MEDICAL PRACTICE

Cytogenetic biodosimetry: what it is and how we do it

KF Wong 黃傑輝
Lisa LP Siu 邵麗平
E Ainsbury
J Moquet

Dicentric assay is the international gold standard for cytogenetic biodosimetry after radiation exposure, despite being very labour-intensive, time-consuming, and highly expertise-dependent. It involves the identification of centromeres and structure of solid-stained chromosomes and the enumeration of dicentric chromosomes in a large number of first-division metaphases of cultured T lymphocytes. The dicentric yield is used to estimate the radiation exposure dosage according to a statistically derived and predetermined dose-response curve. It can be used for population triage after large-scale accidental over-exposure to ionising radiation or with a view to making clinical decisions for individual patients receiving substantial radiation. In this report, we describe our experience in the establishment of a cytogenetic biodosimetry laboratory in Queen Elizabeth Hospital, Hong Kong. This was part of the contingency plan for emergency measures against radiation accidents at nuclear power stations.

Introduction

Estimation of radiation dose after accidental exposure is important for mass casualty triage and also for making clinical decision for individual patients, particularly with respect to the planning and provision of stem cell transplantation.[1] In contrast to measuring radiation in the immediate environment of the individual using personal physical dosimetry with a thermoluminescence dosimeter at the time of radiation exposure, the following triad can give a rough assessment of the radiation dose that has 'penetrated' the individual and its resulting damage: (1) the time to onset of vomiting; (2) the absolute lymphocyte count; and (3) the dicentric assay. Dicentric assay refers to the enumeration of dicentric chromosomes in the first-division metaphase of peripheral blood lymphocytes,[2] and is the gold standard among cytogenetic biodosimetry techniques for radiation dose estimation.

Cytogenetic biodosimetry

Dicentric chromosomes as an indicator of radiation exposure

Exposure to ionising radiation causes DNA strand breaks in living cells, including double-strand breaks. During repair of DNA strand breaks, misrepair of 2 chromosomes and abnormal chromosome replication can lead to the formation of a dicentric chromosome—an unstable aberration with a chromosome having 2 centromeres.[3] It is well established that an increase in radiation dose produces increasing number of dicentrics. Although radiation induces many types of chromosomal changes in addition to dicentrics, the latter are considered

Key words
Acute radiation syndrome; Cytogenetic analysis; Nuclear power plants; Radiation dosage; Radiometry

Hong Kong Med J 2013;19:168-73

Department of Pathology, Queen Elizabeth Hospital, Jordan, Hong Kong
KF Wong, MD, FHKAM (Pathology)
LLP Siu, PhD
Health Protection Agency, Centre for Radiation, Chemical and Environmental Hazards, Oxford, United Kingdom
E Ainsbury, PhD
J Moquet, PhD

Correspondence to: Dr KF Wong
Email: kfwong@ha.org.hk

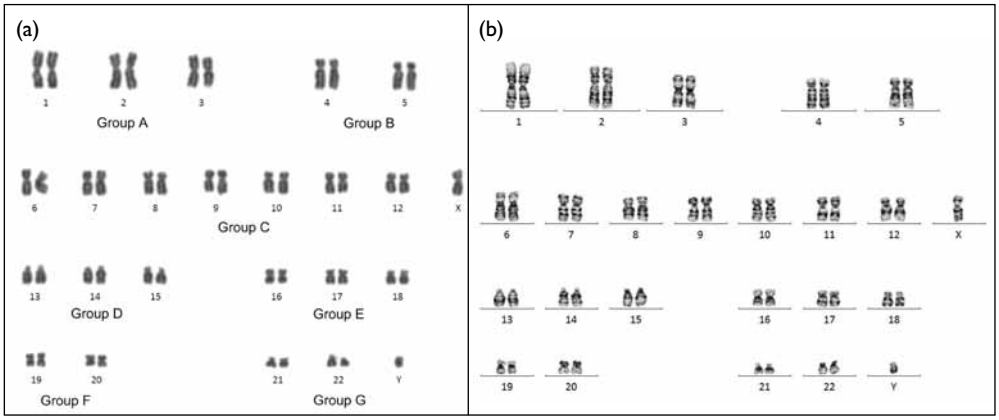

FIG 1. (a) Giemsa-stained karyogram from a male donor, showing group A to G chromosomes. (b) G-banded karyogram from a male donor for comparison (G-band with trypsin-Giemsa)

the most sensitive and specific for assessing radiation dose, even at low doses (≈100 mGy).

Cytogenetic biodosimetry is based on the analysis of aberrations such as dicentrics in solid-stained chromosomes (Fig 1) without the use of a banding technique[4] (ie staining to demonstrate a continuous series of light and dark bands along the chromosomes) in cultured T lymphocytes at their first division. A dicentric is an exchange between the centromeric pieces of 2 radiation-damaged chromosomes which in its complete form is accompanied by an acentric fragment composed of the acentric pieces of the two chromosomes (Fig 2a).[3] A ring is another unstable aberration which is much rarer than the dicentric (Fig 2b). Rings consist of exchanges between two breaks on separate arms of the same chromosome, and are also accompanied by an acentric fragment. Some cytogenetic biodosimetry techniques (eg the Qdr method) combine evaluation for rings with dicentrics.[5] The dicentric is, however, still the main biomarker for chromosome damage used for cytogenetic biodosimetry, as it is known to be almost exclusively radiation-specific, with very little difference in the background rate and little inter-individual variation. Nevertheless, the assay itself is very labour-intensive and expertise-dependent, and its usefulness is affected by the limited lifespan of the unstable aberration. Examples of other cell- or chromosome-based biodosimetry techniques include the evaluation of micronuclei and aberrations of prematurely condensed chromosomes, but recourse to these is less common (Table 1).[3,6,7]

染色體畸變估算生物劑量測定的介紹與運用

儘管費力、費時及高度依賴專門技術，雙著絲粒分析法仍是評估輻射接觸後染色體畸變估算生物劑量測定公認的黃金標準。這種方法涉及辨認大量培養後T淋巴細胞的第一次分裂中期著絲粒、著色染色體的結構及雙著絲粒染色體的計數。雙著絲粒產率可用於根據統計學或預設的劑量反應曲線去估計輻射暴露劑量。此方法在發生大規模意外時過度暴露於電離輻射的傷者分類或為個別接受大量幅射的患者作出臨床決定皆有幫助。本文分享伊利沙伯醫院創立染色體畸變估算生物劑量測定實驗室的經驗，這也是針對核電站發生輻射事故時後備緊急措施的一部份。

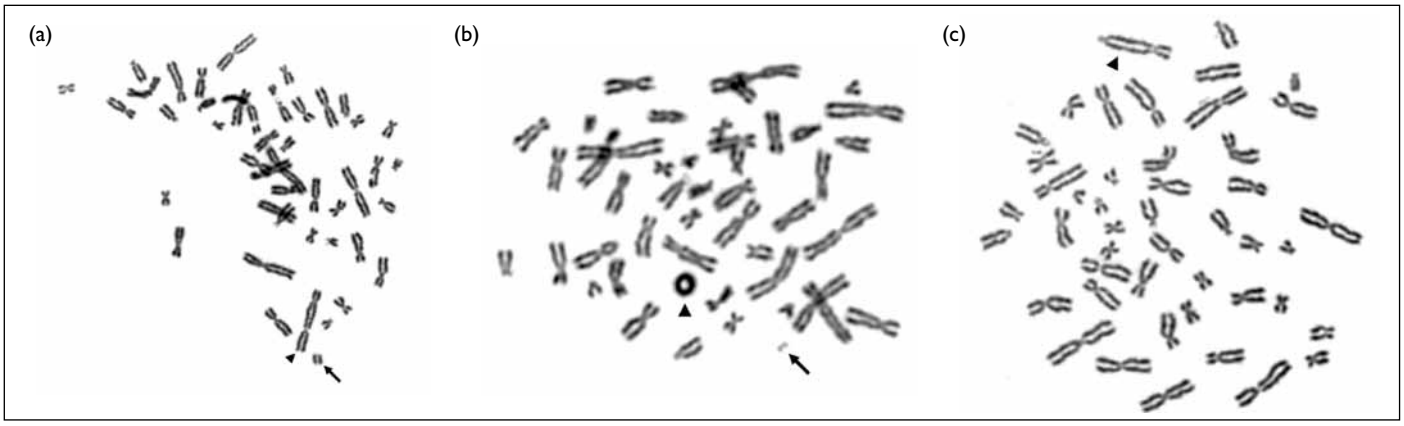

FIG 2. Giemsa-stained metaphase showing (a) a dicentric (arrowhead) and an acentric fragment (arrow); (b) a ring (arrowhead) and an acentric fragment (arrow); and (c) a dicentric (arrowhead) involving group D and possibly group C chromosomes

TABLE 1. Cell- or chromosome-based biodosimetry assays[3,6,7]

Type	Description	Remarks
Micronucleus	In-vitro micronucleus formation in once-divided cells after inhibition of cytokinesis by cytochalasin B	Easier and more rapid, good reliability and reproducibility but limited sensitivity (relatively high and variable spontaneous micronucleus yield); great potential for automation
Stable chromosome translocation	G-banding or fluorescence in-situ hybridisation	Time-consuming and highly expertise-dependent, incomplete calibration of dose-response relationship, not suitable for a quantitative analysis of radiation damage but applicable to old or long-term exposure
Premature chromosome condensation	Fusing and culturing human lymphocytes with Chinese hamster ovary mitotic cells; modifications including the use of chemical induction and interphase in-situ hybridisation with shortened culture time	Useful for low-dose and acute high-dose, and total- and partial-body radiation exposure but requiring the use and maintenance of cell line or carcinogenic chemicals
Multicentric chromosome	Fluorescence in-situ hybridisation with pan-centromeric peptide nucleic acid probes	Easier and more rapid, less labour-intensive but more expensive; great potential for automation

Mass casualty triage and clinical decision-making

There exists a very well-defined dose-effect relationship for dicentric assays in the range of 0.05 to 5.0 Gy for acute exposures to low linear energy transfer (LET) radiation. Factors that affect the results of dicentric assays include radiation source, dose and exposure rate, percent of the body irradiated (whole versus partial body), homogeneity of exposure, as well as the time delay between radiation exposure and blood sampling.[8] Assay results for cytogenetic biodosimetry reported by laboratories include not just a count of dicentrics but an estimated radiation dose (based on a predefined calibration curve). They can assist in clinical decision-making alongside other clinical and laboratory information for patients receiving whole or even partial body radiation by using the Qdr method, which considers the distribution of dicentrics and rings among just the damaged cells.[8] The assay can also be adapted to study the effects of protracted exposure by adding a time-dependent factor (G function) in the dose-squared coefficient of the linear quadratic dose-response relationship (see below).[9] Thus, it is useful for mass casualty triage and counselling of patients of radiation exposure.

Quality assurance and inter-laboratory consistency

In order to accurately carry out the dicentric assay, it is necessary to establish a predetermined in-vitro dose-response curve which is specific to the type of radiation exposure (assessed by clinical situation or otherwise) such as gamma-rays in order to correlate a measured dicentric yield to the dose of exposure from that particular radiation.[3,10] The exposure should cover the range of 0.25 Gy or less to 5 Gy with a minimum of 4 doses in 0.25 to 1 Gy. Around 10 000 metaphases should be analysed for a statistically meaningful curve. The relationship between the dosage of acute exposure to low LET radiation and the dicentric count is given by the linear-quadratic equation:

$$y = c + \alpha D + \beta D^2$$

where y is the dicentric yield; c the control (background frequency); α the linear coefficient, theoretically for exchange aberrations produced by single electron track; β the corresponding quadratic coefficient for aberrations produced as a consequence of two electron tracks, and D the absorbed dose in the cell.

The background level for dicentrics in the population is considered to be low, with about 0.5 to 1 dicentrics in 1000 cells.[3]

Cytogenetic biodosimetry is both time-consuming and technique-dependent.[7] Each laboratory should establish its own dose-response curve and ensure consistency in dose estimation. Only a relatively small number of laboratories have sufficient experience with the rigorous quality control and dose calibration necessary to perform this assay, when it is used to estimate radiation dose. International Organization for Standardization (ISO) 21243 requires the participation of cytogenetic biodosimetry laboratory in inter-laboratory comparison.[11] The latter are complicated by: (1) inter-laboratory and inter-scorer variability, (2) different assays being used in different laboratories, (3) different curve-fitting procedures to construct dose-response curves, and (4) small variations in background aberration yields. However, recent studies demonstrated good consistency for cytogenetic biodosimetry in international collaboration.[12,13]

The situation in Hong Kong

The Hospital Authority of Hong Kong is involved in the formulation of the contingency plan to deal with the emergency measures that should be undertaken in the event of an accident at one of the nuclear power stations near Hong Kong, which may result in the off-site release of radioactive materials. In the unlikely event of such an accident, the Cytogenetic Laboratory of the Department of Pathology, Queen Elizabeth Hospital has been charged with providing biodosimetric assessment for patients exposed to radiation. Dicentric assay was adopted as the default method for developing such a service because of our experience in conventional cytogenetics for blood cancers and the unlikelihood of mass casualties in Hong Kong. In late 2011, the first two authors on this paper went overseas to the Radiation Effects Research Foundation in Hiroshima, Japan and the Health Protection Agency Centre for Radiation, Chemical and Environmental Hazards in Oxford, United Kingdom respectively for training in cytogenetic biodosimetry.

Establishment of the dose-response curve

Sample irradiation and culturing

As most accidental radiation exposures are due to X- or gamma-rays, the dose-response curve for gamma-rays was the first to be established when we embarked upon cytogenetic biodosimetry. Dicentrics were evaluated in cultured peripheral blood T lymphocytes after irradiation by a Cobalt-60 gamma-ray source between 0 and 5.0 Gy. Briefly, 30 mL of blood was taken from a healthy donor, with informed consent and ethics approval from the Berkshire Research Ethics Committee (Ref 09/H0505/87). Aliquots were then irradiated at 37°C with a dose rate of 0.41 Gy/min at the Medical Research Council Radiobiology Unit in Harwell, United Kingdom. The blood samples were left for 2 hours at 37°C after irradiation to allow repair to take place. Lymphocyte cultures were set up according to the micro-culture method.[3] Briefly, phytohaemagglutinin (PHA)-stimulated lymphocytes were grown in Minimum Essential Medium (Life

Technologies Corporation, US) supplemented with 10% foetal bovine serum. The cultures were incubated at 37°C for 48 hours with colcemid (a synthetic analogue of colchicine and a mitosis-arresting agent) added for the final 3 hours. Metaphases were harvested from the cultures by standard hypotonic treatment with potassium chloride, followed by fixation in methanol-to-acetic acid (3:1). Replicate cultures were set up for each radiation dose point (10 dose points of 0-5.0 Gy) to optimise the yield of metaphases at the first in-vitro division (Table 2). Fixed suspensions of the cultured products were dropped onto glass slides to 'spread' the metaphases. The slides were then stained with Giemsa stain after one 'test' slide for each radiation dose had first been checked for the presence of second-division metaphases (using the fluorescence plus Giemsa staining technique).[14]

TABLE 2. Ratio of first- (M1) to second-division (M2) metaphases in relation to radiation dose in irradiated cells

Required dose (Gy)	Actual dose (Gy)	M1:M2 in 100 cells	Minimum No. of cells to analyse
0	0	99:1	2000
0.1	0.1	99:1	2000
0.25	0.26	100:0	1000
0.5	0.51	99:1	1000
0.75	0.75	100:0	1000
1.0	1.0	100:0	1000
2.0	2.01	100:0	500
3.0	2.99	100:0	300
4.0	4.01	100:0	200
5.0	4.99	100:0	100/150

Slide preparation and metaphase analysis

The slides were examined in transmitted light using a motorised microscope (Axio Imager.Z2; Carl Zeiss, Germany) with the software Metafer 4 (version 3.8.6, MetaSystems, Germany) for metaphase scanning and capturing. The metaphase was relocated using either the Metafer4 at the capturing station or the ReloSys (version 3.8.6, MetaSystems, Germany) at an offline station. Dicentric scoring was performed on the metaphases using oil immersion at x1000 magnification. Analysis was only undertaken on metaphases with good morphology and an object count of ≥46. The metaphases scored had to be complete, ie each dicentric should be accompanied by an acentric fragment and the number of excess acentric fragments should correspond to the total object count (some useful tips are provided in the Box). Depending on the morphology, dose, and the yield from individual cultures, around 40 to 800 metaphases were analysed on each slide. The minimum number of metaphases to be analysed for each dose was set at 2000 for 0 and 0.1 Gy; 1000 for 0.25, 0.5, 0.75 and 1.0 Gy, and 500, 300, 200 and 150 for 2.0, 3.0, 4.0 and 5.0 Gy, respectively. Fluorescence plus Giemsa staining that provided information on the relative proportion of cells in the first- and second- in-vitro division metaphases showed ≥99% cells in the first-division metaphase, thus indicating minimal loss of unstable aberrations after culture initiation.

Statistical analysis for dose-response curve

The total number and distribution of dicentrics scored for each dose were analysed by the software Dose Estimate (version 4.0) developed by Health Protection Agency in Oxford, United Kingdom.[15] The

BOX. Some practical tips in cytogenetic biodosimetry

1. Do not drop the cell suspension from too tall a height or slant the slide when dispensing the cells. This is to avoid over-spreading of the metaphase, thus losing some of the smaller chromosome objects.
2. Count all the chromosome objects including acentric fragments such as minutes, double minutes, and acentric rings. Ideally, the centromeres should add up to 46.
3. Any exchange between the centromeric pieces of two damaged chromosomes should be accompanied by an (joined) acentric fragment. Thus, tricentrics are accompanied by two fragments and quadricentric by three. One should always look for a dicentric if an acentric fragment is found and vice versa.
4. A centromere appears as a constriction in the chromosome and is less than one-third of the chromosome width. It has to be distinguished from achromatic gap which is a non-staining or very lightly staining region of chromosome present in one chromatid or in both sister chromatids at apparently identical loci.
5. Beware of D-D, D-G and A/B/C-D/G type* of dicentrics as the short arm is small and can be very lightly stained.
6. An acentric fragment is a block-like or oblong structure. In better stained preparation or long acentric, one may be able to see a clear demarcation between the two chromatids. There should be no constriction.
7. If the presence of an acentric fragment particularly for a small chromosome object is uncertain, one may count the number of D and G group chromosomes to ascertain that they are intact.
8. If the presence of centromere in a ring structure is obscure, count the number of centromeres—it should normally be 46, ie to designate it as an acentric ring if 46 centromeres are already present.
9. It is important to avoid scoring metaphases with long or too many overlapping chromosomes, or metaphases with chromatids that are too separated.

* The non-banded chromosomes are separated into 7 groups (A-G) based on descending order of size and the position of centromere (short-and-long-arm ratio) [Figs 1a and 2c].

TABLE 3. Dicentric yield and its distributions after acute irradiation with various doses of Cobalt-60

Dose (Gy)	No. of cells scored	No. of dicentrics	Dicentric distribution						Variance/ mean ratio	U
			0	1	2	3	4	5		
0	2132	1	2131	1	0	0	0	0	1	0
0.1	2139	5	2134	5	0	0	0	0	0.998	-0.068
0.26	1014	9	1005	9	0	0	0	0	0.992	-0.189
0.51	1018	19	999	19	0	0	0	0	0.982	-0.41
0.75	1016	50	971	41	3	1	0	0	1.19	4.37
1	1020	78	946	70	4	0	0	0	1.03	0.616
2.01	506	96	418	80	8	0	0	0	0.979	-0.337
2.99	306	124	200	88	18	0	0	0	0.888	-1.39
4.01	205	143	93	81	31	0	0	0	0.74	-2.64
4.99	221	251	71	76	52	18	3	1	0.936	-0.67

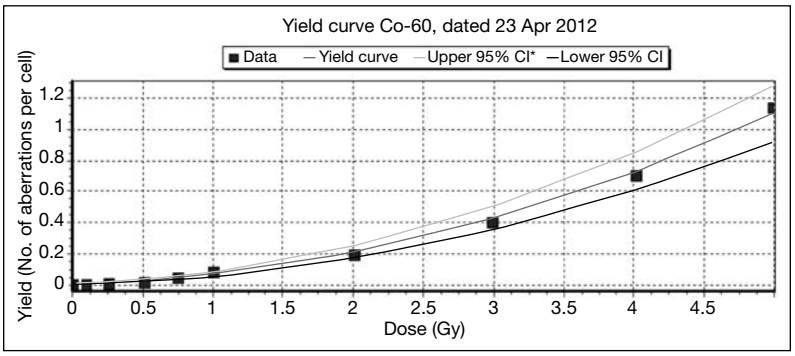

FIG 3. Dose-response curve for Cobalt (Co)-60 gamma-ray irradiation (established by the Cytogenetic Biodosimetry Laboratory, Queen Elizabeth Hospital, Hong Kong)
* CI denotes confidence interval

variance-to-mean ratio and the U-statistic were used to test for Poisson distribution (to predict the degree of spread of cytogenetic data). Data were Poisson distributed if the variance-to-mean ratio was around 1, as under the Poisson model the variance and mean were equal. The data were significantly under- or over-dispersed if the values of U-statistics deviated from ±1.96, as the U-statistic provided a normalised comparison of the variance-to-mean ratio with the expected 95% confidence range. The total number of metaphases analysed for our Cobalt-60 dose-response curve was 9577 (Table 3). The standard error of the curve was automatically adjusted (using the mean variance-to-mean ratio) for deviation from the Poisson distribution. The α and β coefficients were 0.0260 and 0.0386, respectively for the linear quadratic yield curve (Fig 3).

Sample collection, culture, and analysis

Blood should be collected for the dicentric assay as soon as practicable after radiation exposure and certainly within 4 weeks from a suspected exposed person. In case of partial or non-uniform exposure, the blood sample should be taken at least 24 hours after exposure to ensure a more uniform distribution of fractions of irradiated lymphocytes. For suspected radiation doses of >4 Gy, it has been suggested that blood should be drawn at 1 to 6 hours post-event in order to obtain a sufficient population of lymphocytes, as after 6 hours the severe damage may lead to depletion of lymphocytes in the peripheral blood. A total of 10 mL of blood should be collected from the exposed patient after decontamination by aseptic technique using lithium heparin as the anticoagulant. The blood sample was cultured with PHA stimulation for about 48 hours before harvesting. Dicentrics are formed by a misrepair of 2 chromosomes, for which sufficient time should be allowed to permit lymphocytes undergoing and completing the repair. The number of cells to be analysed for the exposed patient should be about 500 cells (or 100 dicentrics), which would require 2 to 3 man-days of manual scoring. Scoring beyond 500 to 1000 cells should also be considered, if clinically indicated.

The way forward

Few countries have more than one cytogenetic laboratory with the primary function of undertaking biodosimetry for radiation emergencies. There may be, however, a lot of cytogenetic expertise in the areas of clinical genetics and genomics. Thus, it has been recommended by the International Atomic Energy Agency to mobilise such expertise under the leadership of a network of reference biodosimetry laboratories.[3] Our cytogenetic laboratory, which is accredited by the National Association of Testing Authorities, Australia in compliance with ISO 15189, embodies one of the few examples of deploying expertise in clinical cytogenetics for the establishment of biodosimetry to prepare for radiation emergencies. We have developed

our techniques according to the International Atomic Energy Agency Manual and in compliance with the ISO standards,[3,11,16] taking reference to our experience in laboratory accreditation and overseas training. As cytogenetic biodosimetry is a rarely requested assay, it is important that we can be part of the network of such a service in other regions, so that our expertise and service quality can be maintained.[17,18] Thus, we have joined the National Biodosimetry Intercomparison Programme (全國生物劑量估算方法比對) organised by the National Institute for Radiological Protection, Chinese Center for Disease Control and Protection. This helps ensure our compliance with international requirements and ISO standards on the participation of intercomparisons.[11,19] By collaborating with other cytogenetic biodosimetry laboratories, we are better equipped to assist in the contingency planning and management of large-scale accidental over-exposure (be it in Hong Kong or the mainland China).[20]

Acknowledgement

Dr KF Wong's training in cytogenetic biodosimetry was supported by the International Exchange Programme of the Radiation Effects Research Foundation, the latter being a bi-national Japan-United States scientific organisation. Dr E Ainsbury and Dr J Moquet were funded by National Institute for Health Research (NIHR) Centre for Research in Health Protection at the Health Protection Agency.

References

1. Waselenko JK, MacVittie TJ, Blakely WF, et al. Medical management of the acute radiation syndrome: recommendations of the Strategic National Stockpile Radiation Working Group. Ann Intern Med 2004;140:1037-51.
2. Radiation Emergency Assistance Center/Training Site (REAC/TS). The Medical Aspects of Radiation Incidents; 2011.
3. International Atomic Energy Agency. Cytogenetic dosimetry: applications in preparedness for response to radiation emergencies. Vienna, Austria: IAEA; 2011.
4. Shaffer LG, Slovak ML, Campbell LJ, editors. ISCN 2009: An International System for Human Cytogenetic Nomenclature. Basel: S Karger; 2009.
5. Sasaki MS, Miyata H. Biological dosimetry in atom bomb survivors. Nature 1968;220:1189-93.
6. Suto Y, Akiyama M, Yamada Y. Unequivocal detection of radiation-induced multicentric chromosomes by fluorescence in situ hybridization using peptide nucleic acid probes. Cytologia 2011;76:1-2.
7. Léonard A, Rueff J, Gerber GB, Léonard ED. Usefulness and limits of biological dosimetry based on cytogenetic methods. Radiat Prot Dosimetry 2005;115:448-54.
8. Vinnikov VA, Ainsbury EA, Maznyk NA, Lloyd DC, Rothkamm K. Limitations associated with analysis of cytogenetic data for biological dosimetry. Radiat Res 2010;174:403-14.
9. Lea DE, Catcheside DG. The mechanism of the induction by radiation of chromosome aberrations in Tradescantia. J Genet 1942;44:216-45.
10. Romm H, Oestreicher U, Kulka U. Cytogenetic damage analysed by the dicentric assay. Ann 1st Super Sanita 2009;45:251-9.
11. International Organization for Standardization. ISO 21243:2008 Radiation protection—performance criteria for laboratories performing cytogenetic triage for assessment of mass casualties in radiological or nuclear emergencies—general principles and application to dicentric assay. Geneva: ISO Office; 2008.
12. Livingston GK, Wilkins RC, Ainsbury EA. Pilot website to support international collaboration for dose assessments in a radiation emergency. Radiat Meas 2011;46:912-5.
13. Wilkins RC, Romm H, Oestreicher U, et al. Biological dosimetry by the triage dicentric chromosome assay—further validation of international networking. Radiat Meas 2011;46:923-8.
14. Perry P, Wolff S. New Giemsa method for differential staining of sister chromatids. Nature 1974;251:156-8.
15. Ainsbury EA, Lloyd DC. Dose estimation software for radiation biodosimetry. Health Phys 2010;98:290-5.
16. International Organization for Standardization. ISO 19238:2004 Radiation protection — performance criteria for service laboratories performing biological dosimetry by cytogenetics. Geneva: ISO Office; 2004.
17. Christie DH, Chu MC, Carr Z. Global networking for biodosimetry laboratory capacity surge in radiation emergencies. Health Phys 2010;98:168-71.
18. 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;83:471-7.
19. 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;169:551-60.
20. Romm H, Wilkins RC, Norman Coleman C, et al. Biological dosimetry by the triage dicentric chromosomes assay: potential implications for treatment of acute radiation syndrome in radiological mass casualties. Radiat Res 2011;175:397-404.