SLOVENSKI STANDARD
kSIST-TS FprCEN/TS 17981-1:2023

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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

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

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European foreword

This document (FprCEN/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.

This document is currently submitted to the Vote on TS.

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

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 (CTC's)). 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 until 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*

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 homogenous 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]

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 shall 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 — Note 4 was deleted and a new 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 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 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: 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.

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 × the number of true positive values (TP) divided by the sum of the number of true positive values (TP) plus the number of false negative values (FP), or 100 × TP/(TP + 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: 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 × the number of true negative values (TN) divided by the sum of the number of true negative plus the number of false positive values (FP), or 100 × TN/(TN + 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: 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]]

3.21
EV
extracellular vesicle
particle naturally released from the cell that are 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]

3.27
in-house examination
laboratory developed examination
examination manufactured or modified and used by health institutions or medical laboratories intended to be used only in their facility, to fulfil specific needs of target patient groups which cannot be met by an equivalent IVD examination available on the market at the appropriate level of performance

Note 1 to entry: In-house examination is also often referred to as a laboratory developed test (LDT).

3.28
intended use
intended purpose
use of a product, process or service intended for medical purposes in accordance with the specifications, instructions and information provided by the manufacturer

Note 1 to entry: The intended purpose indicates the object to be detected/measured, the examination's function (screening, monitoring, diagnosis, prognosis, companion diagnostic), if the examination is automated, specific information to be provided as a component of the device (to determine physiological/pathological state, clinical condition or predisposition, prediction to treatment response/reaction or monitoring of a therapy or non-proprietary name of the medicinal product for companion test; safety to recipients), if the examination results are qualitative or quantitative, the type of specimen required, and the intended population.

Note 2 to entry: The intended purpose directly drives the level of the performance evaluation, the examination methodology, the hardware and software, the examination limitations, positive controls, risk to health (e.g. diagnostic use or screening; consequences of false negatives (FN) or false positives (FP)), the number of samples, and the types of samples.

[SOURCE: ISO 17966:2016, 3.15, modified — Two notes were added.]

3.29
LOD
limit of detection
detection limit
measured quantity value, obtained by a given measurement procedure, for which the probability of falsely claiming the absence of a component in a material is β, given a probability α of falsely claiming its presence

Note 1 to entry: IUPAC recommends default values for α and β equal to 0,05.

Note 2 to entry: The term "sensitivity" is discouraged for this concept.

Note 3 to entry: Adapted from ISO/IEC Guide 99:2007, 4.18.

[SOURCE: ISO 15193:2009, 3.13, modified — Note 2 was deleted.]

3.30
long-read sequencing
subset of MPS methods capable of measuring nucleic acid sequences of ≥2 kbp, that can directly detect DNA bases, RNA bases and nucleic base modifications

Note 1 to entry: Long-read sequencing can be achieved by two approaches at the moment (enzymatic processing or direct reading).