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Does Cell Culture Type And Blood Transport Temperature Affect Dicentric Yield And Radiation Dose Assessment?

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ABSTRACT

Background: The metaphase-spread-based dicentric chromosome assay (DCA) is the "gold standard" biodosimetry method for radiation dose assessment. However, practical issues influencing dose assessment by DCA, such as cell culture type and blood transport temperature are not addressed. **Purpose:** To fill the research gaps, we studied the effects of short-term cell culture type, the use of whole blood versus isolated lymphocyte cultures, and the influence of refrigerated blood transport conditions. **Materials and methods**: Human peripheral blood was irradiated with ^{60}Co (cobalt-60) gamma rays and subjected to different temperature/shipment conditions and to short-term culture procedures. Blood transport temperature was measured using temperature data loggers. Frequency of dicentrics and gamma-H2AX persistent expression were estimated. **Results**: Dicentric yield was identical in both culture types. Immediate exposure of irradiated samples to refrigeration increased dicentric yield, correlating with increased gamma-H2AX positive cells. **Conclusion:** The whole-blood culture method

can be used for DCA instead of the isolated lymphocyte culture to increase throughput in radiation mass casualty events. Storage/shipment of blood under refrigerated conditions immediately after radiation exposure should be avoided because it may result in dose overestimation. An increase in dicentrics was associated with an increase in persistent gamma-H2AX expression, suggesting DNA repair inhibition under refrigeration.

INTRODUCTION

Optimum medical aid to radiation mass casualties requires individual, early, and definitive diagnostic radiation dose assessment within days following a catastrophe [Blakely, 2005]. The dicentric chromosome assay (DCA), which is based on the measurement of dicentric chromosomes in human peripheral blood lymphocytes (HPBL), is the internationally recommended “gold standard” for radiation biodosimetry [International Atomic Energy Agency, IAEA, 2001]. DCA is highly radiation-specific. Radiation specificity of dicentrics is attributed to the induction of two lesions in pre-replicated chromosomes that, in close proximity with respect to time and space, undergo an exchange. DCA shows low background levels (about 1 dicentric in 2000 cells), high sensitivity (a threshold dose of 0.05 Gy, Gray), and known dose dependency of up to 5 Gy for photons [IAEA, 2001]. The sum of the clastogenic action (breakage of chromosomes) from other sources combined, including smoking, alcohol, and drugs accounts for only a very small, if any, proportion of the background rate [Hoffmann 1999]. Dicentrics may be found in pathological conditions. However, the incidence is rare and the fraction of dicentric chromosome is low. The dose estimation procedure involves culturing either whole blood or isolated lymphocytes, harvesting metaphase spreads in first-division metaphase spreads, enumerating dicentric frequency, and comparing the result with an *in vitro* generated calibration curve.

The DCA is gaining renewed importance for quickly assessing dose to potentially irradiated individuals in the early period after a radiological or nuclear incident, when a rapid determination of dose is required for proper medical management [Lloyd, 2000; Prasanna, 2003; Wilkins, 2008] because estimated doses correlate well with the severity of imminent acute radiation syndrome [Sevan'kaev, 2000]. Lloyd [Lloyd, 2000] demonstrated that clinical triage can be accomplished by scoring as low as 20 to 50 metaphase spreads per subject after a simulation of an accident with mass casualties receiving whole- or partial-body irradiation in the 0- to 8-Gy range. He suggested that frequency of metaphase spreads without dicentric aberrations can be used to identify patients suitable for cytokine therapy based on partial-body exposure. Early identification of radiation casualties who will benefit from cytokine therapy is relevant because medical management guidelines encourage early administration of cytokines [Waselenko, 2004].

For the rapid triage of radiation mass casualties, abridged cytogenetic protocols are proposed to guide medical management of the exposed individuals [Lloyd, 2000; Voisin, 2001; Prasanna, 2003]. The International Organization for Standardization (ISO)

workgroup-18 under subcommittee-2 is developing guidelines for applications in mass casualty situations. Several cytogenetic biodosimetry laboratories already have abridged protocols for rapid dose assessment. Abridged cytogenetic protocols for applications in radiation mass casualties are designed, for example, to increase the number of samples processed from 10 to 100 per day and to decrease the number of metaphase spreads analyzed per sample from 500 to 1000 spreads per sample to 20 to 50 spreads, compromising precision on estimated dose but still adequate for stratification of individuals into different dose-based cohorts with implications for treatment. The IAEA suggested that microcultures of whole peripheral blood obtained from hundreds of potentially irradiated individuals can be used to increase throughput [IAEA, 2001]. Dose-based triage is arrived at by scoring 20 to 50 metaphase spreads per subject, compared with the typical 500 to 1000 spreads scored for routine analyses [Lloyd, 2000; Prasanna, 2003; Voisin, 2001]. When more precise dose estimation is essential, additional blood samples from exposed subjects are collected and processed for 500 to 1000 metaphase-spread analysis per subject, so as to improve dose precision for estimating long-term risks. This involves culturing lymphocytes isolated from a larger volume of whole blood from exposed individuals and then harvesting first-division metaphase spreads for chromosome aberration analysis. However, influence of cell culture type on radiation-induced dicentric frequency in HPBL has not been addressed so far.

Because the DCA is conducted in a “reach-back” laboratory, transportation of blood from radiation accident sites is essential. The suggested guidelines for transporting blood for cytogenetic biodosimetry are based on the provisions governing infectious substances, biological products, and diagnostic specimens. These are provided in the following United States regulatory documents: (i) Department of Transportation (DOT) Code of Federal Regulations Title 49 (CFR 49), (ii) Center for Disease Control and Prevention CFR Title 42, (iii) Animal and Plant Health Inspection Service 9 CFR and 7 CFR, (iv) Food and Drug Administration 21 CFR, (v) International Air Transport Association Dangerous Goods Code, (vi) Air Force Interservice Manual 24-204 (AFMAN 24-204), and (vii) the DOT Emergency Response Guidebook. Still needed are clear instructions and standard operating procedures for blood collection and transport, specifically for cytogenetic biodosimetry, in the aftermath of a radiological incident.

In this study, we tested the effects of short-term cell culture methods (whole blood versus isolated lymphocyte cultures), insulation efficiency of certified shipping boxes used for blood transportation, and simulated blood transport temperature conditions on radiation-induced dicentric yield. In addition, we addressed the relationship between the formation of dicentrics, under refrigerated conditions, and double-strand break (dsb) repair, as measured by persistence of gamma-H2AX expression after irradiation.

MATERIALS AND METHODS

Blood samples

After obtaining informed consent, 20 mL of peripheral blood from each of two healthy donors were collected by phlebotomy into vacutainers containing sodium heparin (Becton-Dickinson, Rutherford, NJ, USA) and used separately. The Uniformed Services University of the Health Sciences' Human Use Committee, Bethesda, MD, USA, approved the informed consent form.

Irradiation

Blood-containing vacutainers were irradiated at the Armed Forces Radiobiology Research Institute (AFRRI) ^{60}Co facility in a specially fabricated array. For comparison between cell culture conditions (whole blood and isolated lymphocytes), blood was irradiated at room temperature with 3 Gy at a dose rate of 0.6 Gy min^{-1}. To study the effect of storage temperature on dicentric yield, blood was irradiated with 2 Gy at a dose rate of 0.1 Gy min^{-1}. For dose-dependent expression of gamma-H2AX, blood was irradiated with 0.25, 1, 3 and 5 Gy at a dose rate of 0.6 Gy min^{-1}.

Whole-blood short-term culture

Five hundred microliters of blood were incubated in 4.5 mL of pre-warmed Marrowmax® (Gibco, USA) bone marrow culture medium. Blood cells were stimulated to proliferate by the addition of 10 microliter/mL of phytohemaglutinin (Invitrogen-Gibco, USA). Colcemid (Sigma Chemical Co., USA) was added (0.1 microgram/mL final concentration) to cultures after 24 hours to harvest first-division metaphase spreads at 48 hours, after an additional incubation for 24 hours at 37 °C.

Lymphocyte isolation and short-term culture

Lymphocytes from 3-mL irradiated whole blood were isolated on a density gradient (Histopaque®, Sigma Chemical Co., USA), washed twice with phosphate-buffered saline (PBS) and resuspended in 3-mLMarrowmax®. Lymphocytes were then stimulated to proliferate by addition of 10 microliter/mL of phytohemaglutinin (PHA) for 24 hours at 37 °C. Following incubation with PHA, 0.1 microgram/mL colcemid was added and cells were incubated for an additional 24 hours at 37 °C before the collection of first-division metaphase spreads.

Metaphase spread harvesting, spreading, and staining

Following cell culture, metaphase spreads were harvested, using an automated metaphase harvester (Hanabi PII, ADSTEC Technologies, Japan), after treatment with a hypotonic solution (0.56% potassium chloride) and fixation in 1:3 acetic acid to methanol solution. Cell suspension enriched with mitotic cells was spread on clean glass slides, using a metaphase spreader (Hanabi, ADSTEC Tehnologies, Japan), at 37 °C and 54% relative humidity. The slides were stained in 4% Giemsa in PBS at pH 6.8, using an autostainer (Shandon, Therma, USA), and coverslipped, using an automated coverslipper (Consul, Therma, USA). Metaphase spreads on slides were automatically located at lower magnification (100-X) by a metaphase finder (Loats Associates, USA); about 500 metaphase spreads were manually analyzed by expert scorers at higher magnification (1000-X).

Insulation reliability, blood handling, and simulation of shipment

To test insulation reliability and the effect of blood handling and to simulate shipment conditions, blood was placed in a cooler system (Biofreeze, Inmark, Inc., Austell, GA) with external dimensions of 39 x 34 x 32 cm and internal dimensions of 31 x 29 x 19 cm. The cooler includes a 1.5-L plastic jar, a secondary container suitable for shipping biohazardous material (UN Category 6.2 Infectious Substances) and five cooling shipping gel packs (Thermosafe Polar Pack, PP16 16-oz gel cooler pack Trangent Corp., DeKalb, IL 60115 SCA). Temperature data loggers (Telatemp “Valutemp,” Fullerton, CA) were placed in the box to monitor the temperature over time. Boxes containing gel packs, room temperature equilibrated (24 ºC) or pre-cooled for 24 hours at 4 °C in a refrigerator, were sealed with tape and were either kept on the bench or shipped via FedEx with overnight priority.

Gamma-H2AX measurement for determining DNA double-strand breaks

The double-strand breaks, dsbs, were made visible using gamma-H2AX stain. Immediately after irradiation, or after lymphocyte isolation and mitotic block, lymphocytes were resuspended in 2% paraformaldehyde, permeabilized in 70% methanol and dropped on clean slides. Slides were allowed to air-dry. Cells were rehydrated in PBS and blocked before application of antibodies. Mouse monoclonal gamma-H2AX antibodies (Upstate Biotechnology, VA) were diluted 1:1000, secondary antibodies were goat, anti-mouse IgG (Rodhamine conjugated) diluted 1:200 (Molecular Probes, CA). Slides were counterstained with 4',6-diamidino-2-phenylindole (DAPI) and mounted before examination with a confocal microscope (Olympus BX51). The cells containing large gamma-H2AX foci were counted as positive and reported as a percentage of the total cells counted.

Statistical analysis

Analysis of the yield of dicentrics in metaphase included the determination of the mean ± SE. The chi-square test was used to determine the significance of results.

RESULTS

Comparison of yields of two culture methods

In order to identify any difference between two culture methods, whole-blood short-term microculture and isolated lymphocyte cultures, in terms of radiation-induced dicentric yields, we irradiated peripheral blood with a 3-Gy dose and performed a direct comparison of dicentric yields. After irradiation, blood was divided into two aliquots. One aliquot was immediately processed for whole-blood short-term microculture, and the other aliquot was used for culturing lymphocytes following a density gradient separation as described in the methods section. Metaphase spreads were prepared from these two cell-culture types, and dicentrics were scored as described previously. Five-hundred first-division metaphase spreads were analyzed for each culture type. Figure 1 shows examples of metaphase spreads prepared from whole-blood peripheral blood microculture (panel A) and isolated lymphocyte culture (panel B). Better quality metaphase spreads are seen in the isolated lymphocyte cultures. The frequencies of dicentrics per cell were not significantly different at 0.45 ± 0.02 for whole-blood microculture and 0.43 ± 0.02 for isolated lymphocyte cultures, obtained from the same donor (panel C).

Insulation efficiency of certified shipment boxes

To determine the insulating reliability of certified shipping boxes routinely used for blood transport, we placed a gel pack in each shipping box to measure temperature changes using temperature data loggers. Each gel pack had been equilibrated overnight either at room temperature or at 4 ºC. Boxes were then assembled and sealed in compliance with regulations for biohazardous material shipment. The boxes containing cold insulating packs were left on a laboratory bench for approximately 48 hours; the boxes containing room temperature-equilibrated packs were shipped by FedEx overnight back to the laboratory. Outside minimum and maximum temperatures recorded at the time of the experiment were 1.6 ºC and 10.0 ºC on the day the boxes were shipped and -1.1 ºC and 6.6 ºC on the day they were received. As shown in figure 2 examples of temperatures recorded by the data loggers, within 24 hours the temperature increased by about 10 to 15 ºC in boxes containing pre-cooled gel packs (panel A) while the temperature decreased by about 10 ºC in boxes with insulating gel packs equilibrated at room temperature (panel B). These results suggest that standard insulation is not sufficient to protect samples from external temperature variations and that blood samples packaged to maintain a temperature of about 25 ºC or 4 ºC will likely be subjected to substantial and unpredictable changes in temperature during transport.

Effect of exposure of irradiated whole blood to cold conditions

In the case of radiation mass casualties, it is envisioned that, because of lack of infrastructure or inadequate capacity, samples may not be collected under the suggested controlled temperatures and that sample processing may be delayed. Because, as shown, the temperature inside a shipping box may undergo severe

changes during transport, we determined the effect of storage temperature on dicentric yield. We irradiated blood with a 2-Gy dose and divided the blood into two aliquots. One aliquot was used for control and underwent routine procedures for short-term blood culturing while the second aliquot was immediately subjected to a refrigerated temperature before culturing. Blood was placed for 24 hours in a shipping box and packed in compliance with blood shipment requirements in the presence of precooled (4 ºC) gel packs. Following cold treatment, PHA and culture medium were added and the aliquot was processed for standard short-term blood culturing. After culturing, metaphase spreads were prepared for scoring dicentrics. Our results show that storage at 4 ºC immediately after irradiation significantly ($p < 0.0001$) increases the yield of dicentrics (table 1). Results were confirmed in an independent experiment, where dicentric yield was analyzed on irradiated isolated lymphocytes following storage at 4 ºC and 37 ºC.

We hypothesized that an increase in dicentrics may be due to temperature-dependent inefficient repair of DNA dsbs. To test this hypothesis, we analyzed expression of gamma-H2AX, a recognized rapid marker of DNA dsbs. Persistence of gamma-H2AX is an indication of inefficient DNA repair capability. Persistent expression of gamma-H2AX was analyzed by confocal microscopy in first-division metaphase spreads obtained from lymphocytes two days after exposure to a range of radiation doses (0.25 to 5 Gy; figure 3, panel A). Persistent gamma-H2AX expression was dose-dependent (figure 3, panel B) in a dose range relevant for the formation of dicentrics (Prasanna, 2002). After establishing the presence of gamma-H2AX residual expression 48 hours after irradiation, the same incubation time required to collect metaphase spreads for analysis of dicentrics, we repeated the cold treatment procedure previously done for measuring the effect of temperature on dicentric yield. Only cells containing large gamma-H2AX foci were counted as positive and reported as a percentage of the total number of cells counted. As shown in figure 4, panel A, storage of blood under refrigeration resulted in an increase in the frequency of gamma-H2AX positive cells, indicating a possible inhibition in the repair of dsbs in the cold-challenged blood sample with respect to the control blood. Similarly, the frequency of dicentrics increased as the temperature decreased (figure 4, panel B).

DISCUSSION

Despite the international consensus to use the dicentric assay for biological dosimetry, with recent focus particularly on its mass casualty applications, key logistical and methodological details have yet to be worked out among cytogenetic laboratories. This study addressed critical issues that could influence throughput and dose estimation, including culture methods, blood draw procedures, and transportation.

A primary limitation of the dicentric assay—the fact that it is labor intensive and time consuming during sample preparation and metaphase spread analysis— can be overcome by automation. First, the use of whole blood instead of isolated lymphocytes for a short-term culture will expedite sample processing by simplifying automation and

robotic liquid handling. Second, only a small volume of blood is necessary for a whole-blood cell culture compared with isolated lymphocyte cultures. We have shown that only a small qualitative difference exists between metaphase spreads obtained from whole-blood preparation and spreads obtained from isolated lymphocytes. The yield of dicentrics was the same for both preparations, and the outcome of the analysis was not affected by the culture conditions.

This methodology can be applied to triage dose assessment because a high volume of samples can be quickly processed by analyzing only 20 to 50 spreads per subject for a dose-based stratification of the exposed cohort. However, viable cells cannot be enriched in whole-blood culture, which may result in lower mitotic yield particularly at high dose exposures. However, isolation of lymphocytes provides a large number of metaphase spreads, because of the ability to enrich lymphocytes, and can be used to assess risks for late effects of radiation exposure as in the case of occupational overexposures where analysis of a large number of metaphase spreads is essential. Limitations of this procedure are the large volume of blood and the turnaround time.

In the case of mass casualties, shipment of blood to several cytogenetic laboratories for dose assessment is expected to be the most likely scenario. Guidelines are available for blood collection and transport for cytogenetic dose assessment by the dicentric assay (IAEA, 2001). The current consensus guidelines indicate to collect blood as soon as possible, with the reminder that in case of partial body exposure, about 24 hours are required for circulating lymphocytes to reach the equilibrium, and therefore, dose could be underestimated if blood is sampled too early. Efforts should be made to ensure that samples are obtained within 4 weeks from exposure to avoid uncertainties in dose estimates. Additionally, we recommend that blood should not be collected immediately after irradiation for the dicentric assay and shipped under refrigerated conditions because refrigerated temperature immediately following irradiation will influence dicentric yield resulting in dose overestimation. These recommendations are based on experiments in which blood was irradiated *ex vivo*. Previously, it was shown that irradiation temperature changing from 37°C to 4°C, acts as a dose-modifying factor with regard to the dose-yield relationship for dicentric chromosome aberrations irradiated with 150 kVp X-rays (Gumrich, 1986). The time-course kinetics of signal decay may vary depending on biological endpoint. Therefore, detailed studies are warranted to determine the effect of temperature on a given endpoint before sample shipment guidelines are developed for biological dosimetry.

Insulating features of currently used shipping boxes and packaging recommendations have proven inadequate for both refrigerated and room temperature conditions, as recorded by data loggers placed inside the boxes. Based on our results, storage or shipment of blood under refrigerated conditions immediately after radiation exposure should be avoided because it may result in dose overestimation.

The higher frequency of dicentrics induced by low temperatures may be due to an increase in unrepaired or misrepaired DNA dsbs. Indeed, we found that lymphocytes subjected to cold temperatures showed an increase in persistent gamma-H2AX, a

histone protein that becomes phosphorylated and localizes at sites flanking DNA dsbs (Pilch, 2003) forming of large foci. Not only is gamma-H2AX a reporter of DNA repair but the persistence of residual gamma-H2AX foci has been interpreted as incomplete DNA repair (Rios-Doria, 2006). Thus, this endpoint is a useful tool to study DNA dsbs in a radiation dose range relevant to chromosomal aberrations. Our findings that persistent gamma-H2AX expression is enhanced by cold treatment suggest that a cold-induced increase in dicentrics may be linked to defects in the repair mechanisms. Enzymatic repair processes show a typical dependence on temperature. The kinetics of enzymatic repair of DNA single- and double-strand breaks generally show fast components, on a magnitude of minutes, as well as slower components, which may last for up to an hour or more. One possible explanation for the difference we observed may be that a delay in DNA damage repair triggers alternative mechanisms that are more prone to errors. The two major DNA dsb repair pathways in mammalian cells are homologous recombination (HR) and nonhomologous end joining (NHEJ) (Pfeiffer, 2004). Of these two mechanisms, the one that is thought to be more error prone and, therefore, more likely to participate in creating a dicentric chromosome is NHEJ. The protein responsible for the ligation step in NHEJ is DNA ligase IV (LIG4). Interestingly LIG4 has shown sigmoid-like temperature dependence with maximum efficiency at 4 ºC (Ferretti, 1981). Our laboratory is interested in understanding the mechanisms of radiation-induced dicentric formation.

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TABLES

Table 1: Effect of post-irradiation incubation temperature on dicentric frequency in irradiated whole blood. Blood was exposed *in vitro* to ^{60}Co gamma rays (2 Gy, 0.1 Gy/min) and was immediately incubated at 37ºC for short-term culturing (control) or subjected to cold treatment (4ºC for 24 hours) before culturing

Treatment	Total number of metaphase spreads	No. of dicentrics	No. of dicentrics/spread	S.E.
Control	379	89	0.2348	0.0217
4°C	529	331	0.3743*	0.0210

*Significant at $p < 0.0001$.

FIGURES

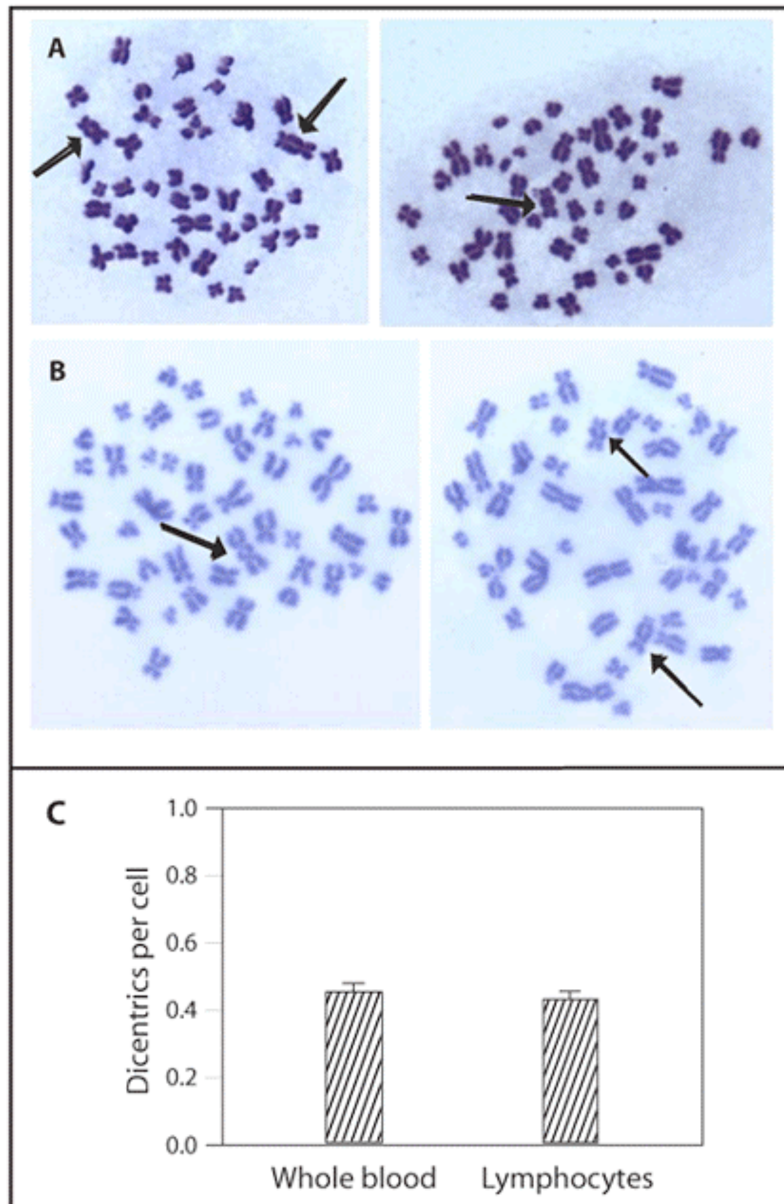

Figure 1: Comparison of radiation-induced dicentric frequencies in first-division metaphase spreads prepared from different procedures. Metaphase spreads prepared from whole blood (panel A) and those prepared from isolated lymphocytes (panel B) show no significant differences in the frequencies of dicentrics per cell. Arrows indicate dicentric chromosomes. Five hundred first-division metaphase spreads were analyzed to estimate the frequency of radiation-induced dicentrics (panel C)

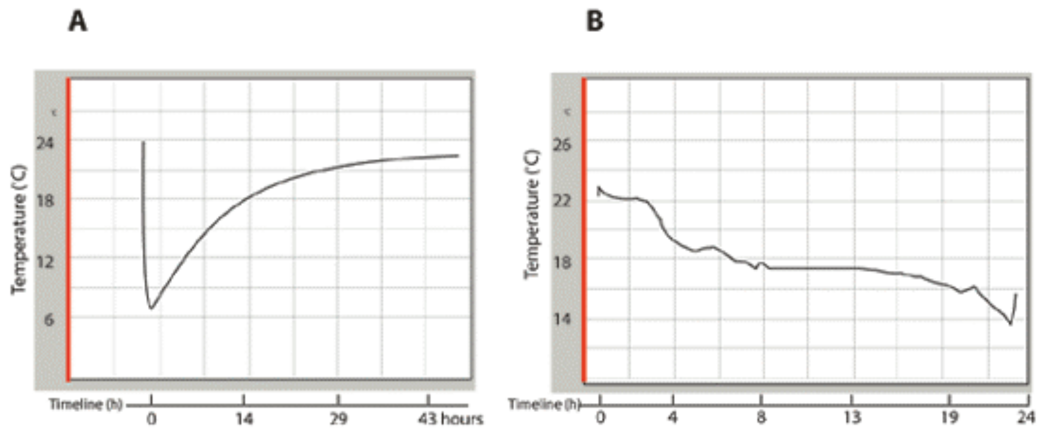

Figure 2: Examination of the insulating properties of boxes approved for shipment of biohazardous materials. Temperature loggers were placed in regulation compliant, insulated shipping boxes. Five cooling packs pre-equilibrated at either 4 ºC (panel A) or room temperature (panel B) were added to each box before it was sealed. The recorded data indicate that blood samples would be subject to significant temperature changes during shipment

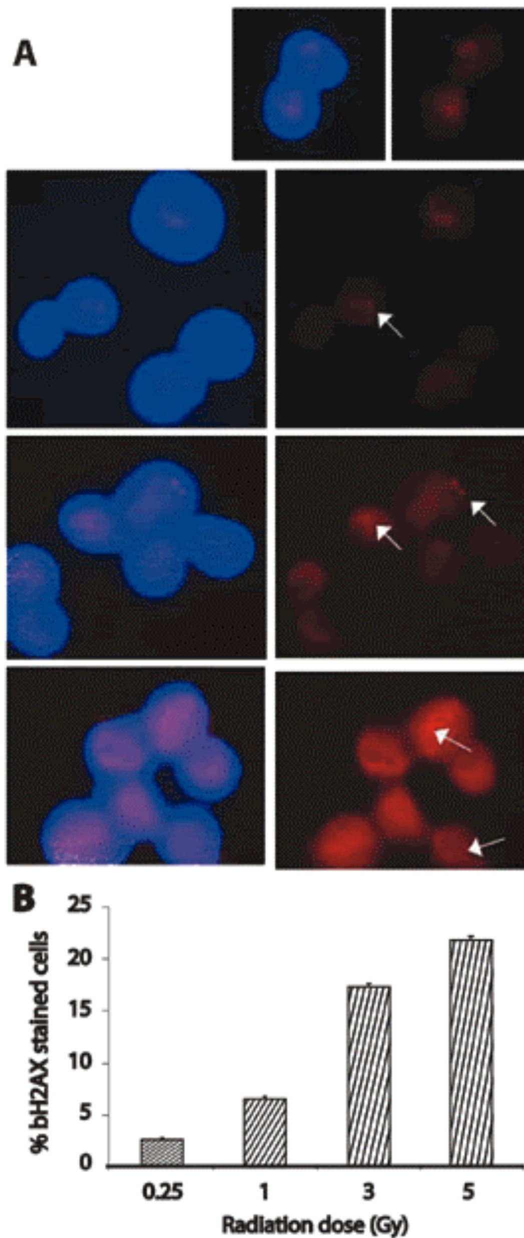

Figure 3: Dose-dependency of gamma-H2AX expression. Blood samples were irradiated with 0.25, 1, 3 and 5 Gy. Lymphocytes were isolated immediately after irradiation, stained with antibodies against gamma-H2AX and analyzed by confocal microscopy. In examples of stained lymphocytes irradiated with increasing doses of radiation (panel A, top to bottom), the red stain represents gamma-H2AX and the blue stain represents the nuclei. Arrows indicate gamma-H2AX foci. Cells with large foci were counted and reported as a percentage of the total number of cells counted (panel B)

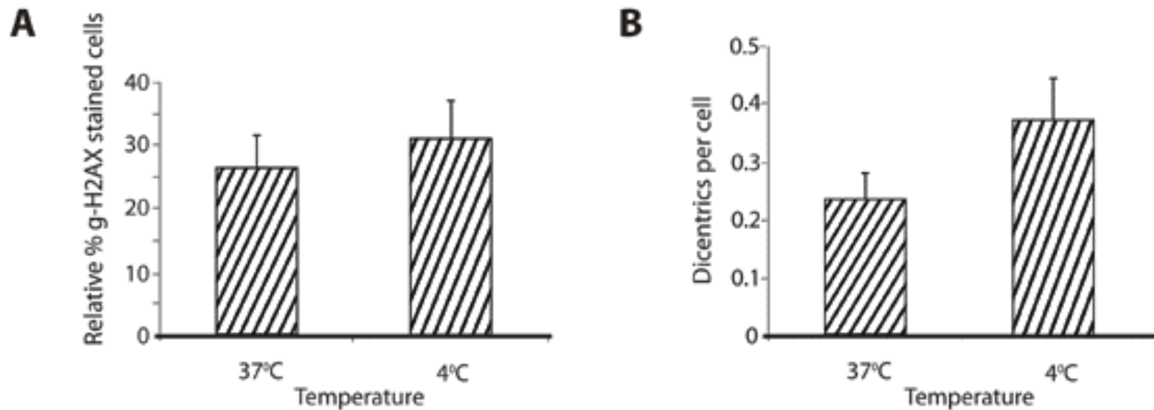

Figure 4: Effect on whole blood subjected to refrigeration immediately following irradiation. Compared with control blood, the percentage of gamma-H2AX positive cells (panel A) and the number of dicentrics (panel B) in refrigerated blood increased significantly as the temperature decreased. Irradiated blood samples either were exposed to 4 ºC for 24 hours prior to short-term culture or were immediately processed (A). Following cell culture, metaphase spreads were prepared. Metaphase spreads were analyzed to determine the formation of double strand breaks as visualized by gamma-H2AX stain (A) and the frequency of dicentrics (B)

REFERENCES

Alexander, G.A., Swartz, H.M., Amundson, S.A., Blakely, W.F., Buddemeier, B., Gallez, B., Dainiak, N., Goans, G.E., Hayes, R.B., Lowry, P.C., Noska, M.A., Okunieff, P., Salner, A.L., Schauer, D.A., Trompier, F., Turteltaub, K.W., Voisin, P., Wiley, A.L., Jr., and Wilkins, R.C. (2007) Acute Dosimetry Consensus Committee Recommendations on Biodosimetry Applications in Events Involving Uses of Radiation by Terrorists and Radiation Accidents. *Radiat. Meas.* **42,** 972-996.

Blakely, W.F., Salter, C.A., and Prasanna, P.G.S. (2005) Early-Response Biological Dosimetry—Recommended Countermeasure Enhancements for Mass-Casualty Radiological Incidents and Terrorism. *Health Phys.* **89,** 494-504.

Ferretti, L. and Sgaramella, V. (1981) Temperature Dependence of the Joining by T4 DNA Ligase of Termini Produced by Type II Restriction Endonucleases. *Nucleic Acids Res.* **9(1),** 85-93.

Gumrich, K., Virsik-Peuckert, R.P., and Harder, D. (1986) Temperature and the formation of radiation-induced chromosome aberrations. II. The temperature dependence of lesion repair and lesion interaction. *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.* **49(4),** 673-681.

Hoffmann W, Schmitz-Feuerhake I. How radiation-specific is the dicentric assay? J. Expo. Anal. Environ. Epidemiol. 1999; 9:113–133.

International Atomic Energy Agency (2001) Cytogenetic Analysis for Radiation Dose Assessment: A Manual. Technical Report Series No. 405. Vienna, Austria: International Atomic Energy Agency.

Lloyd, D.C., Edwards, A.A., Moquet, J.E., and Guerreo-Carbajal, Y.C. (2000) The Role of Cytogenetics in Early Triage Radiation Casualties. *Appl. Radiat. Isotop.* **52,** 1107-1112.

Pfeiffer, P., Goedecke, W., Kuhfittig-Kulle, S., Obe, G. (2004) Pathways of DNA Double-Strand Break Repair and Their Impact on the Prevention and Formation of Chromosomal Aberrations. *Cytogenet. Gen. Res.* **104,** 7-13.

Pilch, D.R., Sedelnikova, O.A., Redon, C., Celeste, A., Nussenzweig, A., and Bonner, W.M. (2003) Characteristics of Gamma-H2AX Foci at DNA Double-Strand Breaks Sites. *Biochem. Cell. Biol.* **81,** 123-129.

Prasanna, P.G.S., Loats, H., Gerstenberg, H.M., Torres, B.N., Shehata, C.W., Duffy, K.L., Floura, R.S., Khusen, A.W., Jackson, W.E., and Blakely, W.F. (2002) AFRRI's Gamma-Ray, X-Ray, and Fission-Neutron Calibration Curves for the Lymphocyte Dicentric Assay: Application of a Metaphase Finder System. Bethesda, MD, and Fort

Belvoir, VA: Armed Forces Radiobiology Research Institute and Defense Technical Information Center.

Prasanna, P.G.S., Subramanian, U., Greenhill, R.G., Jackocks, J.M., Jackson, W.E., and Blakely, W.F. (2003) Cytogenetic Biodosimetry Strategy for Potential Radiation Mass Casualties. In *Radiation Safety Aspects of Homeland Security and Emergency Response* (Ed Health Physics Society) pp. 218-222. San Antonio, TX: Health Physics Society.

Rios-Doria, J., Fay, A., Velkova, A., and Monteiro, A.N. (2006) DNA Damage Response: Determining the Fate of Phosphorylated Histone H2AX. *Cancer Biol. Ther.* **5,** 142-144.

Sevan'kaev, A.V. (2000). Results of cytogenetic studies of the consequences of the Chernobyl accident. *Radiats Biol. Radioecol.* **40** : 589-595.

Voisin, P., Benderitter, M., Claraz, M., Chambrette, V., Sorokine-Durm, I., Delbos, M., Durand, V., Leory, A., and Pailloe, N. (2001) The Cytogenetic Dosimetry of Recent Accidental Overexposure. *Cell Mol. Biol.* **47,** 557-564.

Waselenko, J.K., MacVittie, T.J., Blakely, W.F., Pesik, N., Wiley, A.L., Dickerson, W.E., Hsu, H., Confer, D.L., Coleman, N.C., Seed, T., Lowry, P., Armitage, O., and Dainiak, N. (2004) Medical Management of the Acute Radiation Syndrome: Recommendations of the Strategic National Stockpile Radiation Working Group. *Ann. Inter. Med.* **15,** 1037-1051.

Wilkins, R.C., Romm, H., Kao, T.C., Awa, A.A., Yoshida, M.A., Livingston, G.K., Jenkins M.S., Oestreicher, U., Pellmar, T.C., and Prasanna, P.G.S. (2008). Inter-Laboratory Comparison of the Dicentric Chromosome Assay for Radiation Biodosimetry in Mass Casualty Events. *Radiat. Res.* **169,** 551-560.