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

**Part 2:
Isolated proteins**

*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

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see www.iso.org/iso/foreword.html.

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

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

A list of all parts in the ISO 20166 series can be found on the ISO website.

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.

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

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.

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.

This document is not applicable for protein examination by immunohistochemistry.

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

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.

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

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

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

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

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

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.]

**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

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

**3.23
tissue processor**

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

**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.