# DICENTRIC ASSAY TECHNIQUE FOR THE ASSESSMENT OF WHOLE BODY DOSE TO LOW LET RADIATION

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**ABSTRACT**

*For more than two decades, biodosimetry has been used in biomonitoring of occupational and envir onmental exposure to ionising radiation. Chromosome aberration analysis is a method used to dete ct unstable aberrations in the lymphocytes of irradiated personnel. The Malaysian National Biodosi metry Laboratory is a referance centre for activity relating to biodosimetry in the country. This pap er aims at presenting dicentric assay technique for the assessment of whole body dose to low LET ra diation at the Malaysian National Biodosimetry Laboratory.*

**ABSTRAK**

*Sejak lebih dari dua abad yang lampau, biodosimetri telah diguna dalam pemonitoran biologi dari dedahan sinaran mengion untuk pekerjaan dan alam sekitar. Analisa aberasi kromosom ialah satu kaedah yang digunakan untuk mengesan aberasi yang tidak setabil dalam limposit individu yang tel ah terdedah kepada sinaran. Makmal Biodosimetri Kebangsaan Malaysia merupakan pusat rujukan untuk aktiviti yang berkaitan dengan biodosimetri di negara ini. Kertas kerja ini bertujuan memper sembah teknik asei disentrik untuk penilaian dos seluruh tubuh dari sinaran rendah LET di Makmal Biodosimetri Kebangsaan Malaysia.*

**Keywords:** biodosimetry, dicentric, low LET, whole body dose

## INTRODUCTION

Ionizing radiation is a strong clastogen, causing chromosome breakage, and resulting in cytogenetic aberrations in exposed cells. Since physical dosimetry provides only limited information, when it comes to complex exposures, biodosimetry has increasingly gained in importance. Cytogenetic analysis of peripheral blood lymphocytes can provide a biological estimation of the radiation dose received during exposure to ionizing radiation (Senthamizhchelvan, *et al.,* 2007). Accurate estimation of the level of absorbed dose is important immediately after exposure as a guide for medical treatment and at longer times after exposure to assess possible health consequences. A

number of cytogenetic techniques have been developed to measure radiation exposure and generally produce dose estimation, including Fluorescence *In-Situ* Hybridization (FISH), micronuclei, Premature Chromosome Condensation (PCC) and dicentric assay (IAEA, 2001; IAEA, 1986).

For the past 30 years, chromosome aberration assay and detection of unstable aberrations, dicentrics and acentric fragments, have been used for the estimation of radiation damage . In 1986, the International Atomic Energy Agency (IAEA) published an annual report describing dicentric assay technique as the method of choice in the evaluation of biological effects of ionising radiation (AEA, 1986; Natarajan, 2002). In the years that followed, the dicentric assay technique was shown to be efficient in biodosimetry and cytogenetic biomonitoring of populations occupationally exposed to ionising radiation (Obe, *et al.,* 2002). The technique was later recommended by the World Health Organization (WHO) as a mandatory technique in ionising radiation risk assessment (Albertini, 2000).

It is well known that chromosome aberrations occur as the result of action of ionising radiation on the DNA molecule either directly or indirectly. One of the basic types of chromosome aberration is the doublestrand break (Pfeiffer, 2000). Under certain conditions however, during DNA repair these breaks can transform into dicentric or ring chromosomes accompanied by acentric fragments. These types of chromosome aberrations involve larger DNA rearrangements and are considered to be unstable aberrations. They arise shortly after the exposure of cells to low and high linear energy transfer (LET) radiation. They can also arise from dose accumulation in long-term exposure to low doses of radiation (Natarajan, 2002; Obe, *et al.,* 2002). Misrepair of unstable aberrations leads to stable chromosome.

At present, in the country, a group of radiation worker that is having the highest risk of receiving overexposure are those working in industrial radiography. Those radiation involved are considered as low LET radiation (IAEA, 2001). $^{60}$Co γ- ray is the type of low LET radiation, or spousely ionizing radiation. When interact with cell, the ionization at any particular dose will be randomly distributed between cells, the DNA damage will also be randomly distributed and has the aberration.

Establishing a competent biodosimetry laboratory that is capable of performing cytogenetic analysis for dose estimation is vital in a country like ours, where recently a large use of ionizing radiation are in place. The Malaysian National Biodosimetry Laboratory is a referance centre for activity relating to biodosimetry in the country. This paper aims at presenting dicentric assay technique for the assessment of whole body dose to low LET radiation at the Malaysian National Biodosimetry Laboratory. This is a useful tool for dose reconstruction in medical management of radiation accident victim in the country.

## DICENTRIC ASSAY TECHNIQUE

Fig 1 shows a flowchart for the dicentric assay technique for the assessment of whole body dose to low LET radiation at the Malaysian National Biodosimetry Laboratory.

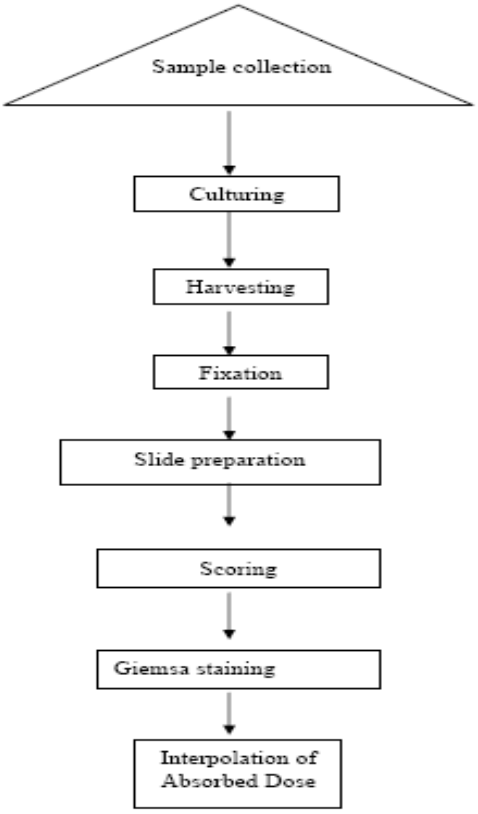


Figure 1. A flowchart for the dicentric assay technique.

***Sample collection***

In Malaysia, until recently, suspected overexposed radiation worker is identified by the Atomic Ener gy Licensing Board, based on results of personnel monitoring devices. Blood sample from the suspe cted overexposed radiation worker is taken by the Malaysian Registered Medical Practicenor. Five ml venipuncture blood were taken soon after the overexposed person has keen identified using plasti c lithium heparin tube

***Culturing***

Blood samples were cultured into the 10 ml of complete culture media in the 25 $cm^2$ flask. Lymphocytes in blood were stimulated to divide by addition of 300 µl of 2.5 mg/ml stock phytohemaglutinin (PHA), and incubated at 37°C with 5% $CO_2$ for 48 hours. After 45 hours of incubation, 100 µl of 10 µg/ml stock colcemid was added to the culture and incubated for an additional 3 hours to arrest cells in metaphase.

***Harvesting***

Following the full incubation period, the cultured blood cell was harvested and fixed. In harvesting lymphocytes, the hypotonic 0.075M potassium chloride solution was applied to break red blood cells.

### *Fixation*

A fixative mixture of 1:3 acetic acid/methanol was added to fix the lymphocytes. The fixative process was repeated 3 times while the first fixer was added extremely slowly to prevent cells clumping. The fixed lymphocytes were then kept in 4°C fixative in fridge for future slide preparation.

### *Slide preparation*

Slide was prepared by dropping 2-3 drops of cells, from a height of at least 10 cm onto the slide, in fixative on grease-free slide and Giemsa stained for observation (IAEA, 2001; IAEA, 1986 ).

### *Giemsa Stainning*

The slides were immersed in 2% Giemsa stain in pH 6.8 phosphate buffers for 5 minute, washed in buffer. Distilled water was used to briefly rinse and allowed to dry, and finally mounted with a cover glass (IAEA, 2001).

### *Scoring*

The slide is coded and scanned to ensure that the entire area is covered. In order to produce a dose estimate with a statistical uncertainty small enough to be of value, a large number of cells needs to be scored. The number of cells needs to be scored however ia a compromise based on the importance of the case, the available labour and the quality of the preparation (IAEA, 2001). In our practice, we aims at scoring 500 cells or 100 dicentrics.

Some of Biodosimetry Laboratories combines centric, ring and acentric with dicentric for scoring. However, we choose to only score dicentric. Fig. 2 shows a picture of dicentric and dicentric ring.

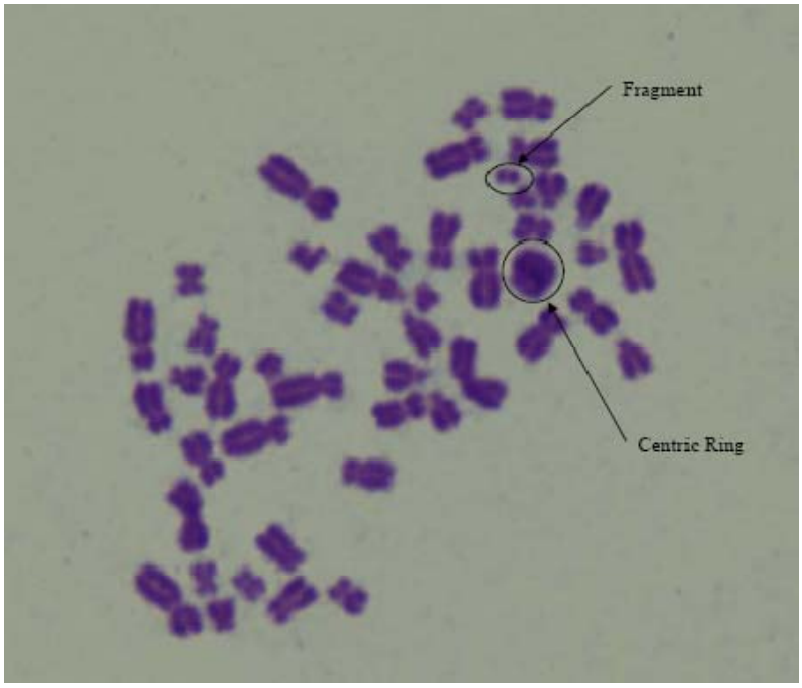


Figure 2. A picture showing fragment and ring dicentric.

The cells are scored by directly viewing the slide under microscope. Only complete metaphase be recorded, namely those with 46 or more pieces (IAEA, 2001). All other abnormalities in the cells are also recorded including number of centric rings and excess acentric fragments. Tricentric aberations are recorded as equivalens to two dicentrics (IAEA, 2001). Fig. 3 shows dicentric, tricentric and fragment.

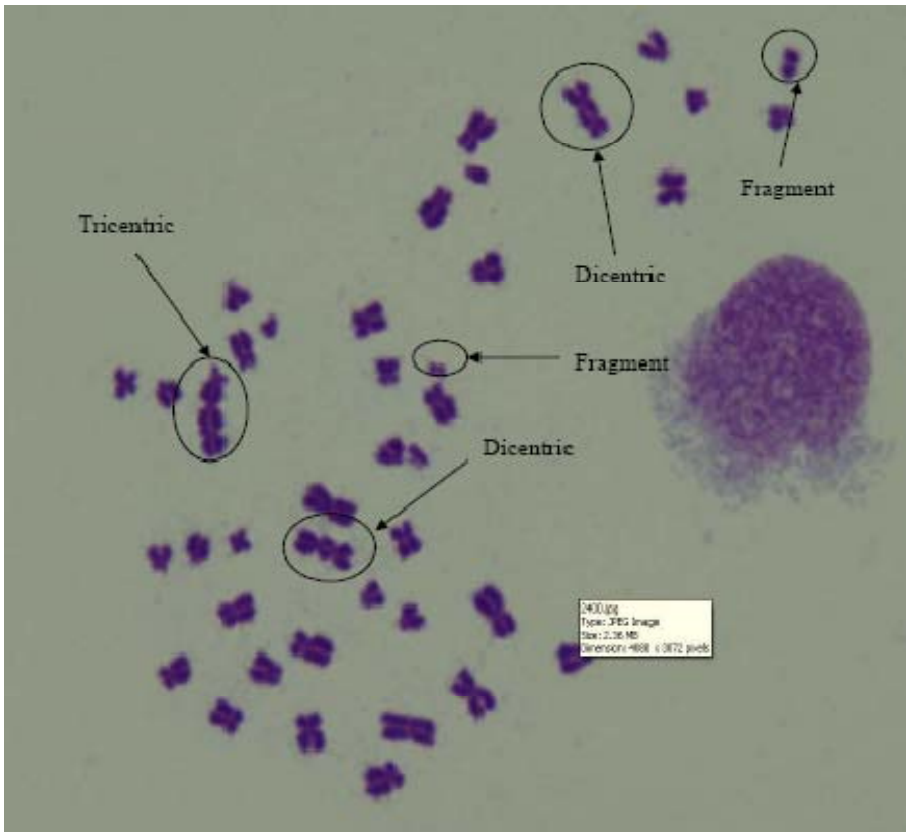


Figure 3. A picture showing dicentric and tricentric.

## ESTIMATION OF WHOLE BODY DOSE

IAEA (2001) reported that there is no difficulty in deriving a dose from a measured yield of dicentri, either no generally accepted way of deriving its uncertainty. The aim is to express uncertainty in terms of a confidence interval, and quite often 95% is chosen as the limit. The 95% confedence limits define an interval that will encompass the true dose on at least 95% of occasions. The dose estimates are made from $^{60}$Co dose response curve described elsewhere (Jamal, *et al.*, 2010). The computation of confidence limits include two components of uncertainty; 1) from the Poisson nature of the yield of aberrations, and 2) from uncertainties associated with the calibration curve which are approximately normally distributed (IAEA, 2001).

The dose value obtained by refering to the frequency of dicentric to a calibration curve represents avarege absorbed dose to the lymphocytes. This represents, an averaged out whole body dose because lymphocytes are widely distributed around the body and are mobile. As the biological end

point being scored is a type of chromosome aberration, this reflects dose to the cells's nuclei. For photon, dose to soft tissue is a good approximation to the dose to the nucleus, because lymphocytes nucleus diameter is small, ~ 6 μm, compared with the ranges of secondary particles produced by photon. In this case, *Bragg–Gray cavity* can be applied (IAEA, 2001).

Biodosimetry based on dicentric calculation is reported to improve radiation protection and supplied data on the correlation between genome damage and other biomarkers related to exposure to ionizing radiation, such as hematological parameters or development of neoplasms (Bonassi, *et. al.,* 2000). Although dicentric and ring chromosomes are considered to be unstable aberrations, a clear correspondence with the development of malignant diseases was found. Unstable chromosome aberrations arise shortly after the exposure of cells to low and high LET radiation. They can also arise from dose accumulation in long-term exposure to low doses of radiation (Bender, *et. al.,* 1988).

The limitation of dicentric assay technique is that the damage is unstable and therefore is eleminated from the peripheral blood lymphocyte pool at the rate that cell renewal occurs. The lower limit of dose detection by dicentric for lower LET radiation is around 0.1-0.2 Gy (IAEA, 2001). Sensitivity to lower doses is a function of the background level of dicentrics, typically about 1/1000 cells, and the limit on the number of metaphases that can realistically be scored.

## SUMMARY

We have presented the dicentric assay technique for the assessment of whole body dose to low LET radiation at the Malaysian National Biodosimetry Laboratory. This may serve in a standardized manner, as the appropriate cytogenetic technique to ensure comparable dose assessment following any accidental exposure to oinizing radiation in the country.

## ACKNOWLEDGEMENT

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