



**International  
Standard**

**ISO 17099**

**Radiological protection —  
Performance criteria for  
laboratories using the cytokinesis-  
block micronucleus (CBMN) assay  
in peripheral blood lymphocytes for  
biological dosimetry**

**Second edition  
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*Radioprotection — Critères de performance pour les  
laboratoires pratiquant la dosimétrie biologique par l'analyse  
des micronoyaux par blocage de la cytotédièrese (CBMN) dans les  
lymphocytes du sang périphérique*

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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)  
Website: [www.iso.org](http://www.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 document 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)).

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](http://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](http://www.iso.org/iso/foreword.html).

This document was prepared by Technical Committee ISO/TC 85, *Nuclear energy, nuclear technologies, and radiological protection*, Subcommittee SC 2, *Radiological protection*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 430, *Nuclear energy, nuclear technologies and radiological protection*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This second edition cancels and replaces the first edition (ISO 17099:2014), which has been technically revised. [standards.iteh.ai/catalog/standards/iso/599393-3532-4aaa-9f0c-ce79fd62f2a5/iso-17099-2024](http://standards.iteh.ai/catalog/standards/iso/599393-3532-4aaa-9f0c-ce79fd62f2a5/iso-17099-2024)

The main changes are as follows:

- minor edits to text throughout;
- reorganization of document to better harmonize with other biological dosimetry standards;
- addition of [7.2.7](#) on data security plan;
- additional requirements added for the report on the conditions of the exposure for the calibration curve in [10.2](#);
- relaxation of the number of individuals required for each age group for establishing background micronucleus frequency, leaving the determination up to the head of the laboratory ([10.3](#));
- addition of details on determining the minimal resolvable dose ([10.4](#)), the absorbed dose ([11.2.4](#)) and the uncertainty ([11.2.5](#));
- removal of reference to coefficient of variance when determining scoring expertise, focussing on the use of 95 % confidence intervals to determine expertise ([11.1.3](#));
- addition of reference to other exposure scenarios ([11.2.8](#));
- removal of Annex on automated micronuclei scoring as it was deemed outside of the scope of the standard;
- addition of a sample group report (see [Annex E](#));

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- addition of a detailed annex (see [Annex F](#)) for calculating the decision threshold and detection limit along with a sample calculation and R script for performing these calculations.

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).

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## Introduction

The purpose of this document is to define the use of the cytokinesis-block micronucleus (CBMN) assay with human peripheral blood lymphocytes for biological dosimetry of exposure to ionizing radiation. This assay is intended to be applied for accidental or malevolent exposures involving

- a) up to a few casualties to provide individual whole-body dose estimates, or
- b) in a triage mode to populations to provide rapid, lower accuracy dose estimates for individuals that can be improved with more accurate analysis at a later time.

The CBMN assay is an alternative cytogenetic technique, which is possibly simpler and faster to perform than the dicentric assay<sup>[1][2]</sup>. It is also routinely used to demonstrate exposure to genotoxic agents, other than ionizing radiation, which is not covered in this document. Although culture of the blood samples is slightly longer than for dicentrics, the scoring of micronuclei (MN) in binucleated lymphocytes is easier.

As was done with the dicentric assay, the CBMN assay has been adapted for the emergency triage of large-scale multi-casualty nuclear or radiological incident. The blood volume required for a sufficient number of scorable binucleated cells (BNCs) is similar to that required for the dicentric assay. Again, the faster counting speed for MN compensates for the extended culture time. However, it has to be considered that factors such as age, sex, diet and environmental mutagens can have an influence on the results particularly after low dose exposures<sup>[3][4][5]</sup>. In addition, the CBMN assay can be performed in an automated mode using various cytometric technologies but these are outside the scope of this document.

This document provides a guideline on how to perform the CBMN assay for dose assessment using documented and validated procedures. Dose assessment using the CBMN assay has relevance in medical management, radiation-protection management, record keeping, and medical/legal requirements. This document is divided into two parts, according to the use of CBMN assay: radiation exposure of a few individuals or population triage in a large radiological or nuclear event.

A part of the information in this document is contained in other international guidelines and scientific publications, primarily in the International Atomic Energy Agency's (IAEA) technical reports series on biological dosimetry. However, this document expands and standardizes the quality assurance and quality control, the criteria of accreditation and the evaluation of performance. This document is generally in conformity with ISO/IEC 17025<sup>[6]</sup> with particular consideration given to the specific needs of biological dosimetry. The expression of uncertainties in dose estimations given in this document complies with ISO/IEC Guide 98-3<sup>[15]</sup> (former GUM) and the ISO 5725 (all parts)<sup>[7]</sup>.





# Radiological protection — Performance criteria for laboratories using the cytokinesis-block micronucleus (CBMN) assay in peripheral blood lymphocytes for biological dosimetry

## 1 Scope

This document gives guidance on

- a) confidentiality of personal information for the customer and the laboratory,
- b) laboratory safety requirements,
- c) calibration sources and calibration dose ranges useful for establishing the reference dose-response curves that contribute to the dose estimation from CBMN assay yields and the detection limit,
- d) performance of blood collection, culturing, harvesting, and sample preparation for CBMN assay scoring,
- e) scoring criteria,
- f) conversion of micronucleus frequency in BNCs into an estimate of absorbed dose,
- g) reporting of results,
- h) quality assurance and quality control, and
- i) informative annexes containing sample instructions for customers, sample questionnaire, a microscope scoring data sheet, and a sample report.

This document excludes methods for automated scoring of CBMN.

## 2 Normative references

There are no normative references in this document.

## 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

#### background frequency

#### background level

spontaneous yield (or number) of MN in BNCs recorded in control samples or individuals who are not abnormally exposed to genotoxins including ionizing radiation

**3.2**  
**binucleated cells**  
**BNCs**

cells that have completed one nuclear division after mitogen stimulation but have been blocked from performing *cytokinesis* (3.6) and are the cell type in which *micronuclei* (3.9) are scored in the CBMN assay

Note 1 to entry: These cells are accumulated in culture using cytochalasin-B which is an inhibitor of cytokinesis.

**3.3**  
**chromosome**

structure that comprises discrete packages of DNA and proteins that carries genetic information which condense to form characteristically shaped bodies during nuclear division

**3.4**  
**confidence interval**

statistical range about an estimated quantity within which the value of the quantity is expected to occur, with a specified probability

**3.5**  
**cytochalasin-B**  
**Cyto-B**

reagent used to block *cytokinesis* (3.6) in dividing cells allowing once-divided cells to be identified as binucleated cells

**3.6**  
**cytokinesis**

physical process of cell division, which divides the cytoplasm of a parental cells into two daughter cells

**3.7**  
**dicentric**

aberrant *chromosome* (3.3) bearing two centromeres derived from the joining of parts from two broken *chromosomes* (3.3)

Note 1 to entry: It is generally accompanied by an acentric fragment.

**3.8**  
**linear energy transfer**  
**LET**

quotient of  $dE/dl$ , as defined by the International Commission on Radiation Units and Measurements (ICRU), where  $dE$  is the average energy locally imparted to the medium by a charged particle of specific energy in traversing a distance of  $dl$

**3.9**  
**micronucleus**  
**MN**

small nucleus that arises from lagging acentric *chromosome* (3.3) fragments or whole chromosomes during nuclear division and *chromosome* (3.3) segregation at mitosis during anaphase/telophase

Note 1 to entry: More than 90 % of the micronuclei induced by ionizing radiation arise from lagging acentric chromosome fragments.

**3.10**  
**non-refractile**

process by which materials do not have the ability to refract or scatter light

**3.11**  
**precision**

dispersion of measurements with respect to a measure of location or central tendency

### 3.12

#### **quality assurance**

planned and systematic actions necessary to provide adequate confidence that a process, measurement, or service has satisfied given requirements for quality

### 3.13

#### **quality control**

part of *quality assurance* (3.12) intended to verify that systems and components correspond to pre-determined requirements

### 3.14

#### **service laboratory**

laboratory performing biological dosimetry measurements

## 4 CMBN assay methodology used in this document

### 4.1 General

In this document, the frequency of MN in cultured human lymphocytes blocked in cytokinesis and scored by microscopy is used for dose estimation after suspected exposure to ionizing radiation.

Lymphocytes are cultured by a method that permits once-divided cytokinesis-block cells to be recognized by their binucleated appearance for analysis. This requires whole blood or isolated lymphocytes to be incubated in culture medium with a mitogen that would enable scoring of MN in first-generation BNCs. A cytokinesis blocking agent, cytochalasin-B, is added at least 6 h, i.e. approximately 24 h after the start of the culture, before the first mitosis commences in order to arrest dividing lymphocytes at the BNC stage after nuclear division is completed. The duration of the cell culture and the timing of addition of the arresting agent are optimised to ensure an adequate frequency of binucleated cells.

BNCs are recovered from the cultures by centrifugation, placing in a hypotonic salt solution and fixing in a mixture of methanol and acetic acid. Fixed cells are placed on microscope slides and stained. In the case of isolated lymphocytes, it is also acceptable to prepare slides by cyto centrifugation of cells onto slides, followed by air-drying, fixation with methanol, and staining. The exact protocol for cell culture, harvesting BNCs and staining employed by a CMBN assay laboratory should be formally documented.

Microscope slides containing stained cells are scanned to identify suitable BNCs. The frequency of MN observed in an appropriate number of scored BNCs is converted to an estimate of radiation dose by reference to calibration data.

### 4.2 Requests for analysis and blood sampling

Depending on national regulations, the request for an analysis should normally be made by a physician representing the patient, or the analysis could be requested by another authority due to legal claims. In all cases where it is normally possible, the blood sampling for MN analysis shall be made with the patient's informed consent. The laboratory head, depending on the national regulations, may be required to maintain the record of the patient's informed consent.

It is the responsibility of the medical staff (e.g. doctor, nurse, etc.) to schedule blood draw and shipping so as to ensure that the blood sample is received by the laboratory in the best possible conditions (see 13.2.4). The purpose is to avoid having the blood sample sit for several hours from time of blood draw and before sample pickup for transportation (see Clause 5 for details).

## 5 Responsibility of the requestor

This clause includes items that are not controlled by the service laboratory. Prior to blood sampling, an initial conversation between the requestor and the service laboratory should occur to co-ordinate the sample collection and shipment. Specific requirements regarding sample collection and shipment should be explained to the requestor including the approximate delivery time for the assay result(s). A standard

instruction sheet (illustrated in [Annex B](#)) explaining the requirements should be sent to the requestor. The requirements include:

- a) Blood sampling shall use Vacutainer®<sup>1)</sup> tubes containing lithium or sodium heparin as the anticoagulant and the Vacutainer® tubes should either be sent or specified by the service laboratory.
- b) Blood shall be collected (ideally about 5 ml), labelled accurately and unambiguously, maintained at room temperature (around 20 °C), and sent to the service laboratory as soon as possible.
- c) Precautions shall be taken to ensure the integrity of the container to prevent leakage during shipment. Blood samples shall be kept at ambient temperature during shipment, i.e. 11° C to 30 °C. A temperature recording device shall be included to ensure that the temperature during shipment is controlled. Packaging and labelling shall conform to national and international regulations. If air transportation is involved, a physical dosimeter shall be included to monitor whether the sample was irradiated in transit.
- d) A questionnaire (see [Annex C](#)) provided by the service laboratory shall be completed and returned prior to the start of blood culturing.
- e) The laboratory shall be alerted of biologically contaminated and/or infectious samples so that extra precautions can be taken when handling the sample.

## 6 Responsibility of the service laboratory

### 6.1 Setup and sustainment of the quality assurance program

The service laboratory shall establish and maintain a quality assurance (QA) program (see [Clause 13](#)), which covers all aspects of the service. The laboratory's QA program shall address the following issues:

- a) It shall include periodic internal checks of equipment operations, reagent suitability, and various performance checks, i.e. intra-laboratory comparison exercises, operator qualifications, sample protocol, scoring, dose estimations, report generation, etc.
- b) It shall include periodic external checks of the laboratory's operations. The external audits shall include a review of the service laboratory's documentation of equipment operations, reagent suitability, and various performance checks, i.e. inter-comparison exercises, operator qualifications, sample transport integrity, etc.

### 6.2 Responsibility during service

The service laboratory shall provide necessary guidance, procedures, and timely reporting to provide dose assessment by the CBMN assay with a request for service. The service activities shall address the following issues:

- a) The service laboratory shall have documentation, reviewed and endorsed by a qualified expert, i.e. service laboratory radiobiologist or equivalent, including the following:
  - 1) an instruction sheet to be sent to the customer describing shipping procedures (see [Annex B](#));
  - 2) a questionnaire that shall elicit patient consent and all available information regarding the patient and the exposure scenario (see [Annex C](#));
  - 3) step by step procedures for processing the blood sample from receipt of the sample to reporting of the dose;
- b) The service laboratory is not responsible for sample transport; however, they should provide advice regarding sample transfer. If required, a kit for the collection of at least 5 ml whole blood in tubes containing lithium or sodium heparin as the anticoagulant shall be sent to the requestor with the

1) *Vacutainer* is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product. Equivalent products may be used if they can be shown to lead to the same results.