



SLOVENSKI STANDARD

SIST-TS CEN/TS 16827-1:2015

01-oktober-2015

Molekularne diagnostične preiskave in vitro - Specifikacije za predpreiskovalne procese za FFPE tkiva - 1. del: Izolirani RNK

Molecular in vitro diagnostic examinations - Specifications for pre-examination processes for FFPE tissue - Part 1: Isolated RNA

Molekularanalytische in-vitro-diagnostische Verfahren - Spezifikationen für präanalytische Prozesse für FFPE-Gewebe - Teil 1: Isolierte RNS

Tests de diagnostic moléculaire in vitro - Spécifications relatives aux processus préanalytiques pour les tissus FFPE - Partie 1: ARN extrait

<https://standards.iteh.ai/catalog/standards/sist/33e87bf-4012-4066-8bcd-6ea3a638932d/sist-ts-cen-ts-16827-1-2015>

Ta slovenski standard je istoveten z: **CEN/TS 16827-1:2015**

ICS:

11.100.10	Diagnostični preskusni sistemi in vitro	In vitro diagnostic test systems
-----------	---	----------------------------------

SIST-TS CEN/TS 16827-1:2015

en,fr,de

iTeh STANDARD PREVIEW
(standards.iteh.ai)

[SIST-TS CEN/TS 16827-1:2015](https://standards.iteh.ai/catalog/standards/sist/33e87fbf-4012-4066-8bcd-6ea3a638932d/sist-ts-cen-ts-16827-1-2015)

<https://standards.iteh.ai/catalog/standards/sist/33e87fbf-4012-4066-8bcd-6ea3a638932d/sist-ts-cen-ts-16827-1-2015>

TECHNICAL SPECIFICATION
SPÉCIFICATION TECHNIQUE
TECHNISCHE SPEZIFIKATION

CEN/TS 16827-1

August 2015

ICS 11.100.10

English Version

**Molecular in vitro diagnostic examinations - Specifications for
pre-examination processes for FFPE tissue - Part 1: Isolated
RNA**

Tests de diagnostic moléculaire in vitro - Spécifications
relatives aux processus préanalytiques pour les tissus
FFPE - Partie 1: ARN extrait

Molekularanalytische in-vitro-diagnostische Verfahren -
Spezifikationen für präanalytische Prozesse für FFPE-
Gewebeproben - Teil 1: Isolierte RNS

This Technical Specification (CEN/TS) was approved by CEN on 6 July 2015 for provisional application.

The period of validity of this CEN/TS is limited initially to three years. After two years the members of CEN will be requested to submit their comments, particularly on the question whether the CEN/TS can be converted into a European Standard.

CEN members are required to announce the existence of this CEN/TS in the same way as for an EN and to make the CEN/TS available promptly at national level in an appropriate form. It is permissible to keep conflicting national standards in force (in parallel to the CEN/TS) until the final decision about the possible conversion of the CEN/TS into an EN is reached.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.

<https://standards.iteh.ai/catalog/standards/sist/33e87bf-4012-4066-8bcd-6ea3a638932d/sist-ts-cen-ts-16827-1-2015>



EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

Contents	Page
European foreword	3
Introduction	4
1 Scope	5
2 Normative references	5
3 Terms and definitions	5
4 General considerations	7
5 Outside the laboratory	8
5.1 Primary tissue collection manual.....	8
5.1.1 Information about the primary sample donor.....	8
5.1.2 Information on the primary tissue sample	8
5.1.3 Information on the primary tissue sample processing.....	8
5.2 Transport requirements	9
6 Inside the laboratory	9
6.1 Information on the primary tissue sample receipt	9
6.2 Formalin fixation of the specimen	9
6.3 Evaluation of the pathology of the specimen and selection of the sample.....	10
6.4 Post-fixation of frozen samples	11
6.5 Processing and paraffin embedding	11
6.6 Storage requirements.....	12
6.7 Isolation of the total RNA.....	12
6.7.1 General.....	12
6.7.2 General information for RNA isolation procedures	12
6.7.3 Using commercial kits.....	13
6.7.4 Using the laboratories' own protocols	13
6.8 Quantity and quality assessment of isolated RNA.....	14
6.9 Storage of isolated RNA.....	14
Annex A (informative) Quality control of RNA extracted from formalin fixed and paraffin embedded tissue samples: implications for RT-qPCR based analyses	15
A.1 Summary.....	15
A.2 Results	15
A.2.1 Time dependency of RNA integrity	15
A.2.2 Impact of formalin-fixation on cDNA synthesis efficiency	16
A.2.3 Fixation and storage introduces major gene-to-gene variations in RT-qPCR	17
A.2.4 Impact of storage conditions of FFPE blocks on RNA Integrity.....	18
A.3 Conclusions	18
A.4 Further reading	19
Bibliography	20

European foreword

This document (CEN/TS 16827-1:2015) has been prepared by Technical Committee CEN/TC 140 “*In vitro* diagnostic medical devices”, the secretariat of which is held by DIN.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to announce this Technical Specification: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

iTeh STANDARD PREVIEW (standards.iteh.ai)

[SIST-TS CEN/TS 16827-1:2015](https://standards.iteh.ai/catalog/standards/sist/33e87fbf-4012-4066-8bcd-6ea3a638932d/sist-ts-cen-ts-16827-1-2015)

<https://standards.iteh.ai/catalog/standards/sist/33e87fbf-4012-4066-8bcd-6ea3a638932d/sist-ts-cen-ts-16827-1-2015>

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 profile generated during the pre-examination process. Therefore, a standardization of the entire process from primary sample collection to RNA analysis is needed. Studies have been undertaken to determine the important influencing factors. This Technical Specification draws upon such work to codify and standardize the steps for formalin fixed and paraffin embedded (FFPE) tissue with regard to RNA analysis in what is referred to as the preanalytical phase.

iTeh STANDARD PREVIEW (standards.iteh.ai)

[SIST-TS CEN/TS 16827-1:2015](https://standards.iteh.ai/catalog/standards/sist/33e87fbf-4012-4066-8bcd-6ea3a638932d/sist-ts-cen-ts-16827-1-2015)

<https://standards.iteh.ai/catalog/standards/sist/33e87fbf-4012-4066-8bcd-6ea3a638932d/sist-ts-cen-ts-16827-1-2015>

1 Scope

This Technical Specification gives recommendations for the handling, documentation and processing of FFPE tissue specimens intended for RNA 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).

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

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

FFPE

formalin fixation and paraffin embedding

3.5

FFPE tissues

formalin fixed and paraffin embedded tissues

3.6

formalin

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

CEN/TS 16827-1:2015 (E)**3.7****formalin fixation**

treatment of a sample with standard buffered formalin solution for stabilization

3.8**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.

3.9**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.]

3.10**quantitative RNA profile****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

3.11**RNA****ribonucleic acid**

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

[SOURCE: EN ISO 22174:2005, 3.1.3]

3.12**room temperature**

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

3.13**sample**

one or more parts taken from a primary sample

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

3.14**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 RNA.

3.15

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.

3.16

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).

The stability of the specific quantitative RNA profile(s) of interest should be investigated throughout the entire preanalytical workflow prior to the development and implementation of an analytical test.

Before tissues are fixed in standard buffered formalin solution, RNA profiles can change significantly depending on the duration of warm and cold ischemia and the temperature before formalin fixation (e.g., gene induction, gene down regulation, RNA degradation). In addition, those changes can vary in tissues from different donors / patients.

Generally, the longer the warm and cold ischemia times 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. 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 time and duration should be documented. When it is not possible to avoid cold ischemia, its time of onset and duration shall be documented and the temperatures of the specimen transport container's surroundings should 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, formalin fixation causes modifications of biomolecules and leads to suboptimal performance of RNA extracted from FFPE tissues that should be considered in quality control and application of molecular assays, especially in the context of gene expression studies (see [1], [2], [3]). Assay optimization for FFPE tissues or the use of non-crosslinking alternatives to standard buffered formalin solution are options to minimize this issue for molecular analyses (see [4]).

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

CEN/TS 16827-1:2015 (E)

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;
- b) the health status of the primary sample donor (e.g., healthy, disease type, concomitant disease);
- c) the information about routine medical treatment and special treatment prior to tissue collection (e.g., anaesthetics, medications, surgical or diagnostic procedures (e.g., biopsy device used for the collection));
- d) the start of ischemia within the body (warm ischemia) by documenting the ischemia-relevant vessel ligation/clamping time point (usually arterial clamping time).

5.1.2 Information on the primary tissue sample

The documentation shall include, but is not limited to:

- a) the time point when tissue is removed from the body;
- b) the description of tissue type, tissue condition (e.g., diseased, unaffected by the disease) and organ tissue of origin, including references to any marking applied in the operating theatre made by surgeon, radiologist or pathologist;
- c) the documentation steps described under 6.2, if the formalin fixation starts outside the laboratory.

5.1.3 Information on the primary tissue sample processing

The following steps shall be performed:

1. the documentation of any additions or modifications to the primary sample after removal from the body (e.g., labelling for the orientation of the specimen (e.g., ink-marking, stitches), incision(s));
2. the selection and use of transport containers and packages (e.g., cooling box, box for storing and transportation, vacuum packaging) fit for transport of formalin fixed tissue samples, if relevant;
3. the selection and use of stabilization procedures (e.g., cooling methods) for transport;

NOTE 1 Accidentally freezing and thawing the tissue (e.g., by using cool packs in a wrong manner) can lead to RNA degradation when the tissue thaws thereafter. It can also impact the morphological characterization.

NOTE 2 This step can be omitted, if the specimen is transferred directly into standard buffered formalin solution (see 6.2).

4. the labelling of the transport container (e.g., registration-number, barcode (1D or 2D), primary sample type, quantity, and organ tissue of origin) and additional documentation (information as specified in 5.1.1, 5.1.2, and 5.1.3, 1. to 3.). If a single sample container contains several aliquots of the same specimen,

and the aliquots represent different features (e.g., tissue type, disease status, location) this shall be documented.

Specimens should be transferred without delay into the transport container after the removal from the body. The container should then be kept on wet-ice or at 2 °C to 8 °C in order to minimize RNA profile changes.

The temperatures of the transport container's surroundings during cold ischemia time (e.g., temperatures in different rooms, transport) should be documented. If the temperature cannot be measured, the temperature range should be estimated by classification as ambient temperature, room temperature, or at 2 °C to 8 °C.

5.2 Transport requirements

The laboratory in partnership with the clinical or surgery department shall establish a protocol for the transport procedure of the specimen.

If the primary tissue sample is not already placed into standard buffered formalin solution, it should be transported on wet-ice or at 2 °C to 8 °C without delay in order to minimize the changes to the RNA profile.

NOTE There is evidence that RNA in tissues can be stabilized in plastic bags under vacuum when kept at 0 °C to 4 °C during transport [5] before the samples are archived for biobanks or used for histopathological evaluation.

If the primary tissue sample is already placed into standard buffered formalin solution outside the laboratory, the temperature during transport should not exceed room temperature.

The compliance with the protocol for the transport procedure shall be documented. Any deviations from the protocol shall be described and documented.

6 Inside the laboratory

6.1 Information on the primary tissue sample receipt

The name of the person receiving the primary tissue sample shall be documented. The tissue sample arrival time and conditions (e.g., labelling, transport conditions including temperature, tissue type and quantity of the primary sample, leaking/breaking of the container) of the received samples shall be documented. Any deviations from the established protocol for the transport procedure (see 5.2) shall be documented.

NOTE Temperature conditions during transport can influence the quantitative RNA profile and RNA quality.

6.2 Formalin fixation of the specimen

The fixative used shall be standard buffered formalin solution.

The pH-value and concentration of the standard buffered formalin solution should be checked regularly as formalin is not stable (e.g., it has a tendency to be oxidized to formic acid [6]).

The following steps shall be performed:

1. the consultation with the manufacturer's material safety data sheet (MSDS) before handling standard buffered formalin solution;

NOTE Formaldehyde is a hazardous compound that chemically modifies biomolecules.

2. the documentation of the time point of placing the tissue sample into standard buffered formalin solution;

NOTE The total formalin fixation duration can impact further analyses e.g., immunohistochemical techniques, nucleic acid based molecular analyses [3]; see also A.2.1 and A.2.2. The optimal formalin fixation duration can vary