SLOVENSKI STANDARD
SIST-TS CEN/TS 16835-1:2015

01-september-2015

Molekularne diagnostične preiskave in vitro - Specifikacije za predpreiskovalne procese za vensko polno kri - 1. del: Izolirana celična RNA

Molecular in vitro diagnostic examinations - Specifications for pre-examination processes for venous whole blood - Part 1: Isolated cellular RNA

Molekularanalytische in-vitro-diagnostische Verfahren - Spezifikationen für präanalytische Prozesse für venöse Vollblutproben - Teil 1: Isolierte zelluläre RNS

Tests de diagnostic moléculaire in vitro - Spécifications relatives aux processus préanalytiques pour le sang veineux total - Partie 1 : ARN cellulaire isolé

Ta slovenski standard je istoveten z: **CEN/TS 16835-1:2015**

ICS:

11.100.10	Diagnostični preskusni sistemi in vitro	In vitro diagnostic test systems
11.100.30	Analiza krvi in urina	Analysis of blood and urine

SIST-TS CEN/TS 16835-1:2015 **en,fr,de**

TECHNICAL SPECIFICATION

SPÉCIFICATION TECHNIQUE

TECHNISCHE SPEZIFIKATION

CEN/TS 16835-1

July 2015

ICS 11.100.10

English Version

Molecular in vitro diagnostic examinations - Specifications for pre-examination processes for venous whole blood - Part 1: Isolated cellular RNA

Tests de diagnostic moléculaire in vitro - Spécifications relatives aux processus préanalytiques pour le sang veineux total - Partie 1 : ARN cellulaire isolé

Molekularanalytische in-vitro-diagnostische Verfahren - Spezifikationen für präanalytische Prozesse für venöse Vollblutproben - Teil 1: Isolierte zelluläre RNS

This Technical Specification (CEN/TS) was approved by CEN on 30 May 2015 for provisional application.

The period of validity of this CEN/TS is limited initially to three years. After two years the members of CEN will be requested to submit their comments, particularly on the question whether the CEN/TS can be converted into a European Standard.

CEN members are required to announce the existence of this CEN/TS in the same way as for an EN and to make the CEN/TS available promptly at national level in an appropriate form. It is permissible to keep conflicting national standards in force (in parallel to the CEN/TS) until the final decision about the possible conversion of the CEN/TS into an EN is reached.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.

EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

Ref. No. CEN/TS 16835-1:2015 E

Contents

Page

Foreword

This document (CEN/TS 16835-1:2015) has been prepared by Technical Committee CEN/TC 140 “In vitro diagnostic medical devices”, the secretariat of which is held by DIN.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to announce this Technical Specification: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Introduction

Molecular *in vitro* diagnostics has enabled a significant progress in medicine. Further progress is expected by new technologies analyzing 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. Therefore, a standardization of the entire process from sample collection to RNA analysis is needed. 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 cellular RNA analysis in what is referred to as the preanalytical phase.

1 Scope

This Technical Specification recommends the handling, documentation and processing of venous whole blood specimens intended for cellular RNA 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 cellular RNA profiles can change significantly after collection. Therefore, special measures need to be taken to secure good quality blood samples for cellular RNA analysis and storage.

Different dedicated measures need to be taken for stabilizing blood cell free circulating RNA and RNA in exosomes circulating in blood, which are not described in this Technical Specification.

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

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

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.

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 cellular RNA
cellular RNA
RNA molecules present in blood cells

3.4
blood cellular RNA profile\s
amounts of different RNA molecules, that are present in blood cells and that can be measured in the absence of any losses, inhibition and interference

3.5
blood cellular RNA profile stabilizers
compounds, solutions or mixtures that are designed to minimize changes of the blood cellular RNA profile

3.6
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

[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.

3.7
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.]

3.8
RNA
ribonucleic acid
polymer of ribonucleotides occurring in a double-stranded or single-stranded form

[SOURCE: EN ISO 22174:2005, 3.1.3]

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

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 30:1992, 2.7]

Note 1 to entry: The measured constituent for the purpose of this document is blood cellular RNA.

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).

Blood cellular RNA profiles can change significantly after collection (e.g. gene induction, gene down regulation, RNA degradation) [3], [4], [5], [6]. These changes can vary individually in different blood donors' / patients' blood [3], [7], [8], [9], [10].

The stability of the specific blood cellular RNA profile of interest should be investigated throughout the complete preanalytical workflow.

Before or during the design of the analytical test system it should be investigated and ensured that the specific blood cellular RNA molecule/s amount/s intended to be analyzed in the analytical test is/are not affected by the envisioned entire preanalytical workflow.

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.

Safety regulations on transport and handling shall be considered (EN ISO 15189:2012, 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, diet, gender, age);

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

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

See also EN ISO 15189:2012, 5.4.4.

5.1.2 Selection of the venous blood collection tube by the laboratory

Due to the high instability of blood cellular RNA profiles in individual patients/donors [3], [7], [8], [9], [10], commercially available blood collection tubes containing blood cellular RNA profile stabilizers should be used [7], [8], [10], [11], [12] (Figure A.1).

Blood collection tubes not containing any blood cellular RNA profile stabilizer should only be used, if the specific blood cellular RNA molecule or the blood cellular RNA profile to be analyzed is stable after blood draw (Figure A.2) or if the requested analytical test allows the use of such tubes.

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

1. The identity of the person collecting the 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.

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 cellular RNA profile stabilizer containing tubes. In this case, the instructions of the collection set and needle holder manufacturer shall be followed.

NOTE There is no known specific effect of venous whole blood draw procedure on the cellular RNA. 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 blood cellular RNA profile quality and the quality of individual cellular RNA molecules 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

The documentation on the primary blood sample shall include the date and time of blood collection [3], [14], [15], [16] as blood cellular RNA profiles can change significantly after blood collection and can thereby affect the validity and reliability of the analytical test result [3], [11], [13],[14].

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

Blood collection tubes without blood cellular RNA profile stabilizers should only be used, if the ordered analytical test specifications allow the usage of such tubes. In these cases, the analytical test providers' instructions on storage conditions shall be followed. This can require documentation of storage conditions.

When using blood collection tubes without blood cellular RNA profile stabilizers and no requirements on the storage conditions are available, the primary blood samples should be transferred immediately to 2 °C to 8 °C or on wet-ice in order to minimize blood cellular RNA profile changes [8], [14], [15], [16] (Figure B.1). The storage conditions (storage duration and temperature) shall be documented. The storage duration allowed at 2 °C to 8 °C or on wet-ice is highly dependent on the stability of the individual RNA molecules and their cellular quantities to be analyzed in the analytical test. This stability can vary between several minutes and over 24 h.

NOTE Under these storage conditions (at 2 °C to 8 °C or on wet-ice) blood cellular RNA profile changes can still occur when no blood cellular RNA profile stabilizers are used (Figure B.1).

For samples dedicated to be archived in a biobank it is usually not known which individual RNA molecules will be analyzed after archiving, therefore tubes without blood cellular RNA profile stabilizers should not be used for biobanking.

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

5.2 Transport requirements

The required transport conditions shall be documented including any deviations.

When using blood collection tubes with blood cellular RNA profile stabilizers, the tubes' manufacturers' instructions on transport conditions shall be followed (e.g. temperature, transport duration). Where the analytical test providers' instructions are more stringent, these shall be followed.

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

When using blood collection tubes without blood cellular RNA profile stabilizers and no analytical test provider's instructions are available, the primary blood sample should be transported at 2 °C to 8 °C or on wet-ice without delay in order to minimize the blood cellular RNA profile changes [16].

See also EN ISO 15189:2012, 5.4.5.

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

6 Inside the laboratory

6.1 Sample reception

The blood sample reception time shall be documented. Nonconformities of labelling, transport conditions and blood volume differences to specifications, leaking/broken tubes, etc. shall be documented.

Where there are nonconformities in labelling, transport conditions, overall storage and transport duration or blood volume that could affect the validity and reliability of the analytical test result, a new sample should be obtained.

6.2 Storage requirements

The storage temperature and time interval between sample receipt and sample processing for cellular RNA isolation shall be documented. Storage temperature and total storage duration shall not exceed specifications identified in 5.1.4 and 5.2.

The primary blood sample total storage duration shall include the duration for storage at the blood collection facility (5.1.4), for transportation to the laboratory (5.2) and for further storage at the laboratory or other institutions.

The stability of specific blood cellular RNA profiles can vary between different RNA molecules (e.g. mRNA, rRNA, miRNA) different blood donors, and different blood storage conditions. Any specified maximum storage duration given by the blood collection tube manufacturer or the analytical test manufacturer shall not be exceeded. If such specifications are not available, the maximum storage duration shall be validated and generally kept to a minimum.

See also Table 1.