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Molecular in vitro diagnostic examinations — Specifications for pre-examination processes in metabolomics in urine, venous blood serum and plasma

Analyses de diagnostic moléculaire in vitro — Spécifications relatives aux processus préanalytiques pour l'analyse du métabolome dans l'urine et le sang veineux (sérum et plasma)

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Contents

Page

Foreword

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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).

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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

Metabolomics is the "-omic" science that deals with the characterization of the metabolome, in turn defined as the whole set of small molecules (molecular mass <2 000 Da) in a certain biological system such as a cell, a tissue, an organ, or an entire organism[1]. The analyses are mainly performed via two major analytical techniques, namely mass spectrometry (MS) and nuclear magnetic resonance (NMR) [2][3][4]. The former has a sensitivity that can be as low as picomolar, requires sample separation and multiple experimental runs targeted to specific classes of compounds. The latter measures metabolites present at concentration above 1 µM and is mainly used for untargeted analyses, where all metabolites above the detection limit are observed simultaneously, independent of their chemical nature, without any separation procedure.

The metabolome is dynamic and quite sensitive to perturbations. The metabolome can change drastically during primary sample collection, transport, storage, and processing. As a result, the outcome from the diagnostic and research measurements could become an unreliable representation of the specific targeted physiological state or point in time, but instead describes an artificial profile generated during the pre-examination process. Pre-analytical variations have been identified to originate from two main sources:

a) enzymatic activity in the samples, mainly related to the presence of cells;

b) chemical reactions (e.g. redox reactions) among metabolites or between metabolites and oxygen, see References [5] to [11].

Moreover, the analyses can be influenced by the use of additives or by the introduction of contaminants, and therefore the selection of appropriate collection tubes and plasticware is also a critical aspect of the pre-examination phase.

Studies have been undertaken to establish the best pre-examination procedures in terms of maintenance of the original sample metabolome by identifying the critical steps and parameters affecting the metabolome composition. Additionally, standardization of the entire pre-examination workflow is needed to ensure comparability in multicentre studies. At the present state of the art, there are no defined pre-examination procedures for metabolomic samples. As a consequence, the procedures adopted by the various centres differentially influence the metabolome of the samples, making their comparison unreliable. The adoption of the present requirements for the pre-examination phase make it possible to compare and evaluate the results obtained from metabolic analysis.

This document draws upon such studies to codify and standardize the steps for urine, serum and plasma metabolomics analysis in what is referred to as the pre-analytical phase.

Molecular in vitro diagnostic examinations — Specifications for pre-examination processes in metabolomics in urine, venous blood serum and plasma

1 Scope

This document specifies requirements and gives recommendations for the handling, documentation and processing of urine, venous blood plasma and serum intended for metabolomics analysis in the pre-examination processes. This document is applicable to metabolomics examinations and can be used by biomedical laboratories, customers of laboratories, in vitro diagnostics developers and manufacturers, institutions and companies performing biomedical research, biobanks, and regulatory authorities.

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
biofluid
biological fluid which can be excreted (such as urine or sweat), secreted (such as breast milk, saliva or bile), obtained with a needle (such as blood or cerebrospinal fluid), or produced as a result of a pathological process (such as blister or cyst fluid)

3.2
examination
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 analyte and include all kinds of parameter testing or chemical manipulation for quantitative or qualitative examination.

Note 2 to entry: For metabolomic analysis, analyte isolation is not necessarily required.

[SOURCE: ISO 20166-1:2018, 3.10, modified — admitted term “analytical test” has been deleted and Note 2 entry has been added.]

3.3
fasting
abstinence from any solid or liquid food excluding water for at least 8 hours

3.4
mass spectrometry
MS
method used to analyse chemical compounds on the basis of their mass to charge ratio

3.5
metabolic profiling
use of analytical platforms to simultaneously measure the ensemble of *metabolites* (3.6) in biological systems that can be measured by the employed (or selected) technique

EXAMPLE Examples for such techniques are NMR and MS.

3.6
metabolites
small molecules (≤ 2 000 Da) that are intermediates and/or products of metabolism of the host organisms, of its microflora, deriving from food, drinks, drugs or pollutants.

Note 1 to entry: For further information see Reference [1].

3.7
metabolome
complete set of *metabolites* (3.6) to be found within an organism or a biological sample

Note 1 to entry: For further information see Reference [1]

3.8
metabolomics
comprehensive analysis of the *metabolome* (3.7) of a biological *specimen* (3.14) (e.g., organism, cell, tissue or *biofluids* (3.1))

3.9
MS-based metabolomics
use of *mass spectrometry* (3.4) to measure *metabolites* (3.6) in biological samples

3.10
nuclear magnetic resonance spectroscopy
NMR
method based on the selective absorption of high frequency radio waves by atomic nuclei subjected to a stationary magnetic field

Note 1 to entry: NMR provides chemical and structural properties of molecules.

3.11
NMR-based metabolomics
use of *NMR spectroscopy* (3.10) to measure *metabolites* (3.6) in biological samples

3.12
plasma
liquid part of unclotted blood

Note 1 to entry: Plasma samples can contain anti-coagulants.

3.13
pre-examination processes
preanalytical phase
preanalytical workflow
processes that start, in chronological order, from the clinician's request and include the *examination* (3.2) request, preparation and identification of the patient, collection of the primary sample(s), temporary storage, transportation to and within the analytical laboratory, aliquoting, retrieval

Note 1 to entry: The preanalytical phase can include preparative processes that can influence the outcome of the intended *examination* (3.2).

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

3.14
primary sample
specimen
discrete portion of a body fluid, breath, hair or tissue taken for *examination* (3.2), 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 are used here without the original notes.]

3.15
room temperature
temperature which is defined as 18 °C to 25 °C for the purpose of this document

3.16
serum
liquid that can be separated from clotted blood

3.17
stability
characteristic of a reference material, when stored under specified conditions, to maintain a specified property value within specified limits for a specified period of time

[SOURCE: ISO Guide 30:2015, 2.1.15 — The term and definition are used here without the original note.]

4 General considerations

For general statements on medical laboratory quality management systems and in particular on specimen 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/IEC 17020 can also apply.

All steps of a diagnostic workflow can influence the final analytical test result and 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. It shall especially be investigated and ensured that the metabolites intended to be analysed are not compromised in a manner impacting the examination performance. This can be done, e.g. by applying the intended examination to specimens/samples which underwent time course studies reflecting the individual pre-examination process steps such as transport and storage and by implementing measures to prevent or reduce impacts by the identified pre-analytical variables.

In the absence of suitable specimen stabilization technologies, regarding the metabolome, the specimen collection shall be carried out in hospital premises or institutions where there are immediate suitable biofluid processing procedures available.

Specifically, for specimens intended to be analysed by metabolomics, the following steps shall be considered:

a) patient pre-treatment (fasting, therapy, etc.);

b) the specimen collection from the patient;

c) the selection of collection containers and packages (e.g. collection tubes, cooling box, box for storing and transportation);

d) the selection of stabilization procedures (e.g. any compounds added for stabilizing the specimen);

e) the recording of any additions or modifications to the specimen;

f) the recording of types and quantity as well as description of specimens.

Safety requirements for facilities, transport and handling shall be considered in accordance with ISO 15189 and ISO 15190. WHO Guidelines for the Safe Transport of Infectious Substances and Diagnostic Specimens[14] should be followed.

5 Urine

5.1 Outside the laboratory

5.1.1 Urine collection

5.1.1.1 General

For the collection of the specimen the requirements (e.g. disease condition, specimen size) for the intended molecular examination shall be considered.

See also ISO 15189.

5.1.1.2 Information about the specimen donor/patient

The documentation shall include the ID 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 health status and relevant lifestyle factors of the urine donor [e.g. healthy, disease type, concomitant disease(s)];

b) demographics (e.g., age, sex);

c) the information about medical treatment and any treatment prior to urine collection (e.g. anaesthetics, medications, diagnostic procedures);

d) the collection time, including information about fasting, previous activities.

e) the appropriate consent from the specimen donor/patient.

5.1.1.3 Selection and labelling of collection containers

The laboratory shall define the container intended for urine collection.

Additives are usually not used, because they can interfere with the analytical method. If they are required for specific purposes, their impact on the analytical performance and outcome shall be analysed. Some additives in collection tubes could present a risk to patients (e.g. toxic or corrosive).

For the labelling (specimen identification) of the urine collection tube a routine procedure (see also ISO 15189) or a procedure with additional information (e.g. 2D-barcode) shall be used.

5.1.1.4 Urine collection and reception from the specimen donor

5.1.1.4.1 General

Instruction for the urine collection shall be given to the donor, including any safety measures that need to be followed while handling collection containers containing harmful additives. All urine collection

devices should be checked for compatibility with metabolomics, e.g. avoiding any interference with the metabolomics profile.

NOTE For non-toilet-trained children, the most popular non-invasive method used is the clean catch, which National Institute for Health and Clinical Excellence (NICE), 2007 defines as a gold standard. This involves catching a sample by holding a sterile specimen bottle in the urine stream. Urine collection bags and urine collection pads can also be used to collect urine. NICE suggests urine collection pads as the next best option to clean catch.

The first midstream urine of the morning should be collected after a minimum of 8 h fasting. Drinking can influence urine metabolite concentrations. This requires a normalization. Specify, if collected at different times, or for 24 h collection. Any variations to instructions shall be validated. Fasting enables to perform the metabolomics analysis of urine where donors are synchronized having similar metabolic conditions. Research or dedicated analytical tests can require different patient conditions.

A sufficient volume of urine shall be collected according to the requirements of the preanalytical preparation steps and the analytical test.

Any clinical procedure affecting the specimen collection shall be documented. The total collected volume shall be documented.

5.1.1.4.2 Information on the urine specimen and storage requirements at the urine collection site

As metabolic profiles can change after urine collection and can thereby affect the validity and reliability of the analytical test result, the documentation on the primary urine specimen shall include the time and date of urine collection.

The whole urine specimen should be kept refrigerated at 2 °C to 8 °C for a maximum of 2 h and shall not be frozen prior to centrifugation and/or filtration to avoid cell disruption upon ice crystal formation, unless specified differently by the analytical test.

The allowed urine specimen total storage duration includes the time for storage at the point of urine collection, transportation to the testing laboratory and further storage at the testing laboratory or other institutions.

5.1.2 Transport requirements

During transport, the specimen should be kept cool (temperature range 2 °C to 8 °C).

Appropriate measures shall be taken to secure temperature specifications and to reduce time for the delivery, which should be completed within 2 h from collection.

The conformity with the protocol for the transport procedure shall be documented. Any deviations from the protocol shall be described and documented.

WHO Guidelines for the Safe Transport of Infectious Substances and Diagnostic Specimens[14] should be followed.

5.2 Inside the laboratory

5.2.1 Specimen reception

The urine specimen reception time and conditions (e.g. labelling, transport conditions, volume, leaking and precipitation) of the received specimens shall be documented. Nonconformities of labelling, transport conditions and urine volume differences to specifications described for the urine collection or specimen preparation requirements shall be documented.