

Blood Transport Method for Chromosome Analysis of Residents Living Near Semipalatinsk Nuclear Test Site

Mohd RODZI¹⁾, Shozo IHDA¹⁾, Masako YOKOZEKI²⁾, Nobuo TAKEICHI^{1, 2)},
 Kimio TANAKA³⁾ and Masaharu HOSHI^{1,*)}

1) *Department of Radiation Biophysics, Research Institute for Radiation Biology and Medicine, Hiroshima University, Kasumi 1-2-3, Minami-Ku, Hiroshima 734-8553, Japan*

2) *Takeichi Clinic, Kojin-machi, Minami-Ku, Hiroshima 732-0806, Japan*

3) *Department of Radiobiology, Institute for Environmental Science, 2-121 Hacchazawa, Takahoko, Rokkasho, Kamikita, Aomori 039-3213, Japan*

ABSTRACT

A study was conducted to compare the storage conditions and transportation period for blood samples collected from residents living in areas near the Semipalatinsk nuclear test site (SNTS). Experiments were performed to simulate storage and shipping environments. Phytohaemagglutinin (PHA)-stimulated blood was stored in 15-ml tubes (condition A: current transport method) in the absence or in 50-ml flasks (condition B: previous transport method) in the presence of RPMI-1640 and 20% fetal bovine serum (FBS). Samples were kept refrigerated at 4°C and cell viability was assessed after 3, 8, 12 and 14 days of storage. RPMI-1640, 20% FBS and further PHA were added to blood samples under condition A in 50-ml flasks for culture. Whole-blood samples under condition B were directly incubated without further sub-culturing process, neither media nor PHA were added, to adopt a similar protocol to that employed in the previous transport method. Samples in condition A and condition B were incubated for 48 hr at 37°C and their mitotic index was determined. The results showed that viable lymphocytes were consistent in both storage conditions but the mitotic index was higher in condition A than in condition B. Although further confirmation studies have to be carried out, previous chromosomal studies and the present experiment have shown that PHA-stimulated blood could be stored without culture medium for up to 8 days under condition A. The present results will be useful for cytogenetic analysis of blood samples that have been transported long distances wherever a radiation accident has occurred.

Key words: *Whole blood, Mitotic index, Chromosome aberrations, Nuclear explosion test, Biodosimetry*

The Semipalatinsk nuclear test site (SNTS) is an area that was contaminated by radioactivity due to Russian nuclear test activities between 1948 and 1989. Investigations and studies on radiation health effects were carried out on people living in this area as they had been exposed to radiation internally and externally after each nuclear test^{8,10,11,17,20-24}. Our earlier studies involved blood samples collected from residents in the areas near the SNTS and brought to the laboratory at the Research Institute for Radiobiology and Medicine (RIRBM), Hiroshima University for chromosome aberration analysis.

An initial method for transportation of blood samples was developed in the 1970s by human

geneticists^{3,4}. Later, Sasaki described his own blood transportation method in the Manual of Cytogenetic Analysis for Radiation Dose Assessment, published by the International Atomic Energy Agency (IAEA 2001). Blood samples can be successfully stored for up to one year using cryopreservation or subzero non-freezing, and can be used for cytogenetic analyses or other purposes^{6,7,13,15}. However, we have found that these enhanced storage methods are not feasible for transportation of large numbers of samples over long distances. In our earlier storage and transport methods, 4-ml samples of whole blood from Semipalatinsk were mixed with heparin, phytohaemagglutinin (PHA), 20% FBS in RPMI-

*Correspondence: M. Hoshi.

Tel: +81 (082) 257 5872, Fax: +81 (082) 257 5873

E-mail: mhoshi@hiroshima-u.ac.jp

1640, stored in 50-ml flasks, and transported cooled to around 4-15°C to Hiroshima University using commercial air transport within 6 days. A modification of this storage and transport method was introduced in 2006: PHA-stimulated blood was stored in medium-free 15-ml tubes and kept at 4-15°C, similar to the temperature during transport. Observations have demonstrated that this modified storage and transport method yields an increased number of metaphases and is able to sustain cell viability for a longer storage time.

It is advantageous to employ a method for whole-blood transport that places few demands on storage and culture resources without compromising cell viability when immediate blood culture processing is not possible. However, any changes and modifications to storage conditions for the improvement of the preservation process need to be validated by testing the reliability of any subsequent chromosome analysis.

MATERIALS AND METHODS

Lymphocyte storage and culture

To simulate the process of blood transfer from the SNTS area to RIRBM Hiroshima Laboratory, four healthy donors, two males and two females, below 30 years of age, were selected. Four millil-

litres of whole blood was taken from each by venipuncture and transferred to a 15-ml sterile tube (Corning Inc., NY, USA) with 200 μ l Novo-Heparin (Mochida, Tokyo, Japan) and 200 μ l phytohaemagglutinin (PHA HA15; Remel Inc., Dartford, UK) (condition A). A second set of samples of whole blood was placed in 50-ml culture flasks (Falcon Becton Dickinson, USA) using a ratio of 4 ml whole blood to 20 ml medium containing RPMI-1640 (+) L-glutamine (Gibco Invitrogen, USA), 20% FBS (Gibco Invitrogen), 200 μ l heparin and 200 μ l PHA (condition B). Four replicates for each donor were set up to allow analysis at time points of 3, 8, 12 and 14 days. All PHA-stimulated blood samples were kept in a refrigerator (Sharp, Model 23TH; Japan) and temperature was maintained at 4°C throughout the experiment. Samples were manually swirled once a day to allow mixing of cell and culture media. During storage, the tubes and flasks were tightly capped and placed upright to prevent any adverse effects on the samples. Figure 1 summarises the overall protocol for the present experiment.

Whole-blood culture and chromosome analysis

Samples of whole blood stored under conditions A and B were taken from the refrigerator after various storage periods and warmed at room tem-

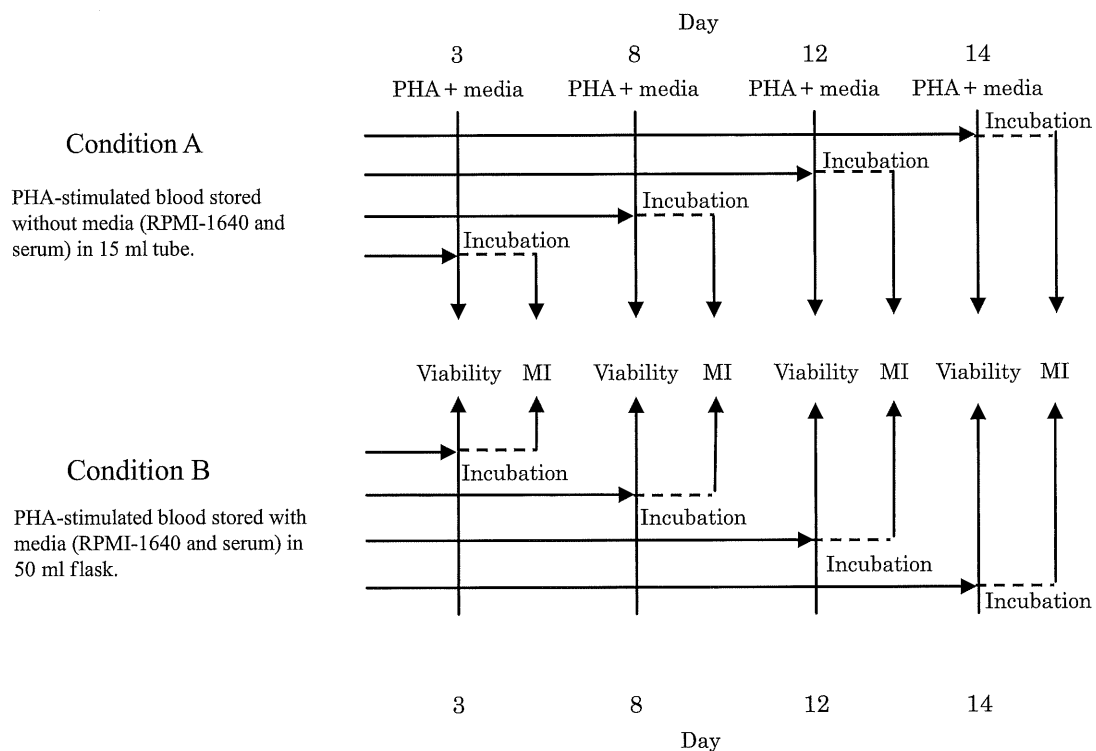


Fig. 1. Summary of experimental protocol

Whole blood samples in condition A were transferred into 15 ml tubes containing PHA without RPMI media. Whole blood samples in condition B were transferred into 50 ml flasks containing PHA and RPMI media. No additional PHA and media were added to samples stored in condition B at various experiment times, 3, 8, 12 and 14 days. Cell viability was assessed before 48 hr incubation period followed by mitotic index count.

perature for 30 min. Whole blood stored under condition A was transferred to a 50-ml culture flask containing 20 ml of RPMI medium, 20% FBS and 200 μ l PHA after 3, 8, 12 and 14 days of storage (Table 1). Whole blood stored under condition B was directly incubated, without further addition of RPMI medium, FBS or PHA. All culture mixtures were mixed well and incubated at 37°C in 5% CO₂ for 48 hr. Colcemid (Demecolcine; Sigma, Irvine, UK) was added to the cultures two hours prior to harvest to arrest the lymphocyte cell cycle at metaphase. Cells were then subjected to hypotonic treatment with potassium chloride (KCl) solution and sodium citrate at a 3:1 ratio, followed by fixing three times with a 3:1 mixture of methanol (Nakarai Tesque, Kyoto, Japan) and glacial acetic acid (Nakarai Tesque). The cells were then sedimented by centrifugation at 1000 rpm for 5 min. Cell pellets at the bottom of the 15-ml tube were vigorously stirred by pipetting at the first fixation stage to remove erythrocytes sufficiently. The cell suspension was then dropped onto clean glass microscope slides (Matsunami Glass Ind., Ltd., Tokyo, Japan), and stained with 3% Giemsa azure eosin methylene blue (Merck, Darmstadt,

Germany) solution for 10 min. The slides were mounted and metaphases were observed under a light microscope (Olympus CX41, Tokyo, Japan).

Scoring of mitotic index

Five chromosome slides were prepared and a total of 5000 cells at metaphase and non-metaphase (rounded cells) from each donor were used for mitotic index scoring. Mitotic indices of lymphocytes stored for each period under conditions A and B were scored and compared. Lymphocytes obtained from two thyroid cancer patients living in Hiroshima and from two non-exposed donors living near the SNTS were scored for comparison. Mitotic index values, indicating the proliferative ability of lymphocytes, were derived from the number of lymphocytes with metaphase spreads divided by the total number of rounded lymphocytes counted on chromosome preparations. Small or large-sized cells and damaged cells were excluded.

Scoring of cell viability

Prior to incubation, cell viability was measured at 3, 8, 12 and 14 days by staining with 0.4% try-

Table 1. Comparison mean values, logarithm transformed, for lymphocyte mitotic index of whole blood stored for various periods under different conditions for the four healthy donors, Hiroshima atomic survivors and non-exposed residents near SNTS.

Culture date	^a Condition A			^b Condition B		
	Mean \pm S.E.	S.D.	N	Mean \pm S.E.	S.D.	N
3 days (control)	0.41 \pm 0.08	0.36	20	^{ns} 0.32 \pm 0.03	0.12	20
8 days	*0.10 \pm 0.10	0.43	20	** -0.62 \pm 0.04	0.18	20
12 days	** -0.41 \pm 0.11	0.44	15	*0.00	0.00	20
14 days	** -0.56 \pm 0.07	0.21	20	*0.00	0.00	20
^c Hiroshima (3 days storage)	^{ns} 0.44 \pm 0.31	0.10	10	n/a	n/a	n/a
^d SNTS (6 days storage)	^{ns} 0.36 \pm 0.09	0.27	10	*0.02 \pm 0.14	0.44	10

^a4 ml of whole blood, heparin and PHA in 15 ml tube. ^b4 ml of whole blood heparin, PHA, 20% FBS and RPMI-1640 medium in 50 ml flask. ^cPeripheral blood from Hiroshima A-bomb exposed thyroid patients, collected in 2008. ^dPeripheral blood from two non-exposed residents near SNTS for storage condition A collected in 2008, and condition B collected in 2005. All parameters and incubation periods under study were the same except that samples from SNTS were kept in a cooled box to maintain temperature at 4°C. n/a, data for Hiroshima A-bomb exposed thyroid patients under condition B were not available. S.D. standard deviation. N sample size. ^{ns} not significant, * significant and ** extremely significant with $p < 0.05$, with reference to the value at 3 day storage under condition A. Z-test was performed for zero values at 12 and 14 instead of Student's t-test using value in control result.

pan blue (Gibco Invitrogen). Each blood sample was diluted 100-fold with Hank's balanced salt solution (Gibco Invitrogen) and subsequently further diluted 1:10 in 0.4% trypan blue. Viable cells were counted by light microscopy using a haemocytometer (Erma Tokyo, Tokyo, Japan).

Statistical analysis

Statistical analysis was performed using GraphPad Software (InStat, California, USA). The software was used to estimate mean, standard deviation and standard error of the mean. Student's t-test and Z-test were performed to compare the significance of differences in metaphase formation in relation to culture delay and storage method. Differences at $p < 0.05$ were considered to be significant.

RESULTS

The logarithmic transformed mean values for the lymphocyte mitotic index of whole blood stored for various periods under different conditions (conditions A and B) for the four healthy donors, Hiroshima atomic survivors and non-exposed residents near SNTS are summarized in Table 1.

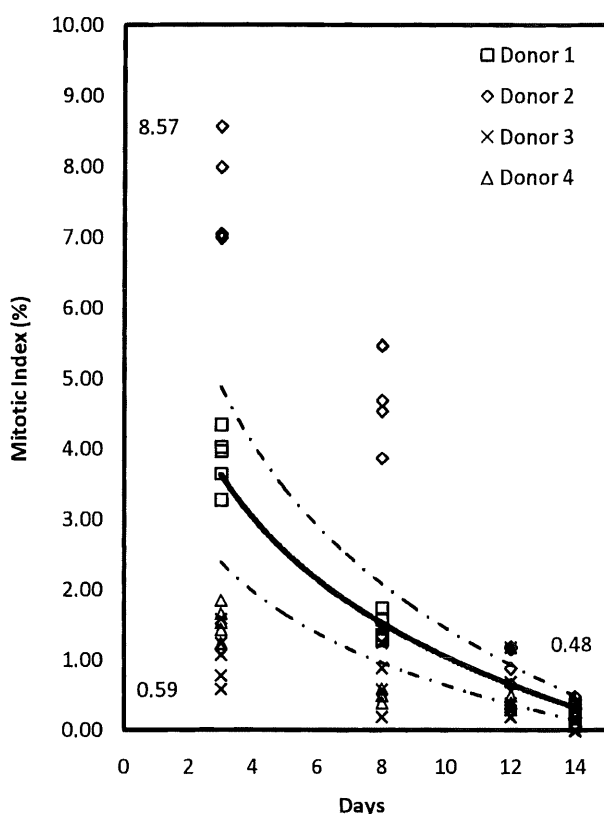


Fig. 2. Individual mitotic indices at various storage times for whole blood stored in condition A.

Five slides from each donor were analysed at day 3 (N=20), 8 (N=20), 12 (N=15) and 14 (N=20). Logarithm curve was derived from mean values; dotted lines represent upper and lower confident limit at 95% confident interval.

These values represent metaphase formation in relation to the success of lymphocyte growth for chromosome analysis. In the present experiment, we used the mitotic index at 3 days of storage (the minimum storage time employed) under condition A (our current storage method, adopted in 2006) as a control. Lymphocyte growth was found to be higher under condition A than under condition B, which was the storage method we had used previously from 1998 to 2005. Mitotic indices after all storage periods under either condition A or condition B differed statistically except in 3 days of storage under condition B. The value obtained at day 3 under condition A from the Hiroshima A-bomb exposed thyroid patients in 2008 showed no significant difference with respect to the control. Similarly, value for non-exposed residents in the controlled area near the SNTS collected in 2008, after 6 days storage under condition A, showed no significant difference with respect to the control. However, under condition B, mitotic index of blood sample collected in 2005 from different non-exposed person living near the SNTS was significantly difference from the control.

The mitotic index showed a wide range among individuals when a shorter storage time was

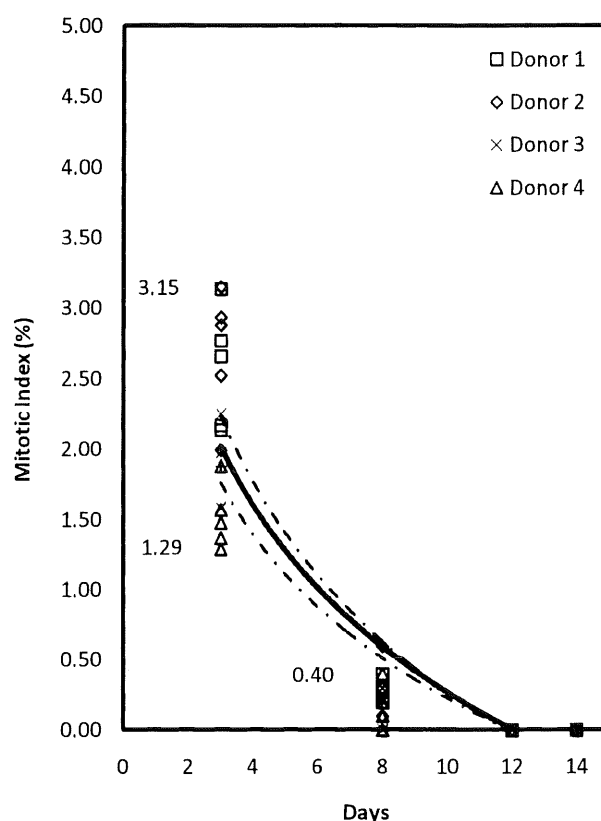


Fig. 3. Individual mitotic indices at various storage times for whole blood stored in condition B.

Five slides from each donor were analysed at day 3 (N=20), 8 (N=20), 12 (N=20) and 14 (N=20). Logarithm curve was derived from mean values; dotted lines represent upper and lower confident limit at 95% confident interval.

used, but became less variable after longer storage times. Figure 2 shows the logarithm curve generated from the mean values, and upper and lower limits, of 20 blood samples from each of four donors stored for different periods under condition A. The maximum and minimum mitotic index at 3 days was 8.57 and 0.59, respectively. Separate observation showed that a sufficient number of well spread metaphases (around 200-1000) were obtained at day 8 under condition A. The range of the mitotic index became smaller at day 14, the maximum value being 0.48 and the minimum zero. A similar trend was observed under condition B, as shown in Fig. 3. The mitotic indices were much lower than under condition A, with a maximum of 3.15 and a minimum of 1.29 at day 3. At day 8, the highest value was 0.40 and the minimum value was zero. Metaphases were completely absent on days 12 and 14 in samples from all donors under condition B.

Data for average cell viability in individual blood samples obtained after storage under conditions A and B for 3, 8, 12, and 14 days prior to culture are summarised in Fig. 4. Cell viability under both storage conditions A and B remained high for all storage periods and there was no statistical significance between condition A and B at each storage time.

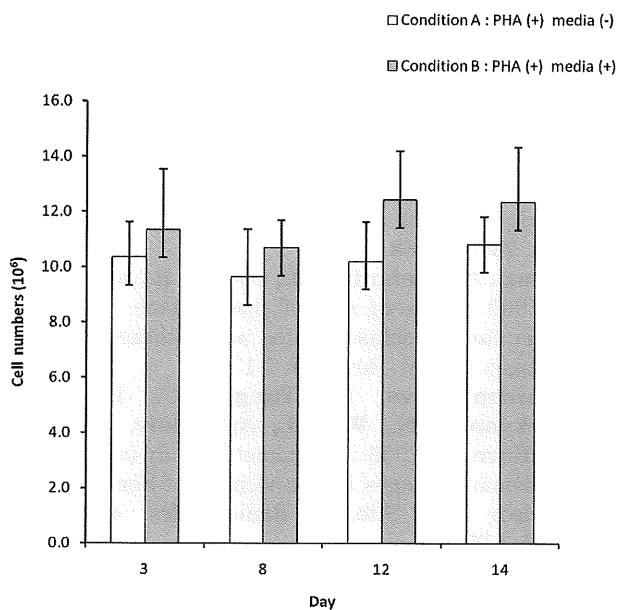


Fig. 4. Mean cell viability (10^6) by trypan blue counted at various times for whole blood samples stored with PHA (+), media (-) (condition A) and PHA (+), media (+) (condition B).

Bars represent standard deviation. There was no statistically significant difference between condition A and condition B at each storage time.

DISCUSSION

Condition B was used for chromosome analysis of residents living near the SNTS between 1998 and 2005, whereas condition A was used between 2006 and 2008. Only 45.8% of samples analysed between 1998 and 2005 yielded successful results, defined as more than 200 well spread metaphases, whereas samples collected between 2006 and 2008 demonstrated a 20.2% yield increase with the use of the new improved method. Our test experiments using condition A for blood samples from thyroid cancer patients among Hiroshima atomic survivors and non-exposed residents living near the SNTS demonstrated that PHA-stimulated whole-blood samples remained viable for 3 and 6 days, respectively, after sampling when stored without culture medium in 15-ml sterile tubes (Table 1), generating a reasonable number of high-quality metaphases (around 200-1000 metaphases per person) for chromosome analysis (data not shown).

The presence or absence of culture medium and the size of the storage container did not significantly influence mitotic potential^{5,18}, but subsequent addition of PHA to the culture had a considerable effect on the rate of cell proliferation. Taking this into consideration, PHA was added twice to blood samples under condition A, both at the beginning of sample storage and prior to incubation, to accelerate further cell division after storage. We did not perform any confirmation experiments or addition of new media and PHA with condition B, since our main study purpose was merely to clarify our experience in blood transport from the SNTS area to Hiroshima University and examine the results for both conditions.

The difference in lymphocyte growth stimulated by PHA under the two storage conditions, A and B, can be explained by changes in the expression of genes located downstream in the signal transduction pathway associated with the cell cycle response¹⁶. Lymphocyte activation by PHA first becomes evident within 2 days after blood has been drawn, though a certain proportion of non-stimulated lymphocytes can remain even during storage at around 4-15°C before culture. Our present results are in agreement with these explanations^{12,14,25}, as further addition of PHA prior to incubation under condition A was found to improve lymphocyte proliferation after a storage period in the absence of culture medium. Furthermore, the presence of culture medium inhibits cell stimulation by PHA at long-term storage. Haemolysis was observed in blood kept in a culture flask for 8 days under condition B, but not under condition A, indicating that some proteins such as haemoglobin released by haemolysis

might affect lymphocytes activated by PHA, thus preventing further lymphocyte proliferation during 48 hr of culture.

There was no clear explanation of why cell viability remained steady under both storage conditions throughout the experimental period, although the mitotic index began to decrease after day 3 and continued to drop until day 14. This finding suggests that resting lymphocytes might be unable to undergo the complete cell cycle after 6 days of storage, and might start to undergo apoptosis despite the addition of PHA at the beginning of culture. It has been reported that PHA-stimulated whole blood stored at 4°C for 96 hr (3 days) and then grown for 56 hr at 37°C shows the highest mitotic index, and that further extension of storage time accelerates apoptosis¹. To some extent, our results are in agreement with those of Belloni et al¹) and Sasaki¹²).

Other factors require to be taken into account for blood transportation as several investigators have demonstrated that human lymphoid cells with radiation induced unstable-type chromosome aberrations are more prone to apoptosis^{2,19}). If this is the case, then the incidence of dicentric chromosomes obtained from individuals exposed to radiation may be lower than expected. Furthermore, a relationship between the detected chromosome aberration rate and shipping temperature has been reported, although the data were conflicting⁹). It was found that a lower temperature (at 4°C) promoted apoptotic cell death in irradiated human peripheral blood during prolonged storage and before stimulation with PHA, suggesting that apoptotic nuclei were increased in the stored samples. The premature chromosome condensation (PCC) index was drastically decreased, particularly when the blood was kept at 4°C. Therefore, it was recommended that whole blood samples should be kept at room temperature during shipping until blood culture and stimulation of lymphocytes when the PCC method is employed for chromosome analysis. We have insufficient data to support these possibilities, except for our results related to stable lymphocyte viability shown in Fig. 4. Therefore, further studies of the relationship between apoptosis and temperature, and also cytogenetic reactions, seem necessary.

Although further confirmation and effects of radiation on chromosome aberration studies have to be done, at least for the meantime, the present data suggest that our currently used method for storage of whole blood in the presence of PHA and the absence of culture medium at 4°C, specifically for unstable chromosome aberration studies, provides reliable preservation and a reasonably high number of lymphocytes with proliferation potential. The blood can be stored for up to 6 days without any adverse effects on the quantity of metaphases produced but prolonged storage

for more than 8 days is not recommended. Our improved method will be helpful to support cytogenetic analysis for biodosimetry and is potentially useful for the storage and transport of whole blood samples over long distances from remote areas after radiation accidents.

ACKNOWLEDGEMENTS

This study was supported by Grants-in-Aid for Scientific Research Nos. 17406001 (Hoshi et al) and 20406002 (Hoshi et al) from the Japan Society for the Promotion of Science.

(Received June 24, 2009)

(Accepted August 25, 2009)

REFERENCES

1. **Belloni, P., Meschini, R. and Palitti, F.** 2008a. Effects of storage conditions of human whole blood on the viability of lymphocytes. *Int. J. Radiat. Biol.* **84**: 613-619.
2. **Belloni, P., Meschini, R., Lewinska, D. and Palitti, F.** 2008b. Apoptosis preferentially eliminates irradiated G0 human lymphocytes bearing dicentric chromosomes. *Radiat. Res.* **169**: 181-187.
3. **Bloom, A.D., Neel, J.V., Choi, K.W., Iida, S. and Chagnon, N.** 1970. Chromosome aberrations among the Yanomma Indians. *Proc. Natl. Acad. Sci. USA.* **66**: 920-927.
4. **Bloom, A.D., Neel, J.V., Tsuchimoto, T. and Meilinger, K.** 1973. Chromosome breakage in leukocytes of South American Indians. *Cytogenet. Cell Genet.* **12**: 175-186.
5. **Bosman, F.T., Van Der Ploeg, M., Schaberg, A. and Van Duijn, P.** 1975. Chromosome Preparations of human blood lymphocytes – Evaluation of techniques. *Genetica.* **45**: 425-433.
6. **Burrill, W., Levine, E.L., Hindocha, P., Roberts, S.A. and Scott, D.** 2000. The use of cryopreserved lymphocytes in assessing inter-individual radiosensitivity with the micronucleus assay. *Int. J. Radiat. Biol.* **76**: 375-382.
7. **Casati, A., Stefanini, M., Giorgi, R. and Nuzzo, F.** 1992. Different rate of chromosome breakage in human fibroblast strains after storage in liquid nitrogen. *Mutat. Res.* **275**: 7-11.
8. **Chaizhunusova, N., Yang, T.C., Land, C., Luckyanov, N., Wu, H., Apsalikhov, K.N. and Madieva, M.** 2006. Biodosimetry study in Dolon and Chekoman villages in the vicinity of Semipalatinsk Nuclear Test Site. *J. Radiat. Res.* **47**: Suppl. A165-A169.
9. **Gotoh, E. and Tanno, Y.** 2005. Simple biodosimetry method for cases of high-dose radiation exposure using the ratio of the longest/shortest length of Giemsa-staining drug-induced prematurely condensed chromosomes (PCC). *Int. J. Radiat. Biol.* **81**: 379-385.
10. **Ilyinskikh, N.N., Eremich, A.V., Ivanchuk, II. and Ilyinskikh, E.N.** 1997. Micronucleus test of erythrocytes and lymphocytes in the blood of the

- Altai region residents living near the Semipalatinsk atomic proving ground. *Mutating Res.* **392**: 223-228.
11. **Ilyinskikh, N.N., Isaeva, T.M., Ivanchuk, II., Rogozin, E.A. and Ilyinskikh, E.N.** 1998. Frequencies of micronucleated lymphocytes and Epstein-Barr virus contamination in Altai region residents living near the Semipalatinsk atomic testing ground. *Environ. Mol. Mutagen.* **31**: 11-17.
 12. **International Atomic Energy Agency (IAEA)** (2001) IAEA Cytogenetic Analysis for Radiation dose assessment. Technical Reports Series No. 405.
 13. **Kondo, K. and Sasaki, M.** 1981. A simple cryopreservation method of human blood for chromosome study. *Jpn. J. Human Genet.* **26**: 255-259.
 14. **Kurnick, J.T., Bell, C. and Grey, H.M.** 1976. PHA-induced activation of suppressor cells in normal peripheral blood lymphocytes. *Scand. J. Immunol.* **5**: 771-778.
 15. **Matsumoto, N., Yoshizawa, H., Kagamu, H., Abe, T., Fujita, N., Watanabe, S., Kuriyama, H., Ishiguro, T., Tanaka, J., Suzuki, E., Kobayashi, K., Gemma, A. and Geyjo, F.** 2002. Successful liquid storage of peripheral blood stem cells at subzero non-freezing temperature. *Bone Marrow Transplantation.* **30**: 777-784.
 16. **Reed, J.A., Reed, J.C., Nowell, P.C. and Hoover, R.G.** 1986. Sequential expression of protooncogenes during lectin-stimulated mitogenesis of normal human lymphocytes. *Proc. Natol. Acad. Sci., USA*, **83**: 3982-3986.
 17. **Salomaa, S., Lindholm, C., Tankimanova, M.K., Mamyrbayeva, Z.Zh., Koivistoinen, A., Hulten, M., Mustonen, R., Dubrova, Y.E. and Bersimbaev, R.I.** 2002. Stable chromosome aberrations in the lymphocytes of a population living in the vicinity of the Semipalatinsk nuclear test site. *Radiat. Res.* **158**: 591-596.
 18. **Schrors, E.** 1987. Transformation capacity, cell cycle and storage of human T lymphocytes: a comparison of two culture media. Paper presented at 10th Meeting of Gesellschaft fur Umweltmutation, Erlangen, Germany, 25-27 March 1987 see *Muta.Res.* **182**: 290 abstract.
 19. **Schwartz, J.L. and Jordan, R.** 1997. Selective elimination of human lymphoid cells with unstable chromosome aberrations by p53-dependent apoptosis. *Carcinogenesis* **18**: 201-205.
 20. **Stephan, G., Pressl, S., Koshpessova, G. and Gusev, B.I.** 2001. Analysis of FISH-painted chromosomes in individuals living near the Semipalatinsk nuclear test site. *Radiat Res.* **155**: 796-800.
 21. **Takeichi, N., Hoshi, M., Iida, S., Tanaka, K., Harada, Y., Zhumadilov, Z., Chaizaunusova, N., Apsalikov, K.N., Noso, Y., Inaba, T., Tanaka, K. and Endo, S.** 2006. Nuclear abnormalities in aspirated thyroid cells and chromosome aberrations in lymphocytes of residents near the Semipalatinsk nuclear test site. *J. Radiat. Res.* **47**: Suppl. A171-A177.
 22. **Tanaka, K., Iida, S., Takeichi, N., Chaizhunusova, N.J., Gusev, B. I., Apsalikov, K. N., Inaba, T. and Hoshi, M.** 2006. Unstable-type chromosome aberrations in lymphocytes from individual living in near Semipalatinsk nuclear test site. *J. Radiat. Res.* **47**: Suppl. A159-A164.
 23. **Tanaka, K., Tchajjunosova, N.J., Takatsuji, T., Gusev, B.I., Sakerbaev, A.K., Hoshi, M. and Kamada, N.** 2000. High incident of micronuclei in lymphocytes from residents of the area near the Semipalatinsk nuclear explosion test site. *J. Radiat. Res.* **41**: 45-54.
 24. **Testa, A., Stronati, L., Ranaldi, R., Spano, M., Steinhausler, F., Gastberger, M., Hubmer, A., Ptiskaya, L. and Akismetov, M.** 2001. Cytogenetic biomonitoring carried out in a village (Dolon) adjacent to the Semipalatinsk nuclear weapon test site. *Radiat. Environ. Biophys.* **40**: 125-129.
 25. **Winger, L.A., Nowell, P.C. and Daniele, R.P.** 1978. Sequential proliferation induced in human peripheral blood lymphocytes by mitogen. II Suppression by PHA-activated cells. *J. Immunol.* **118**: 1767-1773.