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**Molecular in vitro diagnostic  
examinations — Specifications for  
pre-examination processes for frozen  
tissue —**

**Part 2:  
Isolated proteins**

*Analyses de diagnostic moléculaire in vitro — Spécifications relatives  
aux processus préanalytiques pour les tissus congelés —*

*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](http://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](http://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](http://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*.

A list of all parts in the ISO 20184 series can be found on the ISO website.

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](http://www.iso.org/members.html).

## Introduction

Molecular in vitro diagnostics, including molecular pathology, has enabled a significant progress in medicine. Further progress is expected with new technologies analysing 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. Therefore, 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 frozen tissue with regard to protein examination in what is referred to as the pre-examination phase.

Protein profiles and protein–protein interactions in tissues can change drastically before, during (e.g. due to warm ischemia) and after tissue collection (e.g. due to cold ischemia). The changes are caused by 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.

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

Tissues that have undergone chemical stabilization pre-treatment before freezing are not covered in this document. In addition this document is not applicable to protein examination by immunohistochemistry.

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-examination processes for frozen tissue —

## Part 2: Isolated proteins

### 1 Scope

This document gives guidelines on the handling, documentation, storage and processing of frozen 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 any molecular in vitro diagnostic examination performed by medical laboratories and molecular pathology laboratories that evaluate proteins isolated from frozen tissue. It is also intended to be used by laboratory customers, in vitro diagnostics developers and manufacturers, biobanks, institutions and commercial organisations performing biomedical research, and regulatory authorities.

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*

### 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 homogenous 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 the Compendium of Chemical Terminology Gold Book. International Union of Pure and Applied Chemistry. Version 2.3.3., 2014; the PAC, 1990,62,1193 (Nomenclature for sampling in analytical chemistry (Recommendations 1990)) p. 1206; and the PAC 1990, 62, 2167 [Glossary of atmospheric chemistry terms (Recommendations 1990)] p. 2173.

**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]

**3.4  
analytical test performance**

accuracy, precision, and sensitivity of a test to measure the analyte 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 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 — The term and definition is used here without the original notes.]

**3.8  
grossing  
gross examination**

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

**3.9  
homogeneous**

uniform in structure and composition

**3.10  
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, collection of the primary sample(s), transportation to and within the medical or pathology laboratory, isolation of analytes, and end when the analytical examination begins

Note 1 to entry: The pre-examination phase includes preparative processes that influence the outcome of the intended examination.

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



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

**3.12****protein**

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

**3.13****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

**3.14****protein species**

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

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

**3.15****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]

**3.16****room temperature**

temperature which is defined as 18 °C to 25 °C

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

**3.17****sample**

one or more parts taken from a primary sample

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

**3.18****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 30:2015, 2.1.15, modified — The words “reference material” were replaced by “sample material”, “characteristic” has been replaced by “ability” and Note 1 to entry has been changed.]

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

### 3.19

#### **storage**

prolonged interruption of the pre-analytical workflow of a sample or analyte respectively, or of their derivatives e.g., 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.

### 3.20

#### **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: The term “validated” is used to designate the corresponding status.

[SOURCE: ISO 9000:2015, 3.8.13, modified — Note 1 and Note 3 where not taken over.]

### 3.21

#### **verification**

confirmation, through provision of objective evidence, that specified requirements have been fulfilled

Note 1 to entry: The term “verified” is used to designate the corresponding status.

[SOURCE: ISO 9000:2015, 3.8.12, modified — Note 1 and Note 2 where not taken over.]

Note 2 to entry: Confirmation can comprise activities such as:

- performing alternative calculations;
- comparing a new design specification with a similar proven design specification;
- undertaking tests and demonstrations;
- reviewing documents prior to issue.

### 3.22

#### **warm ischemia**

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

### 3.23

#### **workflow**

series of activities necessary to complete a task

## 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.

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 examination test performance shall be performed and mitigation measures shall be established to enable the required examination test performance.

The stability of the specific proteins to be examined and their posttranslational 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 [8]).