INTERNATIONAL STANDARD

ISO 20186-3

First edition
2019-09

Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for venous whole blood —

Part 3: Isolated circulating cell free DNA from plasma

Analyses de diagnostic moléculaire in vitro — Spécifications relatives aux processus préanalytiques pour le sang total veineux —

Partie 3: ADN libre circulant extrait du plasma

Reference number
ISO 20186-3:2019(E)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Fax: +41 22 749 09 47
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

Foreword iv
Introduction v
1 Scope 1
2 Normative references 1
3 Terms and definitions 1
4 General consideration 5
5 Outside the laboratory 5
5.1 Specimen collection 5
5.1.1 Information about the specimen donor/patient 5
5.1.2 Selection of the venous whole blood collection tube by the laboratory 6
5.1.3 Venous whole blood collection from the donor/patient and stabilization procedures 6
5.1.4 Information about the specimen and storage requirements at the blood collection facility 7
5.2 Transport requirements 7
6 Inside the laboratory 8
6.1 Specimen reception 8
6.2 Storage requirements for blood specimens 8
6.3 Plasma preparation 9
6.4 Storage requirements for plasma samples 9
6.5 Isolation of the ccfDNA 10
6.5.1 General 10
6.5.2 Using blood collection tubes with stabilizers 10
6.5.3 Using blood collection tubes without stabilizers 11
6.6 Quantity and quality assessment of isolated ccfDNA 11
6.7 Storage of isolated ccfDNA 11
6.7.1 General 11
6.7.2 ccfDNA isolated with commercially available kits 12
6.7.3 ccfDNA isolated with the laboratory's own protocols 12
Annex A (informative) **Impact of pre-examination process steps on circulating cell free DNA profiles in venous whole blood plasma** 13
Bibliography 16

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*.

A list of all parts in the ISO 20186 series can be found on the ISO website.

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 the pre-examination process, including the specimen collection, transport, storage and processing. Consequently, this makes the outcome from diagnostics or research unreliable or even impossible because the subsequent examination might not determine the real situation in the patient, but an artificial profile generated during the pre-examination processes.

Circulating cell free DNA (ccfDNA) profiles can change significantly after blood collection (e.g. release of genomic DNA from cells in blood, ccfDNA degradation and fragmentation and ccfDNA quantity change). Therefore, special measures need to be taken to secure good quality specimens for ccfDNA examination. Studies have been undertaken to determine the important influencing factors[23].

Standardization of the entire workflow from specimen collection to the ccfDNA examination is needed.

This document standardizes the steps of the pre-examination phase of circulating cell free DNA prepared from plasma of venous whole blood.

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 venous whole blood —

Part 3: Isolated circulating cell free DNA from plasma

1 Scope

This document provides recommendations and requirements on the handling, storage, processing and documentation of venous whole blood specimens intended for circulating cell free DNA (ccfDNA) examination during the pre-examination phase before an analytical test is performed. This document covers specimens collected in venous whole blood collection tubes.

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

Different dedicated measures are taken for stabilizing blood genomic DNA, which are not described in this document. Blood genomic DNA is covered in ISO 20186-2.

Different dedicated measures are taken for preserving DNA in circulating exosomes, which are not described in this document.

NOTE ccfDNA obtained from blood by the procedures cited in this document can contain DNA originally present in exosomes[8][9].

DNA in pathogens present in blood is not covered by 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 15189:2012, *Medical laboratories — Requirements for quality and competence*

3 Terms and definitions

For the purposes of this document, the following terms and definitions 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
analyte
component represented in the name of a measurable quantity

[SOURCE: ISO 17511:2003, 3.2, modified — The example has been deleted.]

3.2
backflow
flow of a liquid opposite to the usual or desired direction

3.3
blood collection set
intravenous device specialized for venipuncture consisting of a stainless steel beveled needle and tube (tubing) with attached plastic wings and fitting connector

Note 1 to entry: The connector attaches to an additional blood collection device, e.g. a blood collection tube.

3.4
blood collection tube
tube used for blood collection, usually with a vacuum which forces blood from the vein through the needle into the tube

3.5
ccfDNA
circulating cell free DNA
extracellular human DNA present in blood and plasma

Note 1 to entry: ccfDNA can include DNA present in vesicles such as exosomes[8][9].

3.6
ccfDNA profile
circulating cell free DNA profile
amount of different ccfDNA molecules, present in blood and plasma that can be measured in the absence of any losses, inhibition and interference

3.7
closed system
non-modifiable system provided by the vendor including all necessary components for the pre-examination and/or examination (i.e. hardware, software, procedures and reagents)

3.8
DNA
deoxyribonucleic acid
polymer of deoxyribonucleotides occurring in a double-stranded (dsDNA) or single-stranded (ssDNA) form

[SOURCE: ISO 22174:2005, 3.1.2]

3.9
DNase
deoxyribonuclease
enzyme that catalyzes the degradation of DNA into smaller components

3.10
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 analyte and include all kinds of parameter testing or chemical manipulation for quantitative or qualitative examination.

[SOURCE: ISO 15189:2012, 3.7, modified —"analytical test" has been added as additional preferred term; Notes to entry have been deleted; new Note 1 to entry has been added.]

3.11
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: ISO/TS 17822-1:2014, 3.2, modified — Two preferred terms have been added.]

3.12
examination provider
analytical test provider
entity that provides the specific analytical test

3.13
needle holder
barrel used in routine venipuncture procedures to hold the blood collection tube in place and to protect the phlebotomist from direct contact with blood

3.14
pre-examination processes
preanalytical phase
preanalytical workflow
processes that start, 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), transportation to and within the medical laboratory, isolation of analytes, and end when the analytical examination begins

Note 1 to entry: The pre-examination phase includes preparative processes, e.g. ccfDNA isolation procedures, which influence the outcome of the intended examination.

[SOURCE: ISO 15189:2012, 3.15, modified — An additional term has been added and more detail have been included in the definition; Note 1 to entry has been added.]

3.15
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 — Notes to entry have been deleted.]

3.16
proficiency testing
evaluation of participant performance against pre-established criteria by means of inter-laboratory comparisons

[SOURCE: ISO/IEC 17043:2010, 3.7, modified — Notes have been deleted.]

3.17
RNA
ribonucleic acid
polymer of ribonucleotides occurring in a double-stranded or single-stranded form

[SOURCE: ISO 22174:2005, 3.1.3]

3.18
RNase
ribonuclease
enzyme that catalyzes the degradation of RNA into smaller components

3.19
room temperature
temperature in the range of 18 °C to 25 °C for the purposes of this document

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

3.20
sample
one or more parts taken from a primary sample

[SOURCE: ISO 15189:2012, 3.24, modified — The example has been deleted.]

3.21
stability
ability of a specimen or sample, 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 words “reference material” have been replaced by “specimen or sample”.]

3.22
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 — Notes 1 and 3 to entry have been deleted, Note 2 to entry has been renumbered as Note 1 to entry.]

3.23
venous whole blood
blood collected after directly puncturing a vein, usually with a needle and syringe, or other collection device

3.24
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 — Notes 1 and Note 2 to entry have been deleted; Note 3 to entry has been renumbered as Note 1 to entry; new Note 2 to entry has been added.]

3.25
workflow
series of activities necessary to complete a task

4 General consideration

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 15189:2012, 4.2, 5.4.4, 5.4.6 or ISO/IEC 17020:2012, Clause 8 and 7.2. The requirements on laboratory equipment, reagents, and consumables according to ISO 15189:2012, 5.3 shall be followed; ISO 15189:2012, 5.5.1.2 and 5.5.1.3 and ISO/IEC 17020:2012, 6.2 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 its impact on the stability of biomolecules intended to be examined shall be verified and validated. Workflow steps which cannot always be controlled shall be documented and their impact on the examination performance shall be investigated and mitigation measures shall be established to allow the required examination performance. In these cases, risk assessment is recommended.

CcfDNA profiles can change significantly after blood collection. The post-collection release of genomic DNA from cells in blood can change the ccfDNA profile significantly (see A.1). Additional post-collection effects can also occur, e.g. ccfDNA fragmentation[10][11][12][13]. All these post-collection changes can vary individually in specimens from different donors or patients, and they can also depend on pathophysiological conditions[10][14][15][16]. This can impact the validity and reliability of the examination results (see A.2).

Before or during the design of an examination, it shall therefore be investigated and ensured that the ccfDNA profile(s) intended to be analysed is/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, e.g. by using blood collection tubes with stabilizers.

Safety procedures for handling and transport shall be in place. Safety requirements on transport and handling shall be considered (see ISO 15189 and ISO 15190).

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

If a commercial product is not used in accordance with the manufacturer's instructions, responsibility for its validation, verification, use and performance lies with the user.

5 Outside the laboratory

5.1 Specimen collection

5.1.1 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 relevant health status of the specimen donor or patient [e.g. healthy, disease type, concomitant disease, demographics (e.g. age and gender)];

   NOTE In particular, e.g. cancer, inflammation, diabetes, hepatic disease, coronary disease, respiratory syndrome, trauma, after exhaustive exercise[10], in elderly patients suffering from acute or chronic disease, first trimester of pregnancy, placental disorders as pre-term labour, pre-eclampsia and malimplantation have been reported to affect both ccfDNA quantity and fragmentation[10][14][15][16].

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