



Technical Specification

ISO/TS 7552-3

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

Analyses de diagnostic moléculaire in vitro — Spécifications relatives aux processus préanalytiques pour les cellules tumorales circulantes (CTC) dans le sang total veineux —

Partie 3: Préparations pour l'analyse par coloration des CTC

**First edition
2024-11**

iTeh Standards
(<https://standards.iteh.ai>)
Document Preview

[ISO/TS 7552-3:2024](https://standards.iteh.ai/catalog/standards/iso/d0dbdbab-cd0c-4c94-896e-1eb36c512b88/iso-ts-7552-3-2024)

<https://standards.iteh.ai/catalog/standards/iso/d0dbdbab-cd0c-4c94-896e-1eb36c512b88/iso-ts-7552-3-2024>



COPYRIGHT PROTECTED DOCUMENT

© ISO 2024

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

| | Page |
|---|-----------|
| Foreword | iv |
| Introduction | v |
| 1 Scope | 1 |
| 2 Normative references | 1 |
| 3 Terms and definitions | 1 |
| 4 General considerations | 5 |
| 5 Activities outside the laboratory | 6 |
| 5.1 Specimen collection..... | 6 |
| 5.1.1 General..... | 6 |
| 5.1.2 Information about the specimen donor/patient..... | 6 |
| 5.1.3 Selection of the venous whole blood collection tube by the laboratory..... | 6 |
| 5.1.4 Venous whole blood specimen collection from the patient/donor..... | 7 |
| 5.2 Specimen storage and transport..... | 7 |
| 5.2.1 General..... | 7 |
| 5.2.2 Storage and transport using blood collection tubes with stabilizers..... | 8 |
| 5.2.3 Storage and transport using blood collection tubes without stabilizers..... | 8 |
| 6 Activities inside the laboratory | 8 |
| 6.1 Specimen reception..... | 8 |
| 6.2 Specimen storage after transport and reception..... | 9 |
| 6.3 Enrichment of CTCs..... | 9 |
| 6.3.1 General..... | 9 |
| 6.3.2 Using a commercial CTC enrichment system intended for diagnostic use..... | 9 |
| 6.3.3 Using the laboratory developed CTC enrichment procedure..... | 10 |
| 6.4 Quality of enriched CTCs..... | 10 |
| 6.5 Storage of enriched CTCs..... | 10 |
| 6.6 Preparation for CTC staining..... | 10 |
| 6.6.1 General..... | 10 |
| 6.6.2 Pretreatment for different staining techniques (antibody, colour staining, in situ techniques)..... | 11 |
| Annex A (informative) Decision guideline for critical steps of the CTC pre-analytical workflow | 12 |
| Bibliography | 14 |

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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).

ISO draws attention to the possibility that the implementation of this document may involve the use of (a) patent(s). ISO takes no position concerning the evidence, validity or applicability of any claimed patent rights in respect thereof. As of the date of publication of this document, ISO had not received notice of (a) patent(s) which may be required to implement this document. However, implementers are cautioned that this may not represent the latest information, which may be obtained from the patent database available at www.iso.org/patents. ISO shall not be held responsible for identifying any or all such patent rights.

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 212, *Medical laboratories and in vitro diagnostic 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 1:10⁷ 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 and ISO 7552-2.

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/>

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, and isolation of *analytes* (3.2), ending 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.]

<https://standards.iteh.ai/catalog/standards/iso/d0dbdbab-cd0c-4c94-896e-1eb36c512b88/iso-ts-7552-3-2024>

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: