Laboratory Intercomparison of Cytogenetic Dosimetry Among 38 Laboratories in China

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Abstract
A nationwide intercomparison exercise for estimating the irradiated dose was organized by the National Institute for Radiological Protection, Center for Disease Control and Prevention of China. Thirty-eight laboratories participated in this program. The main objective of this intercomparison exercise was to compare the participants’ ability of operation and dose assessment basing on the frequencies of dicentrics and centric rings. Whole blood samples were irradiated with different dosages of 60Co γ-rays. Each laboratory collected 2 blind samples and prepared the slides independently. All participants presented the estimated dose reports within 30 days. The doses assessed by the participants were acceptable within the reference dose of ±20%. The mean absolute difference of estimated dose relative to the reference dose was calculated, which reflected the overall accuracy of dose estimates for each laboratory. The overall estimation results of blind blood samples for intercomparison showed a good agreement with the reference dose for each sample, with nearly 75% of the participants producing acceptable results.

Keywords
cytogenetic dosimetry, chromosomal aberration, dicentric chromosome assay, intercomparison

Introduction
With the rapid development of nuclear energy and wide application of radiation technology in the fields of nuclear energy, agriculture, industry, and the medical exposure in China, radiological accidents or unplanned radiation exposures may occur. Moreover, Fukushima Nuclear Power Plant accident told us again that the radiation accident was not distant from us. Biological dosimetry was used to estimate the absorbed dose in the exposed individuals and played a significant role in the triage and medical treatment and management of radiological casualties. The availability of national and regional biodosimetry programs/laboratories will be very important not only in nuclear disaster but also for radiation workers in environments with a certain radiation risk and for the general public.

For many years, the dicentric chromosome assay (DCA) by using blood lymphocytes was the only available biodosimetry method.¹,² To date, this technique has been used most frequently by different laboratories.³⁻⁵ In radiological accidents and suspected overexposures, the DCA was still the “gold standard” biodosimetry method because of its highly standardized and harmonized technique for individual dose assessment.⁶

Several biodosimetry networks have been established,⁷⁻⁹ and biodosimetry laboratory intercomparison of DCA in different countries and regions have been finished.¹⁰⁻¹² To understand the bias and repeatability of techniques in common use in cytogenetic dosimetry in China and improve the current techniques and intensification of collaboration and networking among the different institutes, the National Institute for Radiological Protection (NIRP) of China Center for Disease Control and Prevention (CDC) conducted an interlaboratory comparison for DCA. The intercomparison was based on estimates of dose obtained through the frequency of dicentrics plus centric rings in metaphase lymphocytes. Each laboratory collected 2 blind samples. Slides for chromosomal aberration analyses were prepared by the participating laboratories.

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The main objective of this intercomparison exercise was to compare the participants’ ability of operation and dose assessment basing on the frequencies of dicentrics and centric rings. The 38 laboratories considered such coordinated measures to make them ready to interact in the event of a radiological accident occurring in the region. This study was also intended to establish the Chinese network for estimating the biological dose for radiation-exposed victims.

**Materials and Methods**

**Procedure Common for All Assays**

Blood samples from 2 healthy volunteers aged 26 and 32 years and aliquots of 2-mL whole blood were filled into heparinized tubes. Blood was taken with informed consent and the approval of a local ethics committee. The method of chromosomal aberration analysis for biological dose assessment was performed according to protocols published in the International Atomic Energy Agency (IAEA) and State Standard of the People’s Republic of China (GB/T 28236-2011). A slight variation among participating laboratories was permissible.

**Conditions of Exposure**

Whole blood was irradiated in heparinized tubes with $^{60}$Co $\gamma$-rays at a dose rate of 0.29 Gy/min in the Laboratory of Quality Control for Medical Exposure Equipment (IAEA/World Health Organization Second Standard Dosimetry Laboratory, NIRP). Blood samples were divided into 4 groups irradiated at doses of 1.1, 1.4, 2.1, and 2.8 Gy. After exposure, the blood was kept for 2 hours at 37°C to allow DNA repair.

**Shipping of Samples and Return of Data**

Participants can chose to perform the following steps in the laboratory of NIRP or take the blind blood samples to their respective laboratories. Most of laboratory reported that the estimate dose was lying outside reference dose of ±20%. The results of all 22 laboratories were summarized in Table 1.

**Cell Culture, Sample Processing, and Analysis for the DCA**

The procedure for lymphocyte culture, sample processing, and analysis for the DCA was conducted according to the description in IAEA-2011 and GB/T 28236-2011 of China. The report of the rate of chromosomal aberration ranged from 6.50% to 100% and showed a 4.5-fold difference for the samples at the dose of 1.1 Gy. The estimated doses ranged from 0.60 to 1.61 Gy and showed a 2.68-fold difference. The mean value of all 22 laboratories was 1.12 Gy. Three laboratories reported that the estimate dose was lying outside the reference dose of ±20%.

**Results**

**Result of Submission**

All participants provided triage dose estimate within 30 days after obtaining the blind blood samples. Most of laboratory scored enough metaphase. The number of metaphase and the percent of aberration are summarized in Table 1.

**Statistical Methods**

The accuracy of reported-dose estimates was measured by calculating the mean of the absolute differences (MAD) of estimated doses to their corresponding true doses. We use Wilcoxon test for a 2-group comparison.

**The Number of Metaphase Scored**

Each participant had to score at least 100 dicentrics or 1000 metaphase, or on request of the formula: $n = 100 \frac{(1 - p)^{0.5539}}{p}$, where $p$ is the percent of cell dicentrics or centric rings. The score number accords with the dose estimation.

**Triage Biodosimetry Based on Dicentric Chromosome Analysis**

Each laboratory decided whether manual or automatic scored. For dose assessment based on measured dicentric yields, each participant had to select an appropriate in vitro calibration curve and calculation method.

**Standard Calibration Curves**

All participating laboratories used preexisting standard calibration curves for dose estimations of blind samples. All calibration curves were based on manual dicentric scoring. Standard
calibration curves are listed in Table 2, and the corresponding curves are shown in Figure 1.

**Accuracy of Dose Estimations**

Comparisons of MAD values reflect the overall accuracy of dose estimates for each laboratory. The MAD value of per laboratory ranged from 0.02 to 0.8 and showed a 40-fold difference in MAD. The MAD values of the 9 laboratories ranged from 0 to 0.1 marked with white background. The MAD values of the 16 laboratories ranged from 0.1 to 0.3 marked with light gray background. The MAD values of the 13 laboratories were greater than 0.3 marked with a dark gray background (Table 3).

According to the location of the sample operation, the laboratories were divided into 2 groups (statistical analysis without the data from No. 23 laboratory because the participants of No. 23 laboratory did not make slide by themselves for some reasons). Group A contained the 27 laboratory samples performed and operated in the laboratory of NIRP, and group B contained the 10 laboratory blind samples to their own laboratory performed in the following steps. We used the Wilcoxon test for a 2-group comparison of the MAD distribution. Finally, we found no difference between the 2 groups of MAD distribution.

| Laboratory ID | Number of Metaphase | Dicentrics + Centric Ring/100 Metaphase | Number of Metaphase | Dicentrics + Centric Ring/100 Metaphase | Number of Metaphase | Dicentrics + Centric Ring/100 Metaphase | Number of Metaphase | Dicentrics + Centric Ring/100 Metaphase |
|---------------|---------------------|----------------------------------------|---------------------|----------------------------------------|---------------------|----------------------------------------|---------------------|----------------------------------------|
| 1             | 670                 | 10.15                                  |                     |                                        |                     |                                        |                     |                                        |
| 2             | 1000                | 9.00                                   |                     |                                        |                     |                                        |                     |                                        |
| 3             | 1382                | 11.07                                  |                     |                                        |                     |                                        |                     |                                        |
| 4             | 1000                | 7.80                                   |                     |                                        |                     |                                        |                     |                                        |
| 5             | 1600                | 17.63                                  |                     |                                        |                     |                                        |                     |                                        |
| 6             | 1000                | 13.60                                  |                     |                                        |                     |                                        |                     |                                        |
| 7             | 1000                | 5.80                                   |                     |                                        |                     |                                        |                     |                                        |
| 8             | 970                 | 15.67                                  |                     |                                        |                     |                                        |                     |                                        |
| 9             | 1475                | 13.02                                  |                     |                                        |                     |                                        |                     |                                        |
| 10            | 658                 | 16.90                                  |                     |                                        |                     |                                        |                     |                                        |
| 11            | 770                 | 17.92                                  |                     |                                        |                     |                                        |                     |                                        |
| 12            | 1000                | 5.80                                   |                     |                                        |                     |                                        |                     |                                        |
| 13            | 1000                | 17.60                                  |                     |                                        |                     |                                        |                     |                                        |
| 14            | 775                 | 9.42                                   |                     |                                        |                     |                                        |                     |                                        |
| 15            | 1000                | 17.60                                  |                     |                                        |                     |                                        |                     |                                        |
| 16            | 1500                | 11.00                                  |                     |                                        |                     |                                        |                     |                                        |
| 17            | 1000                | 13.60                                  |                     |                                        |                     |                                        |                     |                                        |
| 18            | 1000                | 20.60                                  |                     |                                        |                     |                                        |                     |                                        |
| 19            | 800                 | 12.63                                  |                     |                                        |                     |                                        |                     |                                        |
| 20            | 658                 | 16.90                                  |                     |                                        |                     |                                        |                     |                                        |
| 21            | 1475                | 13.02                                  |                     |                                        |                     |                                        |                     |                                        |
| 22            | 550                 | 12.40                                  |                     |                                        |                     |                                        |                     |                                        |
| 23            | 1475                | 13.02                                  |                     |                                        |                     |                                        |                     |                                        |
| 24            | 770                 | 17.92                                  |                     |                                        |                     |                                        |                     |                                        |
| 25            | 635                 | 13.86                                  |                     |                                        |                     |                                        |                     |                                        |
| 26            | 350                 | 24.85                                  |                     |                                        |                     |                                        |                     |                                        |
| 27            | 338                 | 17.16                                  |                     |                                        |                     |                                        |                     |                                        |
| 28            | 991                 | 20.29                                  |                     |                                        |                     |                                        |                     |                                        |
| 29            | 1077                | 6.50                                   |                     |                                        |                     |                                        |                     |                                        |
| 30            | 933                 | 10.84                                  |                     |                                        |                     |                                        |                     |                                        |
| 31            | 684                 | 12.13                                  |                     |                                        |                     |                                        |                     |                                        |
| 32            | 200                 | 20.00                                  |                     |                                        |                     |                                        |                     |                                        |
| 33            | 698                 | 17.34                                  |                     |                                        |                     |                                        |                     |                                        |
| 34            | 1000                | 28.40                                  |                     |                                        |                     |                                        |                     |                                        |
| 35            | 550                 | 12.40                                  |                     |                                        |                     |                                        |                     |                                        |
| 36            | 992                 | 24.49                                  |                     |                                        |                     |                                        |                     |                                        |
| 37            | 511                 | 12.92                                  |                     |                                        |                     |                                        |                     |                                        |
| 38            | 700                 | 14.86                                  |                     |                                        |                     |                                        |                     |                                        |

* The number of analyzed metaphase did not meet the requirements.
Of the 13 laboratories with MAD values greater than 0.3, 10 had at least 1 sample, and the estimate dose was lying outside the reference dose of ± 0.2. The estimate dose of 11 laboratories was lying outside the reference dose of ± 0.2, and 10 of them had the MAD values of greater than 0.3. We also observed an increased MAD with increasing absorbed dose per sample.

**Laboratory Distribution for Intercomparison**

Thirty-eight participants from the CDC, Prevention and Treatment Center for Occupational Disease, colleges and universities, Scientific Research Institute, unit of nuclear industry, and a hospital in Hong Kong participated. The region of these participants covered 25 provinces or municipalities in China (Figure 2). Most of these laboratories can individually offer the service of cytogenetic dosimetry to other regions that do not have this service 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 to analyze the causes and conduct the corresponding training and instruction.

The region marked with dark gray shows more than 1 institution taking part in this study. The region marked with light gray shows 1 institute taking part in this study, and the white region shows no participant.

**Discussion**

The dicentric chromosome analysis is considered to represent the gold standard for diagnostic biodosimetry. Dicentric chromosome assay forms a common methodological platform for national, regional, and global biodosimetry networks to enhance the response capacity during large-scale radiological incident. This study also contributes to the further validation of the DCA for network biodosimetry applied in large-scale radiological incidents. To maintain such an assistance network, periodically organized intercomparison between biodosimetry service laboratories are recommended to ensure the accuracy and reliability of their results.

Although many participants need several samples, we restricted each dose of blood taken from the same donors to focus on methodological variance and exclude interindividual variance. The MADs are valid for the study under the fixed specified experimental design, which was identical for all participants and reflect the overall accuracy of dose estimates per

**Table 2. Summary of Standard Calibration Curves.**

| Standard Calibration Curve | Dose Rate (Gy/min) | Number | Laboratory ID |
|----------------------------|--------------------|--------|---------------|
| \( Y = 0.0533D^2 + 0.0756D - 0.0336 \) | 0.32 | 25 | 1, 5, 6, 7, 8, 13, 14, 15, 16, 17, 18, 20, 21, 22, 23, 24, 25, 26, 29, 30, 32, 34, 35, 36, 38 |
| \( Y = 0.0804D^2 + 0.0340D + 0.0735 \) | 1.00 | 3 | 2, 28, 31 |
| \( Y = 0.0695D^2 + 0.0350D \) | 1.00 | 4 | 3, 10, 11, 19 |
| \( Y = 0.0715D^{1.8923} \) | 0.38 | 2 | 4, 33 |
| \( Y = 0.0716D^2 + 0.0259D \) | 1.00 | 1 | 9 |
| Undefined curve^a | | 3 | 12, 27, 37 |

^a The laboratory that they didn’t mark which standard calibration curve that they used.

[Figure 1. Comparison of dose–response calibration curves used for estimating doses.]
laboratory contribution. In this intercomparison, approximately one-quarter of participants were unable to obtain the estimation dose in the range of error, and they need help to improve their analysis ability. As demonstrated in this intercomparison exercise, some sources of potential error directly or indirectly were linked to other results. To this end, we analyzed the concrete reasons.

To obtain accurate estimated dose, the following several factors were required:

1. Accurately analyzing the chromosomal aberration. Unable to accurately judge the aberration would lead to a high or low chromosome distortion rate. Thus, the estimated dose would be high or low surely.

| Laboratory ID | Reference Dose for Each Sample (Gy) | Operation Completed in NIRP | Automated Metaphase Finding |
|---------------|-------------------------------------|-----------------------------|------------------------------|
|               | 1.10 1.40 2.10 2.8 | MAD (Gy) | MAD (SEM) |
| 1             | 1.03 | 2.08 | 0.045 | 0.025 | Yes |
| 9             | 1.40 | 2.76 | 0.020 | 0.020 | Yes |
| 13            | 1.41 | 2.69 | 0.060 | 0.050 | Yes |
| 14            | 0.99 | 2.01 | 0.100 | 0.010 | Yes |
| 21            | 1.16 | 2.73 | 0.065 | 0.005 | Yes |
| 25            | 1.41 | 2.71 | 0.050 | 0.040 | Yes |
| 34            | 1.39 | 2.94 | 0.075 | 0.065 | Yes |
| 35            | 1.08 | 2.05 | 0.035 | 0.015 | Yes |
| 6             | 1.08 | 1.73 | 0.195 | 0.175 | Yes |
| 7             | 1.48 | 2.51 | 0.245 | 0.165 | Yes |
| 16            | 1.30 | 2.70 | 0.150 | 0.050 | Yes |
| 19            | 1.03 | 1.89 | 0.140 | 0.070 | Yes |
| 22            | 1.37 | 3.32 | 0.275 | 0.245 | Yes |
| 24            | 1.18 | 3.16 | 0.220 | 0.140 | Yes |
| 26            | 1.22 | 1.84 | 0.190 | 0.070 | Yes |
| 27            | 1.26 | 2.86 | 0.110 | 0.050 | Yes |
| 28            | 1.23 | 2.65 | 0.140 | 0.010 | Yes |
| 32            | 1.11 | 3.02 | 0.115 | 0.105 | Yes |
| 38            | 1.29 | 2.15 | 0.120 | 0.070 | Yes |
| 12            | 1.61 | 3.36 | 0.535 | 0.025 | Yes |
| 15            | 0.65 | 1.95 | 0.800 | 0.050 | Yes |
| 17            | 0.78 | 1.54 | 0.590 | 0.030 | Yes |
| 18            | 1.21 | 2.29 | 0.350 | 0.160 | Yes |
| 20            | 1.56 | 3.05 | 0.555 | 0.395 | Yes |
| 29            | 1.56 | 2.29 | 0.335 | 0.175 | Yes |
| 33            | 0.83 | 1.28 | 0.695 | 0.125 | Yes |
| 37            | 1.05 | 2.13 | 0.360 | 0.310 | Yes |
| 3             | 0.91 | 2.79 | 0.050 | 0.040 | No |
| 2             | 1.48 | 3.05 | 0.165 | 0.085 | No |
| 4             | 1.51 | 2.44 | 0.225 | 0.115 | No |
| 10            | 1.01 | 1.69 | 0.250 | 0.160 | No |
| 11            | 1.43 | 3.02 | 0.275 | 0.055 | No |
| 5             | 1.74 | 3.75 | 0.645 | 0.305 | No |
| 8             | 0.92 | 3.26 | 0.320 | 0.140 | No |
| 30            | 0.60 | 1.71 | 0.445 | 0.055 | No |
| 31            | 1.00 | 2.28 | 0.460 | 0.060 | No |
| 36            | 1.68 | 2.66 | 0.420 | 0.140 | No |
| 23            | 1.15 | 2.26 | 0.105 | 0.055 | b |

Sample number | 21 17 16 22 |
Average dose  | 1.13 1.32 2.06 2.81 |
MAD (Gy)     | 0.150 0.220 0.373 0.335 |
MAD (SEM)    | 0.031 0.057 0.067 0.058 |

Abbreviations: MAD, mean absolute deviation; NIRP, National Institute for Radiological Protection; SEM, standard error of the mean.

a Dose estimates not falling into the +20% reference dose uncertainty interval accepted for triage are underlined.

b The laboratory did not make slide by themselves for some reasons.
2. Accurate calculation. In the report submitted by some departments, minimal difference was observed between chromosome-type aberration and population mean, but estimated dose would be quite different. For example, the chromosome-type aberration reported by laboratory 17 was basically the same with laboratories 13 and 14, and they used same dose–effect curve, but the estimated doses were quite different due to probably miscalculation. Therefore, effective and accurate calculation was very important.

3. Accurate and effective laboratory dose–effect curve. For the same chromosome distortion rate, we would estimate different doses by using different dose–effect curves. To use the curve that was fit to our laboratory must be effective, or it may obtain inaccurate estimated dose. Nonetheless, we have not found inaccurate estimated dose because the dose–effect curve was no longer applicable in this study.

4. The number of the cells analyzed. We made a principle that dose determines the number of cells needed to be analyzed in this study. However, some laboratories analyzed cells not according to the principle. They also presented estimated doses. Our analysis showed that this circumstance would not influence the accuracy of the estimated dose because of the reduction of the number of cells within a certain range. The finding is the same with the research results published by Beinke et al.

The focus of our study is an intercomparison of various cytogenetic dosimetry laboratories performing individual radiation dose assessment based on the dicentric chromosome analysis.

The 7 participating laboratories used automated metaphase finding system photo in the metaphase and scored the dicentric manually. Garcia et al used electronically transmitted image and compared the DCA interlaboratory. Along with the exaltation of automation degree and digital imaging analysis technology, we need to explore the feasibility and accuracy of scoring the dicentric automatically.

Authors' Note
The studies have been approved by the institutional research ethics committee and have been performed in accordance with the ethical standards in the Declaration of Helsinki. Informed consent was obtained from all individual participants included in the study.

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