FINAL DRAFT
International Standard

ISO/FDIS 17099

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

ISO/TC **85**/SC **2**

Secretariat: **AFNOR**

Voting begins on:
2024-03-28

Voting terminates on:
2024-05-23

ISO/CEN PARALLEL PROCESSING

Reference number
ISO/FDIS 17099:2024(en)

COPYRIGHT PROTECTED DOCUMENT

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

Foreword ..... **v**
Introduction ..... **vii**
1 Scope **1**
2 Normative references **1**
3 Terms and definitions **1**
4 CMBN assay methodology used in this document **3**
4.1 General 3
4.2 Requests for analysis and blood sampling 3
5 Responsibility of the requestor **3**
6 Responsibility of the service laboratory **4**
6.1 Setup and sustainment of the quality assurance program 4
6.2 Responsibility during service 4
7 Confidentiality of personal information **5**
7.1 Overview 5
7.2 Applications of the principle of confidentiality 5
7.2.1 Delegation of responsibilities within the laboratory 5
7.2.2 Requests for analysis 6
7.2.3 Transmission of confidential information 6
7.2.4 Anonymity of samples 6
7.2.5 Reporting of results 6
7.2.6 Storage 6
7.2.7 Data security plan 6
8 Laboratory safety requirements **6**
8.1 Overview 6
8.2 Microbiological safety requirements 7
8.3 Chemical safety requirements 7
8.4 Optical safety requirements 8
8.5 Safety plan 8
9 Sample processing **8**
9.1 Culturing 8
9.2 Staining 10
9.3 Microscopy 10
9.4 Scoring of slides 10
9.4.1 General 10
9.4.2 Criteria for scoring 10
9.4.3 Scoring data sheets 11
9.5 Automated analysis 11
10 Calibration source(s), calibration curve, and minimum detectable dose **11**
10.1 Calibration source(s) 11
10.2 Calibration curve 11
10.3 Background micronucleus frequency 12
10.4 Comparison with the background level: Characterisation of the minimum detectable dose 13
11 Accidental exposure involving few individuals **15**
11.1 Procedure for scoring MN in BNCs 15
11.1.1 Coding of samples and slides 15
11.1.2 Scoring techniques 16
11.1.3 Laboratory scoring expertise 16
11.2 Criteria for converting a micronucleus yield into an estimate of absorbed dose 16
11.2.1 Overview 16

11.2.2 Comparison with controls 16
11.2.3 Confidence limits on the number of MN 16
11.2.4 Calculation of absorbed dose for whole-body exposures 16
11.2.5 Calculation of uncertainty on absorbed dose 17
11.2.6 Acute and non-acute exposure cases 17
11.2.7 Testing the distribution of MN per binucleated cell 18
11.2.8 Other exposure scenarios 18
11.3 Reporting of results 18
11.3.1 General 18
11.3.2 Content of the report (see Annex D for a standard form) 18
11.3.3 Interpretation of the results 19

12 Population triage 19
12.1 General 19
12.2 Use of a CBMN assay network for large scale exposures 19
12.3 Procedure for scoring MN in BNCs 20
12.4 Criteria for converting a micronucleus yield into an estimate of absorbed dose 20
12.5 Reporting of results 20

13 Quality assurance and quality control 20
13.1 Overview 20
13.2 Specific Requirement 20
13.2.1 General 20
13.2.2 Performance checks by laboratory inter-comparisons 20
13.2.3 Periodical performance check of scorer qualification 21
13.2.4 Performance checks of sample transport integrity 21
13.2.5 Performance checks of sample integrity by service laboratory 21
13.2.6 Performance checks for instrumentation 21
13.2.7 Performance checks of sample protocol 22
13.2.8 Performance checks of sample scoring 22
13.2.9 Performance checks of dose and confidence limits estimation 22
13.2.10 Performance checks for result report generation 22

Annex A (informative) **Sample data sheet for recording MN in BNCs 23**

Annex B (informative) **Instructions for requestor (sample) 24**

Annex C (informative) **Sample questionnaire 25**

Annex D (informative) **Sample of report for single assessment 27**

Annex E (informative) **Sample group report 29**

Annex F (informative) **Decision threshold and detection limit 31**

Bibliography 34

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. 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.

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.

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 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.

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.

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].

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 micronuclei 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 that 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 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.

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 vacutainers containing lithium or sodium heparin as the anticoagulant and the vacutainers 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 appropriately labelled and addressed packaging material for the return of the sample to the service