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Molecular in vitro diagnostic examinations - Specifications for pre-examination processes for circulating tumor cells (CTCs) in venous whole blood - Part 1: Isolated RNA

Analyses de diagnostic moléculaire in vitro - Spécifications relatives aux processus préanalytiques pour les cellules tumorales circulantes (CTCs) dans le sang total veineux - Partie 1 : ARN extrait

Molekularanalytische in-vitro-diagnostische Verfahren - Spezifikationen für präanalytische Prozesse für zirkulierende Tumorzellen (CTC) in venösen Vollblutproben - Teil 1: Isolierte RNA

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European foreword

This document (CEN/TS 17390-1:2020) 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 shall not be held responsible for identifying any or all such patent rights.

CEN/TS 17390 consists of the following parts, under the general title *Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for Circulating Tumor Cells (CTCs) in venous whole blood*:

— *Part 1: Isolated RNA*

— *Part 2: Isolated DNA*

— *Part 3: Preparations for analytical CTC staining*

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

Introduction

Solid tumours release cells and bioanalytes into blood and other body fluids. This has opened the option of minimally-invasive tumour detection, diagnosis and characterization from venous whole blood (liquid biopsies). Liquid biopsies are expected to enable earlier detection and diagnosis of cancers and advance personalized patient treatment. These applications have become one of the fastest growing segments of the entire diagnostic market.

Circulating tumour cells (CTCs) in venous whole blood reflect the disease complexity that evolves during tumour progression, with distinct genetic, epigenetic and expression features. Besides the prognostic role of CTC identification and/or enumeration in cancer progression, CTC molecular characterization can improve e.g. disease outcome prediction, therapeutic guidance and post-treatment monitoring of the patient.

CTCs are now considered as a surrogate of tumour tissue in cancer early development, progression and metastatic phase.

Molecular characterization of CTCs can provide for example a strategy for monitoring cancer genotypes during systemic therapies [1], identification of mechanisms of disease progression, identification of novel targets for treatment [2] and to select targeted therapies. Moreover, CTC single-cell sequencing is emerging as an important tool for tumour genomic heterogeneity analysis [3] [4] [5].

CTCs are fragile and tend to degrade within a few hours when collected in conventional blood collection tubes, e.g. EDTA containing tubes, without dedicated CTC stabilizers. CTCs are extremely rare, especially in early disease, e.g. less than 10 cells per 10 ml of blood, representing a ratio of approx. $1:10^7$ CTCs to white blood cells (WBCs). This low ratio represents a significant challenge to CTC enrichment required for examination.

RNA profiles of CTCs resemble gene expression profiles of tumours. For RNA profile analysis, measures need to be taken to get rid of the WBCs in order to obtain sufficiently enriched CTC-specific RNA.

RNA profiles can change significantly after blood collection, during CTC enrichment and isolation. Therefore, special measures need to be taken to obtain good quality CTC samples and good quality isolated RNA for gene expression analysis [4] [6].

Consequently, standardization of all steps of the pre-examination process is required. This includes blood collection and stabilization, transport, storage, CTC enrichment, CTC isolation (if required), and RNA isolation. A decision guideline for the critical steps of the CTC pre-analytical workflow for RNA isolation is provided in Annex A.

This document describes special measures that need to be taken to obtain appropriate quality and quantity of RNA from CTC containing blood specimens for subsequent 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.

1 Scope

This document gives guidelines on the handling, storage, processing and documentation of human venous whole blood specimens intended for the examination of RNA isolated from circulating tumour cells (CTCs) during the pre-examination phase before a molecular examination is performed.

This document is applicable to molecular *in vitro* diagnostic examinations including laboratory developed tests 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.

This document does not cover the isolation of cellular RNA directly from venous whole blood containing CTCs. This is covered in EN ISO 20186-1, *Molecular in vitro diagnostic examinations - Specifications for pre-examination processes for venous whole blood – Part 1: Isolated cellular RNA*.

This document does not cover the isolation of specific white blood cells and subsequent isolation of cellular RNA therefrom.

This document does not cover pre-analytical workflow requirements for viable CTC cryopreservation and culturing.

NOTE 1 The requirements given in this document can also be applied to other circulating rare cells (e.g. fetal cells).

NOTE 2 International, national or regional regulations or requirements can also apply to specific topics covered in this document.

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 and the following terms and definitions apply.

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

- IEC Electropedia: available at http://www.electropedia.org/
- ISO Online browsing platform: available at https://www.iso.org/obp

3.1
aliquot
portion of a larger amount of homogenous material, assumed to be taken with negligible sampling error

Note 1 to entry: The term is usually applied to fluids. Tissues are heterogeneous and therefore cannot be aliquoted.

Note 2 to entry: The definition is derived from References [7], [8] and [9].

[SOURCE: EN ISO 20166-3:2019, 3.1]