



# Technical Specification

**ISO/TS 7552-2**

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

### **Part 2: Isolated DNA**

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

*Partie 2: ADN isolé*

**First edition  
2024-11**

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Document Preview

[ISO/TS 7552-2:2024](https://standards.iteh.ai/catalog/standards/iso/115c8b7f-34ba-47ab-ae55-9e5ad8762f64/iso-ts-7552-2-2024)

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Published in Switzerland

# Contents

	Page
<b>Foreword</b> .....	<b>iv</b>
<b>Introduction</b> .....	<b>v</b>
<b>1 Scope</b> .....	<b>1</b>
<b>2 Normative references</b> .....	<b>1</b>
<b>3 Terms and definitions</b> .....	<b>1</b>
<b>4 General considerations</b> .....	<b>5</b>
<b>5 Activities outside the laboratory</b> .....	<b>6</b>
5.1 Specimen collection.....	6
5.1.1 General.....	6
5.1.2 Information about the patient/specimen donor.....	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
<b>6 Activities inside the laboratory</b> .....	<b>8</b>
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 Isolation of CTCs.....	11
6.6.1 General.....	11
6.6.2 Using a commercial CTC isolation system intended for diagnostic use.....	11
6.6.3 Using the laboratory-developed CTC isolation procedure.....	11
6.7 Isolation of DNA from an enriched CTC sample.....	11
6.7.1 General.....	11
6.7.2 Using a commercial DNA isolation kit intended for diagnostic use.....	12
6.7.3 Using a laboratory-developed CTC DNA isolation procedure.....	12
6.8 Quantity and quality assessment of isolated DNA from enriched or isolated CTCs.....	13
6.8.1 General.....	13
6.8.2 Quantity assessment of CTC DNA.....	13
6.8.3 Quality assessment of CTC DNA.....	13
6.9 Storage of isolated DNA from enriched CTCs.....	13
6.9.1 General.....	13
6.9.2 Storage of DNA isolated with a commercially available kit intended for diagnostic use.....	14
6.9.3 Storage of DNA isolated with the laboratory developed procedure.....	14
<b>Annex A (informative) Decision guideline for critical steps of the CTC pre-analytical workflow</b> .....	<b>15</b>
<b>Bibliography</b> .....	<b>17</b>

## 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](http://www.iso.org/directives)).

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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](http://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](http://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.<sup>[20,22]</sup>

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 gene expression biomarkers.<sup>[22]</sup>

Beside the prognostic role of CTC identification and enumeration in cancer progression, CTC molecular characterization can improve disease outcome prediction, therapeutic guidance and post-treatment monitoring of the patient.<sup>[20]</sup>

CTCs are now considered as a surrogate of tumour tissue in cancer early development, progression, and metastatic phase.<sup>[23]</sup>

Molecular characterization of CTCs can provide a strategy for monitoring cancer genotypes during systemic therapies,<sup>[24]</sup> identifying mechanisms of disease progression, identifying novel targets for biological treatment<sup>[25]</sup> and selecting targeted therapies.<sup>[20]</sup> Moreover, CTC single-cell sequencing is an important tool for tumour genomic heterogeneity analysis.<sup>[26-28]</sup> Molecular examination techniques such as qPCR, dPCR and sequencing methods including next generation sequencing (NGS) enable characterization of the CTC specific DNA features.

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<sup>7</sup> CTCs to white blood cells (WBCs). This low ratio represents a significant challenge to CTC enrichment required for examination. Furthermore, co-enrichment of normal blood cells causes a dilution of CTCs. The challenge is to minimize the amount of co-enriched WBCs for subsequent accurate and sensitive detection of CTC specific genetic and epigenetic alterations, especially when dealing with minor tumour cell clones.

Special measures to remove the WBCs are necessary in order to obtain good quality DNA samples characterized by high purity and thus representative of the mutational pattern within the tumour.<sup>2-2-2024</sup>

Standardization includes all steps of the pre-examination process, including blood collection and stabilization, transport, storage, CTC enrichment, CTC isolation (if included), and DNA isolation. 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.<sup>[29]</sup>

An illustration of critical steps of the CTC pre-analytical workflow is provided in [Annex A](#).

This document describes special measures to obtain appropriate quality and quantity of DNA from CTC-containing blood specimens for subsequent examination.



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

## Part 2: Isolated DNA

### 1 Scope

This document specifies requirements and gives recommendations on the handling, storage, CTC enrichment and isolation, DNA isolation and storage, and documentation of venous whole blood specimens intended for the examination of DNA 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 genomic DNA directly from venous whole blood containing CTCs. This is covered in ISO 20186-2.

This document does not cover the isolation of specific white blood cells and subsequent isolation of genomic DNA therefrom or the 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. 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 type

**3.10**  
**deoxyribonucleic acid**  
**DNA**

polymer of deoxyribonucleotides occurring in a double-stranded (dsDNA) or single-stranded (ssDNA) form

[SOURCE: ISO 22174:2024, 3.1.6]

**3.11**  
**deoxyribonucleic acid proficiency testing program**  
**DNA PT program**

*proficiency testing* (3.19) for DNA based *examinations* (3.13)



### 3.12

#### **diagnosis**

identification of a health or disease state from its signs and symptoms, where the diagnostic process can involve *examinations* (3.13) 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]

### 3.13

#### **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: Processes that start with the isolated *analyte* (3.2) 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.]

### 3.14

#### **examination performance**

##### **analytical test performance**

##### **analytical performance**

ability of an *examination* (3.13) 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* (3.13) procedure to measure or detect a particular *analyte* (3.2).

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]

### 3.15

#### **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* (3.13) manufacturers, collection device manufacturers, *CTC enrichment* (3.8) and isolation manufacturers, nucleic acid isolation manufacturers.

### 3.16

#### **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* (3.13) 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”; “storage, 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* (3.13), 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/115c8b7f-34ba-47ab-ae55-9e5ad8762f64/iso-ts-7552-2-2024>

**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 e.g. 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: