ICS 11.100.10 PREDBEŽNÁ SLOVENSKÁ TECHNICKÁ NORMA Marec 2024

STN P	**Pracovné postupy na diagnostiku *in vitro* metódou sekvenovania novej generácie Časť 1: Vyšetrenie ľudskej DNA**	**STN P CEN/TS 17981-1** 85 1050

In vitro diagnostic Next Generation Sequencing (NGS) workflows - Part 1: Human DNA examination

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. 02/24

Táto predbežná slovenská technická norma je určená na overenie. Prípadné pripomienky pošlite do novembra 2025 Úradu pre normalizáciu, metrológiu a skúšobníctvo Slovenskej republiky.

Obsahuje: CEN/TS 17981-1:2023

138105

TECHNICAL SPECIFICATION

SPÉCIFICATION TECHNIQUE

TECHNISCHE SPEZIFIKATION

CEN/TS 17981-1

November 2023

ICS 11.100.10

English Version

In vitro diagnostic Next Generation Sequencing (NGS) workflows - Part 1: Human DNA examination

Diagnostic *in vitro* Séquençage de nouvelle génération (NGS) - Partie 1 : Examens de l'ADN humain

Next Generation Sequencing (NGS)-Arbeitsabläufe für die *In-vitro*-Diagnostik - Teil 1: Untersuchung von menschlicher DNA

This Technical Specification (CEN/TS) was approved by CEN on 15 October 2023 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, Türkiye 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

Ref. No. CEN/TS 17981-1:2023 E

Contents

Page

European foreword

This document (CEN/TS 17981-1:2023) 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.

A list of all parts in this series can be found on the CEN website: www.cencenelec.eu.

Any feedback and questions on this document should be directed to the users' national standards body. A complete listing of these bodies can be found on the CEN website.

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, Türkiye and the United Kingdom.

Introduction

Molecular *in vitro* diagnostics has enabled significant progress in medicine. Further progress is expected by new technologies analysing profiles of nucleic acids, proteins, and metabolites in human tissues and body fluids. Next Generation Sequencing (NGS) takes a prominent place in the series of molecular techniques used for diagnostics. It facilitates sequence analysis of nucleic acids that can result in precise information for diagnosis and progression of diseases.

The NGS technique, however, has a very complex workflow that contains many steps. The target nucleic acids can originate from different sources, e.g. tissues, blood, and body fluids. The profiles of the isolated DNA or methylated DNA can change during specimen collection, transport, storage and processing (e.g. formalin fixation), 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. The available material can be small, the cells in a tissue can be dispersed heterogeneously (e.g. ratio of tumour to normal), the target nucleic acids can be circulating in blood or body fluids free of cells or in circulating cells (e.g. circulating tumour cells (CTCs)). For a successful and reliable sequence result, a suitable strategy needs to be chosen for every case depending on the available material and disease conditions. Therefore, the NGS workflow can differ from case to case, and the NGS workflow steps need to be carefully considered and chosen to get a sound and reliable result to determine the best available treatment for the patient. In addition, sequence platforms can differ in their technique (e.g. detection of a change in a current or fluorescence) and approach (e.g. panels, short-read sequencing, long-read sequencing) for sequence assessment. The bioinformatics analysis can differ in approach and ability to detect non-conformities and unreliable sequence results. To enable such capabilities, NGS metadata needs to be collected during all workflow steps from the patient to the reporting. In addition, controls and added controls need to be analysed properly. In this way, non-conformities or detected unreliabilities can be reported to the patient and the treating physician. The reporting of diagnostic NGS results can differ in clarity and depth, which can lead to different interpretations.

Standardization of the entire NGS workflow from specimen collection to the reporting of the results to the patient and the treating physician is needed for the development of reliable NGS examinations.

This document draws upon previous work to standardize the steps for NGS examinations from tissues, blood and body fluids in what is referred to as the pre-examination phase (sample collection), the examination phase (library preparation, sequencing), and the post-examination phase (analysis and reporting).

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 next generation sequencing (NGS) workflows for *in vitro* diagnostics and biomedical research. This document covers the pre-examination processes, human DNA (somatic and germline) isolation, sequencing library preparation, sequencing, sequence analysis and reporting of the examination of sequences for diagnostic purposes from isolated DNA from, e.g. formalin-fixed and paraffin embedded tissues, fresh frozen tissues, fine needle aspirates (FNA), whole blood, circulating tumour cells (CTCs), exosomes and other extracellular vesicles, circulating cell free DNA from plasma, and DNA from saliva.

NOTE 1 Typical applications include, but are not limited to, NGS for oncology, pharmacogenomics and clinical genetics; approaches include panels (e.g. disease panels, exome panels, target gene panels and in silico panels), exome and whole genome sequencing, as well as certain epigenetics and certain single-cell analyses.

This document is applicable to molecular *in vitro* diagnostic examinations including laboratory developed tests performed by medical laboratories, molecular pathology laboratories and molecular genetic laboratories. This document is also applicable to laboratory customers, *in vitro* diagnostics developers and manufacturers, biobanks, institutions, and organizations performing biomedical research.

This document is not applicable for *in situ* sequencing, DNA-mediated protein sequencing, forensic sequencing, sequencing of pathogens or microorganisms and microbiome analysis.

NOTE 2 International, national or regional regulations or requirements or multiples of them 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.

CEN/TS 17390-2:2020, *Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for circulating tumor cells (CTCs) in venous whole blood — Part 2: Isolated DNA*

CEN/TS 17688-3, *Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for Fine Needle Aspirates (FNAs) — Part 3: Isolated genomic DNA*

CEN/TS 17747, *Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for exosomes and other extracellular vesicles in venous whole blood — DNA, RNA and proteins*

CEN/TS 17811, *Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for urine and other body fluids — Isolated cell free DNA*

EN ISO 4307, *Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for saliva — Isolated human DNA (ISO 4307)*

EN ISO 13485, *Medical devices — Quality management systems — Requirements for regulatory purposes (ISO 13485)*

EN ISO 15189:2022, *Medical laboratories — Requirements for quality and competence (ISO 15189:2022)*

EN ISO/IEC 17020:2012, *Conformity assessment — Requirements for the operation of various types of bodies performing inspection (ISO/IEC 17020:2012)*

EN ISO/IEC 17025:2017, *General requirements for the competence of testing and calibration laboratories (ISO/IEC 17025:2017)*

EN ISO 20166-3, *Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for formalin-fixed and paraffin-embedded (FFPE) tissue — Part 3: Isolated DNA (ISO 20166-3)*

EN ISO 20184-3:2021, *Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for frozen tissue — Part 3: Isolated DNA (ISO 20184-3:2021)*

EN ISO 20186-2:2019, *Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for venous whole blood — Part 2: Isolated genomic DNA (ISO 20186-2:2019)*

EN ISO 20186-3:2019, *Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for venous whole blood — Part 3: Isolated circulating cell free DNA from plasma (ISO 20186-3:2019)*

ISO 8601-1, *Date and time — Representations for information interchange — Part 1: Basic rules*

ISO 20397-1:2022, *Biotechnology — Massively parallel sequencing — Part 1: Nucleic acid and library preparation*

ISO 20397-2, *Biotechnology — Massively parallel sequencing — Part 2: Quality evaluation of sequencing data*