

STN P	Molekulárno-diagnostické vyšetrenia in vitro Špecifikácie predbežných vyšetrení cirkulujúcich nádorových buniek (CTC) v plnej krvi Časť 2: Izolovaná DNA	STN P CEN/TS 17390-2 85 1027
------------------	---	--

Molecular in vitro diagnostic examinations - Specifications for pre-examination processes for circulating tumor cells (CTCs) in venous whole blood - Part 2: Isolated DNA

Táto norma obsahuje anglickú verziu európskej normy.
This standard includes the English version of the European Standard.

Táto norma bola oznámená vo Vestníku ÚNMS SR č. 03/20

Táto predbežná STN je určená na overenie. Pripomienky zasielajte ÚNMS SR najneskôr do januára 2022.

Obsahuje: CEN/TS 17390-2:2020

130504

TECHNICAL SPECIFICATION
SPÉCIFICATION TECHNIQUE
TECHNISCHE SPEZIFIKATION

CEN/TS 17390-2

January 2020

ICS 11.100.10

English Version

Molecular in vitro diagnostic examinations - Specifications for pre-examination processes for circulating tumor 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 (CTCs) dans le
sang total veineux - Partie 2: ADN extrait

Molekularanalytische in vitro-diagnostische Verfahren
- Spezifikationen für präanalytische Prozesse für
zirkulierende Tumorzellen (CTC) in venösen
Vollblutproben - Teil 2: Isolierte DNA

This Technical Specification (CEN/TS) was approved by CEN on 27 October 2019 for provisional application.

The period of validity of this CEN/TS is limited initially to three years. After two years the members of CEN will be requested to submit their comments, particularly on the question whether the CEN/TS can be converted into a European Standard.

CEN members are required to announce the existence of this CEN/TS in the same way as for an EN and to make the CEN/TS available promptly at national level in an appropriate form. It is permissible to keep conflicting national standards in force (in parallel to the CEN/TS) until the final decision about the possible conversion of the CEN/TS into an EN is reached.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.



EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

CEN/TS 17390-2:2020 (E)

Contents		Page
European foreword		3
Introduction		4
1	Scope	5
2	Normative references	5
3	Terms and definitions	5
4	General considerations	10
5	Outside the laboratory	10
5.1	Specimen collection	10
5.2	Transport requirements	12
6	Inside the laboratory	13
6.1	Specimen reception	13
6.2	Storage requirements for the venous whole blood specimen	13
6.3	Enrichment of CTCs	13
6.4	Quality of enriched CTCs	14
6.5	Storage of enriched CTCs	14
6.6	Isolation of the CTCs	15
6.7	Processing of isolated CTCs	16
6.8	Isolation of DNA from an enriched CTC sample	16
6.9	Quantity and quality assessment of isolated DNA from enriched or isolated CTCs	17
6.10	Storage of isolated DNA from enriched CTCs	17
Annex A (informative) Exemplary complete workflow for the molecular characterization of single CTCs		19
Annex B (informative) Decision guideline for critical steps of the CTC pre-analytical workflow for DNA isolation		22
Bibliography		26

European foreword

This document (CEN/TS 17390-2:2020) has been prepared by Technical Committee CEN/TC 140 “In vitro diagnostic medical devices”, the secretariat of which is held by DIN.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

CEN/TS 17390 consists of the following parts, under the general title *Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for Circulating Tumor Cells (CTCs) in venous whole blood*:

- Part 1: Isolated RNA
- Part 2: Isolated DNA
- Part 3: Preparations for analytical CTC staining

According to the CEN/CENELEC Internal Regulations, the national standards organisations of the following countries are bound to announce this Technical Specification: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

CEN/TS 17390-2:2020 (E)**Introduction**

Solid tumours release cells and bioanalytes into blood and other body fluids. This has opened the option of minimally-invasive tumour detection, diagnosis and characterization from venous whole blood (liquid biopsies). Liquid biopsies are expected to enable earlier detection and diagnosis of cancers and advance personalized patient treatment. These applications have become one of the fastest growing segments of the entire diagnostic market.

Circulating tumour cells (CTCs) in venous whole blood reflect the disease complexity that evolves during tumour progression, with distinct genetic, epigenetic and expression features. Beside the prognostic role of CTC identification and/or enumeration in cancer progression, CTC molecular characterization can improve e.g. disease outcome prediction, therapeutic guidance and post-treatment monitoring of the patient.

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

Molecular characterization of CTCs can provide for example a strategy for monitoring cancer genotype during systemic therapies [1], identification of mechanisms of disease progression, identification of novel targets for biological treatment [2] and to select targeted therapies. Moreover, CTC single-cell sequencing is as an important tool for tumour genomic heterogeneity analysis [3] [4] [5]. Molecular examination techniques such as qPCR, dPCR and sequencing methods including next generation sequencing (NGS) enable to characterize 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 approx. 1:10⁷ 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 need to be taken to get rid of the WBCs in order to obtain good quality DNA samples characterized by high purity and thus representative of the mutational pattern within the tumour.

Standardization of all steps of the pre-examination process is required. This includes blood collection and stabilization, transport, storage, CTC enrichment, CTC isolation (if required), and DNA isolation. An exemplary complete workflow for the molecular characterization of single CTCs is provided in Annex A. A decision guideline for the critical steps of the CTC pre-analytical workflow for DNA isolation is provided in Annex B.

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

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.

1 Scope

This document gives guidelines on the handling, storage, processing and documentation of venous whole blood specimens intended for the examination of human 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 EN ISO 20186-2, *Molecular in vitro diagnostic examinations - Specifications for pre-examination processes for venous whole blood - Part 2: Isolated genomic DNA*.

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

EN ISO 15189:2012, *Medical laboratories - Requirements for quality and competence (ISO 15189:2012, Corrected version 2014-08-15)*

ISO 15190, *Medical laboratories — Requirements for safety*

koniec náhľadu – text ďalej pokračuje v platenej verzii STN