Accreditation of Bio-dosimetry Laboratories for Assessment of Personnel Radiation Exposures

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Atomic Energy Regulatory Board
Niyamak Bhavan, Anushakti Nagar,
Mumbai – 400 094, INDIA.

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Annexures

Foreword

Biological dosimetry based on the chromosomal analysis has been used since mid 1960s for the estimation of accidental external exposures due to x-rays or gamma radiations. The technique has also been extensively used for investigation of suspected overexposure cases. In India, presently, such measurements are being carried out in only two laboratories of the Department of Atomic Energy (DAE). Recently, it was considered that it would be advantageous if such measurements can be carried out in academic and private institutions also. All such institutions have to be accredited by an appropriate authority to ensure use of proper standards and quality assurance in the assessment of exposures. The Atomic Energy Regulatory Board (AERB) is entrusted with the responsibility for laying down safety standards and framing rules, regulations and procedures covering regulatory functions envisaged under the Atomic Energy Act, 1962. AERB has therefore undertaken a programme of accreditation of laboratories carrying out biological dosimetry.

This booklet describes the operational and technical requirements to be met by laboratories desiring accreditation for biodosimetric measurements. It also contains details of sample preparation, culturing, processing, scoring procedures, post accreditation responsibilities of laboratories and a format of the application for seeking accreditation. The booklet has been prepared by a Committee consisting of AERB staff and other experts in the field. In drafting it, extensive use has been made of the information contained in relevant documents of IAEA and the rich experience of DAE in the area over the past several decades. The list of experts who have contributed to the preparation of the booklet is appended to the booklet.

S.K. Sharma

Chairman, AERB

1. Introduction

Biodosimetry is being used during the last four decades for estimating excessive external exposures due to X or gamma radiation under accidental condition. The information on the absorbed dose helps the physicians in planning the course of treatment to the exposed individuals and provides an important input to investigations of the accident by the regulatory authority. Biodosimetry is particularly useful where details of events are not clear and no physical dose measurements are available. Currently such bio-dosimetric assessments are carried out in Bhabha Atomic Research Centre (BARC) in Mumbai and Indira Gandhi Centre for Atomic Research (IGCAR) in Kalpakkam. With the large increase in the use of radiation sources and radiation technology for various applications in the country, it is considered necessary to have more biodosimetry laboratories in different regions of the country. Clearly, such institutions have to be accredited by competent national authorities to ensure proper standards and quality assurance in the assessment of radiation exposures. It is recognized that the reliability of the results from such laboratories depends on the facilities, manpower and methodology followed by the laboratories. It is therefore recommended that such laboratories adopt the procedures laid down in this booklet and participate in inter-laboratory comparisons to establish and maintain the validity of their results.

2. Purpose and Scope

The purpose of this booklet is to provide the details of administrative, operational and technical requirements for the accreditation of laboratories for assessment of radiation exposures by biodosimetry techniques. One of the common and well-recognized techniques is based on the frequency of dicentric chromosomes in peripheral blood lymphocytes of the exposed individual. Hence, the booklet provides the detailed procedure for this analysis. The booklet also gives details of requirements related to infrastructure and trained manpower for a laboratory seeking accreditation. Procedures followed by the accrediting agency for issuance of accreditation are also given in the booklet.

3. Accrediting Agency

In India, the Chairman, Atomic Energy Regulatory Board (AERB) is the Competent Authority for radiation surveillance of radiation workers, members of the public and the environment. He is the Competent Authority for issuance of accreditation to all laboratories (including DAE laboratories) engaged in the measurement of radiation exposures. A Standing Committee appointed by the Chairman, AERB recommends all procedures and terms and conditions for accreditation. It reviews all applications from laboratories. Based on the recommendations of the Committee, accreditation may be issued by the Chairman, AERB to a particular laboratory for carrying out biodosimetry work for the assessment of radiation exposure.

4. Infrastructure Requirements

Accreditation is granted to a laboratory having qualified and trained staff, adequate space, equipment and other requisite facilities, on demonstration of adequate procedures and documentation. The basic requirements to be satisfied by the laboratory are the following:

4.1. Laboratory Layout

Annexure-1 gives a layout of a typical laboratory designed to carry out biodosimetry work for the evaluation of radiation exposure. The laboratory should be designed and built according to the requirements of a normal bio-analytical laboratory with a few additional features such as laminar flow cabinets.

4.2 Facilities and Equipment

The laboratory shall maintain adequate facilities, equipment, chemicals, and services so as to accomplish the required function all the time. Following is the list of essential instruments and equipment needed for biodosimetry laboratory. Details of instruments required for biodosimetry work:

- Sterile hood with laminar flow
- Fume-hood for sample processing
- Microscope with fluorescence attachment

- Automated Metaphase finder system consisting of microscope, motorised stage, CCD camera, computer with software for metaphase finding, Karyotyping and FISH analysis (optional)
- Weighing balance, -70^0 C Freezer
- CO_2 incubator (preferable), otherwise ordinary incubator with a good thermostat,
- Cold centrifuge (4^0 C)
- Hot air oven, Wet and dry sterilizers
- Water distillation unit
- Table top centrifuge

4.3. Standard Methods for Assessment Of Exposure

The internationally recommended method for assessment of radiation exposure is by the analysis of dicentric chromosomes in peripheral blood of the exposed persons. It is important that the biodosimetry laboratories accredited for regulatory purposes follow a standard protocol of analysis. Annexure – 2 is the manual of procedure for carrying out the biodosimetry work for estimation of radiation of doses.

4.4. Qualified Man Power

The laboratory carrying out biodosimetric assessment of exposures shall have at least the following team.

- Laboratory Officer in-charge: M.Sc./ Ph.D degree in life science with five years experience in cytogenetics work – 1 post
- Laboratory Assistant: B.Sc. (Life science)/Diploma in medical laboratory technology – 1 post

At least one of the above two persons should have undergone a minimum three months training in an accredited laboratory doing the similar type of analysis.

5. Procedures for Issuance Of Accreditation

5.1. Submission of Application

The laboratory undertaking biodosimetric measurements for estimation of high radiation doses to persons shall apply to Chairman, AERB in the prescribed format as given in Annexure-3 for issuance of accreditation. The application form shall be completed and signed by the authorized representative (Head of the Institution) of the

laboratory seeking accreditation. The application shall be submitted Chairman, AERB by the laboratory along with the following documents:

a. Procedures for the assessment of radiation doses through CA analysis, laboratory details such as facilities, layout, etc
b. Staff and their bio-data along with the work responsibilities,
c. Quality assurance programme, and
d. Reports on participation in any inter-comparison programmes.

5.2. Pre-Accreditation On-Site Assessment Visit

AERB Committee shall review the applications for the accreditation of bio-dosimetry laboratories and the laboratory may be notified of any additional information, which shall be supplied prior to on-site assessment visit. If the details given in the application form are found to be satisfactory, the AERB Committee shall conduct an on-site assessment visit. The assessment visit shall comprise of:

- A meeting with the management and supervisory personnel responsible for the laboratory activities and discussions with technical personnel to ensure their understanding of the procedures,
- Demonstration of performance testing,
- Examination of major equipment, apparatus and facilities for their appropriateness, capability, adherence to specifications, etc.
- Review of personnel records and job descriptions of key personnel, competency of all the staff members, calibration procedures and test reports (if any) and quality assurance programme.

The Committee shall submit its assessment report to Chairman, AERB.

5.3. Procedure for performance testing

Coded slides from samples irradiated to different doses of radiation will be distributed to the laboratories without disclosing the exact doses. After the analysis of the slides, data sheets along with dose estimate and slides will be returned to the ARRB. The data and doses will be verified and the results will be sent to the various laboratories.

5.4. Quality Assurance (QA) Programme

5.4.1. Internal QA

The biodosimetry laboratory shall have a well-documented programme for quality assurance procedures to be followed for the assessment of exposures. The validity of analytical results should be checked by an internal programme, which incorporates the use of blind duplicates, blanks and standards. The laboratory should keep a record of the mitotic index, % of second cycled scored in 50 h cultures. The samples from control and those exposed to dose of 1 Gy should be processed for this purpose and the details should be kept in a separate logbook.

The laboratory shall maintain adequate backup equipment or systems for key processing steps to be used in the event of failure of a system. Continuity of quality service is a commitment of the laboratory. If it fails to provide the committed service at any point of time, the accreditation shall stand void.

5.4.2. External QA

The biodosimetry laboratory shall participate in the external quality assurance programme conducted by the AERB committee on an annual basis. The laboratory will receive irradiated coded slides from the committee. The laboratory will carry out the analysis of the slides and send the results of the estimated doses to the committee. The laboratory will be informed of the status of its performance and the need for any corrective measures if found necessary. The AERB committee may also conduct surprise spot test to ensure the quality of the service provide by the laboratory.

6. Conditions of accreditation

Based on the information submitted by the laboratory through the application, report on the on-site assessment visit, actions taken by the laboratory to correct any deficiencies and the results of the performance tests, the AERB Committee shall recommend to the Chairman, AERB for issuance of accreditation for the laboratory to undertake assessment of radiation exposures using biodosimetry techniques. Each accreditation shall be valid for a period of three years subject to meeting the terms and conditions of the same. The accredited laboratory shall not partly or fully subcontract the tasks assigned to any other laboratory. Application for renewal should be submitted at

least six months before the expiry of the accreditation. Renewal of accreditation may be granted depending upon the results of periodic quality assurance tests and the performance of the laboratory. During the period of validity of the accreditation, the laboratory shall be responsible for maintaining compliance pertaining to the availability of qualified and trained manpower, adherence to operational procedures as outlined in this booklet and quality assurance programme. In case of any modifications or changes in any of these stipulations, the laboratory shall inform the AERB Committee immediately. The Committee shall review the status of the laboratory in the light of the information submitted and give its recommendations to Chairman, AERB.

7. Post Accreditation Requirements

7.1. Requests for biodosimetry analysis

The laboratory shall carry out the biodosimetry work only on the receipt of a request from AERB or its official nominee. The results of the assessment shall be communicated only to AERB.

7.2. Documentation and record keeping

Physical and biological dose estimate pertaining to cases referred and people reported should be maintained in separate logbooks. These records should be checked by the scientific officer- in – charge of the laboratory / unit and signed by him.

7.3. Culture details aberration analysis and reports

Case history forms, culturing details and the aberration analysis data of the test sample should be verified by the officer. Any aberrant cell observed should be photographed /images stored in the case of image analyzer. Vernier reading should be noted against all the cells analyzed. Cytogenetic dosimetry report should be maintained in the appropriate format.

7.4. Record of QA performance

Record of external and internal QA checks should be maintained properly and separately. Each lab should maintain records of sensitivity check also separately.

7.5. Inter-comparison exercises

Exercises regarding scoring of chromosomal aberrations will be arranged by AERB committee among various accredited laboratories. Blood samples exposed to 0 Gy, 1 Gy,

and 2 Gy of ^{60}Co-gamma rays will be cultured, processed and dispensed on to slides at the central laboratory. The unstained slides along with score sheets and scoring procedure will be dispatched to participating accredited laboratories.

The slides should be stained and analyzed at different labs and data sheets along with slides should be sent back to the designated central laboratory. Different scorers should use different data sheets. Experience of the scorer and the number of the samples analyzed by the scorer should be indicated very clearly on the data sheets. Inter scorer variability and inter laboratory variability will be checked and results will be intimated to various laboratories.

7.6. Training for staff

Three months of training of culturing, processing and scoring of chromosomal aberrations will be provided to one person from each accredited laboratory.

8. References

1. Cytogenetic analysis for Radiation Dose Assessment – A Manual, Technical Report Series, No. 405, IAEA, 2001.

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5. AFRRI's gamma ray, x – ray and fission-neutron calibration curves for the lymphocyte dicentric assay: Application of a metaphase finder system, P.G.S. Prasanna et al., Armed Forces Radiobiology Research Institute, Bethesda, Maryland 20889-5603, USA.

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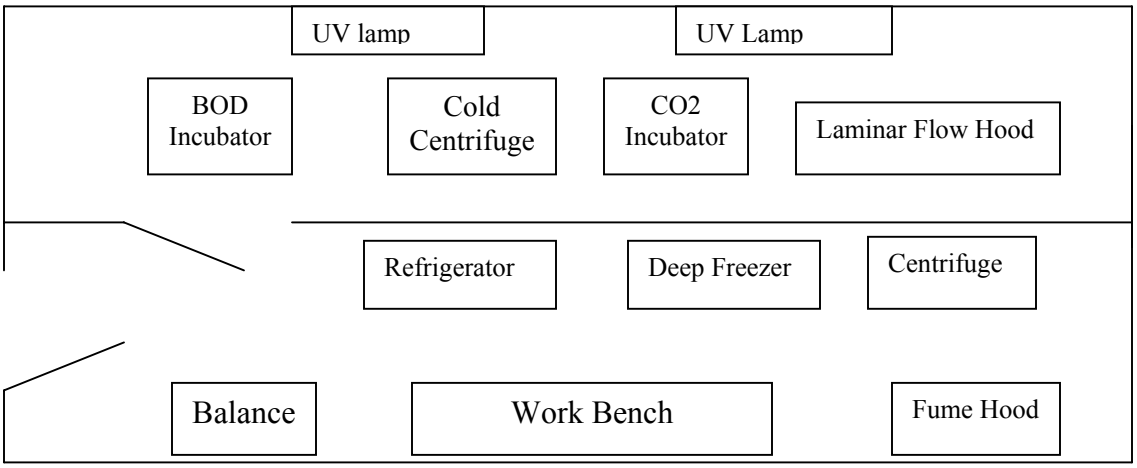
List of Contributors to the Booklet on Accreditation of Bio-dosimetry Laboratories for Assessment of Personnel Radiation Exposures

1.	Shri A.R. Sundararajan	-	Formerly AERB
2.	Dr. B.S. Rao	-	Formerly BARC
3.	Dr. R.K. Jeevanram	-	IGCAR
4.	Dr. B. Sreedevi	-	BARC
5.	Dr. P.R. Bongirwar	-	BARC
6.	Smt. M.V. Inamdar	-	AERB

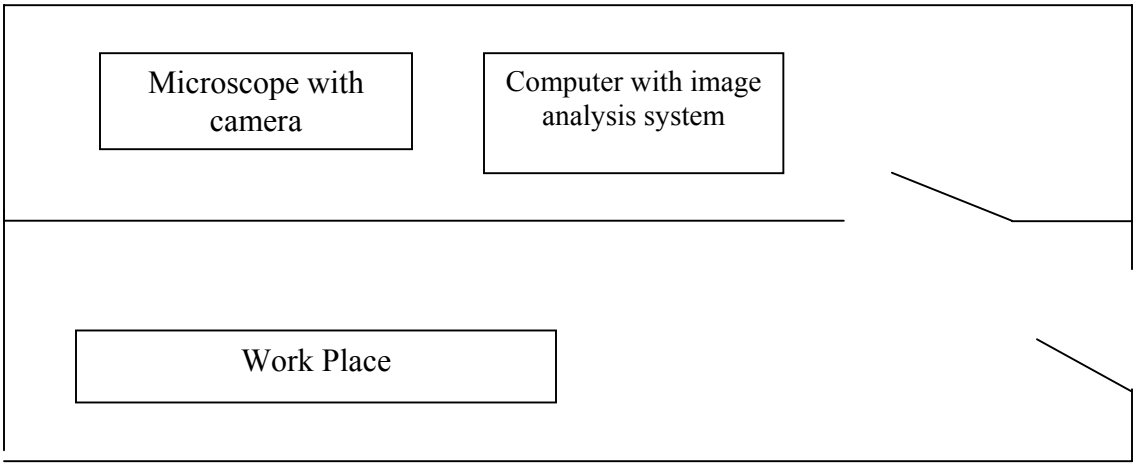
Annexure-1

Layout for a Typical Bio-dosimetry Laboratory

Layout of Cell Culture Facility



Layout of the room for scoring of chromosome aberration



Annexure - 2

Procedure for Assessment of Exposure by Bio-Dosimetry

A.2.1 Introduction

Among various types of chromosome aberrations, the frequency of dicentric chromosomes is studied in peripheral blood lymphocytes to estimate absorbed dose to overexposed persons. 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 as isolated lymphocytes and 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 booklet. Stained microscope slides are methodically scanned to identify dicentric and ring aberrations. The frequency of dicentrics observed in an appropriate number of scored metaphases is converted to an estimate of radiation dose by reference to calibration curve. This curve is generated by exposure of blood *in vitro* to graded doses of the appropriate quality of radiation. It is recommended that laboratories undertaking biological dosimetry should calibrate their procedures in terms of absorbed dose specifying, where appropriate, the radiation type involved.

A 2.2 Experimental Procedure

A.2.2.1 Sample collection

Peripheral blood sample (about 2 ml) is collected from the individual in a vacutainer containing lithium heparin. In the event of partial – body / localized exposure sample may be obtained after 24 hour. In general, most of the accidental exposures and non-uniform and hence, sample collection is ideally done 24 hour after the exposure. Personal details of the worker like Name, IC number, TLD number, age, weight, height etc., are noted in a questionnaire (BD-Form 1, Annexure 2.1)

A.2.2.2 Transport of the sample

The blood specimen may be transported to the laboratory from accident place by providing sufficient coolant packs such that the transportation of the specimen is maintained between 18^0C and 24^0C.

A.2.2.3 Cell culturing

Sterile disposable plastic containers that can be sealed may be used as culture vial. For a 5 mL culture the headspace should be at least 10 mL. 0.5 mL blood sample obtained from the individual is mixed with 4.5 mL cell culture medium (RPMI-1640, Sigma) supplemented with $NaHCO_3$, 20% fetal calf serum, 200mM L-glutamine, 100 units/mL penicillin and 100μg/mL streptomycin taken in a 10 ml culture tube / vial. To this mixture 0.2 mL phytohaemagglutinin-M (PHA-M, Sigma) is added to initiate culture and 100 μL of Bromodeoxyuridine (BrdU) to permit FPG staining. Vials are wrapped in aluminum foil and placed in an incubator maintained at 37^0C at 5% CO_2 atmosphere and humidified condition.

After 47 hours, colcemid is added to a final concentration of 0.1 μg/mL and incubated for a further duration two hours. The contents of the vial are transferred to a 15 mL conical test tube and centrifuged at 1000 rpm for five minutes. The pellet obtained is mixed with 5 ml 0.075M KCl (drop wise) and incubated for 20 minutes at 37^0C. This is followed by centrifugation and three washes with Carnoy's solution (3:1 methanol: acetic acid mixture added drop wise) and finally suspended in 0.5 mL of the same solution. All the above steps are carried out in subdued light, as BrdU is sensitive to light.

A few drops of the above suspension is cast on clean chilled microscope slides, dried and then stained with Hoechst 33258 for ten minutes, washed and mounted with Mcllvaine's buffer, exposed to sunlight for two hours, or long wave UV light (360 nm) from a black ray lamp (distance 2 cm, 20 J/m2/s) for 15 – 20 min on a slide warmer tray kept at 60^0C. Finally the slides are washed in buffer (pH 6.8) and stained with 5% Giemsa solution in Sorenson's buffer, air dried and mounted with DPX mountant. Slides are examined under 10 × 100x oil immersion. The metaphases in first mitosis (M1) having uniform staining are scored for dicentrics and rings. The criteria for scoring are the presence of 46 centromeres. A fragment accompanying each dicentric and centric ring

scored. Metaphases may be captured using an automated metaphase finder system. A format for the score sheet (BD Form – 02) for chromosome aberration is given in Annexure 2.2

A.2.2.4. Preparation of reagents

RPMI - 1640 medium

The medium, RPMI-1640 is in a powder form and contains 30 mg L-glutamine and 25 mM Hepes buffer. The entire content of the vial (16.4 g) is dissolved in about 800 ml of sterile double distilled water. 2 g of sodium bicarbonate is added and mixed well in a vortex mixer for 1 hr. The pH is adjusted to 7.4. The volume is made up to 1000 ml with double distilled water. Medium is sterilized by milli-pore filtration and stored at 4^0C.

Colcemid stock solution

5 mg of colcemid is dissolved in 25 mL of sterile distilled water to obtain a concentration of 200 µg/mL.

Working solution

100 µL of the above stock solution is made up to 10 ml using normal saline to obtain a working solution of 2 µg/mL.

Phytohemagglutinin-M

Stock solution

100 mg is dissolved in 10 mL RPMI medium

Working solution

1 ml of the stock is mixed with 9 mL RPMI medium

0.075 M KCl solution

This is prepared by dissolving 0.56 g of KCl in 100 mL distilled water.

Antibiotics

Preparation of stock solution

50 mL of sterile, PBS is used to dissolve the content of the vial to get a final concentration of 10,000 units/mL of penicillin and 10 mg/mL of streptomycin.

Working solution

1 ml of the above solution is added to 99 mL of RPMI 1640 to get concentrations of 100 U/ml of penicillin and 100 µg/mL of streptomycin.

Carnoy ’s fixative (3:1 Methanol to Acetic acid)

200 ml Carnoy ’s fixative is prepared by mixing 150 mL methanol and 50 mL acetic acid.

Sorensen’s Buffer (pH-6.8)

Solution 1 Na_2HPO_4 0.980 g /100 mL sterile H_2O

Solution 2 KH_2PO_4 0.908 g /100 mL sterile H_2O

Solutions 1and 2 are mixed and adjusted the pH to 6.8. Freshly prepared buffer is used for each experiment.

Hoechst 33258 stock solution

10 mg is dissolved in 100 mL sterile water and stored at -20^0C in dark

Hoechst 33258 working solution

5ml of the stock is diluted to 50 mL with sterile water and prepared fresh.

Bromodeoxyuridine (BrdU) stock solution

15.35 mg of BrdU powder is dissolved in 100 mL of PBS to get a concentration of 0.5mM and stored at -20^0C in dark.

BrdU working solution

100 μL of the stock solution is added to 5 mL of the culture to get a final concentration of 10 μM

Giemsa stain (100ml Merck)

5% Working solution

Is prepared by mixing 2.5 mL Giemsa solution with 47.5 mL Sorensen’s buffer

Mcllvaine’s Buffer (pH 8.25)

Stock solution

Solution A : 1 M, Na_2HPO_4 is prepared by dissolving 14.2g in 100 mL distilled water

Solution B : 0.1M Citric acid is prepared by dissolving 2.1g in 100 mL distilled water.

Solution A and Solution B are stored separately at 4^0C and mixed at suitable proportions just before use.

Working solution

Solution A: 10 mL Solution B: 1.15 mL Distilled water: 38.85 mL

Working solution is prepared by mixing solution A and Solution B and distilled water as mentioned above. pH is adjusted to 8.25

A.2.2.5. Analysis of slides

The slides should be coded to prevent bias in scoring and should be scanned methodically so that the entire area is covered. The scanning should be done at low magnification to prevent a bias towards selecting cells which contain aberrations as at this level it is not possible to detect aberrations and also not possible to count whether all chromosomes are present. Scoring is carried out at higher magnification only when the chromosome images are sharp and without overlapping of chromosomes. With FPG stained material the cell will be rejected if it displays the harlequin effect, as it does not belong to first cycle. It is recommended that only complete metaphases be recorded i.e. those with 46 or more pieces. If the spread contain dicentric then it should have an acentric fragment yet it should count 46 pieces. At the same time the spread contains a centric ring and accompanying fragment then the number of pieces should count to 47. Acentrics not associated with dicentric or centric ring will be counted as excess acentric. Tricentric aberrations are equivalent to two dicentrics and should have two accompanying acentrics while quadricentrics will have three fragments and so on. All abnormalities should be recorded. The x and y coordinates should be recorded for all cells analysed.

A.2.2.6. Recording of Data

The slides prepared for scoring aberrations and the data sheet should be given unique identifier code for future reference. This data sheet for recording aberrations is given in Annexure 2.2.

A.2.2.7. Storage of slides and samples

Laboratories are obliged to store the microscope slides. Giemsa stained preparations have a tendency to fade and FPG stained material creates more difficulty as it frequently fades after several months. Stained slides may be kept in a box in a dry place at room temperature. With conventional Geimsa stain faded slides can be restained by carefully soaking off the cover slip. Attempts to restain with FPG have not succeeded. It may be better to store surplus fixed cells at -20^0C, which can be stained conventionally with FPG.

A.2.2.8. Dose estimate

An important aspect of chromosome aberration dosimetry is the construction of an in vitro dose response curve. Dose response data obtained with low LET radiation have generally been shown to fit the linear quadratic equation $Y = \alpha D + \beta D^2 + C$ where Y is the yield of aberration for dose D, C the base line aberration frequency in control population and α and β are linear and quadratic coefficients respectively. A programme known as "Poly fit" developed by NRPB (UK) can be used to construct the dose – response curve. The essence of this programme is to fit the data points, by a weighted least squares method, thereby taking account of the scoring effort for each point. Using the above programme the dose-response curve for γ radiation is plotted and also the value for α and β coefficients are found out.

A.2.2.8.1 Error of dose estimate

The two important sources of error in the calibration curve are calibration error and Poisson error. These are added to get the total error of the dose estimate.

Calibration error

Ideally 100 dicentrics per data point should be scored for a reliable calibration curve. This is practically impossible at doses < 0.5 Gy. However, a few thousand cells are scored in this dose region. Since most accidents involve doses < 0.5 Gy the calibration curve should have 3-4 data points in the dose range of 0 – 0.5 Gy. Generally, the calibration error is of the order of ± 10 % and does not vary significantly depending upon the dose level.

Poisson error

Poisson error is the most important source of error at doses < 1.0 Gy. For a uniform whole body dose the distribution of dicentrics among the cells follow a perfect poisson distribution. At doses close to the detection threshold, the error of the dose estimate is close to ± 100 % whereas; at 1 Gy the error reduces to ± 15 %. As a result, calibration error makes a negligible contribution to dose estimates < 250 mGy and hence can be ignored.

The interpretation of dose using a calibration curve produced elsewhere may introduce extra uncertainty in the estimation of dose and hence it is recommended that each biodosimetry laboratory should establish its own dose-response data. For construction of dose response curve freshly taken blood specimens in lithium heparin tubes should be used and irradiated as a whole blood at 37^0C. During irradiation the blood specimen should be exposed to x or γ - radiation uniformly. For one cm thick sample the distance between source and sample should be at least 1 m so that the dose between front and back will be less than 2 %. The dose – rate should be chosen such that all doses are given in less than 15 min. This is chosen because the difference in the time of exposure between different doses will be sufficiently small that the β coefficient of yield will be influenced by no more than about 4 %. The irradiated samples should be cultured by a standard method identical to that used for specimens from suspected over exposure cases. Generally for large doses (> 1 Gy) contribution from both the Poisson and calibration errors are of similar magnitude when 500 metaphases are scored. Total uncertainty of the frequency (Y) at 95 % confidence level is obtained by the relationship

$$[\ \mathrm{Var}\ Y_{calib} + (\sqrt{\ n/N\ })^2\ ]^{1/2} \times 1.96$$

Where Var Y_{calib} is the variance of Y at a dose D. Var Y can be obtained from the relationship

$$\mathrm{Var}\ Y = \mathrm{Var}\ C + D^2\ \mathrm{Var}\alpha + D\ \mathrm{Var}\beta$$

Where α and β are the linear and quadratic coefficients respectively and C the base line frequency.

Dose values corresponding to upper and lower Y values are read from the calibration curve.

When the absorbed dose < 0.5 Gy the 95 % of confidence limit of the estimate approaches ± 50 % of the mean value. Generally lower doses primarily provide only qualitative information such as whether the dose received is genuine or not. Whenever only single dicentric chromosome is scored in 500 metaphase cells then there is a possibility that nearly 25 % of the time it could be false positive. Hence, The judgment on the genuineness of the exposure should be based on other supporting observations. It

must be stated that the probability of scoring 2 – 3 dicentrics / 500 metaphases in unexpected individuals is vary small (< 0.05).

A.2.2.9 Non – uniform exposures

Most often accidental exposures are non-uniform involving either partial body or localized irradiation. Since only a part of the lymphocytes are exposed, dicentric aberrations are over dispersed and deviate from Poisson distribution. The degree of over dispersion can be measured by calculating the dispersion index u.

$$U = \frac{[\sigma^2 / Y\text{-}1]\,[N\text{-}1]}{[2\,(N\text{-}1)\,(1 - 1/NY)]^{1/2}}$$

Where N is the number of cells scored, Y the dicentric frequency and σ^2 the variance. U value > 1.96 indicates partial body exposure. The mean dicentric yield Y corresponding to the exposed part of the body is derived by the relationship $Y = (1 - e^{-e})$ n/Nd where n is the number of dicentrics scored in Nd damaged cells.

A.2.2.10 Reporting of the dose

The estimated dose from the chromosomal analysis should be conveyed to AERB in the format prescribed. (BD Form- 03) given in Annexure 2.3

Annexure 2.1

CASE HISTORY FORM FOR BIOLOGICAL DOSIMETRY (BD-Form 01)

Code No: Date:

Name: Male/Female

IC No.

Address of the Institution:

Age: Height: Weight:

Blood Group:

Personal Habits: a. Smoking: Yes/No Light/Heavy

b. Alcoholic

c. Tobacco chewing

d. Any other

Disease and treatment:

Specify disease and drugs taken:

Blood transfusion

Any other

Diagnostic Exposure if any and details

Therapeutic exposure if any and details

Viral infection if any

Exposure details:

TLD Badge Number:

Activity of the radiation source / operating voltage of the X-ray machine etc.

Total cumulative dose:

Year-wise break-up for the last five years:

Possible sources of exposure

External:

Internal:

Details of Accidental Exposure:

Date of accident:

Whole body/Partial body:

Localised exposure:

Duration of exposure

Physical dose (TLD)

Early reaction (signs and symptoms) if any:

Any other relevant information:

Signature
Officer-in-charge
Bio-Dosimetry Laboratory

Annexure 2.2

SCORE SHEET – CHROMOSOMAL ABERRATION (BD-Form 02)													
CULTURE No.						MICROSCOPE NAME							
SAMPLE No.						MAGNIFICATION							
DATE OF COUNTING						PAGE No.							
STAGE CO-ORDINATE		METAPHASE No.	CHROMOSOME PIECES	TYPE OF ABERRATION								REMARKS
X	Y			DC	AC	RG	AR	M	CB	CA	PP	
TOTAL												

DC – DICENTRICS	CA-CHROMATID ABERRATION	RG - CENTRIC RING	AR- ACENTRIC RING
M – MINUTES	CB - CHROMATID BREAK	AC - ACENTRIC	PP - POLYPLOID

Annexure 2.3

Format for Reporting the Estimated Dose by CA analysis (BD Form-03)

1. Name of worker:
2. Code number:
3. Personal monitoring number:
4. Address:
5. Date of blood sampling
6. No. of cells scored:
7. No. of dicentrics observed:
8. Other aberrations: chrb: chtb: chtg:
   minutes: Trans: - inv:
9. Estimated biological dose:
10. Follow up action if any:

Officer-in Charge

Date: Bio-Dosimetry Laboratory

To

Head, Radiological Safety Division
Atomic Energy Regulatory Board
Niyamak Bhavan, Anushaktinagar
Mumbai – 400 094

Annexure – 3

Format of Application Form for obtaining Accreditation of Bio-dosimetry Laboratory

1. Name Designation and Official Address of the applicant (with Tel. Nos. Fax Nos. and e-mail address)
2. Name and Address of Head of the Institution
3. Facility for cell culture (please provide on a separate sheet)
4. List of instruments in bio dosimetry Lab (Please provide the list on a separate sheet)
5. Detailed procedure for dose estimation by chromosome aberration technique including dose response curve (Please provide in Annexure)
6. List of documents submitted along with application (Please enclose a sketch of the laboratory, indicating the location of instruments)
7. Any other relevant information

I hereby certify that all the statements made above are correct to the best of my knowledge and belief.

Place:

Signature:

Date: **Name (Applicant)**

Signature:

Name (Head of the Institution)

Seal of the Institution

Mailing Address:

To
Head, Radiological Safety Division
Atomic Energy Regulatory Board
Niyamak Bhavan, Anushaktinagar, Mumbai – 400 094.