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Report

Cytogenetic Biodosimetry in Radiation Emergency Medicine: 4. Overview of Cytogenetic Biodosimetry

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Dose estimation is performed to provide physicians individual doses of patients exposed to radiation for medical treatment in radiation emergency medicine. Cytogenetic dose assessment plays an important role in radiation medicine because it directly analyzes the *in vivo* response of exposed patients and accurately estimates acute whole-body exposure. The endpoint used as a biological dosimeter in cytogenetic dose assessment fulfils three requirements: (1) specificity, (2) stability, and (3) dose-dependency. Dicentric chromosome (Dic) assay (DCA) is recognized as the gold standard in biodosimetry because Dic is an excellent endpoint that meets all three requirements of a biological dosimeter. In addition to DCA, premature chromosome condensation assay, cytokinesis block micronucleus assay, and translocation assay are used in cytogenetic dose assessment. As the endpoints of each assay are different, the most suitable method is selected according to the exposure scenario in terms of partial/whole-body exposure and applicable dose range. This article outlines the characteristics of cytogenetic dose assessment methods, reagents used for blood culture, and precautions for harvesting and spreading in chromosome preparation.

Key words: cytogenetic biodosimetry, application, selection, blood culture, harvesting, chromosome spreading

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1. Introduction

In radiation medicine, it is important to estimate the radiation dose of an exposed patient to predict possible radiation-induced diseases and prepare a medical treatment plan. External radiation dose can be easily estimated with personal dosimeters. In the case of radiation workers, they are obliged to carry personal dosimeters to monitor and record occupational exposure doses. However, personal dosimeters alone are unable to evaluate doses from internal exposure. If radioactive substances are ingested or inhaled into the body, whole body counters, thyroid monitors, lung monitors, radiochemical analysis, and bioassays are performed instead[1].

On the other hand, if ordinary residents who do not usually equip personal dosimeters are exposed to radiation, different methods are used to assess the external exposure dose due to ionizing radiation. Physical dose assessment is first performed by evaluating the risk of exposure based on radiation sources and/or radioactive substances and calculating external dose from actual measurements of the ambient dose-rate at the accident site. However, as physical dose assessment is unable to directly measure the exposure dose of an exposed patient, biodosimetry is then performed to accurately estimate an individual's exposure as requested by a physician.

Biodosimetry directly measures *in vivo* response induced by radiation exposure. There are two main methods in biodosimetry. The first method is peripheral blood cell count analysis detecting a decrease in the number of peripheral blood leukocytes. In exposed patients, leukocytes, especially radiation-sensitive lymphocytes, decrease in a dose-dependent manner after radiation exposure[2]. Peripheral blood leukocyte count measurement of exposed patients can estimate a rough exposure dose range. However, fluctuations in blood cell count vary greatly among individuals and is therefore unreliable as an accurate dosimeter. The second method is cytogenetic biodosimetry, where the frequency of chromosome aberrations shows a positive correlation with the exposure dose and is effective for precise individual exposure dose assessment. In cytogenetic dose assessment, chromosome aberrations in peripheral blood lymphocytes collected from exposed patients are analyzed, and a pre-constructed dose-response curve is commonly used to calculate an individual's exposed dose[3].

There are various assays analyzing different aberration endpoints in cytogenetic biodosimetry and each assay is selected according to type of radiation exposure scenario. Dicentric chromosome assay (DCA)[3-5] uses dicentric chromosomes (Dic), premature chromosome condensation (PCC) assay[6-10] uses ring chromosomes (rings) in G_1- or G_2/M-PCC cells, cytokinesis block micronucleus (CBMN) assay[11-13] uses micronuclei in binucleated cells, and translocation assay uses chromosome translocations as endpoints[14, 15]. This paper outlines the preparation of blood cultures for cytogenetic biodosimetry, the selection of cytogenetic dose assessment method and the characteristics of each method.

2. Role of biodosimetry in radiation medicine

It is generally accepted that tissues with actively dividing cells are highly sensitive to radiation exposure[16]. The bone marrow, which is a hematopoietic tissue, is very susceptible to radiation because hematopoietic stem cells are constantly undergoing cell division. In addition, the gonads that produce gametes and the intestine, which have a rapid turnover, are also highly radiation-sensitive tissues/organs[17]. On the other hand, as the cell turnover rate and cell division frequency are low in muscles and the nervous system, the radiation sensitivity is lower. Thus, deterministic effects or delayed health outcomes may vary as particular tissue/organ dose thresholds are exceeded.

Dose assessments are performed with the aim of providing physicians individual exposed doses to develop treatment plans for exposed patients. Cytogenetic dose assessment plays an important role in radiation medicine because it directly analyzes the *in vivo* response of exposed patients and accurately estimates the whole-body exposure dose. However, it is difficult to estimate the absorbed dose immediately after the patient is admitted into a medical facility and to estimate an extreme local exposure dose in the event of a partial body exposure. Through dose assessment, exposed patients and their families can better understand their condition and medical treatment. In addition, it should be noted that dose assessment can also reduce unnecessary anxiety when patients and their families are informed that they were not exposed to any health hazards.

3. Peripheral blood culture

Lymphocyte chromosomes needed for cytogenetic dose assessment in radiation emergency medicine can be obtained from peripheral blood, which can be collected from exposed patients using optimal blood collection and management procedures[18]. However, chromosomes cannot be directly observed as peripheral blood lymphocytes are in G_0 phase and are not undergoing cell division. Therefore, in order to analyze chromosome aberrations in peripheral blood lymphocytes, it is necessary to stimulate the lymphocytes with a mitogen such as phytohemagglutinin (PHA) to induce cell division. Whole blood or freshly isolated peripheral blood

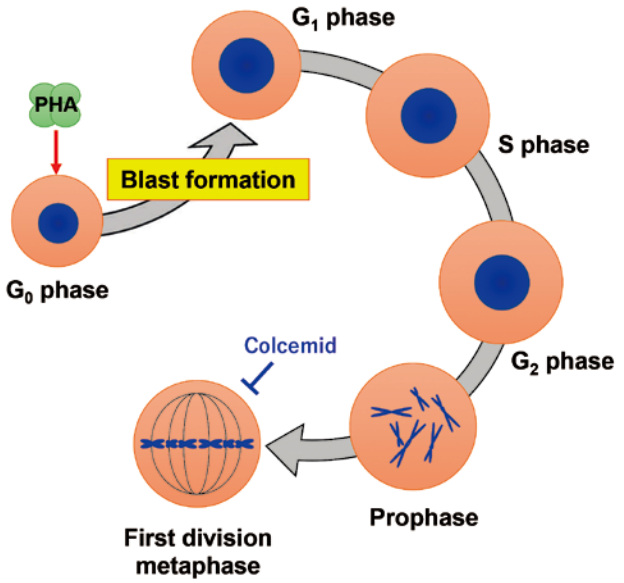

Fig. 1. Cell-cycle progression of peripheral blood lymphocytes during blood culture in DCA.

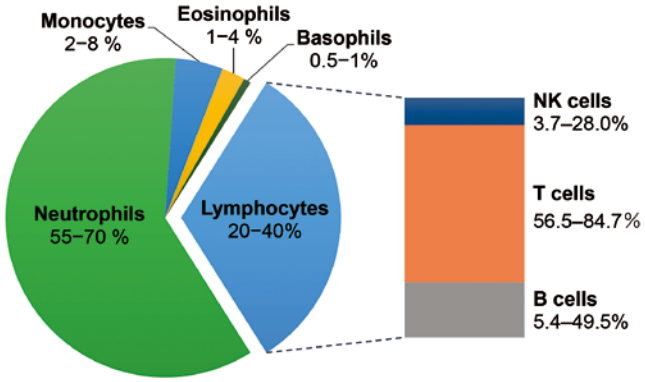

Fig. 2. Reference ranges for leukocyte fraction in human peripheral blood. The reference ranges stated for leukocyte fraction are cited from the literature[21, 22].

mononuclear cells (PBMCs) stimulated by PHA are transformed to blast cells and enter the S phase through the G_1 phase. It takes more than 24 hours after PHA stimulation to enter the S phase[19, 20]. Subsequently, the cell-cycle of lymphocytes progresses to the S phase (6 to 8 hours), the G_2 phase (4 to 6 hours), and then to the metaphase[3]. Therefore, cytogenetic dose assessment requires a blood culture for 34 hours or longer and is generally performed for 48 hours. In DCA, colcemid or colchicine is added to the culture medium to arrest the cell-cycle in metaphase, and metaphases from the first cell-cycle after PHA stimulation are analyzed (Fig. 1).

In blood culture, serum that supports cell growth and antibiotics that suppress bacterial growth are also added to the basal medium suitable for blood culture. An appropriate volume of peripheral blood is added to the supplemented culture medium with a suitable mitogen. Cells can be cultured in two methods with peripheral blood collected using heparin as an anticoagulant. The first is to directly culture whole blood to a concentration of about 10% into culture medium, and the second is to isolate PBMCs from whole blood and culture.

3.1. Isolation of PBMCs from whole blood

Whole blood cultures contain nucleated cells such as neutrophils, lymphocytes and monocytes, and a large number of red blood cells. Lymphocytes make up 20 – 40% of white blood cells (Fig. 2)[21]. In addition, T cells make up 56.5 – 84.7% of peripheral blood lymphocytes, and the proportion of T cells in all nucleated cells is 11.3 – 33.9%[22]. Therefore, if whole blood is cultured, a relatively low frequency of metaphases is observed after DCA. To increase metaphase frequency, isolated PBMCs with density gradient centrifugation with an isotonic density solution with a density of 1.077 g/mL can be cultured under PHA stimulation as reported by Hayata *et al.*[23]. Although the PBMC culture method involves additional separation steps before blood culture, an increase in MI contributes to an improved efficiency of chromosome observation and metaphase image capturing.

3.2. Preparation of culture medium

In cell culture, serum and antibiotics are added to an aseptically prepared basal medium. In general, the prepared culture medium should be stored in 4 °C refrigerator and used within 1 month.

3.2.1. Culture medium

For chromosome analysis in peripheral blood lymphocyte culture, the cell culture medium developed by Roswell Park Memorial Institute (RPMI) modified from McCoy's 5A medium is recommended (RPMI 1640). Sodium bicarbonate in RPMI 1640 helps to maintain osmotic pressure and neutralize culture medium pH during the incubation period (i.e. the bicarbonate salt equilibrates CO_2 gas from CO_2 incubator and carbonate ions in the culture medium). 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid ($C_8H_{18}N_2O_4S$; HEPES), one of the twenty widely used Good's buffering agents, may also be added to increase the buffer capacity of culture media between the pH range of 6.8 to 8.2. The addition of HEPES is useful because the pH of the medium changes due to cell metabolism and aerobic respiration altering CO_2 levels in PBMC culture. It is thus highly recommended to use RPMI 1640 with HEPES for long culture periods (e.g., 72-hour culture) as it is commercially available.

3.2.2. Serum

Animal-derived sera rich in growth factors and nutrients

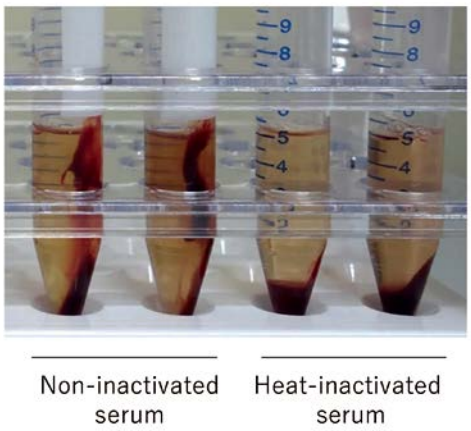

Fig. 3. Comparison of non-inactivated (both leftmost conical tubes) and heat-inactivated (both rightmost conical tubes) sera in whole blood cultures. Displayed is human peripheral blood cultured in using media supplemented with PHA, kanamycin sulfate, and 20% non-inactivated fetal bovine serum (FBS) or heat-inactivated FBS for 48 h.

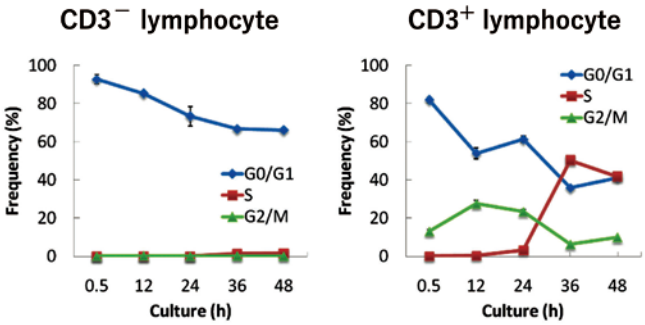

Fig. 4. Differences in PHA responsiveness between lymphocyte subsets during cell culture. PBMCs were cultured in the presence of PHA for indicated periods. After cultivation, cells were stained with propidium iodide and cell-cycle status was analyzed using a flow cytometer.

are used to support lymphocyte growth in eukaryotic cell culture (e.g., fetal bovine serum). Animal-derived sera are provided unique lot numbers from a single manufacturer, and it is highly recommended each lot number is preliminary tested for optimal cell growth prior to purchase. In peripheral blood cultures, serum is added at a final concentration of 15 – 20%[3]. As the commercially available serum used for cell culture has been sterilized, sterilization of the serum is not necessary. If non-sterile serum is used, the serum should be sterilized with a 0.45 or 0.22 μm membrane filter. In addition, as the physiological substances in the serum are inactivated at high temperatures, serum should not be treated at high temperatures with an autoclave.

Furthermore, serum contains complement proteins. As complement proteins have opsonin and cell-lytic activities, it is necessary to inactivate them by heating (56 °C for 30 minutes) before using it for cell culture. Temperature and treatment time must be appropriately controlled because the physiological substances in the serum could be inactivated due to prolonged treatment. In addition, complement proteins are involved in blood coagulation[24]. If whole blood culture is used for chromosome aberration analysis, blood coagulation may occur if serum is untreated. To prevent this from occurring, heat-inactivated serum should be used (Fig. 3).

3.2.3. Antibiotics

Blood is basically sterile *in vivo*. However, when blood is collected from a septic patient or when blood is contaminated at the time of collection, the bacteria will proliferate during the blood culture and hinder cell division, greatly reducing metaphases for chromosome aberration analysis. Therefore, in blood culture, antibiotics are added to the culture medium in order to suppress bacterial growth. Even though antibiotics have their own antibacterial spectrum, antibiotics which show antibacterial activity against a wide range of bacterial species are used in blood cultures. In general, a mixed solution of penicillin G potassium salt, which is effective against gram-positive bacteria due to its cell wall synthesis inhibitory action, and streptomycin sulfate, which is effective against gram-positive and gram-negative bacteria due to its protein synthesis inhibitory action, is used for mammalian cell culture. However, as penicillin and streptomycin are ineffective against mycoplasma, the authors recommend kanamycin sulfate, which has antibacterial activity against gram-positive and gram-negative bacteria and mycoplasma[25]. When using these antibiotics, they should be used at appropriate concentrations which are not cytotoxic.

3.3. Mitogen

A lectin is a protein or glycoprotein that exists in plants, animals, and microorganisms, which has specific binding activity to sugar chains. Some plant-derived lectins bind to sugar chains on the surface of lymphocytes and have lymphocyte stimulating activity. The discovery of phytohemagglutinin (PHA), a type of lectin, greatly facilitated human chromosome analysis[26-28]. PHA has both hemagglutination activity and lymphocyte stimulating activity due to its protein structure[29-31]. PHA is a tetrameric glycoprotein made up of different combinations of E-subunits with hemagglutination activity and L subunits with lymphocyte-stimulating activity.

Although the details of the lymphocyte stimulation mechanism by PHA remain unclear, PHA preferably stimulates T cell division in peripheral blood cultures (Fig. 4). Therefore, in cytogenetic biodosimetry in which peripheral blood is cultured in the presence of PHA, chromosome aberrations in T cells assumed to be

Table 1. Comparison of mitogens with lymphocyte stimulating activity

Mitogens	Stimulated lymphocytes	Sources	Classification
Phytohemagglutinin (PHA)	T cell	Bean of *Phaseolus vulgaris*	Lectin (glycoprotein)
Concanavalin A (ConA)	T cell	Bean of *Canavalia ensiformi*	Lectin (glycoprotein)
Pokeweed mitogen (PWM)	T and B cells	Roots of *Phytolacca americana*	Lectin (glycoprotein)
Lipopolysaccharide (LPS)	T and B cells	Cell wall of gram-negative bacteria, such as *Escherichia coli, Salmonella* etc.	Endotoxin

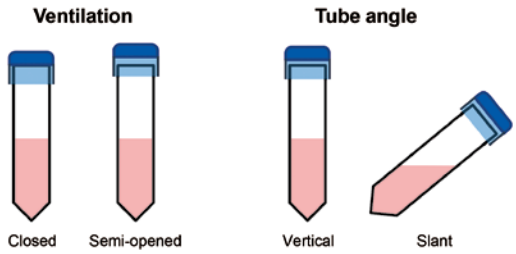

Fig. 5. Ventilation and test tube angle during blood culture.

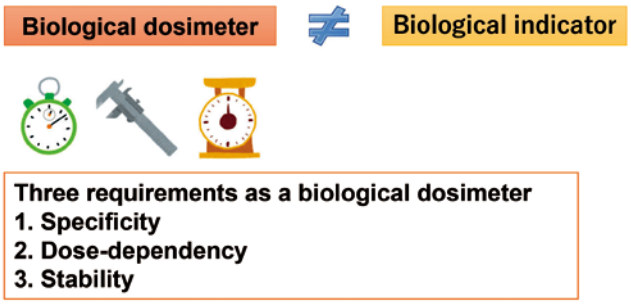

Fig. 6. Three requirements as a biological dosimeter in biodosimetry.

analyzed. In addition to PHA, other mitogens may be used depending on the experiment, such as other lectins of concanavalin A and Pokeweed mitogen, and the cell wall component of gram-positive bacteria lipopolysaccharide (Table 1).

3.4. Culture condition

When performing blood culture for cytogenetic biodosimetry, it is convenient to culture cells in a centrifuge tube (e.g., 15 ml conical tubes) that can be centrifuged immediately after the culture is completed. Blood should also be cultured in a vessel with gas exchange. However, as 15 ml conical tubes with ventilation caps are not commercially available, cells should be cultured in a semi-open state without closing the cap completely. In addition, conical tubes during blood culture can be tilted diagonally to increase the surface area of the culture solution in contact with CO_2 gas to sustain a neutral pH environment suitable for cell culture (Fig. 5).

4. Methods in cytogenetic biodosimetry

The endpoint used as a biological dosimeter in cytogenetic biodosimetry has the following three requirements: (1) specificity: a reaction that occurs specifically in radiation exposure, (2) stability: stable detection is possible for a certain period after accident and (3) dose-dependency: the exposure dose and the reaction amount (frequency) show a correlation (Fig. 6). DCA is recognized as the gold standard in biodosimetry of acute exposures because Dic is an excellent endpoint that meets all three requirements of a biological dosimeter [32].

4.1. Comparison and selection of cytogenetic dose assessment methods

In addition to DCA, CBMN, PCC and chromosome translocation assays are also used as cytogenetic dose assessment methods (Table 2, Fig. 7). As each assay differs in factors such as endpoints analyzed, applicable dose range, and if the assay is suitable for partial body dose estimation, it must be carefully selected according to the exposure scenario. Furthermore, in DCA and CBMN assays, international guidelines recommend scoring a reduced number of cells to report preliminary dose estimates for triage [4, 5, 33]. The authors use the following criteria when selecting a suitable cytogenetic dose assessment method: (1) period from radiation exposure to blood sampling, (2) exposure dose expected from prodromal symptoms and (3) number of exposed persons requiring cytogenetic biodosimetry (Fig. 8).

When performing DCA, blood needs to be collected within 28 days after the accident because Dic is an unstable chromosome aberration. As time passes after the accident, the number of Dic-positive cells decreases and the exposure dose is likely underestimated [3, 34-36]. Therefore, if the blood is collected more than one month after the accident, the chromosome translocation assay is the only applicable method for dose estimation with retrospective dosimetry. On the other hand, in high dose-exposed patients, blood should be collected promptly as the number of peripheral blood lymphocytes decreases

Table 2. Comparison of cytogenetic biodosimetry assays

	Cytogenetic biodosimetry assays			
	Dicentric chromosome assay	PCC-ring assay	CBMN assay	FISH translocation assay
Aberration endpoint	Dicentrics	Rings	Micronuclei	Translocations
Secondary index (Cell-cycle progression)	Mitotic index (MI)	PCC index Cell-cycle progression index (CPI)	Nuclear division index (NDI)	
Radiation scenario application	Acute Protracted Recent exposure	Acute Recent exposure	Acute Protracted Recent exposure	Acute Protracted Old exposure
Dose range for whole-body exposure application	0.1 – 5 Gy	5 – 20 Gy	0.3 – 4 Gy	0.25 – 4 Gy
Staining and labeling	· Giemsa staining · Modified C-banding · PNA-FISH using pan-centromere and telomere probes	· Giemsa staining	· Giemsa staining · Diff-Quik staining · DAPI (DAPI/PI) staining · Acridine Orange staining	· FISH using whole-chromosome painting probes
Blood collection after radiation exposure	24 h – 28 days	Asap	24 h – 28 days	24 h – (few years)
Blood storable period	< 72 h	Asap	< 72 h	< 72 h
Blood culture time	48 h	4-6 h (fusion PCC) 48 h (chemical PCC)	48 or 72 h	48 h
Useful for partial-body exposure application?	Yes	Yes	NA*	Yes
Useful for triage dose assessment?	Yes	Yes	Yes	NA
Status of assay standardization	ISO 19238: 2014[4] ISO 21243: 2008 (triage)[5]	NA	ISO 17099: 2014[33]	ISO 20046: 2019[75]

Table was modified from IAEA EPR-Biodosimetry[3]
*NA: not applicable/not available.

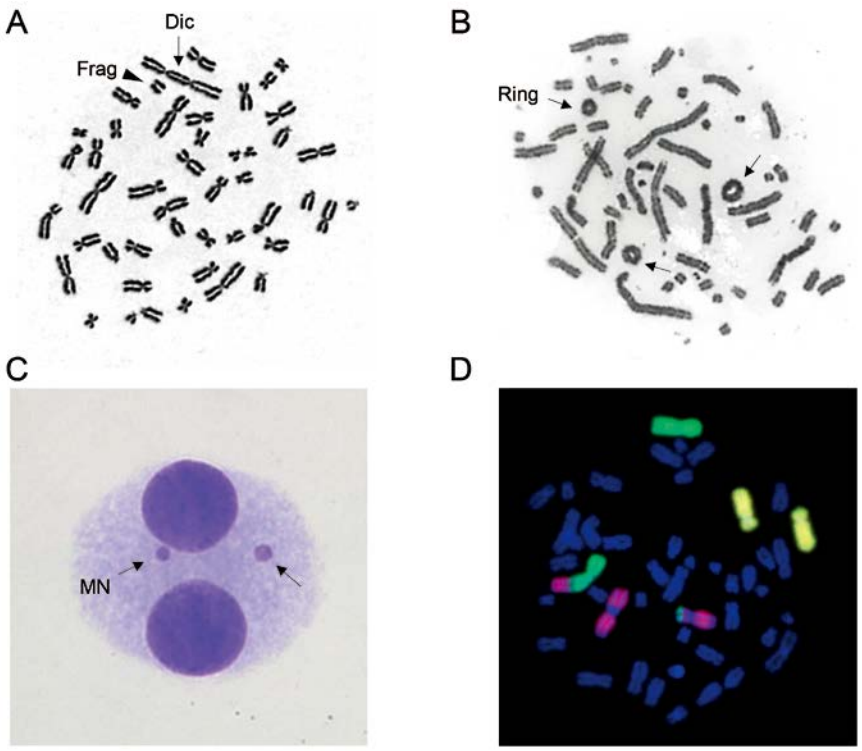

Fig. 7. Representative images of chromosome aberrations observed in the irradiated human peripheral lymphocytes. A: metaphase with dicentric chromosome (Dic) and acentric fragment (Frag), B: G2/M-PCC cell with PCC-rings, C: binucleated cells with 2 micronuclei (MNs), and D: metaphase with reciprocal chromosome translocation between chromosomes #1 and #2.

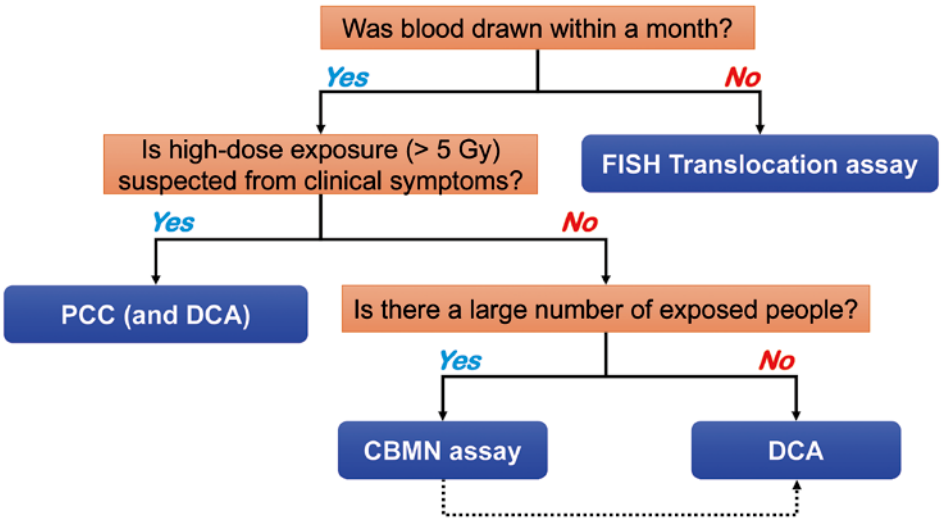

Fig. 8. Flowchart used when selecting a suitable assay for cytogenetic biodosimetry.

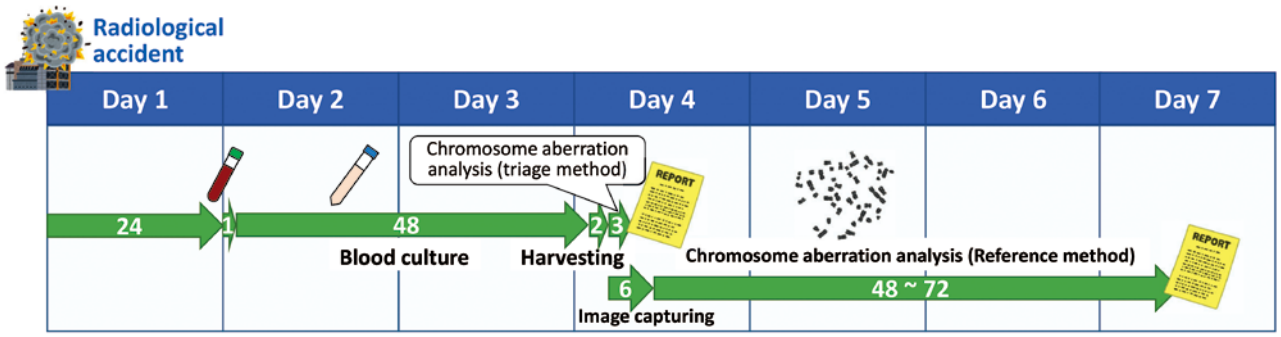

Fig. 9. Estimated time required for DCA if a small number of samples is processed after a radiological accident. The numbers indicate the time (h) required for each process.

immediately[2]. Moreover, cell division tends to arrest at the G_2 phase during the blood culture period, making it difficult to obtain metaphase cells[37]. Therefore, in patients exposed to high dose radiation, the PCC assay is used where chromatin in interphase cells is artificially condensed[6-10].

Unfortunately, the disadvantage in DCA is the longer length of time required for chromosome analysis in individual cells[5]. When a large-scale radiation exposure occurs, the CBMN assay could be more effective as micronuclei are easier to analyze and automated scoring is available[38, 39]. However, the applicable lower limit exposure dose differs depending on the spontaneous frequency of each endpoint and the influence of factors other than radiation exposure. The frequency of spontaneous occurrence of Dic is extremely low and is not affected by age, gender and smoking[40]. The applicable lower limit exposure dose for CBMN assay and chromosome translocation assay is higher than that for DCA because micronuclei and chromosome translocations are affected by age, gender and smoking[41, 42].

The estimated exposure dose assumes that the whole-body is homogeneously exposed to radiation exposure although most exposure cases show non-uniform dose distribution. In extremely biased exposure (partial body exposure), dose calculation suitable for partial exposure can be performed with DCA[43-45] and PCC assay[46]. However, CBMN assay is unable to accurately estimate partial body exposure.

In cytogenetic dose assessment, G_0 phase lymphocytes are stimulated with PHA and cultured for chromosome analysis. Apart from cell fusion-induced PCC (fusion-PCC)[47-49] assay, 48 to 72 hours of blood culture is required for cytogenetic biodosimetry. As a result, 3 to 5 days after blood collection is usually required to report estimated doses.

4.2. Characteristics of DCA

A notable point of DCA is the low frequency of spontaneous Dic occurrence. The number of spontaneous Dic per 1000 metaphases is less than one and the radiation exposure specificity is extremely high. In addition,

consideration of factors such as age, gender, and smoking history, are not necessary because Dic is a consequence typically only induced by radiation exposure unlike CBMN and chromosome translocation assays[40-42].

Understanding the morphological characteristics of human chromosomes is essential for Dic analysis. The number of chromosomes in the chromosome group(s) of chromosomes involved in Dic formation need to be verified to confirm Dic scoring (e.g., if Dic is formed with a D/G group chromosome, the number of D and G group chromosomes need to be verified)[15, 50]. In recent years, centromeres can be labelled with fluorescence *in situ* hybridization (FISH) using a centromere-specific fluorescence-labeled DNA painting probe, and it has become possible to improve the detection accuracy of Dic[51, 52]. However, in order to quickly score Dic by triage and provide physicians with a preliminary estimated dose[5], scoring on Giemsa-stained specimens is preferred and training is essential to ensure accurate scoring.

Figure 9 shows the time required for a typical DCA performed after radiation exposure. Blood is collected 24 hours after exposure[18]. After culturing the blood for 48 hours, the estimated dose is reported to the physician through a process of cell harvesting (hypotonic treatment and fixation), image capturing, chromosome aberration analysis and dose calculation. The preliminary dose report takes at least 78 hours from the occurrence of the radiation exposure accident (54 hours from blood receipt), and the final estimated dose report takes 129 to 153 hours (105 to 129 hours after receipt of blood).

4.3. Characteristics of PCC assay

The DCA is the preferred cytogenetic dose assessment method in most exposure cases, but it is not recommended at high dose exposures above the applicable dose limit of 5 Gy because severe DNA damage causes cell-cycle arrest and/or delay. This causes a marked shortage of metaphase cell numbers, making Dic analysis difficult[53-55]. The PCC assay is thus preferred in high dose exposure situations as chromatin in G_1 and G_2 cells is artificially condensed and chromosome aberrations, such as ring chromosomes (PCC-ring, Fig. 7B), induced by radiation exposure can be observed[10]. While this assay is capable of covering a wide dose range (0.2-20 Gy)[3, 56-58], the applicable dose range depends on the endpoint analyzed. When PCC-ring is used as the endpoint, the applicable dose range is 5 to 20 Gy[57] (Table 2).

There are two types of PCC assays: the fusion-PCC method and the chemically induced PCC (chemical-PCC) method. In the former, a cell fusion agent (polyethylene glycol) is used to fuse peripheral blood lymphocytes with M-phase cells of mammalian cells (i.e., Chinese hamster ovary cells (CHO)) to induce G_1-PCC cells[47-49]. In the latter, phosphatase inhibitors such as calyculin A induce chromosome condensation in blast cells in each cell-cycle. When high-dose exposure is suspected due to the accident description or the patient's prodromal symptoms, both DCA and PCC assay can be performed together for dose assessment of a wide range of exposed doses[6, 7, 10], such as in the Tokai-mura JCO criticality nuclear accident[59].

4.4. Characteristics of CBMN assay

In anaphase of cell division, kinetochore (centromere) microtubules are attached to the centromere sequence-specific protein complex (kinetochore protein) of chromosomes convened on the metaphase plate. The chromatid is pulled to the polar side of the spindle with the help of motor proteins. Chromatids separated into both poles decondense and become the main component of nuclei in daughter cells. However, aberrant chromosomes such as acentric fragments and Dic are not normally distributed and appear as micronuclei (MN) and nucleoplasmic bridges (NPB) in telophase, respectively[60]. MN and NPB are difficult to detect because they are observed in telophase and disappear with multiple cell divisions[61]. By using cytochalasin B to inhibit cytokinesis in CBMN assay, these chromosome abnormalities are easily detected in binucleated cells (Fig. 7C).

As the CBMN assay targets cells after the telophase in which the nucleus is condensed, it is easier to analyze than Dic and the development of automated technology aids in fast analysis for cytogenetic dose assessment[62]. MN also appears with genotoxicity and chromosome mis-separation, and is used as a useful marker in the field of cytotoxicity assessment and oncology[63]. Furthermore, it was discovered that it reflects cell radiosensitivity and is involved in the prognosis of tumor treatment[64, 65]. The radiation exposure specificity in MN is lower than that of Dic, and it is necessary to correct the frequency of MN occurrence due to factors other than radiation exposure[66, 67]. Its applicable dose range is 0.3-4 Gy[3] (Table 2).

4.5. Characteristics of translocation assay

The mis-rejoining of ionizing radiation induced DNA double-strand breaks results in (a) unstable chromosome aberrations (e.g., Dic and ring chromosomes) and (b) stable chromosome aberrations (e.g., translocations). Similar to Dic formations, translocations are proportional to radiation dose, and theoretically, both aberrations should have similar frequencies per cell. However, a higher frequency of translocations occurs in human peripheral blood lymphocytes[68] because of the longer half-life of translocations than unstable aberrations[69-71]. Thus, translocations have been used as an endpoint for retrospective dosimetry and cytogenetic dosimetry for chronic radiation exposure[71-73].

Clinical laboratories routinely use banding methods

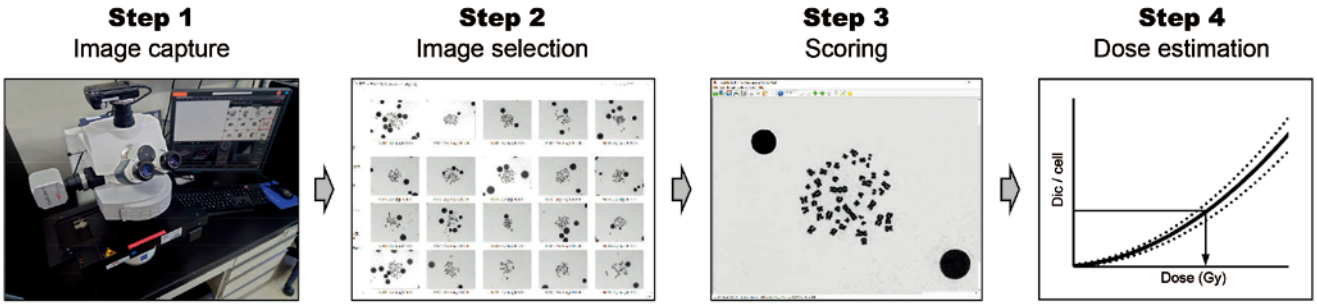

Fig. 10. Main processes from imaging to dose estimation in DCA.

(e.g., G-band or Q-band) to detect translocations in radiation exposed patients. However, as hundreds to thousands of cells need to be analyzed for cytogenetic dosimetry, these banding methods are not recommended. Instead, the FISH method using whole-chromosome painting probes (WCP-FISH) is preferred. WCP for autosomal chromosomes 1-22 and sex chromosomes X and Y have been developed to help detect and distinguish chromosome exchanges[74]. The detectable dose range is 0.25 to 4 Gy[3] and was standardized by the International Organization for Standardization (ISO) in 2019[75] (Table 2).

It should be noted that the WCP-FISH method requires a correction for the number of cells analyzed based on the amount of stained chromosomal DNA as a percentage of total genomic DNA. Furthermore, the genomic DNA per chromosome differs slightly between males and females[76]. This is especially important for inter-laboratory comparisons as the color and number of chromosomes stained may vary. For example, if three-color FISH probes specific for chromosomes 1, 2, and 4 are used[77], the number of cells analyzed would be equivalent to 197-198 cells when 500 cells are available for analysis[78]. Corrections for age and lifestyle habits (e.g., smoking) are necessary because such factors influenced chromosome translocation frequencies[42, 79].

5. Cell harvest and chromosome spreading

Producing high quality chromosome specimens is paramount in cytogenetic dose assessment. Harvesting and chromosome spreading are key processes in chromosome analysis where chromosomes packed in lymphocytes with a diameter of about 8-10 μm are expanded on a slide glass without overlapping.

5.1. Cell harvest: hypotonic treatment and fixation

DCA, PCC and chromosome translocation assays require metaphases of moderately expanded chromosomes. After blood culture, cells are first treated with hypotonic solution for cell swelling and removal of cell membrane and cytoplasm. They are then fixed to stop hypotonic shock and autolysis. It should be noted that the hypotonic treatment conditions are different between whole blood and isolated PBMC culture. In addition, as CBMN assay analyzes binucleated cells, it is necessary to optimize harvest such that the cell membrane and cytoplasm are maintained while still expanding the cell area[13]. Cells with ruptured cell membrane and cytoplasm are excluded from the analysis because the origin of micronuclei induced by radiation exposure cannot be confirmed.

5.2. Chromosome spreading

After fixation, the cell suspension in the fixative is dropped onto a pre-cleaned slide glass. Chromosome expansion is affected by temperature and humidity in the laboratory or humidity chamber, as well as cell concentration and volume of the cell suspension dropped on the slide. As the temperature and humidity vary from region to region and even seasonally, it is recommended to check the optimum condition for chromosome spreading on a regular basis.

In order to adjust the cell concentration in the cell suspension, cell concentration can be measured using a hemocytometer or any other cell counting device. This step can also be substituted by referencing to a standardized suspension. The authors use a standardized suspension by adjusting the McFarland standard solution used in standardize microbial testing to an appropriate concentration. Chromosome spreading should be optimized by confirming the cell concentration and the expanded state of the chromosome with a phase-contrast microscope before air-drying and staining.

6. Chromosome observation and image capture

An upright optical microscope is typically used to observe chromosome specimens stained with Giemsa for DCA. A 60× (63×) or 100× objective lens is used to accurately determine subtle structural changes. Metaphase cells are first searched at a low magnification (usually 10× lens). The chosen metaphase is then viewed at a higher magnification where the number of chromosomes and

centromeres are counted and various chromosome abnormalities are recorded.

To speed up the process of Dic scoring, a device has been developed which scans metaphase cells with a low-magnification objective lens and automatically photographs selected metaphase cells with a high-magnification objective lens. The widespread usage of this device in biodosimetry laboratories globally has significantly reduced the time required to capture metaphase images. In addition, as the image files have been digitized, image files can be easily shared between biodosimetry laboratories around the world.

The main steps from image capturing to dose estimation in DCA are provided in Figure 10. The image files taken with the high-magnification objective lens are expanded on a PC monitor and metaphases that meet the conditions to be analyzed are selected by first checking the thumbnail images. Then, on selected metaphases, centromeres are scored and various chromosome aberrations are recorded. Finally, the estimated dose is calculated from the dose-response curve based on the Dic frequency.

The preparation of good quality chromosome spreads is thus of utmost importance in the accurate analysis of chromosome aberrations. The condition of chromosome spreads should be observed on the glass slide with a phase contrast microscope frequently as the environment of each biodosimetry laboratory could change and affect metaphase quality.

7. Conclusion

In cytogenetic biodosimetry where endpoints analyzed are chromosome aberrations caused by radiation exposure, peripheral blood lymphocytes are stimulated with PHA in blood culture for cell-cycle entry. Therefore, a culture solution suitable for lymphocyte proliferation and reagents for supporting lymphocyte proliferation should be appropriately prepared. Cytogenetic dose assessment can be performed in DCA, PCC, CBMN and chromosome translocation assays. An assay suitable for dose assessment must be selected depending on the radiation exposure scenario, such as the time from accident to blood sampling, exposure dose estimated from prodrome, and number of patients requiring dose assessment.

In cytogenetic dose assessment, it is essential to prepare high quality chromosome spreads for accurate dose estimation. Optimal conditions for preparing chromosome spreads vary with environmental conditions. At the time of specimen preparation, a phase contrast microscope is highly recommended to confirm chromosome spreads, cell concentration and the presence or absence of cytoplasm.

Dose assessment in radiation medicine aims to provide physicians with dose information to develop treatment plans for exposed patients. Exposed patients and their families can understand their condition and treatment using dose assessment. In addition, dose assessment can also reduce anxiety when patients and their families are informed their exposure will not cause deterministic health effects.

Point 1. Role of cytogenetic biodosimetry
- Cytogenetic biodosimetry provides physicians with dose information to develop medical treatment plans for exposed patients.
- Exposed patients and their families can understand their condition and medical treatment through dose assessment.
- Dose assessment can also reduce unnecessary anxiety if patient is assessed to have no/low exposure.

Point 2. Requirements for biological dosimeter
- Specificity: a reaction that occurs specifically in radiation exposure.
- Stability: stable detection is possible for a certain period after accident.
- Dose-dependency: the exposure dose and the endpoint frequency show a correlation.

Point 3. Selection of cytogenetic dose assessment methods
- DCA is the gold standard for biodosimetry.
- Applicable assay(s) is selected from DCA, PCC, CBMN and chromosome translocation assays depending on the type of exposure scenarios (1) to (3).
  (1)Period from accident to blood sampling
  (2)Exposure dose estimated from prodromal symptoms
  (3)Number of patients requiring dose assessment

Point 4. Blood culture
- Blood culture medium is supplemented with serum and antibiotics for blood culture.
- Serum should be inactivated by heating at 56 ℃ for 30 minutes.
- Antibiotics with a broad antibacterial spectrum is recommended.
- PHA is added to stimulate the proliferation of peripheral blood lymphocytes.

Point 5. Harvesting, spreading and chromosome observation
- Hypotonic treatment conditions differ between whole blood culture and isolated PBMC culture.

- CBMN assay requires the preservation of cell membranes and cytoplasm.
- Cell expansion conditions vary depending on the temperature and humidity in the laboratory.
- It is important to adjust the final cell concentration of the fixed cell suspension.
- Good quality specimen preparation is essential for accurate cytogenetic biodosimetry.
- Status of chromosome spreading should be frequently observed under the microscope to ensure consistently good metaphase spreads.

Conflict of Interest

The authors declare that they have no conflict of interests.

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