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

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

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

ISO draws attention to the possibility that the implementation of this document may involve the use of (a) patent(s). ISO takes no position concerning the evidence, validity or applicability of any claimed patent rights in respect thereof. As of the date of publication of this document, ISO had received notice of (a) patent(s) which may be required to implement this document. However, implementers are cautioned that this may not represent the latest information, which may be obtained from the patent database available at www.iso.org/patents. ISO shall not be held responsible for identifying any or all such patent rights.

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 85, *Nuclear energy, nuclear technologies, and radiological protection*, Subcommittee SC 2, *Radiological protection*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 430, *Nuclear energy, nuclear technologies, and radiological protection*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

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

The main changes are as follows:

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

— minor edits to text throughout;

— addition of 8.2.7 on data security plan;

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

— addition of material related to automated analysis;

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

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

— addition of details on determining the minimal resolvable dose.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

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

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

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

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

A part of the information in this document can be found in other international guidelines and scientific publications, primarily in the International Atomic Energy Agency's (IAEA) Technical Reports series on biological dosimetry[2]. However, this document expands and standardizes the quality assurance and quality control, the criteria of accreditation, and the evaluation of performance. This document is generally compliant with ISO/IEC 17025, with particular consideration given to the specific needs of biological dosimetry. The expression of uncertainties in dose estimations given in this document comply with the ISO guide to the expression of uncertainty in measurement (ISO/IEC Guide 98-1[3]) and the ISO 5725-1[4], ISO 5725-2[5] and ISO 5725-3[6] on accuracy (trueness and precision) of measurement methods and results.

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

1 Scope

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

This document is applicable to

a) the confidentiality of personal information, for the requestor and the service laboratory,

b) the laboratory safety requirements,

c) the calibration sources and calibration dose ranges useful for establishing the reference dose-response curves that contribute to the dose estimation from unstable chromosome aberration frequency and the detection limit,

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

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

f) the reporting of results,

g) the quality assurance and quality control, and

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

2 Normative references

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

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

3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
acentric
terminal or interstitial chromosome fragment of varying size, referred to as an excess acentric fragment when it is formed independently of a dicentric or centric ring chromosome aberration

3.2
background frequency
background level
spontaneous frequency (or number) of chromosome aberrations recorded in control samples or individuals

3.3
centric ring
aberrant circular chromosome resulting from the joining of two breaks on separate arms of the same chromosome

Note 1 to entry: It is generally accompanied by an *acentric* (3.1) fragment.

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

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

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

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

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

3.9
interphase
period of a cell cycle between mitotic divisions

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

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

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

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

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

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

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

3.17
service laboratory
laboratory performing biological dosimetry measurements

4 Abbreviated terms

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

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

5 Dicentric assay

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

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

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

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

6 Responsibility of the requestor

This clause includes items that are not controlled by the service laboratory. Prior to blood sampling, an initial conversation between the requestor and the service laboratory should occur to co-ordinate the sample collection and shipment. Specific requirements regarding sample collection and shipment should be explained to the requestor including the approximate delivery time for the assay result(s). A standard instruction sheet (illustrated in Annex A) explaining the requirements should be sent to the requestor. The requirements include:

a) Blood sampling should use vacutainers containing lithium or sodium heparin as the anticoagulant and the vacutainers should either be sent or specified by the service laboratory.

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

c) Precautions should be taken to ensure the integrity of the container to prevent leakage during shipment. Blood samples should not be frozen during shipment and ideally be kept between 11 °C to 30 °C during shipment, although a lower temperature, above freezing, is still acceptable[2]. A temperature recording device should be included to ensure that the temperature during shipment is controlled. Packaging and labelling shall conform to national and international regulations. If air transportation is involved, a physical dosimeter should be included to monitor whether the sample was irradiated in transit.

d) A questionnaire (see Annex B) provided by the service laboratory should be completed and returned prior to the start of blood culturing.

e) The laboratory shall be alerted of biologically contaminated and/or infectious samples so that extra precautions may be taken when handling the sample.

7 Responsibility of the service laboratory

7.1 Setup and sustainment of the QA program

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

a) It shall include periodic internal checks of equipment operations, reagent suitability, and various performance checks (e.g. intra-laboratory comparison exercises, operator qualifications, sample protocol, scoring, dose estimations, report generation, etc.).

b) It shall include periodic external checks of the laboratory's operations. The external audits shall include a review of the service laboratory's documentation of equipment operations, reagent suitability, and various performance checks (e.g. inter-laboratory comparison exercises, operator qualifications, sample transport integrity and time for delivery, etc.).

7.2 Responsibility during service

The service laboratory shall provide necessary guidance, procedures, and timely reporting of the dose assessment by cytogenetics in response to a request for service. The service activities shall address the following issues:

a) the service laboratory shall have documentation, reviewed and endorsed by a qualified expert, for example service laboratory radiobiologist or equivalent), which includes the following:

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

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

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

b) the service laboratory is not responsible for sample transport however, they should provide advice regarding sample transfer. If required, a kit for the collection of at least 10 ml whole blood in tubes containing lithium or sodium heparin as the anticoagulant shall be sent to the requestor with the appropriately labelled and addressed packaging material for the return of the sample to the service laboratory. The packaging shall conform to national and/or international regulations for the transit of potentially infectious pathological specimens (see 14.2.4);

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

1) document the receipt of the blood sample (date, time, recipient);

2) check for conformity of the sample (blood volume, integrity of the tubes);

3) mark the blood sample with a unique code;

4) store samples at room temperature and document the place of storage until the setting up of cultures;

5) set up cultures in parallel as soon as possible and document date, time, and operator;

6) document all the reagents used for culturing with appropriate lot numbers and expiry dates;

7) document the addition of reagents and the end of the culture (date, time, operator);

8) document the short- and long-term storage of the sample until slide making;

9) document the slide codes, number of slides, and location of storage;

10) document the results from scoring;

11) store the slides and case documents in an appropriate place for possible medico-legal re-evaluation of the case;

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

e) the service laboratory shall sustain a dialogue with and provide results to the requestor.

8 Confidentiality of personal information

8.1 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 all of the patient's information including identity, medical data, etc. 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.

8.2 Applications of the principle of confidentiality

8.2.1 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 undertaken appropriate training and 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.

8.2.2 Requests for analysis

Depending on national regulations, the request for an analysis should normally be made by a physician representing the patient, or the analysis could be requested by another authority 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.

8.2.3 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 shall define all processes for information transmission and assurance of confidentiality.

8.2.4 Anonymity of samples

The laboratory head shall 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 analysis. The code is assigned by an authorized person as defined in 7.2. Decoding, interpretation of results, and compiling the report are also to be performed by an authorized person.

8.2.5 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.

8.2.6 Storage

The laboratory head shall define how fixed cells, slides, data and results are stored. Retention periods will be defined by the laboratory head according to national regulations/policies for possible medico-legal re-evaluation of the case. All laboratory records relating to a case, which could permit the patient and/or employer to be identified, shall be stored in a place only accessible to the authorized persons. Final disposal of records shall be conducted by secure means such as shredding.

8.2.7 Data security plan

A data security plan should be established with written procedures for safeguarding data that contains personal identifiable information. This should include provisions for the storage of written and electronic data, results and reports in a secure location accessible only to authorized persons. A plan for secure disposal of data should also be included.

9 Laboratory safety requirements

9.1 Overview

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

9.2 Microbiological safety requirements

Blood specimens shall be unpacked and manipulated in a minimum class 2 microbiological safety cabinet. to minimise culture failure due to microbial contamination.

The legal and ethical position regarding pathogen 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 pathogen negative.

9.3 Chemical safety

Certain chemicals and pharmaceuticals are routinely used in the procedures covered in this document. 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 upon hazard statements (H-Statements) according to the GHS classification system are listed in Table 1 with the key to the H-statements in Table 2. Note that commercially available products may vary depending on the physical form/quantity/composition — always check the hazard and precautionary statements on safety data sheets available from suppliers for your own reagents.

Table 1 — List of reagents and corresponding hazard statement

Reagent	Hazard statement
Acetic acid	H226, H314
Benzylpenicillin	H317, H334
Bisbenzimide (Hoechst stain)	H302, H315, H319
Bromodeoxyuridine (BrdU)	H351
Colcemid	H300
Giemsa stain	H225, H301, H311, H331, H370
Heparin	H315, H319, H334
Methanol	H225, H301, H311, H331, H370
Phytohaemagglutinin (PHA)	H302, H317, H332
Streptomycin sulfate	H302, H361

Table 2 — Key to hazard statements

Hazard statement	Key
H225	Highly flammable liquid and vapour
H226	Flammable liquid and vapour
H300	Fatal if swallowed
H301	Toxic if swallowed
H302	Harmful if swallowed
H311	Toxic in contact with skin
H314	Causes severe skin burns and eye damage

Table 2 *(continued)*

Hazard statement	Key
H315	Causes skin irritation
H317	May cause an allergic skin reaction
H319	Causes serious eye irritation
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

9.4 Optical safety requirements

When ultraviolet lamps are used for 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.

10 Sample processing

10.1 Culturing

The same culturing conditions that were used for establishing the calibration curve shall be used for analysing aberrations in suspected overexposure cases.

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

a) for calibration curves, blood shall be incubated for approximately 2 h at (37 ± 1) °C immediately following irradiation and prior to culture;

b) cells shall be cultured in a calibrated incubator set at (37 ± 1) °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. For example, the most commonly used growth media are RPMI-1640, Ham's F10, MEM or McCoy 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});

e) a mitogen, for example phytohaemagglutinin (PHA), shall be added to the media to stimulate lymphocytes into mitosis;

f) a method such as the use of BrdU to ensure the identification of first-division metaphases shall be used (see 10.2.3);

g) colcemid 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) after culture, cells are centrifuged in order to pellet the cells. Thereafter, cells shall be treated with a hypotonic solution such as 0,075 M KCl for 10 min to 15 min to allow the cells to swell 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 long-term storage of fixed cells is required, then cell suspensions shall be kept in a – 20 °C freezer, preferably in glass tubes;

l) slides shall be prepared to allow an unambiguous identification of chromosomal aberrations. Humidity and temperature conditions can be optimized by each laboratory to improve the quality of the metaphases;

m) if long-term storage of unstained slides is preferred over storage of fixed cells, they should be kept in a -20 °C freezer;

n) slides shall be stained according to the protocols established in each laboratory;

o) for long-term preservation of stained cells, slides should be mounted with an appropriate mounting medium.

10.2 Scoring

10.2.1 Coding of samples and slides

All samples and slides, shall be coded with a unique code as defined by the laboratory head. Complete records of coding shall be maintained.

10.2.2 Scoring techniques

The laboratory head shall establish and implement procedures for the scoring techniques used. Besides manual scoring of dicentric chromosomes, there are some options for automated or semi-automated detection of dicentric chromosomes[2][7]. Criteria for automated or semi-automated scoring shall be established and validated in advance. The system used should have been previously subjected to quality assurance trials with results documented especially when scoring is at least partially performed with computer-assisted metaphase finding and/or image analysis. 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 to record all the chromosomal abnormalities by manual scoring in service laboratories. A standardised scoring sheet shall be used with data recorded such that the aberrations in each cell scored are derivable (see Annex G). When more than one scorer contributes to the analysis for dosimetry purposes, each shall analyse a comparable number of metaphases.

10.2.3 Procedure for scoring first-division metaphases

An important aspect of culturing blood samples for dose estimation by the dicentric assay is the harvest time for metaphase collection. The maximal frequency of unstable chromosomal aberrations in the lymphocytes of radiation-exposed individuals occurs in the first-division, post-exposure metaphase cells. Different methods can be used to ensure that only first-division metaphase cells are scored. The FpG (Fluorescence plus Giemsa) technique requires the addition of BrdU during culturing and colcemid, 2 h or 3 h before terminating the cultures. 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 shall be scored. Early colcemid addition, i.e. 24 h after culture set up, avoids the progression of cells beyond the first metaphase so it can be used as an easier and

faster method than FpG staining but appropriate concentration needs to be optimized to prevent extreme condensation of metaphase chromosomes. Alternative techniques are acceptable as long as the methodology is documented and validated. For long-term storage, stained and mounted slides are recommended.

10.2.4 Laboratory scoring expertise

Metaphase analyses shall be conducted by trained and experienced scorers fully familiar with the scoring of unstable chromosome aberrations and the handling of software tools 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 intra-laboratory and inter-laboratory comparisons regularly.

A scorer is regarded as qualified, if the 95 % confidence intervals of the doses estimated based on the dicentric yield of the test sample and on the calibration curve of the lab, includs the reference doses.

See below additional details for the assessment of scoring expertise by performance checks through laboratory inter-comparison studies (see 14.2.2) and periodical checks of individual scorers (see 14.2.3).

11 Calibration curves

11.1 Calibration source(s)

The service laboratory shall provide a report, reviewed and endorsed by a qualified expert, for example radiation physicist or the service laboratory head, that addresses the following issues:

a) description of radiation quality, for example X-ray machine with a 2,1 mm Cu half-value layer (HVL), peak potential of 250 kV, filament current 12,5 mA, and a source-to-surface distance (SSD) of 50 cm, and irradiation set up for each radiation calibration source 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 including details of the quantity that has been measured, 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.

For more details on irradiation parameters and conditions please refer to the IAEA manual[2].

11.2 Establishment of calibration curve(s)

The selection of the radiation sources for calibration curves should reflect the most likely cases that are analysed. It is important to ensure that the interpretation of the results takes into account the characteristics of the radiation of the calibration curve as the shape of the curve will vary according to radiation type, quality and dose-rate. Typically, acute dose-rates of above 0,3 Gy/min should be chosen. The calibration curve can be fitted for dicentrics or dicentrics and centric rings. The laboratory decides which aberration(s) to score and an appropriate calibration curve shall be established under exactly the same experimental conditions for calibration and dosimetry.

A minimum of 7 doses should be selected with at least 5 doses below 1 Gy including 0 Gy. The typical doses for a calibration curve range from 0 Gy to 5 Gy for low-LET radiation (e.g. Co-60 or Cs-137 gamma

rays)[2]. The selection of these two dose points is important because they define the lowest and highest dose the laboratory is able to report.

To determine the background level of dicentrics (0 Gy dose), at least 5 000 cells should be scored. At doses below 1 Gy, 3 000 to 5 000 cells should be scored per dose point. Above 1 Gy, the laboratory should aim to detect approximately 100 dicentrics (or dicentrics plus rings) at each dose. In all cases, the focus should be on minimising the error in the fitted curve. Increasing numbers of cells scored reduces the uncertainty in the absorbed dose estimates. Calibration curves should be constructed from at least two adult, healthy donors and data may be pooled from these multiple donors. If donor specific effects were observed, further research on the reasons for the differences might be required such as the analysis of a few dose points from additional donors or repeat analysis of a few doses of the original donors.

For high-LET radiation, for example neutrons, the upper dose limit is typically 2 Gy due to the elevated level of damage to cells at higher doses.

The following definitions are used for further calculations:

— y: observed dicentrics: The absolute number of dicentrics observed in n scored cells;

— μ: observed yield of dicentrics: The observed number of dicentrics per cell.

The observed yield of dicentrics, μ, should be fitted to the linear or linear-quadratic models as shown in Formula 1:

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

where $\mu = \frac{y}{n}$

According to the IAEA manual[2], the lower and upper 95 % confidence limits of the curve can be calculated by Formula (2):

$$\mu = C + \alpha D + \beta D^2 \pm \sqrt{R^2\left(\mathrm{var}(C) + \mathrm{var}(\alpha)D^2 + \mathrm{var}(\beta)D^4 + 2\mathrm{covar}(C,\alpha)D + 2\mathrm{covar}(C,\beta)D^2 + 2\mathrm{covar}(\alpha,\beta)D^3\right)} \tag{2}$$

where

D is the absorbed dose;

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

SE is the standard error of the coefficients;

R^2 Is the coefficient of determination, and is the 95 % confidence limit of a chi-square distribution, $\chi 2$ (degrees of freedom, 95 %), with 2 or 3 degrees of freedom. For a linear-quadratic curve (degrees of freedom = 3) R^2 is 7,81, and for a linear curve (degrees of freedom = 2) it is 5,99;

var is the variance of the coefficient in brackets;

covar is the covariance of the two coefficients in brackets, available from most standard software packages.

The statistical significance of the coefficients should be tested with an F-test. For a linear-quadratic model, it is important that both the α and β coefficients are statistically significant. As the linear energy transfer (LET) increases, the β coefficient tends towards zero. If the β coefficient is not statistically significant, the data should be fitted to a linear model, excluding βD^2 from Formula (1).

Two methods are proposed for curve fitting, the iteratively reweighted least squares method and the maximum likelihood method (see Annex D)[8][9]. As a minimum requirement, when the obtained value of

chi-squared is higher than the degrees of freedom, standard errors should be increased by (chi-square/degree of freedom)$^{1/2}$. Other consistent methods can be applied as long they have been validated.

The laboratory should use documented and validated curve fitting software which follow Formulae (1) and (2). Several such software packages have been developed by the international biological dosimetry community and are freely available. Others, including commercially available software tools, can be found through open literature and the internet. Whichever software is used, validation should include testing against published data.

The laboratory shall provide a report, reviewed and endorsed by a designated, competent person that addresses the following:

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

b) detailing the in vitro calibration data and their fit to a calibration curve. Goodness of fit, significance of the fitted linear and quadratic coefficients, and uncertainty estimates should also be reported.

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

12.1 General

The measured dicentric frequency is converted to absorbed dose by reference to an appropriate in vitro calibration curve produced in the same laboratory using radiation of comparable quality (see 11.1). 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 when there is a high abundance of dicentrics but few metaphase spreads, the dose can be reported after scoring fewer than 100 dicentrics.

Where sufficient information is available, the service laboratory shall provide the estimated whole body absorbed dose and confidence limits in reports. Uncertainties should be expressed in terms of the combined standard uncertainty (combined standard deviation) and expanded uncertainty: 95 % confidence limits. Note that uncertainties should be propagated throughout the analysis according to the ISO/IEC Guide 98-3[13]. Other characteristic limits (the decision threshold and detection limit) or confidence intervals should also be reported, if appropriate to each particular case, as discussed below.

12.2 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 over-dispersed (the observed variance is 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 using a standard statistical test, for example the u test which is a normalized unit of the dispersion index $\delta = s^2/\mu$ (variance/mean)[9][10]. For a Poisson distribution, δ is unity. $|\mu|$ values calculated according to Formula (3) to be greater than 1,96 indicate over (or under)-dispersion (two-sided significance level, $\alpha = 0{,}05$) and, therefore, deviation from a Poisson distribution.

$$\mu = (\delta - 1)\sqrt{\frac{n-1}{\left[2\left(1 - 1/\mu\right)\right]}} \tag{3}$$

where

δ is the dispersion index;

n is the number of cells analysed;

μ is the mean number of dicentrics detected.

12.3 Comparison with the background level: Characterisation of the minimum detectable dose

In ISO 11929-1[11], two characteristic limits are defined:

— the decision threshold (y_d) or minimum resolvable number of dicentrics, which is the threshold above which the physical effect is assumed to be quantifiably present. In other words, the decision threshold can be understood as the minimum number of dicentrics that is significantly different from the expected background number of dicentrics for a given significance level α (e.g. α = 0,05);

— the detection limit (y_z) is the smallest true value of the measurand (number of dicentrics), which can be detected. It gives information on the practical operating range of the assay and is thus used to assess whether the measurement procedure is appropriate in a particular set of circumstances.

The decision threshold and the detection limit should be calculated before the measurement is performed.

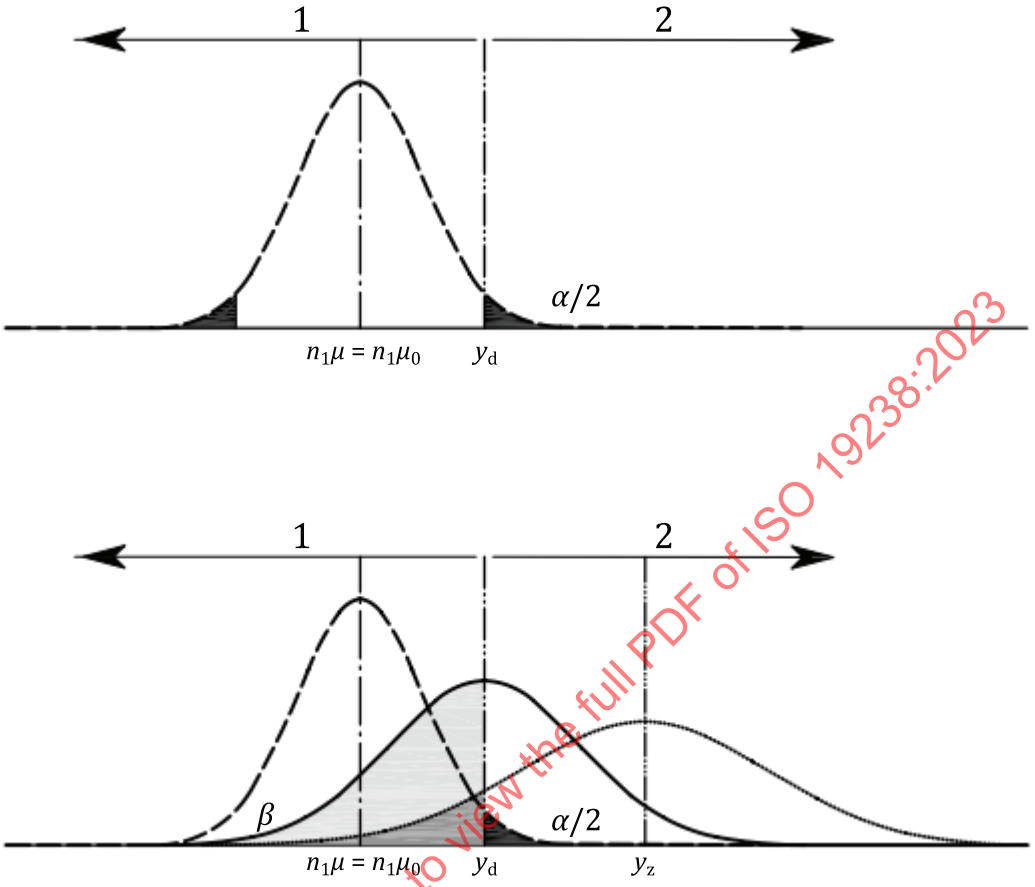
Table 3 — Error rates for the inference of positive and negative conclusions

		Inferred conclusion	
		Test person exposed H_0 rejected	Test person not exposed H_0 accepted
True state	Test person exposed H_0 false	True positive	Type 2 error (β) False negative
	Test person not exposed H_0 true	Type 1 error $\left(\frac{\alpha}{2}\right)$ False positive	True negative

If a test person shows a number of dicentrics greater than the decision threshold (y_d), we could conclude that the patient was exposed and a dose estimate should be made. In other terms, the null hypothesis (H_0) that the test person was not exposed or that the observed dicentric yield is equal to the background dicentric yield can be rejected with a type 1 error rate $\frac{\alpha}{2}$, and it is concluded that the physical effect is present. However, an important parameter to consider is the fact that the dose value of zero should not be present within the confidence interval of 95 % from the dose-effect curve used. If this were to be the case, one could not conclude that the person has been exposed. If the observed number of dicentrics, y, is below the decision threshold, y_d, it is decided that the result cannot be attributed to the physical effect, i.e. it cannot be concluded that the person was exposed. Nevertheless, depending on the unknown true state (see Table 3) the probability for a false negative result might still be relatively high and, therefore, it cannot be concluded that the person was not exposed (see Figure 1).

In cases where the observed number of dicentrics is below the decision threshold, the detection limit shall be reported. The detection limit provides valuable information about the true dose (or number of dicentrics) above which fewer than 100·β % false negative results are expected. The detection limit can be interpreted in the following way: given that a test person was exposed (true state, see Table 3), if the true dose or number of dicentrics of the test person was higher than the detection limit, the probability

of observing a number of dicentrics lower than the decision threshold and thus inferring a false negative conclusion would be less than β.



Key

1	H_0 accepted	——	sampling distribution under H_1 (mean at y_d)
2	H_0 rejected	——	sampling distribution under H_1 (mean at y_z)
----	sampling distribution under H_0	——	detection limit y_z
——	decision threshold y_d		

Figure 1 — Illustration of the decision threshold and the detection limit (μ_0 = const)

Top panel: Given the null hypothesis H_0 (test person not exposed) is true (i.e. dicentric yield μ of the test person is equal to the background dicentric yield μ_0), the probability for wrongly rejecting H_0 or for observing a dicentric number that exceeds the decision threshold (y_d) is less than $\frac{\alpha}{2}$.

Bottom panel: Given that the alternative hypothesis H_1 (test person exposed) is true, if the true dose or the true number of dicentrics, respectively, is equal to the decision threshold (y_d), the probability for wrongly accepting H_0 is approximately 0,5 (light gray area under the curve). If the true dose or the true number of dicentrics, respectively, is greater than the detection limit, the probability for wrongly accepting H_0 is lower than a predefined type 2 error rate β (e.g. β = 0,1).

To define the decision threshold, it is assumed that the test person has not been exposed, i.e. the dicentric yield of the test person μ is equal to the background dicentric yield μ_0. The null hypothesis is given by $\mu = \mu_0$ [or $n\mu = n\mu_0$], respectively. Assuming that the background dicentric yield is constant, i.e. has no uncertainty, the decision threshold, y_d, can be defined by the following probability:

$$P(y > y_d \,|\, n_1\mu = n_1\mu_0) = \sum_{k=y_d+1}^{\infty} \frac{e^{-(n_1\mu_0)}(n_1\mu_0)^k}{k!} = 1 - \sum_{k=0}^{y_d} \frac{e^{-(n_1\mu_0)}(n_1\mu_0)^k}{k!} \leq \frac{\alpha}{2} \quad (4)$$

where

n_1 is the number of cells scored for the test person;

α is the probability for a type 1 (false positive) error, which is generally taken to be 5 %.

Again, assuming that the number of dicentrics of the test person follows a Poisson distribution, the detection limit for the number of dicentrics, y_z, is defined by the following formula:

$$P(y \le y_d \,|\, n_1\mu = y_z) = \sum_{k=0}^{y_d} \frac{e^{-y_z} y_z^k}{k!} = \beta \tag{5}$$

The latter (Formula 5) can be solved analytically using

$$y_z = \frac{\chi^2_{2(y_d+1),1-\beta}}{2} \tag{6}$$

where

χ^2 is the chi-squared quantile (the inverse of the chi-squared distribution);

β is the type 2 (false negative) error, which would generally be between 5 % and 20 %;

$2(y_d+1)$ is the degree of freedom.

A detailed example for the calculation of the decision threshold and detection limit can be found in Annex F.

It is necessary to assess the detection limit and decision threshold on a case-by-case basis. The detection limit can be converted to a 'minimum detectable dose' by application of the calibration curve, as shown in Annex F. However, in all cases, the minimum detectable dose is limited to the lowest dose used in the appropriate calibration curve.

If the observed number of dicentrics exceeds the decision threshold, this means that there is evidence to refute the null hypothesis of no significant difference between the background and observed numbers of dicentrics. The calculation of observed absorbed dose should then be carried out and reported according to 13.2. If not, then the detection limit should be reported.

If the true number of dicentrics is higher than the detection limit (or the true dose is higher than the minimum detectable dose), the probability of observing a lower number of dicentrics than the decision threshold (or the probability to infer false negative conclusions) is lower than β.

12.4 Confidence limits on the number of dicentrics

There are several methods for deriving confidence limits on an observed number of dicentrics. Confidence limits on Poisson observations may be obtained from standard tables, by exact or approximate calculations. If a measured aberration is over-dispersed with respect to Poisson, the Poisson-derived uncertainty should be increased by the square root of the ratio of variance to mean or a more appropriate model should be used to describe the distribution of aberrations. Alternatively, for over-dispersed data, more appropriate distributions can be applied, such as the Negative Binomial, Hermite or Neyman type A distributions, in order to take into account both the mean yield of aberrations and the dispersion coefficient[12]. The laboratory shall provide justification for and validation of the chosen model(s).

The exact Poisson confidence limit on the observed number of dicentrics can be calculated as:

$$y_L = \frac{\chi^2_{2y,\alpha/2}}{2} \tag{7}$$

$$y_U = \frac{\chi^2_{2(y+1),1-\alpha/2}}{2} \quad (8)$$

where

χ^2	is the chi-squared quantile (the inverse of the chi distribution);
$\alpha/2$	is the lower tail probability (usually 0,025 for α = 0,05);
1- $\alpha/2$	is the upper tail probability (usually 0,975);
2y or 2(y+1)	are the respective degrees of freedom.

12.5 Calculation of absorbed dose for whole-body exposures

If the observed excess number of dicentrics satisfactorily exceeds the decision threshold as defined above, then the absorbed dose should be calculated by comparison with an appropriate calibration curve, created as described in 11.2, and defined by Formula (1). The absorbed dose, D, is calculated by solving the linear or quadratic equations, which is possible using freely or commercially available software tools. The information on how the calculations are performed within the software should be documented and updates performed periodically.

The absorbed dose can be obtained by solving Formula (9):

$$D = \frac{-\alpha + \sqrt{\alpha^2 + 4\beta(\mu - C)}}{2\beta} \quad (9)$$

where

C, α, β	are the coefficients of the calibration curve;
μ	is the observed dicentric yield of the test person.

12.6 Calculation of uncertainty on absorbed dose

The resulting estimated absorbed dose represents the best estimate possible given the associated dispersion, which arises from the experimental and intrinsic uncertainties. It is thus necessary to estimate measurement uncertainty using appropriate methods.

The general procedure for assessing uncertainty relies on formal combination of all the sources of experimental uncertainty according to standard methods of error propagation (ISO/IEC Guide 98-3) [13]. In brief, the relationship between the absorbed dose and the input quantities should first be clearly defined, the sources of uncertainty relevant to the particular case should be identified and quantified and then the combined uncertainty should be calculated (see ISO 5725-1[4]).

In practice, the recommended methodology to calculate uncertainty on absorbed dose in the context of the dicentric assay is to combine the confidence limits on the dicentric frequency with the uncertainties on the calibration curve coefficients (see Formula 1). In many cases, these represent the dominant sources of uncertainty. This can be achieved by using Merkle's method[8] based on Formulae (5) to (8) or using available software which performs its calculations based on Formula (9).

In all cases, the laboratory head shall define the methods used to determine confidence limits. The service laboratory shall report the method used to determine the standard uncertainty (the standard deviation) and the expanded uncertainty (which gives the 95 % confidence interval). The laboratory should also retain records of the uncertainty budget (a list of the uncertainty components and how they were evaluated) together with details of any systematic uncertainties accounted for and all other corrections and constants used.

12.7 Acute and non-acute exposure cases

If an overexposure is known to have occurred acutely, i.e. with a time frame of 30 minutes or less, the absorbed dose may be estimated by reference to an acute in vitro calibration curve. For a linear-quadratic dose-response relationship, the absorbed dose is estimated by solving the quadratic equation utilising the calibration curve coefficients and the observed yield.

If an overexposure results from a protracted exposure, the absorbed dose estimate may be obtained by reference to just the background level and linear coefficients of the acute calibration curve. For radiation exposures that range from 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. Detailed information on the G-function can be found in the IAEA manual[2]. 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 interfraction factor should be estimated using a 2 h time constant[2].

As with curve fitting, most of the freely available bespoke software packages developed by the international biological dosimetry community include tools for dose estimation and further information and examples are given in the IAEA manual[2].

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

12.8 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 Qdr or contaminated Poisson methods[8][9].

In the contaminated Poisson method, the frequency of dicentrics of the irradiated fraction (μ) is estimated using Formula (10) and solving the μ value by iteration.

$$\frac{\mu}{\left(1-e^{-\mu}\right)}=\frac{y}{(n-n_0)} \tag{10}$$

where

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

$e^{-\mu}$ represents the undamaged cells from the irradiated fraction;

n is the number of cells scored;

y is the number of dicentrics observed;

n_0 is the number of cells free of dicentrics.

To calculate the 95 % confidence limits for μ, the approach indicated in 12.4 shall be adopted.

The fraction of cells scored which were irradiated (f) can be calculated using Formula (11):

$$\mu f=\frac{y}{n} \tag{11}$$

To estimate the fraction of cells originally exposed (F), Formula (12) should be used:

$$F=\frac{f/p}{(1-f)+f/p} \tag{12}$$

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 (13):

$$p = \exp\left(\frac{-D}{D_{37}}\right) \tag{13}$$

where

D is the estimated dose;

D_{37} 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_{37} shall be assumed for photon irradiation.

With the Qdr method, Formula (14) is used to estimate the dose received by the irradiated fraction.

$$\frac{y}{C_u} = \frac{\mu_1}{\left(1 - e^{(\mu_1 - \mu_2)}\right)} \tag{14}$$

where

y is the number of dicentrics;

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

μ_1 and μ_2 are the expected yields of dicentrics and excess acentrics, respectively.

μ_1 and μ_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 dicentrics among unstable cells. By the Qdr 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 (12) and (13).

Exposures occurring more than approximately 6 months prior to analysis tend to be underestimated by the dicentric assay. In this case, scoring translocations by FISH (fluorescent in situ hybridization) should also be proposed to the requestor. If the timing and duration of an old exposure is known, the measured dicentric frequency should be adjusted by assuming a disappearance half-life time of approximately 3 years[2]. In the case of prior acute exposures greater than approximately 1 Gy whole-body equivalent photon dose, a shorter half-life 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.

12.9 Other exposure scenarios

When there is a high degree of uncertainty in the conversion of the measured yield to dose, calculation of the dose may not be prudent. In this case, it is possible to use the comparison of radiation induced frequency of dicentrics to the background to give an indication of whether or not an exposure has occurred (e.g. in the case of internal exposures).

Detailed examples of a number of different exposure scenarios in which the dicentric assay has been applied are given in the IAEA manual[2].

13 Reporting of results

13.1 General

The report should only be sent to the requestor unless otherwise instructed by the requestor. Routinely, the report should contain relevant information provided by the requestor 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 subclauses.

13.2 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. “Dicentric assay report”;

b) full contact information of the laboratory performing the analysis;

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

d) full contact details of the requestor, 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 and date of birth of the subject;

g) description of the case, i.e. all relevant information provided by the requestor 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 13.3);

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

13.3 Interpretation of the results

This varies depending on the circumstances of each case but the report should include as many as possible of the following:

a) a dose assessment and its 95 % confidence interval 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 95 % confidence interval;

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. e.g. due to heterogeneous exposure;

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 requestor to contact the laboratory if he/she requires further clarification or explanation about the results and/or the assay;

k) if required, the regulatory body should be informed of the results according to national or local processes.

14 Quality assurance and quality control

14.1 Overview

Quality management shall ensure a continued improvement of operations. This document requires that the quality assurance (QA) and quality control (QC) procedures required by ISO/IEC 17025 shall be followed in each laboratory. This document defines quality assurance and quality control procedures specific for laboratories performing biological dosimetry by cytogenetics.

14.2 Specific requirements

14.2.1 General

QA and QC programs comparing the proficiency and competency with other certified or suitably qualified cytogenetic biodosimetry laboratories shall be established and these evaluations have to be performed at regular intervals (e.g. annually or bi-annually).

ISO 5725[4][5][6][14] is dedicated to statistical analysis to test the reliability and the precision of a technique. The laboratory head is responsible for deciding which statistical tests are appropriate according to the design of the inter-laboratory comparison.

14.2.2 Performance checks by laboratory inter-comparisons

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 viewpoints.

Variations in assessment values of individual laboratories that appear to be inconsistent with all other laboratories may change the outcome of assay results (dose estimates). To discard or correct inconsistent values, two approaches can be used (ISO 5725-2/ISO 5725-5[5][14]):

— numerical outlier tests, for example Cochran and Grubbs tests: to discard data that after a statistic test exceeds the critical value of the test at the 1 % significance level;

— 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 inter-laboratory comparison performance requires a minimum of five laboratories for statistical robustness (ISO 5725-1[4]);

b) estimation of the mean value and the standard deviation once the inter-laboratory 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).

14.2.3 Periodical performance check of scorer qualification

The laboratory is responsible for ensuring that the individuals carrying out the assays are appropriately trained. All individuals should periodically participate in intra- and inter-laboratory comparisons. A set of calibration samples should be used to verify that the accuracy of results is well within the expected range.

A list of qualified scorers is established at least every second year by intra-comparison of laboratory personnel.

To be qualified, each scorer shall score a sample of lymphocytes exposed to a radiation dose above 1 Gy (acute photons) and a sample of lymphocytes exposed to a radiation dose below 1 Gy (acute photons) according to the standard practice of the laboratory. Scoring of 500 cells is preferable depending on the practice of each service laboratory.

A scorer is regarded as qualified, if the 95 % confidence intervals of the doses estimated based on the dicentric yield of the test sample and on the calibration curve of the lab, include the reference doses.

For example, if a scorer finds that his/her measured absorbed dose in a test sample is 0,40 Gy with corresponding 95 % confidence limits of 0,32 Gy to 0,58 Gy for a reference sample irradiated with 0,50 Gy; the reference dose is included in the confidence interval and the scoring was therefore performed according to the calibration curve. The formal statistical methodologies described in 14.2.2 can also be applied for intra-laboratory comparisons.

14.2.4 Performance checks of sample transport integrity

In many cases, blood collection occurs at sites distant from the service laboratories and therefore transportation is necessary. The requestor is responsible for assuring the blood samples are transported in optimal temperature conditions (from 11 °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 whether or not the sample is exposed to X-rays. For international transport, the appropriate permits shall be obtained in advance and included in the shipment to avoid delays at customs including declaration about the biosafety of samples. For international shipments a Pro Forma or commercial invoice is also required. All details concerning blood collection and storage should be recorded.

14.2.5 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 barcoded samples (a barcode can be assigned for the sample(s) either by the requestor or by the designated service laboratory) is critical to maintain impartiality while scoring. If possible, chain of custody can be established for sample tracking.

14.2.6 Performance checks for instrumentation

Performance of all the equipment used for the assay shall be checked and evaluated at regular intervals while the equipment is in use.

Examples of critical equipment include incubators, water baths, weighing machines, thermometers, pipettes, centrifuges and freezers.

For example, the stability of the temperature control and humidity of the incubators has to be monitored and registered in a logbook on a daily basis. If used, the weighing machine has to be checked periodically for accuracy. These regular checks shall be sufficient to demonstrate that the assay equipment is properly calibrated and that all the components are functioning properly.

14.2.7 Performance checks of sample protocol

As an internal quality assurance, negative controls from unexposed individuals and, where possible, internal positive controls may periodically (at least annually) be processed 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 groups (exposed and unexposed) 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 to determine whether lymphocyte counts are in the normal range. 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 expiry dates for growth media and reagents need to be checked prior to their use. The composition of all reagents shall be described as accurately as possible. A quality check for lymphocyte proliferation may be required if different batches of serum are used.

14.2.8 Performance checks of sample scoring

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

14.2.9 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.

14.2.10 Performance checks for result report generation

The test reports to requestors (preferably physicians who order the cytogenetic biodosimetry testing) shall be prepared in a manner to ensure that they contain the necessary information defined in this document (see Clause 13), namely: subject and requestor 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 requestor

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 persons' 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.

— Notify laboratory before the blood sample so that we can prepare for its arrival and pick up.

— Collect at least 10 ml of blood into lithium or sodium heparin tubes. Gently rock the tubes for 2 min to ensure proper mixing. Label the tubes unambiguously and complete the questionnaire.

— Package the blood sample carefully to prevent breakage of the tubes in transit.

  — Blood samples should be packaged to avoid extreme heating or cooling (11 °C up to 30 °C). 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 to ensure that the samples do not freeze during transportation. If temperature extremes are likely to be encountered, a minimum-maximum thermometer should be included in the package. Blood samples shall not be frozen.

— Mark on the external packaging and the shipping documents UN 3373, Biological Substances-Category B- DO NOT FREEZE

— Avoid X-irradiation at the security checkpoints when air transportation is used. A physical dosimeter should 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 documents to avoid delays at customs. For air transport, packaging and labelling should be conform to the current International Air Transport Association (IATA) regulations for dangerous goods, class 6.2 infectious substances. These require that blood samples should be marked and packed to conform to UN 3373, Biological substances, Category B. The package itself and the 'Nature and Quantity of Goods' box of the air waybill should show the following wording: "UN 3373, Biological Substances – Category B packed in compliance with IATA packing instruction 650".

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

— Ship the sample immediately after blood collection, by special transportation using overnight air express so that we can receive the blood early in the morning following sample collection.

— Contact the laboratory to confirm the shipment and inform us of the Way Bill number. THIS IS IMPORTANT FOR TRACKING THE SAMPLE.

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

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

Annex B
(informative)

Sample questionnaire

EXPOSURE INFORMATION FOR CHROMOSOME ABERRATION ANALYSIS

(TO BE FILLED OUT BY THE REQUESTOR)

I ..... (Name), born ..... (YYYY-MM-DD) 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: ..... (YYYY-MM-DD) Volume of blood ..... ml

Specify anticoagulant: .....

<u>Exposure data:</u> **Radiation worker** **or** **Non-radiation worker**

Occupation: .....

Date and time of overexposure: ..... (YYYY-MM-DD hh:mm)

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

1. Brief description of overexposure scenario:

2. 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 .....

3. Type of radiation:

X - ray	O	Energy ?	
γ	O	Origin ?	
α	O	Origin ?	
Neutrons	O	Origin ?	Energy ? .....
Electrons	O	Origin?	Energy ? .....

<u>Patient data:</u>

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 of 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

Laboratory name, address

Contact data

Test report of biological dose assessment/dicentric analysis
(Report form should be in accordance with the requirements of ISO/IEC 17025)

ID No. of report (unambiguous) and date of issue of report:

Requestor name, contact data:

Date of request:

Sample received

Sample	Sampling date/location	Arrival date	Condition of a sample
Unambiguous sample code			

Description of case

Method(s) of analysis: dicentric assay according to Standard Procedure no. issued *date* in accordance with ISO 19238. The dose is estimated using a calibration curve for *type of radiation*.

Date of beginning of cell culture and aberrations analysis

All tests followed a standard method and there were no environmental conditions which may bear upon the results (any deviations shall be described in the report).

Results

Sample code	Number of cells analysed	No of dicentric (and centric rings)	Others aberrations

Dose assessment with units and uncertainties:

Results reported by:
position, name
date, signature

Dose assessment by:
position, name
date, signature

Approved by:
position, name
date, signature

End of report

This test report may only be published and copied in full, except with a prior written permission by *the name of service laboratory*. The test results only apply to the tested samples.

(Information in the footer: ID No. of report (unambiguous) and date of issue of report, page 1 of ....)

Interpretation of results

Results interpreted by:

position, name

date, signature

Annex D
(informative)

Fitting of the low-LET dose-response curve by the method of maximum likelihood and calculating the error of dose estimate

D.1 Fitting procedure

Consider exposing blood lymphocytes to N doses of radiation x_i (i = 1, 2 ... N). The following data are obtained:

r_i number of aberrations for i dose;

n_i number of scored cells for i dose;

$y_i = r_i/n_i$ frequency of aberrations per cell for i dose.

It is assumed that cells are exposed to radiation of low LET, where the distribution of aberrations is Poissonian. Hence:

$$P(r_i) = \frac{(n_i\lambda_i)^{r_i}}{r_i!} e^{-n_i\lambda_i} \tag{D.1}$$

where $n_i\lambda_i = E(r_i)$ and $\lambda_i = E(y_i)$ (the expected frequency of aberrations per cell). The variance for y_i is $V(y_i) = \lambda_i / n_i$.

Thus, for the data $x_1, x_2, \ldots, x_N, r_1, r_2, \ldots, r_N$ and $y_1, y_2, \ldots, y_N$, the probability, $P(r_1, r_2, \ldots, r_N) = \prod_{i=1}^{N} \frac{(n_i\lambda_i)^{r_i}}{r_i!} e^{-n_i\lambda_i}$

.

This is the so-called likelihood function.

The observed relationship between the dose and the aberration frequency can be fitted by a linear-quadratic formula $y_i = \beta_2 x_i^2 + \beta_1 x_i + \beta_0$, where $\beta_0, \beta_1, \beta_2$ are not known.

The aim of the fitting procedure is to find the coefficients, $\beta_0, \beta_1, \beta_2$, for which the likelihood function reaches a maximum. This maximum can be calculated by the iterative method of maximum likelihood as described below:

Let $l = \ln(P(r_1, r_2, \ldots, r_N))$ (D.2)

Thus $l = \ln\left(\prod_{i=1}^{N} \frac{(n_i\lambda_i)^{r_i}}{r_i!} e^{-n_i\lambda_i}\right) = \sum_{i=1}^{N} (r_i \ln\lambda_i - n_i\lambda_i + r_i \ln n_i - \ln r_i!)$ (D.3)

The function l reaches a maximum for $(\beta_k)_{k=0,1,2}$ such that $\frac{\partial l}{\partial \beta_k} = 0$. These are the so-called likelihood equations. The formulae can only be solved by iteration. The succeeding approximations of the parameter $\beta^T = (\beta_k)_{k=0,1,2}$ are designated as ${}_j\beta$ where $j = 0, 1, \ldots p-1$ and the value p is a natural number denoting the last approximation of the estimated coefficients, $(\beta_k)_{k=0,1,2}$. The first approximation of the parameter ${}_0\beta$ results from estimation or from earlier experiments. The second