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

Part 1: Isolated RNA

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

Partie 1: ARN extrait

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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.

The committee responsible for this document is ISO/TC 212, *Clinical laboratory testing and in vitro diagnostic test systems*.

A list of all parts in the ISO 20166 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 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 formalin-fixed and paraffin-embedded (FFPE) tissue with regard to RNA examination in what is referred to as the pre-examination phase.

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

RNA profiles in tissues can change drastically before, during and after collection and change differently in different donors'/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.

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

Part 1: Isolated RNA

1 Scope

This document gives guidelines on the handling, documentation, storage and processing of formalin-fixed and paraffin-embedded (FFPE) tissue specimens intended for RNA examination 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.

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, modified — 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
cDNA
complementary DNA
single-stranded DNA that is complementary to a given RNA synthesized in the presence of reverse transcriptase to serve as a template for synthesis of DNA copies

[SOURCE: ISO 17822-1:2014, 3.12]

3.7
diagnosis
identification of a health or disease state from its signs and/or symptoms, where the diagnostic process can involve *examinations* (3.10) 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

3.8
DNA
deoxyribonucleic acid
polymer of deoxyribonucleotides occurring in a double-stranded (dsDNA) or single-stranded (ssDNA) form

[SOURCE: ISO 22174:2005, 3.1.2]

3.9
DNase
deoxyribonuclease
enzyme that catalyzes the degradation of *DNA* (3.8) into smaller components

3.10
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.]

3.11
formalin
saturated aqueous formaldehyde solution which at 100 % contains 37 % formaldehyde by mass (corresponding to 40 % by volume)

3.12
formalin fixation
treatment of a sample with *standard buffered formalin solution* (3.25) for stabilization

3.13
grossing
gross examination
inspection of pathology specimens with the bare eye to obtain diagnostic information, while being processed for further microscopic examination

3.14
interfering substances
endogenous substances of a *specimen* (3.17)/*sample* (3.23) or exogenous substances (e.g. stabilization solution) that can alter an examination result

3.15
paraffin embedding
process in which a tissue sample is placed in paraffin to generate a hard surrounding matrix so that thin microscopic sections can be cut

3.16
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 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.]

3.17
primary sample
specimen
discrete portion of a body fluid, breath, hair or tissue taken for *examination* (3.10), 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.]

3.18
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.]

3.19
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 or interference

3.20
RNA
ribonucleic acid
polymer of ribonucleotides occurring in a double-stranded or single-stranded form

[SOURCE: ISO 22174:2005, 3.1.3]

3.21
RNase
ribonuclease
enzyme that catalyzes the degradation of RNA into smaller components

3.22
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.

3.23
sample
one or more parts taken from a *primary sample* (3.17)

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

3.24
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 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.]

3.25
standard buffered formalin solution
neutral buffered formalin
NBF
10 % *formalin* (3.11) 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.

3.26
storage
prolonged interruption of the pre-analytical workflow of a sample or analyte, 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.

3.27
tissue processor
automated instrument where tissue fixation, dehydration, clearing and paraffin infiltration occurs

3.28
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.]

3.29
warm ischemia
condition before the tissue is removed from the body, but deprived of its normal blood supply

3.30
workflow
series of activities necessary to complete a task

3.31
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.

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 analyzed is/are not compromized 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 fixed in standard buffered formalin solution, the RNA profile(s) can change significantly depending on the warm and cold ischemia duration and the temperature before formalin fixation (e.g. gene induction, gene down regulation, RNA degradation). In addition, those changes 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 RNA profile can occur.

NOTE Intraoperative warm ischemia can result in more pronounced changes of RNA profiles than in postoperative cold ischemia[7][8]. RNA profiles can also vary, depending on the origin and type of tissue, the underlying disease, the surgical procedure, 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 (e.g. transport to the laboratory before formalin fixation), 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.

In addition, the formalin fixation itself as well as the subsequent FFPE tissue storage duration and storage temperature cause modifications of biomolecules and leads to suboptimal analytical test performance of RNA extracted from FFPE tissues (see A.2.1 and A.2.2). This should be considered in the quality control and application of molecular analytical tests, especially in the context of gene expression studies [9][10][11] [12]. These effects can limit the size of amplifiable target cDNA and/or influence the cDNA target sequence of primers used for amplification. 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[13][14].

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.

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.

5.1.3 Information about the specimen

The documentation shall include, but is not limited to:

a) the start of ischemia within the body (warm ischemia) by documentation of the ischemia-relevant vessel ligation/clamping time point (usually arterial clamping time);

b) the time and date when tissue is removed from the body and the method of removal (e.g. core-needle biopsy, resection, biopsy device used for the collection);

c) the description of tissue type and origin, tissue condition (e.g. diseased, unaffected by the disease), including references to any marking applied in or outside the operating theatre made by surgeon, radiologist or pathologist;

d) the documentation steps described under 6.2, if the formalin fixation starts outside the laboratory, and also the documentation steps described under 6.3, if the evaluation of the pathology of the specimen and selection of the sample(s) is also done outside the laboratory.

The documentation should also include the ID of the responsible person for collecting the specimen.

5.1.4 Specimen processing

The following steps shall be performed:

a) the documentation of any addition or modification to the specimen after removal from the body (e.g. labelling for the orientation of the specimen [e.g. ink-marking, stitches, incision(s)]);