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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

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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 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

stored in concentrated stock solutions. The main reagents of concern and their internationally agreed hazard statements (H-Statements) according to the GHS classification system are listed below:

Acetic acid	H226, H290, H314
Benzylpenicillin	H317, H334
Bromodeoxyuridine (BrdU)	H351
Colcemid	H300, H361
Cytochalasin B	H300, H310, H330, H361
Giemsa stain	H225, H301, H311, H331, H370
Heparin	H315, H319, H334
Hoechst stain (Bisbenzimide)	H302, H315, H319
Methanol	H225, H301, H311, H331, H370
Phytohaemagglutinin	H302, H317, H332
Streptomycin sulphate	H302, H332, H317, H334, H361

Keys

H225:	Highly flammable liquid and vapour
H226	Flammable liquid and vapour
H290:	May be corrosive to metals
H300:	Fatal if swallowed
H301:	Toxic if swallowed
H302:	Harmful if swallowed
H310:	Fatal in contact with skin
H311:	Toxic in contact with skin
H314:	Causes severe skin burns and eye damage
H315:	Causes skin irritation
H317:	May cause an allergic skin reaction
H319:	Causes serious eye irritation
H330:	Fatal if inhaled
H331:	Toxic if inhaled
H332:	Harmful if inhaled
H334:	May cause allergy or asthma symptoms or breathing difficulties if inhaled
H351:	Suspected of causing cancer
H361:	Suspected of damaging fertility or the unborn child
H370:	Causes damage to organs

7.4 Optical safety requirements

When ultraviolet lamps are used in sterilising the interior of microbiological safety cabinets or exposing slides during the fluorescence plus Giemsa (FPG) staining procedure, shielding and working procedures shall be in place to avoid direct irradiation of the skin or eyes of laboratory staff.

7.5 Safety plan

The laboratory head shall define written safety procedures for protection against microbiological, chemical, and optical hazards.

The laboratory head shall maintain a record of accidents and protocols or procedures to avoid repeating similar accidents.

8 Calibration curve(s)

8.1 Culturing

The same culturing conditions shall be used for establishing the calibration curve as for analysing aberrations in a case of suspected overexposure.

The exact protocol for the dicentric assay shall be established by each service laboratory, and there are several critical aspects that shall be adhered to as listed below:

a) Blood shall be incubated for a minimum of 2 h at 37 °C immediately following irradiation and prior to culture.

b) Cells shall be cultured at 37 °C ± 0,5 °C either as whole blood, as an enriched lymphocyte suspension (buffy coat), or as isolated lymphocytes.

c) The culture vessel shall be sterile and used in a way to avoid microbial contamination.

d) Specific culture media that allow peripheral blood lymphocytes to proliferate shall be used. These are commonly supplemented with Foetal Bovine Serum (FBS) (between 10 % and 20 %), 200 mM L-glutamine, and Penicillin/Streptomycin (100 IU·ml–1/100 µg·ml–1)(see Reference[8]).

e) The mitogen (e.g. phytohaemagglutinin or PHA) shall be added to the media to stimulate lymphocytes into mitosis.

f) A method to ensure the scoring of first-division metaphases shall be used (see 9.1).

g) Colcemid or colchicine shall be added, at a time and concentration determined by the laboratory, to the cell culture to block cells in mitosis.

h) The timing of harvest is crucial to maximize the number of cells in first-division metaphase and shall be adapted according to the standard culture conditions for that service laboratory. The recommended culture time is 48 h, but under certain conditions where mitotic delay is anticipated, longer time might be required.

i) Cells are centrifuged in order to separate the cells from the medium. Thereafter, cells shall be treated with a hypotonic solution such as 0,075 M KCl for 10 min to 15 min to swell the cells prior to fixation.

j) After centrifugation, the supernatant shall be removed and cells shall be fixed in freshly prepared fixative solution (i.e. 1:3 acetic acid:methanol) and washed three or four times with the same fixative until the cell suspension is clear.

k) If storage of fixed cells is required, then cell suspensions shall be kept in a − 20 °C freezer.

l) Slides shall be prepared to allow an unambiguous identification of chromosomal aberrations. Humidity and temperature conditions can be adjusted to increase the quality of the spreading.

m) Slides should be allowed to dry for at least 1 h prior to staining. Slides shall be stained with Giemsa. If the FPG method is being used for the identification of first-division metaphases, FPG staining has to be performed with Hoechst 33258, followed by UV exposure.

n) To avoid fading, stained slides shall be stored in the dark at room temperature. For better conservation, slides should be mounted with an appropriate mounting medium.

8.2 Calibration source(s)

The service laboratory shall provide a report, reviewed and endorsed by a qualified expert (i.e. radiation physicist or the service laboratory head), that addresses the following issues:

a) description [e.g. Philips X-ray machine with a 2,1 mmCu half value layer (HVL), 250 kVp, filament current 12,5 mA, and a source-to-surface distance (SSD) of 50 cm] for all radiation calibration source(s) used to generate *in vitro* calibration curves;

b) characterization of the radiation calibration source(s) used to generate each *in vitro* calibration curve and traceability to a national/international radiation standard;

c) description of the dosimetry protocol, the procedure to certify that the dosimetry method is calibrated to a standard, the method used to measure dose uniformity in the experimental array, and the written procedures and documentation to verify dose and dose-rate determinations for individual experiments;

d) provision of a summary dosimetry report for each calibration-source dose-response curve.

8.3 Establishment of calibration curve(s)

In the case of both low-LET and high-LET photon radiation, a minimum of seven doses should be selected, distributed equally among the linear and quadratic components of the dose response curve. The typical doses for a calibration curve range from 0,1 Gy to 4 Gy.

Observed frequencies of dicentrics (Y) should be fitted to the linear or linear-quadratic models [Formula (1)]. For most high-LET radiation types, a linear model should be more appropriate.

$$Y = C\left(SE_C\right) + \alpha D_T\left(\pm SE_\alpha\right) + \beta {D_T}^2\left(\pm SE_\beta\right) \tag{1}$$

where

D_T is the dose;

α, β, C are coefficients of the fit to the linear-quadratic model;

SE is the standard error of the coefficients.

Two methods are proposed for curve fitting, iteratively reweighted least squares and maximum likelihood (see Annex D). For overdispersed non-Poisson data (i.e. for high-LET radiation types), the weights shall take into account the overdispersion. When the obtained value of chi-squared is higher than the degrees of freedom, standard errors should be increased by

$$(\text{chi-square/degree of freedom})^{1/2}$$

The service laboratory shall provide a report, reviewed and endorsed by a qualified expert (i.e. service laboratory radiobiologist or equivalent), that addresses the following issues:

a) describing the experimental exposure setup (sample holder, temperature control, etc.) and procedures to verify reproducibility of exposure setup for individual experiments;

b) detailing the *in vitro* calibration data and their fitting to a calibration. Goodness of fit and significance of the fitted linear and quadratic coefficients should be quoted.

The service laboratory should use documented and validated curve fitting software. Examples of such software have been developed by the international biological dosimetry community and are available freely. Probably, some others can be found through the open literature and the Web. Whatever the software used, validation should include testing against published data.

8.4 Minimum resolvable dose measurement

The minimum resolvable dose is a function of the laboratory's measured control background levels of dicentrics, the calibration curve coefficients, and the number of cells scored in an analysis, and is limited to the lowest dose used in the appropriate calibration curve. An accredited laboratory shall be able to provide a report mentioning the minimum resolvable dose; this limit should be addressed to the medical doctor requiring the dose estimation. A minimum resolvable dose around 100 mGy is recommended.

When the suspected radiation dose is low, the odds ratio calculation could be added in the report (see Annex E).

9 Scoring unstable chromosome aberrations

9.1 Procedure for scoring first-division metaphases

An important aspect of culturing blood samples for dose estimation by the dicentric chromosome aberrations bioassay is the harvest time for metaphase collection. The maximal frequency of unstable chromosomal aberrations in lymphocytes collected from radiation-exposed individuals occurs in the first-generation, post-exposure metaphase cells. The standard method used to ensure that only first-generation metaphase cells are scored is based on the FPG technique which requires the addition of BrdU during culturing. An acceptable procedure is to check a replicate slide of the same culture with FPG and if the frequency of the second or later metaphases is low (below 5 %), a replicate slide stained with Giemsa alone may be scored. For cultures containing more than 5 % second divisions, only the FPG-stained material should be scored. Alternative techniques are acceptable as long as the methodology is documented and validated. For long-term storage mounting, stained slides are recommended.

9.2 Criteria for scoring

9.2.1 Coding of samples and slides

All samples, slides, and intralaboratory or interlaboratory validation standards shall be coded. Complete records of coding shall be maintained.

9.2.2 Scoring techniques

The laboratory head shall establish and implement procedures for the scoring techniques used. When scoring is at least partially performed with computer-assisted metaphase finding and/or image analysis, the system used should have been previously subjected to quality assurance trials with results documented.

Methodical scanning of slides is crucial to ensure complete analysis without scoring any cell more than once. It is recommended that more than one slide be scored for each sample.

While dicentrics are invariably used for dose estimation, it is standard practice in service laboratories for all chromosomal abnormalities to be recorded. A standardised scoring sheet shall be used with data recorded such that the aberrations in each cell scored are derivable (Annex F). When more than one scorer contributes to the analysis, each shall analyse a comparable number of metaphases.

9.2.3 Laboratory scoring expertise

Metaphase analyses are to be conducted by trained and experienced observers fully familiar with the scoring of unstable chromosome aberrations used in biological dosimetry. Documentation validating their expertise shall be maintained.

The laboratory head is responsible for maintaining the scoring criteria and the qualifications of the individual scorers. All scorers shall participate in intralaboratory and interlaboratory comparisons.

A scorer is regarded as competent if his/her measured yield is within the Poisson 95 % confidence limits of the test reference yield expected from the laboratory's calibration curve.

See below additional details for scoring expertise by performance checks through laboratory inter-comparison studies (12.2.2) and periodical checks of individual scorers (12.2.3).

10 Criteria for converting a measured aberration frequency into an estimate of absorbed dose

10.1 Overview

The measured dicentric frequency is converted to absorbed dose by reference to an appropriate *in vitro* calibration curve produced in the same laboratory with radiation of comparable quality. This provides an estimate of the mean whole-body dose. In conventional cases, at least 500 cells should be scored from the case specimen, unless the aberration yield is high, in which case it is not necessary to proceed beyond 100 dicentrics. In the special case where there exists a high abundance of dicentrics but few metaphase spreads, the dose can be reported after scoring fewer than 100 dicentrics.

10.2 Comparison with controls

The service laboratory shall provide its background dicentric level in case reports (an example is provided in Annex C). If the measured aberration yield is not significantly different from the control frequency, the best estimate of dose should be quoted as zero with its upper confidence limit. If the measured aberration yield is significantly higher than the control level, then a dose estimate with its uncertainties is derived and reported.

10.3 Testing the distribution of aberrations per cell

Dicentrics formed by a homogeneous exposure to human lymphocytes in G_0 by low-LET radiation are distributed among cells following the Poisson distribution. In cases of non-homogeneous irradiation or after exposure to high-LET radiation, the dicentric cell distribution tends to be overdispersed (variance greater than the mean). Because curve fitting methods are based on the Poisson distribution, the dicentric cell distribution shall be tested for all doses used to construct the dose-effect curve. This should be done by the *u* test which is a normalized unit of the dispersion index $D = s^2/y$ (variance/mean). For a Poisson distribution, *D* should be unity, *u* values higher than 1,96 indicate an overdispersion (significance level, $\alpha = 0{,}025$).

$$u = (D-1) \times \sqrt{\frac{N-1}{\left[2 \times \left(1 - \frac{1}{X}\right)\right]}} \qquad (2)$$

where

D is the dispersion index;

N is the number of cells analysed;

X is the number of dicentrics detected.

10.4 Determination of estimated whole-body dose and confidence limits

10.4.1 General

The service laboratory shall provide in result reports the estimated whole body dose and confidence limits. Uncertainties would usually be expressed as 95 % confidence limits although other percentage

values may be quoted, if judged appropriate to a particular case. If the lower confidence limit falls below zero dose, only the upper limit needs to be quoted.

10.4.2 Confidence limits on the yield of dicentrics

There are several methods for deriving confidence limits on an observed yield of dicentrics. Confidence limits on Poisson observations may be obtained from standard tables, by an exact or approximates calculation. If a measured aberration is overdispersed with respect to Poisson, the Poisson-derived uncertainty should be increased by the square root of the ratio of variance to mean (see 10.3).

If not using a table, Formulae (3) and (4) can be used as soon as the number of cells exceeds 15:

$$a_1 = \frac{1}{4}(\sqrt{4N-1} - u_{1-\alpha/2})^2 \tag{3}$$

$$a_2 = \frac{1}{4}(\sqrt{4N+1} + u_{1-\alpha/2})^2 \quad \text{with } u_{1-\alpha/2} = 1.96 \tag{4}$$

10.4.3 Confidence limits on the dose

The most formal method to estimate the uncertainties on the dose is by combining the confidence limits on the frequency to the uncertainties on the curve. Then, the higher value of the confidence limit should be reported in the lower dose-effect curve and the lower confidence value reported in the higher dose-effect curve.

The service laboratory describes in result reports the method used to determine the expanded uncertainty, e.g. the 95 % confidence interval, as defined in ISO 5725-1.

The laboratory head shall define the methods used to determine confidence limits.

10.5 Acute and non-acute exposure cases

If an overexposure is known to have been received acutely, i.e. below 0,5 h, the dose estimate may be obtained by reference to an acute *in vitro* calibration curve. If an overexposure is known to have been protracted beyond 24 h, the dose estimate may be obtained by reference to just the background level and linear coefficients of the acute calibration curve. For exposures of 0,5 h to 24 h, if available, the measured yield may be interpreted from an appropriate non-acute calibration curve. Alternatively, the full acute curve may be used but with a reduction of the dose-squared coefficient. This may be calculated by the G-function method. Further explanations of the G-function can be found in the IAEA technical reports.

If an overexposure is known to have been intermittent, its individual fractions may be assumed to be independent, i.e. their effects are additive, if the interfraction interval is above 5 h. If below 5 h, an interaction factor should be estimated using a 2 h time constant.

The service laboratory shall state in the result reports the method used to correct for non-acute exposure dose estimates and, when appropriate, also justify its assumptions.

10.6 Partial-body and prior-exposure cases

In the event of a partial-body exposure to low-LET radiation, it may be possible, depending on the particular circumstances, to interpret the measured aberration yield in terms of an irradiated fraction and its mean dose. These are derived using the Qd[1)] or contaminated Poisson techniques.

1) The Qdr method was originally devised for dicentrics and rings. However, only dicentrics are being considered here.

In the contaminated Poisson method, the frequency of dicentrics of the irradiated fraction (Y) is estimated using Formula (5) and solving the Y value by iteration.

$$\frac{Y}{\left(1-e^{-y}\right)}=\frac{X}{\left(N-n_0\right)} \tag{5}$$

where

Y is the mean yield of dicentrics of the irradiated fraction;

e^{-y} is the undamaged cells from the irradiated fraction;

N is the number of cells scored;

X is the number of dicentrics observed;

n_0 is the number of cells free of dicentrics.

To calculate the 95 % confidence limits for Y, the approach indicated in 10.4.2 shall be adopted.

The fraction of cells scored which were irradiated (f) can be calculated using Formula (6):

$$Yf=\frac{X}{N} \tag{6}$$

To estimate the fraction of cells originally exposed (F), Formula (7) should be used:

$$F=\frac{\frac{f}{p}}{\left(1-f\right)+\frac{f}{p}} \tag{7}$$

where

f is the fraction of cells scored which were irradiated;

p is the fraction of irradiated cells which reached metaphase, taking into account mitotic delay and interphase death. The p values are estimated using Formula (8).

$$p=\exp\left(\frac{-D_T}{D_{T0}}\right) \tag{8}$$

where

D_T is the estimated dose;

D_{T0} is the dose for which 37 % of cell killing is expected.

In the absence of specific data, a default value of 3,5 Gy for D_{T0} shall be assumed for photon irradiation.

With the Qd method, Formula (9) is used to estimate the dose received by the irradiated fraction.

$$\frac{X}{Cu} = \frac{Y_1}{\left(1 - e^{(Y_1 - Y_2)}\right)} \tag{9}$$

where

X is the number of dicentrics (dic);

Cu is the number of cells with unstable chromosome aberrations (dicentrics or acentric fragments);

Y_1 and Y_2 are the expected yields of dic and excess acentrics, respectively.

Y_1 and Y_2 are functions of the dose and derivable from the *in vitro* dose-response curves. Estimated doses are obtained by an iterative process. In this case, standard errors are calculated taking into account the variance of dic among unstable cells. By the Qd method, information on the fraction of cells scored that were irradiated and the fraction of cells originally exposed are derived by converting the estimated dose to yield and then using Formulae (6) and (7).

Exposure occurring a long time prior to analysis tends to be underestimated by the dicentric assay. If the timing and duration of an old exposure is known, the measured dicentric frequency should be adjusted by assuming a disappearance half time of 3 years. In the case of prior exposures, sufficient to have caused deterministic reactions, a shorter half-time assumption may be appropriate.

In all the cases, the service laboratory shall state in the result reports the method used to correct for partial-body and prior-exposure cases and, when appropriate, also justify its assumptions.

11 Reporting of results

11.1 General

Routinely, the report should contain relevant information provided by the customer because this may influence the interpretation of the findings in the service laboratory. All observed aberrations shall be listed and interpreted based on the current understanding of mechanisms for radiation-induced chromosome aberration formation.

The report should be subdivided into the following sections.

11.2 Content of the report (see Annex C for a standard form)

The report should include information on the following points:

a) title of the report, i.e. “Test Report”;

b) name and address of the laboratory performing the analysis;

c) identification of the report by a unique number, i.e. a specific document number provided by the institutional registry;

d) name and address of the customer, date of order;

e) identification of the method of analysis, i.e. providing the number and name of the method as described in the in-house quality system, and where relevant, any deviations from the test method;

f) unambiguous identification of the sample, i.e. name, internal code, and date of birth of the subject;

g) description of the case, i.e. all relevant information provided by the customer that is relevant to the interpretation of the result shall be stated (may also be dealt with in the interpretation of the results);

h) date and location of blood sampling, date of sample arrival in the laboratory, date of setting up of cultures (if different), and date of completed analysis;

i) test results, i.e. number of cells scored, numbers and types of aberrations found;

j) interpretation of test results (see 11.3);

k) name(s), title(s), position(s), and signature(s) authorizing the report and contact information.

11.3 Interpretation of the results

This varies depending on the circumstances of each case but the report should include one or more of the following:

a) a dose estimate based on the frequency of dicentric aberrations expressed in SI units of absorbed dose (Gy);

b) a statement on the likelihood that any aberrations used in dose estimation relate to this particular radiological incident;

c) the dicentric background of the laboratory and the coefficients of the calibration curve used for converting the dose from the aberration yield;

d) a quantification of the uncertainties on the dose estimate. This would normally be an upper, and where appropriate, a lower confidence limit, and the percent level of confidence;

e) a statement on whether the dose estimate was made assuming acute or protracted irradiation and, if the latter, how protraction had been accounted for;

f) if appropriate, the interpretation needs to consider partial-body irradiation and excessive delay between the accident and blood sampling;

g) a comment on, and if appropriate, a dosimetric interpretation of, cells observed with multiple damage;

h) comments regarding the frequencies of other aberration types scored but not used for dose estimation;

i) a summary of the essential key elements from the points above. This would normally include the best estimate of dose based on the cytogenetic findings;

j) at the end of the report, an invitation for the customer to contact the laboratory if he/she requires further clarification or explanation about the results and/or the assay.

12 Quality assurance and quality control

12.1 Overview

Quality management shall ensure a continued improvement of operations. The quality assurance and quality control procedures required by ISO/IEC 17025 have to be followed in the laboratory according to this International Standard. This International Standard defines quality assurance and quality control procedures specific for laboratories performing biological dosimetry by cytogenetics.

12.2 Specific requirements

12.2.1 General

Comparison programs with other certified or suitably qualified cytogenetic biodosimetry laboratories have to be established and periodic measurements have to be performed.

ISO 5725 is dedicated to statistical analysis to test the reliability and the precision of a technique. The tests proposed can be applied only if many samples are analysed.

12.2.2 Performance checks by laboratory intercomparisons

Proficiency tests are essential tools for the quality assurance of the laboratories as they constitute an objective evaluation of its performance, from both human and technical point of view.

The presence of individual laboratories or values that appear to be inconsistent with all other laboratories may change the estimates. To discard or correct inconsistent values, two approaches can be used (ISO 5725-2/ISO 5725-5):

a) numerical outlier tests (Cochran and Grubbs tests): To discard data that give rise to a test statistic that exceeds the critical value of the test at the 1 % significance level;

b) robust methods for data analysis: To yield robust values of the average and standard deviation of the data.

The procedure is as follows:

a) the outlier test for laboratory intercomparison performance requires a minimum of five laboratories for statistical robustness (ISO 5725-1:1994);

b) estimation of the mean value and the standard deviation interlaboratory once outliers are discarded or corrected. The preferred method is the calculation of the robust parameters;

c) determination of the laboratory's performance by calculating z-score parameter from the laboratory results, the reference value, and the estimated standard deviation. To determine u-score parameter (this evaluation includes both participant measurements and reference value uncertainties).

12.2.3 Periodical performance check of operator qualification

A list of qualified observers is established at least every second year by intracomparison.

To be qualified, each observer shall score a sample of lymphocytes exposed to a dose above 1 Gy (acute photons) and a sample of lymphocytes exposed to a dose below 1 Gy (acute photons) according to the standard practice of the laboratory.

A scorer is regarded as qualified if his/her measured yield is within the Poisson 95 % confidence limits of the test reference yield expected from the laboratory's calibration curve. For example, a scorer finds that his/her measured yield in a test sample is 48 dicentrics in 1 000 cells (95 % LCL: 0,035, UCL: 0,063) for a reference level test and agrees with the laboratory's calibration curve that predicts a yield of 0,05 dicentrics per cell.

12.2.4 Performance checks of sample transport integrity

In many cases, blood collection occurs at sites distant from the processing laboratory and transportation is necessary. The customer is responsible for assuring the blood samples are transported in optimal temperature conditions (6 °C up to 30 °C). When air transportation is used, the X-irradiation at the security checkpoints should be avoided. A physical dosimeter may be included in the shipping package to verify this. For international transport, the appropriate permits shall be obtained in advance and included in the shipment to avoid delays at customs. All details concerning blood collection and storage should be recorded.

12.2.5 Performance checks of sample integrity by service laboratory

A system for recording the collection, transport, and storage of the blood samples shall be established so that sample integrity is guaranteed. The use of coded samples is critical to avoid potential bias in the scoring.

12.2.6 Performance checks of instrumentation

Performance of the measurement equipment shall be checked and evaluated at regular intervals while the equipment is in use.

Examples of critical equipment include incubators, balances, thermometers, pipettes, and freezers.

For example, the stability of the temperature control of the incubators has to be controlled. If used, the balance has to be checked periodically.

These checks shall be sufficient to demonstrate that the measurement equipment is properly calibrated and that all components are functioning properly.

12.2.7 Performance checks of sample protocol

As internal quality assurance, negative controls from unexposed individuals and, where possible, internal positive controls have to be included in the study to prove the reliability of the procedure. Blood from both exposed and unexposed individuals shall be handled in the same manner. The samples of both populations have to be taken concurrently and not successively.

For the interpretation of results, it can be useful to prepare a slide for differential count from each blood sample before starting the cultures. The culture, fixation, and staining procedures shall be described in detail. It is recommended that the same lot of media and reagents be used throughout the study. The composition of all reagents shall be described as accurately as possible.

12.2.8 Performance checks of sample scoring

Uniform criteria for scoring shall be used. Scoring shall be performed by trained and experienced observers. If different scorers are involved, a balanced scoring design shall be used. Each scorer should analyse the same number of metaphases from the slides of all subjects rather than different scorers analysing all cells from different subjects. Cross-checking of scoring results is required. The identity of the scorer of the slides shall be recorded.

12.2.9 Performance checks of dose and confidence limits estimation

Non-parametric tests should be used for univariate statistical analysis. The confidence interval of the exposure has to be calculated from the uncertainty on the dicentric yields and the variation of the dose-response relationship among individuals, typically determined in a prior study. The dose-response relationship used for chronic and acute exposures has to be appropriate. The results of the negative and positive internal quality assurance controls are used to demonstrate the reliability of the methodology and scoring.

12.2.10 Performance checks of result report generation

The study reports to customers shall be examined to ensure that they contain the necessary information defined in this International Standard (see Clause 11), namely: subject and customer identifiers, exposure information, exposure and sampling dates, scoring results, interpretation of the results in terms of dose and its uncertainty, and information on how these were derived.

Annex A
(informative)

Sample instructions for customer

PROCEDURES FOR COLLECTING BLOOD FOR CHROMOSOMAL ANALYSIS

Analysis of chromosomal aberrations in human peripheral blood lymphocytes is the present-day standard for the biological assessment of radiation exposure. It is used when a person`s physical dosimeter is absent or inoperative or when the reading of the physical dosimeter is missing or in dispute. To optimize the recovery of lymphocytes from the blood, it is very important that the blood be collected and shipped according to protocol outlined below.

X Before the blood sample is taken, please notify us so that we can prepare for its arrival and pick up.

X All blood samples are to be collected into **lithium heparin tubes**, at least 10 ml (2 ml × 5 ml tubes). Gently rock the tubes for 2 min to ensure proper mixing. Label the tubes unambiguously and complete the questionnaire.

X Package the blood sample carefully to prevent breakage of the tubes in transit. The customer is responsible for ensuring that the blood samples are transported in optimal temperature conditions (6 °C up to 30 °C). If temperature extremes are likely to be encountered, a minimum-maximum thermometer can be included in the package. **Blood samples shall not be frozen**. One method of maintaining blood at room temperature is to place the tubes on a gel pack that has been allowed to stay at room temperature for several hours. Ensure also that the samples do not freeze during transportation (e.g. airmail).

X Mark the external packaging and the shipping documents: **URGENT DIAGNOSTIC SAMPLES - NOT TO BE FROZEN.**

X When air transportation is used, the X-irradiation at the security checkpoints should be avoided. A physical dosimeter may be included in the shipping package to verify this. For international transport, the appropriate permits shall be obtained in advance and included in the shipment to avoid delays at the customs. For air transport, packaging and labelling should conform to the current International Air Transport Association (IATA) regulations. These require that blood samples be packed to conform to United Nations Regulation 602 for infectious materials. The package itself and the 'Nature and Quantity of Goods' box of the air waybill should show the following wording: "Diagnostic specimen packed in compliance with IATA Packing Instruction 650".

X Mark the package and shipping documents: **DO NOT X-RAY**.

X Immediately after blood collection, ship the sample by **special transportation** and **use overnight air express so we can receive the blood early in the morning following sample collection.** Contact the laboratory to confirm the shipment and inform us of the **waybill** number. THIS IS IMPORTANT FOR TRACKING THE SAMPLE.

X For best results, blood shall be received within 24 h of sampling.

X All details concerning blood collection and storage should be recorded.

(Service Laboratory Head)

(Service Laboratory address)

Phone: (XXX) XXX-XXX

Fax: (XXX) XXX-XXX

E-mail: name@company.com

Annex B
(informative)

Sample questionnaire

EXPOSURE INFORMATION FOR CHROMOSOME ABERRATION ANALYSIS

(TO BE FILLED OUT BY THE REQUESTOR)

I,..... (Name), born ..... (dd/mm/yy) consent to giving a blood sample for the purpose of estimating chromosome aberrations induced by exposure to ionizing radiation.

.....

Signature

.....

Blood sample taken by:..... Laboratory name: .....

Laboratory Address.....

Telephone #: ..... Fax: ..... E-mail:.....

Date and time blood sample taken:..... (dd/mm/yy) Specify anticoagulant: .....

Exposure Data: Radiation Worker or Non-Radiation Worker

Date and time of overexposure:..... (dd/mm/yy - time)

Place: ..... Company: .....

3. Brief description of overexposure:

4. Whole-body exposure O Partial-body exposure O Internal contamination O

Dose value :..... Part of body: .....Nuclide .....

Dose value: .....Dose value .....

How was this dose value obtained.....

5 Type of radiation: x - ray O Energy ?.....

γ O Origin ? .....

α O Origin ? .....

Neutrons O Origin ?.....Energy ? .....

Electrons O Origin ?.....Energy ? .....

Patient Data:

1. Previous exposure through medical practice:

   Radiation therapy O Date, Part of Body.....

   x - ray diagnoses O Date, Part of Body.....

   Nuclear medicine O Date, Part of Body.....

2. Illness within the last 4 weeks before taking the blood sample.....

3. Intake of medication: O

Name of medication: ..... Dose: ..... Duration: .....

4. Smoker: no: O yes: O number cigarettes / day: .....

5. Other diseases:

   HIV O Hepatitis O

.....

Results of chromosomal analyses to be sent to:

Name: .....

Address: .....

.....

Telephone # : .....

Annex C
(informative)

Sample of report

Requestor name, address, phone, e- mail
Date of order

BIOLOGICAL DOSE ASSESSMENT / CHROMOSOME ANALYSIS

Sample	Sampling date/location	Arrival date	Date of analysis
Code, name and date of birth of exposed subject			

Method(s) of analysis

Additional sampling Information

Results

Table. Test results

		Number of chromosome aberrations			
Sample	**Number of cells analysed**	**Dicentrics**	**Rings**	**Fragments**	**Others**

Interpretation of results

Signatures and contact information