



BSI Standards Publication

***In vitro* diagnostic Next Generation Sequencing (NGS) workflows**

Part 2: Human RNA examination

National foreword

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die *In-vitro*-Diagnostik - Teil 2: Untersuchung von
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Contents	Page
European foreword	4
Introduction	5
1 Scope.....	6
2 Normative references.....	6
3 Terms and definitions.....	7
4 General requirements	20
4.1 General.....	20
4.2 Examination design.....	21
4.3 Examination development	25
4.4 Examination performance verification and validation.....	25
4.5 Technical examination performance characteristics.....	30
5 Pre-examination processes for examination development	31
5.1 General.....	31
5.2 Human RNA isolation	33
5.2.1 General.....	33
5.2.2 Isolation from formalin fixed and paraffin embedded (FFPE) tissue	33
5.2.3 Isolation from fresh frozen tissue.....	33
5.2.4 Isolation from fine needle aspirates (FNA)	33
5.2.5 Isolation from whole blood	33
5.2.6 Isolation of circulating cell free RNA from plasma	34
5.3 RNA sample quality and quantity evaluation	34
6 Examination processes for examination development.....	36
6.1 Sequencing library preparation for examination development.....	36
6.1.1 General.....	36
6.1.2 Sequencing library preparation steps.....	36
6.1.3 RNA sequencing (RNA-Seq)	40
6.2 Sequencing examination development.....	43
6.2.1 General.....	43
6.2.2 Techniques	43
6.2.3 Sequencing quality control.....	44
6.3 Data analysis requirements for examination development.....	44
6.4 Quality control (QC) requirements for examination development.....	45
6.4.1 General.....	45
6.4.2 RNA Sequencing.....	45
7 Requirements for the development of the examination reporting tool	46
7.1 General.....	46
7.2 Report attributes	47
7.3 Report content	47
8 Implementation of the <i>in vitro</i> diagnostic NGS workflow into routine practice	48
9 Reporting and interpretation of results	49
10 Quality assurance procedures	50
10.1 General.....	50

10.2	Performance monitoring, optimization of the examination and interlaboratory comparison	50
Annex A (normative) <i>in vitro</i> diagnostic NGS workflow for single-cell analyses.....		51
A.1	General information and requirements on single-cell analyses	51
A.2	Pre-examination processes for examination development	52
A.2.1	General information of applicable procedures	52
A.2.2	Requirements for CTCs from blood specimen collection to CTC isolation	52
A.2.3	Requirements for fresh frozen/FFPE human tissue from specimen collection to isolation of single cells.....	52
A.2.4	Human RNA isolation	54
A.2.5	RNA sample quality evaluation.....	55
A.3	Examination phase for examination development	55
A.3.1	Sequencing library preparation for examination development for CTCs and single cells from tissues	55
A.3.2	Sequencing examination development for CTCs and single cells from tissues.....	55
A.3.3	Data analysis requirements for examination development for CTCs and single cells from tissues	56
A.3.4	QC requirements for examination development	57
A.4	Implementation of the <i>in vitro</i> diagnostic NGS workflow into routine practice.....	57
A.5	Reporting and interpretation of results.....	57
A.6	Quality assurance procedures	57
Annex B (normative) Exemplary <i>in vitro</i> diagnostic NGS workflow for spatial transcriptomics		58
Annex C (informative) <i>in vitro</i> diagnostic NGS workflow scheme for the examination of RNA		59
Bibliography		60

European foreword

This document (CEN/TS 17981-2:2023) 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 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 RNA 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. whole transcriptome, 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. 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 RNA isolation, sequencing library preparation, sequencing, sequence analysis and reporting of the examination of sequences for diagnostic purposes from isolated RNA 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, and circulating cell free RNA from plasma.

NOTE 1 Typical applications include, but are not limited to, NGS for oncology and clinical genetics, 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 organisations performing biomedical research.

This document is not applicable for in situ 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 17390-1, *Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for circulating tumor cells (CTCs) in venous whole blood — Part 1: Isolated RNA*

CEN/TS 17688-1:2021, *Molecular in vitro diagnostic examinations - Specifications for pre-examination processes for Fine Needle Aspirates (FNAs) — Part 1: Isolated cellular RNA*

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 17742, *Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for venous whole blood — Isolated circulating cell free RNA from plasma*

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 20166-1:2018, *Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for formalin-fixed and paraffin-embedded (FFPE) tissue — Part 1: Isolated RNA (ISO 20166-1:2018)*

EN ISO 20184-1:2018, *Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for frozen tissue — Part 1: Isolated RNA (ISO 20184-1:2018)*

EN ISO 20186-1, *Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for venous whole blood — Part 1: Isolated cellular RNA (ISO 20186-1)*

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 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 (SOURCE: 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

ccfRNA

circulating cell free RNA

extracellular human RNA present in blood and plasma

Note 1 to entry: ccfRNA includes RNA present in vesicles such as exosomes.

3.9**clinical accuracy****diagnostic accuracy**

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

3.10**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.

3.11**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: Also defined as 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.]

3.12

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 $100 \times \text{TN}/(\text{TN} + \text{FP})$. 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.]

3.13

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]]

3.14

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.

3.15

closed system

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

3.16

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

3.17**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]

3.18**DNase****deoxyribonuclease**

enzyme that catalyzes the degradation of DNA into smaller components

[SOURCE: EN ISO 20186-3:2019, 3.9]

3.19**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: [6]]

3.20**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.]

3.21**examination manufacturer****analytical test manufacturer**

entity that manufactures and/or produces a specific analytical test

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

3.22**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]

3.23

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]

3.24

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]

3.25

in vitro diagnostic NGS workflow

all activities required to generate the NGS examination result including the *pre-examination processes* (3.39), *examination* (3.20) and *post-examination processes* (3.38)

3.26

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).

3.27

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.]

3.28

isoform

member of a set of highly similar RNA transcripts or proteins that originate from a single gene or gene family

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 (3.33) 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).

3.31**no template control****NTC**

control reaction containing all reagents except the extracted test sample template nucleic acid

[SOURCE: ISO 20395:2019, 3.20]

3.32**MPS****massively parallel sequencing**

nucleic acid sequencing techniques that detect millions or billions of sequences from individual input nucleic acid molecules in parallel

Note 1 to entry: For DNA sequencing, alleles are analysed separately. Combining allele sequences with each other is required to determine a genotype of a donor/patient at any tested position of the genome. For RNA sequencing, locus-specific sequences are analysed by counting and/or by determining exon coverage or exon usage to determine splicing.

Note 2 to entry: Massively parallel sequencing technology can provide millions or billions of reads per run.

3.33**NGS****next generation sequencing**

high-capacity single molecule sequencing techniques

EXAMPLE MPS (3.32) including *long-read sequencing* (3.30) and *short-read sequencing* (3.61); *in situ* sequencing, etc.

3.34

panel

sequencing panel

selected set of regions of a genome enriched for sequencing focused on associations with a particular subject

3.35

performance characteristic

metrological property

one of the parameters used to define the minimum acceptable levels of critical properties of an IVD medical device

EXAMPLE Detection limit, precision, specificity.

Note 1 to entry: Information about more than one performance characteristic is usually required to evaluate the suitability of an IVD medical device for its intended medical use.

[SOURCE: EN ISO 18113-1:2009, 3.50 modified — Replaced “performance” in the definition with “minimum acceptable levels of critical properties” to avoid a circular definition]

3.36

performance specification

document that defines what the customer desires as a product, its operational environments and all required minimum acceptable levels of critical properties characteristics

[SOURCE: ISO 14621-1:2019, 3.1.5 modified — Replaced “performance” in the definition with “minimum acceptable levels of critical properties” to avoid a circular definition]

3.37

pharmacogenomics

approach to establish the correlation of genetic differences among individuals that cause varied responses to the same therapeutic drug and with developing drug therapies to compensate for these differences

3.38

post-examination processes

post-examination phase

processes following the examination including review of results, formatting, releasing, reporting and retention of examination results, retention and storage of clinical material, sample and waste disposal

[SOURCE: EN ISO 15189:2022, 3.23, modified — The alternative term “post-examination phase” was added.]

3.39

pre-examination processes

pre-examination phase

processes that start, in chronological order, from the user's request and include the examination request, preparation and identification of the patient, collection of the primary sample(s), transportation to and within the laboratory, isolation of analytes, ending when the examination begins

Note 1 to entry: The pre-examination phase includes preparative processes that influence the outcome of the intended examination.

[SOURCE: EN ISO 15189:2022, 3.24, modified — Note 1 was added, the alternative term “pre-examination phase” was added, and “, isolation of analytes,” was added.]

3.40**proficiency testing**

evaluation of participant performance against pre-established criteria by means of inter-laboratory comparisons

[SOURCE: EN ISO/IEC 17043:2010, 3.7, modified — Term and definition are used here without the original notes.]

3.41**protein**

type of biological macromolecules composed of one or more chains with a defined sequence of amino acids connected through peptide bonds

[SOURCE: EN ISO 20166-2:2018, 3.14]

3.42**proteinase**

enzyme that catalyses the degradation of proteins into smaller components

3.43**reference material****RM**

material, sufficiently homogeneous and stable regarding one or more properties, used in calibration, assignment of a value to another material, or quality assurance

Note 1 to entry: “Reference material” comprises materials embodying quantities as well as nominal properties.

Note 2 to entry: Adapted from ISO/IEC Guide 99:2007, 5.13.

EXAMPLE 1 Human serum with an assigned quantity value for the amount-of-substance concentration of cholesterol, used only as a calibrator, embodies a quantity.

EXAMPLE 2 DNA compound containing a specified nucleic acid sequence embodies a nominal property.

Note 3 to entry: In this definition, value covers both “quantity value” and “nominal property value”.

Note 4 to entry: Some reference materials have quantities which are metrologically traceable to a measurement unit outside a system of units. Such materials include those containing antibodies to which International Units (IU) have been assigned by the World Health Organization.

Note 5 to entry: A reference material is sometimes incorporated into a specially fabricated device, e.g.

- glass of known optical density in a transmission filter holder;
- spheres of uniform particle size mounted on a microscope slide; and
- calibration plate for microtiter plate reader.

[SOURCE: ISO 15194:2009, 3.4]

3.44

reference method

reference measurement procedure

measurement procedure accepted as providing measurement results fit for their intended use in assessing measurement trueness of measured quantity values obtained from other measurement procedures for quantities of the same kind, in calibration, or in characterizing reference materials

[SOURCE: ISO/IEC Guide 99:2007, 2.7]

3.45

reference range

reportable sequence variations that the examination can detect and that are considered benign or non-pathogenic based on accepted scientific evidence

3.46

repeatability

measurement repeatability

measurement precision under a set of *repeatability conditions of measurement* (3.47)

[SOURCE: ISO 17123-1:2014, 3.2.5]

3.47

repeatability condition of measurement

repeatability condition

condition of measurement that includes the same measurement procedure, same operators, same measuring system, same location (laboratory or usual building), and replicate measurements on the same object over a short period of time

[SOURCE: ISO/IEC Guide 99:2007, 2.20, modified — The Notes have been deleted. In the definition, “out of a set of conditions” and “same operating conditions” have been removed and “(laboratory or usual building)” has been added after “location”.]

3.48

reportable range

span of sequences covering the region(s) targeted by the examination that fulfil the performance characteristics

3.49

reproducibility

measurement reproducibility

measurement precision under *reproducibility conditions of measurement* (3.50)

Note 1 to entry: Relevant statistical terms are given in ISO 5725-1:2023 and ISO 5725-2:2019.

[SOURCE: ISO/IEC Guide 99:2007, 2.25]

3.50

reproducibility condition of measurement

reproducibility condition

condition of measurement that includes different laboratories, operators, measuring systems, and replicate measurements on the same or similar objects

[SOURCE: ISO/IEC Guide 99:2007, 2.24, modified — The Notes have been deleted. In the definition, “out of a set of conditions” has been removed and “locations” has been replaced by “laboratories”.]

3.51**reverse transcription****RT**

process of making cDNA from an RNA template, using the enzymatic activity of a reverse transcriptase associated with one or more oligonucleotide primers under a suitable set of conditions

[SOURCE: ISO 20395:2019, 3.27]

3.52**RT-dPCR****reverse transcription dPCR**

process by which an RNA strand is first reverse transcribed into its DNA complement (complementary DNA or cDNA) using reverse transcriptase and the resulting cDNA is amplified using dPCR

Note 1 to entry: This process can be one- or two-step.

Note 2 to entry: In one-step RT-dPCR, RT and dPCR amplification steps are performed sequentially, in the same tube with gene-specific primers.

Note 3 to entry: In two-step RT-dPCR, RT and dPCR stages are performed as two independent reactions. In this case, the RT step can use non-specific primers (i.e. a blend of oligo-dT primers and/or random oligonucleotides) to produce a global cDNA population from all transcripts in the RNA sample. The cDNA is then used for subsequent analysis by dPCR and interrogated for the sequences of interest using gene-specific PCR primers.

[SOURCE: ISO 20395:2019, 3.30]

3.53**RT-qPCR****reverse transcription qPCR**

process by which an RNA strand is first reverse transcribed into its DNA complement (complementary DNA or cDNA) using reverse transcriptase and the resulting cDNA is amplified using qPCR

Note 1 to entry: This process can be one- or two-step as is the case for RT-dPCR.

[SOURCE: ISO 20395:2019, 3.31]

3.54**RNA****ribonucleic acid**

polymer of ribonucleotides occurring in a double-stranded or single-stranded form

[SOURCE: EN ISO 22174:2005, 3.1.3]

3.55**RNase****ribonuclease**

enzyme that catalyses the degradation of RNA into smaller components

[SOURCE: EN ISO 20186-2:2019, 3.21]

3.56

**RNA-Seq
transcriptome sequencing
RNA sequencing**

technique that determines the complete or partial RNA sequence

Note 1 to entry: The RNA profiles vary in different tissues of the same organism and can be influenced by physiopathological circumstances.

[SOURCE: ISO/TS 22693:2021, 3.16, modified — “of an organism's genome” was removed and Note 1 was added.]

3.57

sample

one or more parts taken from a primary sample

[SOURCE: EN ISO 15189:2022, 3.28]

3.58

scientific validity

scientifically proven association of an analyte with a clinical condition or a physiological state

EXAMPLE Scientific publication, results from clinical performance studies, expert opinions.

3.59

sequencing library

library

DNA, cDNA or RNA that has been prepared for NGS within a specific size range and containing adapters, which are designed to be appropriate for (a) specific sequencing platform(s)

3.60

sequencing library preparation

library preparation

procedures used to prepare DNA or RNA fragments containing tags, and sequencing primer binding regions for NGS

3.61

short-read sequencing

subset of MPS methods where nucleic acid sequences on the length-order of up to several hundred bases are measured

Note 1 to entry: Short-read sequencing can be achieved by enzymatic processing.

Note 2 to entry: Short-read sequencing or MPS typically generates sequences < 2 kbp.

3.62**specimen
primary sample**

discrete portion of a body fluid or tissue or other sample associated with the human body taken for examination, study or analysis of one or more quantities or characteristics to determine the character of the whole

Note 1 to entry: The International Medical Device Regulators Forum (IMDRF) uses the term specimen in its harmonized guidance documents to mean a sample of biological origin intended for examination by a medical laboratory.

[SOURCE: EN ISO 15189:2022, 3.25]

3.63**stability**

ability of a sample material, when stored under specified conditions, to maintain a stated property value within specified limits for a specified period of time

[SOURCE: ISO Guide 30:2015, 2.1.15, modified — The phrase “reference material” has been replaced by “sample material”.]

3.64**validation**

confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled

Note 1 to entry: The term “validated” is used to designate the corresponding status.

[SOURCE: ISO 9000:2015, 3.8.13, modified — Note 1 and Note 3 have been omitted.]

3.65**venous whole blood**

blood collected after directly puncturing a vein, usually with a needle and syringe, or other collection device

[SOURCE: EN ISO 20186-2:2019, 3.26]

3.66**verification**

confirmation, through the provision of objective evidence, that specified requirements have been fulfilled

Note 1 to entry: The term “verified” is used to designate the corresponding status.

Note 2 to entry: Confirmation can comprise activities such as:

- performing alternative calculations;
- comparing a new design specification with a similar proven design specification;
- undertaking tests and demonstrations;
- reviewing documents prior to issue.

[SOURCE: ISO 9000:2015, 3.8.12, modified — Note 1 and Note 2 have been omitted.]