ISO/FDIS 17099~~:2024(en)~~

~~Date: 2024-02-26~~

ISO/TC 85/SC 2~~/WG 18~~

Secretariat: AFNOR

Date: 2024-xx

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

FDIS stage

ISO copyright office
CP 401 • Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel.Phone: + 41 22 749 01 11

Fax + 41 22 749 09 47

E-mail: copyright@iso.org
Website: www.iso.org

Published in Switzerlandwww.iso.org

Formatted: Font: Bold
Formatted: Font: Bold
Formatted: HeaderCentered, Line spacing: single
Formatted: Left: 1.5 cm, Right: 1.5 cm, Header distance from edge: 1.27 cm, Footer distance from
Commented [eXtyles1]: The reference "ISO 2024" is to a withdrawn standard
Commented [eXtyles2]: The reference "ISO 2024" is to a withdrawn standard
Formatted: Default Paragraph Font
Formatted: Default Paragraph Font
Formatted: Indent: Left: 0 cm, Right: 0 cm, Space Before: 0 pt, No page break before, Adjust space between Latin and Asian text, Adjust space between Asian text and numbers
Formatted: French (France)
Formatted: French (France)
Formatted: zzCopyright address, Indent: Left: 0 cm, First line: 0 cm, Right: 0 cm, Adjust space between Latin and Asian text, Adjust space between Asian text and numbers
Formatted: Font: 11 pt
Formatted: FooterPageRomanNumber, Space Before: 0 pt, Line spacing: single, Tab stops: Not at 17.2 cm

Contents

Formatted: Font: 11 pt, Bold
Formatted: Font: Bold
Formatted: HeaderCentered, Left, Line spacing: single
Formatted: Adjust space between Latin and Asian text, Adjust space between Asian text and numbers
Formatted: Font: 10 pt
Formatted: Font: 10 pt
Formatted: Font: 10 pt
Formatted: FooterCentered, Left, Line spacing: single
Formatted: Font: 11 pt
Formatted: FooterPageRomanNumber, Left, Space After: 0 pt, Line spacing: single

Formatted: Font: Bold
Formatted: Font: Bold
Formatted: HeaderCentered, Line spacing: single
Formatted: Font: 11 pt
Formatted: FooterPageRomanNumber, Space Before: 0 pt, Line spacing: single, Tab stops: Not at 17.2 cm

Formatted: Font: 11 pt, Bold
Formatted: Font: Bold
Formatted: HeaderCentered, Left, Line spacing: single
Formatted: Font: 10 pt
Formatted: Font: 10 pt
Formatted: Font: 10 pt
Formatted: FooterCentered, Left, Line spacing: single
Formatted: Font: 11 pt
Formatted: FooterPageRomanNumber, Left, Space After: 0 pt, Line spacing: single

Formatted: Font: Bold
Formatted: Font: Bold
Formatted: HeaderCentered, Line spacing: single
Formatted: Font: 11 pt
Formatted: FooterPageRomanNumber, Space Before: 0 pt, Line spacing: single, Tab stops: Not at 17.2 cm

Formatted: Font: 11 pt, Bold
Formatted: Font: Bold
Formatted: HeaderCentered, Left, Line spacing: single
Formatted: Font: 10 pt
Formatted: Font: 10 pt
Formatted: Font: 10 pt
Formatted: FooterCentered, Left, Line spacing: single
Formatted: Font: 11 pt
Formatted: FooterPageRomanNumber, Left, Space After: 0 pt, Line spacing: single

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).

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.~~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~~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*~~*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.

The main changes are as follows:

— ~~—~~minor edits to text throughout;

— ~~—~~reorganization of document to better harmonize with other biodosimetry standards

— ~~—~~addition of ~~7.2.7~~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.~~10.2:

Formatted: Font: Bold
Formatted: Font: Bold
Formatted: HeaderCentered, Line spacing: single
Formatted: Adjust space between Latin and Asian text, Adjust space between Asian text and numbers
Formatted: English (United Kingdom)
Formatted: Font: Italic
Formatted: Default Paragraph Font
Formatted: Default Paragraph Font
Formatted: Default Paragraph Font
Formatted: Adjust space between Latin and Asian text, Adjust space between Asian text and numbers, Tab stops: Not at 0.7 cm + 1.4 cm + 2.1 cm + 2.8 cm + 3.5 cm + 4.2 cm + 4.9 cm + 5.6 cm + 6.3 cm + 7 cm
Formatted: Font: 11 pt
Formatted: FooterPageRomanNumber, Space Before: 0 pt, Line spacing: single, Tab stops: Not at 17.2 cm

— 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);~~(10.3);

— addition of details on determining the minimal resolvable dose ~~(10.4),~~(10.4), the absorbed dose ~~(11.2.4)~~(11.2.4) and the uncertainty ~~(11.2.5);~~(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);~~(11.1.3);

— addition of reference to other exposure scenarios added ~~(11.2.8);~~(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);~~(Annex E);

— addition of a detailed annex ~~(Annex F)~~(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~~www.iso.org/members.html.

Formatted: Font: 11 pt, Bold
Formatted: Font: Bold
Formatted: HeaderCentered, Left, Line spacing: single
Formatted: Adjust space between Latin and Asian text, Adjust space between Asian text and numbers
Formatted: Font: 10 pt
Formatted: Font: 10 pt
Formatted: Font: 10 pt
Formatted: FooterCentered, Left, Line spacing: single
Formatted: Font: 11 pt
Formatted: FooterPageRomanNumber, Left, Space After: 0 pt, Line spacing: single

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.[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 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 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][4][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 in conformity with ISO/IEC 17025[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 ISO/IEC Guide 98-3[15] (former GUM) and the ISO 5725- (all parts)[7].

Formatted: Font: Bold
Formatted: Font: Bold
Formatted: HeaderCentered, Line spacing: single
Formatted: Numbered + Level: 1 + Numbering Style: a, b, c, … + Start at: 1 + Alignment: Left + Aligned at: 0 cm + Indent at: 0 cm, Adjust space between Latin and Asian text, Adjust space between Asian text and numbers, Tab stops: Not at 0.7 cm + 1.4 cm + 2.1 cm + 2.8 cm + 3.5 cm + 4.2 cm + 4.9 cm + 5.6 cm + 6.3 cm + 7 cm
Formatted: Adjust space between Latin and Asian text, Adjust space between Asian text and numbers
Formatted: Default Paragraph Font
Formatted: Default Paragraph Font
Commented [eXtyles3]: Invalid reference: "ISO 5725-all parts"
Commented [eXtyles4]: Invalid reference: "ISO 5725-all parts"
Formatted: Default Paragraph Font
Formatted: Default Paragraph Font
Formatted: Default Paragraph Font
Formatted: Default Paragraph Font
Formatted: Default Paragraph Font
Formatted: Default Paragraph Font
Formatted: Default Paragraph Font
Formatted: Font: 11 pt
Formatted: FooterPageRomanNumber, Space Before: 0 pt, Line spacing: single, Tab stops: Not at 17.2 cm

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 terminologicalterminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obphttps://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/https://www.electropedia.org/

Formatted: Left: 1.5 cm, Right: 1.5 cm, Section start: New page, Header distance from edge: 1.27 cm, Footer distance from edge: 0.5 cm

Formatted: Main Title 1, Space After: 0 pt, Adjust space between Latin and Asian text, Adjust space between Asian text and numbers

Formatted: Adjust space between Latin and Asian text, Adjust space between Asian text and numbers

Formatted: Numbered + Level: 1 + Numbering Style: a, b, c, … + Start at: 1 + Alignment: Left + Aligned at: 0 cm + Indent at: 0 cm, Adjust space between Latin and Asian text, Adjust space between Asian text and numbers, Tab stops: Not at 0.7 cm + 1.4 cm + 2.1 cm + 2.8 cm + 3.5 cm + 4.2 cm + 4.9 cm + 5.6 cm + 6.3 cm + 7 cm

Formatted: Adjust space between Latin and Asian text, Adjust space between Asian text and numbers

Commented [eXtyles5]: The URL https://www.iso.org/obp has been redirected to https://www.iso.org/obp/ui. Please verify the URL.

Formatted: Adjust space between Latin and Asian text, Adjust space between Asian text and numbers, Tab stops: Not at 0.7 cm + 1.4 cm + 2.1 cm + 2.8 cm + 3.5 cm + 4.2 cm + 4.9 cm + 5.6 cm + 6.3 cm + 7 cm

Formatted: Font: 11 pt

Formatted: Left, Space After: 0 pt, Line spacing: single

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

3.~~3~~2
binucleated cells
BNCs
cells that have completed one nuclear division after mitogen stimulation but have been blocked from performing *cytokinesis* ~~(3.7)~~(3.6) and are the cell type in which *micronuclei* ~~(3.10)~~(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.~~4~~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.~~5~~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.~~6~~5
cytochalasin-B
Cyto-B
reagent used to block *cytokinesis* ~~(3.7)~~(3.6) in dividing cells allowing once-divided cells to be identified as binucleated cells

3.~~7~~6
cytokinesis
physical process of cell division, which divides the cytoplasm of a parental cells into two daughter cells

3.~~8~~7
dicentric
aberrant *chromosome* ~~(3.4)~~(3.3) bearing two centromeres derived from the joining of parts from two broken *chromosomes* ~~(3.4)~~(3.3)

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

Formatted: Font: Bold
Formatted: Font: Bold
Formatted: HeaderCentered, Line spacing: single
Formatted: Adjust space between Latin and Asian text, Adjust space between Asian text and numbers
Commented [eXtyles6]: The term "acentric" is used only in terms and definitions section
Formatted: Adjust space between Latin and Asian text, Adjust space between Asian text and numbers
Formatted: Adjust space between Latin and Asian text, Adjust space between Asian text and numbers, Tab stops: Not at 0.7 cm + 1.4 cm + 2.1 cm + 2.8 cm + 3.5 cm + 4.2 cm + 4.9 cm + 5.6 cm + 6.3 cm + 7 cm
Formatted: Adjust space between Latin and Asian text, Adjust space between Asian text and numbers
Formatted: Adjust space between Latin and Asian text, Adjust space between Asian text and numbers, Tab stops: Not at 0.7 cm + 1.4 cm + 2.1 cm + 2.8 cm + 3.5 cm + 4.2 cm + 4.9 cm + 5.6 cm + 6.3 cm + 7 cm
Formatted: Font: 11 pt
Formatted: FooterPageRomanNumber, Space Before: 0 pt, Line spacing: single, Tab stops: Not at 17.2 cm