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STN P	**Molekulárno-diagnostické vyšetrenia in vitro Špecifikácie postupov pred vyšetrením ľudskej vzorky Izolovaná mikrobiómová DNA**	**STN P CEN/TS 17626** 85 6576

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

Táto norma obsahuje anglickú verziu európskej normy.
This standard includes the English version of the European Standard.

Táto norma bola oznámená vo Vestníku ÚNMS SR č. 08/21

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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

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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.

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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*

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