INTERNATIONAL STANDARD

ISO 19238

First edition
2004-08-01

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

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

Reference number
ISO 19238:2004(E)

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Published in Switzerland

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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

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

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

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

Introduction

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

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

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

The International Standard is written in the form of procedures to be adopted for biological dosimetry for overexposures involving at most a few casualties. The criteria required for such measurements will usually depend upon the application of the results: radiation protection management, medical management when appropriate, record keeping and legal requirements. In the special situation of a mass radiation casualty and limited resources, the technique can be applied for emergency triage analysis. The standard recommended scoring criteria would then be relaxed as appropriate to the situation.

A part of the information in this International Standard is contained in other international guidelines and scientific publications, primarily in the International Atomic Energy Agency's (IAEA) Technical Reports Series on Biological Dosimetry. However, this International Standard expands and standardizes the quality assurance and quality control, the criteria of accreditation and the evaluation of performance. This International Standard is generally compliant with ISO/IEC 17025, with particular consideration given to the specific needs of biological dosimetry. The expression of uncertainties in dose estimations given in this International Standard comply with the ISO Guide to the expression of uncertainty in measurement (GUM) and the ISO 5725 on accuracy (trueness and precision) of measurement methods and results.

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

1 Scope

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

This International Standard addresses

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

b) the laboratory safety requirements,

c) the calibration sources and calibration dose ranges useful for establishing the reference dose-effect curves allowing the dose estimation from chromosome aberration frequency, and the minimum detection levels,

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

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

f) the reporting of results,

g) the quality assurance and quality control,

h) informative annexes containing examples of a questionnaire, instructions for customers, a data sheet for recording aberrations and a sample report.

2 Terms and definitions

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

2.1
acentric
terminal or interstitial chromosome fragment of varying size

NOTE When it is formed independently of a dicentric or centric ring chromosome aberration, it is usually referred to as an excess acentric

2.2
background level
spontaneous frequency (or number) of chromosome aberrations recorded in control samples or individuals

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

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

NOTE It is generally accompanied by an acentric fragment.

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

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

2.7
chromosome
structure that carries genetic information

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

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

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

NOTE It is generally accompanied by an acentric fragment.

2.10
FISH
fluorescence *in situ* hybridization
technique that uses specific sequences of DNA as probes to particular parts of the genome, allowing the chromosomal regions to be highlighted or “painted” in different colours by attachment of various fluorochromes

NOTE This technique permits the detection of damage involving exchanges between differently painted pieces of DNA (usually whole chromosomes)

2.11
interphase
period of a cell cycle between the mitotic divisions

2.12
LET
linear energy transfer
quotient of $\mathrm{d}E/\mathrm{d}l$, as defined by the International Commission on Radiation Units and Measurements (ICRU), where $\mathrm{d}E$ is the average energy locally imparted to the medium by a charged particle of specific energy in traversing a distance of $\mathrm{d}l$

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

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

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

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

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

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

2.18
service laboratory
laboratory performing biological dosimetry measurements

3 Dicentric assay

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

Lymphocytes are cultured by a method that permits first-division metaphases to be recognised for analysis (see 9.1). This requires whole blood, or lymphocytes separated from the other blood components, to be incubated in culture medium that would enable scoring of first-generation metaphase cells. A mitotic blocking agent, colcemid or colchicine, is added to arrest dividing lymphocytes in metaphase. The duration of the cell culture and the timing of addition of the arresting agent is optimised to ensure an adequate mitotic index and predominance of first-division metaphases.

Metaphases are recovered from the cultures by centrifugation, placing in a hypotonic salt solution and fixing in a mixture of alcohol and acetic acid. Fixed cells are placed on microscope slides and stained. The exact protocol for cell culture, harvesting metaphases and staining employed by a service laboratory should be formally documented (see Clause 12).

Stained microscope slides are methodically scanned to identify dicentric aberrations (see 9.2). The frequency of dicentrics observed in an appropriate number of scored metaphases is converted to an estimate of radiation dose by reference to calibration data (see Clause 10).

4 Confidentiality of personal information

4.1 Overview

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

This requirement extends to

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

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

4.2 Applications of the principle of confidentiality

4.2.1 Delegation of responsibilities within the aboratory

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

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

4.2.2 Requests for analysis

Depending on national regulations, the request for an analysis should normally be made by a doctor representing the patient, by the patient him/herself or could be requested due to legal claims. In all cases, the blood sampling for chromosome analysis must be made with the patient’s informed consent. The laboratory head, depending on the national regulations, may be required to maintain the record of the patient's informed consent.

4.2.3 Transmission of confidential information

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

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

4.2.4 Anonymity of samples

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

4.2.5 Reporting of results

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

4.2.6 Storage

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

5 Laboratory safety requirements

5.1 Overview

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

5.2 Microbiological safety requirements

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

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

5.3 Chemical safety requirements

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

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

Heparin	R 36; 37; 38;
Hoechst stain	R23; 24; 25; 36; 37; 38;
Phytohaemagglutinin	R20, 21; 22; 43;
Streptomycin sulfate	R 20; 21; 61.

Keys

R20	Harmful by inhalation;
R21	Harmful in contact with skin;
R22	Harmful if swallowed;
R23	Toxic by inhalation;
R24	Toxic in contact with skin;
R25	Toxic if swallowed;
R26	Very toxic by inhalation;
R27	Very toxic in contact with skin;
R28	Very toxic if swallowed;
R36	Irritating to eyes;
R37	Irritating to respiratory system;
R38	Irritating to skin;
R40	Possible risk of irreversible effects;
R41	Risk of serious damage to eyes;
R42	May cause sensitisation by inhalation;
R43	May cause sensitisation by skin contact;
R46	May cause heritable genetic damage;
R61	May cause harm to the unborn child;
R63	Possible risk of harm to the unborn child.

5.4 Optical safety requirements

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

5.5 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.

6 Calibration source(s), calibration curve(s) and minimum detection levels

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

b) 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;

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

6.2 Calibration curve(s)

The selection of the calibration dose range will depend on the radiation quality. In the case of low-LET photon radiation, more than 7 doses should be selected, distributed equally among the linear and quadratic component of the dose response curve. The typical doses for a low-LET calibration curve range from 0,25 to 4 Gy, although data at lower doses are highly desirable, e.g. 0,1 or 0,15 Gy. Any substantial deviations from this dose range shall be justified.

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) description of 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 fitting to a calibration curve.

6.3 Minimum detection levels

The minimum testing or detection level of 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. An accredited laboratory may be able to detect a dose as low as 100 mGy.

7 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 standardized instruction sheet as illustrated in Annex A. The essential features are the following.

a) Blood sampling should use the collection system, containing lithium heparin as an 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. Packaging and labelling shall conform with national and international regulations. If air transportation is involved, a sheet of X-ray film should be included to monitor whether the sample was exposed in transit.

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

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

8 Responsibility of the service laboratory

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) Written procedures shall include an instruction sheet (see Annex A) and pro-forma questionnaire (Annex B) for the customer, plus all steps from receipt of the sample at the service laboratory through preparation and scoring of slides for chromosome analysis.

b) This questionnaire should elicit 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).

c) Send the instruction sheet and the questionnaire to the customer.

d) Send, if required, a blood collection system (10 ml) containing lithium heparin as the anticoagulant to the customer, and if necessary also include the appropriately labelled and addressed packaging material for the return of the sample to the service laboratory. The packaging should conform to national and/or international regulations for the transit of potentially infectious pathological specimens (refer to 12.3.2).

e) Handling the blood sample after receipt:

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

   2) code the blood sample;

   3) document the place of storage until setting up cultures;

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

   5) document with batch numbers, as appropriate, all reagents used for culturing;

   6) document end of culture (date, time, operator);

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

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 plus ring 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 fluorescence plus Giemsa staining (FPG) technique. An acceptable procedure is to check a replicate slide of the same culture with FPG and, if the frequency of 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 the stained slides is required.

9.2 Criteria for scoring

9.2.1 Coding of samples and slides

All samples, slides, and intra- or inter-laboratory 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 duplication.

Whilst dicentrics (or dicentrics and centric rings) are invariably used for dose estimation, it is standard practice in service laboratories for all chromosomal abnormalities to be recorded. A standardized scoring sheet shall be used with data recorded such that the aberrations in each cell scored are derivable (Annex C). 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 must 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- and inter-laboratory comparisons.

For an observer to be considered qualified, he/she should normally achieve a dicentric yield that falls within 20 % of the test reference value.

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

10.1 Overview

The measured dicentric (or dicentric plus centric ring) 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 average whole body dose. 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 (or dicentric plus centric rings).

10.2 Comparison with controls

The service laboratory shall provide in-case reports (an example is provided in Annex D) of the laboratory's background dicentric (or dicentric plus centric ring) level. 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, a dose estimate with its uncertainties is derived and reported.

10.3 Determination of estimated dose and confidence limits

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.

There is no absolute method for deriving confidence limits; the method is always an approximation because the uncertainties on the calibration curve are distributed as a normal probability function whilst those on the measured aberration yield are usually Poissonian or overdispersed. Confidence limits on Poisson observations may be obtained by calculation or from standard tables. 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.

The most formal method is to express algebraically the derived dose in terms of the measured yield and the coefficients of the calibration curve. The uncertainty of the dose can then be related to the variances and covariance of the yield and the coefficients. Such a formula would apply only when the standard error on curves are small i.e., below 10 %. This procedure is in accordance with the ISO Guide to the expression of uncertainty in measurement (GUM), for determining combined standard uncertainties.

In practice, this procedure may be simplified by ignoring the uncertainty of the calibration curve, provided that the standard error is less than 30 % of the standard error on the measured yield. If the curve's uncertainty is 30 % or more, it should be included when deriving the uncertainty of the dose. This is achieved by combining the percentage standard errors of the yield and the curve.

The service laboratory will describe, 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.

10.4 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 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 result reports, the method used to correct for non-acute exposure dose estimates and, when appropriate, also justify its assumptions.

10.5 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 can be derived by using one or both of two techniques, the Qdr and the Contaminated Poisson methods. These techniques are detailed in the IAEA technical reports.

Exposure occurring a long time prior to analysis may be underestimated by the dicentric assay. The scoring of stable chromosome aberrations painted by FISH might be considered as an additional or alternative technique in this situation. 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. Again, IAEA reports discuss these points more deeply.

In all the cases, the service laboratory shall state, in 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

Routinely, the report should contain relevant information provided by the customer, since 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 (a standard format should be used: for a sample see Annex D).

11.1 Identification of the exposed subject

All records of the name or code of the exposed subject, date of birth, and internal code number of the service laboratory shall be stated in the report.

11.2 Description of the case

11.3 Task of the service laboratory

According to the contract between the service laboratory and the customer, the report should include as a minimum: name and address of customer, date of order, the reason for the order and the expectation of the customer.

11.4 Results of the service laboratory

The report should include: date of blood sampling, date of its arrival in the service laboratory, date of setting up cultures (if different), number of cells scored, numbers and types of aberrations found.

11.5 Interpretation of the results

This will vary 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 (or dicentric plus ring) 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, if cells are observed with multiple damage;

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

11.6 Contact person

The report should indicate the person responsible for its issue, his/her position in the service laboratory and contact information (see Annex D).

11.7 Summary

Essential key elements from the points addressed above in the report. This would normally include the best estimate of dose based on the cytogenetic findings.

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

As a minimum, the quality assurance and quality control practices cited below apply to service laboratories performing biological dosimetry by cytogenetics.

12.2 Quality assurance

The fundamental requirements for a full measurement quality assurance (MQA) programme include:

a) compliance with general operational requirements stated in accepted written criteria;

b) documented in-house quality assurance (QA) programme;

c) periodic performance evaluations, including proficiency measurement tests and on-site expert assessments;

d) documented procedures and quality assurance programme for services provided to customers.

To assure the quality of a laboratory's output over extended periods of time, its production process shall be solidly based on sound scientific principles, method validation, and product verification. The four fundamental requirements for a full MQA program as described above provide the strategy for safeguarding the quality of the laboratory's product, whether it is a measurement or a service. Furthermore, these requirements assure periodic comparison of the laboratory's measurement capabilities with those of other certified or suitably qualified cytogenetic biodosimetry laboratories, continued stability of the laboratory process, and periodic evaluation of the final product to confirm that it meets specifications.

Operating within the guidance of the documented criteria under an in-house quality assurance program, periodic peer assessments and documented quality procedures for customer services assure stable operation between proficiency evaluations.

The in-house quality assurance programme shall provide for programme assessments, adequate operational environment, personnel qualifications, procedure manual, instrumentation, calibration, data reduction, record system and data reporting. Control over the cytogenetic process between proficiency evaluations provides another assurance of end products with reproducible quality. Adoption of a total quality management approach would assure continued improvement of operations.

The objective of periodic on-site expert evaluations, i.e., assessments, is to assure the technical and scientific soundness of the laboratory's methods and processes. The experts will review and evaluate the laboratory's service procedures (including validation of all key steps, and product and/or service verification), documentation, quality assurance programme, and results of proficiency tests (pre- and post-start-up) to see that the operations are based on solid scientific grounds to meet the operational criteria. The expert evaluation assures that the process to be used by the laboratory is capable of yielding services that meet technical and quality specifications.

The proficiency tests periodically evaluate measurement consistency with certified or suitably qualified cytogenetic biodosimetry laboratories (traceability), and test the laboratory and its capabilities to verify their ability to produce high quality products and/or services. Although the term "traceability" has many facets, for this International Standard it is interpreted as the demonstrated lineage of measurement quality. As discussed in the second and third paragraphs above, one component of traceability is verified through appropriate periodic proficiency testing by a hierarchical metrology system. An essential element of the state of being traceable is successful completion of tests within specified limits of accuracy. Finally, the traceable measurement process can be used to verify the quality of a laboratory's service/product output. For the specific area of biological dosimetry by cytogenetics measurements, two measurement proficiency testing strategies are available: 1) samples are exposed *in vitro* to a known radiation dose, dose rate, and quality of radiation, and are sent to the laboratory for analysis (implicit traceability); and 2) the laboratory engages in an intercomparison study of samples sent to a certified or suitability qualified laboratory for analysis (explicit traceability). In both cases, analyses are carried out and comparisons are made between the value obtained by the laboratory and that obtained by its testing laboratory. The laboratory is then notified of the percentage difference through a report. For implicit traceability testing, only the laboratory's measurement capabilities are being tested. On the other hand, when the laboratory assays its own product and also sends an aliquant to the testing laboratory for conformational and explicit traceability measurements, both the laboratory's analytical processes and measurement capabilities are tested.

Through the combination of all these MQA strategies, the quality and integrity of the laboratory's measurements or services can be assured. Of these strategies, major emphasis should be placed on strong in-house quality assurance programs, active and thorough on-site expert evaluations, strict adherence to the documented operational criteria, and laboratory evaluation by "blind" testing. This combination of checks will assure that the analytical processes will remain in control within specified precision objectives. Although periodic end-product evaluation remains a necessary precaution, its frequency can be minimal when the analytical processes remain in control.

Quality assurance plans for service laboratories performing biological dosimetry by cytogenetics shall include the following elements: identification and preparation of samples, validation of procedures or methods, measurement, data reduction, and documentation. Systematic actions shall be included in the quality assurance plan to provide adequate confidence that a measurement or cytogenetic procedure will be performed satisfactorily.

12.2.1 Quality assurance plan

The service laboratory shall have a written quality assurance plan to ensure conformance to policies, procedures, and instructions. The plan shall include the following:

a) organization structure, management and operational responsibilities;

b) instructions and procedures, including procedure and software validation;

c) qualification and training of laboratory personnel;

d) document control;

e) procurement of materials;

f) identification and control of material and samples (chain of custody);

g) inspection and testing of material and equipment;

h) control and maintenance of calibration standards;

i) corrective action;

j) review of procedures, specifications and operating logs;

k) observation of operations and evaluation of quality control data;

l) quality assurance records;

m) documentation of MDL, relative bias, relative precision, and methods of calculating results for periodic quality control determinations.

12.2.2 Responsible quality assurance person or organization

The quality assurance plan shall designate an organization or person, typically the laboratory head, with sufficient knowledge to identify quality assurance problems, and with sufficient authority to initiate or recommend corrective actions and to provide verification of deficiency corrections.

12.3 Quality control

Performance checks shall be conducted to ensure the conformance of analytical processes, measurement equipment and procedures, and the facilities to predetermined operational requirements.

12.3.1 Quality control procedures

The laboratory shall verify that the estimation of absorbed dose measurements complies with the accuracy requirements specified in Clause 10. Procedures should include quality control performance checks on the following:

a) measurement systems and use of traceable reference standards;

b) review of procedures, specifications and operating logs;

c) observation of operations and evaluation of quality control data to ensure the long-term consistency of analytical results;

d) evaluating conformance to the performance criteria of this standard;

e) verification of MDL determinations.

12.3.2 Performance checks of sample transport integrity

In many cases, blood collection occurs at sites distant from the processing laboratory and transportation is necessary. A minimum-maximum thermometer in the shipping container will provide information on the temperature range during transport. If air transportation is used, one has to avoid the X-irradiation at the security checkpoints. A sheet of X-ray film 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 to avoid delays at the customs. All details concerning blood collection and storage should be recorded. Because of the risk of infectious diseases (hepatitis, HIV), appropriate precautions must be followed when handling the blood samples.

12.3.3 Performance checks of sample integrity by service laboratory

A system for recording the collection, transport and storage of the blood samples should be established so that sample integrity is guaranteed. The use of coded samples is critical to avoid potential bias in the scoring. 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 must be handled in the same manner. The samples of both populations have to be taken concurrently and not successively.

12.3.4 Performance checks of instrumentation

Performance of the measurement equipment shall be checked and evaluated at regular intervals while the equipment is in use. For example, the stability of the temperature control of the incubators has to be controlled. These checks shall be sufficient to demonstrate that the measurement equipment is properly calibrated and that all components are functioning properly. Replicate *in vitro* measurements should also be made periodically. Techniques such as quality control or tolerance charts shall be used for the evaluation of instrument performance. A quality control measurement shall be performed prior to use of the instrument, and the number of quality control measurements should represent at least 5 % of the total number of measurements.

12.3.5 Performance checks of sample protocol

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 in the quality handbook. 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 in the quality handbook.

Samples containing known exposures of specific radiation dose and quality of interest shall be analysed to determine bias and precision of the analytical procedures. Replicate samples should also be processed periodically. Statistical techniques, such as quality control charts, shall be used to evaluate performance data for biological dosimetry cytogenetic procedures. The number of quality control samples shall be equal to at least 5 % of the total samples analysed.

12.3.6 Performance checks of sample scoring

Before analysis, the microscopic slides or fixed cell suspensions should be stored in a manner that maintains their high quality. Uniform criteria for scoring shall be used. Scoring shall be performed by a trained and experienced observer. 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. The identity of the scorer of the slides shall be recorded. Within the set of slides of the study, a positive quality assurance standard should be included. Independently of the service activity, internal quality assurance periodically involves the comparison of the scoring results of replicate samples between scorers. External quality assurance steps involve the sharing of replicate samples with other laboratories. Furthermore, external quality assurance requires the organization of multicenter intercomparisons at regular time intervals to guarantee the uniformity of the scoring results among the laboratories.

12.3.7 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.

12.3.8 Performance checks of result-report generation

The study reports provided 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, the scoring results, the interpretation of the results in terms of dose and its uncertainty and information on how this was 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 × 5 ml tubes). Gently rock the tubes for 2 minutes 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. Also, the blood should be maintained at about 20 °C. If temperature extremes are likely to be encountered, a minimum-maximum thermometer can be included in the package. **Blood samples must 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. To further ensure that the samples do not freeze during transportation (e.g. Air-mail), please mark on the external packaging and the shipping documents **URGENT DIAGNOSTIC SAMPLES — NOT TO BE FROZEN.** For air transport, packaging and labelling should conform with the current International Air Transport Association (IATA) regulations. These require that blood samples should be packed to conform with 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** and include a piece of X-ray film in the package.

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, must be received within 24 h of sampling.

(Service Laboratory Head)
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