INTERNATIONAL STANDARD

ISO 20166-2

First edition
2018-12

Molecular in vitro diagnostic examinations — Specifications for pre-examinations processes for formalin-fixed and paraffin-embedded (FFPE) tissue —

Part 2: Isolated proteins

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Analyses de diagnostic moléculaire in vitro — Spécifications relatives aux processus préanalytiques pour les tissus fixés au formol et inclus en paraffine (FFPE) —

Partie 2: Protéines extraites

ISO 20166-2:2018
https://standards.iteh.ai/catalog/standards/sist/cd3dd877-033d-420d-bd02-965e0169f75d/iso-20166-2-2018

Reference number
ISO 20166-2:2018(E)

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Published in Switzerland

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Contents

Page

iTeh STANDARD PREVIEW
(standards.iteh.ai)
ISO 20166-2:2018
https://standards.iteh.ai/catalog/standards/sist/ed3dd877-033d-420d-bd02-96960169175d/iso-20166-2-2018

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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This document was prepared by Technical Committee ISO/TC 212, *Clinical laboratory testing and in vitro diagnostic test systems*.

ISO 20166-2:2018
https://standards.iteh.ai/catalog/standards/sist/cd3dd877-033d-420d-bd02-965a0169f75d/iso-20166-2-2018

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Introduction

Molecular in vitro diagnostics, including molecular pathology, has enabled a significant progress in medicine. Further progress is expected with new technologies analyzing nucleic acids, proteins, and metabolites in human tissues and body fluids. However, the profiles and/or integrity of these molecules can change drastically during specimen collection, transport, storage, and processing, thus making the outcome from diagnostics or research unreliable or even impossible because the subsequent examination 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 isolation techniques from 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 isolation procedures[5][6]. Currently, most investigators accept that proteins isolated from FFPE tissue are suitable for downstream proteomic examination[7].

Protein profiles, protein integrities, and protein–protein interactions in tissues can change drastically before, during and after collection (due to, e.g. gene induction, gene down regulation, protein degradation). Protein species amounts can change differently in different donors'/patients' tissues. 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 environmental conditions after the tissue removal from the body.

Furthermore, the formalin-fixation and paraffin-embedding processes lead to modifications of the protein molecules, which can impact the validity and reliability of the examination test results.

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

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A standardization of the entire process from specimen collection to the protein examination is needed. Studies have been undertaken to determine the important influencing factors. This document draws upon such work to codify and standardize the steps for FFPE tissue with regard to protein examination in what is referred to as the pre-examination phase.

In this document, the following verbal forms are used:

— "shall" indicates a requirement;

— "should" indicates a recommendation;

— "may" indicates a permission;

— "can" indicates a possibility or a capability.

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Molecular in vitro diagnostic examinations — Specifications for pre-examinations processes for formalin-fixed and paraffin-embedded (FFPE) tissue —

Part 2: Isolated proteins

1 Scope

This document gives guidelines on the handling, documentation, storage and processing of formalin-fixed and paraffin-embedded (FFPE) tissue specimens intended for the examination of isolated proteins during the pre-examination phase before a molecular assay is performed.

This document is applicable to molecular in vitro diagnostic examinations including laboratory developed tests performed by medical laboratories and molecular pathology laboratories. It is also intended to be used by laboratory customers, in vitro diagnostics developers and manufacturers, biobanks, institutions and commercial organizations performing biomedical research, and regulatory authorities.

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This document is not applicable for protein examination by immunohistochemistry.

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NOTE International, national or regional regulations or requirements can also apply to specific topics covered in this document.

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2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 15189:2012, *Medical laboratories — Requirements for quality and competence*

ISO 15190, *Medical laboratories — Requirements for safety*

ISO/IEC 17020:2012, *Conformity assessment — Requirements for the operation of various types of bodies performing inspection*

3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
aliquot
portion of a larger amount of homogeneous material, assumed to be taken with negligible sampling error

Note 1 to entry: The term is usually applied to fluids. Tissues are heterogeneous and therefore cannot be aliquoted.

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Note 2 to entry: The definition is derived from References [28], [29], and [30].

3.2
ambient temperature
unregulated temperature of the surrounding air

3.3
analyte
component represented in the name of a measurable quantity

[SOURCE: ISO 17511:2003, 3.2 — EXAMPLE has been removed.]

3.4
analytical test performance
accuracy, precision, and sensitivity of a test to measure the *analyte* (3.3) of interest

Note 1 to entry: Other test performance characteristics such as robustness, repeatability can apply as well.

3.5
cold ischemia
condition after removal of the tissue from the body until stabilization or fixation

3.6
diagnosis
identification of a health or disease state from its signs and/or symptoms, where the diagnostic process can involve *examinations* (3.7) and tests for classification of an individual's condition into separate and distinct categories or subclasses that allow medical decisions about treatment and prognosis to be made

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3.7
examination
analytical test
set of operations having the object of determining the value or characteristics of a property

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Note 1 to entry: Processes that start with the isolated analyte and include all kinds of parameter testing or chemical manipulation for quantitative or qualitative examination.

[SOURCE: ISO 15189:2012, 3.7, modified — Notes to entry 1 to 3 have been removed, Note 1 to entry has been added and “analytical test” has been added as a preferred term.]

3.8
formalin
saturated aqueous formaldehyde solution which at 100 % contains 37 % formaldehyde by mass (corresponding to 40 % by volume)

3.9
formalin fixation
treatment of a sample with *standard buffered formalin solution* (3.21) for stabilization

3.10
grossing
gross examination
inspection of pathology specimens with the bare eye to obtain diagnostic information, while being processed for further microscopic examination

3.11
paraffin embedding
process in which a tissue *sample* (3.19) is placed in paraffin to achieve a hard surrounding matrix so that thin microscopic sections can be cut

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3.12
pre-examination process
pre-analytical phase
pre-analytical workflow
process that starts, in chronological order, from the clinician's request and includes the examination request, preparation and identification of the patient, collection of the primary sample(s), transportation to and within the medical or pathology laboratory, isolation of analytes, and ends when the analytical examination begins

Note 1 to entry: The pre-examination phase includes preparative processes, e.g. protein isolation procedures, which influence the outcome of the intended examination.

[SOURCE: ISO 15189:2012, 3.15, modified — "pre-analytical workflow" has been added as a preferred term, Note 1 to entry has been added and the definition has been extended.]

3.13
primary sample
specimen
discrete portion of a body fluid, breath, hair or tissue taken for examination (3.7), study or analysis of one or more quantities or properties assumed to apply for the whole

[SOURCE: ISO 15189:2012, 3.16, modified — Notes to entry 1 to 3 have been removed.]

3.14
protein
type of biological macromolecule composed of one or more chains with a defined sequence of amino acids connected through peptide bonds

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3.15
protein profile
amounts of the individual *protein* (3.14) molecules that are present in a sample and that can be measured in the absence of any losses, inhibition and interference

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3.16
protein species
amounts of a chemically clearly-defined protein corresponding to one spot on a high-performance two-dimensional gel electrophoresis pattern

Note 1 to entry: The definition is taken from Reference [7].

3.17
post-translational modification
chemical alterations to a primary protein structure, often crucial for conferring biological activity on a protein

Note 1 to entry: The definition is taken from Reference [8].

3.18
room temperature
for the purposes of this document, temperature in the range of 18 °C to 25 °C

Note 1 to entry: Local or national regulations can have different definitions.

3.19
sample
one or more parts taken from a *primary sample* (3.13)

[SOURCE: ISO 15189:2012, 3.24, modified — EXAMPLE has been removed.]

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3.20
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

Note 1 to entry: The analyte for the purpose of this document is isolated protein.

[SOURCE: ISO Guide 30:2015, 2.1.15, modified — "reference material" has been replaced by "sample material", "characteristic" has been replaced by "ability" and Note 1 to entry has been changed.]

3.21
standard buffered formalin solution
neutral buffered formalin
NBF
10 % *formalin* (3.8) solution in water with 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 small amounts of methanol to inhibit oxidation and polymerization of formaldehyde.

3.22
storage
prolonged interruption of the *pre-analytical workflow* (3.12) of a sample or analyte respectively, or of their derivatives, such as stained sections or tissue blocks, under appropriate conditions in order to preserve their properties

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Note 1 to entry: Long-term storage typically occurs in laboratory archives or in biobanks.

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3.23
tissue processor
automated instrument where tissue fixation, dehydration, clearing and paraffin infiltration occurs

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3.24
validation
confirmation, throughout the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled

Note 1 to entry: "Validated" is used to designate the corresponding status.

[SOURCE: ISO 9000:2015, 3.8.13, modified — Notes to entry 1 and 3 have been removed.]

3.25
warm ischemia
condition before the tissue is removed from the body, but where it is deprived of its normal blood supply

3.26
workflow
series of activities necessary to complete a task

3.27
homogeneous
uniform in structure and composition

4 General considerations

For general statements on medical laboratory quality management systems and in particular on specimen collection, reception, and handling (including avoidance of cross contaminations) see ISO 15189:2012, 4.2, 5.4.4, 5.4.6 or ISO/IEC 17020:2012, Clause 8 and 7.2. The requirements on laboratory equipment, reagents, and consumables in accordance with ISO 15189:2012, 5.3 shall be followed; ISO 15189:2012, 5.5.1.2 and 5.5.1.3, and ISO/IEC 17020:2012, 6.2 can also apply.

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All steps of a diagnostic workflow can influence the final analytical test result. Thus, the entire workflow including biomolecule stability and sample storage conditions shall be verified and validated. Workflow steps which cannot always be controlled (e.g. warm ischemia) shall be documented. A risk assessment of non-controllable workflow steps including their potential impact on the analytical test performance shall be performed and mitigation measures shall be established to enable the required analytical test performance.

The stability of the specific protein(s) of interest and their post-translational modifications (if important for the assay) should be investigated throughout the complete pre-examination process prior to the development and implementation of an examination test (e.g. by performing a time course experiment or study; see also Annex A and Reference [9]).

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 different donors'/patients' tissues.

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

NOTE Prolonged cold ischemia results in changes of protein (e.g. cytokeratin 18) and phosphoprotein (e.g. phospho-p42/44) amounts[9][10]. Keeping the specimen on wet-ice diminishes this effect[11]. Protein amounts as well as the protein modifications can also 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.

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As warm ischemia cannot be easily standardized, its duration shall be documented. When it is not possible to avoid cold ischemia, its duration shall be documented and the temperatures of the specimen container's surroundings shall 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.

ISO 20166-2:2018
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In addition, the formalin fixation itself, as well as the subsequent FFPE tissue storage duration and storage temperature causes modifications of biomolecules and leads to suboptimal performance of protein isolated from FFPE tissues[12]. This should be considered in the quality control and application of molecular analytical tests. Analytical test optimization for FFPE tissues or the use of non-crosslinking alternatives to standard buffered formalin solution are options to minimize this issue for molecular examinations.

Safety instructions on transport and handling shall be considered and followed in accordance with ISO 15189:2012, 5.2.3 and 5.4.5, and ISO 15190.

During the whole pre-examination process precautions shall be taken to avoid cross contamination between different specimens/samples, e.g. by using single-use material whenever feasible or appropriate cleaning procedures between processing of different specimens/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 Specimen collection

5.1.1 General

For the collection of the specimen, the requirements (e.g. disease condition, specimen size) for the intended molecular examination (see also Clause 6) should be considered.

See also ISO 15189:2012, 5.4.4.

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