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

**Part 1:
Isolated RNA**

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

Partie 1: ARN extrait

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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*.

A list of all parts in the ISO 20184 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 profile generated during the pre-examination process.

Therefore, a standardization of the entire process from specimen collection to the RNA 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 RNA examination in what is referred to as the pre-examination phase.

RNA profiles in tissues can change drastically before, during and after collection (due to e.g. gene induction or gene down regulation). RNA species can change differently in different donor's patients' tissues.

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

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-examination processes for frozen tissue —

Part 1: Isolated RNA

1 Scope

This document gives guidelines on the handling, documentation, storage and processing of frozen tissue specimens intended for RNA examination 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 RNA extracted 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.

Tissues that have undergone chemical stabilization pre-treatment before freezing are not covered in this document.

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*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 15189 and the following terms and definitions 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 References [22], [23] and [24].

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

DNA

deoxyribonucleic acid

polymer of deoxyribonucleotides occurring in a double-stranded (dsDNA) or single-stranded (ssDNA) form

[SOURCE: ISO 22174:2005, 3.1.2]

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3.8

DNase

deoxyribonuclease

enzyme that catalyzes the degradation of DNA into smaller components

3.9

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.]

3.10

grossing

gross examination

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

3.11

homogeneous

uniform in structure and composition

3.12**interfering substances**

endogenous substances of a specimen/sample or exogenous substances (e.g. stabilization solution) that can alter an examination result

3.13**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 — “pre-analytical workflow” has been added as a preferred term, Note 1 to entry has been added and the definition has been extended.]

3.14**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 — Notes to entry 1 to 3 have been removed.]

3.15**proficiency test**

evaluation of participant performance against pre-established criteria by means of inter-laboratory comparisons

[SOURCE: ISO 17043:2010, 3.7, modified — Notes to entry 1 and 2 have been removed.]

3.16**RNA profile**

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

3.17**RNA****ribonucleic acid**

polymer of ribonucleotides occurring in a double-stranded or single-stranded form

[SOURCE: ISO 22174:2005, 3.1.3]

3.18**RNase****ribonuclease**

enzyme that catalyses the degradation of RNA into smaller components

3.19**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.

3.20

sample

one or more parts taken from a primary sample

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

3.21

stability

characteristic of a sample material, when stored under specified conditions, to maintain a specified 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 RNA.

[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.]

3.22

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.

3.23

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 — Notes to entry 1 and 3 have been removed.]

3.24

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.

3.25

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

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

Before or during the design of an analytical test, it should therefore be investigated and assured that the RNA profile(s) intended to be analysed is/are not compromised in a manner impacting the analytical test performance (e.g. by performing a time course experiment or study; see also [Annex A](#)).

Before tissues are stabilized by freezing, the RNA profile(s) can change e.g. by gene induction, gene down regulation and RNA degradation. These effects depend on the warm and cold ischemia duration 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 RNA profile can occur.

NOTE Intraoperative warm ischemia can result in more pronounced changes of RNA profiles than during postoperative cold ischemia^{[2][8]}. RNA profiles can also vary, 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, duration shall be documented and temperatures of the specimen container's surroundings should 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 instructions on transport and handling shall be considered (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 the intended molecular examination (see also [Clause 6](#)) should be considered.

See also ISO 15189:2012, 5.4.4.