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Molekularne diagnostične preiskave in vitro - Specifikacije za predpreiskovalne procese za FFPE tkiva - 3. del: Izolirani DNK

Molecular in vitro diagnostic examinations - Specifications for pre-examination processes for FFPE tissue - Part 3: Isolated DNA

Molekularanalytische in-vitro-diagnostische Verfahren - Spezifikationen für präanalytische Prozesse für FFPE-Gewebe - Teil 3: Isolierte DNS

Tests de diagnostic moléculaire in vitro - Spécifications relatives aux processus préanalytiques pour les tissus FFPE - Partie 3: ADN

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Tests de diagnostic moléculaire in vitro - Spécifications relatives aux processus préanalytiques pour les tissus FFPE - Partie 3: ADN isolé

Molekularanalytische in-vitro-diagnostische Verfahren - Spezifikationen für präanalytische Prozesse für FFPE-Gewebeproben - Teil 3: Isolierte DNS

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European foreword

This document (CEN/TS 16827-3:2015) has been prepared by Technical Committee CEN/TC 140 "*In vitro* diagnostic medical devices", the secretariat of which is held by DIN.

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Introduction

Molecular *in vitro* diagnostics has enabled a significant progress in medicine. Further progress is expected by new technologies analysing signatures of nucleic acids, proteins, and metabolites in human tissues and body fluids. However, the profiles and/or integrity of these molecules can change drastically during primary sample collection, transport, storage and processing thus making the outcome from diagnostics or research unreliable or even impossible because the subsequent analytical 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 DNA analysis from formalin fixed and paraffin embedded (FFPE) tissue. These studies demonstrated that a standardization of the entire process from primary sample collection to DNA analysis is needed. This Technical Specification draws upon such work to codify and standardize the steps for FFPE tissue with regard to DNA analysis in what is referred to as the preanalytical phase.

1 Scope

This Technical Specification gives recommendations for the handling, documentation and processing of FFPE tissue specimens intended for DNA analysis during the preanalytical phase before a molecular assay is performed. This Technical Specification is applicable to molecular *in vitro* diagnostic examinations (e.g., *in vitro* diagnostic laboratories, laboratory customers, developers and manufacturers of *in vitro* diagnostics, institutions and commercial organizations performing biomedical research, biobanks, and regulatory authorities).

DNA integrity in tissues can change before and during formalin fixation, processing and storage. Chemical modifications introduced into DNA during tissue fixation might lead to fragmentation and sequence alterations [1], changes in the methylation status or even structural changes which can lead to e.g., spurious copy number changes in array-CGH profiles [2]. These modifications of the DNA molecules can impact the validity and reliability of the analytical test results. Therefore, it is essential to take special measures to minimize the described modifications for subsequent DNA analysis.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN ISO 15189:2012, *Medical laboratories — Requirements for quality and competence (ISO 15189:2012, Corrected version 2014-08-15)*

ISO 15190, *Medical laboratories — Requirements for safety*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN ISO 15189:2012 and the following apply.

3.1
ambient temperature
unregulated temperature of the surrounding air

3.2
analytical phase
processes that start with the isolated analyte and include all kinds of parameter testing or chemical manipulation for quantitative or qualitative analysis

3.3
cold ischemia
condition after removal of the tissue from the body until its stabilization or fixation

3.4
DNA
deoxyribonucleic acid
polymer of deoxyribonucleotides occurring in a double-stranded (dsDNA) or single-stranded (ssDNA) form

[SOURCE: EN ISO 22174:2005, 3.1.2]

3.5
FFPE
formalin fixation and paraffin embedding

3.6
FFPE tissues
formalin fixed and paraffin embedded tissues

3.7
formalin
saturated formaldehyde solution containing a mas fraction of 37 % (corresponding to a volume fraction of 40 %) formaldehyde, termed 100 % formalin

3.8
formalin fixation
treatment of a sample with standard buffered formalin solution for stabilization

3.9
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, surgical procedure, collection of the primary sample(s), temporary storage, transportation to and within the analytical laboratory, aliquoting, retrieval, isolation of analytes, and end when the analytical examination begins

[SOURCE: EN ISO 15189:2012, definition 3.15, modified — An additional term was added and more details were included.]

Note 1 to entry: The preanalytical phase may include preparative processes that may influence the outcome of the intended examination.

3.10
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: EN ISO 15189:2012, 3.16, modified — The term and definition is used here without the original notes.]

3.11
room temperature
temperature which is defined as 18 °C to 25 °C for the purposes of this document

3.12
sample
one or more parts taken from a primary sample

[SOURCE: EN ISO 15189:2012, 3.24, modified — The example was not taken over.]

3.13
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 30:1992, 2.7]

Note 1 to entry: The measured constituent for the purpose of this document is DNA.

3.14
standard buffered formalin solution
10 % formalin solution containing 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 methanol to inhibit oxidation and polymerization of formaldehyde.

3.15
warm ischemia
warm Ischemia is the condition where the tissue is deprived of its normal blood supply containing oxygen and nutrients while the tissue is at body temperature

4 General considerations

For general statements on primary sample collection and handling (including avoidance of cross contaminations) see EN ISO 15189:2012, 5.4.4, 5.2.6. Consumables including kits shall be verified before use in examination (see EN ISO 15189:2012, 5.3.2.3); EN ISO 15189:2012, 5.5.1.2 and 5.5.1.3 can also apply.

As all steps of a diagnostic workflow can influence the final analytical performance, the entire workflow comprising the preanalytical steps, including information on biomolecule stability and storage conditions, and analytical steps should be verified and validated (see EN ISO 15189).

For samples intended to be analysed for DNA, the following specific aspects shall be considered.

In contrast to RNA or proteins, DNA in tissue is relatively stable during warm and cold ischemia times. Changes of DNA, sequence or copy numbers (e.g., CGH profiles) due to an extended duration of warm and cold ischemia are unknown. 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 [3].

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 sample 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 [1], [2], [4], [5].

Safety regulations on specimen transport and handling shall be considered (see EN ISO 15189:2012, 5.2.3 and 5.4.5 and ISO 15190).

During the whole preanalytical workflow precautions shall be taken to avoid cross contamination between different 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 Primary tissue collection manual

5.1.1 Information about the primary sample donor

The documentation should include, but is not limited to:

a) the primary donor / patient ID, which can be in the form of a code;