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Molecular in vitro diagnostic examinations - Specifications for pre-examination processes for circulating tumor cells (CTCs) in venous whole blood - Part 3: Preparations for analytical CTC staining

Analyses de diagnostic moléculaire in vitro - Spécifications relatives aux processus préanalytiques pour les cellules tumorales circulantes (CTC) du sang total veineux - Partie 3 : Préparations pour l'analyse par coloration des CTC

Molekularanalytische in-vitro-diagnostische Verfahren - Spezifikationen für präanalytische Prozesse für zirkulierende Tumorzellen (CTC) in venösen Vollblutproben - Teil 3: Vorbereitungen für die analytische CTC-Färbung

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EUROPEAN COMMITTEE FOR STANDARDIZATION
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European foreword

This document (CEN/TS 17390-3:2020) 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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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 identification and analysis can improve e.g. disease outcome prediction, therapeutic guidance and post-treatment monitoring of the patient.

CTCs are now considered as a surrogate sample of tumour tissue, both in cancer early development 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 identification and examination as tumour-derived cells.

Furthermore, CTC morphology and biomolecules can change during the pre-examination process. These can lead to changes in protein quantity, integrity, modification, conformation and localization within the cell. This can impact the validity and reliability of the examination result.

CTC examination usually requires a CTC enrichment step (e.g. based on biological properties, such as expression of surface molecules, or physical properties, such as size and density, of the CTCs or their combination) prior to cytomorphological examination or immunofluorescent staining. CTC enrichment technologies can provide CTCs attached on a solid surface, ready for cytological examination, or CTCs in suspension requiring extra processing steps prior to the examination. This can lead to potential cell loss. [6]

CTC enrichment is usually followed by their identification by conventional cytochemical or protein-targeted staining procedures that allow detection of the cell traits.

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

This document describes measures to standardize the pre-examination process to obtain appropriate CTC staining.

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 specifies guidelines on the handling, storage, processing and documentation of human venous whole blood specimens intended for staining of 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 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 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.

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 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 bibliographical references [7], [8] and [9].

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

3.2
ambient temperature
unregulated temperature of the surrounding air

[SOURCE: EN ISO 20166-3:2019, 3.2]

3.3
analyte
component represented in the name of a measurable quantity

[SOURCE: EN ISO 17511:2003, 3.2, modified — EXAMPLE has been removed.]

3.4
analytical test performance
accuracy, precision, specificity and sensitivity of a test to measure the analyte of interest

Note 1 to entry: Other test performance characteristics such as robustness, repeatability can apply as well.

[SOURCE: EN ISO 20184-1:2018, 3.4, modified — "specificity" was added.]

3.5
blood collection set
intravenous device specialized for venipuncture consisting of a stainless steel bevelled 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.

3.6
blood collection tube
tube used for blood collection, usually in a vacuum which forces blood from the vein through the needle and into the tube

3.7
backflow
flow of a liquid opposite to the usual or desired direction

3.8
circulating tumor cells
CTCs
cells present in blood, which are derived from a primary and/or metastatic site of a tumor

3.9
diagnosis
identification of a disease from its signs and symptoms, where the diagnostic process can involve examinations and tests for classification of an individual's condition into separate and distinct categories or subclasses that allow medical decisions about treatment and prognosis to be made

[SOURCE: EN ISO 20184-1:2018, 3.6]

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 CTC staining and include all kinds of parameter testing or chemical manipulation for quantitative or qualitative examination.

[SOURCE: EN ISO 15189:2012, 3.7, modified — Notes to entry 1 to 3 have been removed, Note 1 to entry has been added and "analytical test" has been added as a preferred term.]