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

Part 3: Preparations for analytical CTC staining

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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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).

A list of all parts in the ISO 7552 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Solid tumours 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 tumour detection, diagnosis and characterization. Liquid biopsies can enable earlier detection and diagnosis of cancers and advance personalized patient treatment.[19,20]

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

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

Besides the prognostic role of CTC identification and enumeration in cancer progression, CTC identification and analysis can improve disease outcome prediction, therapeutic guidance and post-treatment monitoring of the patient.[19]

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

Molecular characterization of CTCs can provide a strategy for monitoring cancer during systemic therapies,[23] identifying mechanisms of disease progression, identifying novel targets for treatment[24] and selecting targeted therapies[19].

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 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 tumour-derived cells.

Furthermore, CTC morphology and biomolecules can change during the pre-examination process. This 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, 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]

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

Standardization includes all steps of the pre-examination process, including blood collection and stabilization, transport, storage, CTC enrichment, and CTC isolation (if included). 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]

An illustration of 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.

Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for circulating 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 7552-1[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*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

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/

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.

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.

[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 — 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 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
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 tumour cells
CTCs
cells present in blood, originating from a primary or metastatic site(s) of a tumour

3.7
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]

3.8
CTC enrichment
method that is able to increase the ratio of *CTCs* (3.6) to other cells including white blood cells in a *sample* (3.21)

3.9
CTC isolation
method resulting in a *sample* (3.21) containing *CTCs* (3.6) without any other cell types

3.10
diagnosis
identification of a health or disease state from its signs and symptoms, where the diagnostic process can involve *examinations* (3.11) 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: ISO 20184-1:2018, 3.6]

3.11
examination
analytical test
set of operations having the objective of determining the numerical value, text value or characteristics of a property

Note 1 to entry: An examination includes the processes that start with CTC staining and include all kinds of parameter testing or chemical manipulation for quantitative or qualitative examination.

[SOURCE: ISO 15189:2022, 3.8, modified — the original Notes to entry have been removed, and a new Note 1 to entry has been added; "analytical test" has been added as a preferred term.]

3.12
examination performance
analytical test performance
analytical performance
ability of an *examination* (3.11) procedure to measure or detect a particular *analyte* (3.2)

Note 1 to entry: Analytical performance is determined from analytical performance studies used to assess the ability of an in vitro diagnostic examination procedure to measure or detect a particular analyte.

Note 2 to entry: Analytical performance includes such characteristics as analytical sensitivity, detection limit, analytical specificity (interference and cross-reactivity), trueness, precision and linearity.

[SOURCE: ISO 20186-3:2019, 3.11]

3.13
immunocytochemistry
in situ detection technique that uses the principle of antibodies binding specifically to antigens in or on cells to detect the antigens (e.g. proteins) using brightfield microscopy

3.14
manufacturer
entity that is legally responsible for manufacturing a specific *workflow* (3.26) component

Note 1 to entry: For the purpose of this document, manufacturers can be *examination* (3.11) manufacturers, collection device manufacturers, *CTC enrichment* (3.8) and isolation manufacturers, nucleic acid isolation manufacturers.

3.15
needle holder
barrel used in routine venipuncture procedures to hold the *blood collection tube* (3.5) in place and to protect the phlebotomist from direct contact with blood

[SOURCE: ISO 20186-1:2019, 3.16]

3.17
pre-examination process
pre-analytical phase
pre-analytical workflow
process that starts, in chronological order, from the clinician's request and includes the *examination* (3.11) request, preparation and identification of the patient, collection of the *primary sample(s)* (3.18), transportation to and within the laboratory, cell enrichment, isolation of *analytes* (3.2), and ends when the analytical examination begins

Note 1 to entry: The pre-examination phase includes preparative processes that influence the outcome of the intended examination.

[SOURCE: ISO 15189:2022, 3.24, modified — "pre-analytical phase" and "pre-analytical workflow" have been added as preferred terms; in the definition, "user's request" has been changed to "clinician's request"; "cell enrichment, isolation of analytes" has been added to the definition; Note 1 to entry has been added.]

3.18
primary sample
specimen
discrete portion of a body fluid or tissue or other *sample* (3.21) associated with the human body taken for *examination* (3.11), study or analysis of one or more quantities or characteristics to determine the character of the whole

[SOURCE: ISO 15189:2022, 3.25, modified — Note 1 to entry has been removed.]

3.19
proficiency testing
PT
evaluation of participant performance against pre-established criteria by means of interlaboratory comparisons

[SOURCE: ISO/IEC 17043:2023, 3.7, modified — Note 1 to entry has been removed.]

3.20
room temperature
temperature in the range of 18 °C to 25 °C

Note 1 to entry: Local or national regulations can have different definitions.

3.21
sample
one or more parts taken from a *primary sample* (3.18)

[SOURCE: ISO 15189:2022, 3.28.]

3.22
stability
ability of a *sample* (3.21) 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”; “specified” replaced by “stated” before “property value”. Note 1 to entry has been removed.]

3.23
storage
prolonged interruption of the pre-examination *workflow* (3.26) of a *sample* (3.21) or *analyte* (3.2) respectively, or of their derivatives, such as stained sections or tissue blocks, under appropriate conditions in order to preserve their properties

Note 1 to entry: Long-term storage typically occurs in laboratory archives or in biobanks.

[SOURCE: ISO 20166-3:2018, 3.21]

3.24
validation
confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled

Note 1 to entry: The term “validated” is used to designate the corresponding status.

[SOURCE: ISO 9000:2015, 3.8.13, modified — the original Notes 1 to 3 to entry have been removed.]

3.25
verification
confirmation, through the provision of objective evidence, that specified requirements have been fulfilled

Note 1 to entry: The term “verified” is used to designate the corresponding status.

Note 2 to entry: Confirmation can comprise activities such as: