



# FINAL DRAFT Technical Specification

## ISO/DTS 7552-1

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

#### Part 1: Isolated RNA

ISO/TC 212

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CP 401 • Ch. de Blandonnet 8  
CH-1214 Vernier, Geneva  
Phone: +41 22 749 01 11  
Email: [copyright@iso.org](mailto:copyright@iso.org)  
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## 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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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](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>[20,21]</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>[41]</sup>

Besides 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>[39]</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>[39]</sup>.

Moreover, CTC single-cell sequencing is emerging as an important tool for tumour genomic heterogeneity analysis.<sup>[26-28]</sup> 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.

RNA profiles of CTCs resemble gene expression profiles of tumours. For RNA profile analysis, measures to remove the WBCs are necessary in order to obtain sufficiently enriched CTC-specific RNA.

RNA profiles can change significantly after blood collection, during CTC enrichment and isolation. Therefore, special measures are necessary to obtain CTC samples of adequate quality and isolated RNA of appropriate quality for ensuring the specified RNA examination performance.<sup>[29]</sup>

Standardization includes all steps of the pre-examination process, including blood collection and stabilization, transport, storage, CTC enrichment, CTC isolation (if included), and RNA 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>[30]</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 RNA 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 1: Isolated RNA

### 1 Scope

This document specifies requirements and gives recommendations on the handling, storage, CTC enrichment and isolation, RNA isolation and storage, and documentation of venous whole blood specimens intended for the examination of RNA 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 cellular RNA directly from venous whole blood containing CTCs. This is covered in ISO 20186-1.

This document does not cover the isolation of specific white blood cells and subsequent isolation of cellular RNA therefrom. 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. 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 terms and definitions given in ISO 15189 and the following 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 tumor cells**  
**CTCs**

cells present in blood, originating from a primary or metastatic site(s) of a tumor

**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* (3.24)

**3.9**  
**CTC isolation**

method resulting in a *sample* (3.24) containing *CTCs* (3.6) without any other cell type

**3.10**  
**deoxyribonuclease**  
**DNase**

enzyme that catalyzes the degradation of DNA into smaller components

[SOURCE: ISO 20186-1:2019, 3.11]



### 3.11

#### **diagnosis**

identification of health or a disease state from its signs and symptoms, where the diagnostic process can involve *examinations* (3.12) 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]

### 3.12

#### **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 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.]

### 3.13

#### **examination performance**

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

##### **analytical performance**

ability of an *examination* (3.12) 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]

### 3.14

#### **manufacturer**

entity that is legally responsible for manufacturing a specific *workflow* (3.29) component

Note 1 to entry: For the purpose of this document, manufacturers can be *examination* (3.12) 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]

### 3.16

#### **pre-examination process**

##### **pre-analytical phase**

##### **pre-analytical workflow**

process that starts, in chronological order, from the clinician's request and include the *examination* (3.12) request, preparation and identification of the patient, collection of the *primary sample(s)* (3.17), transportation to and within the laboratory, *storage* (3.26), cell enrichment, isolation of *analytes* (3.2), ending when the 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.]

**3.17**

**primary sample specimen**

discrete portion of a body fluid or tissue or other *sample* (3.24) associated with the human body taken for *examination* (3.12), 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.]

**3.18**

**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.]

**3.19**

**ribonucleic acid**

**RNA**

polymer of ribonucleotides occurring in a double-stranded or single-stranded form

[SOURCE: ISO 22174:2005, 3.1.3]

**3.20**

**ribonucleic acid proficiency testing program**

**RNA PT program**

proficiency testing for RNA-based *examinations* (3.12)

**3.21**

**RNA profile**

amounts of different *RNA* (3.19) molecules present in a *specimen* (3.17) or *sample* (3.24), that can be measured in the absence of any losses, inhibition, and interference

**3.22**

**RNase**

**ribonuclease**

enzyme that catalyzes the degradation of *RNA* (3.19) into smaller components

[SOURCE: ISO 20186-1:2019, 3.24]

**3.23**

**room temperature**

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

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

**3.24**

**sample**

one or more parts taken from a *primary sample* (3.17)

[SOURCE: ISO 15189:2022, 3.28]

**3.25**

**stability**

ability of a *sample* (3.24) 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.]