



**BBMRI.at**

Biobanking and  
BioMolecular resources  
Research Infrastructure  
Austria

# Meeting CEN/TS & ISO Standard Requirements – Self Assessment

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**bmwfw**  
Bundesministerium für  
Wissenschaft, Forschung und Wirtschaft

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# CEN/TS & ISO Standards

**CEN Technical Specifications & ISO Standards**  
for molecular in vitro diagnostic examinations  
– Specifications for pre-examination processes

- ✓ Why?
- ✓ Which?
  - Where to get?
  - For whom?
  - What they are about?
  - Are you conform?




# CEN/TS & ISO Standards

**CEN Tech Spec**  
(published) 

**ISO Standards**  
(under devel.) 



**Sample type - Analyte**

FFPE tissue – RNA	<a href="#">CEN/TS 16827-1:2015</a>	ISO/DIS 20166-1	FFPE - RNA	
FFPE tissue – Proteins	<a href="#">CEN/TS 16827-2:2015</a>	ISO/DIS 20166-2	FFPE - Prot.	
FFPE tissue – DNA	<a href="#">CEN/TS 16827-3:2015</a>	ISO/DIS 20166-3	FFPE - DNA	
Frozen tissue – RNA	<a href="#">CEN/TS 16827-3:2015</a>	ISO/DIS 20184-1	Frozen - RNA	
Frozen tissue – Proteins	<a href="#">CEN/TS 16827-3:2015</a>	ISO/DIS 20184-2	Frozen - Prot.	
Blood – cellular RNA	<a href="#">CEN/TS 16835-1:2015</a>	ISO/DIS 20186-1	Blood - RNA	
Blood – genomic DNA	<a href="#">CEN/TS 16835-2:2015</a>	ISO/DIS 20186-2	Blood - DNA	
Blood – circul. cell free DNA	<a href="#">CEN/TS 16835-3:2015</a>	ISO/DIS 20186-3	Blood – ccfDNA	
Metabolomics in urine, serum & plasma	<a href="#">CEN/TS 16835-3:2015</a>	ISO/AWI 23118	Metabolomics	

**=> Available at National Standardization Bodies**



Molecular in vitro diagnostic examinations -- Specifications for preexamina fixed and paraffin-embedded (FFPE) tissue -- Part 1: Isolated RNA

ISO 20166-1:2016 06 28

Produkttyp: Internationale Norm, Entwurf



~€ 45 – 70

 **IN DEN WARENKORB**





## ISO/DIS 20166-1(en) Molecular in vitro diagnostic examinations — Specifications for preexamination processes for formalin-fixed and paraffin-embedded (FFPE) tissue — Part 1: Isolated RNA



This document is now under preparation for its final publication.

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Available in: en fr

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## 1 Scope

This International Standard recommends 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 International Standard 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, as well as 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 examination test results.

RNA profiles in tissues can change drastically during collection and change differently in different tissue 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.

**NOTE** International, national or regional regulations or requirements may also apply to specific topics covered in this International Standard.

## 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.

ISO 15189:2012, *Medical laboratories — Requirements for quality and competence*

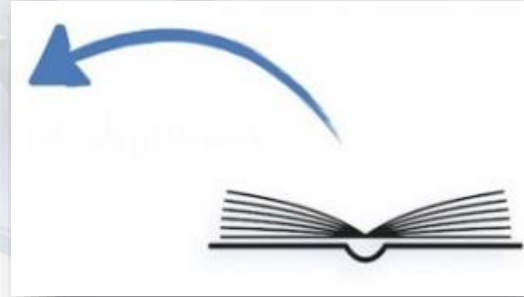
ISO 15190, *Medical laboratories — Requirements for safety*

ISO 17020:2012, *Conformity assessment – Requirements for the operation of various types of bodies performing inspection*

# Scope



... recommends 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.



## Target groups

- ✓ *In-vitro* diagnostic laboratories
- ✓ *In-vitro* diagnostics developers & manufacturers
- ✓ Research institutions /commercial organizations
- ✓ Biobanks
- ✓ Regulation authorities



... **accredited: ISO 15198 Med. Lab., ISO 20387 Biobanking, ...**



# CEN/TS & ISO Standard Family

## Sample type - Analyte

FFPE tissue – Part 1: **RNA**

FFPE tissue – Part 2: **Proteins**

FFPE tissue – Part 3: **DNA**

Frozen tissue – Part 1: **RNA**

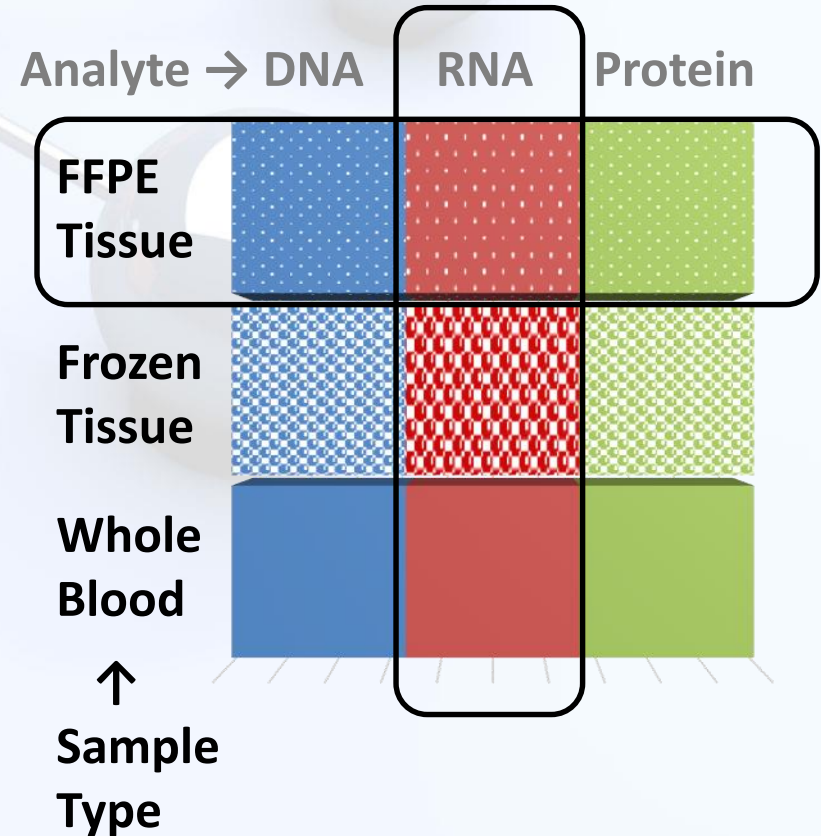
Frozen tissue – Part 2: **Proteins**

Blood – cellular **RNA**

Blood – genomic **DNA**

Blood – circul. cell free DNA

Metabolomics in  
urine, serum & plasma



# Structure

## FFPE Tissue - RNA

### Outside the laboratory

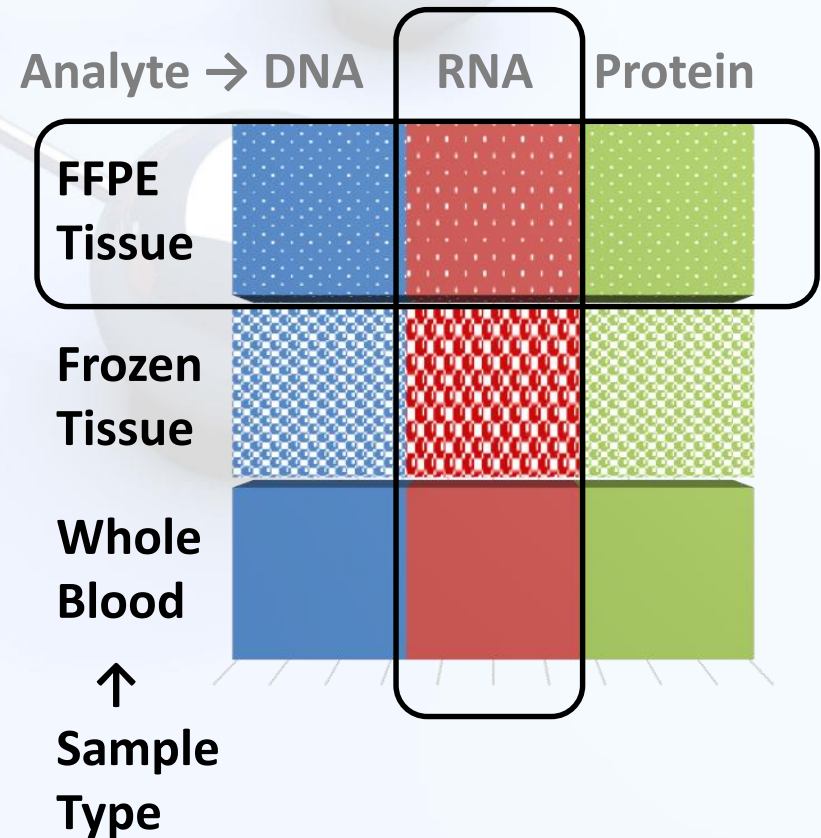
Collection of biospecimen  
Transport requirements

### Inside the laboratory

Primary tissue sample receipt  
Fixation of the specimen  
Evaluation of the pathology  
Processing and paraffin embedding  
Storage requirements (paraffin blocks & sections)

### Isolation of total RNA

General information (FFPE)  
Quantity and quality assessment of RNA  
Storage of isolated RNA



**=> They cover the whole preanalytical phase**



# Structure/Content

## FFPE Tissue - RNA

### Outside the laboratory

Collection of primary tissue  
Transport requirements

### Inside the laboratory

Primary tissue sample receipt

Fixation of the specimen

Evaluation of the pathology

Processing and paraffin embedding

Storage requirements (paraffin blocks & sections)

### Isolation of total RNA

General information (FFPE)

Quantity and quality assessment of RNA

Storage of isolated RNA

## Frozen Tissue - RNA

### Outside the laboratory

Collection of tissue  
Transport requirements

### Inside the laboratory

Primary tissue sample receipt

Evaluation of the pathology

Cryo storage (stabilization)

Storage requirements (cryo)

### Isolation of total RNA

General information (cryo)

Quantity and quality assessment of RNA

Storage of isolated RNA



## Examples of pre-analytical variables -- FFPE tissue - RNA

### Outside the laboratory

Collection: Donor (healthy/diseased, medication, phys. activ., ...)

Specimen (tissue/organ, surgery/biopsy, warm/cold ischemia, ...)

Transport (container, labeling, temp., duration, ....)

### Inside the laboratory

Receipt (loss of sample, mixing up, ...)

Fixation (formalin formula, pH/conc., duration, temp., volume, tissue size, ...)

Evaluation (selection of sample, ...)

Processing & embedding (method/protocol, paraffin ...)

Storage (duration, humidity, temp., retrieval, ..)

### Isolation of total RNA

General (method/protocol incl. RNase, protease, intermediate storage ... )

Quantity and quality assessment of RNA (method)

Storage of isolated RNA (duration, temp., freeze/thaw cycles, ...)

## Examples of pre-analytical variables -- FFPE tissue - RNA

### Outside the laboratory

Collection: Donor (healthy/diseased, medication, phys. activ, ...)

Transport ( ...)

### Inside the laboratory

Receipt (los ...)

Fixation (fo ...)

Evaluation (selection of sample, ...)

Processing

Storage (duration, humidity, temp., recovery, ...)

### Isolation of total RNA

General (method/protocol incl. RNase, protease, intermediate storage ...)

Quantity and quality assessment of RNA (method)

Storage of isolated RNA (duration, temp., freeze/thaw cycles, .)

**"shall"** indicates a **requirement**;  
**"should"** indicates a **recommendation**;  
**"may"** indicates a permission;  
**"can"** indicates a possibility or a capability

**Documentation!**

# BBMRI Self-Assessment Survey



MEDIZINISCHE  
UNIVERSITÄT  
INNSBRUCK

SERVICES ▾ DIRECTORY

<http://www.bbmri-eric.eu/services/self-assessment-survey/>

## SELF-ASSESSMENT SURVEY

Please fill in your contact information:

\*Name

\*E-mail address

\*Affiliation

\*Address/Country

Please provide us with some information by answering the following questions:

\* Is your organisation located in a BBMRI-ERIC Member/Observer State?

Yes  No

\* Are you in contact with the coordinating office from the National Node nodes/

Yes  No

\* Have you purchased the required CEN Technical Specifications as a basis for your laboratory? <http://www.bbmri-eric.eu/services/standardisation/>

Yes  No

\* Please select the required BBMRI-ERIC Self-Assessment Surveys from the list below:

Specifications for Pre-examination processes for snap frozen tissue – Part 1: Isolated RNA; CEN/TS 16826-1:2015

Specifications for Pre-examination processes for snap frozen tissue – Part 2: Isolated proteins; CEN/TS 16826-2:2015

### 1. Apply for Access

- BBMRI-ERIC website
- Request form
- Fulfill preconditions
- Email to BBMRI-ERIC (A. Wutte), contacts you & National Node
- Login (for a collection)

### Self Assessment

- Perform self-assessment
- Option 1: Internal use
- Option 2: Submit to BBMRI-ERIC for uptake of collection in BBMRI-ERIC Directory

# HandsOn BBMRI Self Assessment Survey

**Select one of the following:**

- Snap frozen tissue – Isolated RNA
- Snap frozen tissue – Isolated protein
- FFPE tissue – Isolated RNA
- FFPE tissue – Isolated DNA
- Venous whole blood – Isolated genomic DNA



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**THANK YOU!**

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**Medizinische Universität Graz**



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.



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

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

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A.3 Conclusions .....

A.4 Further reading .....

Bibliography .....

## 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

▼ **Molecular in vitro diagnostic examinations - Specifications for pre-examination processes for FFPE tissue -**

**OUTSIDE THE LABORATORY**

**Primary tissue collection**

**Information about sample donor** **y/n** **Information/details documented**

should	<b>Donor/patient ID</b> documented?	yes	(e.g. code)
		no	

should	<b>Health status</b> of donor/patient documented?	yes	(e.g. healthy, disease type, concomitant disease)
		no	

should	<b>Medical treatment</b> prior to tissue collection documented?	yes	(e.g. anaesthetics, medications, surgical or diagnostic procedures)
		no	

**Start of warm ischemia**

should	- Date of vessel ligation/arterial clamping time documented?	yes	
		no	

should	- Time of vessel ligation/arterial clamping time documented?	yes	
		no	

**Information on primary tissue sample**

**Start of cold ischemia**

shall	- Date of removal of tissue from the body documented?	yes	
		no	

shall	- Time of removal of tissue from the body documented?	yes	
		no	

shall	<b>Tissue type and condition</b> documented?	yes	
		no	

*If fixation started outside the biobank*

shall	<b>Type and start of fixation</b> documented?	yes	
		no	

"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 FFPE tissue -

**OUTSIDE THE LABORATORY**

**Primary tissue collection**

**Information about sample donor**

y/n Information/details documented

should	Donor/patient ID documented?	yes	(e.g. code)
should	Health status documented?		(e.g. case)
should	Medical treatment documented?		
should	Start of work documented?		
should	- Date of work documented?		
should	- Time of work documented?		
	<b>Information about fixation</b>		
shall	Start of collection documented?		
	- Date of removal of tissue from the body documented?	no	
shall	- Time of removal of tissue from the body documented?	yes	
		no	
shall	Tissue type and condition documented?	yes	
		no	
	<i>If fixation started outside the biobank</i>		
shall	Type and start of fixation documented?	yes	
		no	

Documentation,  
documentation,  
documentation!

"shall" indicates a requirement;  
 "should" indicates a recommendation;  
 "may" indicates a permission;  
 "can" indicates a possibility or a capability

# CEN/TS & ISO Standards

## CEN Technical Specifications & ISO Standards for molecular in vitro diagnostic examinations – Specifications for pre-examination processes

- |                        |                     |
|------------------------|---------------------|
| ✓ Why?                 | Need                |
| ✓ Which?               | Types               |
| • Where to get?        | Source              |
| • For whom?            | Scope               |
| • What they are about? | Structure & Content |
| • Are you conform?     | Self-Assessment     |



**OUTSIDE THE LABORATORY**

y/n Information/details documented

**S = Surgery**

Primary tissue collection

Information about sample donor

Information on primary tissue sample

Information on primary tissue sample processing

Transport Requirements

**INSIDE THE LABORATORY**

Primary tissue sample receipt at the laboratory

Formalin fixation

Evaluation of the pathology

Processing and paraffin emb

Storage

Isolation of total RNA

General Information

Using a commercial kit

Using a laboratories own pro

Quantity and quality assessm

Storage of isolated RNA

**"shall"** indicates a requirement;  
**"should"** indicates a recommendation;  
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Documentation,  
 documentation,  
 documentation!

 S  
S  
S  
S  
S

**P B = Biobank**
**P B = Patho**

P B

P B

P B

P B

P B

**Lab**

P B

L

P B

L

P B

L

P B

L

P B

L

**=> Calculation:**

- Warm ischemia time	= "warm ischem start" until "cold ischemia start"
- Cold ischemia time	= "cold ischemia start" until "start of fixation"
- Fixation time	Formol: '= "fixation start" until "start of dehydration"
- Total storage time of tissue until RNA isolation	FFPE: "end of paraffin embedding" until "use"
- Storage time of sections until RNA isolation	= "preparation of sections" until "RNA isolation"
- Time of RNA on ice prior to use	= "dilution of RNA after isolation" until "use"
- Time of RNA on ice prior to storage	= "dilution of RNA after isolation" until "storage at -70°C"
- Storage time of RNA at -70°C prior to shipping/use	= "freezing of RNA aliquot until retrieval for shipping/use"
- Number of freeze and thaw cycles of an RNA aliquot	= "1x freezing + 1x thawing = 1 cycle"

# Structure

## FFPE Tissue - RNA

### Outside the laboratory

Collection of primary tissue

Transport requirements

### Inside the laboratory

Primary tissue sample receipt

Fixation of the specimen

Evaluation of the pathology

Processing and paraffin embedding

Storage requirements (paraffin blocks & sections)

### Isolation of total RNA

General information (FFPE)

Quantity and quality assessment of RNA

Storage of isolated RNA

### Calculation:

Warm ischemia time

= "warm ischem start" until "cold ischemia start"

Cold ischemia time

= "cold ischemia start" until "start of fixation"