



SLOVENSKI STANDARD
SIST-TS CEN/TS 17981-1:2024

01-marec-2024

**In vitro diagnostični delovni postopki Sekvenciranje naslednje generacije (NGS) -
1. del: Preiskava človeškega DNK**

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

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

Diagnostic in vitro Séquençage de nouvelle génération (NGS) pour des examens de l'ADN/ARN humain

Ta slovenski standard je istoveten z: CEN/TS 17981-1:2023

SIST-TS CEN/TS 17981-1:2024

<http://standards.sist.si/standards/sist-ts/2024/01/marec/17981-1-2024>

ICS:

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

SIST-TS CEN/TS 17981-1:2024

en,fr,de

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.

(<https://standards.iteh.ai>)
Document Preview

[SIST-TS CEN/TS 17981-1:2024](https://standards.iteh.ai/catalog/standards/sist/b552087a-afce-4b89-b186-b0e7d3a30c43/sist-ts-cen-ts-17981-1-2024)

<https://standards.iteh.ai/catalog/standards/sist/b552087a-afce-4b89-b186-b0e7d3a30c43/sist-ts-cen-ts-17981-1-2024>



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.....	7
4 General requirements.....	19
4.1 General.....	19
4.2 Examination design.....	20
4.3 Examination development	24
4.4 Examination performance verification and validation.....	24
4.5 Technical examination performance characteristics.....	30
5 Pre-examination processes for examination development	30
5.1 General.....	30
5.2 Human DNA isolation	32
5.3 DNA sample quality and quantity evaluation.....	33
6 Examination processes for examination development.....	35
6.1 Sequencing library preparation for examination development.....	35
6.2 Sequencing examination development.....	40
6.3 Data analysis requirements for examination development.....	41
6.4 Quality control (QC) requirements for examination development.....	42
7 Requirements for the development of the examination reporting tool	43
7.1 General.....	43
7.2 Report attributes	44
7.3 Report content.....	44
8 Implementation of the <i>in vitro</i> diagnostic NGS workflow into routine practice	45
9 Reporting and interpretation of results.....	46
10 Quality assurance procedures	47
10.1 General.....	47
10.2 Performance monitoring, optimization of the examination and interlaboratory comparison	47
Annex A (normative) <i>in vitro</i> diagnostic NGS workflow for single-cell analyses.....	48
A.1 General information and requirements on single-cell analyses	48
A.2 Pre-examination processes for examination development	49
A.3 Examination phase for examination development.....	53
A.4 Implementation of the <i>in vitro</i> diagnostic NGS workflow into routine practice	54
A.5 Reporting and interpretation of results.....	54
A.6 Quality assurance procedures	54

Annex B (informative) NGS workflow scheme for the examination of DNA..... 55
Bibliography 56

iTeh Standards
(<https://standards.iteh.ai>)
Document Preview

[SIST-TS CEN/TS 17981-1:2024](https://standards.iteh.ai/catalog/standards/sist/b552087a-afce-4b89-b186-b0e7d3a30c43/sist-ts-cen-ts-17981-1-2024)

<https://standards.iteh.ai/catalog/standards/sist/b552087a-afce-4b89-b186-b0e7d3a30c43/sist-ts-cen-ts-17981-1-2024>

CEN/TS 17981-1:2023 (E)**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.

iTeh Standards
(<https://standards.itih.ai>)
Document Preview

[SIST-TS CEN/TS 17981-1:2024](https://standards.itih.ai/catalog/standards/sist/b552087a-afce-4b89-b186-b0e7d3a30c43/sist-ts-cen-ts-17981-1-2024)

<https://standards.itih.ai/catalog/standards/sist/b552087a-afce-4b89-b186-b0e7d3a30c43/sist-ts-cen-ts-17981-1-2024>

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.

CEN/TS 17981-1:2023 (E)

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*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN ISO 15189:2022 and the following apply.

ISO and IEC maintain terminology 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/>

3.1

aliquot

portion of a larger amount of homogeneous material, assumed to be taken with negligible sampling error

Note 1 to entry: The term is usually applied to fluids. Tissues are heterogeneous and therefore cannot be aliquoted.

Note 2 to entry: The definition is derived from [1] [2] [3].

[SOURCE: EN ISO 20184-3:2021, 3.1, modified — Note 2 to entry was added.]

3.2

amplicon

specific DNA fragment produced by a DNA-amplification technology, such as the polymerase chain reaction (PCR)

[SOURCE: ISO 13495:2013, 3.3.1]

3.3

analyte

component represented in the name of a measurable quantity

[SOURCE: EN ISO 17511:2021, 3.2]

CEN/TS 17981-1:2023 (E)**3.4****analytical accuracy**

closeness of the agreement between the result of an examination and a true value

Note 1 to entry: For NGS-based examinations, accuracy represents the degree of concordance (or agreement) of results between a sequence obtained from the examination and the same sequence determined by a valid comparator method, or between a reference sample run on an NGS-based examination and the high confidence sequence of the reference.

3.5**analytical sensitivity****sensitivity of a measurement procedure**

quotient of the change in a measurement indication and the corresponding change in a value of a quantity being measured

Note 1 to entry: The sensitivity of a measurement procedure can depend on the value of the quantity being measured.

Note 2 to entry: The change considered in the value of the quantity being measured must be large compared with the resolution.

Note 3 to entry: The analytical sensitivity of a measuring system is the slope of the calibration curve.

Note 4 to entry: Analytical sensitivity is often confused with positive percentage agreement (*PPA*), because similar calculations are used for both the analytical sensitivity and the *PPA*. However, the term *PPA* instead of analytical sensitivity is only used, if a reference method is not available or is not used. The *PPA* represents merely an estimation of the analytical sensitivity. *PPA* is applicable only to measurements of quantities that have the nature of a count.

[SOURCE: ISO/IEC Guide 99:2007, 4.12, modified — definition slightly changed, new Note 3 and Note 4 was added.]

3.6**analytical specificity**

capability of a measuring system, using a specified measurement procedure, to provide measurement results for one or more measurands which do not depend on each other nor on any other quantity in the system undergoing measurement

Note 1 to entry: Lack of analytical specificity is called analytical interference (see EN ISO 18113-1:2009, A.3.2).

Note 2 to entry: Specificity of a measurement procedure is not to be confused with clinical specificity (see EN ISO 18113-1:2009, A.3.16).

Note 3 to entry: VIM; JCGM 200:2012 uses the term selectivity for this concept instead of specificity.

Note 4 to entry: For qualitative and semiquantitative examination procedures, analytical specificity is determined by the ability to obtain negative results in concordance with negative results obtained by the reference method.

Note 5 to entry: Analytical specificity is often confused with negative percentage agreement (*NPA*), because the same calculations are used for both the analytical specificity and the *NPA*. However, the term *NPA* instead of analytical specificity is only used, if a reference method is not available or is not used. The *NPA* represents merely an estimation of the analytical specificity.

[SOURCE: EN ISO 18113-1:2009, A.3.4, modified — “should” was replaced by “is” and Note 5 was added.]

3.7**bioinformatics pipeline**

suite of different bioinformatics tools that process the NGS data

3.8**ccfDNA****circulating cell free DNA**

extracellular human DNA present in blood and plasma

Note 1 to entry: ccfDNA includes DNA present in vesicles such as exosomes.

[SOURCE: EN ISO 20186-3:2019, 3.5]

3.9**cfDNA****cell free DNA**

extracellular human DNA present in body liquids such as urine

Note 1 to entry: cfDNA can include DNA present in vesicles such as exosomes.

[SOURCE: CEN/TS 17811:2022, 3.8]

3.10**clinical accuracy****diagnostic accuracy**

extent of agreement between the outcome of the new examination and the reference method

3.11**clinical performance**

ability of an examination to yield results that are correlated with a particular clinical condition, physiological or pathological state in accordance with the target population and intended use(r)

Note 1 to entry: Although sometimes referred to as diagnostic performance or clinical validity; clinical performance is the harmonized term endorsed by the Global Harmonization Task Force (GHTF) and its successor, the International Medical Devices Regulators Forum (IMDRF).

Note 2 to entry: Evaluation of clinical performance often relies on the outcome of other types of clinical examinations to define “true positive or true negative” results.

CEN/TS 17981-1:2023 (E)**3.12****clinical sensitivity****diagnostic sensitivity**

ability of an *in vitro* diagnostic examination procedure to identify the presence of a target marker associated with a specific disease or condition

Note 1 to entry: percent positivity in samples where the target marker is known to be present.

Note 2 to entry: Diagnostic sensitivity is expressed as a percentage (number fraction multiplied by 100), calculated as $100 \times \frac{\text{number of true positive values (TP)}}{\text{number of true positive values (TP)} + \text{number of false negative values (FN)}}$, or $100 \times \frac{\text{TP}}{\text{TP} + \text{FN}}$. This calculation is based on a study design where only one sample is taken from each subject.

Note 3 to entry: The target condition is defined by criteria independent of the examination procedure under consideration.

Note 4 to entry: Clinical sensitivity is often confused with positive percentage agreement (*PPA*), because the same calculations are used for both the clinical sensitivity and the *PPA*. However, the term *PPA* instead of clinical sensitivity is only used, if a reference method is not available or is not used. The *PPA* represents merely an estimation of the clinical sensitivity.

[SOURCE: EN ISO 18113-1:2009, A.3.15, modified — “<laboratory medicine>” was deleted and Note 4 was added.]

3.13**clinical specificity****diagnostic specificity**

ability of an *in vitro* diagnostic examination procedure to recognise the absence of a target marker associated with a specific disease or condition

Note 1 to entry: Also defined as percent negativity in samples where the target marker is known to be absent.

Note 2 to entry: Clinical specificity is expressed as a percentage (number fraction multiplied by 100), calculated as $100 \times \frac{\text{number of true negative values (TN)}}{\text{number of true negative values (TN)} + \text{number of false positive values (FP)}}$, or $100 \times \frac{\text{TN}}{\text{TN} + \text{FP}}$. This calculation is based on a study design where only one sample is taken from each subject.

Note 3 to entry: The target condition is defined by criteria independent of the examination procedure under consideration.

Note 4 to entry: Clinical specificity is often confused with negative percentage agreement (*NPA*), because the same calculations are used for both the clinical specificity and the *NPA*. However, the term *NPA* instead of clinical specificity is only used, if a reference method is not available or is not used. The *NPA* represents merely an estimation of the clinical specificity.

[SOURCE: EN ISO 18113-1:2009, A.3.16, modified — “<laboratory medicine>” was deleted and Note 4 was added.]

3.14**clinical utility**

ability of a screening or diagnostic examination to prevent or ameliorate adverse health outcomes such as mortality, morbidity, or disability through the adoption of efficacious treatments conditioned on examination results

Note 1 to entry: Clinical utility can be part of scientific validity and clinical performance [4] [5].

[SOURCE: [4]]

3.15**clinical validity**

predictive value of a test for a given clinical outcome

Note 1 to entry: Clinical validity is primarily determined by the sensitivity and specificity with which a test identifies individuals with a defined clinical condition within a given population. The clinical validity of a genetic test is the likelihood that, e.g. cancer will develop in someone with a positive test result.

3.16**closed system**

non-modifiable system provided by the vendor including all necessary components for the examination (i.e., hardware, software, procedures and reagents)

3.17**diagnosis**

identification of a disease from its signs and symptoms, where the diagnostic process can involve examinations and tests for classification of an individual's condition into separate and distinct categories or subclasses that allow medical decisions about treatment and prognosis to be made

3.18**DNA****deoxyribonucleic acid**

polymer of deoxyribonucleotides occurring in a double-stranded (dsDNA) or single-stranded (ssDNA) form

[SOURCE: EN ISO 22174:2005, 3.1.2]

3.19**DNA sequencing**

determining the order of nucleotide bases (adenine, guanine, cytosine and thymine) in a molecule of DNA

Note 1 to entry: Sequence is generally described from the 5' end.

[SOURCE: ISO 17822:2020, 3.19]

3.20**epigenetics**

study of changes in expression or gene function that are mitotically and/or meiotically heritable and that do not entail a change in DNA sequence

[SOURCE: [6]]

CEN/TS 17981-1:2023 (E)**3.21****EV****extracellular vesicle**

particle naturally released from the cell that is delimited by a lipid bilayer and cannot replicate, i.e. does not contain a functional nucleus

EXAMPLE Exosomes, endosomes, oncosomes, apoptotic bodies.

[SOURCE: [7]]

3.22**examination****analytical test**

set of operations having the objective of determining the numerical value or characteristics of a property

[SOURCE: EN ISO 15189:2022, 3.8, modified — Notes to entry 1 to 3 have been removed. “analytical test” has been added as a preferred term.]

3.23**examination manufacturer****analytical test manufacturer**

entity that manufactures and/or produces a specific analytical test

[SOURCE: EN ISO 20166-4:2021, 3.16]

3.24**examination performance****analytical test performance****analytical performance**

ability of an examination procedure to measure or detect a particular analyte

Note 1 to entry: Analytical performance is determined from analytical performance studies used to assess the ability of an *in vitro* diagnostic examination procedure to measure or detect a particular analyte.

Note 2 to entry: Analytical performance includes such characteristics as analytical sensitivity, detection limit, analytical specificity (interference and cross-reactivity), trueness, precision and linearity.

[SOURCE: EN ISO 20186-3:2019, 3.11]

3.25**formalin**

saturated aqueous formaldehyde solution which at 100% contains 37% formaldehyde by mass (corresponding to 40 % by volume)

[SOURCE: EN ISO 20166-1:2018, 3.11]

3.26**FNA****fine needle aspirate**

specimen withdrawn by a non-operative procedure that uses a thin, hollow-bore needle

[SOURCE: CEN/TS 17688-1:2021, 3.18]