4 General considerations

For general statements on primary sample collection and handling (including avoidance of cross contaminations) see EN ISO 15189:2012, 5.4.4, 5.2.6. Consumables including kits shall be verified before use in examination (see EN ISO 15189:2012, 5.3.2.3); EN ISO 15189:2012, 5.5.1.2 and 5.5.1.3 can also apply.

As all steps of a diagnostic workflow can influence the final analytical performance, the entire workflow comprising the preanalytical steps, including information on biomolecule stability and storage conditions, and analytical steps should be verified and validated (see EN ISO 15189).

The stability of the specific protein(s) of interest and their posttranslational modifications (if important for the assay) should be investigated throughout the complete preanalytical workflow prior to the development and implementation of an analytical test.

Before tissues are fixed in standard buffered formalin solution, protein amounts, conformations and binding status can change e.g. by protein degradation and altered synthesis following gene induction, gene down regulation, RNA degradation, and changes of the biochemical pathway and energy status. These effects depend on the duration of warm and cold ischemia and the ambient temperature before formalin fixation. In addition, the described effects can vary in tissues from different donors / patients.

Generally, the longer the warm and cold ischemia times and the higher the ambient temperature before fixation the tissue specimen, the higher is the risk that changes in the protein profile can occur.

NOTE Prolonged cold ischemia times result in changes of protein (e.g., cytokeratin 18) and phosphoprotein (e.g., phospho-p42/44) amounts [4], [5]. Keeping the specimen on wet-ice diminishes this effect [6]. Proteins amounts as well as the protein modifications can vary, depending on the origin and type of tissue, the underlying disease, the surgical procedure, the drug regime, and drugs administered for anaesthesia or treatment of concomitant disease, and on the different environmental conditions after the tissue removal from the body.

As warm ischemia cannot be easily standardized, its time and duration should be documented. When it is not possible to avoid cold ischemia, its time of onset and duration shall be documented and the temperatures of the specimen transport container's surroundings should be documented. Where the specimen is transported to another facility for formalin fixation, the transport duration shall be documented and the ambient conditions should also be documented.

In addition, formalin fixation causes modifications of biomolecules and leads to suboptimal performance of protein extracted from FFPE tissues [7] that should be considered in quality control and application of molecular assays. Assay optimization for FFPE tissues or the use of non-crosslinking alternatives to standard buffered formalin solution is an option to minimize this issue for molecular analyses.

Safety regulations on transport and handling shall be considered (see EN ISO 15189:2012, 5.2.3 and 5.4.5 and ISO 15190).

During the whole preanalytical workflow precautions shall be taken to avoid cross contamination between different samples.

If a commercial product is not used in accordance with the manufacturers' instructions, responsibility for its use and performance lies with the user.

5 Outside the laboratory

5.1 Primary tissue collection manual

5.1.1 Information about the primary sample donor

The documentation should include, but is not limited to:

a) the primary donor / patient ID, which can be in the form of a code;

b) the health status of the primary sample donor (e.g., healthy, disease type, concomitant disease);

c) the information about routine medical treatment and special treatment prior to tissue collection (e.g., anaesthetics, medications, surgical or diagnostic procedures (e.g., biopsy device used for the collection));

d) the start of ischemia within the body (warm ischemia) by documenting the ischemia-relevant vessel ligation/clamping time point (usually arterial clamping time).

5.1.2 Information on the primary tissue sample

The documentation shall include, but is not limited to:

a) the time point when tissue is removed from the body;

b) the description of tissue type, tissue condition (e.g., diseased, unaffected by the disease) and organ tissue of origin, including references to any marking applied in the operating theatre made by surgeon, radiologist or pathologist;

c) the documentation steps described under 6.2, if the formalin fixation starts outside the laboratory.

5.1.3 Information on the primary tissue sample processing

The following steps shall be performed:

1. the documentation of any additions or modifications to the primary sample after removal from the body (e.g., labelling for the orientation of the specimen (e.g., ink-marking, stitches), incision(s));

2. the selection and use of transport containers and packages (e.g., cooling box, box for storing and transportation, vacuum packaging) fit for transport of formalin fixed tissue samples, if relevant;

3. the selection and use of stabilization procedures (e.g., cooling methods) for transport;

   NOTE 1 Accidentally freezing and thawing the tissue (e.g., by using cool packs in a wrong manner) can lead to protein degradation when the tissue thaws thereafter. It can also impact the morphological characterization.

   NOTE 2 This step can be omitted, if the specimen is transferred directly into standard buffered formalin solution (see 6.2).

4. the labelling of the transport container (e.g., registration-number, barcode (1D or 2D), primary sample type, quantity, and organ tissue of origin) and additional documentation (information as specified in 5.1.1, 5.1.2, and 5.1.3, 1. to 3.). If a single sample container contains several aliquots of the same specimen, and the aliquots represent different features (e.g., tissue type, disease status, location) this shall be documented.

Specimens should be transferred without delay into the transport container after the removal from the body. The container should then be kept on wet-ice or at 2 °C to 8 °C in order to minimize protein profile changes.

The temperatures of the transport container's surroundings during cold ischemia time (e.g., temperatures in different rooms; transport) should be documented. If the temperature cannot be measured, the temperature range should be estimated by classification as ambient temperature, room temperature, or at 2 °C to 8 °C.

5.2 Transport requirements

The laboratory in partnership with the clinical or surgery department shall establish a protocol for the transport procedure of the specimen.

If the primary tissue sample is not already placed into standard buffered formalin solution, it should be transported on wet-ice or at 2 °C to 8 °C without delay in order to minimize changes to the protein profile.

NOTE There is evidence that proteins in tissues can be stabilised in plastic bags under vacuum when kept at 0 °C to 4 °C during transport [8] before the samples are archived for biobanks or used for histopathological evaluation.

If the primary tissue sample is already placed into standard buffered formalin solution outside the laboratory, the temperature during transport should not exceed room temperature.

The compliance with the protocol for the transport procedure shall be documented. Any deviations from the protocol shall be described and documented.

6 Inside the laboratory

6.1 Information on the primary tissue sample receipt

The name of the person receiving the primary tissue sample shall be documented. The tissue sample arrival time and conditions (e.g., labelling, transport conditions including temperature, tissue type and quantity of the primary sample, leaking/breaking of the container) of the received samples shall be documented. Any deviations from the established protocol for the transport procedure (see 5.2) shall be documented.