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Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for saliva — Isolated human DNA

Analyse de diagnostic moléculaire in vitro — Spécifications relatives aux processus préanalytiques pour la salive — ADN humain extrait

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 212, *Clinical laboratory testing and in vitro diagnostic test systems*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 140, *in vitro diagnostic medical devices*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Molecular in vitro diagnostics has enabled a 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. However, the profiles of these molecules can change drastically during specimen collection, transport, storage and processing thus 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.

Genetic examination of DNA is commonly used in clinical practice. This includes e.g. predisposition testing, pharmacogenomics, analysis of genetic disorders with the perspective used in precision medicine. This is a fast-growing field in molecular diagnostics.

Saliva is increasingly used as a non-invasive alternative specimen to blood for the examination of human DNA. Saliva naturally contains microorganisms and extraneous substances (e.g. food debris), which make the composition of saliva more complex and unique among patients/donors. Dedicated measures are therefore needed for informing and preparing patients/donors for the collection and to check compliance with the instructions, in order to reduce the specimen variability. In contrast to invasive specimen collection, saliva collection does not require trained and educated professionals or dedicated facilities. By good instruction and verified collection device safety claims, saliva specimens can be self-collected at home; however, home collection also contributes to high variability in specimen quality. Similarly, medical laboratories/in vitro manufacturers need to be aware of specimen variability when performing design verification and validation.

DNA in saliva can fragment or degrade after collection. In addition, bacteria present in the saliva specimen can continue to grow, thus diluting the human DNA. DNases secreted by these bacteria can also accelerate the DNA degradation. This can impact the sensitivity and reliability of DNA examination.

Standardization of the entire process from specimen collection to the DNA examination is needed to minimize pre-examination impacts such as DNA degradation and fragmentation after saliva collection. This document describes special measures which need to be taken to obtain good quality saliva specimen/samples and isolated DNA therefrom for human DNA examination.

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.

Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for saliva — Isolated human DNA

1 Scope

This document specifies requirements and recommendations on the handling, storage, processing and documentation of saliva specimens intended for human DNA examination during the pre-examination phase before a molecular examination is performed.

This document is applicable to molecular in vitro diagnostic examination including laboratory developed tests performed by medical laboratories. It can also be used by laboratory customers, in vitro diagnostics developers and manufacturers, biobanks, institutions and commercial organizations performing biomedical research, and regulatory authorities.

Dedicated measures that need to be taken for saliva collected on absorbing material or by mouth washes are not described in this document. Neither are measures for preserving and handling of native saliva cell-free DNA, pathogens, and other bacterial or whole microbiome DNA in saliva described.

NOTE International, national or regional regulations or requirements can also apply to specific topics covered in this document.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 15189, *Medical laboratories — Requirements for quality and competence*

ISO 15190, *Medical laboratories — Requirements for safety*

3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
ambient temperature
unregulated temperature of the surrounding air

[SOURCE: ISO 20184-1:2018, 3.2]

3.2
analyte
component represented in the name of a measurable quantity

[SOURCE: ISO 17511:2003, 3.2, modified — The examples were not taken over.]

3.3
examination performance
analytical test performance
analytical performance
accuracy, precision, and sensitivity of a test to measure the *analyte* (3.2) of interest

Note 1 to entry: Other test performance characteristics such as robustness, repeatability can apply as well.

[SOURCE: ISO 20184-1:2018, 3.4]

3.4
DNA stabilizers
compounds, solutions or mixtures that are designed to minimize degradation and fragmentation of *DNA* (3.6)

[SOURCE: ISO 20186-2:2019, 3.5, modified — The term "genomic DNA in blood" has been replaced with "DNA".]

3.5
closed system
non-modifiable system provided by the vendor including all necessary components for the analysis (i.e. hardware, software, procedures and reagents)

[SOURCE: ISO 20186-2:2019, 3.6]

3.6
DNA
DNA
polymer of deoxyribonucleotides occurring in a double-stranded (dsDNA) or single-stranded (ssDNA) form

[SOURCE: ISO 22174:2005, 3.1.2]

3.7
examination
analytical test
set of operations having the object of determining the value or characteristics of a property

Note 1 to entry: Processes that start with the isolated measurand and include all kinds of parameter testing or chemical manipulation for quantitative or qualitative examination.

[SOURCE: ISO 15189:2012, 3.7, modified — The term and definition is used here without the original notes.]

3.8
examination provider
analytical test provider
entity that provides the specific analytical test

3.9
interfering substance
endogenous or exogenous substance (e.g. stabilization solution) that can be present in specimens and that can alter an examination result

[SOURCE: ISO 20184-1:2018, 3.12]

3.10
microorganism
entity of microscopic size, encompassing bacteria, fungi and protozoa

[SOURCE: ISO 11139:2018, 3.176, modified — The term "viruses" was deleted from the definition.]

3.11
pre-examination process
pre-analytical phase
pre-analytical workflow
process that starts, in chronological order, from the clinician's request and include the examination request, preparation and identification of the patient, collection of the *primary sample(s)* (3.12), transportation to and within the analytical laboratory, isolation of *analytes* (3.2), and ends when the analytical examination begins

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

[SOURCE: ISO 15189:2012, 3.15, modified — An additional term was added, and more detail was included.]

3.12
primary sample
specimen
discrete portion of a body fluid, breath, hair or tissue taken for examination, study or analysis of one or more quantities or properties assumed to apply for the whole

[SOURCE: ISO 15189:2012, 3.16, modified — The term and definition is used here without the original notes.]

3.13
proficiency testing
evaluation of participant performance against pre-established criteria by means of interlaboratory comparisons

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

3.14
room temperature
temperature in the range of 18 °C to 25 °C, for the purpose of this document

Note 1 to entry: Local or national regulations can have different definitions.

[SOURCE: ISO 20186-2:2019, 3.22]

3.15
saliva
whole saliva
bio-fluid of the mouth composed mainly of secretion originating from the three major salivary glands (parotids, submandibular and sublingual glands) and from salivary glands present in the oral cavity

3.16
saliva collection device
tube or other container in which the *saliva* (3.15) *specimen* (3.12) is collected

3.17
sample
one or more parts taken from a *primary sample* (3.12)

[SOURCE: ISO 15189:2012, 3.24, modified — The examples were not taken over.]

3.18
stability
ability of a *specimen* (3.12)/*sample* (3.17) material, when stored under specified conditions, to maintain a defined property value within specified limits for a specified period of time

Note 1 to entry: The measurand constituent for the purpose of this document is isolated *DNA* (3.6).

[SOURCE: ISO Guide 30:2015, 2.1.15, modified — Note 1 was not taken over. The following words were replaced: “characteristic” by “ability”; “reference material” by “sample material”; “specified” by “defined”.]

3.19
storage
prolonged interruption of the *pre-analytical workflow* (3.11) of a *sample* (3.17) or *analyte* (3.2) respectively, or of their derivatives, under appropriate conditions in order to preserve their properties

Note 1 to entry: Long-term storage typically occurs in laboratory archives or in biobanks.

[SOURCE: ISO 20184-1:2018, 3.22, modified — Example in the definition was deleted.]

3.20
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 word “validated” is used to designate the corresponding status.

[SOURCE: ISO 9000:2015, 3.8.13, modified — Note 1 and 3 were not taken over.]

3.21
verification
confirmation, through provision of objective evidence, that specified requirements have been fulfilled

Note 1 to entry: The word “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; and

— reviewing documents prior to issue.

[SOURCE: ISO 9000:2015, 3.8.12, modified — Note 1 and Note 2 were not taken over.]

3.22
workflow
structured series of activities necessary to complete a task

[SOURCE: ISO 20184-1:2018, 3.26, modified — The word “structured” was added]

4 General considerations

For general statements on medical laboratory quality management systems and in particular on primary sample collection, reception and handling (including avoidance of cross contaminations) see ISO 15189 or ISO/IEC 17020. The requirements on laboratory equipment, reagents, and consumables according to ISO 15189 shall be followed; ISO 15189 and ISO/IEC 17020 can also apply. For general considerations on specimen collection, transport, receipt, handling, and storage, see ISO/TS 20658. For biobanking, ISO 20387 can also apply.

All steps of a diagnostic workflow can influence the final examination result. Thus, the entire workflow, including specimen/sample storage and transport conditions, and their impact on the stability of biomolecules intended to be examined shall be specified, verified and validated for its intended use. This includes the development of in vitro diagnostic (IVD) medical devices. The stability of the human DNA should be investigated throughout the complete pre-examination process development. The verification of performance claims as well as the validation of the intended examination shall take into account the variability of the saliva specimen's quality.

During the design and development of a saliva DNA based examination, a risk assessment shall be performed (see also ISO 14971). Mitigation measures for eliminating or reducing identified risks shall be established where required for ensuring the performance of the examination. This shall include the pre-examination workflow steps.

Before or during the design of an examination, it should be investigated and ensured that the human DNA quality parameters such as minimum DNA amount, size and purity required for the examination is/are not compromised in a manner impacting the examination performance.

Safety requirements on specimen collection, transport and handling shall be in accordance with relevant ISO standards such as ISO 15189 and ISO 15190.

During the whole pre-examination process, precautions shall be taken to avoid cross contamination between different specimens/samples, for example by using single-use material whenever feasible or appropriate cleaning procedures between processing of different specimens/samples.

For all pre-examination steps, the examination manufacturer's instructions shall be followed, if provided.

Where, for justified reasons (e.g. unmet patient needs), a commercial product is not used in accordance with the manufacturer's instructions, responsibility for its verification, validation, use and performance lies with the laboratory.

The manufacturer's material safety data sheet should be considered before first use of any potentially hazardous material (e.g. chemicals in stabilizers).

5 Activities outside the laboratory

5.1 Specimen collection

5.1.1 Information about the specimen donor/patient

The documentation shall include the identity of the specimen donor/patient, which can be in the form of a code.

The documentation should include, but is not limited to:

a) the relevant health status of the primary sample donor/patient [e.g. healthy, disease type, concomitant disease, demographics (e.g. age and sex)];

b) the information about medical treatment and special treatment prior to saliva collection (e.g. anaesthetics, medications);

c) the type and the purpose of the examination requested;

d) the appropriate consent from the specimen donor/patient. See also ISO 15189.

5.1.2 Selection of the saliva collection device by the laboratory

The Saliva DNA examination manufacturer instructions should contain specifications on the saliva collection device(s) to be used. Where the examination manufacturer specifies usage of dedicated saliva collection device(s), these shall be used.

Where the examination manufacturer does not provide such specifications (e.g. due to former less stringent legal frameworks), the saliva collection device(s) shall be specified, verified, validated and documented by the laboratory.

The quality and quantity of human DNA can be influenced by inadequate saliva collection procedures, inappropriate storage/shipping conditions as well as DNA isolation procedures[6],[7].