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
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 AREMSCs. 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), salivary alpha-amylase (sAA), FMS-like tyrosine kinase 3 ligand (FLT3L), and citrulline 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. 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. 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
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 °C 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
packaging with biohazard marker

(3) Sturdy outer packaging (Biological Substance Category B (UN 3373))

*The storage temperature of heparinized blood is 18 to 24°C. Heparinized blood partially aggregates when exposed to cold conditions. 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 (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.

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.
(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 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