



Intercomparison in Cytogenetic Dosimetry among 22 Laboratories in China

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ABSTRACT

As part of a regional International Atomic Energy Agency-coordinated research project with the support from the National Health and Family Planning Commission of China, 22 laboratories participated in the intercomparison in cytogenetic dosimetry in China. Slides for chromosomal aberrations were prepared by the Department of Radiation Epidemiology, National Institute for Radiological Protection, which organized the exercise. Slides were sent to the other participating laboratories through Express Mail Service. For estimates of dose, each laboratory scored the frequency of dicentrics plus centric rings chromosomes. The whole blood samples were irradiated with ⁶⁰Co γ -rays (1.3 Gy, 2.4 Gy and 1.5 Gy, 2.6 Gy). Each laboratory got one group of the slides. Ten of the 44 estimates of dose fell within $\pm 5\%$ of the true physical dose, 12 fell within $\pm 5\text{--}10\%$, 9 fell within $\pm 10\text{--}15\%$, 12 fell within $\pm 15\text{--}20\%$, while only one sample fell $\pm >20\%$. The evaluation of the respective dose was achieved by 21 laboratories.

Key words: Chromosomal aberration, cytogenetic dosimetry, intercomparison

Introduction

To strengthen radiation biodosimetry services, an intercomparison exercise in cytogenetic biodosimetry was arranged. Twenty-two laboratories from different regions, including Hong Kong, in China participated. The intercomparison was based on determining the dose calculated from the frequency of dicentrics plus centric rings (dic + r) in metaphase lymphocytes. The exercise was organized by one laboratory where irradiation of the whole blood, culturing of the lymphocytes, and preparation of metaphase spreads on the slides were performed. The slides were then sent to the other participating laboratories for dicentric analysis. The main objective of this exercise was to compare the participating laboratory's ability to score chromosomal aberrations and their use in dose estimation. It was also intended to establish the Chinese network for estimating the biological dose for radiation exposure.

Materials and Methods

Conditions of exposure

Blood samples from two healthy donors aged 26 and 32 years (male and female, respectively) were collected in heparinized tubes. Each blood sample was divided into two aliquots, each for irradiation at 1.3, 2.4 Gy (first sample) and 1.5, 2.6 Gy (second sample). The samples were irradiated in tubes with ⁶⁰Co γ -rays at a dose rate of 0.32 Gy/min in the Laboratory of Quality Control for Medical Exposure Equipment (International Atomic Energy Agency [IAEA]/WHO Second Standard Dosimetry Laboratory, National Institute for Radiological Protection [NIRP]). The irradiated blood was placed at 37°C for 2 h to allow DNA repair.^[1]

Cell culture

Lymphocyte cultures were performed according to the description in the IAEA-405 report and IAEA-2011.^[2,3] In brief, lymphocytes were cultured in RPMI 1640 medium (Sigma, USA) enriched with fetal calf serum (20%). To 4 ml of culture medium containing 10 $\mu\text{g}/\text{ml}$ phytohemagglutinin (Sigma), 0.04 $\mu\text{g}/\text{ml}$ colchicine (Sigma), 100 IU/ml penicillin, and 100 IU/ml streptomycin,

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0.8 ml of whole blood was added and mixed. The culture tubes were incubated for 52 h at 37°C. After hypotonic treatment with KCl (0.075 mol/L), the cells were harvested by Genial Cellsprint (Genial, UK). The lymphocytes were fixed with three fixative steps of methanol/acetic acid mixture (3:1, v: v). The slides were stained using Giemsa. Stained slides in simple blind method were sent to the participating laboratories through Express Mail Service.

Results and Discussion

Laboratory distribution for intercomparison

Twenty-two participants from the Center for Disease Control and Prevention, Prevention and Treatment Center for Occupational Disease, Colleges and Universities, Scientific Research Institute, Unit of Nuclear Industry, and a hospital in Hong Kong participated in this exercise. The locations of these participating laboratories covered 19 provinces or municipalities of China [Figure 1]. In the figure, the number delegated the laboratory number. Most of these laboratories are individually capable of offering cytogenetic/biological dosimetry services in the event of a situation when individuals are overexposed to ionizing radiation. As for the laboratories that need to further improve the estimated ability, NIRP will assist them in analyzing the causes and conduct the corresponding training and instruction.

Comparison of dicentric scoring

Initially, the yield of dicentrics scored for each sample was compared.^[4] Two out of four samples were sent to the participating laboratories for intercomparison. Consequently, each sample was analyzed independently by 11 laboratories. Twenty-two laboratories participated in the scoring of dic + r and acentric aberrations, wherein each laboratory was requested to score a minimum of 100 dic + r or 1000 metaphases, or depending on the formula
$$n = \frac{(1-p) \times 96.04}{p}$$
 the score number was requested for dose estimation. Most of the laboratories followed these guidelines.

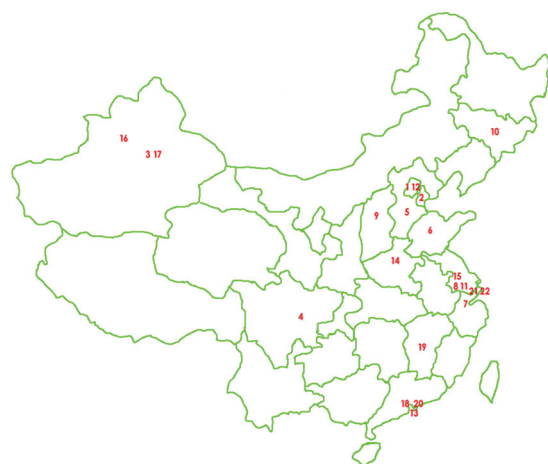


Figure 1: The regional map showing location of laboratories that participated in the intercomparison exercise in China. The numbers are the participants' laboratory ID

The yield of dic + r per 100 cells was calculated by each laboratory [Figure 2]. The minimum value for L1 sample was obtained by laboratory 7 with 26.33 dic + r per 100 cells (dic + r/100 cells) and the highest value was observed by laboratory 3 (52.49 dic + r/100 cells). The mean value of all the 11 laboratories is 39.04 ± 6.41 dic + r/100 cells. Only two laboratories (3 and 7) reported a yield that exceeded 39.05 ± 6.41 . The minimum value for C2 sample was obtained by laboratories 4 and 9 with 14 dic + r per 100 cells (dic + r/100 cells) and the highest value was observed by laboratory 3 (22.52 dic + r/100 cells). The mean value of all the 11 laboratories is 17.37 ± 2.75 dic + r/100 cells with a standard deviation (SD) of 2.75. Only three laboratories (3, 4, and 9) reported a yield that exceeded the magnitude of 17.37 ± 2.75 .

The minimum value for L2 sample was obtained by laboratory 21 with 8.57 dic + r per 100 cells (dic + r/100 cells) and the highest value was observed by laboratory 17 (18.67 dic + r/100 cells). The mean value of all the 11 laboratories is 13.36 ± 2.97 dic + r/100 cells with a SD of 2.97. Four laboratories (12, 15, 17, and 21) reported a yield that exceeded the magnitude of 13.36 ± 2.97 . The minimum value for C1 sample was obtained by laboratory 19 with 32.5 dic + r per 100 cells (dic + r/100 cells) and the highest value was observed by laboratory 13 (53.8 dic + r/100 cells). The mean value of all the 11 laboratories is 42.29 ± 7.95 dic + r/100 cells with a SD of 7.95. Five laboratories (13, 14, 16, 19, and 21) reported a yield that exceeds the value of 42.29 ± 7.95 .

There were some discrepancies among the participating laboratories. Two types of intercomparisons were performed in this study: (1) The entire experimental process was evaluated, where blood samples were distributed to 22 participants. Processes such as blood culturing, lymphocyte harvesting, and chromosome preparation and analysis were handled by the individual laboratories or (2) partial experimental process was compared as timely delivery of the blood samples was not practically possible. In this case, the samples were processed on-site at NIRP to obtain fixed cell suspension and slide preparation less critical for transportation. Therefore, variation

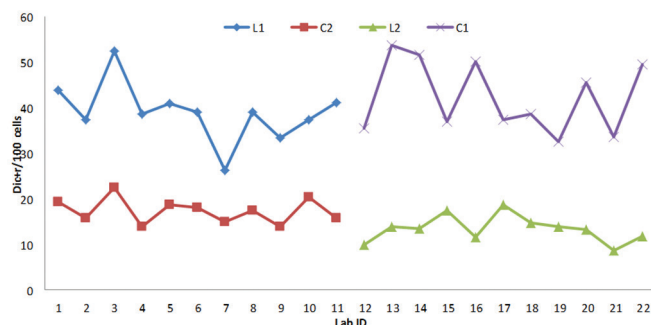


Figure 2: Comparison of the observed yield of dicentrics for different laboratories. L1 samples were irradiated by ^{60}Co γ -rays to 2.6 Gy (39.05 ± 6.41 dicentric per 100 cells). C2 samples were irradiated by ^{60}Co γ -rays to 1.5 Gy (17.37 ± 2.75 dicentric per 100 cells). L2 samples were irradiated by ^{60}Co γ -rays to 1.3 Gy (13.36 ± 2.97 dicentric per 100 cells). C1 samples were irradiated by ^{60}Co γ -rays to 2.4 Gy (42.29 ± 7.95 dicentric per 100 cells)

in culture conditions was expected by only one laboratory. Some discrepancies in the rate of dic + r were detected among participants. The main complaint from the different laboratories was on the poor quality of cells/metaphases as there was difficulty in scoring. The guidelines used by each laboratory in choosing scorable metaphases were found to be different.

Comparison of the estimated dose

The dose was derived from the yield of dic + r obtained from a dose-effect relationship plot. This calculation was done by all the laboratories for γ -ray exposure. The estimation of dose of γ -ray is shown in Figures 3-6 together with the 95% confidence limit of the dose estimated by each laboratory. The horizontal lines represent the physical dose delivered in each sample at $\pm 20\%$.

From the second step of this intercomparison, it can be seen, as displayed in Figure 3, that the estimation of the dose for all the laboratories except one laboratory for sample L1 was within this

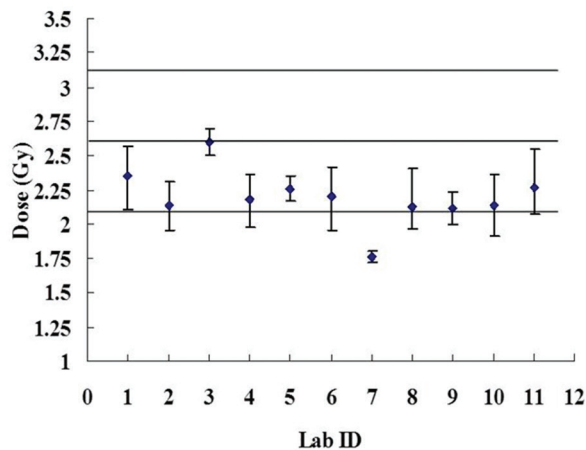


Figure 3: Comparison between laboratories for dose estimation for L1 samples. Each point corresponds to a dose estimation \pm confident interval. The two horizontal lines represent the physical dose 2.6 Gy $\pm 20\%$

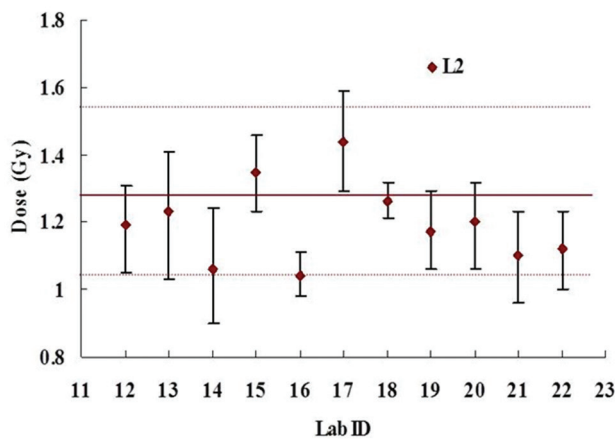


Figure 5: Comparison between laboratories for dose estimation for L2 samples. Each point corresponds to a dose estimation \pm confident interval. The two horizontal lines represent the physical dose 1.3 Gy $\pm 20\%$

range. Most of the data fell within the exposed dose of 2.6 Gy within 20% range. Data for samples C2, L2, and C1 are shown in Figures 4-6, respectively, where the dose estimations were within $\pm 20\%$ range. Even though the methods used to prepare chromosomes for biological dosimetry appear relatively similar to those described in the IAEA manual, each laboratory followed their own routine technique in which they are comfortable with. This adaptation may be a more critical component of sample preparation (from blood samples to slides) than for the dicentric scoring. It is possible that these factors might have contributed to the specific shape of the dose-effect calibration curve generated by individual laboratories. Distributing the prepared slides may significantly reduce the effect of blood preparation in this intercomparison. Less variations between the laboratories are anticipated based on the scoring methods. The scoring process depends on three main factors: Quality of chromosome spreads, selection of metaphases, and identification of dicentrics. Quality of metaphases decreases with increasing dose in our experience. It is possible that some metaphases with dicentrics might have been discarded by the scorers which could lead to an altered chromosome aberration distribution.^[4-6]

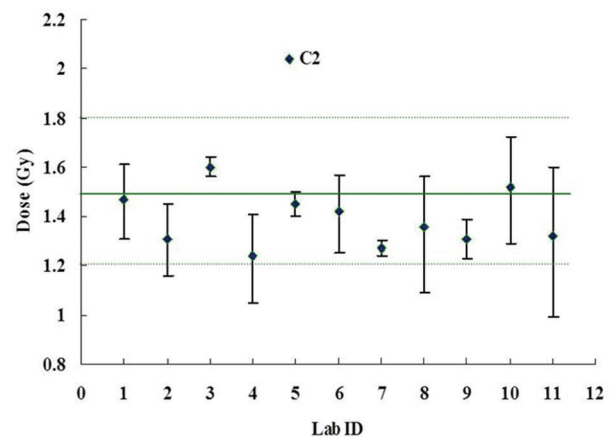


Figure 4: Comparison between laboratories for dose estimation for C2 samples. Each point corresponds to a dose estimation \pm confident interval. The two horizontal lines represent the physical dose 1.5 Gy $\pm 20\%$

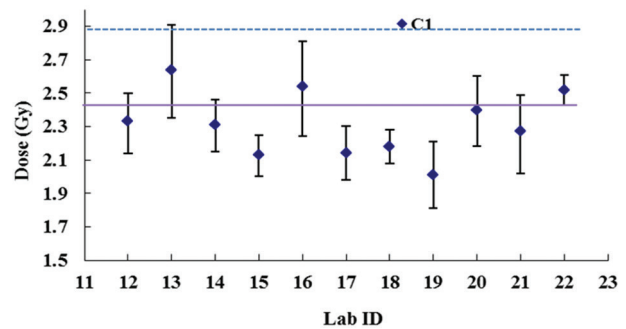


Figure 6: Comparison between laboratories for dose estimation for C1 samples. Each point corresponds to a dose estimation \pm confident interval. The two horizontal lines represent the physical dose 2.4 Gy $\pm 20\%$

There were several international intercomparisons reported earlier.^[4-12] While Roy *et al.*^[4] have also used the dic + r as indicator, the main criteria for their comparison was to evaluate the ability of the laboratories to distinguish between neutron and γ -ray exposures. Garcia *et al.*^[5] used the dicentric and micronuclei as biomarkers in their comparison exercise among five laboratories. Lindholm *et al.*^[6] performed an interlaboratory comparison of FISH chromosome painting and to study the time course of translocations and dicentric in three accident victims exposed to radiation. Few groups^[9-12] conducted several kinds of intercomparisons of different biomarkers such as chromosome aberrations, micronucleus, γ -H2AX, and gene expression. However, in our exercise, the ability of 22 laboratories to estimate the radiation dose was evaluated using dic + r which are biomarkers.

Conclusion

In our intercomparison exercise, 43 of 44 dose estimates were correctly calculated based on the dic + r frequencies by allowing 20% deviation. Only 1 of the 22 laboratories produced a result, which was beyond 20% range. In summary, the scoring from all the 21 participated laboratories were quite acceptable. In a large-scale radiation accident, the participating laboratories can facilitate the scoring of blood samples for dose estimation. Here, we successfully report the establishment of a Chinese biological dosimetry network for the first time.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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