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Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for circulating tumortumour cells (CTCs) in venous whole blood— —

Part 3:
Preparations for analytical CTC staining

DTS stage

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Foreword

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This document was prepared by Technical Committee ISO/TC 212, *Clinical laboratory testing and in vitro diagnostic test systems*~~.~~, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 140, *In vitro diagnostic medical devices*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

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Introduction

Solid tumorstumours release cells and bioanalytes into blood and other body fluids. This has opened the option of utilizing such body fluids (liquid biopsies) for a minimally-invasive procedure for tumortumour detection, diagnosis and characterization. Liquid biopsies can enable earlier detection and diagnosis of cancers and advance personalized patient treatment.[19,20] [1,2].

These applications have become one of the fastest growing segments of the entire diagnostic market.

Circulating tumortumour cells (CTCs) in venous whole blood can reflect the disease complexity that evolves during tumortumour progression, with distinct genetic, epigenetic and expression features.[21] 3.

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.[19] [1].

CTCs are now considered as a surrogate sample of tumortumour tissue, both in cancer early development, progression and metastatic phase.[22] [4].

Molecular characterization of CTCs can provide for example a strategy for monitoring cancer during systemic therapies,[23] [5], identification of identifying mechanisms of disease progression, identification ofidentifying novel targets for treatment[24] [6] and to selectselecting targeted therapies[19] [1].

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.approximately 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 tumortumour-derived cells.

Furthermore, CTC morphology and biomolecules can change during the pre-examination process. TheseThis 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 of the CTCs, 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.[25] [7].

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

Standardization ofincludes all steps of the pre-examination process is required. This includes, including blood collection and stabilization, transport, storage, CTC enrichment, and CTC isolation (if requiredincluded). This pre-examination standardization is crucial to ensure reliable examination results in current clinical use and is also critical to develop new CTC based diagnostic examinations and to establish these in clinical healthcare.[26] [8].

An illustration of critical steps of the pre-analytical workflow for CTC staining is provided in Annex AAnnex 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.

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Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for circulating ~~tumor~~tumour cells (CTCs) in venous whole blood~~—~~ —

Part 3: Preparations for analytical CTC staining

1 Scope

This document specifies requirements and gives recommendations on the handling, storage, CTC enrichment, preparation for CTC staining, and documentation of venous whole blood specimens intended for staining of CTCs during the pre-examination phase before an 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.

Different dedicated measures are taken for stabilizing CTCs genomic DNA and RNA that are not described in this document; they are covered in ISO ~~7552, *Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for circulating tumor cells (CTCs) in venous whole blood* — Part 1 and Part 2.~~ 7552-1[1] and ISO 7552-2[2].

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

ISO 15189, *Medical laboratories — Requirements for quality and competence*

ISO 15190, *Medical laboratories — Requirements for safety*

~~ISO 14971, *Medical devices — Application of risk management to medical devices*~~

~~ISO 35001, *Biorisk management for laboratories and other related organisations*~~

3 Terms and definitions

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

[1] Under preparation. Stage at the time of publication: ISO/DTS 7552-1.
[2] Under preparation. Stage at the time of publication: ISO/DTS 7552-2.

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

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

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 [9], [10] and [11].~~

[SOURCE: ISO 20166-3:2018, 3.1]

3.2
analyte
component represented in the name of a measurable quantity

[SOURCE: ISO 17511:2020, 3.1, modified — ~~EXAMPLE~~the example has been removed.]

3.3
backflow
flow of a liquid opposite to the usual or desired direction

3.4
blood collection set
intravenous device specialized for venipuncture consisting of a stainless steel ~~bevelled~~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* (3.5~~(3.5).~~).

3.5
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

3.6
circulating ~~tumor~~tumour cells
CTCs
cells present in blood, originating from a primary ~~and/~~or metastatic site(s) of a ~~tumor~~ tumour

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

[SOURCE: ISO 20186-2:2019, 3.6]

3.~~80~~8
CTC enrichment
method that is able to increase the ratio of *CTCs* (3.6~~[3.6]~~) to other cells including white blood cells in a *sample* (3.21~~(3.21)~~)