Dose-Response:
An International Journal
January-March 2019:1-7

DOI: 10.1177/1559325819833473
journals.sagepub.com/home/dos
SAGE

Laboratory Intercomparison of Cytogenetic Dosimetry Among 38 Laboratories in China

Yan Pan[1], Jianlei Ruan[1], Gang Gao[1], Lina Wu[1], Chunnan Piao[1], and Jianxiang Liu[1]

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 ^{60}Co 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 $\pm$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.

[1] China CDC Key Laboratory of Radiological Protection and Nuclear Emergency, National Institute for Radiological Protection, Chinese Center for Disease Control and Prevention, Beijing, China

Corresponding Author:
Jianxiang Liu, China CDC Key Laboratory of Radiological Protection and Nuclear Emergency, National, Institute for Radiological Protection, Chinese Center for Disease Control and Prevention, Xinkang Street, Deshengmenwai, XiCheng District, Beijing 100088, China.
Email: liujianxiang@nirp.chinacdc.cn

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).[6,13] A slight variation among participating laboratories was permissible.

Conditions of Exposure

Whole blood was irradiated in heparinized tubes with ^{60}Co γ-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 own laboratory according to the distance and the transportation. Transportation temperature ranged from 18°C to 24°C. Transportation time ranged from minutes to 12 hours. All participants provided triage dose estimate within 30 days after obtaining the blind blood samples.

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.[6,13]

The Number of Metaphase Scored

Each participant had to score at least 100 dicentrics or 1000 metaphase, or on request of the formula: $n = \frac{[(1-p)\times 96.04]}{p}$, where p is the percent of cell dicentrics or centric rings. The score number accorded 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.

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.

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.

The report of the rate of chromosomal aberration ranged from 6.50% to 24.85% and showed a 3.8-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 $\pm 20\%$.

The report of the rate of chromosomal aberration ranged from 5.80% to 26.00% and showed a 4.5-fold difference for the samples at the dose of 1.4 Gy. The estimated doses ranged from 0.65 to 1.74 Gy and showed a 2.68-fold difference. The mean value of all 18 laboratories was 1.33 Gy. Five laboratories reported that the estimate dose was lying outside the reference dose of $\pm 20\%$.

The report of the rate of chromosomal aberration ranged from 20.90% to 69.34% and showed a 3.32-fold difference for the samples at the dose of 2.1 Gy. The estimated doses ranged from 1.28 to 3.05 Gy and showed a 2.38-fold difference. The mean value of all 18 laboratories was 2.05 Gy. Four laboratories reported that the estimate dose was lying outside reference dose of $\pm 20\%$.

The report of the rate of chromosomal aberration ranged from 42.00% to 100% and showed a 2.38-fold difference for the samples at the dose of 2.8 Gy. The estimated doses ranged from 1.95 to 3.75 Gy and showed a 1.92-fold difference. The mean value of all 22 laboratories was 2.81 Gy. Three laboratories reported that the estimate dose was lying outside reference dose of $\pm 20\%$.

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

Table 1. Summary of the Part of the Analytic Results for the Samples at the Different Reference Doses.

	Actual Dose for Each Sample (Gy)							
	1.10		1.40		2.10		2.8	
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[a]	10.15			376	35.37		
2			696	23.42			520	86.16
3	1000	9.00					300	63.60
4			970	15.67	436	38.76		
5			400	26.00			200	100.00
6	1382	11.07			1445	25.67		
7			1750	19.60	1855	49.27		
8	1000	7.80					100	86.00
9			1600	17.63			1000	61.70
10	348[a]	10.63			252	25.80		
11	322[a]	19.25					100	74.00
12	300[a]	17.70					141	71.66
13			557	17.95			189	55.55
14	775[a]	9.42			102[a]	33.33		
15			1000	17.60			800	54.40
16	1500	11.00					400	52.30
17			1000	5.80	1000	20.90		
18			1000	13.60			238	42.00
19	339[a]	10.91			318	36.47		
20			1000	20.60	300	69.34		
21	800	12.63					300	57.00
22			658	16.90			200	80.50
23	550[a]	12.40			164	40.80		
24	1475	13.02					756	73.81
25			770	17.92			581	56.46
26	635	13.86			370	28.65		
27	350	24.85					140	93.57
28	338[a]	17.16					150	66.00
29			991	20.29			980	64.08
30	1077	6.50			1106	28.40		
31			684[a]	12.13			228	50.44
32	933	10.84					200	68.00
33			200[a]	20.00	540	23.88		
34			698	17.34			205	64.88
35	450[a]	11.11			180[a]	32.80		
36			992	24.49	549	54.46		
37	511[a]	12.92					176	82.00
38	700	14.86			600	37.00		

[a] The number of analyzed metaphase did not meet the requirements.

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.

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 $\pm 20\%$. The estimate dose of 11 laboratories was lying outside the reference dose of $\pm 20\%$, 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.

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.

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

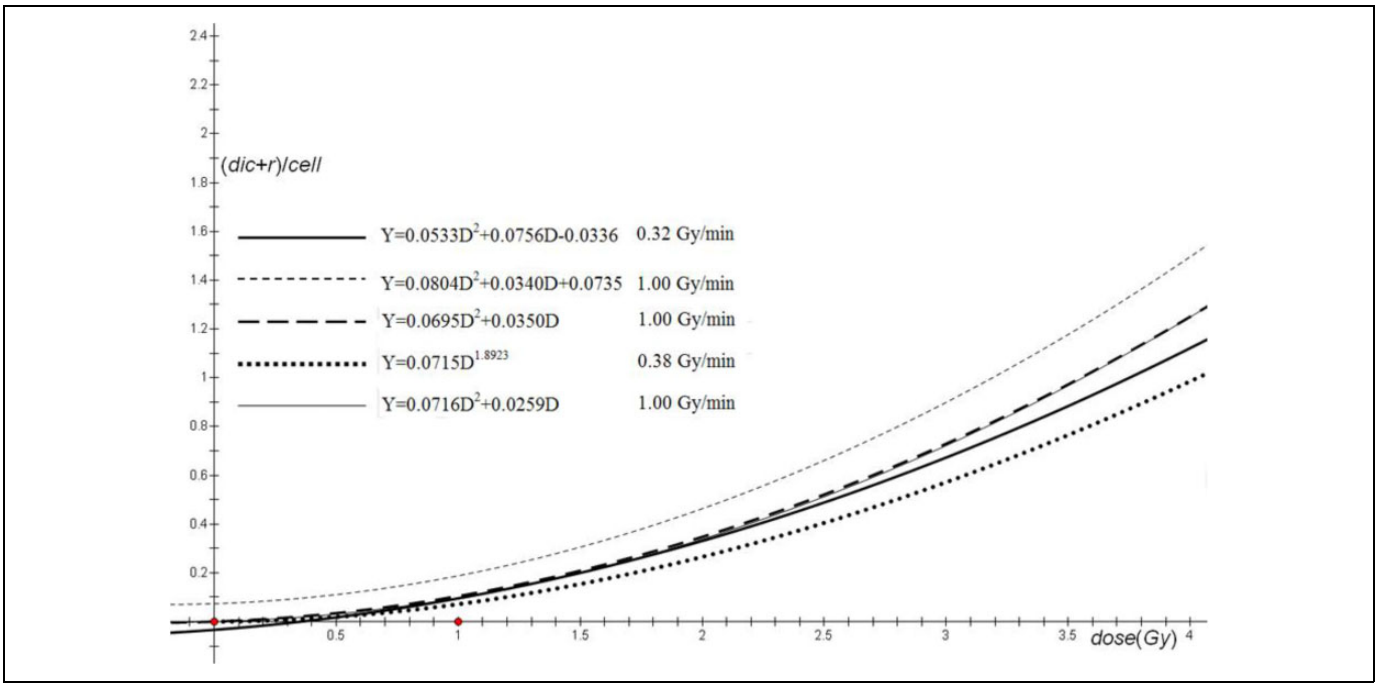

Figure 1. Comparison of dose–response calibration curves used for estimating doses.

Table 3. Comparison of MAD Between Different Participants and Different Samples.[a]

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

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 the 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.

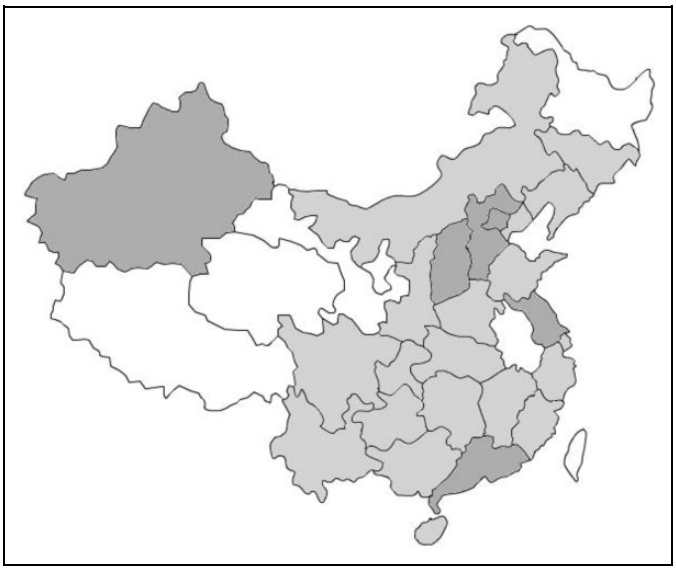

Figure 2. Region of laboratories that participated in the intercomparison in the map of China.

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.[14] 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.

Acknowledgments

The authors are grateful to National Health and Family Planning Commission and NIRP, China CDC, for sponsoring the project. Also, the authors are grateful to the participants and laboratories participating in this proficiency test. Thanks are also given to staffs from section of science & technology of NIRP for their support to this work.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of the article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work is conducted as part of project IAEA (Research Contract No. 17092).

References

1. International Atomic Energy Agency. *Biological Dosimetry: Chromosomal Aberration Analysis for Dose Assessment Technical Report 260*. Vienna, Austria: IAEA; 1986.
2. Lloyd DC. Chromosomal analysis to assess radiation dose. *Stem Cells*. 1997;15(suppl 2):195-201.
3. Feng L, He L, Wang Y, et al. Eight-year follow-up study of three individuals accidentally exposed to (60)Co radiation: chromosome aberration and micronucleus analysis. *Mutat Res Genet Toxicol Environ Mutagen*. 2015;784-785:10-14.
4. Lee JK, Han EA, Lee SS, et al. Cytogenetic biodosimetry for Fukushima travelers after the nuclear power plant accident: no evidence of enhanced yield of dicentrics. *J Radiat Res*. 2012; 53(6):876-881.
5. Suto Y, Hirai M, Akiyama M, et al. Biodosimetry of restoration workers for the Tokyo Electric Power Company (TEPCO) Fukushima Daiichi nuclear power station accident. *Health Phys*. 2013;105(4):366-373.
6. International Atomic Energy Agency. *Cytogenetic Dosimetry: Applications in Preparedness for and Response to Radiation Emergencies, Emergency Preparedness and Response Series*. Vienna, Ausrtia: IAEA; 2011.
7. Miller SM, Ferrarotto CL, Vlahovich S, Wilkins RC, Boreham DR, Dolling JA. Canadian cytogenetic emergency network (CEN) for biological dosimetry following radiological/nuclear accidents. *Int J Radiat Biol*. 2007;83(7):471-477.

8. Realizing the European Network in Biodosimetry (RENEB). *Project funded within the 7th EU Framework Programme*. 2012. https://cordis.europa.eu/project/rcn/94420/reporting/en.
9. Christie DH, Chu MC, Carr Z. Global networking for biodosimetry laboratory capacity surge in radiation emergencies. *Health Phys*. 2010;98(2):168-171.
10. Beinke C, Barnard S, Boulay-Greene H, et al. Laboratory intercomparison of the dicentric chromosome analysis assay. *Radiat Res*. 2013;180(2):129-137.
11. Bhavani M, Tamizh Selvan G, Kaur H, et al. Dicentric chromosome aberration analysis using giemsa and centromere specific fluorescence in-situ hybridization for biological dosimetry: an inter- and intra-laboratory comparison in Indian laboratories. *Appl Radiat Isot*. 2014;92:85-90.
12. Wilkins RC, Romm H, Kao TC, et al. Interlaboratory comparison of the dicentric chromosome assay for radiation biodosimetry in mass casualty events. *Radiat Res*. 2008;169(5):551-560.
13. State Standard of the People's Republic of China. *Method of Chromosome Aberration Analysis for Biological Dose Assessment. GB/T 28236-2011*. Beijing, China: State Standard of the People's Republic of China. 2011.
14. García O, Di Giorgio M, Vallerga MB, et al. Interlaboratory comparison of dicentric chromosome assay using electronically transmitted images. *Radiat Prot Dosim*. 2013;154(1):18-25.