
Molekularne diagnostične preiskave in vitro - Specifikacije za predpreiskovalne procese za tkiva, ki so fiksirana v formalinu ter položena v parafin - 4. del: Tehnike detekcije in situ (ISO/DIS 20166-4:2020)

Molecular in vitro diagnostic examinations - Specifications for preexamination processes for formalin-fixed and paraffin-embedded (FFPE) tissue - Part 4: In situ detection techniques (ISO/DIS 20166-4:2020)

Molekularanalytische in-vitro-diagnostische Verfahren - Spezifikationen für präanalytische Prozesse für formalinfixierte und paraffineingebettete (FFPE-) Gewebeproben - Teil 4: In-situ-Detektionstechniken (ISO/DIS 20166-4:2020)

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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) - Partie 4: Titre manque (ISO/DIS 20166-4:2020)

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Part 4: In situ detection techniques

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Contents

	Page
Foreword.....	iv
Introduction.....	v
1 Scope.....	1
2 Normative references.....	1
3 Terms and definitions.....	1
4 General considerations.....	8
5 Outside the laboratory.....	10
5.1 Specimen collection.....	10
5.1.1 General.....	10
5.1.2 Information about the patient/specimen donor.....	10
5.1.3 Information about the specimen.....	10
5.1.4 Specimen processing, intermediate storage and preparation for transport.....	11
5.2 Transport.....	12
6 Inside the laboratory.....	12
6.1 Specimen reception.....	12
6.2 Formalin fixation of the specimen or sample(s).....	12
6.3 Evaluation of the pathology of the specimen and selection of the sample(s).....	13
6.3.1 Macroscopic evaluation.....	14
6.3.2 Selection of the sample(s).....	14
6.4 Post-fixation of frozen samples from intraoperative consultation.....	15
6.5 Sample decalcification/softening.....	15
6.6 Tissue processing and paraffin embedding.....	16
6.6.1 General.....	16
6.6.2 Dehydration and clearing.....	16
6.6.3 Impregnation with paraffin and paraffin embedding.....	17
6.7 Storage of FFPE tissue blocks.....	17
6.8 Sectioning of FFPE tissue blocks and storage of slide-mounted sections.....	18
6.9 Deparaffinization and rehydration of slide-mounted sections.....	19
6.10 Pretreatment of slide-mounted sections for <i>in situ</i> detection techniques.....	19
6.10.1 General.....	19
6.10.2 Pretreatment for antibody- or other affinity binder-based <i>in situ</i> detection techniques.....	19
6.10.3 Pretreatment for hybridization-based <i>in situ</i> detection techniques.....	20
6.10.4 Pretreatment for other <i>in situ</i> detection techniques.....	20
6.11 Quality assessment of the pre-analytical part of <i>in situ</i> detection.....	20
Annex A (informative) Recommendations relating to verification and validation of laboratory developed <i>in situ</i> detection tests.....	21
Annex B (informative) Example protocol for tissue processing *.....	22
Bibliography.....	23

ISO/DIS 20166-4:2020(E)

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).

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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 Committee 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 by new technologies analyzing tissue morphology and biomolecules, such as (e.g. proteins, DNA, RNA and/or metabolites (e.g. glucose) in human tissues and body fluids.

In pathology, the majority of diagnoses are based on *in situ* staining of formalin-fixed and paraffin-embedded (FFPE) tissue sections. In the context of personalized medicine classical histological staining (e.g., hematoxylin and eosin) for morphological evaluation is increasingly complemented by additional *in situ* detection techniques, such as immunohistochemistry or *in situ* hybridization, as well as molecular analysis of isolated biomolecules. For example, many regulatory bodies approved companion diagnostics in oncology are based on *in situ* detection techniques applied on FFPE tissue sections. Developments in personalized medicine and new technologies, such as multi-label immunostaining and computer-based analysis of digital images pose new requirements on standardization of pre-analytical procedures to obtain reproducible qualitative and quantitative results.

Profiles and/or integrity of biomolecules and their *in situ* localization, amount and accessibility for *in situ* detection in tissues can change drastically during the pre-examination process comprising specimen collection, tissue processing, embedding, transport, storage, sectioning and pretreatment for *in situ* detection. This makes the outcome from *in situ* detection in diagnostics or research unreliable or even impossible because the subsequent examination will not represent the *in vivo* state of molecules, but an artificial profile or morphology generated during the pre-examination process.

Therefore, a standardization of the entire pre-examination process of FFPE tissue specimens intended for *in situ* examinations of morphology and biomolecules on FFPE tissue sections by using different *in situ* detection techniques, is needed.

There is multiple scientific evidence that several factors of the pre-examination phase influence the outcome (e.g. quality or quantity in terms of specificity or sensitivity) of *in situ* detection and, thus, can have major impact on the diagnostic results.

This document draws upon such work to organize and standardize the steps for formalin-fixed and paraffin-embedded (FFPE) tissue with regard to various *in situ* detection techniques in what is referred to as the pre-examination phase. This standard is for the pre-examination phase of *in situ* detection techniques and is applicable to the whole spectrum of *in situ* detection techniques.

These include but are not limited to:

- Classical histological staining, e.g. Hematoxylin & Eosin staining (H&E)
- Histochemical techniques, e.g. Lipid staining, Periodic Acid Schiff (PAS) reaction, Perls' Prussian Blue reaction, Feulgen's reaction, enzyme histochemistry
- Immunohistochemical staining (IHC) or immunofluorescence staining using antibodies (polyclonal, monoclonal or recombinant antibodies) or other affinity binders
- Hybridization-based techniques such as RNA or DNA *in situ* hybridization (ISH) techniques, e.g. fluorescence *in situ* hybridization (FISH), chromogenic *in situ* hybridization (CISH), or silver enhanced *in situ* hybridization (SISH)
- Molecular analysis of isolated biomolecules that can be mapped to a defined region of an FFPE section (by e.g. *in situ* sequencing, imaging mass spectrometry).

In this document, the following verbal forms are used:

- "shall" indicates a requirement;
- "should" indicates a recommendation;

ISO/DIS 20166-4:2020(E)

- "may" indicates a permission;
- "can" indicates a possibility or a capability.

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

Part 4: In situ detection techniques

1 Scope

This document gives requirements and recommendations for the collection, handling, documentation, transport, storage and processing during the pre-examination phase of formalin-fixed and paraffin-embedded (FFPE) tissue specimens intended for qualitative and/or (semi-)quantitative *in situ* examination of the morphology and of biomolecules, such as metabolites, proteins, DNA and/or RNA, on FFPE tissue sections by using different *in situ* detection techniques.

This document is applicable to *in vitro* diagnostic examinations using *in situ* detection techniques. These include laboratory developed tests performed by pathology laboratories (histopathology laboratories) as well as by molecular pathology laboratories and other medical laboratories. It is also intended to be used by laboratory customers, *in vitro* diagnostics developers and manufacturers, biobanks, as well as institutions and commercial organizations performing biomedical research, and regulatory authorities.

This document is not applicable to the pre-examination phase of RNA, proteins and DNA isolated from FFPE tissue for examination. These are covered in ISO 20166-1, -2 and -3, *Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for isolated RNA, proteins and DNA*, respectively.

Different dedicated measures are taken for pre-examination processes for fine needle aspirates (FNAs). These are covered in CEN WI 00140128, CEN WI 00140126, and CEN WI 00140129, *Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for Fine Needle Aspirates (FNAs) isolated cellular RNA, isolated proteins, and isolated genomic DNA*, respectively.

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, *Medical laboratories — Requirements for quality and competence*

ISO 15190, *Medical laboratories — Requirements for safety*

ISO/IEC 17020, *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/DIS 20166-4:2020(E)

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
affinity binder**
molecules including affibodies, peptides, *antibody* (3.3) fragments or other small molecules that interact with *biomolecules* (3.6) and structures in a cell, and can be used in *in situ detection* (3.27) techniques

**3.2
ambient temperature**
unregulated temperature of the surrounding air

[SOURCE: ISO 20166-1:2018, 3.2]

**3.3
antibody**
protein (3.37) (immunoglobulin) produced and secreted by B lymphocytes in response to a molecule recognized as foreign (*antigen* (3.4)) and which is capable of binding to that specific *antigen* (3.4)

[SOURCE: ISO 16577:2016, 3.10, modified — “Note 1 to entry” has been deleted.]

**3.4
antigen**
substance that stimulates the production of *antibodies* (3.3) and reacts with them

[SOURCE: ISO 15089:2000, 3.5]

**3.5
antigen retrieval
epitope retrieval**
procedure(s) to unmask *antigens* (3.4)/*epitopes* (3.14) and restore their binding properties for *antibodies* (3.3) used in *immunohistochemistry* (3.25) by neutralizing the modifications introduced by *formalin fixation* (3.19), *tissue processing* (3.47) and *paraffin embedding* (3.33) of tissue

**3.6
biomolecule**
organic molecule produced in living organisms that is involved in the maintenance and metabolic processes of organisms

Note 1 to entry: The examples of organic molecule are protein (3.37), carbohydrate, lipid, or nucleic acid.

**3.7
clearing**
process step in *tissue processing* (3.47) in which formalin-fixed tissue is transferred from *dehydration* (3.10) reagent to clearing agent (e.g. xylene) to prepare the tissue for *impregnation* (3.26)

**3.8
cold ischemia**
condition after removal of the tissue from the body until its stabilization or fixation

[SOURCE: ISO 20166-1:2018, 3.5]

**3.9
decalcification**
technique using chemical agents for removal of mineral (inorganic calcium) from bone or other calcified tissue to adjust the hard tissue components to the softness of *paraffin* (3.32) for sectioning

3.10**dehydration**

process step in *tissue processing* (3.47) for removal of water from formalin-fixed tissue by immersing the tissue in a series of dehydrating reagent solutions of increasing concentration finishing with water free (100%) solution

3.11**deviation**

departure from an approved instruction, procedure and/or method or established standard

[SOURCE: ISO 15378:2017(en), 3.7.5 modified — The words “[approved \(3.7.1\)](#) [standard operating procedure \(SOP\) \(3.7.10\)](#)” were replaced by “instruction, procedure and/or method”]

3.12**diagnosis**

identification of a health or disease state from its signs and/or symptoms, where the diagnostic process can involve *examinations* (3.15) 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

[SOURCE: ISO 20166-1:2018, 3.7]

3.13**DNA****deoxyribonucleic acid**

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

[SOURCE: ISO 22174:2005, 3.1.2]

3.14**epitope**

antibody (3.3) [binding site on a biomolecule \(3.6\)](#) that is an *antigen* (3.4)

3.15**examination****analytical test**

set of operations with the objective of determining the value or characteristics of a property

Note 1 to entry: Processes that start with the *in situ detection* (3.27) using *antibodies* (3.3), nucleic acid probes or dyes 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.]

3.16**examination manufacturer****analytical test manufacturer**

entity that manufactures and/or produces the specific *analytical test* (3.15)

3.17**FFPE tissue****formalin-fixed, paraffin-embedded tissue**

tissue *specimens* (3.36)/*samples* (3.42) having undergone fixation in *formalin* (3.18, 3.19), *tissue processing* (3.47), and *paraffin embedding* (3.33) in a tissue cassette

3.18**formalin**

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

[SOURCE: ISO 20166-1:2018, 3.11]

ISO/DIS 20166-4:2020(E)

3.19

formalin fixation

treatment of a *sample* (3.42) with *standard buffered formalin solution* (3.45) for stabilization

[SOURCE: ISO 20166-1:2018, 3.12]

3.20

formalin pigment**acid formalin haematin pigment acid hematin**

black to brown amorphous to microcrystalline granules representing an artefact in histologic sections prepared from tissues fixed in *formalin* (3.18, 3.19) having an increased formic acid concentration, which is produced by acid acting upon haemoglobin

3.21

grossing**gross examination**

inspection of pathology specimens with the bare eye to obtain diagnostic information, while being processed for further microscopic *examination* (3.15)

[SOURCE: ISO 20166-1:2018, 3.13]

3.22

histochemical technique(s)

in situ detection (3.27) technique(s) for the visualization and characterization of *biomolecules* (3.6) that involves chemical reactions with specific groups, radicals or chemical bonds in *biomolecules* (3.6) and provides information on the *biomolecules'* (3.6) *in situ* localization in *tissue sections* (3.49)

Note 1 to entry: The examples of biomolecules (3.6) are carbohydrates, lipids, other metabolites, proteins (3.37), amino acids, nucleic acids, pigments, or enzymes etc.

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3.23

histological staining

in situ detection (3.27) technique undertaken to prepare *tissue sections* (3.49) by using histological stains (e.g. Haematoxylin-Eosin (HE), Chromotrop-Anilinblue (CAB)) to highlight features of the *tissue section* (3.49) and enhance *tissue section* (3.49) contrast

3.24

homogeneous

uniform in structure and composition

[SOURCE: ISO 20166-1:2018, 3.31]

3.25

immunohistochemistry**IHC**

in situ detection (3.27) technique that uses the principle of *antibodies* (3.3) binding specifically to *antigens* (3.4) / *epitopes* (3.14) in biological tissues or cells to visualize *antigens* (3.4) (e.g. *proteins* (3.37)) using brightfield microscopy

3.26

impregnation**impregnation with paraffin**

process step in *tissue processing* (3.47) for replacement of clearing agent and infiltration of tissue with molten *paraffin* (3.32)