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SIST-TS CEN/TS 16835-2:2015

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Molekularne diagnostične preiskave in vitro - Specifikacije za predpreiskovalne procese za vensko polno kri - 2. del: DNA, izoliran iz genoma

Molecular in vitro diagnostic examinations - Specifications for pre-examination processes for venous whole blood - Part 2: Isolated genomic DNA

Molekularanalytische in-vitro-diagnostische Verfahren - Spezifikationen für präanalytische Prozesse für venöse Vollblutproben - Teil 2: Isolierte genomische DNA

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Molecular in vitro diagnostic examinations - Specifications for pre-examination processes for venous whole blood - Part 2: Isolated genomic DNA

Tests de diagnostic moléculaire in vitro - Spécifications relatives aux processus pré-analytiques pour le sang total veineux - Partie 2: ADN génomique extrait

Molekularanalytische in-vitro-diagnostische Verfahren - Spezifikationen für präanalytische Prozesse für venöse Vollblutproben - Teil 2: Isolierte genomische DNS

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European foreword

This document (CEN/TS 16835-2:2015) 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 a significant progress in medicine. Further progress is expected by new technologies analysing signatures of nucleic acids, proteins, and metabolites in human tissues and body fluids. However, the profiles of these molecules can change drastically during primary sample 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.

A standardization of the entire process from primary sample collection to genomic DNA analysis is needed due to genomic DNA degradation and fragmentation after blood collection. Studies have been undertaken to determine the important influencing factors. This Technical Specification draws upon such work to codify and standardize the steps for venous whole blood genomic DNA analysis in what is referred to as the preanalytical phase.

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1 Scope

This Technical Specification recommends the handling, documentation and processing of venous whole blood specimens intended for genomic DNA analysis during the preanalytical phase before a molecular assay is performed. This Technical Specification covers specimens collected by venous whole blood collection tubes. This Technical Specification is applicable to molecular *in vitro* diagnostic examinations (e.g. *in vitro* diagnostic laboratories, laboratory customers, *in vitro* diagnostics developers and manufacturers, institutions and commercial organizations performing biomedical research, biobanks, and regulatory authorities).

Blood genomic DNA can fragment or degrade after blood collection. Therefore, special measures need to be taken to secure good quality blood samples for genomic DNA analysis. This is particularly relevant for analytical test procedures requiring high molecular weight DNA.

Different dedicated measures need to be taken for preserving blood circulating cell free DNA, which are not described in this Technical Specification. Circulating cell free DNA in blood is covered in CEN/TS 16835-3, *Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for venous whole blood — Part 3: Isolated circulating cell free DNA from plasma*.

Different dedicated measures need to be taken for collecting, stabilizing, transporting and storing capillary blood as well as for blood collected and stored by paper based technologies. These are not described in this Technical Specification.

DNA from pathogens present in blood is not covered by this Technical Specification.

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2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

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EN ISO 15189:2012, *Medical laboratories — Requirements for quality and competence (ISO 15189:2012, Corrected version 2014-08-15)*

ISO 15190, *Medical laboratories — Requirements for safety*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN ISO 15189:2012 and the following apply.

3.1
ambient temperature
unregulated temperature of the surrounding air

3.2
analytical phase
processes that start with the isolated analyte and include all kind of parameter testing or chemical manipulation for quantitative or qualitative analysis

3.3
blood genomic DNA stabilizers
compounds, solutions or mixtures that are made to minimize degradation and fragmentation of genomic DNA in a blood sample

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3.4
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]

3.5
genomic DNA
DNA from the genome containing all coding (exon) and non-coding (intron and other) sequences

Note 1 to entry: In this document it is always only referred to genomic DNA present in blood cells, excluding circulating cell free DNA.

3.6
high molecular weight DNA
HMW DNA
DNA larger than 50 kb for the purpose of this document

3.7
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), temporary storage, transportation to and within the analytical laboratory, aliquotting, retrieval, isolation of analytes, and end when the analytical examination begins

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

Note 1 to entry: The preanalytical phase may include preparative processes that may influence the outcome of the intended examination.

3.8
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: EN ISO 15189:2012, 3.16, modified — The term and definition is used here without the original notes.]

3.9
room temperature
temperature which is defined as 18 °C to 25 °C for the purpose of this document

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3.10
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 words “reference material” were replaced by “sample material".]

Note 1 to entry: The measured constituent for the purpose of this document is genomic DNA.

4 General considerations

For general statements on primary sample collection and handling (including avoidance of cross contaminations), see EN ISO 15189:2012, 5.2.6, 5.4.4. Consumables including kits shall be verified before use in examination (see EN ISO 15189:2012, 5.3.2.3); EN ISO 15189:2012, 5.5.1.2 and 5.5.1.3 can also apply.

As all steps of a diagnostic workflow can influence the final analytical performance, the entire workflow, comprising the preanalytical steps, including information on sample stability and storage conditions, and the analytical steps should be verified and validated (see EN ISO 15189).

The stability of the genomic DNA should be investigated throughout the complete pre-analytical workflow.

Before or during the design of the analytical test system it should be investigated and ensured that the genomic DNA minimum amount and size required for the analytical test are not affected by the envisioned entire preanalytical workflow.

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

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Safety regulations on facilities, transport and handling shall be considered (EN ISO 15189:2012, 5.2.3 and 5.4.5, and ISO 15190).

5 Outside the laboratory

5.1 Primary venous whole blood collection manual

5.1.1 Information about the primary sample donor

The documentation should include, but is not limited to:

a) the primary donor / patient ID, which can be in the form of a code;

b) the health status and relevant lifestyle factors of the blood donor (e.g. healthy, disease type, gender, age);

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

d) the type and the purpose of the analytical test requested.

See also EN ISO 15189:2012, 5.4.4.

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5.1.2 Selection of the venous whole blood collection tube by the laboratory

The quality of genomic DNA can be influenced (e.g., DNA fragmentation), by inadequate blood collection procedures, inappropriate storage/shipping conditions and DNA isolation procedures, [3], [4], [5], [6], [7], [8], [9], [10].

Blood should be collected in appropriate venous whole blood collection tubes containing an anticoagulant such as EDTA or Acid Citrate Dextrose (ACD) [11].

NOTE Blood collection tubes containing EDTA as an anticoagulant are preferable for most genomic DNA analysis. Blood collection tubes containing heparin as an anticoagulant can impact the purity of the isolated genomic DNA, when using genomic DNA isolation methods not eliminating the heparin. Carrying over of heparin into the genomic DNA eluate can cause inhibitions in analytical test technologies, such as PCR.

Specifically developed blood collection tubes, containing genomic DNA stabilizing reagents, are also available aimed to standardize blood collection, transport and storage of venous whole blood.

5.1.3 Primary venous whole blood sample collection from the patient and stabilization procedures

1. The identity of the person collecting the primary sample and the time of blood collection according to EN ISO 15189:2012, 5.4.4.3, f) shall be documented.

2. For the labelling (sample identification) of the blood collection tube a routine procedure (EN ISO 15189:2012, 5.4.4.3, e)) or a procedure with additional information (e.g. 2D-barcode) shall be used.

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3. Standard venepuncture technique can be used. Steps for preventing possible backflow may be required. The manufacturers' instructions for using the blood collection tubes shall be followed. A blood collection set and needle holder can be required when using blood genomic DNA stabilizer containing tubes. In this case, the instructions of the collection set and needle holder manufacturer shall be followed.

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   NOTE There is no known specific effect of venous whole blood draw procedure on the genomic DNA. Routine procedures can therefore be used.

4. Blood collection tubes shall be filled in accordance to the manufacturers' instructions and attention should be drawn to the correct positioning of the collection tube during the blood draw as well as the required volume.

5. Blood collection tube manufacturers' instructions for mixing or inverting the tube immediately after blood collection shall be followed.

   NOTE Unless additives are homogenously mixed with the blood sample, the genomic DNA quality can be compromised, which can impact the validity and reliability of the analytical test results.

6. Any tampering with and/or additions to the primary sample shall be documented.

5.1.4 Information on the primary blood sample and storage requirements at the blood collection facility

5.1.4.1 General

As blood genomic DNA can fragment or degrade after blood collection (Figure A.1) and can thereby affect the validity and reliability of the analytical test result (Figure A.3), the documentation on the primary blood sample shall include the date and should also include the time of blood collection [11].

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For samples dedicated to be archived in a biobank it is usually not known which individual genomic DNA tests will be performed after archiving, therefore either tubes with genomic DNA stabilizers should be used or, if using tubes without genomic DNA stabilizers, the recommendations for HMW DNA should be followed (see Table 1 and 5.1.4.3.2).

The temporary storage duration in the blood collection facility contributes to the total duration for storage.

5.1.4.2 Using blood collection tubes with stabilizers

For storing the primary blood samples collected in blood collection tubes with blood genomic DNA stabilizers, the blood collection tube manufacturers' instructions on storage conditions shall be followed (including the storage duration and temperature). Where the analytical test providers' instructions are more stringent, these shall be followed. The storage conditions (including the storage duration and temperature) shall be documented.

5.1.4.3 Using blood collection tubes without stabilizers

5.1.4.3.1 When using blood collection tubes without blood genomic DNA stabilizers, the analytical test providers' instructions on storage conditions shall be followed. This can require documentation of storage conditions.

5.1.4.3.2 When using blood collection tubes without blood genomic DNA stabilizers and no requirements on the storage conditions are available from the analytical test provider, the primary venous whole blood samples should be processed as soon as possible.

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As blood collection tubes containing EDTA as an anticoagulant are most wide spread for genomic DNA analysis the following recommendations (see also Table 1) refer to this blood collection tube type. For analytical tests requiring High Molecular Weight DNA, the blood sample should be stored at room temperature for not longer than one day or at 2 °C to 8 °C for not longer than three days. For a longer storage the sample should be kept at −20 °C for not longer than 1 month, or at −70 °C or below for longer storage. For the analysis of DNA variants not requiring HMW DNA analyses, the blood sample should be stored at room temperature for up to 3 days or at 2 °C to 8 °C for up to 7 days (Figure A.4). For a longer storage the sample should be kept at −20 °C for up to 3 months or at −70 °C or below for longer storage.

The storage conditions (including the storage duration and temperature) shall be documented.

5.2 Transport requirements

The required transport conditions shall be documented including any deviations therefrom.

When using blood collection tubes with blood genomic DNA stabilizers, the tubes' manufacturers' instructions on transport conditions shall be followed (including the transport duration and temperature). Where the analytical test providers' instructions are more stringent, these shall be followed.

When using blood collection tubes without genomic DNA stabilizers, the analytical test providers´ instructions on transport conditions shall be followed. This can require the documentation of transport conditions (including the transport duration and temperature).

When using blood collection tubes without genomic DNA stabilizers and no analytical test provider's instructions are available, the primary blood sample should be transported at either room temperature, at 2 °C to 8 °C, or at −20 °C or below within the specifications given in 5.1.4.3.2 and Table 1 in order to minimize the degradation and fragmentation of the blood genomic DNA [11], [12].

See also EN ISO 15189:2012, 5.4.5.

The transport duration to the laboratory contributes to the total duration for storage.