

English Version

**Molecular in vitro diagnostic examinations - Specifications
for pre-examination processes for human specimen -
Isolated microbiome DNA**

Analyses moléculaires de diagnostic in vitro -
Spécifications relatives aux processus préanalytiques
pour les échantillons humains - ADN du microbiote
isolé

Molekularanalytische in-vitro-diagnostische Verfahren
- Spezifikationen für präanalytische Prozesse für
menschliche Proben - Isolierte Mikrobiom-DNA

This Technical Specification (CEN/TS) was approved by CEN on 19 March 2021 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, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, 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: Rue de la Science 23, B-1040 Brussels

Contents

Page

European foreword.....	4
Introduction	5
1 Scope	6
2 Normative references	6
3 Terms and definitions	6
4 General considerations	11
5 Outside the laboratory	12
5.1 Specimen collection.....	12
5.1.1 General.....	12
5.1.2 Information about the patient/specimen donor	13
5.1.3 Specific information about the patient/specimen donor	13
5.1.4 Selection of specimen collection method and device(s)	15
5.1.5 Specimen collection from the patient/specimen donor and specimen stabilization.....	16
5.2 Specimen storage and transport	18
5.2.1 General.....	18
5.2.2 Using collection devices with stabilizers.....	19
5.2.3 Using collection devices without stabilizers	19
6 Inside the laboratory	20
6.1 Specimen reception.....	20
6.2 Processing of specimens.....	20
6.3 Specimen storage before microbiome DNA isolation	21
6.4 Isolation of microbiome DNA.....	21
6.4.1 General.....	21
6.4.2 Using a commercial kit.....	22
6.4.3 Using a laboratory developed procedure.....	23
6.5 Quantity and quality assessment of isolated microbiome DNA	23
6.5.1 General.....	23
6.5.2 Quantity assessment	24
6.5.3 Quality assessment.....	24
6.6 Storage of isolated microbiome DNA	24
6.6.1 General.....	24
6.6.2 Microbiome DNA isolated using a commercial kit.....	25
6.6.3 Microbiome DNA isolated using a laboratory developed procedure.....	25
Annex A (informative) Impact of various pre-analytical variables on microbiome DNA quantity, quality and profile	26
A.1 Introduction	26
A.2 Result – Impact of specimen stabilization method on isolated microbiome DNA quantity.....	26
A.2.1 General.....	26
A.2.2 Method	26
A.2.3 Result/conclusion	27

A.3	Result – Impact of specimen to stabilizer mass/volume ratio on isolated microbiome DNA quantity and quality.....	28
A.3.1	General	28
A.3.2	Method.....	28
A.3.3	Result/conclusion	28
A.4	Result – Impact of different microbiome DNA isolation methods on microbiome DNA profile.....	29
A.4.1	General	29
A.4.2	Method.....	29
A.4.3	Result/conclusion	30
A.5	Results – Impact of stabilization status and storage of collected specimens/samples on microbiome DNA profile	31
A.5.1	General	31
A.5.2	Method.....	31
A.5.3	Result/conclusion	32
Annex B (informative)	Importance of using an in-process quality control material	33
B.1	Introduction	33
B.2	Results	33
B.2.1	General	33
B.2.2	Method.....	33
B.2.3	Result	35
B.3	Conclusions	35
Bibliography	36

European foreword

This document (CEN/TS 17626:2021) 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 shall not be held responsible for identifying any or all such patent rights.

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

Introduction

Molecular *in vitro* diagnostics has enabled significant progress in medicine. Further progress is expected using new technologies analysing the microbiome (e.g. bacteria, fungi, viruses, yeasts, archaea) in human specimens.

The human microbiome has come into focus in many medical disciplines such as gastroenterology, dermatology, or gynaecology as a potential biomarker for diagnosis and management of diseases, and even as a therapeutic agent. Technologies analysing microbiome DNA such as shotgun metagenome or amplicon-based sequencing (e.g. 16S or 18S rRNA gene sequencing) have accelerated this process and are being increasingly performed in research and clinical practice.

However, the human microbiome profile can change drastically during the pre-examination process, which includes the specimen collection, transport, storage, and processing. These changes can, for example, be due to contamination of specimens with microbial cells or DNA from other sources than the sampling site or due to undesired growth and/or instability of individual microorganisms and viruses. Consequently, this makes the outcome from diagnostics or research unreliable or even impossible because the subsequent microbiome DNA examination might not determine the real situation in the patient but an artificial profile generated during the pre-examination processes. Therefore, special measures have to be taken to secure the stability of the microbiome profile.

Specimens for microbiome analysis are often collected by donors/patients. Therefore, dedicated measures are needed for informing donors/patients about and preparing them for the collection, storage and transport of specimens, and to check the compliance with the instructions, in order to reduce specimen variability.

In addition, isolation of microbiome DNA, which is representative in composition of the *in vivo* microbiome of the respective body site, is critical. This can be especially challenging e.g. due to different lysis requirements of the microorganisms (e.g. Gram-negative versus Gram-positive bacteria, or versus fungi) as well as inhibitory compounds (e.g. PCR inhibitors) in the specimen, which can impact the examination if not removed during the DNA isolation. The presence of high amounts of human host DNA, in addition to DNA introduced by reagents such as remnant plasmid DNA from generation of recombinant enzymes and/or DNA isolation kits, can further impact the examination result.

Therefore, standardization of the entire pre-examination workflow from specimen collection to the microbiome DNA examination is needed.

Studies have been undertaken to determine the important influencing factors. This document draws upon such work to codify and standardize the steps for microbiome DNA examination in what is referred to as the pre-examination phase.

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.

1 Scope

This document specifies requirements and gives recommendations for the pre-examination phase of human specimens, such as stool, saliva, skin and urogenital specimens, intended for microbiome DNA examination. The pre-examination phase includes but is not limited to specimen collection, handling, transport, storage, processing, isolation of DNA, and documentation.

This document is applicable to molecular *in vitro* diagnostic examinations performed by medical 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.

Different dedicated measures are taken for pre-examination processes for infectious disease examination (e.g. targeted pathogen identification) and for microbiome DNA examination from tissue (e.g. biopsies). These are outside of the scope of this document.

Different dedicated measures are taken for pre-examination processes for saliva for human genomic DNA examination. These are not described in this document but are covered in CEN/TS 17305, *Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for saliva — Isolated DNA*.

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.

EN ISO 15189, *Medical laboratories — Requirements for quality and competence (ISO 15189)*

ISO 15190, *Medical laboratories — Requirements for safety*

ISO/TS 20658, *Medical laboratories — Requirements for collection, transport, receipt, and handling of samples*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN ISO 15189 and the following ones 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 <https://www.electropedia.org/>