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

**Part 3:
Isolated DNA**

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

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Partie 3: ADN 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).

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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 ISO/TC 212, *Clinical laboratory testing and in vitro diagnostic test systems*.

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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 molecular pattern generated during the pre-examination process. Studies have been undertaken to determine the influencing factors for the DNA examination from formalin-fixed and paraffin-embedded (FFPE) tissue. These studies demonstrated that a standardization of the entire process from specimen collection to the DNA examination is needed. This document draws upon such work to codify and standardize the steps for FFPE tissue with regard to DNA examination in what is referred to as the pre-examination phase.

DNA integrity in tissues can change before, during and after formalin fixation, processing and storage. Chemical modifications introduced into DNA during tissue fixation might lead to fragmentation and sequence alterations, changes in the methylation status or even structural changes which can lead to, for instance, spurious copy number changes in array-CGH profiles. These modifications of the DNA molecules can impact the validity and reliability of the examination test results. Therefore, it is essential to take special measures to minimize the described DNA changes and modifications 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 formalin- fixed and paraffin-embedded (FFPE) tissue —

Part 3: Isolated DNA

1 Scope

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

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2 Normative references

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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*

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 [25], [26], and [27].

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, specificity 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* (3.8) 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

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.]

3.9

formalin

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

3.10

formalin fixation

treatment of a *sample* (3.18) with *standard buffered formalin solution* (3.20) for stabilization

3.11

grossing

gross examination

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

3.12**interfering substances**

endogenous substances of a *specimen* (3.15)/*sample* (3.18) or exogenous substances (e.g. stabilization solution) that can alter an examination result

3.13**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

3.14**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)* (3.15), transportation to and within the medical or pathology laboratory, isolation of *analytes* (3.3), 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.]

3.15**primary sample specimen**

discrete portion of a body fluid, breath, hair or tissue taken for *examination* (3.8), 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.]

3.16**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.]

3.17**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.

3.18**sample**

one or more parts taken from a *primary sample* (3.15)

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

3.19**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 DNA.

[SOURCE: ISO Guide 30:2015, 2.1.15, modified — “reference material” has been replaced by “sample material” and Note 1 to entry has been changed.]

3.20

standard buffered formalin solution

neutral buffered formalin

NBF

10 % *formalin* (3.9) 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 polymerisation of formaldehyde.

3.21

storage

prolonged interruption of the *pre-analytical workflow* (3.14) of a *sample* (3.18) or *analyte* (3.3) 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.

3.22

tissue processor

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

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: "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

warm ischemia

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

3.25

workflow

series of activities necessary to complete a task

3.26

homogeneous

uniform in structure and composition

4 General considerations

For general statements on medical laboratory quality management systems and in particular on specimen collection 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 for 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.

In contrast to RNA or proteins, DNA in tissue is relatively stable during warm and cold ischemia. Changes of DNA sequence or copy numbers [e.g. comparative genomic hybridization (CGH) profiles] due

to longer warm and cold ischemia durations are unknown[7]. However, DNA methylation patterns may change in response to ischemia[6]. The duration until the specimen is placed into standard buffered formalin solution should be kept as short as possible in order to avoid enzymatic degradation of DNA. The duration before fixation shall be documented and the temperature before fixation should be documented[5].

During the fixation, processing and storage, the DNA integrity can change depending on the kind of fixative, fixation time and temperature, storage or archiving of the fixed paraffin-embedded tissue as well as the method used for DNA isolation and purification. When using a fixative based on formaldehyde, temperature and fixation duration have a significant impact on DNA integrity. The longer the fixation duration and the higher the temperature, the more chemical modifications and crosslinks are introduced, which can lead to degradation or sequence alterations[9][10][11][12][13][14]. These effects can limit the size of amplifiable target DNA and/or influence the target sequence of primers used for amplification.

Safety instructions on specimen 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 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:

- the relevant health status of the specimen donor/patient [e.g. healthy, disease type, concomitant disease, demographics (e.g. age and gender)];
- the information about routine medical treatment and special treatment prior to tissue collection (e.g. anaesthetics, medications, surgical or diagnostic procedures);
- the appropriate consent from the specimen donor/patient.

5.1.3 Information about the specimen

The documentation shall include, but is not limited to:

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