



# SLOVENSKI STANDARD

## oSIST prEN ISO 19238:2022

01-november-2022

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**Radiološka zaščita - Merila za delovanje laboratorijev, ki izvajajo biološko dozimetrijo s citogenetiko - Diecentrična analiza (ISO/DIS 19238:2022)**

Radiological protection - Performance criteria for service laboratories performing biological dosimetry by cytogenetics - The dicentric assay (ISO/DIS 19238:2022)

Strahlenschutz - Durchführungskriterien für Dienstleistungslaboratorien zur Anwendung der biologischen Dosimetrie mittels zytogenetischer Verfahren - Dizentrischer Test (ISO/DIS 19238:2022)

Radioprotection - Critères de performance pour les laboratoires de service pratiquant la dosimétrie biologique par cytogénétique (ISO/DIS 19238:2022)

**Ta slovenski standard je istoveten z: prEN ISO 19238**

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**ICS:**

13.280	Varstvo pred sevanjem	Radiation protection
17.240	Merjenje sevanja	Radiation measurements

**oSIST prEN ISO 19238:2022**

**en,fr,de**



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## Radiological protection — Performance criteria for service laboratories performing biological dosimetry by cytogenetics - The dicentric assay

ICS: 17.240; 13.280

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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](http://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](http://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](http://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 19238:2004), of which it constitutes a minor revision.

## 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 proved 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 (e.g. 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.

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. 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) and the ISO 5725 on accuracy (trueness and precision) of measurement methods and results.



# Radiological protection — Performance criteria for service laboratories performing biological dosimetry by cytogenetics - The 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 addresses

- 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-effect 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,
- h) informative annexes containing sample instructions for requestor, sample questionnaire, sample report, fitting of the low dose-response curve by the method of maximum likelihood and calculating the error of the dose estimate, odds ratio method for cases of suspected exposure to a low dose, a sample calculation and sample data sheet for recording aberrations.

## 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

**ISO/DIS 19238:2022(E)****3.2****background frequency/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 fragment.

**3.4****centromere**

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

**3.5****confidence interval**

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

**3.6****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

**3.7****chromatid**

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

**3.8****cytogenetics**

A branch of genetics that deals with the study of chromosomes

**3.9****dicentric**

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

**3.10****interphase**

period of a cell cycle between mitotic divisions

**3.11****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

**3.12****metaphase**

stage of mitosis when the nuclear membrane is dissolved and the chromosomes are condensed to their minimum lengths and aligned for division

**3.13****mitotic index**

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

**3.14****precision**

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

**3.15****quality assurance (QA)**

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

**3.16****quality control (QC)**

planned and systematic actions intended to verify that systems and components conform with predetermined requirements

**3.17****Qdr**

Chromosome aberration yield in cells with a chromosome aberration, typically calculated as the number of dicentrics and/or rings divided by the number of metaphase spreads with either a dicentric or ring

**3.18****service laboratory**

laboratory performing biological dosimetry measurements

**4 Abbreviated terms**

BrdU	Bromodeoxyuridine
Co	Cobalt
covar	Covariance
Cs	Cesium
FBS	Foetal bovine serum
FpG	Fluorescence plus Giemsa
Gy	Gray
$H_0$	Null hypothesis
$H_1$	Alternative hypothesis
HVL	Half value layer
IAEA	International Atomic Energy Agency
IEC	International Electrochemical Commission
ISO	International Organization for Standardization
IU	International units
KCl	Potassium chloride
LCL	Lower confidence limits
MEM	Minimum essential medium

**ISO/DIS 19238:2022(E)**

PHA	Phytohaemagglutinin
$R^2$	Coefficient of determination
SE	Standard error
SSD	Source-to-surface distance
TBT	Technical Barriers to Trade
TC	Technical Committee
UCL	Upper confidence limits
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 employed 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.<sup>[1]</sup> 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 (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-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 (i.e. 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](#));
  - 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 10 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