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# DRDC Ottawa Working Standard for Biological Dosimetry

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**Defence R&D Canada – Ottawa**
TECHNICAL REPORT
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Canada

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# Abstract

This Standard provides quality assurance, quality control, and evaluation of the performance criteria for the purpose of accreditation of the Radiation Biology laboratory at Defence Research & Development Canada - Ottawa (DRDC Ottawa) using biological dosimetry to predict radiation exposure doses. The International Standard (ISO 19238) and the International Atomic Energy Association (IAEA) Technical Report Series No. 405 are used as guiding documents in preparation of this working document specific to the DRDC Ottawa Radiation Biology Laboratory.

This Standard addresses:

1. The confidentiality of personal information, for the customer and the service laboratory;
2. The laboratory safety requirements;
3. 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;
4. Transportation criteria for shipping of test samples to the laboratory;
5. Preparation of samples for analysis;
6. The scoring procedure for unstable chromosome aberrations used for biological dosimetry;
7. The criteria for converting a measured aberration frequency into an estimate of absorbed dose;
8. The reporting of results;
9. The quality assurance and quality control plan for the laboratory; and
10. Informative annexes containing examples of a questionnaire, instructions for customers, a data sheet for recording aberrations, a sample report and other supportive documents.

# Résumé

Ces normes fournissent les critères d'assurance de la qualité, du contrôle, ainsi que l'évaluation de rendement nécessaires à l'accréditation du laboratoire de radiobiologie de RDDC Ottawa utilisant la dosimétrie biologique pour prédire les doses d'irradiation. La norme internationale (ISO 19238) et le rapport technique no. 405 de l'Agence internationale de l'énergie atomique ont servi de guide dans la préparation de ce document spécifique au groupe de radiobiologie de RDDC Ottawa.

Ces normes adressent :

1. la confidentialité des renseignements personnels, pour le client et le laboratoire;

2. les exigences pour les règles de sécurité du laboratoire;

3. les sources d'étalonnage et les gammes de doses d'étalonnage utilisées pour établir les courbes de référence permettant d'estimer les doses à partir de la fréquence des aberrations chromosomiques, et les niveaux minimums de détection;

4. les critères de transport pour l'expédition des échantillons au laboratoire;

5. la préparation des échantillons pour l'analyse;

6. la méthode de notation des aberrations chromosomiques instables utilisée pour la dosimétrie biologique;

7. les critères pour convertir la fréquence mesurée d'aberrations en dose absorbée;

8. le rapport des résultats;

9. le plan d'assurance de la qualité et du contrôle de la qualité du laboratoire; et

10. les annexes pertinentes qui offrent des exemples de questionnaire, d'instructions pour les clients, les feuilles de contrôle pour inscrire les aberrations, un rapport-échantillon et autres documents auxiliaires.

# Executive summary


Introduction

Over the past 60 years scientists have been working towards establishing biological methods that would be able to accurately predict the radiation doses received by irradiated individuals. Up until 2004, many of the available assays for biodosimetry have been used, but only as research tools. In 2004, the International Organization for Standardization (ISO) accepted the dicentric assay as the International Standard and published guidelines for service laboratories performing biological dosimetry by cytogenetics. This new standard now allows laboratories to be certified in accordance with ISO standards and provide biological dosimetry as a medical tool that takes into consideration medico-legal concerns. In response to these guidelines and in preparation for laboratory accreditation, the DRDC Ottawa Radiation Biology team developed a Working Standard for Biological Dosimetry. This standard will be used to acquire and maintain accreditation of the DRDC Ottawa Biological Dosimetry laboratory.

Significance

As a collaborating partner in the National Biological Dosimetry Response Plan (NBDRP) the DRDC Ottawa Radiation Biology team is building capacity to be able to effectively respond to a radiological/nuclear emergency affecting the Canadian Forces or the Canadian public. In preparation for responding to a mass casualties disaster involving radiological/nuclear agents we have prepared this laboratory-specific standard as required by the ISO. The protocols described in this document and their implementations are necessary for acquiring accreditation for biological dosimetry for individuals that are suspected of having received a radiation dose.



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# Sommaire

Introduction

Durant les dernières soixante années, les scientifiques ont tenté d'établir des méthodes biologiques qui permettraient de prédire avec précision les doses d'irradiation reçues par des individus. Jusqu'en 2004, plusieurs méthodes de dosimétrie biologique ont été utilisées mais uniquement comme outil de recherche. En 2004, l'Organisation internationale de normalisation (ISO) a accepté l'analyse dicentrique comme norme internationale et publié des directives pour les laboratoires de service pratiquant la dosimétrie biologique par cytogénétique. Cette nouvelle norme permet la certification des laboratoires en accord avec les normes ISO et permet l'utilisation de la dosimétrie biologique comme outil médical tout en tenant compte des préoccupations médico-légales. En fonction de ces directives et en préparation de l'accréditation de notre laboratoire, le groupe de radiobiologie de RDDC Ottawa a développé un étalon de travail pour la dosimétrie biologique. Cet étalon sera utilisé pour obtenir et maintenir la certification du laboratoire de dosimétrie biologique de RDDC Ottawa.

Importance

En tant que partenaire du plan national de dosimétrie biologique, le groupe de radiobiologie de RDDC Ottawa veut bâtir sa capacité à répondre à une urgence radiologique/nucléaire visant les Forces armées canadiennes ou la population canadienne. Afin de se préparer à répondre à un sinistre impliquant des agents radiologiques/nucléaires et causant des pertes massives, nous avons préparé cet étalon spécifique à notre laboratoire tel qu'exigé par l'ISO. Les protocoles décrits dans ce document et leur mise en application sont nécessaires pour l'obtention de l'accréditation en dosimétrie biologique pour des individus soupçonnés d'avoir reçu une dose d'irradation.

Segura, TM; Prud'homme-Lalonde, L; Thorleifson, E; Lachapelle, S; Mullins, D; Qutob, S; Wilkinson, D. 2005. DRDC Ottawa Working Standard for Biological Dosimetry. DRDC Ottawa TR 2005-106. R & D pour la défense Canada - Ottawa..

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# Acknowledgements

First of all we wish to thank CRTI for support funding for a Research and Development project (CRTI 0027RD) that facilitated this work on biological dosimetry. We would also like to thank DRDC Toronto Human Research Ethics Committee for providing us with Human Research Ethics Document Approvals so that we could conduct the necessary research to produce the required dose response curves. We are also very grateful to our volunteers who willingly gave samples for the purpose of generating *in vitro* dose response curves. And finally, and with greatest appreciation, we wish to thank Ms. Susan Miller of Health Canada's Consumer and Clinical Radiation Protection Bureau for in-depth discussions and input, Dr. David Wilkins, Ottawa Regional Cancer Centre, for providing radiation exposures and necessary physics expertise and Ms. Leonora Marro of the Environmental Health Science Bureau's Biostatistics and Epidemiology Division for her guidance and advice on statistics.

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# 1. Dicentric Assay

The dicentric assay is the internationally recommended method for biological dosimetry (ISO 19238 and IAEA Technical Report Series No. 405). This assay relies on the frequency of dicentric and ring chromosome aberrations found in metaphases from cultured human peripheral blood lymphocyte samples. These blood samples are cultured either as whole blood or isolated lymphocytes and culture conditions are controlled to ensure an adequate mitotic index and a predominance of first division metaphases. Metaphase spreads are prepared for analysis by standard methods outlined in this manual (Section 6).

Stained microscope slides are methodically scanned to identify dicentric and ring aberrations (Section 7.2). For the remainder of this document, "dicentric" will be used to refer to dicentrics or rings. 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 section 8). These data follow a Poisson distribution. Detailed protocols described in this document enable the DRDC Ottawa Laboratory to predict doses of potentially exposed individuals.

# 2. Confidentiality of Personal Information

## 2.1 Overview

Biological dosimetry investigations made by a service laboratory must be undertaken in accordance with Canadian regulations regarding confidentiality (DRDC Human Research Ethics Committee Document No. L444). This would normally include the maintenance of confidentiality of the patient's identity, medical data and social status. In addition, the commercial confidentiality of the patient's employer and any other organizations involved in a radiological accident /incident should be observed.

This requirement extends to:

1. Written, electronic or verbal communications between the laboratory and the person/organization requesting the analysis and receiving the report, and

2. The secure protection of confidential information held within the organization where the service laboratory is located.

## 2.2 Applications of the Principle of Confidentiality

### 2.2.1 Marking documents

All documents with identifiers such as names, are to be designated protected and must be marked “Protected” and “Medical-Confidential” in the upper and lower right corner of each page of the document.

### 2.2.2 Delegation of Responsibilities within the Laboratory

The head of the laboratory may authorize a limited number of laboratory staff to deal with documents related to the analysis. Annex A contains the Organizational Chart for the Radiation Biology Laboratory listing all authorized personnel. Persons with this authority shall have signed a commitment to confidentiality (Annex B) regarding their duties within the laboratory.

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

These documents are to be handled on a “need-to-know” basis, all employees must have permission from the laboratory head to view any protected documents. The laboratory head shall ensure the security and safety of all protected documents.

### 2.2.3 Requests for Analysis

The request for analysis should be made by a doctor representing the patient, the patient himself or herself, or by the patient's employer. In all cases the blood sampling for chromosome analysis must be made with the patient's informed consent (See Annex C). The laboratory head shall maintain the record of the patient's informed consent.

### 2.2.4 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. Reports must be sent by courier, not email, unless there is an appropriate level of encryption.

### 2.2.5 Anonymity of Samples

In order to maintain anonymity of samples, all samples must be coded by authorized personnel upon arrival in the laboratory as outlined in section 6.3.

Code numbers shall be assigned to samples as they are received. Identifying information shall be recorded on a prepared sheet. The sample will be referred to by this code for the entire analysis and not decoded until the dose estimation is complete and the report is being written. Only designated individuals will have access to the decoding information.

All questionnaires and sample codes shall be placed in the "Records" binder (in a locked filing cabinet) under the responsibility of the laboratory head or designate (see section 2.2.2).

### 2.2.6 Reporting of Results

All reports will include as much relevant information as possible because they may be used as medico-legal documents. All reports will routinely contain relevant sample information provided by the customer since this may influence the interpretation of the findings in the service laboratory. All observed aberrations would be listed and interpreted based on the current understanding of mechanisms for radiation-induced chromosome aberration formation.

The distributions of these reports will be tightly controlled with only the requestor receiving the official report. Depending on appropriate approvals copies may be passed to other responsible persons.

As described in section 2.2.4, secure courier service will be used to notify the requestor of the final laboratory results. A copy of the report will also be held in a secure file cabinet by the laboratory.

### 2.2.7 Storage

All laboratory documents relating to a case and which could permit the patient and/or employer to be identified will be stored in the "Records" binder (in a locked filing cabinet) under the responsibility of the laboratory head or designate. Documents will be retained for at least 30 years for possible medico-legal re-evaluation of the case. Final disposal of documents will be by secure means such as shredding.

# 3. Laboratory Safety Requirements

## 3.1 Overview

Staff shall conform to Canadian legislation and DRDC regulations regarding safety in the laboratories. There are some particular considerations for safety that are described here.

## 3.2 Microbiological Safety Requirements

Handling human blood poses some risk of blood borne parasites and infections being transmitted to laboratory staff. All samples should be regarded as being potentially infectious even if they are derived from apparently healthy persons.

1. Samples must be unpacked and manipulated in a class II Biological Safety Cabinet. Setting up cultures in such a cabinet has the added benefit of minimizing culture failure due to microbial contamination.

2. Use of sharps, e.g. hypodermic needles, should be kept to a minimum to reduce the risk of injuries.

3. Suitable disinfectants (e.g. sodium hypochlorite 6% 1/10 dilution) must be available to deal with spills.

4. All biological waste and used disposable plastic-ware must be sterilized, for example by autoclaving or incineration, before final disposal.

5. Staff should be offered available vaccinations against blood-borne diseases.

6. It should be noted that when blood samples are accepted from abroad, depending on the country of origin, the airlines may require the sender to provide a certificate confirming that the samples have been tested and are HIV negative.

## 3.3 Chemical Safety Requirements

Certain chemicals and pharmaceuticals are used routinely in the procedures covered in this 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 sulphate | 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 sensitization by inhalation;
R43 May cause sensitization 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.

## 3.4 Optical Safety Requirements

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

## 3.5 Safety Plan

1. All staff shall follow the Departmental General Safety Standards referenced in the "Safety Handbook for DND Managers and Supervisors in the NCR."

2. All staff shall follow the "Laboratory Biosafety Guidelines", Health Canada 1996.

3. All staff shall have active WHMIS certification.

4. Staff members responsible for shipping and receiving shall maintain active Transportation of Dangerous Goods (TDG) certification.

5. All potential hazards and accidents are to be recorded in "Hazard Report Log" and "Personnel Operating Error Log" respectively, located near the First Aid Kit in room 17.

6. The Laboratory First Aid Kit shall be routinely checked for contents and expiration dates.

7. The in-house Health and Safety Committee shall routinely inspect the laboratory.

# 4. Calibration Source(s), Calibration Curve(s) and Minimum Detection Levels

## 4.1 Calibration Source(s)

A report will be placed in the Annex section D for each source used in a Calibration Curve. These reports will be provided or endorsed by a qualified expert (i.e., radiation physicist or the service laboratory head) and will address the following issues:

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

2. Description of the dosimetry protocol, the procedure to certify that the dosimetry method is calibrated to a standard, and the method used to measure dose uniformity in the experimental array;

3. Written procedures and documentation to verify dose and dose-rate determinations for individual experiments;

4. Provision of a summary dosimetry report for each calibration-source dose-response curve.

Currently, we have a completed calibration report of the source used for our X-ray dose response curve. This report can be found in Annex D-1. As new calibration curves are created, new calibration reports will be mentioned here and added to Annex D.

## 4.2 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.

A dose response graph will be placed in Annex E for each Calibration Curve created. All supporting reports will be provided and endorsed by a qualified expert (i.e., radiobiologist or equivalent) and will address the following issues:

1. Description of the experimental exposure set-up (sample holder, temperature control, etc.) and procedures to verify reproducibility of exposure set-up for individual experiments (Annex D);
2. Detailing the *in vitro* calibration data and their fitting to a calibration curve. Our laboratory uses the statistical method for calibrating our dose response curves found in Annex F.

3. Referencing the Calibration Source Report that was created for the Calibration Curve (Annex D).

Currently, we have a completed calibration report of the source used for our X-ray dose response curve. This report can be found in Annex E and contains the experimental details and calibration data for the calibration curve. As new calibration curves are created, new calibration reports will be mentioned here and added to Annex D, E, and F that will contain the experimental details and calibration data.

### 4.2.1 Number of Cells Scored for Dose Response Curves

At higher doses scoring should be aimed at about 100 dicentrics at each dose. This may not be possible at lower doses, thus it is recommended that a minimum of 1000, but ideally 2000-3000 cells be scored if the number of dicentrics is less than 100.

## 4.3 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.

The service laboratory shall provide a report, reviewed and endorsed by a qualified expert (i.e., service laboratory radiobiologist or equivalent) that describes the laboratory's chromosome aberrations levels for the reference controls and measured radiation-induced levels for its proposed minimum detection level and the number of cells scored per sample.

### 4.3.1 Number of Cells Scored for Analysis

For a low or zero dicentric yield, the improved confidence limits resulting from 500 scored cells are worthwhile and are usually sufficient. The decision to extend scoring beyond 500 cells depends on whether there is evidence of a serious over-exposure, or if the continued employment of a radiation worker is in jeopardy in which case there may be a scientific justification to score up to 1000 or more cells.

# 5. Responsibility of the Customer

Prior to blood sampling, coordination between the customer and the service laboratory will occur. The following essential requirements will be explained to the customer and included on a standardized instruction sheet (Annex G):

1. Before the blood sample is taken the customer shall notify our laboratory in order for us to prepare for the sample arrival and pick up. At the same time, the customer will also be issued (faxed or emailed) consent forms and a questionnaire (Annex C and Annex H) to be completed and returned with the specimen sample.

2. Preferably, all blood samples are to be collected into lithium heparin tubes (sodium heparin is acceptable in an emergency), and are to contain at least 3 mL (but ideally 2 x 5 mL tubes). The tubes shall be gently rocked for 2 minutes to ensure proper mixing. The tubes shall be labelled unambiguously.

3. A questionnaire is to be completed for each patient and returned to the service laboratory with the corresponding blood sample.

4. The customer shall package and ship the blood samples to the laboratory as per the protocol described in Annex G. It is intended that the guidelines listed in Annex G comply with the current regulations of the transportation of dangerous goods act. This document will be updated as necessary.

5. Immediately after blood collection, the customer shall ship the sample and the completed questionnaire by special transportation and use overnight air express in order for our laboratory to receive the blood as early as possible the morning following sample collection. It is recommended that samples be shipped using FedEx or Purolator since these couriers do not X-ray their packages. Since regulations may change, this shipping requirement should be confirmed on regular basis.

6. The customer shall then contact the laboratory to confirm the shipment and inform us of the Way Bill number. This is important for tracking the sample.

7. For best results blood must be received within 24 hours of sampling.

8. The service laboratory should be alerted of biologically contaminated samples.

# 6. Responsibility of the Service Laboratory

When a sample is expected for dicentric analysis, the following steps should be followed to ensure efficient processing of the sample. Details of each step will follow throughout this section:

1. Upon request, send the instructions for sample collection (Annex G), informed consent forms (Annex C) and the questionnaire (Annex H) to the customer. Ensure that the customer is aware of the importance of completing all forms as accurately as possible.

2. If required, send a blood collection kit (2 x 5 mL) containing lithium heparin as the anticoagulant to the customer, and if necessary also send appropriately labelled and addressed packaging material for the return of the sample to the service laboratory.

3. Specific protocols for sample preparation and analysis are detailed in this section:

    1. Preparation for blood sample arrival at the laboratory;

    2. Arrival of blood at the laboratory;

    3. Coding of samples;

    4. Culture setup for dose assessment;

    5. Slide preparation protocol;

    6. Staining of slides;

    7. Slide coding; and

    8. Storage of slides.

## 6.1 Preparation for Blood Sample Arrival at the Laboratory

Before the arrival of the blood sample at the service laboratory there are anticipatory steps that must be undertaken by the laboratory:

1. Ensure that all supplies necessary for processing the anticipated samples (see section 6.4.1.1 or 6.4.2.1) are readily available in the laboratory.

2. Prepare appropriate amount of complete culture media (see section 6.4.1.3 or 6.4.2.3) and test for sterility prior to adding Pen-strep.

3. Contact a volunteer for “methods control” and arrange for sample acquisition. It is advisable that a control sample from a healthy, unexposed volunteer be set up as a methods control at the same time with the test samples.

4. Label and prepare culture flasks for all diagnostic and quality control samples.

5. If the number of diagnostic samples is small (< 10) positive control samples (1 Gy irradiated) may be prepared. To do this, the diagnostic samples will be split in four parts. Two of these will be irradiated with 1 Gy and the other two aliquots will remain as the diagnostic samples. The double cultures, beside providing backups, will also provide an opportunity to sample at two different time points, thus ensuring the capture of optimal conditions for first division metaphases.

6. If the number of diagnostic samples is large (≥ 10) positive control samples will not be prepared for every sample. The sample will only be split in two parts for replicate cultures.

7. In addition to the split diagnostic samples (small or large) also prepare for two unirradiated and two 1 Gy irradiated cultures from one healthy "methods control" volunteer. This volunteer must also provide completed consent forms and questionnaire (Annex C and H). The irradiated samples may provide information on individual radiation responsiveness.

8. The final decision on the number of control and replicate samples set up would rest with the head of the laboratory. The protocols for the dicentric assay in sections 6.4.1 and 6.4.2 reflect the processing of 2 ml blood samples in 20 mL cultures (final volume). If it is decided that the samples will be split for replicates or positive controls the protocols should be adapted to reflect 1 mL blood samples in 10 mL cultures (final volume).

## 6.2 Arrival of Blood at the Laboratory

The following protocol shall be followed upon receipt of samples for biological dose estimate:

1. All received packages should be checked by a TDG certified individual for damage or leakage and the shipper notified if damage is observed.

2. Properly trained personnel, wearing appropriate protective gear shall open all packages inside a certified biosafety cabinet.

3. Any damaged or haemolysed blood samples should be noted and a request initiated for their replacement. Damaged samples should not be set up for culturing and will be disposed of appropriately.

## 6.3 Coding of samples

1. Code numbers (Five digit, i.e. 00001) shall be assigned sequentially to samples as they are received. Those samples that are divided into 4 parts as stated in 8.1, point 4 shall also be labeled with a letter. For example, the two diagnostic samples would be labeled as 00001A and 00001B and the two 1 Gy positive control samples would be

labeled 00001C and 00001D. Identifying information for the samples shall be recorded on a prepared sheet (Annex I).

2. For the remaining sample analysis time all samples will be referred to by their new assigned number. The sample will not be decoded until the dose estimation is complete and the report is being written.

3. Only designated individuals will have access to the decoding information.

4. All questionnaires and sample codes shall be placed in the "Records" binder (in a locked filing cabinet) under the responsibility of the laboratory head or designate in charge.

## 6.4 Setting up Cultures for Dose Assessment

Two possible methods for culture set up and dose estimation are listed below. The method of choice should be supported by the corresponding dose response curves. In dose estimate studies using the dicentric assay, it is important to analyze cells in their first metaphase after mitogenic stimulation to ensure that all terminal mutations are captured. When using the bromodeoxyuridine (BrdU) method a fixation time should be chosen for which a high proportion of analyzable cells are at the first division stage. Unfortunately, it is not always possible to predict the ideal fixation time due to differences in individuals and especially in response to a high-dose radiation exposure where lymphocyte division may be stalled. Generally, this BrdU method requires the setup of a series of cultures to optimize capturing the highest frequency of cells in first mitosis. Because of the added work, time and expenses using this protocol, Cytochalasin B (Cyto B), a cytokinesis inhibitor, can replace BrdU in the traditional dicentric assay method. To show that the two assays are comparable our laboratory generated two dose response curves in parallel using the same healthy donor.

The detailed protocols for the cytochalasin B (6.4.1) and bromodeoxyuridine (6.4.2) Methods are outlined below:

### 6.4.1 Cytochalasin B Materials and Methods

It is imperative that aseptic technique be maintained throughout this procedure. Be sure to wipe down the biosafety cabinet with 70% ethanol before and after use, and dispose of blood and containers in biohazard waste.

#### 6.4.1.1 Materials Required

1. Vacutainer® brand sterile test tubes with Lithium heparin (or Sodium heparin), from Becton Dickinson Systems (3, 5 or 10 mL)

2. 15 mL polypropylene tubes

3. 25 $cm^2$ culture flasks

4. RPMI complete media:

- ❖ RPMI Medium 1640 (Sigma, Catalogue number R8758)
- ❖ Fetal Bovine Serum (Sigma, Catalogue number F1051)
- ❖ L-glutamine (Life Technologies, Catalogue number 25030-081)
- ❖ Pen-strep (Life Technologies, Catalogue number 15140-148)

5. PHA (Life Technologies, Catalogue number 10576-015)

   - ❖ Supplied at 100X concentration and added to the culture so final concentration is 2X (2%).

6. 37°C, 5% $CO_2$ incubator

7. Viability stain:

   - ❖ Hank's Balanced Salt Solution (Life Technologies, Catalogue number 14025-092)
   - ❖ Fluorescein diacetate (Sigma, Catalogue number F7378)
   - ❖ Ethidium bromide (Sigma, Catalogue number E4391)

8. Haemocytometer

9. KaryoMAX Colcemid® Solution (Life Technologies, Catalogue number 15210-040)

10. Cytochalasin B (Sigma, Catalogue number C6762): 2.5 mg/mL cytochalasin B in DMSO

11. 0.075 M KCl (0.56 g/100 mL)

12. Methanol

13. Glacial Acetic Acid

14. Clean cold slides

15. Giemsa stain

#### 6.4.1.2 Safety Precautions

1. A lab coat and gloves must be worn during the preparation of all solutions and procedures. At all times, aseptic technique must be followed to ensure personnel safety.

2. All pipette tips and centrifuge tubes used to hold blood must be disposed of in the Sharps bucket or biohazard bag inside the biological safety cabinet and sealed before removal from cabinet.

#### 6.4.1.3 Cytochalasin B Protocol

**DAY 1**

1. Obtain at least 3 mL (or applicable volume) Lithium heparin (or sodium heparin) Vacutainer® of blood (one per treatment/case).

2. Samples to be used in generating dose response curves will be transported in a cooler with 37°C heat packs. All other samples, to be used for diagnostic purposes, will be shipped at approximately 20°C (Annex G).

   **Donor ID number: _____**

   **Time of blood draw: _____**

3. Prepare a blood smear for future optional differential leukocyte counts (for methods, see section 6.6.3, 'Wright Staining for Differential Leukocyte Counts').

4. Warm Media to RT.
   Per 100 mL complete media:

   - 84 mL RPMI Medium 1640
   - 15 mL Fetal Bovine Serum
   - 1 mL 200 mM L-glutamine
   - 1 mL Pen-strep

5. Check viability at TIME 0 h (dilute blood 1:20) – 5 µL blood + 95 µL viability stain (1.2 mL Hank's Balanced Salt Solution + 7.5 µL fluorescein diacetate @ 5 mg/mL + 100 µL ethidium bromide @ 100µg/mL).

   **Time**:_____

6. Label a 25 $cm^2$ flask and put 18 mL of media into it.

7. Transfer 2.0 mL of blood from the appropriate tube into the flask.

8. Add 400 µL PHA (final concentration 2X stock) to the flask.

   **Time: _____**

9. Incubate 48 h at 37°C in $CO_2$ incubator at a 30º angle.

**DAY 2**

1. At TIME 24 h add 16 μL 2.5 mg/mL cytochalasin B to the flask and gently mix (for a final concentration of 2.0 μg/mL). Return to incubator at 37°C $CO_2$ for 24 h (at a 30° angle).

**Time: _____**

**DAY 3**

1. Take hypotonic solution (0.075 M KCl) out of fridge and warm to 37°C.

2. At TIME 48 h add 200 μL of 10 μg/mL colcemid to the flask (final concentration of 0.10 μg/mL). Incubate (at a 30° angle) for 4 h at 37°C in $CO_2$ incubator.

**Start Time: _____**
**End Time: _____**

3. After incubation (TIME 52 h) transfer suspension to two 15 mL polypropylene tubes per flask.

4. Spin at 200*g* for 8 min.

5. Remove supernatant, and store 3 x 2 mL aliquots in –80°C for future cytokine work. Gently resuspend pellet in 10 mL hypotonic solution (37°C). At this step and all of the following resuspensions, ensure that the pellet is completely resuspended before moving to the next step.

6. Incubate for 12-15 min at 37°C.

7. Gently resuspend the cells and add 2 mL of freshly prepared cold fixative (3:1 methanol:acetic acid). Let stand for 10 min at RT.

8. Spin at 200*g* for 8 min.

9. Aspirate supernatant. Gently resuspend pellet. Add 10 mL freshly prepared cold fixative to cell suspension. Let stand for 10 min at RT.

10. Spin at 200*g* for 8 min.

11. Aspirate supernatant. Gently resuspend pellet. Add 10 mL freshly prepared cold fixative to cell suspension. Let stand for 10 min at RT.

12. Spin at 200*g* for 8 min.

13. Aspirate supernatant. Gently resuspend pellet. Add 10 mL freshly prepared cold fixative to cell suspension.

14. Store suspension at -20°C for at least 1 h before making slides.

### 6.4.2 Bromodeoxyuridine Materials and Methods

It is imperative that aseptic technique be maintained throughout this procedure. Be sure to wipe down the biosafety cabinet with 70% ethanol before and after use, and dispose of blood and containers in biohazard waste.

#### 6.4.2.1 Materials Required

1. Vacutainer® brand sterile test tubes with Lithium heparin (or Sodium heparin), from Becton Dickinson Systems (3, 5 or 10 mL).

2. 15 mL polypropylene tubes.

3. 25 $cm^2$ culture flasks

4. BrdU/RPMI complete media:

   - ❖ RPMI Medium 1640 (Sigma, Catalogue number R8758)
   - ❖ Fetal Bovine Serum (Sigma, Catalogue number F1051)
   - ❖ L-glutamine (Life Technologies, Catalogue number 25030-081)
   - ❖ Pen-strep (Life Technologies, Catalogue number 15140-148)
   - ❖ Bromodeoxyuridine (BrdU) (Sigma, Catalogue number B9285)

5. PHA (Life Technologies, Catalogue number 10576-015)

   - ❖ Supplied at 100X concentration and added to the culture so final concentration is 2X (2%).

6. 37°C, 5% $CO_2$ incubator

7. Viability stain:

   - ❖ Hank's Balanced Salt Solution (Life Technologies, Catalogue number 14025-092)
   - ❖ Fluorescein diacetate (Sigma, Catalogue number F7378)
   - ❖ Ethidium bromide (Sigma, Catalogue number E4391)

8. Haemocytometer

9. KaryoMAX Colcemid® Solution (Life Technologies, Catalogue number 15210-040)

10. 0.075 M KCl (0.56 g/100 mL)

11. Methanol

12. Glacial Acetic Acid

13. Clean Cold slides

14. Giemsa stain

#### 6.4.2.2 Safety Precautions

1. A lab coat and gloves must be worn during the preparation of all solutions and procedures. At all times, aseptic technique must be followed to ensure personnel safety.

2. All pipette tips and centrifuge tubes used to hold blood must be disposed of in the Sharps bucket or biohazard bag inside the biological safety cabinet and sealed before removal from cabinet.

#### 6.4.2.3 Bromodeoxyuridine (BrdU) Protocol

**DAY 1**

1. Obtain at least 3 mL Lithium heparin (or sodium heparin) Vacutainer® of blood (one per treatment/case).

2. Samples to be used in generating dose response curves will be transported in a cooler with 37°C gel packs. All other samples, to be used for diagnostic purposes, will be shipped at approximately 20°C (Annex G).

   **Donor ID number: _____**

   **Time of blood draw: _____**

3. Prepare a blood smear for future optional differential leukocyte counts (for methods, see section 6.6.3, 'Wright Staining for Differential Leukocyte Counts').

4. Warm BrdU media to RT.
   Per 100 mL BrdU media:

   - 84 mL RPMI Medium 1640
   - 15 mL Fetal Bovine Serum
   - 1 mL 200 mM L-glutamine
   - 1 mL Pen-strep
   - 450 µL Bromodeoxyuridine

5. Check viability at TIME 0 h (dilute blood 1:20) – 5 µL blood + 95 µL viability stain (1.2 mL Hank's Balanced Salt Solution + 7.5 µL fluorescein diacetate @ 5 mg/mL + 100 µL ethidium bromide @ 100µg/mL).

**Time**: _____

6. Label a 25 $cm^2$ flask and put 18 mL of media into the flask.

7. Transfer 2 mL of blood from the Vacutainer® into the flask.

8. Add 400 μL PHA (final concentration 2%) to the flask.

**Time: _____**

9. Incubate 44 h at 37°C in $CO_2$ incubator at a 30° angle.

## DAY 3

1. Take hypotonic (0.075 M KCl) out of fridge and warm to 37°C.

2. At TIME 44 h add 200 μL of 10 μg/mL colcemid to the flask (final concentration of 0.10 μg/mL). Incubate (at a 30° angle) for 4 h at 37°C in $CO_2$ incubator.

**Start Time: _____**

**End Time: _____**

3. After incubation (TIME 48 h) transfer cell suspension to two 15 mL polypropylene tubes per flask.

4. Spin at 200*g* for 8 min.

5. Remove supernatant, and store 3 x 2 mL aliquots in −80°C for future cytokine work, gently resuspend pellet in 10 mL hypotonic solution (37°C). At this step and all of the following resuspensions, ensure that the pellet is completely resuspended before moving to the next step.

6. Incubate for 12-15 min at 37°C.

7. Gently resuspend the cells and add 2 mL of freshly prepared cold fixative (3:1 methanol:acetic acid). Let stand for 10 min at RT.

8. Spin at 200*g* for 8 min.

9. Aspirate supernatant. Gently resuspend pellet. Add 10 mL freshly prepared cold fixative to cell suspension. Let stand for 10 min at RT.

10. Spin at 200*g* for 8 min.

11. Aspirate supernatant. Gently resuspend pellet. Add 10 mL freshly prepared cold fixative to cell suspension. Let stand for 10 min at RT.

12. Spin at 200*g* for 8 min.

13. Aspirate supernatant. Gently resuspend pellet. Add 10 mL freshly prepared cold fixative to cell suspension.

14. Store suspension at –20°C for at least 1 h before making slides.

## 6.5 Slide Preparation Protocols

1. Use clean slides:
   - ❖ Soak for a few hours in 1% HCl in EtOH
   - ❖ Wipe clean
   - ❖ Store in EtOH at –20°C
2. Prepare fresh cold fixative. Methanol: Acetic acid (3:1); 50 mL for each sample.
3. Spin cells for 8 min at 200*g*.
4. Remove most of supernatant, leaving about 200 µL.
5. Resuspend pellet in ~ 8 mL fixative.
6. Repeat steps 3 to 5.
7. Spin for 8 min at 200*g*.
8. Remove most of fixative, leaving 0.5 – 1 mL (slightly cloudy appearance).
9. Swish slide into beaker of ice water until water runs off smoothly.
10. Pipet cell suspension 2-3 times and drop about 15 µL onto an angled, still wet, slide.
11. Stream a Pasteur pipette of fixative over the slide three times.
12. Wipe the back of the slide.
13. Hold slide over steaming water bath for 20 s, cell side up.
14. Give the slide one vigorous shake and wipe the back of the slide.
15. Hold over steaming water bath for 20 s.
16. Wipe the back and label the slide.
17. Move slide to slidewarmer set at about 40°C.
18. Label the slides (using a xylene resistant pen) with the sample code and identifying number as well as the date prepared (e.g. 00001A, 17May03; where 00001 is the

sample code, A is the slide Id number and 17May03 is the date the slide was prepared).

19. After the excess water has dried (about 2 min), check the slide under the microscope. If the cells are too dense, add more fixative to cell suspension and prepare new slides.

20. Let the slides dry on the slidewarmer for at least 15 min. If leaving overnight cover loosely with aluminum foil.

21. Turn off the slidewarmer at the end of the day.

## 6.6 Staining of Slides

Fluorescence plus Giemsa (FPG) staining is recommended for BrdU preparations as this permits the analysis to be confined to the first *in vitro* division metaphase (M1). However, for triage purposes and to avoid certain limitations of the FPG staining technique, it is acceptable to use the conventional Giemsa staining, providing that the level of M2 cells is less than 5%. For cytochalasin B preparations the Giemsa staining protocol is recommended. For the differential leukocyte counts, Wright staining is appropriate.

### 6.6.1 Giemsa Staining

#### 6.6.1.1 Materials Required

1. Giemsa Stain (Sigma, catalogue number GS-1L)

2. Coverslips (Grace Biolabs, catalogue number HS6024-CS)

#### 6.6.1.2 Method

1. Prepare Giemsa Stain in a staining dish and remove the oxidation film with an absorbent tissue before inserting slides.

2. Put slides into Giemsa stain for 20 min at room temperature (4 mL stain in 200 mL dd$H_2O$).

2. Place the staining dish in the sink with the end of dd$H_2O$ tubing inserted in the dish and rinse for 30–60 s, or until the water runs clear.

3. Move the slides to a drying rack for ~ 15 min.

4. Place the slides on the slidewarmer and let dry.

5. Mount slides with a glass coverslip with mounting media (optional).

### 6.6.2 Fluorescence plus Giemsa (FPG) Staining

#### 6.6.2.1 Materials Required

1. Hoechst 33342/33258

   Storage solution
   1 mg/mL in PBS pH 6.8. Stored at -20°C. Protected from light in aliquots (avoid repeated freeze/thaw cycles).

   Stock solution
   0.1 mg/mL: 1mL of 1mg/mL storage solution, 9 mL PBS pH 6.8. Stored at 4°C for up to one month. Protected from light.

   Working solution
   10 µg/mL: 4 mL 0.1 mg/mL stock solution, 36 mL PBS in a coplin jar covered in foil.

2. Disodium Phosphate ($Na_2HPO_4$, pH 9.0)

   - 8.52 g $Na_2HPO_4$
   - 70 mL $ddH_2O$
   - pH to 9.0. Add $ddH_2O$ to 100 mL. Filter sterilize.

3. Giemsa Stain (Sigma, catalogue number GS-1L).

4. Coverslips (Grace Biolabs, catalogue number HS6024-CS).

5. UV light (8 W – 365 nm).

#### 6.6.2.2 Method

1. Put slides in a coplin jar containing the Hoechst working solution (wrapped in foil) for 10 min at RT.

2. Take out slides and blot backs dry.

3. Put 150 µL 0.6 M $Na_2HPO_4$ on the slide and cover with soft plastic 'coverslips' (Grace Biolabs catalogue number HS6024-CS).

4. Place the slides on a 60°C hotplate with a UV light (8 W – 365 nm) 8 cm above the slides for 40 min.

5. Carefully remove the coverslips to prevent cells from scratching off.

6. Rinse 3 times with $ddH_2O$.

7. Blot back of slide dry.

8. Put slides into Giemsa stain (oxidation film removed) for 10 min at RT (1 mL stain in 50 mL $ddH_2O$).

9. Rinse with $ddH_2O$ by gently overflowing the staining dish.

10. Dry slides.

11. Mount slides with a glass coverslip using mounting media (optional).

### 6.6.3 Wright Staining for Differential Leukocyte Counts

This protocol is recommended for acquiring general information on the tested samples. It is not a requirement for the dicentric assay.

#### 6.6.3.1 Materials Required

1. Clean slides

2. Wright Stain (Fisher Scientific, catalogue number CS432D)

3. Methanol

4. PBS (pH 6.8)

#### 6.6.3.2 Method

1. Using a pencil, clearly label a clean slide with the corresponding information from the coded tubes of whole blood.

2. Pipette a 10 μL drop of whole blood onto the slide and prepare the smear using a second glass slide at a 45° angel.

3. After the smear has dried fix the slide in methanol for 1-2 min.

4. Transfer slide into a Wright stain for 15 min.

5. Transfer staining rack to PBS (pH 6.8) and dip 10 times.

6. Transfer staining rack to $ddH_2O$ and dip 10 times.

7. Remove slides from staining rack and stand in rack to air dry (no need to coverslip).

## 6.7 Slide Coding

Slides must be blinded for analysis and the coding must not be revealed until analysis of all slides is complete. One method of blinding is to cover the slide labels with a piece of opaque tape, mix up the slides and number the tape with the date (day only) of blinding and sequential numbers (i.e. 12-1, where 12 is the day of the month and 1 is the sequential slide number). Ideally, two independent people should apply the tape and complete the numbering to ensure the integrity of the blinding.

## 6.8 Storage of Slides

1. All slides shall be mounted for long term storage.

2. The slides shall be stored in a secure facility allowing long term storage for at least 30 years.

# 7. Scoring Unstable Chromosome Aberrations

## 7.1 Procedure for Scoring First Division Metaphases

An important aspect of culturing blood samples for dose estimation by the dicentric assay is the harvest time for metaphase collection. The maximal frequency of unstable chromosomal aberrations in irradiated lymphocytes is observed in first-generation metaphase cells. A standard procedure is to estimate the frequency of second metaphases by the Fluorescence Plus Geimsa (FPG) method and if that frequency is below 5% it is acceptable to score slides stained with Giemsa only (accepting an error of <5%). For cultures containing >5% of cells in second division, only FPG stained slides shall be scored. An alternative techniques described in section 6.4.1 is to culture the cells in cytochalasin B (cytokinesis inhibitor), and only score cells in M1 (a complement of 2N or 46 chromosomes). Cells in M2 will have a chromosome complement of 4N therefore about 92 chromosomes and shall not be scored.

## 7.2 Criteria for Scoring

### 7.2.1 Coding of Samples and Slides

All samples, slides, and intra-laboratory or inter-laboratory validation standards must be coded. Complete records of coding will be maintained. See section 6.3 and 6.7 for details.

### 7.2.2 Mitotic Index

Examine at least 1000 cells per sample. To insure that the cells are representative of the cells in the entire culture, it is recommended that cells from replicate slides be scored. Record the number of metaphases per total number (metaphases plus interphases) of stimulated cells and express this as a percentage.

#### 7.2.2.1 Scoring Mitotic Index

1. To avoid bias score along the center down the entire length of the slide.

2. Turn the eyepiece scale so that it is vertical.

3. Using a 10x objective lens and the scale as guide, count the number of M1, M2, and M3 metaphase cells and interphase lymphocyte nuclei that pass through the scale. If any portion of the nuclei/metaphase spread passes through the scale it is counted. If it is ambiguous, nuclei should be counted as a lymphocyte.

4. Count the number of metaphase and interphase cells until a combined total of 1000 has been reached and that complete row has been counted.

5. The mitotic index is calculated by dividing the number of cells in mitosis into the total number of cells counted.

### 7.2.3 Scoring Techniques

All slides will be coded as per 6.7. The individuals scoring for chromosomal aberrations will not have access to the code thus ensuring blind analysis. There are two options for slide analysis and they are both acceptable (Reminder: "dicentric" refers to dicentrics or rings):

#### 7.2.3.1 Manual Scanning

1. Slides should be scanned methodically so that no area is rescanned.

2. The scanning should be done at low magnification (20x objective) to prevent a bias towards selecting cells that contain aberrations.

3. Having found a likely metaphase, the scorer should switch to high magnification (100x oil immersion objective). The scorer must make a snap judgment on whether the chromosomes are of a suitable quality for scoring and ignore the presence of any aberrations. This will be based on the sharpness of the images and the amount of twisting and overlapping of chromosomes. With FPG stained material the cell should be rejected if it displays the harlequin effect, indicating that it is not an M1 spread. Cells cultured in cytochalasin B containing 4N chromosomal complement are also to be rejected as they are in second mitosis.

4. Once the decision is taken to analyze the spread, then the number of individual chromosome pieces must be counted and the presence of aberrations noted. The cells should be scored using the microscope rather than counting only using the computer screen image.

5. Only complete metaphases are to be recorded, i.e. those with 46 or more pieces. If the cell contains unstable aberrations, then it should balance. For example, a spread containing a dicentric should also have an accompanying acentric fragment and still have 46 pieces. By contrast, a centric ring will also have an accompanying fragment, but the total number of objects in the cell will be 47. When high radiation doses are involved there may be more than one aberration in the spread, but the pieces should still balance. Tricentric aberrations are equivalent to two dicentrics and should have two accompanying fragments, while quadricentrics will have three fragments, and so on.

6. Each excess acentric, i.e. one not associated with a dicentric or centric ring, will increase the count of pieces beyond 46 and must be recorded.

7. A standardized scoring sheet must be used with data recorded such that the aberrations in each cell scored are derivable (Annex J-1).

8. When recording the aberrations, the fragments associated with a dicentric or ring must not be included with the count of excess acentrics.

9. All abnormalities in the cell should be recoded, although for dosimetry purposes only the data on dicentrics plus rings will normally be used.

10. The x and y stage co-ordinates of all complete cells analyzed, including those free from aberrations, should be recorded for possible future reference.

11. At least 500 cells must be scored from each case, unless the aberration yield is high, in which case 100 aberrations (dicentrics) are sufficient for statistical analysis. In the event of a low dose exposure and few incident samples for analysis it is recommended that a minimum of 1000 be scored. For the purposes of generating a dose response curve, it is recommended that at least 100 dicentrics be scored. If this number is impossible to achieve at lower dose exposures, it is recommended that a total of 2-3000 cells be examined.

12. When more than one scorer contributes to the analysis, each must analyze a comparable number of metaphases. In order to ensure this equalization of analysis a maximum of 250 spreads will be scored per slide by one individual, or up to 50 scorable aberrations.

#### 7.2.3.2 Automated Scanning

1. Metaphase finding by automated pattern recognition is an acceptable method for scanning the slides.

2. Some metaphase finders also include semi-automated analysis of digitized images that assist with locating aberrant chromosomes; however, no system is fully automatic, all must incorporate steps where the operator's judgment and decision are required.

3. Use of these instruments should be such that the same recommended criteria as outlined above (7.2.3.1) are maintained, namely, selection of candidate metaphases for scoring should not introduce bias likely to distort aberration yields and only complete spreads of chromosomes should be scored.

4. In contrast to manual scoring, it is not necessary to record the x and y stage co-ordinates of all cells analyzed. It is sufficient that a unique cell identifier be recorded for future reference.

5. A standardized scoring sheet must be used with data recorded such that the aberrations in each cell scored are derivable (Annex J-2).

6. The automated system must be subjected to quality assurance trials and the results documented (See Section 10.1.2).

### 7.2.4 Laboratory Scoring Expertise

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

The laboratory head is responsible for maintaining the scoring criteria and the qualifications of the individual scorers. All scorers must 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.

# 8. Criteria for Converting a Measured Aberration Frequency into an Estimate of Absorbed Dose

## 8.1 Overview

The measured dicentric frequency is converted to absorbed dose by reference to an appropriate *in vitro* calibration curve. Ideally, these calibration curves should be produced by the laboratory that is performing the scoring and analysis; however, under circumstances where a laboratory has been validated through an inter-comparison program, it is acceptable to share the calibration curves.

This analysis provides an estimate of the average whole body dose. At least 500 cells will be scored from the case specimen, unless the aberration yield is high, in which case it is not necessary to proceed beyond 100 dicentrics.

## 8.2 Comparison with Controls

The service laboratory will provide in reporting of results (Annex K) the laboratory's background dicentric level. If the measured aberration yield, upon analyzing 1000 spreads, is not significantly different from the control frequency, the best estimate of dose will be quoted as zero with its upper confidence limit. If the measured aberration yield is significantly higher than the control level, then a dose estimate with its uncertainties will be derived and reported.

## 8.3 Determination of Estimated Dose and Confidence Limits

The following guidelines will be followed to determine a dose estimate and confidence limits:

1. Dose estimates will be made using the equations derived from the calibration curves (Annex F).

2. When possible, a calibration curve of the same radiation quality as the exposure being examined should be used to estimate radiation dose. When such a curve is not available, the closest radiation quality shall be used and a special note will be made regarding the isotope used.

3. An estimated equivalent whole body dose and confidence limits are interpolated from the appropriate calibration curve.

4. Uncertainties are to be expressed as 95% confidence limits. There is no definitive method for determining the uncertainty of dose estimates; three methods are outlined in the IAEA Technical Report Series No. 405. Our laboratory uses the statistical method found in Annex L to calculate confidence limits.

5. If the measured aberration yield in 1000 spreads analyzed is not significantly different from the control frequency, the best estimate of dose should be quoted as zero with its upper confidence limit.

6. If the measured aberration yield is significantly higher than the control level, then a dose estimate with its uncertainties is derived from the calibration curve.

## 8.4 Acute and Non-Acute Exposure Cases

If an overexposure is known to have been received acutely i.e., in < 0.5 hours, 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 hours, 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 hours, 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 No. 405.

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 inter-fraction interval is > 5 hours. If < 5 hours, an interaction factor should be estimated using a two-hour time constant.

The service laboratory shall state in reporting of results (Annex K) the method used to correct for non-acute exposure dose estimates.

## 8.5 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/or the Contaminated Poisson methods. These techniques are detailed in the IAEA technical report No. 405.

Exposure occurring a long time prior to analysis may be underestimated by the dicentric assay. The scoring of stable chromosome aberrations painted by FISH (so-called FISH assay) 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.

The service laboratory shall state in result reports the method used to correct for partial-body and prior-exposure cases.

# 9. Reporting of Results

All reports will include as much relevant information as possible because they may be used in legal settings. All reports will routinely contain relevant sample information provided by the customer since this may influence the interpretation of the findings in the service laboratory. All aberrations observed shall be listed and interpreted based on the current understanding of mechanisms for radiation-induced chromosome aberration formation.

The distributions of these reports will be tightly controlled with only the requestor receiving the official report. With appropriate approvals, copies may be passed to other responsible persons.

Secure courier service will be used to notify the requestor of the final laboratory results. A copy of the report will be held in a secure file cabinet as described in section 2.2.6.

A standard form is to be used for reporting the results (Annex K). The report should be subdivided into the following sections:

## 9.1 Identification of the Exposed Subject

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

## 9.2 Description of the Case

All information provided by the customer that is relevant to the interpretation of the results shall be stated. This will include relevant information about the exposure incident. This could include the time, date, duration, dose rate, source of exposure, estimate of exposure dose based on physical dosimetry, and prediction of exposure direction (partial or whole body exposure).

## 9.3 Task of the Service Laboratory

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

## 9.4 Results of the Service Laboratory

The report will include: date of blood sampling, date of its arrival in the service laboratory, culture set-up date(s), number of cells analyzed, and number and type of chromosomal aberrations found.

## 9.5 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:

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

2. A statement on the likelihood that any aberration used in the dose estimation is related to this particular radiological incident;

3. The dicentric background of the laboratory and the coefficients of the calibration curve used for converting the dose from the aberration yield;

4. A quantification of the uncertainties on the dose estimate. This should include the upper and lower confidence limits and the percent level of confidence;

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

6. If appropriate, the interpretation will consider partial-body irradiation and excessive delay between the accident and blood sampling;

7. If appropriate, a comment on the interpretation of the distribution of chromosomal aberrations (cells with multiple damage);

8. Comments regarding the frequencies of other aberration types observed, but not used for dose estimation.

## 9.6 A Summary

This will comprise 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 there shall be an invitation for the customer to contact the laboratory if he/she requires further clarification or explanation about the results and/or the assay.

## 9.7 A Contact Person

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

# 10. Quality Assurance and Quality Control Plan

## 10.1 Overview of Quality Plan

Our service laboratory, as a minimum, will follow the quality assurance and quality control practices cited below for performing biological dosimetry by cytogenetics. This is an overview of the quality plan, policies, procedures, and instructions.

Performance checks shall be conducted to ensure the conformance of analytical processes, measurement equipment and procedures, and the facilities to predetermined operational requirements. The laboratory will verify that the estimation of absorbed dose measurements complies with the accuracy requirements specified in Section 8.

Procedures should include quality performance checks on the following:

### 10.1.1 Organization Structure, Management and Operational Responsibilities

The organizational structure of the laboratory is described in Annex A. The head of the laboratory is responsible for ensuring that conformity to this standard is met and that the quality plan is followed implicitly. In the event that the head of the laboratory will not be available he or she will designate an alternate.

### 10.1.2 Software Validations

All software shall be purchased from reputable companies with approved quality control standards. Software for metaphase finding equipment shall be validated in-house on an annual basis or when new upgrades are implemented. The Software validations will be conducted on each metaphase finder through comparative analysis. One or two slides from a group of designated calibration slides will be used for this process. The results of the validation studies will be maintained in the quality records binder.

1. Run calibration slide on each bay 5 times, for each microscope.

2. Compare the total resulting number of ‘metaphase’ hits and good ‘metaphase’ hits between all of the runs.

3. If there are any significant differences in the number of good hits (greater than 25%) between the replicate runs, various bays, or between the two microscopes; then a full recalibration of the instrument (according to the Applied Imaging Manual for the instrument) should be completed.

Criteria for calibration slides:

1. The slide has a significant number of metaphase spreads.

2. The slide has previously been scanned on each microscope with the current classifier and software several times (minimum of three scans).

3. The slide has been set-aside for this purpose.

### 10.1.3 Training and Qualification of Laboratory Personnel

Staff will be trained using validated protocols. Each individual's qualifications shall be maintained by the laboratory head (Annex M). Listed below are the requirements for training and qualification of personnel:

**Receiving:** The individual must be TDG certified and appropriately vaccinated.

**Coding:** The qualified individual will have signed the Confidentiality Agreement (Annex B) and has agreed to follow the coding protocol in section 6.3. They should also be TDG certified and appropriately vaccinated.

**Culturing to Fixing:** Each trainee will independently process a sample that will be compared to a sample prepared by a qualified individual. The prepared cell suspension will be checked for mitotic index and suspension quality.

**Irradiation of cells for Positive Controls:** The qualified individual will be the Deputy Radiation Safety Officer or a certified Radiation Worker wearing a physical dosimeter while in the radioactive area.

**Preparing Slides:** The qualified individual has demonstrated capability for making slides suitable for analysis.

**Blinding of Slides:** The qualified individual has agreed to follow the blinding protocol in section 6.7.

**Scoring of Slides:** The qualified individual will have participated in an intra-comparison or has successfully analyzed training slides. Successful scorer will fall within 20% of the laboratory reference standard.

**Conversion to Dose:** A qualified statistician or a validated software program will conduct all statistical analysis.

**Decoding:** The qualified individual has agreed to follow the blinding protocols (Sections 6.3 and 6.7) and will have signed the Confidentiality Agreement (Annex B).

**Report Writing:** The qualified individual has agreed to follow the report writing protocol (Section 9) and will have signed the Confidentiality Agreement (Annex B).

### 10.1.4 Document Control

Documents shall be maintained as stated throughout this manual. All Annexes shall be updated as required. Any revisions or additions to this manual must be submitted to the Quality Manager with a 'Document Creation or Revision Form', Annex N.

### 10.1.5 Procurement of Materials

Procured materials are subjected to the manufacturer's tests in their quality control laboratory. Where this does not meet our standards, additional in-house testing shall be conducted. At all times the laboratory shall ensure that necessary materials for processing 200 samples (in duplicate) are readily accessible.

### 10.1.6 Identification and Control of Material and Samples (Chain of Custody)

Each specimen must be traceable from the point of acquiring the sample to the point of issuing of the final analysis report; therefore, chain of custody procedures will be an integral part of both the sampling and analytical activities followed for all samples collected for analysis. The final custody procedures will document each sample from the time of its collection until its receipt by our laboratory. Internal laboratory records will then document the custody of the sample through its final deposition.

Standard chain of custody procedures will be used. Each sample will be labeled with a unique identification number that will be recorded on a sample data sheet along with other information such as sampling date, location of the sample, size of the sample (volume), and conditions (temperature) of sample shipping. The sample 'Chain of Custody' form can be found in Annex O.

### 10.1.7 Inspection and Testing of Equipment

All laboratory equipment will be inspected and tested periodically and subjected to preventative maintenance on regular basis. All maintenance should be implemented in accordance with the manufacturer's specifications. A logbook recording the date of purchase, model, and serial number of the equipment and all inspections, testing and maintenance will be maintained. The equipment is to be maintained in a clean and operational state at all times. All spills are to be cleaned up immediately.

#### 10.1.7.1 Laboratory Equipment Checklist for Inspection and Preventative Maintenance

- Analytical Balances
- Autoclaves

- Biological Safety Cabinets
- Incubators & Fyrite
- Microscope
- Pipettes & Pippetters
- pH meter
- Thermometers
- Temperature Monitoring (Refrigerators & Freezers)
- Vacuum pump
- Water baths
- Water Filtration System

Analytical Balances

Balances will be verified once daily before each use and after every time they are moved. If necessary, adjustments are made by the use of leveling screws to ensure that the indicator bubble is in the middle of the circle. The balance can then be switched on, tared and the calibrations performed (weighing range, integration time and stability detector changes may be applied if needed). Analytical balance is equipped with built-in calibration weight thus calibrations can be performed while the balance is in use. Verification information including date, experiment number (if applicable), reading provided by the balance, and a statement indicating whether the reading is acceptable shall be recorded in the balance logbook.

If a balance is off specification, it must be recalibrated before re-use. A tag indicating that the balance is off specification shall be attached to the balance in a visible location. The analytical balance is to be serviced once a year, through a service contract, and the report shall be added to the logbook.

Autoclaves

A sterilization indicator will accompany each use of the autoclave. This indicator will function by clearly indicating when the temperature of 121°C has been reached. There are a number of commercially available products that can be used (i.e. Diack sterilization monitors, VWR, catalogue number: 55710-400). The indicator should be placed within the load in such a way that it is the last to reach 121°C. For example, if six 500 mL bottles of water are being autoclaved, an extra 500 mL bottle of water should be included with the indicator suspended inside the volume.

If a load fails because of machine error, a Corrective Action Report (CAR, Section 10.1.10, Annex P) should be made and the autoclave should be serviced. If a load fails for other reasons such as operator error or a power outage, the load should be re-autoclaved with a fresh indicator. The temperature and pressure shall be calibrated once a year by certified contractor or in reference to local rules or regulations. The Calibration Report shall be added to the logbook. Maintenance instructions are described in the manufacturer's operation and maintenance manual and are also posted in the laboratory.

Biological Safety Cabinets

The biological safety cabinet fan shall run continuously. If the cabinet is off it shall be turned on for at least 30 minutes before use. Before and at the end of each usage the cabinet will be thoroughly cleaned with 70% ethanol. A certified contractor will conduct annual inspections of filter integrity and airflow rate. All reports will be added to the logbook.

Incubators

All incubators shall be connected to an emergency electrical circuit and emergency alarms; a back-up $CO_2$ source is recommended. Daily remote sensing temperature readings, digital $CO_2$ readings and once a month Fyrite ($CO_2$ gas analyzer) readings shall be maintained in a logbook. The water level in the incubator humidity pan shall be checked daily and reservoir cleaned once a month. The incubators shall be disinfected every 3 months using a 1% benzalchonium chloride solution. All parts are disinfected for 10 to 15 minutes and rinsed twice with purified water. Filters and outside dusting shall be completed annually. All readings and scheduled cleanings and disinfections shall be recorded in the logbook.

Fyrite ($CO_2$ gas analyzer)

Fyrite strength may be checked using the following method:

- A zero calibrated Fyrite unit is attached to the incubator sample port.
- The inject bulb in the connection tubing of the Fyrite is compressed 20 times.
- Do not vent Fyrite after reading, but reabsorb sample gas by inverting and turning upright. Repeat the inversion step once more.
- If reading increases by more then 0.5% $CO_2$ as compared to initial reading, replace fluid.

Fyrite should be changed approximately every 350 uses.

Any fluid strength checks or change of Fyrite fluid shall be recorded in the incubator logbook.

Microscopes

The lenses and stage should be cleaned at the end of each microscope session to ensure removal of any remaining immersion oil. If the microscope is not in use it shall be turned off and covered with a dust cover. Before covering the microscope with a dust cover, the user will ensure that the camera is also turned

off. Microscopes shall be professionally serviced on an annual basis and the reports will be recorded in the logbook.

Pipettes & Pippeters

Pipettes will be calibrated in-house at least once yearly. Pipetter filters should be changed as needed or at least biannually. Pippettes and Pippetters will be used as per the manufacturer's instructions.

pH Meters

The pH meters will be standardized before each use. To standardize the pH meter two primary standard buffers should be used.

Thermometers

Thermometers will be validated against a standardized digital thermometer that is traceable to an international standard and will be used as per the manufacturer's instructions.

Temperature Monitoring

In order to assure accurate and precise temperature for all freezers, refrigerators, and incubators, continuous remote sensing temperature recorders shall be attached to each freezer, refrigerator, and incubator. The monitors record a 30-day cycle, and then the paper cartridges must be replaced. The paper cartridge monitor records shall be kept in the Instrument Maintenance Logbook. The remote sensing temperature recorder shall be calibrated every 6 months and certificate of calibration and testing shall be kept in the Instrument Maintenance Logbook. If the temperature is outside of acceptable limits (refrigerator: 4°C ± 4; freezer: -20°C ± 4; incubator: 37°C ± 1.0, 5.0%$CO_2$ ± 0.5%), a corrective action report must be made and the instrument must be adjusted until the proper temperature setting is reached. Contact information for repair services should be readily available in the logbook and/or on the individual instruments. (The only exception to this continuous style of temperature monitoring will be the -70°C Freezer. This freezer has a built in alarm for any rise or fall in temperature and is also hooked up to a generator in the event of a power outage.)

Vacuum Pump

Exhaust filters and oil changes will be performed as per the manufacturer's instructions.

Water Baths

Water baths will be monitored for temperature when in use. Water baths shall be maintained clean and water changes shall be conducted as necessary.

Water Filtration System

When the resistivity of the filtered water drops below 17 megaohm-cm the cartridges need to be replaced. The date of cartridge replacement with the individual's initials shall be recorded on the cartridges.

### 10.1.8 Inspection and Testing of Materials

Reagents and Supplies
Receipt of Reagents: The date of receipt and initials of the individual receiving shall be recorded. Once the reagent is opened that date shall also be recorded on the label.

Preparation of Reagents: The contents, concentration, date prepared, expiration date, lab book reference page for preparation and identification of the individual who prepared the solution shall be clearly labelled for each reagent. Ensure consistent good water quality at all times.

A rigorous quality control protocol shall be followed for new batches of Fetal Bovine Serum (FBS). New batches of FBS (with new lot numbers) shall be tested against the existing batch known for its efficacy and growth potential and all records maintained in the DRDC Ottawa Working Standard quality control manuals. All culture media with sera shall be tested for sterility for at least 24 hours prior to use.

Maintenance and Storage of Supplies
Adequate supplies of all materials, including medium and reagents, shall be kept on hand at all times. All stock is to be rotated, using the oldest first and discarding the expired (if expiration dates available), on regular monthly basis. Stock that should be routinely checked includes all reagents and supplies. All reagents must be stored according to manufacturer's instructions.

### 10.1.9 Control and Maintenance of Calibration Standards

Thermometers
Thermometers and remote sensing temperature recorders should come serialized, with a certificate to indicate instrument traceability to the national standards. The remote sensing temperature recorder shall be calibrated every 6 months and certificate of calibration and testing should be kept in the Instrument Maintenance Logbook. Non-certified thermometers may be used if they are validated once a year against a certified thermometer and these results will also be kept in the Instrument Maintenance Logbook. If a thermometer cannot be validated against the national standard it will be removed from circulation for these DRDC Ottawa Working Standard purposes.

### 10.1.10 Corrective Action

Corrective Action Reports (Annex P) are issued when non-conformity (Annex Q) has been detected in regard to the standards and procedures outlined in this manual. They

are used to define and identify the origins of non-conformity, identify the root causes of the non-conformity, outline an action plan, and to verify that the corrective action has taken place.

The Report of Non-Conformity and subsequent CAR is typically issued following an external or an internal review. They may also be issued at any other time that non-conformity has been detected. The Action Plan Issue Date refers to the date when the CAR is first issued. Each CAR is given a date by which the actions specified in the Action Plan should be completed. This is referred to as the Action Plan Due Date. The CAR is then delivered to the affected personnel, and the Action Plan is then implemented. Once the action plan has been verified by either the laboratory head or quality plan manager, the CAR can be officially closed. The Action Plan Verification Date refers to the date that Management has verified that the Action plan has been implemented.

### 10.1.11 Review of Procedures, Specifications and Operating Logs

The laboratory shall review its procedures, specifications and operating logs on a semi-annual basis and implement updates accordingly. An external review shall be conducted every three years.

### 10.1.12 Intra- and Inter- Laboratory Comparisons

To ensure maintenance of the Laboratory Quality Program the laboratory shall participate in conducting periodic comparisons of the laboratory's capability. There are two separate components in this verification process, an intra- and an inter-laboratory comparison. These programs will be set up and followed as per agreed schedules.

The laboratory's certified staff shall participate in an intra-laboratory comparison to test tissue culture and chromosome scoring expertise. This intra-comparison shall be conducted every three years and will provide an opportunity for certification of new staff. Each participant will carry out the whole process of culturing, preparing slides and scoring for aberrations in a coded *in vitro* irradiated blood sample. The individual's performance will be evaluated by comparing their predicted dose estimate against the established delivered dose. All predicted values within 95% confidence limits of the actual dose would be considered acceptable. All individuals whose predicted dose estimates fail to be within this acceptable range will repeat the evaluation process with a new coded *in vitro* irradiated blood sample. The outcome of this intra-comparison will then be used for updating the list of Laboratory Qualified Individuals (Annex M) and for implementing of any necessary corrective actions.

The laboratory shall also participate in an inter-laboratory cytogenetic proficiency-testing program to be conducted every five years. If the inter-laboratory testing coincides to the same year as the intra-laboratory testing it is not necessary to repeat the latter. This proficiency test evaluates each laboratory's capability to produce

consistency in radiation dose predictions within each participating laboratory and compare this to performance of other laboratories. Each participating laboratory will carry out the whole process of culturing, preparing slides and scoring for aberrations in a coded *in vitro* irradiated blood sample. Only one dose will be used for testing. The blood sample should be sufficiently large enough to allow testing of all certified staff within the laboratory (1 mL / individual tested plus an additional 2 mL for other tests and spillage backup). This blood sample will originate from the laboratory coordinating the intra-comparison and be distributed to all participating laboratories. All laboratories, including the coordinating laboratory, are to participate in each proficiency test. Only the individual who conducted the blood *in vitro* irradiations and coded the samples will be exempt from participating in the test. Participating laboratories will take turns in coordinating the intra-comparisons. Each laboratory's performance will be evaluated by comparing their predicted dose estimate against the established delivered dose. All predicted values within 95% confidence limits of the actual dose would be considered acceptable. Those individuals or laboratories whose predicted dose estimates fail to be within this acceptable range will be required to conduct an intra-comparison within six months of participating in the inter-comparison program. The successful results of this intra-comparison will be forwarded to the coordinating laboratory that conducted the inter-comparison. Unsuccessful results will require a repeat of the intra-comparison for those laboratories or individuals that still require further training. A successful participation in an inter-comparison will be used for laboratory accreditation. Successful individual results may also be used for updating the list of Laboratory Qualified Individuals (Annex M). Unsuccessful participation in this proficiency-testing program will offer the participating laboratories an opportunity to review procedures and implement corrective actions.

DRDC Ottawa, Health Canada, Chalk River Laboratories (AECL) and McMaster University are the four laboratories presently participating in the National Biology Dosimetry Response Plan and would be expected to participate in the annual inter-comparisons. As other laboratories join the program they would also be expected to participate in the proficiency-testing program.

### 10.1.13 Quality Plan Records

The Quality Plan Manager will be the primary individual responsible for identifying quality assurance and control problems. The Quality Plan Manager will initiate or recommend corrective actions and provide verification of deficiency corrections. The manager will also update this manual after the receipt and acceptance of any new or revised documents or annexes (Annex N).

### 10.1.14 Quality Plan Manager

The Quality Plan Manager will be the primary individual responsible for identifying quality assurance and control problems. The major responsibilities are outline in section 10.1.13, 'Quality Plan Records'. The Quality Plan Manager is identified in the Organizational Chart in Annex A.

### 10.1.15 Performance Checks of Sample Transport Integrity

For sample shipping purposes we will use couriers that do not X-ray their packages, such as Fed-Ex and/or Purolator. To test their transport integrity two trials will be conducted at times of the year when extreme temperatures are expected (January and July). Blood samples will be shipped according to shipping instructions in Annex G, to a Canadian location requiring at least a 3 hour one-way air transport. The shipment will also contain a min-max thermometer and 2 thermoluminescent detectors (TLDs). Two negative control TLDs will be kept at the site of origin. The package will be returned unopened to the originator for analysis. Only the temperature data recorded during the first one-way portion of the trip will be used for analysis since all of our blood shipments will only be required to travel one-way to our service laboratory. If the quality of transport is not acceptable, a corrective action request will be submitted to the Quality Plan Manager. 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.

### 10.1.16 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. An internal negative control from unexposed individuals, and where possible, an internal positive control shall be included in the study to prove the reliability of the procedures. Blood from both exposed and unexposed individuals must be handled in the same manner. All samples (test samples, positive and negative controls) are to be processed concurrently and not successively.

### 10.1.17 Performance Checks of Sample Protocol

For the interpretation of results it can be useful to prepare a slide for differential leukocyte count from each blood sample before starting the cultures. The culture, fixation and staining procedures are described in detail in section 6. The same lot of media and reagents will be used throughout the same dose estimate or experiment. Section 6 also describes the accurate composition of all experimental reagents used.

Positive and negative controls will be included with dose estimate cases, where possible, and shall be analyzed to determine bias and precision of the analytical procedures. Replicate samples should also be processed at least once a year to test reproducibility.

### 10.1.18 Performance Checks of Sample Scoring

Before analysis, the microscopic slides or fixed cell suspensions should be stored in a manner that maintains their highest quality. Uniform criteria for scoring must be used. A trained and experienced observer must perform chromosomal aberrations

analysis in metaphase spreads and each scorer's identity will be recorded. If multiple scorers are involved they must all be validated through an intra-laboratory comparison. Within the set of slides for the study, a positive and a negative control slide may be included. Independent of the service activity, the internal quality plan involves annual comparison of the scoring results of replicate samples between scorers.

### 10.1.19 Performance Checks of Dose and Confidence Limits Estimation

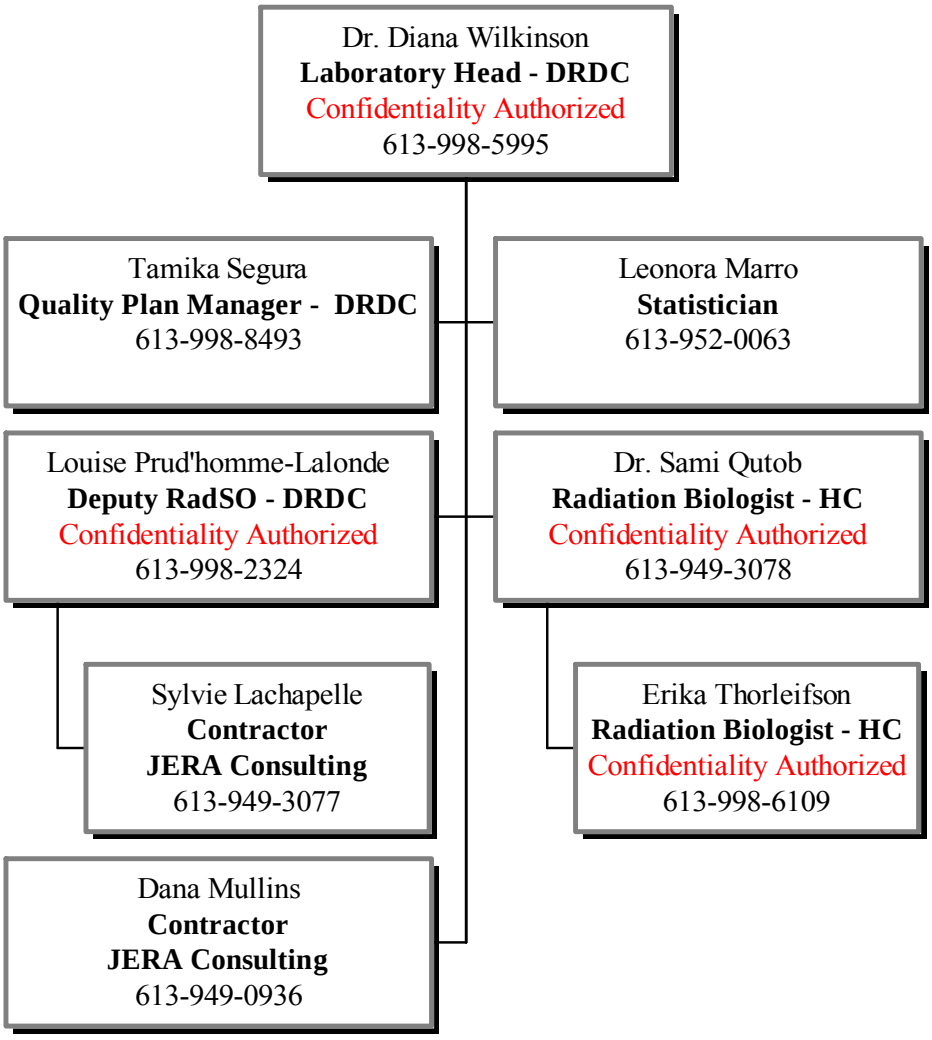
Non-parametric tests should be used for univariate statistical analysis. The confidence interval of the exposure has to be calculated from the uncertainty on the dicentric yields and the variation of the dose-response relationship among individuals, typically determined in a prior study. The dose-response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

### 10.1.20 Performance Checks of Result Report Generation

The study reports to customers shall be examined to ensure that they contain the necessary information defined in this standard (see section 9) 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: Organizational Chart

# DRDC Radiation Biology Organization Chart for Biological Dosimetry




- This is an organizational chart for the purpose of Radiological / Nuclear Biodosimetry Response only.

# Annex B: Confidentiality Agreement

I,……..(name), born……..(dd/mm/yy) consent to maintain all personal information, samples and sample coding, data analysis and reports confidential and locked under appropriate security conditions. I shall not disclose any volunteer related information, nor will I allow access of such to a third party. Only authorized personnel will be privy to this confidential information.

…… ……
Signature Date

I,……(name), born……(dd/mm/yy) have witnessed the

signing of this confidential agreement in ……(location).

…… ……
Signature Date

# Annex C: Consent Forms

There are multiple forms in Annex C. These forms are presently in use, but are subject to biannual updates through the Human Research Ethics Committee approvals. They are as follows:

Annex C-1: "Critical Personnel" and "Case Studies" Consent Form

Annex C-2: Volunteer Invasive Consent Form

# Annex C-1: "Critical Personnel" and "Case Studies" Consent Form

**Protocol Number**: **L-444**

**Research Project Title**: Biological Response and Radiation Biodosimetry At-Ground and At-Altitude

**Principal Investigator**: Dr. D. Wilkinson, DRDC Ottawa

**Co-Investigator(s)**: Dr. T. Cousins, DRDC Ottawa
Dr. B.J. Lewis, Royal Military College of Canada
Dr. L.G.I. Bennett, Royal Military College of Canada
Dr. H. Ing, Bubble Technology Industries
Ms. T. Segura, DRDC Ottawa
Ms. L. Prud'homme-Lalonde, DRDC Ottawa

I, _____ (name) of _____ _____ _____(address and phone number) hereby volunteer to participate in the above named study (Protocol # L-444). I have read the information package on the research protocol, and have had the opportunity to ask questions of the Investigator(s), DGNS and Medical Operations Officers. All of my questions concerning this study have been fully answered to my satisfaction. However, I may obtain additional information about the research project and diagnostic analysis used in this study by contacting Dr. Diana Wilkinson at 613-998-5995.

I have been told that I will be asked to participate in 1 session of approximately 30 minutes to fill out the questionnaire form, and 1 session of approximately 5 minutes for a venous blood draw. I am freely volunteering to give a venous blood sample (2 x 10mL) and a saliva sample for this project. A qualified nurse or a phlebotomist will perform the venipuncture. A small needle will be used to pierce the skin and withdraw a sample into two 10mL vacutainer tubes. I understand that there are almost no risks associated with blood donation and that it is not possible to acquire any disease through donating blood because disposable, sterilized equipment is used for each donation. I acknowledge that the principal risks of the research protocol other than the discomfort from the blood sampling procedure are complications that include a very low risk of infection of the wound site, bruising and the small possibility of a hematoma (or localized swelling due to bruising). Occasionally, fainting due to nervous reflexes may occur during the venipuncture.

I understand that **at no time will I personally be exposed to radiation as a part of this study.**

Only the assays specified in this document and approved by the Human Research Ethics Committee will be conducted on the acquired samples. Samples will not be used for any other research purposes. Any remaining, unused samples will be destroyed.

I acknowledge that there are no risks associated with the saliva donation using the rinse method where 2mL of sterile water will be taken into my mouth, swished and returned to a sterile sample cup.

I hereby consent to the medical screening assessment outlined in the protocol and agree to provide responses to questions that are to the best of my knowledge, truthful and complete. Furthermore, I agree to advise the Investigators of any health status changes since my initial assessment (including, but not limited to, viral illness or new prescription or "over-the-counter" medications). I have been advised that the medical information I reveal and the experimental data concerning me will be treated as confidential, and not revealed to anyone other than the Investigators. The final report relating the dose estimates and associated implications will be related to me by the Radiation Safety Officer who may solicit advice from DGNS and Medical Operations Officers. I am aware of the requirement to sign a separate consent form for invasive medical procedures. In the highly unlikely event that I become incapacitated during my participation, I understand that every necessary medical treatment will be instituted even though I am unable to give my consent at that time. I will go with the Investigators to seek immediate medical attention that either the Investigators or I consider necessary. Every effort will be made to contact a family member or the designated person indicated below should that be necessary. Any records bearing my name will be kept by the responsible Defence Scientist and DRDC Human Research Ethics Committee. Only in the event that the study results suggest that I have received a dose will the Radiation Safety Officer, the DGNS and the Medical Operations Officer become involved.

I understand that I am free to refuse to participate and may withdraw my consent to participation in this study at any time without prejudice or hard feelings for any reason. Should I withdraw my consent, my participation as a subject would cease immediately, unless the Investigators determine that such action would be dangerous or impossible (in which case my participation will cease as soon as it is safe to do so). I also understand that the Investigators, their designate, or the physician(s) responsible for the research project may terminate my participation at any time, regardless of my wishes.

I understand that I will not receive any type of remuneration for my participation in this study.

Volunteer's Name: _____
Signature:_____
Date: _____

Name of Witness: _____
Signature:_____
Date:_____

Family Member or Contact Person (name, address, daytime phone number & relationship):
_____

Principal Investigator:_____
Signature: _____
Date: _____

**FOR SUBJECT ENQUIRY IF REQUIRED:**

This protocol has been approved by DRDC Human Research Ethics Committee. Should I have any questions or concerns regarding the project before, during, or after participation, I understand that I am encouraged to contact either or both Defence R&D Canada – Toronto (DRDC Toronto), P.O. Box 2000, 1133 Sheppard Avenue West, Toronto, Ontario M3M 3B9, or Defence R&D Ottawa (DRDC-Ottawa), 3701 Carling Ave., Ottawa, Ontario K1A 0Z4. This contact can be made by surface mail at this address or in person, by phone or email, to any of the DRDC Toronto or Ottawa numbers and addresses listed below:

Principal DRDC Ottawa Investigator:

Dr. Diana Wilkinson, 613-998-5995, Diana.Wilkinson@drdc-rddc.gc.ca

Chair, DRDC Human Research Ethics Committee (HREC):

Dr. Jack Landolt, 416-635-2120, Jack.Landolt@drdc-rddc.gc.ca

I understand that I will be given a copy of this consent form so that I may contact any of the above-mentioned individuals at some time in the future should that be required.

# Annex C-2: Volunteer Invasive Consent Form

**Protocol Number**: **L-444**

**Research Project Title**: Biological Response and Radiation Biodosimetry At-Ground and At-Altitude

**Principal Investigator**: Dr. D. Wilkinson, DRDC Ottawa

**Co-Investigator(s)**: Dr. T. Cousins, DRDC Ottawa
Dr. B.J. Lewis, Royal Military College of Canada
Dr. L.G.I. Bennett, Royal Military College of Canada
Dr. H. Ing, Bubble Technology Industries
Ms. T. Segura, DRDC Ottawa
Ms. L. Prud'homme-Lalonde, DRDC Ottawa

**For the Subject**: Initial beside all procedures to which you consent.

_____ **Venipuncture:** A venous blood sample (2 x 10mL) is required for this project. A qualified nurse or a phlebotomist will perform intravenous catheterisation. A small needle will be used to pierce the skin and withdraw a sample into two 10mL vacutainer tubes. I understand that there are almost no risks associated with blood donation and that it is not possible to acquire any disease through donating blood because disposable, sterilized equipment is used for each donation. I acknowledge that the principal risks of this research protocol other than the discomfort from the blood sampling procedure are complications that include a very low risk of infection of the wound site, bruising and the small possibility of a hematoma (or localized swelling due to bruising). Occasionally, fainting due to nervous reflexes may occur during the venipuncture.

_____ **5 monthly Venipuncture samples:** A venous blood sample (2 x 10mL) is required once a month for 5 months. Same procedures and precautions will be observed as for a single donation described above.

**SUBJECT'S DECLARATION:**

I hereby consent to the procedures that I have initialed above. The Investigators have explained the procedures and any possible complications to me to my satisfaction, and I have had the opportunity to ask questions both of the Investigators and of the Medical Professionals.

Subject's Name: _____ Signature: _____
Date: _____

Name of Witness: _____ Signature: _____
Date:_____

Principal Investigator: _____ Signature: _____
Date: _____

I understand that I shall be given a copy of this consent form for my records.

# Annex D: Calibration Reports

There will be multiple reports in Annex D for calibration reports from the various radiation qualities as more dose response curves are generated. The current annexes are as follows:

Annex D-1: Dosimetry of Blood Samples Irradiated for the Dicentric Chromosome Assay, 200 kVp X-Ray Beam

# Annex D-1: Dosimetry of Blood Samples Irradiated for the Dicentric Chromosome Assay, 200 kVp X-Ray Beam

**Beam Characteristics**

The samples were irradiated using a Pantak Therapax HF300DT Orthovoltage Unit, which is in clinical use at the Ottawa Regional Cancer Centre, General Campus. This machine is equipped with an unsealed transmission monitor chamber. The accelerating potential was 200 kV, the added filtration was a Thoreus filter consisting of 0.1 mm Cu and 2.5 mm Al, resulting in a X-ray beam with a measured Half Value Layer (HVL) of 0.64 mm Cu.

The irradiations were conducted in a clinical set-up using a 10x10 cm cone with a Focus to Surface Distance (FSD) of 50 cm. Percentage Depth Dose (PDD) curves were generated at the time of clinical commissioning of the treatment unit using a plane parallel ionization chamber in a scanning water tank. The output of the treatment machine was calibrated using a Farmer chamber and electrometer calibrated by the Institute for National Measurement Standards, National Research Council of Canada, Ottawa, Canada, according to the ICRU 23 protocol for kilovoltage X-ray beam dosimetry (1). The constancy of output and beam energy was verified daily using a quality assurance chamber in an acrylic phantom.

External verification of absolute dosimetry has been performed for this treatment unit using a mailed TLD service (Radiation Dosimetry Services, MD Anderson, Houston).

**Experimental Set-up**

Blood samples were irradiated in Becton-Dickinson Vacutainer® tubes, with an inside diameter of 10.5 mm, and a glass wall thickness of 0.5 mm. These were inserted into a hole drilled in a solid water phantom (30 cm x 30 cm x 9 cm), to provide full scatter conditions. The centre of the sample tube was at a depth in phantom of 2.0 cm, with a field size at depth of 10.4 cm x 10.4 cm. The end of the cone was in contact with the surface of the phantom. Samples were irradiated one at a time, with the sample centered in the field. This irradiation geometry allowed excellent inter-sample reproducibility.

This irradiation geometry produced a dose variation across the sample diameter of $\pm$6.5%, due to the decrease in the PDD from the proximal to the distal side of the sample tube. To ensure uniform irradiation, the sample tube was remotely rotated about its longitudinal axis through approximately 4 revolutions during the time of irradiation. This ensured uniformity of irradiation, and the dose to the sample was taken to be the dose to the centre, i.e. at a depth of 2.0 cm in the phantom.

**Verification of Sample Dose by Measurement**

The calculation of dose to the sample based on our tabulated clinical data was verified through an independent measurement using a calibrated ionization chamber. A RK chamber (Scanditronix model 8305, S/N 2100, connected to a Farmer Dosemeter 2570/1, S/N 472) was placed in a Vacutainer® tube containing water to replace the blood sample. This was

inserted into the phantom and irradiated in the experimental setup described above. The ionization reading was converted to dose using the AAPM TG-61 protocol (2). The measured dose agreed to within 0.5% with the dose calculated using clinical data.

Based on these calculations and measurements, the dose rate to the blood sample irradiated in the described experimental setup was 0.979 cGy/MU, with an estimated overall uncertainty of $\pm$4.9%. In this irradiation set-up, the temporal dose rate was 126 cGy/min, which means that 4 Gy is delivered in 3.2 minutes.

## Qualifications

David E. Wilkins, Senior Physicist, Ottawa Regional Cancer Centre, performed the measurements and calculations described in this report. Dr. Wilkins has a PhD in Medical Physics from Carleton University in Ottawa, is a Fellow of the Canadian College of Physicists in Medicine, and has 10 years experience as a radiotherapy physicist.

## References

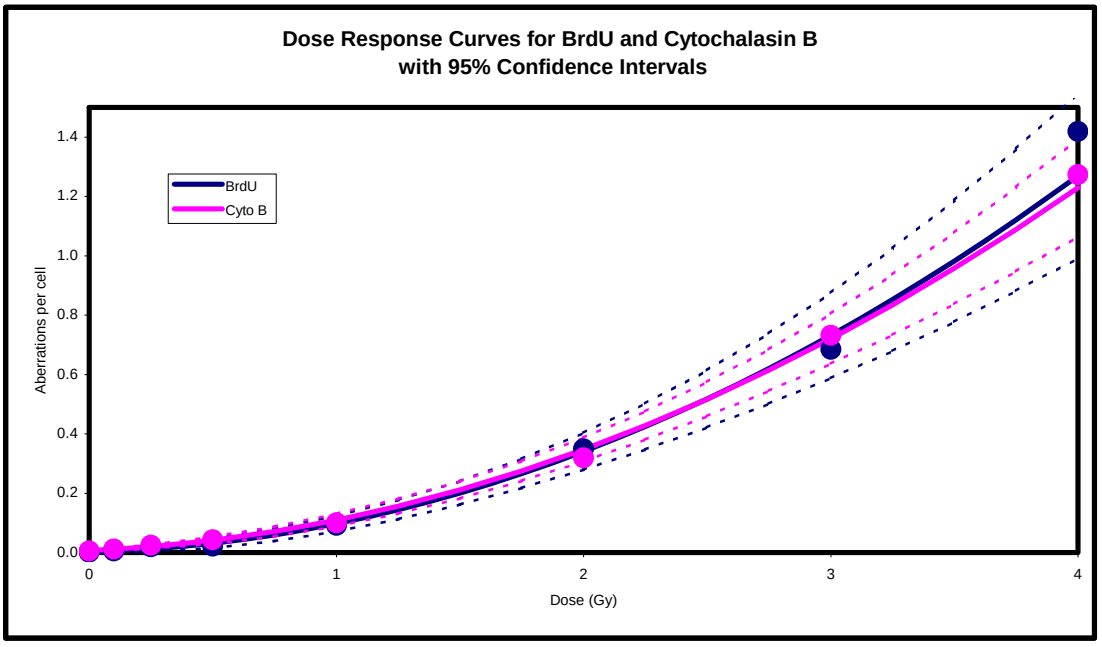

1. Measurement of absorbed dose in a phantom irradiated by a single beam of X or gamma rays. ICRU Report #23 (Bethesda, MD: ICRU).
2. C-M. Ma, C.W. Coffey, L.A. DeWard, C. Liu, R. Nath, S.M. Seltzer, J.P. Seuntjens. AAPM Protocol for 40-3000 kV x-ray beam dosimetry in radiotherapy and radiobiology. Medical Physics 28(6): 868-893, June 2001.

# Annex E: Calibration Curve Reports

There will be multiple reports in Annex E for calibration curve reports from each of the various radiation qualities as each is generated. The current annexes are as follows:

Annex E-1: 200 kVp X-ray Calibration Curve

# Annex E-1: 200 kVp X-ray Calibration Curve




The dose equation for the BrdU curve is $Y = 7.4x10^{-2} * D^2 + 2.3x10^{-2} * D + 1.31x10^{-3}$.

The dose equation for the Cytochalasin B curve is $Y = 6.77x10^{-2} * D^2 + 3.5x10^{-2} * D + 6.3x10^{-3}$.

# Annex F: Statistical Analysis for Calculating the Calibration Curves

The number of chromosome aberrations, spontaneous or radiation induced, follows a Poisson distribution. An important application of dose response curves in cytogenetics is their use as calibration curves for estimating dose from aberration yield. In this analysis we are interested in fitting a dose response curve to the counts of dicentrics, tricentrics and rings, which follow a Poisson distribution. The model is:

$$y_{ij} = \beta_0 + \beta_1 x + \beta_2 x^2 + \varepsilon_{ij},$$

(1)

where, $y_{ij} = r_{ij} / n_{ij}$ the proportion of aberrations (dicentrics + tricentric + rings) to the total number of cells scored in the $j^{th}$ replicate of the $i^{th}$ dose group; $x$ is the dose level; $\varepsilon_{ij}$ is the error of the model distributed as a Poisson random variable with mean and variance equal to $\lambda_i = E(y_i)$ (the expected value of the proportion of aberrations in the $i^{th}$ dose group). The regression curve is then used for calibration analysis (inverse estimation). For the regression equation given in (1), the point estimate of dose corresponding to an observed aberration frequency $y^* = r^* / n^*$ is given by $\hat{x}(y^*)$

$$\hat{x}(y^*) = \frac{-\hat{\beta}_1 + \sqrt{\hat{\beta}_1^2 - 4\hat{\beta}_2(\hat{\beta}_0 - y^*)}}{2\hat{\beta}_2}.$$

**References**

Merkle, W. (1983). Statistical methods in regression and calibration analysis of chromosome aberration data. Radiation and Environmental Biophysics 21:217-233.

**Qualification of the Statistician**

The statistics described in this report were performed by Leonora Marro, a statistician with the Biostatistics and Epidemiology Division of the Healthy Environments and Consumer Safety Branch at Health Canada. She has a BA in Mathematics, and MSc in Statistics with 5 years applied statistical experience, as well as research in applied statistical methodology.

# Annex G: Instructions for Customers

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 the protocol outlined below.

- Before the blood sample is taken please notify us so that we can prepare for its arrival and pick up.
- All blood samples are to be collected into lithium heparin tubes (if not available sodium heparin tubes may be used), and are to contain at least 3 mL (ideally 2 x 5 mL tubes). Gently rock the tubes for 2 minutes to ensure proper mixing. Label the tubes unambiguously using the coding system identified by the receiving laboratory and complete the questionnaire.
- Package the blood sample carefully to prevent breakage of the tubes in transit. Also, the blood should be maintained at about 20°C. **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.
- 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.
- For best results blood must be received within 24 h of sampling.
- The "Case Studies" questionnaire must be completed and enclosed with the sample shipment or faxed to our laboratory.
- For air transport, packaging and labeling should conform to the current International Air Transport Association (IATA) regulations. These require that blood samples should be packed to conform to United Nations Regulation 650 for biological substances. Saf-T-Pak manufactures packaging that meets these requirements (STP 210) (www.saftpak.com). Other packaging is acceptable providing it meets the requirements stated below.
- Packaging:
  - leakproof primary container (Vacutainer)
  - leakproof secondary container (e.g. Ziplock bag)
  - absorbent material placed between the primary and the secondary container
  - must be marked with **TC-125-1B** (e.g. STP 210 packaging)
  - if the shipper is making his own packaging, it must be a rigid outer packaging, and the exterior must be marked with **125-1B**
- Marking and labelling on outer package for air transport:
  - **name, address and telephone number** of receiver and shipper
  - **Biological substances, category B UN3373**
  - diamond shaped **UN3373 label**
  - **2 orientation arrows** placed on opposite sides of the package
  - **DO NOT X-RAY**
  - **DO NOT FREEZE**
- Waybill:
  - in "Description", enter: **Biological substances, category B UN3373**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Defence R&D Canada - Ottawa
3701 Carling Avenue
Ottawa, Ontario, Canada, K1A 0Z4

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E-mail: diana.wilkinson@drdc-rddc.gc.ca

# Annex H: Questionnaire

**Protected**
**Medical - Confidential**

Study ID Sticker

**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

1. Date and time of overexposure: .....…… (dd/mm/yy - time)
2. Place……. Company:……
3. Brief description of overexposure: …….
   ……..
4. Whole body exposure O Partial body exposure O Internal contamination O
   Dose value: ..... Part of body: ..... Nuclide:……...
   Dose value: ..... Dose value:…….
   How was this dose value obtained……
5 Type of radiation:

| | | |
|---|---|---|
| x - ray | O | kV |
| γ | O | nuclide? …… |
| α | O | nuclide? …… |
| Neutrons | O | source? ……. |

**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 / day: .....

---

**Results of chromosomal analyses to be sent to :** Name: .....…

Address:.....……

**Protected**
**Medical - Confidential**

Telephone # : .....……

# Annex I: Sample Coding

Coding of Samples

A, B are used to label 2 separate flasks for a sample
C, D are used to label 2 separate flasks for a sample given a 1 Gy dose.

| | **Donor Identification** | **Code** | **A** | **B** | **C** | **D** | **Comments** |
|---|---|---|---|---|---|---|---|
| | Mary Jane | 00001 | X | X | | | i.e. 00001A, 00001B |
| 1 | | | | | | | |
| 2 | | | | | | | |
| 3 | | | | | | | |
| 4 | | | | | | | |
| 5 | | | | | | | |
| 6 | | | | | | | |
| 7 | | | | | | | |
| 8 | | | | | | | |
| 9 | | | | | | | |
| 10 | | | | | | | |
| 11 | | | | | | | |
| 12 | | | | | | | |
| 13 | | | | | | | |
| 14 | | | | | | | |
| 15 | | | | | | | |
| 16 | | | | | | | |

# Annex J: Sample Data Sheets

There are two types of data recording sheets, one for manual scoring and one for automated. The following are two examples of recording sheets for each method:

Annex J-1: Sample Data Sheet for Recording of Aberrations Manually
Annex J-2: Sample Data Sheet for Recording of Aberrations using the Automated Microscope

# Annex J-1: Sample Data Sheet for Recording of Aberrations Manually

Slide ID:

Scorer:

Microscope N° :

Date:

| Cell N°. | Stage Co-ordinates | | N°. of chromosomal pieces | Dicentrics | Centric rings | Excess acentrics | Remarks | Aberration checked by |
|---|---|---|---|---|---|---|---|---|
| | X | Y | | | | | | |
| 1 | 100.1 | 1.2 | 46 | | | | | |
| 2 | 103.4 | 1.5 | 47 | 1 | | 1 | | |
| 3 | 105.4 | 1.2 | 49 | 2 | 1 | 2 | | |
| 4 | 112.4 | 1.6 | - | | | | Endo-reduplication | |
| 5 | 112.7 | 1.8 | 48 | | | 2 | | |
| 6 | 120.1 | 1.2 | 46 | 1 | | | | |
| 7 | 122.7 | 1.5 | 47 | | 1 | | | |
| 8 | 124.1 | 1.4 | 46 | | | | Chromatid exchange | |
| 9 | 126.8 | 1.7 | 46 | 2* | | | *= 1 tricentric | |
| etc. | | | | | | | | |

# Annex J-2: Sample Data Sheet for Recording of Aberrations using the Automated Microscope

Slide ID:

Case Name:

Scorer:

Microscope N° :

Date:

| D | D+AF | R | R+AF | TRQ | AF | CT | 47chr | Other | # pieces | Comment | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | | | 1 | | | | 47 | #14 (record cell # from software) | 1 |
| | 2 | | 1 | | 2 | | | | 49 | #116 | 2 |
| | | | | | 2 | | | | 48 | #59 | 3 |
| | 1 | | | | | | | | 46 | #22 | 4 |
| | | | 1 | | | | | | 47 | #219 | 5 |
| | | | | | | | | 1 | 46 | #149 chromatid exchange | 6 |
| | 2 | | | | | | | | 46 | #89 1 tricentric | 7 |
| | | | | | | | | | | | 8 |
| | | | | | | | | | | | 9 |
| | | | | | | | | | | | 10 |
| | | | | | | | | | | | 11 |
| | | | | | | | | | | | 12 |
| | | | | | | | | | | | 13 |
| | | | | | | | | | | | 14 |
| | | | | | | | | | | | 15 |
| | | | | | | | | | | | 16 |
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| | | | | | | | | | | | 32 |

# Annex K: Sample Report

**Mr. Y**
**General Hospital**
**1 Main Street**
**Anytown, ON**
**A1A 1A1**

**Protected**
**Medical - Confidential**

Phone : **(555) 555-5555**
Fax : **(555) 555-4444**

Dose Estimates for **Mr. John Doe**:

Dear **Mr. Y**

This report is to inform you of our cytogenetic assessment of the radiation dose received by **Mr. John Doe**, born **May 17, 1952**. This cytogenetic assessment was requested **June 28, 1999** for the following reasons: **Mr. John Doe is an electronics technician at the General Hospital and while in the process of repairing a faulty hinge on a Co60 irradiator he may have been irradiated. Unfortunately, he was not wearing a TLD at the time.**

The dicentric assay is used to estimate the acute whole body radiation dose received by an individual. The assay was performed on blood samples received by our Laboratory on **June 29, 1999** (blood collected from **Mr. Doe June 28 1999**) and processed under the case code number **00001** by using the routine culture method of our laboratory. The expected incidence of dicentric or ring chromosomes in a normal, unexposed individual is 1 aberration (range 0 to 2) in 1000 lymphocyte cells at first metaphase as reported in literature and confirmed by our laboratory. These types of aberrations are representative of damage observed after exposure to radiation or radiomimetic drugs.

We randomly assessed **801** metaphase cells and found the equivalent of **18** dicentrics, **1** centric ring and **15** excess acentric fragments. Based on interpolation from a standard dose-response curve for **Iridium-192** and 95% confidence levels, we estimate **Mr. Doe** received no more than **0.52** Gy (95% upper confidence level) and no less than **0.26** Gy (95% lower confidence level) with a mean dose of **0.40** Gy.

Should you have any further questions about this assessment please do not hesitate to contact me.

Yours sincerely,

Dr. Diana Wilkinson, Defence Scientist

DRDC Ottawa
Radiological Analysis and Defence Group
3701 Carling Ave.
Ottawa, ON, K1A 0Z4

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E-mail : Diana.Wilkinson@drdc-rddc.gc.ca

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**Medical - Confidential**

# Annex L: Statistical Analysis for Calculating the Confidence Limits

Upper and lower confidence limits of an approximate (1-α) 100% confidence interval ($x_L$, $x_U$) are given by the positive real roots of

$$d^2(x)/V[d(x)] = \chi^2_{1,1-\alpha}, \tag{2}$$

where $\chi^2_{1,1-\alpha}$ is the (1-α) percentile of the chi-square distribution with 1 degree of freedom;

$d(x) = y^* - \hat{y}^*(x) = y^* - (\hat{\beta}_0 + \hat{\beta}_1 x + \hat{\beta}_2 x^2)$ is the estimated residual, and is distributed asymptotically normal with variance $V[d(x)] = V[y^*] + V[\hat{y}^*(x)]$.

Estimates of the variances $V[y^*]$ and $V[\hat{y}^*(x)]$ are obtained as follows: as $r^*$, the number of aberrations in $n^*$ cells irradiated with unknown dose $x$ is assumed to follow a Poisson distribution, the variance of $y^*$ is $\hat{V}(y^*) = (\hat{\beta}_0 + \hat{\beta}_1 x + \hat{\beta}_2 x^2)/n^*$; and an estimate of the variance of the expected yield is $\hat{V}[\hat{y}^*] = x'\Sigma x$, where $\mathbf{x}' = (1\ x\ x^2)$, and Σ is the variance covariance matrix of the parameter estimates of the linear quadratic model. Equation (2) reduces to solving for the positive roots of the following fourth power polynomial

$$ax^4 + bx^3 + cx^2 + dx + e = 0, \text{ where,}$$

$$a = \hat{\beta}_2^2 - s_{22}\chi^2_{1,1-\alpha},$$

$$b = 2\hat{\beta}_1\hat{\beta}_2 - 2s_{12}\chi^2_{1,1-\alpha},$$

$$c = \hat{\beta}_1^2 + 2\hat{\beta}_2(\hat{\beta}_0 - y^*) - (s_{11} + 2s_{02} + \hat{\beta}_2 / n^*)\chi^2_{1,1-\alpha},$$

$$d = 2\hat{\beta}_1(\hat{\beta}_0 - y^*) - (2s_{01} + \hat{\beta}_1 / n^*)\chi^2_{1,1-\alpha}, \text{ and}$$

$$e = (y^*)^2 + \hat{\beta}_0(\hat{\beta}_0 - 2y^*) - (s_{00} + \hat{\beta}_0 / n^*)\chi^2_{1,1-\alpha}.$$

The terms $s_{00}$, $s_{01}$, $s_{02}$, $s_{11}$, $s_{12}$, and $s_{22}$ are the variance covariance terms of the matrix Σ.

## References

Merkle, W. (1983). Statistical methods in regression and calibration analysis of chromosome aberration data. Radiation and Environmental Biophysics 21:217-233.

## Qualifications of the Statistician

The statistics described in this report were performed by Leonora Marro, a statistician with the Biostatistics and Epidemiology Division of the Healthy Environments and Consumer Safety Branch at Health Canada. She has a BA in Mathematics, and MSc in Statistics with 5 years applied statistical experience, as well as research in applied statistical methodology.

# Annex M: Laboratory Member Qualifications

| | Lachapelle, Sylvie | Mullins, Dana | Prud'homme -Lalonde, Louise | Qutob, Sami | Segura, Tamika | Thorleifson, Erika | Wilkinson, Diana |
|---|---|---|---|---|---|---|---|
| **Receiving** | | | X | | | X | X |
| **Coding** | | | X | X | | X | X |
| **Culturing-Fixing** | X | X | X | | X | X | X |
| **Positive Control** | X | X | X | X | X | X | X |
| **Preparing Slides** | X | X | X | X | X | X | X |
| **Blinding Slides** | X | X | X | X | X | X | X |
| **Scoring Slides** | X | X | X | X | X | X | X |
| **Conversion to Dose** | X | X | X | X | X | X | X |
| **Decoding** | | | X | X | | X | X |
| **Report Writing** | | | X | X | | X | X |

# Annex N: Document Creation or Revision Form

**SECTION A – CHANGE REQUEST**

**A. TYPE of DOCUMENT**

☐ SECTION : _____ ☐ ANNEX : _____

**B. NATURE of CHANGE**

☐ EXISTING DOCUMENT ☐ NEW DOCUMENT

**INDICATE CHANGE:**_____

_____

_____

_____

(Attach sample if changes are extensive.)

**REQUEST BY:** _____

**DATE:** _____

**SECTION B – REQUEST AUTHORIZATION**

**ISO QUALITY MANAGER:** _____

**LABORATORY HEAD:** _____

**EFFECTIVE DATE:** _____

# Annex O: Sample Chain of Custody Form

DEFENCE R&D DÉFENSE

Defence Research Establishment Ottawa

Radiation Analysis & Defence
Radiation Biology
3701 Carling Ave
Ottawa, Ontario K1A 0Z4
Phone: 613-998-5995 Fax: 613-998-4560

**SAMPLER (PRINT & SIGN NAME):** _____
**HOSPITAL OR LABORATORY:** _____
**ADDRESS:** _____
**CITY:** _____ **PROVINCE:** _____ **POSTAL CODE:** _____
**PHONE NO.:** _____ **FAX NO.:** _____
**E-mail:** _____

**TOTAL NO. SAMPLES:** _____ **RESEARCH SAMPLE** ☐ **RUSH/EMERGENCY*** ☐

*If this is an Emergency sample, please print a 24-hour contact name and number.
**NAME:** _____ **TELEPHONE NO.:** _____

**Date Submitted:** _____ DD/MM/YYYY

**Please fill all appropriate boxes to assure accurate contacting and submission of sample(s).**

**Please fill Submitter's name in Chain-of-Custody box. Thank you.**

**SAMPLE DATA**

| SAMPLE NO. | SAMPLE DESCRIPTION | SAMPLE VOLUME | DATE SAMPLED | TIME SAMPLED | ANTI-COAGULANT | SHIPPING CONTAINER TYPE | REMARKS |
|---|---|---|---|---|---|---|---|
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |

| RELINQUISHED BY | DATE | TIME | RECEIVED BY | DATE | TIME |
|---|---|---|---|---|---|
| | / / | AM / PM | | / / | AM / PM |
| | / / | AM / PM | | / / | AM / PM |
| | / / | AM / PM | | / / | AM / PM |

**NOTE**: Until the sample has been relinquished to someone else, you are responsible to ensure that no one is able to tamper with the contents.

**FOR ANALYTICAL LAB USE ONLY**

| | | | | |
|---|---|---|---|---|
| **TEMP:** | **Frozen** | **Cold** | **R. Temp** | **Warm** |
| **INTACT:** | | | **YES / NO** | |
| **SAMPLE SEALED:** | | | **YES / NO** | |

**COMMENTS:**

# Annex P: Corrective Action Report (CAR)

**SECTION A: IDENTIFIED NON-CONFORMITY AND RESOLUTION**

**1.0 DEFINITION AND ORIGIN OF THE NON-CONFORMITY**

**2.0 ROOT CAUSE(S) OF THE NON-CONFORMITY**

**3.0 ACTION PLAN**

_____ _____

**QUALITY REVIEW MANAGER** **DATE**

**SECTION B: DELIVERY OF ACTIONS**

I acknowledge that the required actions(s) specified by the Quality Review Manager in Section A, have been completed by the date indicated below.

_____ _____

**EMPLOYEE** **DATE**

**SECTION C: VERIFICATION**

I authorize the immediate closure of Internal Action Report, since:

_____ _____

**QUALITY REVIEW MANAGER** **DATE**

# Annex Q: Report of Non-Conformity

**SECTION A – REPORTING and NOTIFICATION of NON-CONFORMITIES**

DATE _____

REPORTING PERSONNEL _____

SUSPECTED NON-CONFORMITY _____

**ISSUED AGAINST**

A. REQUISITIONED MATERIAL ☐
B. THIRD PARTY SUPPLIER ☐
C. DRDC PERSONNEL ☐
D. OTHER ☐

**PARTIES NOTIFIED BY**

A. CLIENT ☐
B. SUPPLIER ☐
C. LABORATORY HEAD ☐
D. QUALITY MANAGER ☐
E. OTHER ☐

**SECTION B - DISPOSITION**

**CORRECTIVE ACTION**

A. LABELLED AS NON-CONFORMING ☐
B. SEGREGATED FROM OTHER INVENTORIES ☐
C. CLIENT CONCESSION ☐
D. OTHER _____

**PREVENTATIVE ACTION**

A. EXPERIMENT REVIEW ☐
B. DRDC WORKING STANDARDREVIEW ☐
C. CHANGE IN PURCHASING STATUS ☐
D. OTHER _____

_____

**ROUTE of DISPOSITION**

_____
LABORATORY HEAD

_____
QUALITY REVIEW MANAGER

# Bibliography


1. DOLPHIN, G.W. "Biological dosimetry with particular reference to chromosome aberration analysis. A review of methods", Handling of Radiation Accidents (Proc. Int. Symp. Vienna, 1969), IAEA, Vienna (1969) 215-224.

2. EVANS, H.J. Chromosome aberrations induced by ionizing radiations. *Int. Rev. Cytol*. 13 (1962) 221-321.

3. GUM: "BIPM/IEC/IFCC/ISO/IUPAC/IUPAP/OIML Guide to the expression of uncertainty in measurement" (1995).

4. LAMBERT, B., HANSSON, K., LINDSTEN, J., STEN, M., WERELIUS. B. Bromodeoxyuridine- induced sister chromatid exchanges in human lymphocytes. *Hereditas* 93 (1976) 163-174.

5. LLOYD, D.C., EDWARDS, A.A. "Chromosome aberrations in human lymphocytes: Effect of radiation quality, dose and dose rate", Radiation-induced Chromosome Damage in Man (ISHIHARA, T., SASAKI, M.S., Eds), Alan R. Liss, New York (1983) 23-49.

6. LLOYD, D.C., EDWARDS, A.A., MOQUET, J.E., GUERRERO-CARBAIAL, Y.C. The role of cytogenetics in early triage of radiation casualties. *Appl. Radiat. Isotopes*. 52 (2000) 1107-1112.

7. MOORHEAD, P.S., NOWELL, P.C., MELLMANN, W.J., BATTIPS, D.M., HUNGERFORD, D.A. Chromosome preparations of leukocytes cultured from human peripheral blood. *Exp. Cell Res*. 20 (1960) 613-616.

8. PAPWORTH, D.G. Curve fitting by maximum likelihood. *Radiat. Bot*. 15 (1975) 127-140.

9. PERRY, P., WOLFF, S. New Giemsa method for the differential staining of sister chromatids. *Nature* (London) 2SI (1974) 156-158.

10. PROSSER. J.S., MOQUET. J.E. The effect of blood storage on differential chromosome staining of human lymphocytes. *Experientia* 39 (1983) 778-780.

11. PURROTT, R.J., VULPIS, N., LLOYD, D.C. The influence of incubation temperature on the rate of human lymphocyte proliferation in vitro. *Experientia* 37 (1981) 407-408.

12. PURROTT, R.J., VULPIS, N., LLOYD, D.C. Chromosome dosimetry. The influence of culture media on the proliferation of irradiated and unirradiated human lymphocytes. *Radiat. Prot. Dosim*. 1 (1981) 203-208.

13. SASAKI, M.S., MIYATA, H. Biological dosimetry in atomic bomb survivors. *Nature* (London) 220 (1968) 1189-1193.

14. SAVAGE, J.R.K. Classification and relationships of induced chromosomal structural changes. *J. Med. Genet*. 13 (1976) 103-122.

15. VOISIN, P., BENDERITTER, M., CLAARAZ, M., CHAMBRETTE, V., SOROKINE-DURM, I., DELBOS, M., DURAND, V., LEROY, A., PAILLOLE, N. The cytogenetic dosimetry of recent accidental overexposure. *Cell. Mol. Biol*. 47 (2001) 557-564.

16. IAEA:1986, Chromosomal Aberration Analysis for Dose Assessment — Technical Reports Series N°260.

17. IAEA:2001, Cytogenetic Analysis for Radiation Dose Assessment, A Manual — Technical Reports Series No. 405.

18. ISO 5725-1, Accuracy (trueness and precision) of measurement methods and results -- Part 1: General principles and definitions.

19. ISO 19238-1, Radiation protection – Performance criteria for service laboratories performing biological dosimetry by cytogenetics; International Standard.

# List of symbols/abbreviations/acronyms/initialisms

| | |
|---|---|
| 2N | Diploid cell (46 chromosomes) |
| 4N | Cell finishing synthesis, 92 chromosomes |
| AECL | Atomic Energy Canada Limited |
| BrdU | Bromodeoxyuridine |
| C | Celsius |
| CAR | Corrective Action Report |
| CBRN | Chemical Biological Radiological and Nuclear |
| cm | Centimeter(s) |
| $CO_2$ | Carbon dioxide |
| CRTI | CBRN Research and Technology Initiative |
| dd | Double distilled |
| DND | Department of National Defence |
| DRDC | Defence Research and Development Canada |
| EtOH | Ethanol |
| FBS | Fetal Bovine Serum |
| FISH | Fluorescent in situ hybridization |
| FPG | Fluorescence plus Giemsa |
| Gy | Gray |
| h | Hour(s) |
| $H_2O$ | Dihydrogen oxide, water |
| HCl | Hydrochloric acid |
| HIV | Human immunodeficiency virus |

| | |
|---|---|
| HREC | Human research ethics committee |
| IAEA | International Atomic Energy Agency |
| ISO | International Organization for Standardization |
| KCl | Potassium Chloride |
| LET | Linear energy transfer |
| M | Molar |
| M1 | First metaphase |
| M2 | Second metaphase |
| M3 | Third Metaphase |
| mg | Milligram(s) |
| min | Minute(s) |
| mM | Millimolar(s) |
| mL | Milliliter(s) |
| $Na_2HPO_4$ | Disodium Phosphate |
| NBDRP | National Biological Dosimetry Response Plan |
| NCR | National Capitol Region |
| nm | Nanometer(s) |
| PBS | Phosphate buffered Saline |
| Pen-strep | Penicillin - Streptomycin |
| PHA | Phytohaemagglutinin |
| Qdr | Quadratic |
| RPMI | RPMI was developed at Roswell Park Memorial Institute, hence the acronym RPMI |
| RT | Room temperature |
| s | second(s) |

| | |
|---|---|
| SI | International System of Units |
| TDG | Transportation of Dangerous Goods |
| TLD | Thermoluminescent dosimeters |
| μg | Microgram(s) |
| μl | Microliter(s) |
| UV | Ultraviolet |
| W | Watts |
| WHMIS | Workplace Hazardous Materials Information System |

# Glossary

| Technical term | Explanation of term |
|---|---|
| acentric | Terminal or interstitial chromosome fragment of varying size. When it is formed independently of a dicentric or centric ring chromosome aberration, it is usually referred to as an excess acentric. |
| background level of aberrations | Spontaneous frequency (or number) of chromosome aberrations recorded in control samples or individuals. |
| bias | A statistical sampling or testing error caused by systematically favouring some outcomes over others. |
| centric ring | Aberrant circular chromosome resulting from the joining of two breaks on separate arms of the same chromosome (generally accompanied by an acentric fragment). |
| centromere | Specialized constricted region of a chromosome that appears during mitosis joining together the chromatid pair. |
| 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. |
| chromosome | 46 of these structures that carry genetic information are normally contained in the human cell nucleus. During nuclear division they condense to form characteristically shaped bodies. |
| Colcemid® | Methylated derivative of colchicines. A drug isolated from the *Autumn crocus* that blocks microtubule assembly, and as a result will block mitosis at metaphase, preventing the completion of cell division. |
| confidence interval | Statistical range about an estimated quantity within which the value of the quantity is expected to occur, with a specified probability. |
| cytochalasin B | A mold metabolite that inhibits cell division by blocking formation of contractile microfilament structures, resulting in multinucleated cell formation. |
| dicentric | Aberrant chromosome bearing two centromeres derived from the joining of parts from two broken chromosomes. It is generally accompanied by an acentric fragment. |
| FISH (Fluorescence in | Technique that uses specific sequences of DNA as probes to |

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|---|---|
| situ Hybridization) | particular parts of the genome, allowing the chromosomal regions to be highlighted or “painted” in different colours by attachment of various fluorochromes. This technique permits the detection of damage involving exchanges between differently painted pieces of DNA (usually whole chromosomes). |
| harlequin chromosome | Chromatids that stain differently, so that one appears dark and the other light (harlequin-like). |
| interphase | Period of a cell cycle between the mitotic divisions. |
| LET (Linear Energy Transfer) | Defined by the International Commission on Radiation Units and Measurements (ICRU) as the quotient of dE/dl, where dE is the average energy locally imparted to the medium by a charged particle of specific energy in traversing a distance of dl. In other words, it is the rate at which the energy of the radiation is transferred to a medium (i.e. tissue). |
| metaphase | Stage of mitosis when the nuclear membrane has dissolved, the chromosomes condensed to their minimum lengths and aligned for division. |
| pH | A measure of the acidity or alkalinity of a solution, numerically equal to 7 for neutral solutions, increasing with increasing alkalinity and decreasing with increasing acidity. The pH scale commonly in use ranges from 0 to 14. |
| precision | Concept employed to describe dispersion of measurements with respect to a measure of location or central tendency. |
| 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. |
| quality control | Part of quality assurance intended to verify that systems and components conform to predetermined requirements. |
| service laboratory (in this standard) | Laboratory performing biological dosimetry measurements. |

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This Standard provides quality assurance, quality control, and evaluation of the performance criteria for the purpose of accreditation of our laboratory using biological dosimetry to predict radiation exposure doses. The International Standard (ISO 19238) and the International Atomic Energy Association (IAEA) Technical Report Series No. 405 are used as guiding documents in preparation of this working document specific to Defence Research & Development Canada - Ottawa (DRDC Ottawa) Radiation Biology Laboratory.

This Standard addresses:

1. The confidentiality of personal information, for the customer and the service laboratory;
2. The laboratory safety requirements;
3. 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;
4. Transportation criteria for shipping of test samples to the laboratory;
5. Preparation of samples for analysis;
6. The scoring procedure for unstable chromosome aberrations used for biological dosimetry;
7. The criteria for converting a measured aberration frequency into an estimate of absorbed dose;
8. The reporting of results;
9. The quality assurance and quality control plan for the laboratory;
10. Informative annexes containing examples of a questionnaire, instructions for customers, a data sheet for recording aberrations, a sample report and other supportive documents.

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