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Radiological protection — Performance criteria for service laboratories performing biological dosimetry by cytogenetics — Dicentric assay

Radioprotection — Critères de performance pour les laboratoires de service pratiquant la dosimétrie biologique par cytogénétique — Dénombrement des dicentriques

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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 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).

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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 third edition cancels and replaces the second edition (ISO 19238:2014), of which it constitutes a minor revision.

The main changes are as follows:

— title changed from "*Radiological Protection — Performance criteria for service laboratory performing biological dosimetry by cytogenetics*" to "*Radiological protection — Performance criteria for service laboratory performing biological dosimetry by cytogenetics — Dicentric assay*";

— minor edits to text throughout;

— addition of 8.2.7 on data security plan;

— simplification of laboratory safety requirements including deletion of safety plan to demonstrate that each laboratory shall meet the requirements of their country;

— addition of material related to automated analysis;

— addition of detail in 10.2.3 on scoring first-division metaphases;

— addition of detail in 11.2, Establishment of calibration curve(s);

— addition of details on determining the minimal resolvable dose.

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 widening use of ionising radiations for medical, industrial, agricultural, research, and military purposes increases the risk of overexposure of radiation workers and individuals of the general population. Biological dosimetry, based on the study of chromosomal aberrations, mainly through the dicentric assay, has become a routine component of accidental dose assessment. Experience with its application in hundreds of cases of suspected or verified overexposures has proven the value of this method and also defined its limitations. It should be emphasized that dicentric chromosome analysis is used as a dosimeter and provides one input into the compendium of information needed for assessment of a radiological incident.

Many studies on animals and humans have shown that one can establish a good correlation between the results obtained in vivo and in vitro, so that in vitro established dose-effect relationships from irradiated blood samples can be used to form calibration curves. The dicentric yield is dependent on radiation quality and dose rate, as well as the circumstances of exposure, for example time since exposure, homogeneity, so information about these variables is important for each investigation. If known, these exposure characteristics are important for refining the aberration dose estimates. The specificity of this technique is enhanced by the fact that generally 1 dicentric is observed per 1 000 metaphase spreads in the normal population, and that this frequency is essentially independent of age and sex. The precision of the technique thus depends on the number of cells observed, the background level, and the calibration curve used. Theoretically, it is possible to detect exposure as low as 0,01 Gy, however, for such low doses, it is necessary to analyse tens of thousands of metaphase spreads. In practice, this level of detection is neither feasible nor necessary. The upper dose detection limits extend well into the range of doses that are lethal to humans.

The primary purpose of this document is to provide a guideline to all laboratories in order to perform the dicentric assay using documented and validated procedures. Secondly, it facilitates the comparison of results obtained in different laboratories, particularly for international collaborations or interlaboratory comparisons. Finally, laboratories newly commissioned to carry out the dicentric assay should conform to this document in order to perform the assay reproducibly and accurately.

This document is written in the form of procedures to be adopted for biological dosimetry for overexposures involving, at most, a few casualties. The criteria required for such measurements usually depends upon the application of the results: radiation protection management, medical management when appropriate, record keeping, and legal requirements. In the special situation of a mass radiation casualty and limited resources, the technique can be applied for emergency triage analysis as described in ISO 21243[1].

A part of the information in this document can be found in other international guidelines and scientific publications, primarily in the International Atomic Energy Agency's (IAEA) Technical Reports series on biological dosimetry[2]. 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, with particular consideration given to the specific needs of biological dosimetry. The expression of uncertainties in dose estimations given in this document comply with the ISO guide to the expression of uncertainty in measurement (ISO/IEC Guide 98-1[3]) and the ISO 5725-1[4], ISO 5725-2[5] and ISO 5725-3[6] on accuracy (trueness and precision) of measurement methods and results.

Radiological protection — Performance criteria for service laboratories performing biological dosimetry by cytogenetics — Dicentric assay

1 Scope

This document provides criteria for quality assurance and quality control, evaluation of the performance and the accreditation of biological dosimetry by cytogenetic service laboratories using the dicentric assay performed with manual scoring.

This document is applicable to

a) the confidentiality of personal information, for the requestor and the service 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 unstable chromosome aberration frequency and the detection limit,

d) the scoring procedure for unstable chromosome aberrations used for biological dosimetry,

e) the criteria for converting a measured aberration frequency into an estimate of absorbed dose,

f) the reporting of results,

g) the quality assurance and quality control, and

h) informative annexes containing sample instructions for requestor (see Annex A), sample questionnaire (see Annex B), sample report (see Annex C), fitting of the low dose-response curve by the method of maximum likelihood and calculating the error of the dose estimate (see Annex D), odds ratio method for cases of suspected exposure to a low dose (see Annex E), a method for determining the decision threshold and detection limit (see Annex F) and sample data sheet for recording aberrations (see Annex G).

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO/IEC 17025, *General requirements for the competence of testing and calibration laboratories*

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
background level
spontaneous frequency (or number) of chromosome aberrations recorded in control samples or individuals

3.3
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* (3.1) fragment.

3.4
confidence interval
range within which the true value of a statistical quantity lies with a specified probability

3.5
chromosome
structure that comprises discrete packages of DNA and proteins that carry genetic information, and which condenses to form characteristically shaped bodies during nuclear division

3.6
chromatid
either of the two strands of a duplicated *chromosome* (3.5) that are joined by a single centromere and which separate during cell division to become individual *chromosomes* (3.5)

3.7
cytogenetics
branch of genetics that deals with the study of *chromosomes* (3.5)

3.8
dicentric
aberrant *chromosome* (3.5) having two centromeres derived from the joining of parts from two broken *chromosomes* (3.5), generally accompanied by an *acentric* (3.1) fragment

3.9
interphase
period of a cell cycle between mitotic divisions

3.10
linear energy transfer
LET
quotient of the mean energy lost by the charged particles due to electronic interactions in traversing a distance in the material, minus the mean sum of the kinetic energies in excess of the maximum energy of electrons locally deposited, of all the electrons released by the charged particles and the distance traversed

3.11
metaphase
stage of mitosis when the nuclear membrane is dissolved and the *chromosomes* (3.5) are condensed to their minimum lengths and aligned for division

3.12
mitotic index
percentage of cells of a cell population under division at a particular time of observation

3.13
precision
concept used to describe dispersion of measurements with respect to a measure of location or central tendency

3.14
quality assurance
QA
planned and systematic actions necessary to provide adequate confidence that a process, measurement, or service satisfies given requirements for quality

3.15
quality control
QC
planned and systematic actions intended to verify that systems and components conform with predetermined requirements

3.16
Qdr method
chromosome (3.5) aberration yield in cells with a *chromosome* (3.5) aberration, typically calculated as the number of dicentrics and/or rings divided by the number of *metaphase* (3.11) spreads with either a dicentric or ring

3.17
service laboratory
laboratory performing biological dosimetry measurements

4 Abbreviated terms

BrdU	Bromodeoxyuridine
Co	Cobalt
covar	Covariance
Cs	Cesium
Cu	Copper
DNA	Deoxyribonucleic acid
FBS	Foetal bovine serum
FpG	Fluorescence plus Giemsa
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
Gy	Gray
H_0	Null hypothesis
H_1	Alternative hypothesis
HVL	Half-value layer
IAEA	International Atomic Energy Agency
IATA	International Air Transport Association
IEC	International Electrochemical Commission

ISO	International Organization for Standardization
IU	International units
KCl	Potassium chloride
LET	Linear energy transfer
MEM	Minimum essential medium
PHA	Phytohaemagglutinin
R^2	Coefficient of determination
SE	Standard error
SSD	Source-to-surface distance
TBT	Technical Barriers to Trade
TC	Technical Committee
var	Variance
WTO	World Trade Organization
y_d	Decision threshold
y_z	Detection limit

5 Dicentric assay

Determining the frequency of unstable chromosomal aberrations at metaphase in cultured human peripheral blood lymphocytes is the recommended method for biological dosimetry. The chromosome aberrations to be used are either dicentrics only or dicentrics plus centric rings. For the application of this document, the service laboratory shall choose which type of aberrations to score for the purpose of assessing dose estimates and shall be consistent throughout. Hereafter, chromosome aberrations are referred to as dicentrics but may include centric rings if determined by the service laboratory.

Lymphocytes are cultured by a method that permits first-division metaphases to be recognized for analysis (see 10.1). This requires either whole blood, or lymphocytes separated from the other blood components, to be incubated in a culture medium that enables the scoring of first-generation metaphase cells. A mitotic blocking agent, colcemid or colchicine, is added to arrest dividing lymphocytes in metaphase. The duration of the cell culture and the timing of addition of the arresting agent are optimised to ensure an adequate mitotic index and predominance of high quality, first-division metaphases.

Metaphases are recovered from the cultures by centrifugation, placing in a hypotonic salt solution and fixing in a mixture of alcohol and acetic acid. Fixed cells are placed on microscope slides and stained. The exact protocol for cell culture, harvesting metaphases, and staining used by a service laboratory shall be formally documented (see Clause 10).

Microscope slides containing stained cells are scanned to identify suitable first-division metaphases to score chromosome aberrations (see 10.2). The frequency of dicentrics observed in an appropriate number of scored metaphases is converted to an estimate of radiation dose by reference to calibration data (see Clause 11).

6 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 A) explaining the requirements should be sent to the requestor. The requirements include:

a) Blood sampling should 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 should be collected (ideally about 10 ml), labelled accurately and unambiguously, maintained at room temperature (around 20 °C), and sent to the service laboratory as soon as possible.

c) Precautions should be taken to ensure the integrity of the container to prevent leakage during shipment. Blood samples should not be frozen during shipment and ideally be kept between 11 °C to 30 °C during shipment, although a lower temperature, above freezing, is still acceptable[2]. A temperature recording device should 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 should be included to monitor whether the sample was irradiated in transit.

d) A questionnaire (see Annex B) provided by the service laboratory should 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 may be taken when handling the sample.

7 Responsibility of the service laboratory

7.1 Setup and sustainment of the QA program

The service laboratory shall establish and maintain a QA program (see Clause 14), which covers all aspects of the service. The laboratory's QA program should address the following issues:

a) It shall include periodic internal checks of equipment operations, reagent suitability, and various performance checks (e.g. 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 (e.g. inter-laboratory comparison exercises, operator qualifications, sample transport integrity and time for delivery, etc.).

7.2 Responsibility during service

The service laboratory shall provide necessary guidance, procedures, and timely reporting of the dose assessment by cytogenetics in response to 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, for example service laboratory radiobiologist or equivalent), which includes the following:

   1) an instruction sheet to be sent to the requestor describing the shipping procedure (see Annex A);

   2) a questionnaire that shall elicit patient consent and all available information regarding the patient and the exposure scenario (see Annex B);