In-vitro ^{60}Co dose calibration curve using dicentrics assay technique for the Malaysian National Biodosimetry Laboratory

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It has been recommended that each laboratory, intended to carry out biological dosimetry analysis should have its own *in-vitro* dose calibration curve for dose reconstruction. The purpose of this study was to establish an *in-vitro* ^{60}Co dose calibration curve using dicentric assay technique for the Malaysian National Biodosimetry Laboratory. Sample from five volunteers were irradiated using ^{60}Co at doses ranging from 0 to 4 Gy. The blood samples were surrounded by 4 mm Polyvinyl chloride and irradiated at a dose rate of 0.98 Gy min^{-1}. Blood specimens were then cultured and processed using dicentric assay technique. The observed dose calibration data were fitted to a linear quadratic model using Dose Estimate Ver 2.0, established by Health Protection Agency, UK. The calibration curve parameters were compared with results from other studies. The dose calibration curve for dicentrics yield is: Yield = $(0.0010 \pm 0.0000) + (0.0208 \pm 0.0013)D + (0.0551 \pm 0.0005)D^2$, (Weighted $\chi^2 = 15.6500$, on 7 df, P = 0.02). The linear and quadratic coefficients were comparable with results from other recent studies. We have established the *in-vitro* ^{60}Co dose calibration curve using dicentric assay technique for the Malaysian National Biodosimetry Laboratory. This curve may be useful for *in-vitro* dose reconstruction.

I. INTRODUCTION

Ionizing radiation is a strong clastogen, causing chromosome breakage, and resulting in cytogenetic aberrations in exposed cells. 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 the possible health consequences. Cytogenetic analysis of peripheral blood lymphocytes can provide a biological estimation of the dose received in exposure to ionizing radiation [1]. A number of cytogenetic assay techniques have been developed for dose estimation, including dicentric, Fluorescence In–Situ Hybridization (FISH), micronuclei and Premature Chromosome Condensation (PCC) [2,3].

Dicentric is the aberration type that is most frequently used in biological dosimetry. This is because it clearly involves an interaction (or exchange) between two chromosomes. This assay technique is generally accepted as the most specific, sensitive and currently available method for determining doses from recent (i.e. within days to about 6 months) exposure to ionizing radiation [4]. It could be especially useful in providing evidence of non-uniform exposure and conformation of individuals in a high dose exposure triage category, and the most sensitive method of quantifying the radiation dose in the absence of physical measurements due to its ability to estimate the average whole-body dose [2]. It is a reliable and useful tool in medical management of radiation accident victims [4].

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. It has been recommended that each laboratory intended to carry out biological dosimetry should have its own *in-vitro* dose calibration curve for dose reconstruction [2].

The purpose of this study was to establish an *in-vitro* ^{60}Co dose calibration curve using dicentric assay technique for the Malaysian National Biodosimetry Laboratory. This curve is a useful tool for dose reconstruction in medical management of radiation accident victim in the country.

II. EXPERIMENTAL

IIa. Irradiation

Ten ml of freshly taken blood specimens were collected from five healthy volunteers; Malay (n = 2), Chinese (n = 2) and Indian (n = 1) respectively. Those volunteers are aged between 26 to 49 years old. Blood was collected and divided into 1 ml aliquots, in lithium heparin tube.

γ-ray from a ^{60}Co teletherapy unit ELDORADO 8 # 104 located at the Secondary Standard Dosimetry Laboratory (SSDL), Malaysian Nuclear Agency is used to irradiate the sample. Sample from each volunteer was irradiated at 0, 0.1, 0.15, 0.25, 0.5, 1.0, 2.0, 3.0, 3.5 and 4.0 Gy respectively. They were irradiated at a dose rate of 0.98 Gy min^{-1}. Four mm Polyvinyl chloride is used as an absorbing material surrounding the lithium heparin tube. The experimental set-up is shown in Fig. 1. After

irradiation, blood samples were kept at 37ºC for one hour to allow for any chromosomal repair to take place [3].

IIb, Culturing

Blood samples were cultured into the 10 ml complete culture media in the 25 cm^2 flask. Lymphocytes in blood were stimulated to divide by addition of 2.5 mg/ml stock phytohemaglutinin (PHA), and incubated at 37ºC with 5% CO_2 for 48 hours. After 45 hours of incubation, 10 µg/ml stock colcemid was added to the culture and incubated for an additional 3 hours to arrest cells in metaphase. Following the full incubation period, the cultured blood cell was harvested and fixed. In harvesting lymphocytes, the hypotonic 0.075 M potassium chloride solution was applied to break red blood cells and fixative mixture of 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 was prepared by dropping 2-3 drops of cells in fixative on grease-free slide and Giemsa stained for observation [1-3].

IIc. Scoring

Slides from each culture were scored by a single scorer. At least 100 first division metaphase cells or 50 dicentrics were scored per sample. In addition, number of centric rings and excess acentric fragments were also recorded. The conventional research microscope, Olympus BH2-BHT (Olympus, Japan) was used in this study.

IId. Curve Fitting

The pooled observed dose calibration data were fitted to a linear quadratic model using Cytogenetics Dose Estimation software program Ver 2.0, Health Protection Agency, UK [5]. The method is based on weighted least square fitting. The standard *u*-test was used to test the yield of chromosome aberrations with dose for poisson probabilities. If the value of *u* is greater than ±1.96, the under or over dispersion of chromosome aberration yield is significant at 5% [1,5]. The method is also based on the concept that for poisson distribution, the ratio of variance (σ^2) to mean (y) is equal to 1. The calibration curve parameters were compared with results from other studies.

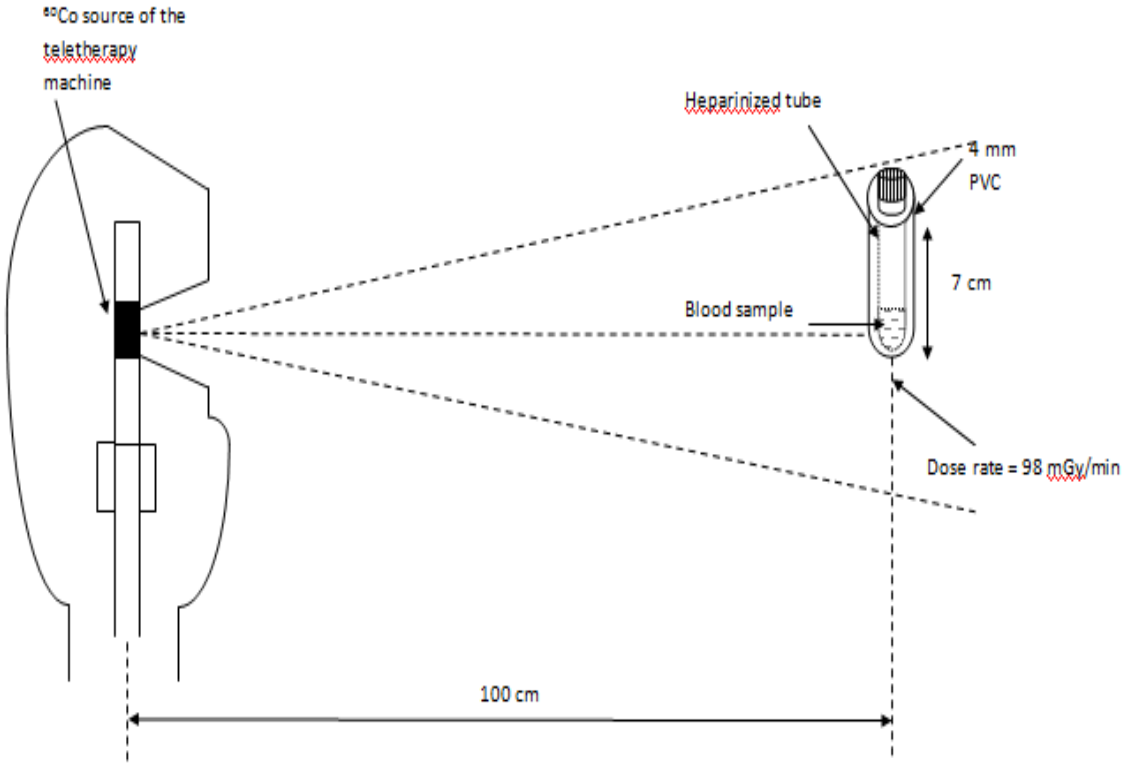

FIG. 1. Experimental set-up.

III. RESULT AND DISCUSSION

Establishing the ^{60}Co *in-vitro* dose calibration curve is important for the reconstruction of radiation dose of an exposed radiation worker in any standard biodosimetry laboratory. The *in-vitro* dose calibration curve is comparable to the effect of *in-vivo* irradiation [6]. The yield of chromosome aberration with dose was fitted to a linear quadratic model [1-5]:

$$Y = C + \alpha D + \beta D^2 \quad (1)$$

where Y is the yield of aberration for dose D, C is the baseline aberration frequency in the control population, α and β are linear and quadratic coefficients respectively.

Fig. 2 shows a dose calibration curve for dicentrics in human lymphocytes exposed to incremental radiation doses from ^{60}Co γ-ray. The 95% confidence intervals are also shown on the curves. The dose calibration curve for dicentrics yield is: Yield = $(0.0010 \pm 0.0000) + (0.0208 \pm 0.0013)D + (0.0551 \pm 0.0005)D^2$, (Weighted $\chi^2 = 15.6500$, on 7 df, P = 0.02).

Our ^{60}Co *in-vitro* dose response curve was fitted by the linear quadratic model. Table I shows pooled data of dicentrics frequency in lympocytes of blood samples exposed to *in-vitro* ^{60}Co γ-ray. The value of *u* range from -1.970 to +1.960 with only a single value greater than ±1.96, at dose point of 4.0 Gy. It shows that the distribution of dicentrics follows a Poisson distribution except at 4.0 Gy where $u > \pm 1.96$. This over dispersion at dose of 4.0 Gy may be explained by the nonuniformity of irradiation involving a small volume of sample at a high dose [1].

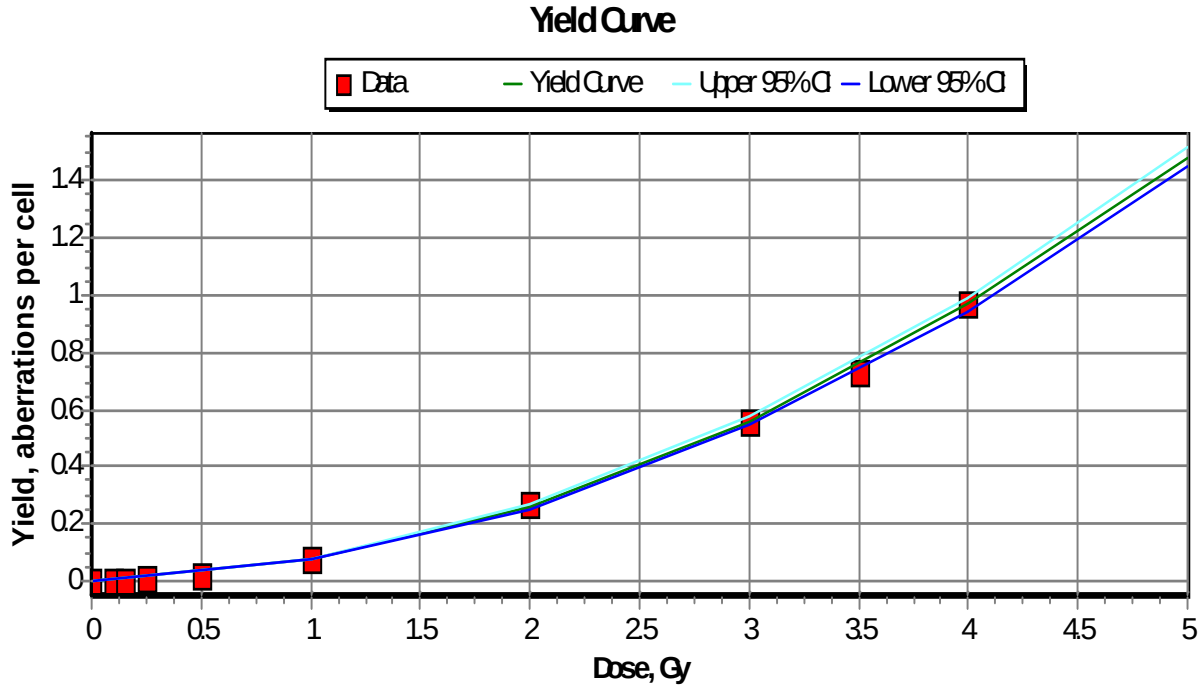

FIG. 2. Dose calibration curve for dicentrics in human lymphocytes exposed to incremental radiation doses from ^{60}Co γ-ray.

TABLE I. Pooled data of dicentrics frequency in lymphocytes of blood samples exposed *in-vitro* to ^{60}Co γ-ray.

Dose (Gy)	No. of cell scored	Dicentric per cell ± SE	Total Dicentric	Dicentric distribution per cell						*U*	σ^2/y
				0	1	2	3	4	5		
0	5000	0.0006 ± 0.000	3	4997	3	0	0	0	0	- 0.024	1.500
0.1	5000	0.0016 ± 0.001	8	4992	8	0	0	0	0	- 0.075	1.490
0.15	5000	0.0052 ± 0.001	26	4974	26	0	0	0	0	- 0.255	1.490
0.25	4325	0.0104 ± 0.002	45	4280	45	0	0	0	0	- 0.478	1.480
0.5	5000	0.0240 ± 0.002	120	4882	116	2	0	0	0	+ 0.479	1.510
1.0	4885	0.0762 ± 0.004	372	4528	342	15	0	0	0	+ 0.233	1.500
2.0	2647	0.2667 ± 0.010	706	2043	513	80	11	0	0	+ 1.960	1.570
3.0	1235	0.5603 ± 0.021	692	719	360	136	20	0	0	0.173	1.510
3.5	976	0.7275 ± 0.027	710	466	338	147	22	3	0	- 1.670	1.380
4.0	740	0.9730 ± 0.036	720	276	257	166	33	8	0	- 1.970	1.340

The (σ^2/y) value of dicentric yield was close to 1, ranging form 1.340 to 1.570 to 1.570. This indicates that the calibration curve is following poisson distribution. The data are under or over dispersed if the value (>) of 'u' deviate from ± 1.96 [5].

In order to produce a dicentric aberration, DNA damage must be induced in the two unreplicated chromosomes involved such that the damaged chromosome can undergo exchange. For low LET radiation, such as used in this study, two ionizations at minimum are necessary to produce damage in the two-chromosomes involved in dicentric. Dicentric produced by one track will have frequency that is α to the linear function of dose. Whereas dicentric induced in two tracks will have a frequency β to the square of the dose. The ratio of α/β of the two coefficients is equal to the dose at which the linear and quadratic components contribute to the formation of dicentrics.

Table II shows results of comparison of the calibration curve parameters for dicentric yield, using ^{60}Co with results from other recent studies. It shows that α, which is a linear coefficient is comparable with the values reported by Edwards, 1997 [7], Voisin *et al*. [8] Lloyd *et al*. [9]. However, our α value is larger than that has been reported by Bauchinger *et al*. [10]. While β, which is a quadratic coefficient is agreeable with the values reported by Edwards, 1997 [7], Voisin *et al*. [8], Lloyd *et al*. [9], Bauchinger *et al*. [10].

Table II. Comparison of calibration curve parameters for dicentric yield using ^{60}Co with results from other recent studies.

Source	α ± SE (Gy^{-1})	β ± SE (Gy^{-2})
Edwards [7]	0.018 ± 0.003	0.060 ± 0.006
Voisin *et al*., [8]:		
Laboratory A	0.0187 ± 0.0056	0.0527 ± 0.0046
Laboratory B	0.0371 ± 0.0085	0.0547 ± 0.0039
Laboratory C	0.0128 ± 0.0031	0.0640 ± 0.0022
Lloyd *et al*., [9]	0.016 0 ±.005	0.065 ± 0.003
Bauchinger *et al*., [10]	0.011 ± 0.004	0.056 ± 0.003
Present study	0.0208 ± 0.0013	0.0551 ± 0.0005

IV. CONCLUSION

We have established a dose calibration curve for the induction of chromosome aberrations in human lymphocytes from blood irradiated with ^{60}Co γ-ray in a dose range of 0.0-4.0 Gy. This curve may be useful for *in-vitro* dose reconstruction.

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