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Radiation protection — Performance criteria for service laboratories performing biological dosimetry by cytogenetics

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

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ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

ISO 19238 was prepared by Technical Committee ISO/TC 85, *Nuclear energy*, Subcommittee SC 2, *Radiation protection*.

Introduction

The wide 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 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 emphasised that cytogenetic analysis is used as a dosemeter and provides one input into the compendium of information needed for assessment of a radiological accident.

Many studies on animals and man have shown that one could 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 as calibration curves. The dicentric yield is dependent on radiation quality and dose rate so that information about these variables needs to be established for each investigation. If known, these exposure characteristics are important for refining the 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 approximatively 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 these very 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 limits to dose detection extend well into the range of doses that are lethal to humans.

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

The International Standard 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 will usually depend 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. The standard recommended scoring criteria would then be relaxed as appropriate to the situation.

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, with particular consideration given to the specific needs of biological dosimetry. The expression of uncertainties in dose estimations given in this International Standard comply with the ISO Guide to the expression of uncertainty in measurement (GUM) and the ISO 5725 on accuracy (trueness and precision) of measurement methods and results.

Radiation protection — Performance criteria for service laboratories performing biological dosimetry by cytogenetics

1 Scope

This International Standard provides criteria for quality assurance and quality control, evaluation of the performance and the accreditation of biological dosimetry by cytogenetic service laboratories.

This International Standard addresses

a) the confidentiality of personal information, for the customer 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 allowing the dose estimation from chromosome aberration frequency, and the minimum detection levels,

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 examples of a questionnaire, instructions for customers, a data sheet for recording aberrations and a sample report.

2 Terms and definitions

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

2.1
acentric
terminal or interstitial chromosome fragment of varying size

NOTE 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 frequency (or number) of chromosome aberrations recorded in control samples or individuals

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

2.4
centric ring
aberrant circular chromosome resulting from the joining of two breaks on separate arms of the same chromosome

NOTE It is generally accompanied by an acentric fragment.

2.5
centromere
specialized constricted region of a chromosome that appears during mitosis joining together the chromatid pair

2.6
confidence interval
statistical range about an estimated quantity within which the value of the quantity is expected to occur, with a specified probability

2.7
chromosome
structure that carries genetic information

NOTE Normally, 46 such structures are contained in the human cell nucleus. During nuclear division, they condense to form characteristically shaped bodies.

2.8
chromatid
either of the two strands of a duplicated chromosome that are joined by a single centromere and separate during cell division to become individual chromosomes

2.9
dicentric
aberrant chromosome bearing two centromeres derived from the joining of parts from two broken chromosomes

NOTE It is generally accompanied by an acentric fragment.

2.10
FISH
fluorescence *in situ* hybridization
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 This technique permits the detection of damage involving exchanges between differently painted pieces of DNA (usually whole chromosomes)

2.11
interphase
period of a cell cycle between the mitotic divisions

2.12
LET
linear energy transfer
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

NOTE In other words, it is the rate at which the energy of the radiation is transferred to tissues.

2.13
metaphase
stage of mitosis when the nuclear membrane is dissolved, the chromosomes condensed to their minimum lengths and aligned for division

2.14
minimum detection level
MDL
smallest measurable amount (e.g. frequency or dose) that will be detected with a probability β of non-detection (Type II error) while accepting a probability α of erroneously deciding that a positive (non-zero) quantity is present in an appropriate background sample (Type I error)

2.15
precision
concept employed to describe dispersion of measurements with respect to a measure of location or central tendency

2.16
quality assurance
planned and systematic actions necessary to provide adequate confidence that a process, measurement or service will satisfy given requirements for quality in, for example, those specified in a licence

2.17
quality control
part of quality assurance intended to verify that systems and components conform with predetermined requirements

2.18
service laboratory
laboratory performing biological dosimetry measurements

3 Dicentric assay

The frequency of dicentric chromosomal aberrations seen at metaphase in cultured human peripheral blood lymphocytes is the recommended method for biological dosimetry.

Lymphocytes are cultured by a method that permits first-division metaphases to be recognised for analysis (see 9.1). This requires whole blood, or lymphocytes separated from the other blood components, to be incubated in culture medium that would enable 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 is optimised to ensure an adequate mitotic index and predominance of 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 should be formally documented (see Clause 12).

Stained microscope slides are methodically scanned to identify dicentric aberrations (see 9.2). 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 10).

4 Confidentiality of personal information

4.1 Overview

Biological dosimetry investigations made by a service laboratory must be undertaken in accordance with national regulations regarding confidentiality. This would normally include the maintenance of confidentiality of the patient's identity, medical data and social status. In addition, the commercial confidentiality of the patient's employer and any other organizations involved in a radiological accident/incident should be observed.

This requirement extends to

a) written, electronic or verbal communications between the laboratory and the person/organization requesting the analysis and receiving the report, and

b) the secure protection of confidential information held within the organization where the service laboratory is located.

4.2 Applications of the principle of confidentiality

4.2.1 Delegation of responsibilities within the aboratory

The head of the laboratory may authorise a limited number of laboratory staff to deal with documents related to the analysis. Persons with this authority shall have signed a commitment to confidentiality regarding their duties within the laboratory.

The laboratory head shall maintain the signed confidentiality agreements and ensure the security and safety of all confidential documents.

4.2.2 Requests for analysis

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, the blood sampling for chromosome analysis must 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.

4.2.3 Transmission of confidential information

Whatever the chosen means of communication, confidentiality must be ensured during the exchange of information and reports between the service laboratory and the requestor of the analysis.

The laboratory head needs to define all processes for information transmission and assurance of confidentiality.

4.2.4 Anonymity of samples

The laboratory head needs to have established protocols for maintaining the anonymity of samples. To avoid the identification of the patient while guaranteeing the tracability of the analysis, the blood samples should be coded upon arrival in the service laboratory. The coding is performed in an unambiguous way according to a standard procedure. The same code is to be used for all the stages of the analysis. The code is assigned by an authorized person as defined in 4.2.1. Decoding, interpretation of results and compiling the report are also to be performed by an authorized person.

4.2.5 Reporting of results

The final report containing the results and their interpretation (when needed*)* is communicated to the requestor of the analysis. Depending on national regulations, further copies may, with appropriate approvals, be passed to other responsible persons.

4.2.6 Storage

The laboratory head shall define how data and results are stored. All laboratory documents relating to a case, and which could permit the patient and/or employer to be identified, must be stored in a place only accessible to the authorized persons. Documents must be retained in an appropriate place for at least 30 years for possible medico-legal re-evaluation of the case. Final disposal of documents must be by secure means such as shredding.

5 Laboratory safety requirements

5.1 Overview

Staff shall conform to their national legislation and institutional regulations regarding safety in the laboratories. There are some particular features concerning safety in service laboratories that are worth highlighting. These include microbiological, chemical and optical considerations.

5.2 Microbiological safety requirements

Handling human blood poses some risk of blood-borne parasites and infections being transmitted to laboratory staff. All specimens should be regarded as being potentially infectious, even if they are known to be derived from apparently healthy persons. Specimens must be unpacked and manipulated in a class 2 microbiological safety cabinet. Setting up cultures in such a cabinet has the added benefit of minimising culture failure due to microbial contamination. Use of sharps, e.g. hypodermic needles, should be kept to a minimum to reduce the risk of injuries. Suitable disinfectants must be available to deal with spills. All biological waste and used disposable plastic ware must be sterilised, for example by autoclaving or incineration, before final disposal.

Staff should be offered available vaccinations against blood-borne diseases. The legal and ethical position regarding HIV testing of blood samples upon receipt differs between countries and researchers should follow their national requirements. It should be noted that, when blood samples are accepted from abroad, depending on the country of origin, airlines might require the sender to provide a certificate confirming that the samples have been tested and are HIV negative.

5.3 Chemical safety requirements

Certain chemicals and pharmaceuticals are used routinely in the procedures covered in this International Standard. When present in cultures or used in staining procedures, they are mostly used in small volumes and in dilutions that generally present no health hazard. They are, however, prepared and stored in concentrated stock solutions. The main reagents of concern and their internationally agreed risk phrases (R numbers) are listed below :

Benzylpenicillin	R 42; 43;
Bromodeoxyuridine	R 20; 21; 22; 46; 61;
Colcemid	R 25;63;
Cytochalasin B	R 26; 27; 28; 63;
Giemsa stain	R 20; 21; 22; 40; 41;