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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 analyse des micronoyaux par blocage de la cytokinèse (CBMN) dans les lymphocytes du sang périphérique

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Foreword

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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 meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT), see the following URL: Foreword — Supplementary information.

The committee responsible for this document is ISO/TC 85, *Nuclear energy, nuclear technologies, and radiological protection*, Subcommittee SC 2, *Radiological protection*.

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Introduction

The purpose of this International Standard 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 full 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 (ISO 19238:2014, ISO 21243:2008). It is also routinely used to demonstrate exposure to genotoxic agents, other than ionizing radiation, which is not covered in this International Standard. 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 sufficient number of scorable binucleated cells is similar than required for the dicentric assay. Again, the faster counting speed for micronuclei compensates for the extended culture time. In addition, the CBMN assay can be performed in an automated mode.

This International Standard 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 International Standard 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 event.

A part of the information in this International Standard 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 International Standard expands and standardizes the quality assurance and quality control, the criteria of accreditation and the evaluation of performance. This International Standard is generally compliant with ISO/IEC 17025 “*General requirements for the competence of testing and calibration laboratories*” with particular consideration given to the specific needs of biological dosimetry. The expression of uncertainties in dose estimations given in this International Standard complies with the “ISO-guide for the expression of uncertainty in measurement” (former GUM) and the ISO 5725-all parts.

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

1 Scope

This International Standard addresses the following:

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

b) laboratory safety requirements;

c) radiation sources, dose rates, and ranges used for establishing the calibration reference dose-effect curves allowing the dose estimation from CBMN assay yields and the minimum resolvable dose;

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

e) scoring criteria;

f) conversion of micronucleus frequency in binucleated cells into an estimate of absorbed dose;

g) reporting of results;

h) quality assurance and quality control;

i) informative annexes containing examples of a questionnaire, instructions for customers, a microscope scoring data sheet, a sample report and advice on strengths and limitations of current automated systems for automated micronucleus scoring.

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2 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

2.1
acentric
chromosome fragment of varying size

Note 1 to entry: When it is formed independently of a dicentric or centric ring chromosome aberration, it is usually referred to as an excess acentric.

2.2
background level
spontaneous yield (or number) of micronuclei recorded in control samples or individuals

2.3
bias
statistical sampling or testing error caused by systematically favouring some outcomes over others

2.4
binucleated cells
cells that have completed one nuclear division after mitogen stimulation and cell type in which micronuclei are scored

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

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2.5
CBMN laboratory
laboratory performing biological dosimetry measurements using the CBMN assay

2.6
centric ring
aberrant circular chromosome resulting from the joining of two breaks on separate arms of the same chromosome, generally accompanied by one acentric fragment

2.7
centromere
specialized constricted region of a chromosome that appears during mitosis joining together the two sister chromatids

2.8
chromosome
structure that carries genetic information

Note 1 to entry: Normally, 46 such structures are contained in the human cell nucleus. During nuclear division, they condense to form characteristically-shaped bodies.

2.9
chromatid
either of the two strands of a duplicated chromosome that are joined by a single centromere

Note 1 to entry: Chromatids separate during mitosis to become individual chromosomes.

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2.10
confidence interval
statistical range about an estimated quantity within which the value of the quantity is expected to occur, with a specified probability

2.11
cytochalasin-B
Cyto-B
reagent used to block cytokinesis in dividing cells allowing once-divided cells to be identified as binucleated cells

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Note 1 to entry: The binucleated cells are the cells in which micronuclei are specifically scored.

2.12
dicentric
aberrant chromosome bearing two centromeres derived from the joining of parts from two broken chromosomes, generally accompanied by an acentric fragment

2.13
fluorescence in situ hybridization
FISH
technique that uses specific sequences of DNA as probes to particular parts of the genome, allowing the chromosomal regions to be highlighted or “painted” in different colours by attachment of various fluorochromes

Note 1 to entry: This technique permits the detection of damage involving exchanges between differently painted pieces of DNA (usually whole chromosomes).

2.14
interphase
period of the cell cycle between the mitotic divisions

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2.15
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

2.16
metaphase
second stage of mitosis when the nuclear membrane is dissolved, the chromatids are condensed to their minimum lengths and are aligned for division at the metaphase plate

2.17
micronucleus or 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.

2.18
minimum detection level
MDL
smallest measurable amount (e.g. yield or dose) that is detected with a probability β of non-detection (Type II error) while accepting probability *α* of erroneously deciding that a positive (non-zero) quantity is present in an appropriate background sample (Type I error)

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2.19
minimum resolvable dose
lowest additional dose for which the lower 95 % poisson confidence limit is greater than 0, so that there is a 97,5 % chance that the dose received in excess of normal background is greater than 0

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2.20
nuclear division index
index in the CBMN assay that is calculated from the relative frequencies of mononucleated, binucleated, and multinucleated cells

Note 1 to entry: This index provides a measure of inhibition of nuclear division.

2.21
precision
dispersion of measurements with respect to a measure of location or central tendency

2.22
quality assurance
planned and systematic actions necessary to provide adequate confidence that a process, measurement, or service has satisfied given requirements for quality

EXAMPLE Dose specified in a licence.

2.23
quality control
part of quality assurance intended to verify that systems and components correspond to pre-determined requirements

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3 Micronucleus assay methodology used in this standard

3.1 General

In this International Standard, the frequency of micronuclei in cytokinesis block binucleated lymphocytes in cultured human peripheral blood lymphocytes 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 lymphocytes separated from the other blood components to be incubated in culture medium with a mitogen that would enable scoring of micronuclei in first-generation binucleated cells. A cytokinesis blocking agent, cytochalasin-B, is added at least 6 h before the first mitosis commences 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 binucleated cells and staining employed by a CBMN laboratory should be formally documented.

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

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3.2 General requirement of the laboratory

The laboratory should be well-equipped with the required bio-hazard units, tissue culture, and standard laboratory equipment for lymphocyte tissue culture, cell separation, slide preparation, and microscopy scoring of cells and subcellular structures, such as micronuclei. The laboratory should maintain quality assurance documents including those describing periodic calibration of the equipment used for cell culture such as laminar flow hoods, pipettes, incubator, etc.

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3.3 Requests for analysis and blood sampling

Depending on national regulations, the request for an analysis should normally be made by a doctor representing the patient, by the patient him/herself, or could be requested due to legal claims. In all cases where it is normally possible, the blood sampling for micronuclei analysis shall be made with the patient's informed consent. It is advisable that the laboratory head maintain the record of the patient's informed consent and the patient should also indicate who they will allow to receive the data. For minors, the informed consent should be obtained from the parent/guardian.

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. The purpose is to avoid having the blood sample sit for several hours from time of blood draw and before sample pickup for transportation.

The blood sample is collected using lithium or sodium heparin anticoagulant, maintained at room temperature (at approximately 20 °C) and cultured as soon as possible, but before 72 h. In some unavoidable circumstances involving a delay beyond 72 h, good sample preparation is still possible if the blood samples are stored with due precautions, such as using room temperature gel packs to maintain a temperature of 20 °C.

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3.4 Cell culturing

The protocol for the CBMN assay shall be established and documented by each CBMN laboratory. The protocol used for the calibration curve and for dose estimates of patient samples shall be identical. There are several critical aspects that shall be adhered to.

a) Blood used to establish the calibration curves shall be incubated for 2 h at 37 °C immediately following irradiation and prior to culture of samples.

b) Cultures should be set up in duplicate to allow the determination of the intra-experimental coefficient of variation.

c) Cells shall be cultured at 37 °C ± 0,5 °C either as whole blood, enriched lymphocyte suspension (buffy coat), or isolated lymphocytes.

d) Culture vessel shall be sterile and handled in a way to avoid microbial contamination.

e) Specific culture media that allow peripheral blood lymphocytes to proliferate shall be used.

   EXAMPLE RPMI-1640, Ham's F10, MEM, or McCoy supplemented with Foetal Bovine Serum (FBS) between 10 % and 20 %, 200 mM L-glutamine, and penicillin/Streptomycin (100 IU ml^{-1}/100 $\mu g \cdot ml^{-1}$) is commonly used.

f) Mitogen [e.g. phytohaemagglutinin (PHA)] shall be added to the media to stimulate lymphocytes into mitosis.

g) Cytochalasin-B (Cyto-B) shall be added, 24 h to 44 h after mitogen stimulation at a concentration of at least 3,0 µg/ml and no more than 6,0 µg/ml to the cell culture to block cytokinesis in cells during their first nuclear division after mitogen stimulation.

h) The timing of harvest is crucial to maximize the number of binucleated cells and minimize the number of mononucleated and multinucleated cells. It shall be adapted according to the standard culture conditions for each CBMN laboratory. The recommended culture time after mitogen stimulation for cell harvest is 72 h but under certain conditions (e.g. where mitotic delay is anticipated), longer time might be required. Typically, binucleated cells are harvested 24 h to 48 h after addition of cytochalasin-B.

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i) Cells may be treated with a hypotonic solution such as 0,075 M KCl for 10 min to 15 min to swell the cells prior to fixation.

j) Cells may be fixed in suspension and then transferred to slides or alternatively, they may be transferred to slides by cyto-centrifugation and then fixed on the slide after air drying. In the former case, cells shall be fixed in freshly prepared fixative solution (i.e. 5:1 methanol:acetic acid) while agitating the cells to prevent clump formation and washed three times or four times with the same fixative until the cell suspension is clear. In the latter case, cells shall be fixed in absolute methanol.

k) If storage of fixed cells is required, then cell suspensions shall be kept in a −20 °C freezer.

l) Slides shall be prepared to ensure integrity of the cell membrane and allow an unambiguous identification of micronuclei in binucleated cells. Humidity and temperature conditions can be adjusted to increase the quality of the spreading.

m) Duplicate cultures should be performed from each blood sample per individual.

3.5 Staining

Cells shall be stained appropriately so that nuclei and micronuclei can be clearly visualized. Commonly used stains include, but are not limited to, Giemsa (for brightfield microscopy), DAPI, and acridine orange (for fluorescence microscopy). The stain used shall be specific for nuclei and micronuclei to avoid artefactual staining of other cellular structures that might resemble micronuclei (e.g. centrioles).

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