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Radiological protection — Performance criteria for laboratories using Fluorescence In Situ Hybridization (FISH) translocation assay for assessment of exposure to ionizing radiation

Radioprotection — Critères de performance pour les laboratoires utilisant l'analyse des translocations visualisées par hybridation in situ fluorescente (FISH) pour évaluer l'exposition aux rayonnements ionisants

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Foreword

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

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on 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 the following URL: 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*.

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 purpose of this document is to define the use of fluorescent in situ hybridization (FISH) for chromosome translocation analysis on human peripheral blood lymphocytes for biological dosimetry of exposure to ionizing radiation. Biological dosimetry, based on the study of chromosomal aberrations, mainly the dicentric assay, has become a routine component of accidental dose assessment. Dicentric aberrations, however, disappear with time after exposure, making this assay useful only in the short term after exposure. Translocations, however, are more stable, allowing dose estimates to be made long times after exposure or after protracted exposures.

This document provides a guideline for performing the translocation assay by FISH for dose assessment using documented and validated procedures. The minimum requirements for testing translocation yield in peripheral blood lymphocytes, by precisely defining the technical aspects of staining chromosomes (number of chromosomes and types of painting), selecting types of aberrations and cells, scoring aberrations, converting aberration yield to dose, statistical considerations, problems related to heterogeneous, chronic or delayed exposures and extrapolation to full genome are described. Dose assessment using the FISH assay has relevance in medical management, radiation-protection management, record keeping, and medical/legal requirements.

A part of the information in this document 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 document expands and standardizes the quality assurance and quality control and the evaluation of performance.

Radiological protection — Performance criteria for laboratories using Fluorescence In Situ Hybridization (FISH) translocation assay for assessment of exposure to ionizing radiation

1 Scope

The purpose of this document is to provide criteria for quality assurance (QA), quality control (QC) and evaluation of the performance of biological dosimetry by cytogenetic service laboratories.

This document addresses:

a) the responsibilities of both the customer and the laboratory;

b) the confidentiality of personal information, for the customer and the laboratory;

c) the laboratory safety requirements;

d) sample processing; culturing, staining and scoring, including the criteria for scoring for translocation analysis by FISH;

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

f) the scoring procedure for translocations stained by FISH used for evaluation of exposure;

g) the criteria for converting a measured aberration frequency into an estimate of absorbed dose (also appears as "dose");

h) the reporting of results;

i) the QA and QC;

j) Annexes A to F containing sample instructions for the customer, sample questionnaire, sample datasheet for recording aberrations, sample of report and fitting of the low dose-response curve by the method of maximum likelihood and calculating the uncertainty of dose estimate.

2 Normative references

There are no normative references in this document.

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
absorbed dose
D
quantity of ionizing radiation energy imparted per unit mass of a specified material

3.2
acentric
terminal or interstitial chromosome fragment of varying size lacking a centromere, referred to as an excess acentric fragment when it is formed independently of a dicentric or centric ring chromosome aberration

3.3
anticoagulant
drug which prevents blood from clotting

3.4
background frequency/level
spontaneous frequency (or number) of chromosome aberrations recorded in a general population

3.5
buffy coat
layer of an anticoagulated blood sample after centrifugation that contains most of the white blood cells

3.6
calibration curve
graphical or mathematical description of the dose effect relation derived by the in vitro irradiation of blood samples to known absorbed doses

Note 1 to entry: The curve is used to determine, by interpolation, the absorbed radiation dose to a potentially exposed individual.

3.7
centromere
specialized constricted region of a chromosome that appears during mitosis and joins together the chromatid pair

3.8
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

3.9
chromosome
structure comprised of discrete packages of DNA and proteins that carries genetic information, which condense to form characteristically shaped bodies during nuclear division

3.10
chromosome aberration
change in the normal structure of a chromosome involving both chromatids of a single chromosome at the same locus as observed in metaphase

3.11
colcemid
alkaloid compound that inhibits spindle formation during cell division

Note 1 to entry: It is used to collect a large number of metaphase cells by preventing them from progressing to anaphase.

3.12
complex aberration
aberration involving three or more breaks in two or more chromosomes and is characteristically induced after exposure to densely ionizing radiation or high doses of sparsely ionizing radiation

3.13
confidence interval
range within which the true value of a statistical quantity lies with a specified probability

3.14
covariance
measure of the correlation of the variance between two (or more) dependent sets of data or parameters

3.15
decision threshold
value of the estimator of the measurand, which when exceeded by the result of the actual measurement using a given measurement procedure of a measurand quantifying a physical effect, one decides that the physical effect is present

Note 1 to entry: The decision threshold is defined such that in cases where the measurement result, y, exceeds the decision threshold, y^*, the probability that the true value of the measurand is zero is less or equal to a chosen probability, α.

Note 2 to entry: If the result, y, is below the decision threshold, y^*, the result cannot be attributed to the physical effect; nevertheless it cannot be concluded that it is absent.

3.16
detection limit
smallest true value of the measurand which ensures a specified probability of being detectable by the measurement procedure

Note 1 to entry: With the *decision threshold* (3.15), the detection limit is the smallest true value of the measurand for which the probability of wrongly deciding that the true value of the measurand is zero is equal to a specified value, β, when, in fact, the true value of the measurand is not zero

3.17
dicentric
aberrant chromosome bearing two centromeres derived from the joining of parts from two broken chromosomes, generally accompanied by an acentric fragment

3.18
fluorescence in situ hybridization
FISH
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

3.19
fluorochrome
molecules that are fluorescent when appropriately excited

Note 1 to entry: They are used for FISH cytogenetics to highlight specific chromosomal regions.

3.20
genome equivalent
number of translocations that would be observed with all chromosomes painted, calculated from the number of translocations detected with a limited number of painted chromosomes

3.21
insertion
chromosome rearrangement in which a piece of one chromosome has been inserted within another chromosome

3.22
interphase
period of a cell cycle between the mitotic divisions

3.23
linear energy transfer
LET
radiation energy lost per unit length of path through a biological material

3.24
metaphase
stage of mitosis when the nuclear membrane is dissolved, the chromosomes condensed to their minimum lengths and aligned for division

3.25
protocol for aberration identification and nomenclature terminology
PAINT
terminology used in FISH analysis for describing chromosomal aberrations

3.26
precision
concept employed to describe dispersion of measurements with respect to a measure of location or central tendency

3.27
proficiency test
evaluation of participant performance against pre-established criteria by means of inter-laboratory comparisons

3.28
quality assurance
QA
planned and systematic actions necessary to provide adequate confidence that a process, measurement or service satisfies given requirements for quality

3.29
quality control
QC
part of quality assurance intended to verify that systems and components conform with predetermined requirements

3.30
radiation-induced translocation
among the observed translocations, the ones that can be attributed to a radiation exposure i.e. not translocations induced by other sources (e.g. age, lifestyle factors)

3.31
ring
aberrant circular chromosome resulting from the joining of two breaks within one chromosome

Note 1 to entry: Rings can be centric or acentric.

3.32
service laboratory
laboratory performing biological dosimetry measurements

3.33
stable aberration
aberration which is not lethal to the cell and can be passed on to daughter cells (e.g. simple translocation)

3.34
stable cell
cell without unstable aberrations, that may be entirely undamaged or contain stable type aberrations only, and are likely to survive division

3.35
translocation
stable chromosome aberration in which parts of two or more chromosomes are exchanged

3.36
unstable aberration
aberration which is lethal to the cell (e.g. dicentrics/centric rings/acentric fragments)

4 Translocation assay by FISH

4.1 General

The frequency of chromosomal aberrations seen at metaphase in cultured human peripheral blood lymphocytes is used for absorbed dose estimation after suspected exposure to ionizing radiation. This document focuses on retrospective evaluation of exposures which occurred in the past or protracted exposures where the dicentric assay (see ISO 19238) and the cytokinesis block micronucleus assay (see ISO 17099) are not applicable due to the decrease in this type of damage over time. The aberrations to be used are translocations and insertions in stable cells. For the application of this document, the service laboratory shall choose which type of aberrations to score for the purpose of assessing absorbed dose estimates and shall be consistent throughout.

It has been well established that the background translocation frequency in individuals varies with age due to various confounding factors (i.e. nutritional status, genotoxic exposures, lifestyle factors, malsegregation of sex chromosomes). This is an important consideration to take into account for absorbed dose estimations using translocation analysis.

Hereafter, chromosome aberrations are referred to as translocations but may include insertions if determined by the service laboratory.

4.2 Culturing and fixation

Similarly to ISO 19238, lymphocytes are cultured by a method that maximizes first-division metaphases. This requires whole blood, or lymphocytes separated from the other blood components, to be incubated in a culture medium containing a mitogen that stimulates lymphocyte cycling into mitosis. For translocation analysis, cell cycle control is recommended. A mitotic blocking agent, colcemid, is added to arrest and collect dividing lymphocytes in metaphase. The duration of the cell culture and the timing of the addition of the arresting agent are optimized to ensure an adequate mitotic index.

Metaphases are recovered from the cultures, using a hypotonic salt solution and fixing in a mixture of methanol and acetic acid. Fixed cells are dropped on microscope slides and stained. The exact protocol for cell culture, harvesting metaphases, and staining employed by a service laboratory shall be formally documented (See Clause 9).

4.3 Types of staining

Whole chromosome FISH staining shall be conducted and can involve one colour painting for all selected chromosome pairs or two or three different colours, one for each pair of chromosomes selected. Painting of three of the larger chromosomes covers about 20 % to 24 % of the total human DNA content, however there are many choices of the number of fluorochromes and which chromosomes could be selected for painting. It is recommended that chromosomes selected cover a large amount of DNA.

Some examples are described below:

a) one colour painting of three chromosome pairs [e.g. chromosome pairs 1, 4 and 8 all painted with FITC (green)];

b) three colour painting of three chromosome pairs [e.g. chromosome pair 1 painted with Texas Red (red), chromosome pair 2 painted with FITC (green) and chromosome pair 4 painted with FITC and Texas Red (yellow)].

It is possible to increase the number of painted chromosome pairs to analyse a higher proportion of the genome, but the staining of a few chromosome pairs is an efficient method in most cases.

All chromosomes should be counterstained with a fluorescent DNA dye such as DAPI (4',6-diamidino-2-phenylindole). The use of a pan-centromeric probe may also be added but is not necessary for translocation analysis, when the centromeres can be clearly identified.

4.4 Scoring

Microscope slides containing stained cells are methodically scanned to identify suitable metaphase cells. The frequency of translocations observed in an appropriate number of scored metaphase cells is converted to an estimate of radiation dose by reference to calibration data.

There are many types of chromosomal aberrations visible with whole chromosome FISH including stable and unstable, which should all be recorded during the scoring procedure. For the application of this document, the focus is on the scoring of translocations in stable cells so that this method can be applied to retrospective analysis of exposures that have occurred in the past. Therefore, it is recommended that unstable aberrations detected in counterstained chromosomes should also be recorded to determine whether the translocation occurs in a stable or unstable cell. To avoid slowing down the scoring, the analysis of all the counterstained chromosomes can be performed only in cells with stable aberrations visible in the painted chromosomes.

The service laboratory shall choose which chromosome pairs and type of aberrations to score for the purpose of assessing absorbed dose estimates and shall be consistent throughout.

Metaphases selected for scoring should be well-spread and appear to be complete.

There are several aberration classification systems currently in use (e.g. PAINT)[3]. For the application of this document, the service laboratory shall choose which type of scoring system to be used and shall be consistent throughout. It is recommended that all visible damage be recorded but only translocations in stable cells are to be counted for generating the calibration curve and providing absorbed dose estimates. The information about the frequencies of all observed aberrations allows an opportunity for further interpretation of the exposure conditions.

4.5 General requirement of the laboratory

The laboratory shall be well-equipped with the required standard laboratory equipment for lymphocyte culture, cell processing, slide preparation, and fluorescence microscopy scoring of cells. The laboratory should maintain QA documents, including those describing periodic calibration and maintenance of the equipment used for cell culture as required.

5 Responsibility of the customer

This clause includes items that are not controlled by the 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 should be by a standardised instruction sheet as illustrated in Annex A. The essential features are:

a) blood sampling shall use a collection system, containing lithium or sodium heparin as the anticoagulant, which has been sent or specified by the service laboratory;

b) blood shall be collected (ideally about 10 ml), labelled accurately and unambiguously, maintained between 6 °C and 30 °C and sent to the laboratory as soon as possible (ideally within 48 h);

c) precautions to ensure the integrity of the container and to prevent leakage during shipment shall be observed. Blood samples should be kept between 6 °C and 30 °C during shipping. A temperature recorder should be included to monitor the temperature during shipment. Packaging and labelling shall conform to national and international regulations. If air transportation is involved, a physical dosimeter shall be included to monitor whether the sample was irradiated in transit;

d) a questionnaire provided by the laboratory shall be completed accurately and returned promptly; particularly listing previous medical or occupational radiation exposures (see Annex B). Accurate biological dosimetry is limited for patients who have received a previous unknown medical exposure;

e) the laboratory shall be alerted of biologically contaminated and/or infectious samples.

6 Responsibility of the laboratory

6.1 Setup and sustainment of the QA program

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

a) the laboratory's QA program shall include periodic internal checks of equipment operations, reagent suitability, and various performance checks (i.e., intra-laboratory comparisons, operator qualifications, sample protocol, scoring, absorbed dose estimations, report generation, etc.);

b) the laboratory's QA program shall include periodic external checks of the laboratory's operations. The external audits shall include a review of the service laboratory's documentation of equipment operations, reagent suitability, and various performance checks (i.e., inter-laboratory comparisons, operator qualifications, sample transport integrity, etc.).

The laboratory shall establish the frequency of these QA checks.

6.2 Responsibility during service

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

a) the laboratory shall have documentation, reviewed and endorsed by a designated, competent person including the following:

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

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

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

b) if required, a blood collection system (10 ml) containing lithium or sodium heparin as the anticoagulant shall be sent to the customer, also including 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, consignee);

   2) blind code the blood sample;

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

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

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

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

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

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

   9) document the results from scoring;

   10) store slides and case documents in an appropriate place for a time period defined by the laboratory for possible medico-legal re-evaluation of the case;

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

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

7 Confidentiality of personal information

7.1 Overview

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

This requirement extends to

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.

7.2 Applications of the principle of confidentiality

7.2.1 Delegation of responsibilities within the laboratory

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

7.2.2 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 most cases the blood sampling for chromosome analysis shall be made with the patient's informed consent. The laboratory, depending on the national regulations, may be required to maintain the record of the patient's informed consent.

7.2.3 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 needs to define all processes for information transmission and assurance of confidentiality.

7.2.4 Anonymity of samples

The laboratory needs to have established protocols for maintaining the anonymity of samples. To avoid the identification of the patient while guaranteeing the traceability of the analysis, the blood samples should be coded upon arrival in the service laboratory. The coding is performed in an unambiguous way according to a standard procedure. The same code is to be used for all stages of the analysis. The code is assigned by an authorized person as defined in 7.2.1. Decoding, interpretation of results and compiling the report are also to be performed by an authorized person.

7.2.5 Reporting of results

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

7.2.6 Storage of data and results

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

8 Laboratory safety requirements

8.1 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.

WARNING — The use of this document can involve hazardous materials, operations and equipment. It does not purport to address all of the safety or environmental problems associated with its use. It is the responsibility of users of this document to take appropriate measures to ensure the safety and health of personnel and the environment prior to application of the document and fulfil statutory and regulatory requirements for this purpose.

8.2 Microbiological safety requirements

Handling human blood poses some risk of blood-borne pathogens being transmitted to laboratory staff. All specimens shall be regarded as being potentially infectious even if they are known to be derived from apparently healthy persons. Staff should be offered available vaccinations against appropriate blood-borne diseases.

8.3 Chemical safety requirements

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
4',6-diamidino-2-phenylindole (DAPI)	H315, H317, H335
Acetic Acid	H226, H314
Benzylpenicillin	H317, H334
Colcemid	H300
Ethanol	H225, H319
Formamide	H351, H360, H373
Hoechst stain (Bisbenzimide)	H302, H315, H319
Methanol	H225, H301, H311, H331, H370
Propidium iodide	H315, H319, H335, H341
Phytohaemagglutinin (PHA)	H302, H317, H332
Streptomycin sulphate	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
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
H335	May cause respiratory irritation
H341	Suspected of causing genetic defects

Table 2 *(continued)*

Hazard Statement	Key
H351	Suspected of causing cancer
H360	May damage fertility or the unborn child
H361	Suspected of damaging fertility or the unborn child
H370	Causes damage to organs
H373	Causes damage to organs through prolonged or repeated exposure

8.4 Optical safety requirements

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

8.5 Safety plan

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

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

9 Sample processing

9.1 Culturing and staining

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

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

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

b) cells shall be cultured at 37 °C ± 1 °C either as whole blood, as enriched lymphocyte suspension (buffy coat) or isolated lymphocytes;

c) the culture vessels shall be sterile and used in a way to avoid microbial contamination;

d) specific culture media that allows peripheral blood lymphocytes to proliferate shall be used. For example 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}) is commonly used;

e) a mitogen (e.g. PHA) shall be added to the media to stimulate lymphocytes into mitosis;

f) colcemid shall be added, at a time and concentration determined by the laboratory, to the cell culture to block cells in mitosis;

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

h) 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 3 or 4 times with the fixative until the cell suspension is clear;

i) if storage of fixed cells is required then cell suspensions shall be kept in a −20 °C freezer;

j) slides shall be prepared to allow an unambiguous identification of chromosomal aberrations. Humidity and temperature conditions can be adjusted to improve the quality of the metaphases;

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

l) slides shall be stored below 4 °C.

9.2 Scoring

9.2.1 Criteria for scoring

9.2.1.1 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.

9.2.1.2 Scoring techniques

The laboratory 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 QA trials with results documented.

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

It is standard practice in service laboratories for all chromosomal aberrations to be recorded regardless of whether the cell is stable or unstable. It is recommended that only translocations in stable cells be used for creating the calibration curve if it is to be used for retrospective biodosimetry. Alternatively laboratories can use their own established procedures suitable for the case being analysed.

Translocations are used for absorbed dose estimation and can be described as simple or complex, the latter defined as when three or more breaks in two or more chromosomes are required to produce the observed chromosome aberration(s). However, painting a subset of chromosomes does not allow the origin of all the rejoined portions to be identified; therefore simple aberrations can only be considered as "apparently" simple. All types of translocations in stable cells may be used in the absorbed dose estimate as long as the same types of translocations are consistently used. If complex aberrations are included in the calibration curve, they shall be converted into the equivalent number of simple translocations according to the defined laboratory rules.

There are several aberration classification systems currently in use. For example, in the PAINT system, a two-way translocation between a painted chromosome and a counterstained one is described as t(Ab) plus t(Ba) and scored as one translocation where A and a represent the counterstained material and B and b represent the painted material. In this scoring system the capital letter refers to the part of the chromosome containing the centromere and the small letter refers to the part of the chromosome not containing a centromere. Sometimes all exchanges are not visible, and only a t(Ba) or a t(Ab) is visible in the metaphase. These aberrations can also be stable and are named as incomplete or one-way translocations. The main reason of this type of one-way aberration is the resolution limit of the painting techniques, where the corresponding piece is too small to visualize. A dicentric formed by a painted and a counterstained chromosome is described as dic(AB) ace(ab).

A standardised scoring sheet shall be used with data recorded such that the number of aberrations in each cell scored is derivable. An example is shown in Annex D.

9.2.2 Conversion of translocation frequencies to genome equivalence

In order to be able to compare data from different sets of chromosomes, it is appropriate to apply genome equivalent corrections to the translocation frequencies. Depending on the data available at the

laboratory level this should be done to both the calibration curve data (before curve fitting) and also to the observed yield of excess (radiation induced) translocations. In this case, the Lucas formula[4] should be applied, to calculate the proportional number of aberrations that would have been observed if the full genome had been painted, according to Formula (1):

$$f_P / f_G = 2{,}05\left[\sum_i F_i\left(1-F_i\right)-\sum_{i<j} F_i F_j\right] \quad (1)$$

where

f_P is the translocation frequency measured by FISH;

f_G is the full genome equivalent translocation frequency;

F_i is the fraction of the genome painted each colour *i*, which can be taken from IAEA (2011)[5].

The calculation can be implemented in freely available software tools produced in the community[6][7].

In each case, a consistent approach between calibration and scoring of a suspected overexposure case should always be applied.

10 Background levels of translocations

For the purpose of radiation biodosimetry using translocation frequency in lymphocytes, it is assumed that an individual's base-line translocation frequency value prior to the ionizing radiation exposure event is equivalent to the mean translocation frequency value of unirradiated cells for their age. Ideally the data set of background translocation frequencies would include results for at least three age groups separated into 1 to 25, 26 to 50, 51 to 80 years and include at least 5 individuals in each group to include base-line values across different age groups.

In the absence of a laboratory having their own local age-background information, a comprehensive meta-analysis published by Sigurdson, *et al.* 2008[8] currently provides the best international database, broken down by age, gender, race and smoking habits. From this study, it appears clearly that age is the major factor that determines the background frequency of translocations. This study includes a large number of individuals for each age group. Therefore they can be used as a control value assuming:

— the mean frequency of translocation of this publication has been confirmed at least by two samples from two different age groups. If no deviation is detected between the published data and the sample tested, the published data can be used as the control by the service laboratory;

— the reference to such published data is mentioned in the report.

Whatever the approach used, it is recommended to score at least 500 genome equivalent cells per donor or 100 translocations [see Formula (1)].

As many confounding factors can create translocations which persist over time, a precise questionnaire including any known relevant genotoxic exposures, particularly prior medical radiological exposures, shall be established. The retrospective absorbed dose estimation is not possible when a potential exposed person has undergone radiotherapy in the past, and due consideration of accumulated past exposures should be undertaken for absorbed dose assessment for radiation workers.

11 Calibration curves

11.1 Calibration source(s)

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

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

b) characterization of the radiation 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[5].

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 quality of radiation of the calibration curve. Typically acute dose-rates of above 0,1 Gy/min should be chosen and the calibration source shall be traceable to national standards. The calibration curve can be fitted for simple observed or genome equivalent translocations (see 9.2.2), whether one or two way translocations, or total translocations present in stable cells. The laboratory decides which type of translocation is used assuming the calibration curve is established under the same experimental conditions and identical expertise assessment.

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 0,1 Gy to 3 Gy for low-LET radiation (e.g. photons). The selection of these two dose points is important because they define the lowest and highest dose the laboratory is allowed to report.

For high-LET radiation (e.g. neutrons), the upper dose limit is typically 1 Gy due to the high level of damage to cells at higher doses. It is recommended that 500 genome equivalent cells be scored.

For low-LET radiation, it is recommended that for absorbed doses under 1 Gy, 2 000 genome equivalent cells be scored. For absorbed doses over 1 Gy, scoring 500 genome equivalent cells is recommended. Increasing numbers of cells scored reduces the uncertainty in the absorbed dose estimates.

In order to minimise the uncertainties associated with age correction (see 12.1.4), the simplest recommended method is to fit multiple calibration curves based on individuals from multiple age groups.

The following definitions are used for further calculations:

— y: observed translocations: The absolute number of translocations observed in n_1 genome equivalent cells

— μ: observed translocation yield: The observed number of translocations per genome equivalent cell without background correction

— y': excess translocations: The absolute number of translocations in n_1 genome equivalent cells corrected for background translocations

— μ': excess translocation yield: The observed number of translocations induced by radiation per equivalent cell; the translocation yield corrected for background translocations

The observed yield of translocations, μ, (or μ', if preferred) should be fitted to the linear or linear-quadratic models [Formula (2)].

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

with $\mu = \frac{y}{n_1}$.

According to the IAEA manual, the lower and upper 95 % confidence limits of the curve can be calculated by the equation:

$$\mu = C + \alpha D + \beta D^2 \pm$$

$$\sqrt{R^2\left[var(C) + var(\alpha)D^2 + var(\beta)D^4 + 2covar(C,\alpha)D + 2covar(C,\beta)D^2 + 2covar(\alpha,\beta)D^3\right]} \tag{3}$$

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, χ^2 (degrees of freedom, 95 %), with 2 or 3 degrees of freedom. For a linear-quadratic curve (degrees of freedom = 3) χ^2 is 7,81, and for a linear curve 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 LET increases, the β coefficient tends towards zero, once it is no longer statistically significant the linear model should be used, in which case the data should be fitted to a linear model, excluding βD^2 from Formula (2).

Two methods are proposed for curve fitting, the iteratively reweighted least squares method and the maximum likelihood method (see Annex E maximum likelihood method)[9][10]. 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. Several such software packages have been developed by the international biological dosimetry community and are freely available[6][7]. Others, including commercially available software tools, can be found through open literature and the Web. 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 issues:

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 Determination of estimated whole-body absorbed dose and confidence limits

12.1.1 General

Several factors need to be taken into account when determining the absorbed dose using translocation frequency including age, time delay between exposure and analysis, occupational exposures to chemicals and other confounding factors. All of these factors can increase the uncertainty of the absorbed dose estimate and shall be considered on a case by case basis.

Where sufficient information is available, the service laboratory shall provide the estimated whole body absorbed dose and confidence limits in result 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[11]. Other characteristic limits (the decision threshold and detection limit) or confidence percentage values should also be reported, if appropriate to each particular case, as discussed below.

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

In ISO 11929 two characteristic limits are defined:

— the decision threshold (y_d) or minimum resolvable number of translocations, 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 translocations that is significantly different from the expected background number of translocations for a given significance level α (e.g. α = 0,05).

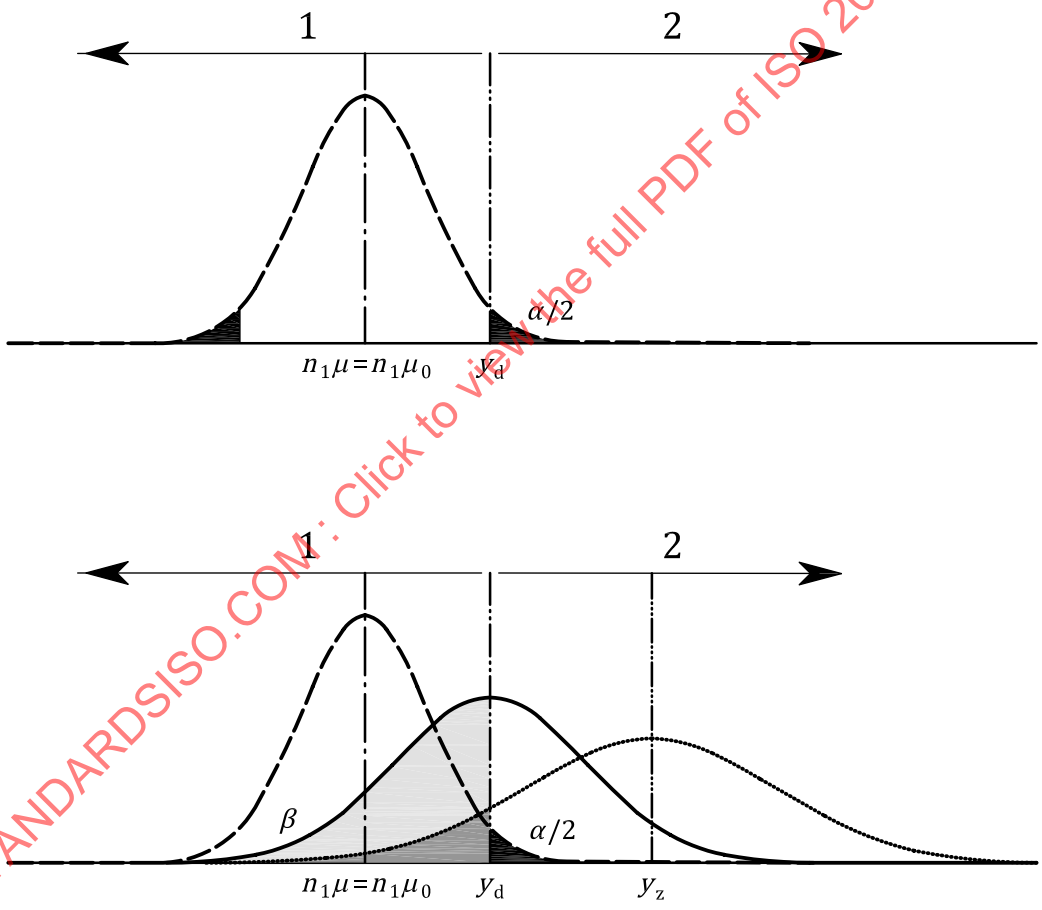
— the detection limit (y_z) is the smallest true value of the measurand (number of translocations), which can be detected. It gives information on the practical operating range of the assay and is thus used to assess whether the measurement procedure is appropriate in a particular set of circumstances.

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 translocations greater than the decision threshold, the null hypothesis (H_0) that the test person was not exposed or that the observed translocation yield is equal to the background translocation yield, can be rejected with a type 1 error rate $\frac{\alpha}{2}$, and it is concluded that the physical effect is present, therefore a dose estimate should be made. If the observed number of translocations, 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 translocations is below the decision threshold, the detection limit shall be reported. The detection limit provides valuable information about the true dose (or number of translocations) above which fewer than $100 \cdot \beta$ % 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/translocation number of the test person was higher than the detection limit, the probability of observing a translocation number 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. translocation yield μ of the test person is equal to the background translocation yield μ_0), the probability for wrongly rejecting H_0 or for observing a translocation 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 translocations, 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 translocations, 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 translocation yield of the test person μ is equal to the background translocation yield $\mu_0(j)$ for a person of j years old. The null hypothesis is given by $\mu = \mu_0(j)$ [or $n_1\mu = n_1\mu_0(j)$], respectively. Assuming that the background translocation yield is constant, i.e. has no uncertainty, the decision threshold, y_d, can be defined by the following probability:

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

where

n_1 is the number of genome equivalent cells for the test person;

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

Assuming that the number of background translocations is Poisson distributed, i.e. the background has an uncertainty, the null hypothesis can be defined by $\frac{\mu}{\mu_0(j)} = 1$ and the decision threshold can be obtained by solving the following formula for y_d.

$$P\left[y > y_d \middle| \mu = \mu_0(j)\right] = 1 - \sum_{k=0}^{y_d} \begin{bmatrix} n_0\mu_0(j) + y_d + 1 \\ k \end{bmatrix} \left(\frac{n_1}{n_1 + n_0}\right)^k \left(1 - \frac{n_1}{n_1 + n_0}\right)^{n_0\mu_0(j) + y_d + 1 - k} \leq \frac{\alpha}{2} \quad (5)$$

where

n_0 is the number of genome equivalent cells for the background data;

$\begin{bmatrix} n_0\mu_0(j) + y_d + 1 \\ k \end{bmatrix}$ is the relevant binomial coefficient in this case.

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

$$P\left[y < y_d \middle| n_1\mu = y_z\right] = \sum_{k=0}^{y_d} \frac{e^{-y_z} y_z^k}{k!} = \beta \quad (6)$$

The latter [Formula (6)] can be solved analytically using

$$y_z = \frac{\chi^2_{2(y_d+1),1-\beta}}{2} \quad (7)$$

where

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

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

$2(y_d + 1)$ are the respective degrees of freedom.

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

Indeed, for the FISH translocation assay, the decision threshold and detection limit are a function of number of factors including the laboratory's measured or chosen control background levels of translocations, the age of the suspected exposed individual and the number of cells chosen for analysis. It is thus 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, according to 12.1.5 below. 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 excess translocations does exceed the decision threshold, this means that there is evidence to refute the null hypothesis of no significant difference between the expected and observed numbers of translocations. The calculation of observed absorbed dose should then be carried out and reported according to 12.1.5. If not, then the detection limit should be reported.

If the true number of translocations 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 translocations than the decision threshold (or the probability to infer false negative conclusions) is lower than β.

12.1.3 Confidence limits on the number of translocations

There are several methods for deriving confidence limits on an observed number of translocations. Confidence limits on Poisson observations may be obtained from standard tables, by exact or approximate calculations. If a measured aberration is over-dispersed (see ISO 19238) 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 (see 11.2). Alternatively, for over dispersed data, more appropriate distributions can be applied, such as the negative binomial or Neyman type A distribution, in order to take into account both the mean yield of aberrations and the dispersion coefficient[13]. The laboratory shall provide justification for and validation of the chosen model(s).

The exact Poisson observed number can be calculated as:

$$y_L = \frac{\chi^2_{2y,\alpha/2}}{2} \quad (8)$$

$$y_U = \frac{\chi^2_{2(y+1),1-\alpha/2}}{2} \quad (9)$$

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.

The following formulae for the normal approximation to the 95 % confidence limits of the translocation yield and number of translocations for a test person can be used if the number of cells is large.

$$\mu_L = \mu - 1{,}96\sqrt{\frac{\mu}{n_1}} \text{ and } y_L = n_1\mu_L \tag{10}$$

$$\mu_U = \mu + 1{,}96\sqrt{\frac{\mu}{n_1}} \text{ and } y_U = n_1\mu_U \tag{11}$$

The confidence limits for the adjusted (excess) number of translocations of a test person at age j years can then be calculated according to Formula (13).

12.1.4 Adjustment for background yield

As described in Clause 10, the background levels of translocations increase with age due to confounding factors. For this reason, once the measurement has been carried out, the background level of translocations should be adjusted for the age of the individual and this value is required to convert the observed yield to absorbed dose (see 12.1.5).

As described above, ideally, the calibration curve data should be based on one or several individuals of a similar age. Then, it is strongly recommended, that age adjustments are performed after the curve fitting and after uncertainty estimation for the unadjusted number of translocations of the test person. If uncertainties are directly estimated on the adjusted (excess) number of translocations, y', the uncertainties are underestimated. If the data for the calibration curve and the test person are based on different age groups, the data shall be adjusted to ensure that the dose estimates are being made based on a curve that is appropriate to the age of the test person. This can be accomplished by making age adjustments for both the calibration curve and the test person or by making age adjustments only to the curve to match the age of the test person (as in example in Annex F). The laboratory should decide on the most appropriate analysis method: ideally, age should be included into the calibration model as an additional covariate.

Further, ideally, the uncertainties of the age specific background should be included into the process of uncertainty estimation. However, assuming that a large enough amount of information is available on age-specific background rates, so that the uncertainty on the age specific background yield tends to zero, the background yield at the age corresponding to the data used to obtain the calibration curve (= k years) and at the age (= j years) of the test person can be approximated by constants, i.e. $\mu_0(k) = const$ and $\mu_0(j) = const$.

In the ideal situation, the adjustment for age of the test individual should be based on the laboratories' own local age-background information, otherwise on the information from Sigurdson, et. al., 2008[8].

Either data from Table 3 of Sigurdson, et. al., 2008[8] or the given fitted relationship between age, j, and background yield of translocations can be used:

$$\mu_0(j) = e^{-7,925} + e^{-9,284}\left(j \cdot e^{j \cdot 0,010\,62}\right) \tag{12}$$

where $\mu_0(j)$ is the age specific background translocation yield per cell equivalents (including both one-way and two-way translocations) and age, j, is in years.

For converting the number of translocations of the test person to dose, the age adjusted (excess) translocation number, y', is then calculated according to:

$$y' = n_1\left[\mu - \mu_0(j)\right] \tag{13}$$

with the lower and upper confidence limits of the adjusted (excess) number of translocations:

$$y'_{\mathrm{L}} = y_{\mathrm{L}} - n_1 \cdot \mu_0(j) \text{ and } y'_{\mathrm{U}} = y_{\mathrm{U}} - n_1 \cdot \mu_0(j) \tag{14}$$

where

$y_{\mathrm{L}}, y_{\mathrm{U}}$ are the lower and upper confidence limits of the unadjusted number of translocations in n_1 genome equivalent cells;

$y'_{\mathrm{L}}, y'_{\mathrm{U}}$ are the lower and upper confidence limits of the adjusted (excess) number of translocations in n_1 genome equivalent cells.

Based on the fit of the unadjusted calibration curve from Formula (2), we obtain the age adjusted calibration curve:

$$\mu' = \mu - \mu_0(k) = C + \alpha D + \beta D^2 - \mu_0(k) \tag{15}$$

where the lower and the upper confidence limits can be approximated by

$$\mu' = C + \alpha D + \beta D^2 - \mu_0(k) \pm \sqrt{R^2\left[var(\mathrm{C}) + var(\alpha)D^2 + var(\beta)D^4 + 2covar(C,\alpha)\mathrm{D} + 2\mathrm{covar}(C,\beta)D^2 + 2covar(\alpha,\beta)D^3\right]} \tag{16}$$

12.1.5 Calculation of absorbed dose

If the observed excess number of translocations 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 (2). It shall be ensured that the data used for the estimation of the calibration curve is representative for the test person and that confounding factors (e.g. factors influencing the background translocation rate) have been accounted for appropriately as described in 12.1.4. The absorbed dose, D, is calculated by solving the linear or quadratic equations which is possible using the freely or commercially available software tools[6][7]. The information on how the calculations are performed within the software should be documented and updates performed periodically.

It is important to reiterate that the calibration curve and the number of translocations of the test person shall be adjusted for the corresponding age (and any other appropriate factor) related background levels as described in Formulae (13) and (15), respectively, according to the documented standard procedures of the laboratory.

A point estimate for the absorbed dose based on age adjusted data can be obtained by solving the equation:

$$D = \frac{-\alpha + \sqrt{\alpha^2 + 4\beta\left\{\left[\mu - \mu_0(j)\right] - \left[C - \mu_0(k)\right]\right\}}}{2\beta} \tag{17}$$

where

C, α, β are the coefficients of the unadjusted calibration curve;

μ is the observed unadjusted translocation yield of the test person;

$\mu_0(j)$ is the background translocation yield at the age of the test person (= j years);

$\mu_0(k)$ is the background translocation yield at the age corresponding to the data used to obtain the calibration curve (= k years).

If $C - \mu_0(k) < 0$, zero or a small positive value shall be used instead [e.g. $C - \mu_0(k) = 0{,}001$].

In normal circumstances, calibration curves should be fitted according to the standard scoring practice of the laboratory, i.e. using total numbers of aberrations amongst the painted chromosomes.

12.1.6 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). 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, then the combined uncertainty should be calculated (see ISO 5725-1).

In practice, the recommended methodology to calculate uncertainty on absorbed dose in the context of the FISH translocation assay is to combine the confidence limits on the translocation frequency with the uncertainties on the calibration curve coefficients [see Formula (2)] which in many cases represent the dominant sources of uncertainty. This can be achieved by using Merkle's method[5] based on Formulae (13) to (16) or using the current version of Dose Estimate which is based on Formula (17). However, the uncertainty associated with an assessment varies widely depending on a large number of factors, including the necessary age adjustment. As such, it is recommended that the uncertainty is assessed according to the above procedure on a case by case basis. The laboratory 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 employed (See Annex F for example).

12.1.7 Acute and non-acute exposure cases

If an overexposure is known to have been received acutely i.e., below 0,5 h, the absorbed dose estimate may be obtained by reference to an acute in vitro calibration curve where one is scoring translocations in stable cells. For a linear-quadratic dose-response relationship, the absorbed dose is estimated by solving the quadratic Equation utilising the calibration curve coefficients and observed yield. If an overexposure is known to have been protracted, the absorbed dose estimate may be obtained by reference to just the background level and linear coefficients of the acute calibration curve. As with curve fitting, most of the freely available bespoke software packages developed by the international

biological dosimetry community include tools for dose estimation[6][7] and further information and examples are given in IAEA 2011[5].

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.

12.1.8 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 translocations 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 translocation assay has been applied are given in IAEA 2011[5].

13 Reporting of results

13.1 General

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.

13.2 Content of the report (see Annex C for an example of a standard form)

The report should include information on the following:

a) title of the report, e.g. “test report”;

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

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

d) name and address of the customer;

e) date of request;

f) 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;

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

h) description of the case: all relevant information provided by the requester that is relevant to the interpretation of the result shall be stated (may also be dealt with in the interpretation of the results);

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

j) test results: number of cells scored, numbers and types of aberrations found;

k) interpretation of test results: see 13.3;

l) name(s), title(s), position(s) and signature(s) of those authorizing the report and their contact information.

13.3 Interpretation of the results

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

a) a comparison of the translocation frequency to the background level, specifying if using the background translocation frequency from the laboratory results or the Sigurdson, et. al. 2008 meta-analysis[8], together with the appropriate characteristic limits — confidence limits and decision threshold or detection limit, as described in 12.1.1;

b) an absorbed dose estimate based on the frequency of translocations expressed in SI units of absorbed dose (Gy) along with a quantification of the uncertainties on the absorbed dose estimate. This would normally be an upper, and where appropriate, a lower confidence limit, and the percent level of confidence;

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

d) the coefficients of the calibration curve used for converting the absorbed dose from the aberration yield;

e) a statement on whether the absorbed 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, and if appropriate a dosimetric interpretation, on cells observed with multiple damage;

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

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

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

14 Quality assurance and quality control

14.1 Overview

Quality assurance (QA) and quality control (QC) shall be established with elements as described in Clause 14.

14.2 Specific requirements

14.2.1 General

Performance checks with other accredited or suitably qualified cytogenetic biodosimetry laboratories and networks shall be established through periodic inter-laboratory comparisons.

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

14.2.2 Performance checks by inter-laboratory comparisons

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

The inclusion of individual laboratories or results in an inter-laboratory comparison that appear to be inconsistent with all other laboratories may change the average absorbed dose estimates. To discard or correct inconsistent data, two approaches can be used (ISO 5725-2, ISO 5725-5):

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

b) robust methods for data analysis: To yield robust values of the average and standard deviation of the data (see ISO 13528);

The robust methods procedure is as follows:

a) the outlier test for laboratory inter-laboratory comparison performance requires a minimum of five laboratories for statistical robustness (see ISO 5725-1);

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

c) determination of the laboratory's performance: Calculation of the z-score parameter from the laboratory results, the reference value and the estimated standard deviation. Determination of the u-score parameter (this evaluation includes both participant measurements and reference value uncertainties)[5].

14.2.3 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 observers is established at least every second year by intra-laboratory comparison.

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

A scorer is regarded as qualified if both of their measured absorbed doses are within the 95 % confidence limits of the absorbed dose from the laboratory's calibration curve. For example, if a scorer finds that his/her measured absorbed dose in a test sample is 0,40 Gy for a reference sample irradiated with 0,50 Gy; this agrees with the laboratory's calibration curve which, for an absorbed dose of 0,50 Gy, has confidence limits of 0,34 Gy to 0,63 Gy. Alternatively, the formal statistical methodologies described in 14.2.2 can also be applied for intra-laboratory comparisons.

Automatic scoring systems may be used if appropriate QA and QC procedures have been defined.

14.2.4 Performance checks of sample transport integrity

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

It is recommended that blood samples be shipped using an experienced express service for diagnostic material and the samples declared as UN 3373 Biological Substance Category B. Packaging and labelling shall conform to national and international regulations. A temperature logger and a physical dosimeter to monitor the temperature and any absorbed dose received by the samples during transport are advisable. A standardized sample instruction sheet, (see Annex A), can be used to inform customers of the correct procedure.

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 coded samples is critical to avoid potential bias in the scoring.

14.2.6 Performance checks of instrumentation

The laboratory's QA and QC program should include periodic internal checks of equipment operations.

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

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

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

14.2.7 Performance checks of sample protocol

As internal QA, negative controls from unexposed individuals and where possible internal positive controls shall be included in the study to prove the reliability of the procedure. Blood from both samples and internal controls shall be handled in the same manner. The samples of both populations shall be taken as concurrently as practically possible.

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

14.2.8 Performance checks of sample scoring

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

14.2.9 Performance checks of result report generation

The reports to customers shall be examined to ensure that they contain the necessary information as defined in this document (see Clause 13) namely: subject and customer identifiers, exposure information, exposure and sampling dates, the scoring results, the interpretation of the results in terms of absorbed 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 min to ensure proper mixing. Label the tubes unambiguously and complete the questionnaire.

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

X Mark on the external packaging and the shipping documents **Biological Substances — Category B — DO NOT FREEZE**.

X When air transportation is used, the X-irradiation at the security checkpoints should be avoided. A physical dosimeter may be included in the shipping package to verify this. For international transport, the appropriate permits shall be obtained in advance and included in the shipment to avoid delays at the customs. For air transport packaging and labelling should conform to the current International Air Transport Association (IATA) regulations. These require that blood samples should be packed to conform to UN 3373 infectious materials. The package itself and the 'Nature and Quantity of Goods' box of the air waybill should show the following wording: "Biological Substances — Category B packed in compliance with IATA packing instruction 650".

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

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

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

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

(Service Laboratory Head)
(Service Laboratory address)
Phone: (XXX) XXX-XXX
Fax: (XXX) XXX-XXX
E-mail

Annex B
(informative)

Sample questionnaire

EXPOSURE INFORMATION FOR CHROMOSOME ABERRATION ANALYSIS

(TO BE FILLED OUT BY THE REQUESTOR)

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

.....

Signature

.....

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

Laboratory address: .....

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

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

Exposure Data: Radiation Worker or Non-Radiation Worker

Occupation: .....

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

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

1. Brief description of:

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? .....

Patient Data:

1. Previous exposure through medical practice:

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

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

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

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

3. Intake of medication: O

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

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

5. Other diseases:

   HIV O Hepatitis O

.....

Results of chromosomal analyses to be sent to:

Name: .....

Address: .....

.....

Telephone #: .....

Annex C
(informative)

Sample of report

Address of lab performing analysis

ID No of report

Requestor name, address, phone, e-mail

Date of request

BIOLOGICAL DOSE ASSESSMENT/CHROMOSOME ANALYSIS (FISH analysis)

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

Method(s) of analysis

Additional sampling

Description of case

Results

Table. Test results

Sample	Number of cells analysed	Translocations	Others

Interpretation of results

Signatures and contact information

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

Annex D
(informative)

Sample data sheets for recording painted aberrations

Three colour painting:

Slide Code: Scorer:

Microscope N° Date:

Cell Number	Coordinates	Description of all observed aberrant chromosome pieces	Aberrations affecting						Stable cell	C
			Painted Chromosomes (Rr,Gg,Yy): 1, 2, 4				Unpainted chromosomes (Aa)[a]			
			T_2	T_1	Dic/R	ace	Dic/R	ace		
1		t(Ra) t(Ar) t(Ga) dic(YA) ace(ay) dic(AA) ace(a)	1	1	1	1	1	1	No	0
2		t(Ga) t(Ag) t(Ya)	1	1	0	0	0	0	Yes	0
3		t(Ra) t(Ar) t(Ga) ace(ag) r(R) ace(r) ace(a)	1	1	1	2	0	1	No	1(3)
4		t(Yay)	0	0	0	0	0	0	Yes	1(3)
5		t(Ag) ace(g) ace(a)	0	1	0	1	0	1	No	0

T_2 = two-way simple translocation

T_1 =one-way simple translocation

Dic = dicentric

R = ring

C = complex aberration with the minimal number of breaks to produce the observed complex aberrations in parentheses

[a] Refers to completely unpainted chromosomes.