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Report

Human Resource Development for Cytogenetic Biodosimetry at Hirosaki University

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Hirosaki University has been designated by the Nuclear Regulation Authority as an Advanced Radiation Emergency Medical Support Center (AREMSC) and as a hospital which accepts radiation emergency medical patients in Japan. In radiation emergency medicine, blood analysis is required to check the patient's health and estimate radiation dose. As the medical staff in Nuclear Emergency Core Hospitals and Nuclear Emergency Medical Cooperative Institutions do not have much experience in requesting biodosimetry laboratories for chromosome analysis, they are often unsure about which blood collection tubes to use and how blood should be stored after collection. Thus, AREMSC in Hirosaki University has prepared and provided guidelines for blood collection, management and shipping. Furthermore, AREMSC in Hirosaki University has also been developing young human resources as one of AREMSCs in Japan. AREMSC in Hirosaki University has provided training materials for developing human resources in biological dose evaluation, which is one of the main missions in AREMSC. This article introduces an overview of the guidelines for blood collection, management and shipping, and an excerpt of training materials in cytogenetic biodosimetry at AREMSC in Hirosaki University.

Key words: Cytogenetic biodosimetry, blood collection, Dic assay, chromosome classification, human resource

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1. Introduction

In 2015, the Nuclear Regulatory Authority in Japan certified five institutions: Hirosaki University, Fukushima Medical University, Hiroshima University, Nagasaki University, and National Institutes for Quantum and Radiological Sciences (QST) as Advanced Radiation Emergency Medical Support Centers (AREMSCs). AREMSC is responsible for medical care in the event of a nuclear-related exposure accident. In 2019, QST was elected as the Center for Advanced Radiation Emergency Medicine (AREM) to manage and support the four AREMs. One of the key roles of AREM and AREMSC is to estimate radiation exposure dose of exposed patients.

Similar to general medical care, serum biochemical tests are performed in radiation emergency medicine, and blood collection tubes for serum separation are frequently used. It has been reported that C-reactive protein (CRP)[1-3], salivary alpha-amylase (sAA)[4, 5], FMS-like tyrosine kinase 3 ligand (FLT3L)[2, 5-7] and citrulline[8-11] in serum are reliable biomarkers in radiation emergency medicine. In addition, blood cell counts are also commonly used as endpoints for biological dose assessment as they have been reported to decrease after radiation exposure[12]. As EDTA is generally used as an anticoagulant for blood cell counting, all hospitals, not limited to radiation emergency medicine, are familiar with EDTA blood collection tubes. On the other hand, heparin, another anticoagulant, is used for emergency tests such as biochemical tests, blood pH measurement and blood gas analysis. Emergency tests are performed promptly in the hospital, eliminating the need for blood management or transport. Heparin tubes are also often used for clinical chromosome aberration analysis, such as leukemia diagnosis or testing for spontaneous chromosomal abnormalities. Unfortunately, many hospitals have little experience in blood management and shipping as chromosome analysis is often outsourced to external laboratories and blood shipment is usually handled by other specialized courier services. Therefore, the understanding of blood management and shipping after blood collection for biodosimetry is usually insufficient in Nuclear Emergency Core Hospitals and Nuclear Emergency Medical Cooperative Institutions. In order for biological dose assessments of radiation-exposed patients to be reliably performed in cytogenetic biodosimetry laboratories, hospitals involved in radiation emergency medicine should be well educated in blood management and shipping.

In cytogenetic biodosimetry, exposed doses are often estimated with radiation-induced dicentric chromosomes (Dic), using the gold standard Dic assay[13-15]. However, dose estimation with Dic assay requires a high level of expertise and consistent training. The shortage of experienced young human resources in cytogenetic biodosimetry has been acknowledged as a problem, both internationally and in Japan. AREMSC in Hirosaki University has incorporated biodosimetry in undergraduate and graduate school education, and provided teaching materials for effective human resource development. Furthermore, as the COVID-19 pandemic from March 2020 made it difficult to provide face-to-face instructions, we prepared chromosome grouping and karyotyping training files that can be performed online for continuous human resource development.

This paper introduces an overview of the guidelines for blood collection, management and shipping, which can be shared with medical institutions involved in radiation emergency medicine. In addition, an excerpt of cytogenetic biodosimetry training materials used in AREMSC in Hirosaki University is shown. Training in chromosome grouping and karyotyping for normal human metaphases and metaphases with Dic analysis are presented.

Blood collection, management and shipping guidelines

In addition to blood collection for chromosome aberration analysis, biodosimetry requires blood collection for biomarker analysis and blood cell counting. However, in Japan, medical staff in Nuclear Emergency Core Hospitals and Nuclear Emergency Medical Cooperative Institutions are often unsure about which blood collection tubes to use and how blood should be stored after collection. As a result, AREMSC in Hirosaki University has prepared and provided the following guidelines for blood collection, management and shipping.

1. Materials for blood collection
   - Blood collection tube for serum separation (5 to 6 ml, plain or coagulation accelerator)*1
   - Blood collection tube for blood cell count (2 ml, anticoagulant: EDTA)*2
   - Blood collection tube for cytogenetic biodosimetry (anticoagulant: heparin)*3
   - Vacutainer holder
   - Wing needle/syringe

   *1Blood chemistry is needed to diagnose a patient's physiological condition. Some test items can also be measured in plasma.

   *2In emergency medicine, heparin blood can be used to blood cell count. Blood cell count with heparin blood should be performed immediately after blood collection.

   *3Heparin is the preferred anticoagulant in cytogenetic biodosimetry. If there is a shortage of heparin blood collection tubes, substitute with EDTA blood collection tubes and report to the biodosimetry laboratory. An over-capped blood collection tube

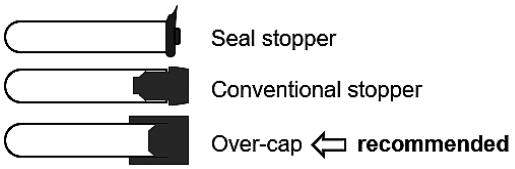

Fig. 1. The type of cap used for blood collection tubes.

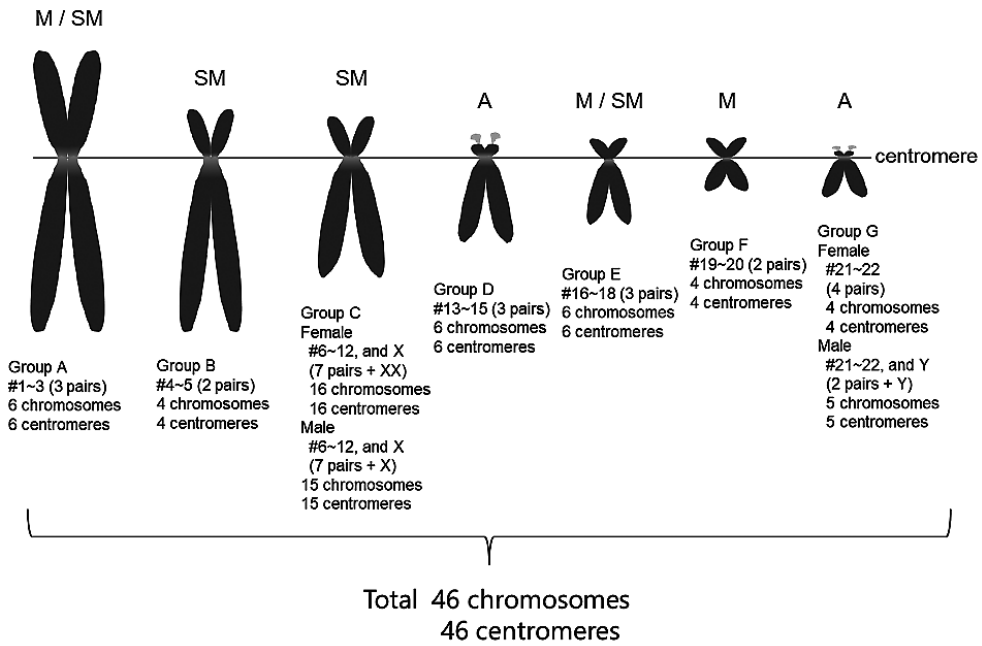

Fig. 2. Chromosome classification and morphological characteristics in human peripheral blood lymphocyte. M: metacentric chromosome, SM: submetacentric chromosome, A: acrocentric chromosome.

is recommended to minimize the infection risk of blood-borne diseases during blood culture (Fig. 1).

2. Blood volume and timing of blood collection for biodosimetry

The timing of blood collection depends on the exposure dose estimated by clinical symptoms. Furthermore, it is desirable to prepare about 10 ml of blood in case it is necessary to select a different cytogenetic biodosimetry method or in case of inadequate blood culture due to some reasons.

(1) Case where 5 Gy or less is estimated

Volume: 4 to 6 ml×2 tubes

Timing: 24 h after radiation exposure*

*Since it is assumed that the patient is not uniformly exposed, peripheral blood needs to be homogeneously circulated in the body before blood collection. After blood collection, blood should be stored at 18 to 24℃ until shipment to biodosimetry laboratory.

(2) Case where 5 Gy or more is estimated

Volume: 4 to 6 ml×2 tubes

Timing: as soon as possible*

*At high dose exposure (5 Gy or more), peripheral blood lymphocytes decrease rapidly and remarkably[12, 16, 17], thus blood should be collected as soon as possible for chromosome analysis. If it is difficult to collect the patient's blood, the exposure dose can be estimated from the clinical symptoms of acute radiation syndrome. In cases where the boundaries of the following dose categories are estimated, it is desirable to collect blood at both timings (1) and (2).

3. Blood storage and transportation

As blood is treated as a biohazard specimen (Biological Substance Category B (UN 3373)), the following packaging is required. The packaging for transportation should consist of three components.

(1) The primary receptacle(s): blood collection tube

*Blood collection tube(s) should be protected with absorbent material.

(2) The secondary packaging: a leak-proof secondary

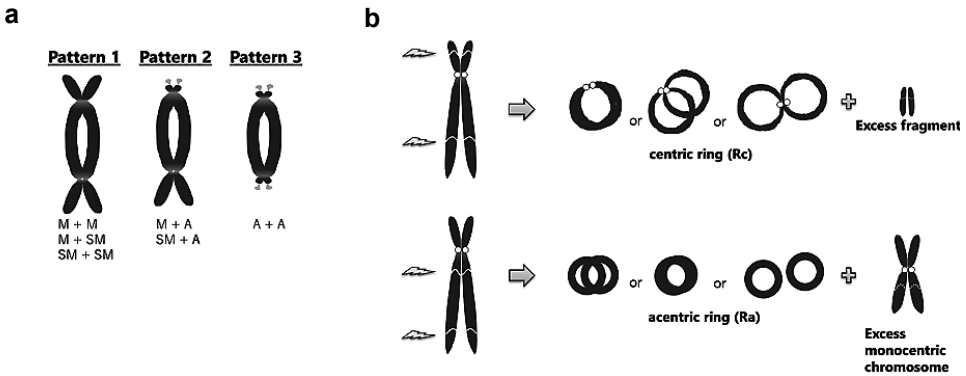

Fig. 3. Dicentric and ring chromosomes observed in human peripheral blood lymphocyte.
a, Dicentric chromosomes. M: metacentric chromosome, SM: submetacentric chromosome, A: acrocentric chromosome. b, Ring chromosomes.

packaging with biohazard marker
(3) Sturdy outer packaging (Biological Substance Category B (UN 3373))[18]

*The storage temperature of heparinized blood is 18 to 24 ℃. Heparinized blood partially aggregates when exposed to cold conditions[19]. In order to obtain sufficient cells for chromosome analysis, it is necessary to regulate the temperature in a temperature-controlled box.

Educational materials for dicentric chromosome assay

In order to master the Dic assay, which is the gold standard for cytogenetic dose evaluation, it is necessary to master human karyotyping techniques. Humans have 46 chromosomes. These chromosomes are classified into seven groups, A to G, according to chromosome length and centromere position[20] (Fig. 2). There is no gender difference in the 44 autosomes, and the sex chromosomes differ between males and females (XX for females, XY for males). The centromeres of chromosomes in groups D (#13 to #15) and G (#21 and #22) are located terminally, and all of these chromosomes are satellite chromosomes. On the other hand, the Y chromosome is morphologically classified into the G group, but it is larger than the #21 and #22 chromosomes and has no satellite. Furthermore, it has been reported that Y chromosome length differs among individuals[21, 22].

Dic observed in human peripheral blood lymphocytes is classified into three patterns as shown in Figure 3a. By confirming the number of chromosomes in groups D and G, analysis of Dic patterns 2 and 3 will be more reliable as chromosomes in groups D or G are involved in Dic formation. In addition, the type of chromosome abnormality associated with the ring chromosome differs depending on the presence or absence of the centromere (centric ring: Rc or acentric: Ra, respectively, Fig. 3b). Moreover, as it is difficult to determine if a small ring chromosome is truly a ring chromosome, calibration curves and dose estimation have been performed based only on the Dic frequency instead of Dic+Rc frequency in the recent years.

At AREMSC in Hirosaki University, training files for human karyotyping and Dic analysis were used in online undergraduate classes during the COVID-19 pandemic. In Figure 4, students were first trained in chromosome grouping and human karyotyping in a normal human metaphase, by moving individual chromosomes around into groups based on morphological characteristics. In Figure 5, karyotyping and chromosome abnormality identification were trained using a human metaphase with Dic. The usage of these files is shown below. Other than unstable chromosomes, stable chromosome abnormalities also occur in radiation-exposed cells. Group D and G chromosomes are also involved in chromosome translocations, but retain the morphological features of bearing very short arms and satellites. In order to confirm Dic patterns 2 and 3, comprehensive judgment including marker chromosomes is required.

Chromosome classification and karyotyping (Fig. 4)
(1) Count the chromosome number of a metaphase spread.
(2) Copy all chromosomes and paste them on the template.
(3) Classify chromosomes into group A-G based on chromosome length, centromere position (centromere index or arm ratio), and presence or absence of satellite.
(4) Rotate each chromosome and pair homologous chromosomes.
(5) Copy identified chromosomes and paste on karyotype template.

Classification of chromosomes including Dic (Fig. 5)
(1) Count the chromosome number of a metaphase spread.

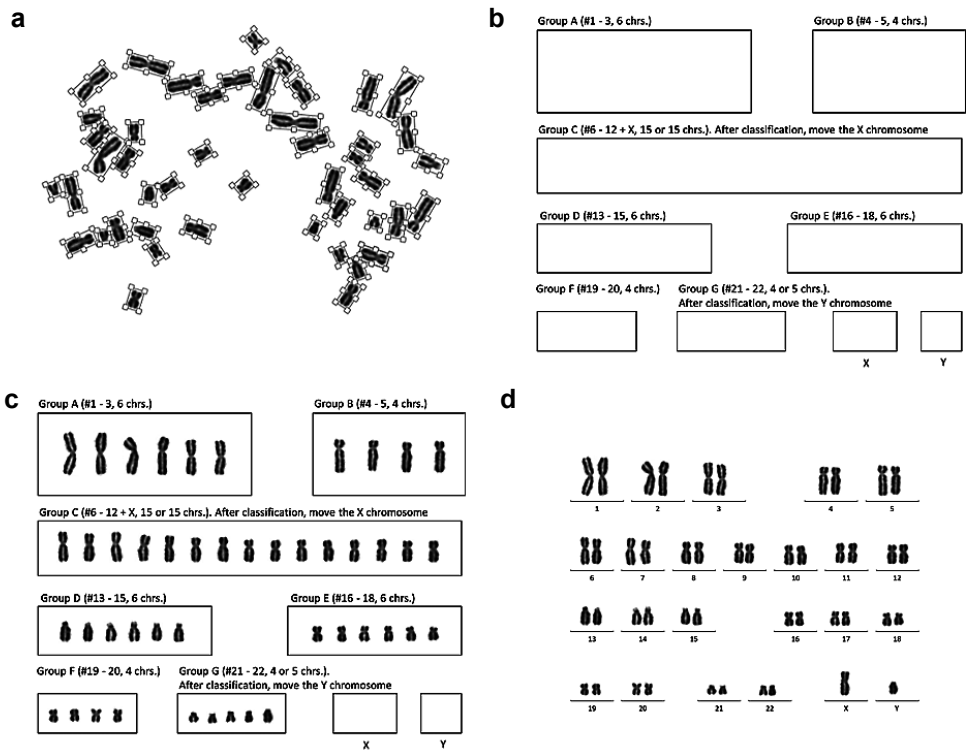

Fig. 4C. Example of normal human chromosome classification training.
a, Normal human metaphase, in which each individual chromosome is an independent image file. b, Template for classifying cropped chromosomes. c, Chromosomes classified into groups A to G based on morphological characteristics such as size and centromere position. d, Normal human karyotype confirmed by G-banding after destaining.

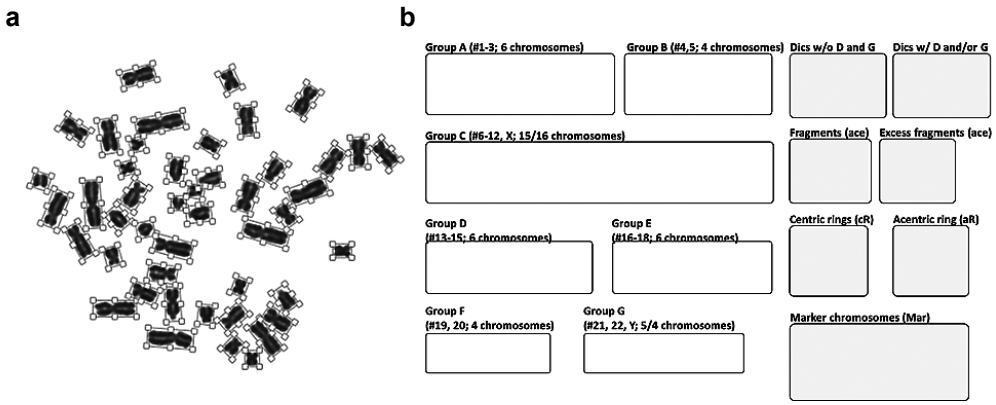

Fig. 5. Example of Dic judgment training in Dic assay.
a, Metaphase spread with Dic. b, Template for classifying cropped chromosomes.

(2) Copy all chromosomes and paste them on the template.
(3) Classify chromosomes into group A to G based on chromosome length, centromere position (centromere index or arm ratio), and presence or absence of satellite.
(4) Rotate each chromosome and pair homologous chromosomes.
(5) Place the Dic involving chromosomes of group D or G in "Dic w/ D and/or G".
(6) Check the number of chromosomes in group D or G.

2. Summary

We have developed guidelines to share and educate medical staff on the management and transportation of blood collected in radiation emergency medicine. This

guideline was distributed to participants at a workshop held at Hirosaki University. We hope that that this information will contribute to the construction of a biological dose evaluation system for exposed patients. Furthermore, we have provided various materials, including chromosome classification training files, for human resource development activities in cytogenetic biodosimetry.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Mal'tsev VN, Strel'nikov VA, Ivanov AA. C-reactive protein in the blood serum as an indicator of the severity of a radiation lesion Dokl Akad Nauk SSSR. 1978;239(3):750–2(in Russian).
2. Ossetrova NI, Sandgren DJ, Blakely WF. Protein biomarkers for enhancement of radiation dose and injury assessment in nonhuman primate total-body irradiation model. Radiat Prot Dosim. 2014;159(1-4):61–76.
3. Hayashi T, Morishita Y, Khattree R, Misumi M, Sasaki K, Hayashi I, *et al*. Evaluation of systemic markers of inflammation in atomic-bomb survivors with special reference to radiation and age effects. FASEB J. 2012;26(11):4765–73.
4. Dubray B, Girinski T, Thames HD, Becciolini A, Porciani S, Hennequin C, *et al*. Post-irradiation hyperamylasemia as a biological dosimeter. Radiother Oncol. 1992;24(1):21–6.
5. Balog RP, Bacher R, Chang P, Greenstein M, Jammalamadaka S, Javitz H, *et al*. Development of a biodosimeter for radiation triage using novel blood protein biomarker panels in humans and non-human primates. Int J Radiat Biol. 2020;96(1):22–34.
6. Bertho JM, Demarquay C, Frick J, Joubert C, Arenales S, Jacquet N, *et al*. Level of Flt3-ligand in plasma: a possible new bio-indicator for radiation-induced aplasia. Int J Radiat Biol. 2001;77(6):703–12.
7. Guipaud O, Holler V, Buard V, Tarlet G, Royer N, Vinh J, Benderitter M. Time-course analysis of mouse serum proteome changes following exposure of the skin to ionizing radiation. Proteomics. 2007;7(21):3992–4002.
8. Lutgens LC, Deutz NE, Gueulette J, Cleutjens JP, Berger MP, Wouters BG, *et al*. Citrulline: a physiologic marker enabling quantitation and monitoring of epithelial radiation-induced small bowel damage. Int J Radiat Oncol Biol Phys. 2003;57(4):1067–74.
9. Jones JW, Bennett A, Carter CL, Tudor G, Hankey KG, Farese AM, *et al*. Citrulline as a biomarker in the non-human primate total- and partial-body irradiation models: correlation of circulating citrulline to acute and prolonged gastrointestinal injury. Health Phys. 2015;109(5):440–51.
10. Bujold K, Hauer-Jensen M, Donini O, Rumage A, Hartman D, Hendrickson HP, *et al*. Citrulline as a biomarker for gastrointestinal-acute radiation syndrome: species differences and experimental condition effects. Radiat Res. 2016;186(1):71–8.
11. Ye F, Ning J, Fardous Z, Katsube T, Li Q, Wang B. Citrulline, a potential biomarker of radiation-induced small intestine damage. Dose Response. 2020 Sep 22;18(3):1559325820962341.
12. IAEA. Diagnosis and Treatment of Radiation Injuries. Safety Reports Series No. 2. Vienna: International Atomic Energy Agency; 1998.
13. IAEA. Cytogenetic analysis for radiation dose assessment: A manual. Technical reports series no. 405. Vienna: International Atomic Energy Agency; 2001.
14. IAEA. Cytogenetic Dosimetry: Applications in Preparedness for and response to radiation emergencies. Vienna: International Atomic Energy Agency; 2011.
15. Blakely WF, Carr Z, Chu MC, Dayal-Drager R, Fujimoto K, Hopmeir M, *et al*. WHO 1st Consultation on the Development of a Global Biodosimetry Laboratories Network for Radiation Emergencies (BioDoseNet). Radiat Res. 2009;171(1):127–39.
16. Moroni M, Lombardini E, Salber R, Kazemzedeh M, Nagy V, Olsen C, *et al*. Hematological changes as prognostic indicators of survival: similarities between Gottingen minipigs, humans, and other large animal models. PLoS One. 2011;6(9):e25210.
17. Blakely WF. Multiple parameter biodosimetry of exposed workers from the JCO criticality accident in Tokai-mura. J Radiol Prot. 2002;22(1):5–6.
18. WHO. Guidance on regulations for the transport of infectious substances 2019–2020. Geneva: World Health Organization; 2019.
19. Fujishima Y, Kanahama S, Hagino S, Natsubori S, Saito H, Azumaya A, *et al*. Influence of anticoagulants and storage temperatures on blood counts and mitotic index of blood samples collected for cytogenetic biodosimetry. Int J Radiat Biol. 2019;95(2):186–92.
20. International Standing Committee on Human Cytogenomic Nomenclature. ISCN 2020: an international system for human cytogenomic nomenclature (2020). Basel; Hartford: Karger, 2020.
21. Lubs HA, Ruddle FH. Chromosome polymorphism in American Negro and White populations. Nature. 1971 Sep 10;233(5315):134–6.
22. McKay RD, Bobrow M, Cooke HJ. The identification of a repeated DNA sequence involved in the karyotype polymorphism of the human Y chromosome. Cytogenet Cell Genet. 1978;21(1-2):19–32.