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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

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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).

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*.

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.

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]).

Before tissues are stabilized by freezing, 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 freezing. In addition, the described effects can vary in different donors'/patients' tissues.

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

NOTE Prolonged cold ischemia durations result in changes of protein (e.g. cytokeratin 18) and phosphoprotein (e.g. phospho-p42/44) amounts[8][9]. Keeping the specimen on wet-ice diminishes this effect[10]. Protein amounts as well as posttranslational modifications can also vary during the pre-examination phase, depending on the origin and type of tissue, the underlying disease, the surgical procedure, the drug regimen, 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 duration shall be documented. When it is not possible to avoid cold ischemia, its duration shall be documented and temperatures of the specimen container's surroundings shall be documented. Where the specimen is transported to another facility for freezing, the transport duration shall be documented and the ambient conditions should also be documented.

Safety regulations on transport and handling shall be followed (see 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 manufacturer's 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 intended molecular examination (see also Clause 6) should be considered.

See also ISO 15189:2012, 5.4.4.

5.1.2 Information about the specimen donor/patient

The documentation shall include the ID of the specimen donor/patient, which can be in the form of a code.

The documentation should include, but is not limited to:

a) the relevant health status of the specimen donor/patient (e.g. healthy, disease type, concomitant disease, demographics [e.g. age and gender]);

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

c) the appropriate consent from the specimen donor/patient.