SLOVENSKI STANDARD
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Radiološka zaščita - Merila za delovanje laboratorijev, ki za biološko dozimetrijo uporabljajo analizo tvorjenja mikrojeder s citokinetskim blokom (CBMN) v perifernih krvnih limfocitih (ISO/DIS 17099:2023)

Radiological protection - Performance criteria for laboratories using the cytokinesis block micronucleus (CBMN) assay in peripheral blood lymphocytes for biological dosimetry (ISO/DIS 17099:2023)

Strahlenschutz - Leistungskriterien für Laboratorien, die den Zytokineseblock-Mikronukleustest (CBMN) in peripheren Blutlymphozyten für die biologische Dosimetrie verwenden (ISO/DIS 17099:2023)

Radioprotection - Critères de performance pour les laboratoires pratiquant la dosimétrie biologique par l'analyse des micronoyaux par blocage de la cytodiérèse (CBMN) dans les lymphocytes du sang périphérique (ISO/DIS 17099:2023)

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

Radioprotection — Critères de performance pour les laboratoires pratiquant la dosimétrie biologique par le test des micronoyaux avec blocage de la cytodiérèse (CBMN) dans les lymphocytes du sang périphérique

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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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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

For an explanation on 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 the following URL: 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*.

This second edition cancels and replaces the first edition (ISO 17099:2014), which has been technically revised.

The main changes compared to the previous edition are as follows:

— minor edits to text throughout;

— reorganization of document to better harmonize with other biodosimetry 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 added (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 (Annex E);

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

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 interim dose estimates for individuals.

The CBMN assay is an alternative cytogenetic technique, which is possibly simpler and faster to perform than the dicentric assay.[1,2] 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 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 radiation accidents. 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 micronuclei 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 [3-5] 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 compliant with ISO/IEC 17025 "*General requirements for the competence of testing and calibration laboratories*"[6]with particular consideration given to the specific needs of biological dosimetry. The expression of uncertainties in dose estimations given in this document complies with the "ISO-guide for the expression of uncertainty in measurement" (former GUM) and the ISO 5725-all parts [7].

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

1 Scope

This document addresses the following:

a) the confidentiality of personal information for the customer and the laboratory;

b) the laboratory safety requirements;

c) the 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) the performance of blood collection, culturing, harvesting, and sample preparation for CBMN assay scoring;

e) the scoring criteria;

f) the conversion of micronucleus frequency in BNCs into an estimate of absorbed dose;

g) the reporting of results;

h) quality assurance and quality control;

i) informative annexes containing sample instructions for customers, sample questionnaire, a microscope scoring data sheet, and a sample report.

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 terminological 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
acentric
terminal or interstitial chromosome fragment of varying size, referred to as an excess acentric fragment when it is formed independently of a dicentric or centric ring chromosome aberration

3.2
background frequency/level
spontaneous yield (or number) of micronuclei in BNCs recorded in control samples or individuals who are not abnormally exposed to genotoxins including ionising radiation

3.4
binucleated cells
BNCs
cells that have completed one nuclear division after mitogen stimulation but have been blocked from performing cytokinesis and are the cell type in which micronuclei 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.

3.5
centric ring
aberrant circular chromosome resulting from the joining of two breaks on separate arms of the same chromosome

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

3.6
centromere
specialized constricted region of a chromosome that appears during mitosis and joins together the chromatid pair

3.7
chromosome
structure that comprises discrete packages of DNA and proteins that carries genetic information which condense to form characteristically shaped bodies during nuclear division

3.8
chromatid
either of the two strands of a duplicated chromosome that are joined by a single centromere and separate during cell division to become individual chromosomes

3.9
confidence interval
statistical range about an estimated quantity within which the value of the quantity is expected to occur, with a specified probability

3.10
cytochalasin-B
Cyto-B
reagent used to block cytokinesis in dividing cells allowing once-divided cells to be identified as binucleated cells

3.11
cytokinesis
the physical process of cell division, which divides the cytoplasm of a parental cells into two daughter cells

3.12
dicentric
aberrant chromosome bearing two centromeres derived from the joining of parts from two broken chromosomes

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

3.13
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

3.14
micronucleus
micronuclei
MN
small nucleus that arises from lagging acentric chromosome fragments or whole chromosomes during nuclear division and chromosome segregation at mitosis during anaphase/telophase

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

3.17
non-refractile
process by which materials do not have the ability to refract or scatter light

3.19
precision
concept employed to describe dispersion of measurements with respect to a measure of location or central tendency

3.20
quality assurance
planned and systematic actions necessary to provide adequate confidence that a process, measurement, or service has satisfied given requirements for quality

3.21
quality control
part of quality assurance intended to verify that systems and components correspond to pre-determined requirements

3.22
service laboratory
laboratory performing biological dosimetry measurements

4 CMBN assay methodology used in this document

In this document, the frequency of micronuclei 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 binucleated cell 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.

Binucleated cells 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 cytocentrifugation 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 CBMN 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.

Methods for automated scoring of CBMN have been validated but are outside the scope of this document.