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Radiological 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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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. www.iso.org/directives

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The committee responsible for this document is 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 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 emphasized that cytogenetic analysis is used as a dosimeter and provides one input into the compendium of information needed for assessment of a radiological accident.

Many studies in animals and man 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 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 intercomparisons. Finally, laboratories newly commissioned to carry out the dicentric assay should conform to this International Standard in order to perform it reproducibly and accurately.

This 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 (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

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 that contribute to the dose estimation from chromosome aberration frequency and the minimum resolvable doses,

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 customer, sample questionnaire, sample of report, fitting of the low dose-response curve by the method of maximum likelihood and calculating the error of dose estimate, odds ratio method for cases of suspected exposure to a low dose, and sample data sheet for recording aberrations.

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, referred to as an excess acentric fragment when it is formed independently of a dicentric or centric ring chromosome aberration

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

2.5
centromere
specialized constricted region of a chromosome that appears during mitosis and joins 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 comprises discrete packages of DNA and proteins that carries genetic information which condense to form characteristically shaped bodies during nuclear division

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 1 to entry: 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

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

2.13
lower threshold of dose
smallest measurable amount (e.g. frequency or dose) that is 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)

2.14
metaphase
stage of mitosis when the nuclear membrane is dissolved, the chromosomes condensed to their minimum lengths and aligned for division

2.15
minimum resolvable dose
lowest additional dose for which the lower 95 % Poisson confidence limit is greater than 0, so that there is a 97,5 % chance that the dose received in excess of normal background is greater than 0

2.16
precision
concept employed to describe dispersion of measurements with respect to a measure of location or central tendency

2.17
quality assurance
planned and systematic actions necessary to provide adequate confidence that a process, measurement, or service satisfies given requirements for quality in, for example, those specified in a licence

2.18
quality control
part of quality assurance intended to verify that systems and components conform to predetermined requirements

2.19
service laboratory
laboratory performing biological dosimetry measurements

3 Dicentric assay

The frequency of unstable chromosomal aberrations seen at metaphase in cultured human peripheral blood lymphocytes is the recommended method for biological dosimetry. The chromosome aberrations to be used are dicentrics or dicentrics and centric rings. For the application of this International Standard, 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 9.1). This requires whole blood, or lymphocytes separated from the other blood components, to be incubated in a 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 are 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 shall be formally documented (see Clause 12).

Microscope slides containing stained cells are methodically scanned to identify suitable first-division metaphases to score 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 Responsibility of the customer

This clause includes items that are not controlled by the service laboratory. Prior to blood sampling, coordination between the customer and the service laboratory should occur. Essential requirements should be explained to the customer and this may be by a standardised instruction sheet as illustrated in Annex A. The essential features are:

a) Blood sampling should use the collection system containing lithium heparin as anticoagulant which has been 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 to ensure the integrity of the container and prevent leakage during shipment shall be observed. Blood samples should be kept cool during shipping (i.e. 6° C to 30 °C). A temperature recording could be included to document 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 could be included to monitor whether the sample was irradiated in transit.

d) A questionnaire provided by the service laboratory should be completed and returned promptly.

e) The service laboratory should be alerted of biologically contaminated samples.

5 Responsibility of the service laboratory

5.1 Setup and sustainment of the QA program

The service laboratory shall establish and maintain a QA program (see Clause 12), which covers all aspects of the service. The QA program should address the following issues:

a) The laboratory's QA program shall include periodic internal checks of equipment operations, reagent suitability, and various performance checks (i.e. intracomparison exercises, operator qualifications, sample protocol, scoring, dose estimations, report generation, etc.).

b) The laboratory's QA program 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. intercomparison exercises, operator qualifications, sample transport integrity, etc.).

5.2 Responsibility during service

The service laboratory shall provide necessary guidance, procedures, and reporting to provide 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 customer describing shipping procedures (Annex A);

   2) a questionnaire that shall elicit patient consent and information on whole or partial body exposure, source and quality of the radiation, circumstances of the exposure, exposure location (country, city, company, etc.), date and time of exposure, previous occupational or medical exposures to radiation, intake of pharmaceuticals, infection, smoking habit, and significant exposures to any other DNA damaging agents (such as organic solvents or heavy metals) (Annex B);

   3) step-by-step procedures for processing the blood sample from receipt of the sample to reporting of the dose.

b) If required, a blood collection system (10 ml) containing lithium heparin as the anticoagulant shall be sent to the customer with the appropriately labelled and addressed packaging material for the return of the sample to the service laboratory. The packaging shall conform to national and/or international regulations for the transit of potentially infectious pathological specimens (see 12.2.4).

c) After receipt of the blood sample, the following steps shall be performed:

   1) Document the receipt of the blood sample (date, time, consignee).

   2) Code the blood sample.

3) Document the place of storage until the setting up of cultures.

4) Set up cultures in parallel as soon as possible and document date, time, and operator.

5) Document all the reagents used for culturing with appropriate lot numbers.

6) Document the addition of reagents and the end of the culture (date, time, operator).

7) Document the short- and long-term storage of the sample until slide making.

8) Document the slide codes, number of slides, and location of storage.

9) Document the results from scoring.

10) Store the slides and case documents in an appropriate place for at least 30 years for possible medico-legal re-evaluation of the case.

d) The service laboratory shall interpret the results and prepare reports (Annex C).

e) The service laboratory shall sustain a dialogue with the requestor, reprioritizing cases as required, and providing results to the requestor.

6 Confidentiality of personal information

6.1 Overview

Biological dosimetry investigations made by a service laboratory shall 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 1) written, electronic, or verbal communications between the laboratory and the person/organization requesting the analysis and receiving the report, and 2) the secure protection of confidential information held within the organization where the service laboratory is located.

6.2 Applications of the principle of confidentiality

6.2.1 Delegation of responsibilities within the laboratory

The head of the laboratory may authorize 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.

6.2.2 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 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.

6.2.3 Transmission of confidential information

Whatever the chosen means of communication, confidentiality shall 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.

6.2.4 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 traceability 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 6.2.1. Decoding, interpretation of results, and compiling the report are also to be performed by an authorized person.

6.2.5 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.

6.2.6 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 shall be stored in a place only accessible to the authorized persons. Documents shall be retained in an appropriate place for at least 30 years for possible medico-legal re-evaluation of the case. Final disposal of documents shall be by secure means such as shredding.

7 Laboratory safety requirements

7.1 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.

7.2 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 shall 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 shall be available to deal with spills. All biological waste and used disposable plasticware shall 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.

7.3 Chemical safety

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

