# Diagnostic Biodosimetry Response for Radiation Disasters: Current Research and Service Activities at AFRRI

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## *ABSTRACT*

*Diagnostic Biodosimetry Response for Radiation Disasters Using Advanced Molecular Cytogenetic, Molecular Biology, and a Biodosimetry Assessment Tool: Current Research and Service Activities at the Armed Forces Radiobiology Research Institute*

*This paper addresses the importance of diagnostic radiation dose assessment to help develop a treatment strategy within days of a catastrophe. The long-range goals of the Armed Forces Radiobiology Research Institute (AFRRI) Biological Dosimetry Team are to develop validated radiation biodosimeters and to equip medical personnel with diagnostic information (clinical signs and symptoms, physical dosimetry, etc.) germane to the medical management of human radiation casualties. Our specific objectives are (a) to establish definitive, rapid, high-throughput clinical bioassays for radiation dose assessments, (b) to develop complementary triage-type radiation dose assessment bioassays, and (c) to transition the Biodosimetry Assessment Tool (BAT) software program to facilitate the collection, integration, and arching of biodosimetry data to support medical treatment decisions of radiation-exposed individuals.*

*The experimental approach involves three steps: (a) to establish a "reach-back reference laboratory" that uses conventional bioassays for definitive analyses of biological samples; (b) to develop a validated and forward-deployable biological dosimetry capability for rapid radiation dose assessment, with an emphasis on the use of molecular biology-based diagnostic platforms; and (c) to integrate the biodosimetry data in a suitable software platform to assist in medical management, for example, BAT software.*

*AFRRI researchers established the conventional lymphocyte metaphase-spread dicentric assay in accordance with international harmonized protocols and have been applying it in order to estimate radiation doses in several overexposure accidents. The researchers seek to validate a novel interphase, cell-based cytological bioassay that detects cells with chromosomal-type aberrations and radiation-responsive molecular biomarkers (e.g., gene expression, protein) and to perfect it for rapid radiation dose assessment applications. The BAT software program was released at the AFRRI website (www.afrri.usuhs.mil) in June 2002. Designed primarily for prompt use after a radiation incident, the user-friendly program facilitates collection, integration, and archiving of data obtained from exposed persons. Data collected in templates, using the Microsoft Windows-compatible, user-friendly software program, are compared with established radiation dose responses obtained from the literature to provide multiparameter dose assessment. The program*

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*archives additional clinical information (e.g., extent of contamination, wounds, infection) that is useful for casualty management, and it displays useful information in a concise format. The program is designed for both civilian and military applications, the latter illustrated by fulfilling the requirements of proposed NATO STANAG 2474 NBC/MED "Determination and Recording of Ionizing Radiation Exposure for Medical Purposes."*

*Our research and service efforts contribute to an improved diagnostic responses to mass casualty situations and enhance force protection and survivability in adverse ionizing radiation mission environments.*

## 1.0 INTRODUCTION

Mettler [2002] reviewed potential radiation exposure scenarios, which included detonation of nuclear weapons, terrorist attacks on nuclear reactors, and dispersal of radioactive substances with the use of conventional explosives, resulting in mass casualties. These disasters can result in different radiation exposure types: whole body, localized or partial body, internal contamination, external contamination, and contaminated burns and wounds. Strategies for triage and for the evacuation of the injured, contaminated, and noncontaminated casualties are proposed herein. The onset, nature, severity, and duration of clinical symptoms following radiation exposure are determined primarily by the casualty's absorbed dose but are also influenced by the radiation field and quality, the dose rate, and the individual's inherent radiosensitivity and general medical health status. In reaction to prompt total-body ionizing radiation with a dose range of 0.5 to 30 Gy (photons), the typical symptoms of radiation exposure in humans include nausea, vomiting, diarrhea, and peripheral blood lymphocyte depletion [Anno 1989]. The duration of initial or prodromal symptoms and the latent phase of radiation syndrome is anywhere from 1 h to 2 weeks. Without appropriate medical care, the median lethal dose of radiation, the $LD_{50/60}$ (the dose that kills 50% of the exposed population within 60 days after exposure), is estimated to be 4.5 Gy [Mole 1984]. However, the likelihood of survival can be significantly increased with appropriate aggressive medical intervention and care [Anno 2003].

Nonavailability or inaccurate initial dose estimates, within hours to weeks after exposure, could result in suboptimal medical intervention. In all potential radiation exposure scenarios, it is unlikely that physical dosimeters will be available for dose assessment to aid clinical management of mass casualties. For early treatment of radiation victims, it is recommended that medical personnel rely heavily on clinical signs and biological dose assessments [Goans 1997]. However, early dose estimates may be required in radiation disasters that involve a large number of victims and a finite amount of medical resources available to responders and healthcare providers.

The focus of present research is based on the following factors.

- Radiation dose to the exposed individual is the primary determinant of the nature, onset, severity, and duration of acute radiation syndrome (ARS).
- Early diagnostic estimation of the absorbed dose is essential for effective clinical management.
- Medical personnel rely heavily on clinical signs and biological assessments of radiation exposure for clinical treatment.
- With appropriate medical and intensive care, the likelihood of near term survival can be increased significantly.

In all potential radiation disasters, a single population is likely to encounter a number of complex radiation exposure scenarios, including different dose ranges and dose rates. Therefore, a single biodosimetry assay

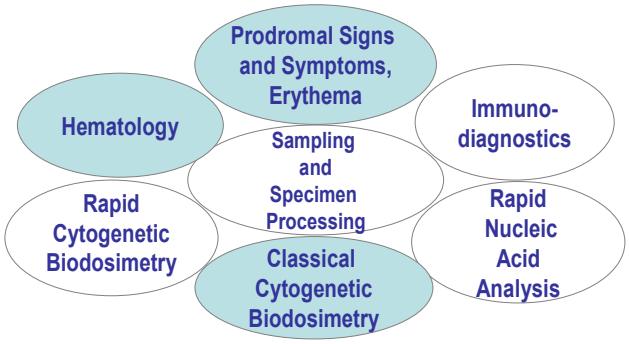
cannot fully address the biodosimetry requirement in complex exposure scenarios. Triage, clinical, and definitive radiation biodosimetry will all require multiple bioassays. Table 1, adapted from AFRRI's Medical Management of Radiological Casualties Handbook [AFRRI 2003], suggests validated biodosimetry methods for different dose ranges, expected prodromal effects, manifest symptoms, and survival expectancies.

**Table 1: Proposed validated biodosimetry methods for different dose ranges, expected prodromal effects, manifest symptoms, and survival expectancies.**

| Dose range (Gy) | Proposed validated biodosimetry methods | Prodromal effects | Manifest symptoms | Survival expectancy |
|---|---|---|---|---|
| 0.1 – 1.0 | Dicentric/premature chromosome condensation (PCC) | None to mild (from 3 h to 48 h) | None to slight decrease in blood count | Almost certain |
| 1.0 – 3.5 | Lymphocyte depletion kinetics/dicentric/PCC | Mild to moderate (from 1 h to 48 h) | Mild to severe bone marrow damage | 0 to 10% death |
| 3.5 – 7.5 | Lymphocyte depletion kinetics/PCC | Severe (from 1 h to 48 h) | Pancytopenia, mild to moderate GI damage | 10 to 100% death (within 2 to 6 weeks) |
| 7.5 – 10.0 | Lymphocyte depletion kinetics/PCC | Severe (from <1 h to 48 h) | Combined BM and GI damage | 90 to 100 % death (within 1 – 3 weeks) |
| >10.0 | PCC | Severe (from minutes to <48 h) | GI, neurological and cardiovascular damage | 100% death (within 2 to 12 days) |

At AFRRI, we are developing a multifaceted and integrated biodosimetry system to fully address the need for triage, based on early physical assessments, bioindicators, and biological assessments, in order to aid clinical management of radiation accident victims (Figure 1). This system will help differentiate between exposed and nonexposed but concerned individuals. Our long-range goal is to develop and integrate a battery of validated radiation bioassays to equip medical personnel with diagnostic information (clinical signs and symptoms, physical and biological dosimetry, etc.) germane to the medical management of human radiation casualties. Our specific objectives are to (a) establish definitive, rapid, high-throughput clinical bioassays for radiation dose assessments; (b) develop, for triage purposes, complementary triage-type radiation dose assessment bioassays, such as molecular-biology based forward-deployable diagnostic platforms; and (c) transfer the Biodosimetry Assessment Tool (BAT) software program and complementary tools to healthcare professionals to facilitate the collection, integration, and archival of relevant biodosimetry information.

**Figure 1. Illustration of an integrated and multiparameter diagnostic biodosimetry system.**




# 2.0 DEFINITIVE RAPID, HIGH-THROUGHPUT CLINICAL BIOASSAYS

## 2.1 The Dicentric Assay

Radiation exposure induces many types of chromosomal aberrations in the exposed individual's peripheral blood lymphocytes. The presence of dicentrics, a chromosomal structural aberration, in an individual's peripheral blood lymphocytes indicates radiation exposure. Dicentrics are considered relatively radiation specific; only a few chemicals are known to interfere with the assay. Low background levels (about 1 dicentric in 2000 cells), high sensitivity (a threshold dose of 0.05 Gy), and known dose dependency of up to 5 Gy (for photons) make this assay robust and a "gold standard" biodosimetry method. This cytogenetic chromosome aberration bioassay is a thoroughly investigated biodosimetry method. The dicentric assay is conventionally used to provide definitive radiation dose assessment. Because exposure of human peripheral blood lymphocytes (HPBL) *in vitro* and *in vivo* produces similar levels of dicentrics per unit dose, dose estimates to an exposed individual can be made by comparing the observed frequencies of dicentrics to an *in vitro* generated dose-effect calibration curve [IAEA 2001].

### 2.1.1 Reference Cytogenetic Biodosimetry Laboratory

AFRRI supports the U.S. Department of Defense's medical readiness by providing a limited cytogenetic biodosimetry service capability for radiation dose assessment conforming to international guidelines following the establishment of "a reach-back" cytogenetic biodosimetry laboratory. Blood samples (10 to 15 ml) are collected from the exposed individuals as soon as practical, generally 1-day after exposure, and are transported to the laboratory where lymphocytes are isolated from whole blood and stimulated to grow in culture, metaphase spreads are harvested, and chromosome aberration analyses are performed using internationally accepted laboratory protocols [IAEA 2001].

The blood collection procedure for cytogenetic biodosimetry is described in AFRRI's Medical Management of Radiological Casualties Handbook [AFRRI 2003]. This medical management doctrine can be downloaded from AFRRI's website, www.afrri.usuhs.mil. Since 2000, AFRRI's cytogenetic laboratory has analyzed more than 15 cases from radiation incidents and accidents, using dose-response calibration curves in HPBL [Prasanna 2002a] by dicentric assay, and 12 cases from a radiological accident that occurred in Thailand in February 2002 using the Rapid Interphase Chromosome Aberration (RICA) assay [Boreham personal communication].

### 2.1.2 International Efforts on Cytogenetic Biodosimetry Method Harmonization

The biodosimetry scientific community realized the need to harmonize the cytogenetic dosimetric methodology because there is no universally adopted laboratory protocol and important variations occur between the laboratories, often influencing the quality of results. AFRRI scientists are involved in these international efforts to harmonize the biodosimetry cytogenetic methods to address this problem. The International Atomic Energy Agency (IAEA) published a technical manual, involving the efforts of AFRRI scientists, on cytogenetic biodosimetry that provides a harmonized methodology for various cytogenetic assays. This manual [IAEA 2001] provides information necessary for selecting and implementing, in a standardized manner, the appropriate cytogenetic method to ensure accurate dose assessments following an accidental exposure to ionizing radiation.

An International Organization for Standardization (ISO) working group, comprised of 13 scientists from 11 countries and an IAEA representative, was established to standardize biological dosimetry by cytogenetics. Under the auspices of the ISO, regulatory compliance and validation efforts are being made for the dicentric assay. The scope and structure of the working draft, ISO TC-85/SC-2, Radiation Protection – Performance Criteria for Service Laboratories Performing Biological Dosimetry, provides the guidelines for conducting the biological dosimetry by cytogenetics [Voisin 2002]. The "reach-back" cytogenetic biodosimetry laboratory in AFRRI is implementing good laboratory procedures (GLP) for quality control and quality assurance.

## 2.2 Premature Chromosome Condensation (PCC) Assay

Conventional metaphase-spread chromosome-aberration biodosimetry techniques are robust, but they are laborious, time-consuming, and, more importantly, require an *in vitro* stimulation of resting HPBL to cause proliferation. For potential high-dose irradiation above the median lethal dose, such as in a radiation disaster, it is expected that radiation-induced cell death and delay in cell cycle progression into mitosis will interfere with dose estimation [Prasanna 2002b]. In addition, high-dose radiation accident victims will also suffer from lymphopenia; therefore, few cells will be available for cytogenetic studies. In order to overcome this limitation, quantitative analysis of radiation-induced damage may be performed using resting HPBL in lieu of metaphase spreads. Use of interphase cytological assays, such as the PCC assay, could eliminate these inherent problems associated with the use of metaphase-spread cytogenetic assays. The PCC assay is useful to determine exposure to low doses as well as to life-threatening acute high doses of low-LET (linear energy transfer) [Prasanna 1997, 2000] and high-LET radiation [Prasanna 1997]. Moreover the PCC assay can discriminate between total- and partial-body exposures [Blakely 1995].

It was shown that PCC can be induced in resting HPBL through signal transduction mechanisms by a simple incubation of cells in a culture medium containing a protein phosphatase inhibitor, okadaic acid, mitosis-promoting factor $p34^{cdc2}$/cyclin B kinase [Prasanna 2000]. The interphase-based rapid interphase chromosome aberration (RICA) assay, is a simple alternative to the metaphase-spread based dicentric assay. In RICA assay, damage involving specific chromosomes is analyzed in chemically induced PCC spreads after fluorescence *in situ* hybridization (FISH) with specific whole-chromosome DNA hybridization probes (Prasanna and Blakely, international patent pending). In the RICA assay, the cells that display two chromosome spots are considered normal and cells with more than two chromosome spots are considered aberrant. The frequency of aberrant cells [Prasanna 2000] and the number of aberrations per cell [Prasanna 2002b] are shown to increase with radiation dose over a broad dose range encompassing those well above the median lethal dose. This invention in cytogenetics has wide applications across biotechnology and biomedical fields.

Recently, the RICA assay was used to assess chromosome damage in individuals accidentally exposed to gamma radiation in Samutprakarn, Thailand, in studies designed to validate this method for assessing radiation dose to human subjects. This study used a cohort of several individuals accidentally exposed to gamma rays from an unshielded $^{60}$Co source; they received acute, chronic, or fractionated exposures. The frequencies of aberrant chromosome 1 showed a good correlation with clinical symptoms of acute radiation syndrome: mainly nausea, vomiting, severe headache, fever, and depletion in white blood cell counts [Boreham submitted].

## 2.3 Cytogenetics in Mass Casualty Scenarios

Cytogenetic biological dosimetry can also make valuable contributions to the medical management of patients in the early period after a radiation disaster, where a rapid confirmation of dose is required. At such a time, all that is needed is a rapid triage of casualties, based on approximate dose estimation using biological and clinical endpoints, rather than precise dose estimations for a vast number of individuals. Recently, a consensus document generated by the Dosimetry Sub Panel of the Radiological/Nuclear Threat Countermeasures Work Group of the Office of Science and Technology Policy (OSTP, U.S. Homeland Security Council) recommended that sufficient supplies for cytogenetic biodosimetry procedures be available in a national stockpile for emergency radiation disaster management. AFRRI scientists contributed to the document.

The utility of cytogenetic assays to assess health risks and to guide medical treatment decisions was demonstrated in several radiation accidents involving mass casualties, such as those referred to as Chernobyl, Goiania, and Tokaimura. Table 2 summarizes the information on the use of cytogenetic methods in radiation accidents. Estimated doses using cytogenetic methods correlate well with the severity of acute radiation syndrome [Sevan'kaev 2000]. In the Chernobyl, Russia, accident, an approximate dosimetry was achieved by rapid preliminary examination of 50 lymphocyte metaphases per person for several individuals [Pyatkin 1989]. Ramalho [1991] investigated 129 exposed or potentially exposed individuals from the Goiania, Brazil, accident cohort immediately after the radiological emergency. Dose estimates exceeded 1 Gy for 21 subjects from this cohort and 4 Gy for 8 individuals. More recently, dose estimation was done using the dicentric and PCC assays in the Tokaimura, Japan, criticality accident in 3 severely exposed workers [Kanda 2002, Hayata 2001] and 43 resident workers [Sasaki 2001]. These radiation accidents highlight the importance of the cytogenetic methods in early dose assessment after a radiological event and demonstrate their ability to influence medical treatment decisions.

Recently, it was suggested that the dicentric assay could be adapted for the triage of mass casualties [Lloyd 2000, Voisin 2001, Prasanna 2003]. Lloyd [2000] described an *in vivo* simulation of an accident with mass casualties receiving whole- or partial-body irradiation in the 0- to 8-Gy range. Faced with an urgent need for rapid results, clinical triage was accomplished by scoring as low as 20 metaphase spreads per subject, compared with the typical 500 to 1000 spreads scored in routine analyses for estimating dose. However, Lloyd [2000] suggested increasing the analyses to 50 metaphase spreads when there is disagreement with the initial assessment or when there is evidence of significant inhomogeneous exposure. After the initial results are communicated to the treating physician, additional scoring is recommended to resolve potential conflicts in dose assessment and, in the case of high doses, to assist physicians considering marrow-stem-cell transfusions to mitigate bone marrow ablation. Using the dicentric assay in this triage mode, a reasonable throughput of 500 or more samples per week per laboratory is achievable [Prasanna 2003].

**Table 2. Cytogenetic assays in radiation mass casualties.**

| Accident location | Year of accident | Number of people exposed | Number of samples analyzed using cytogenetic methods | | Reference |
|---|---|---|---|---|---|
| | | | Dicentrics | PCC*# | |
| Chernobyl, Russia | 1986 | ~ 116,000 | 158 | NA | Sevan'kaev [2000] |
| Goiania, Brazil | 1987 | ~ 250 | 129 | NA | Ramalho [1991] |
| Tokaimura, Japan | 1999 | 3**<br>Unknown*** | 1**<br>43*** | 3** | Kanda [2002]<br>Hayata [2001]<br>Sasaki [2001] |

*#Premature chromosome condensation assay, **criticality exposed workers, ***resident and other workers, #the PCC assay permits dose assessment above 4 Gy.

### 2.3.1 Cytogenetic Laboratory Automation and High-Throughput Analysis

The use of commercially available off-the-shelf instruments, such as liquid-handling robotic devices, automated metaphase finders, and multiple satellite chromosome aberration analysis stations, is recommended for triaging radiation mass casualties for a rapid turnover of results. Efforts are under way at AFRRI to automate, for military and civilian applications, assays based on chromosome damage. The automated cytogenetic biodosimetry process is outlined in Table 3. The throughput of the cytogenetic laboratory can be increased by using liquid-handling robotic devices for handling blood and for isolating lymphocytes, by using microprocessor-controlled multi-pipettes for transferring reagents, by using automated metaphase harvesters and spreaders with modules for 20 to 50 slides for simultaneous staining, and by using automated instruments for DNA hybridization of centromere/whole-chromosome-specific probes and immunoenzymatic chromosome painting. These methods will also ensure quality control and quality assurance under the good laboratory practice environment for conducting assays.

Specialized cytogenetic laboratories ususally rely on automated metaphase-finder systems for locating suitable chromosome spreads for analysis [Lloyd 1990, Prasanna 2002a]. A typical metaphase finder consists of a standard binocular microscope equipped with a stage that accommodates a few hundred slides and motorized x- y- and z-axis computer-controlled positioning with specially adapted autofocus capabilities. The system includes specialized software utilities (Loats Associates Inc., Westminster, MD) that permit user control of image recognition parameters and relocation of metaphase spreads on the metaphase finder as well as multiple satellite chromosome aberration analysis stations for rapid analyses by multiple scorers to increase throughput.

**Table 3. Components of automated cytogenetic radiation bioassay.**

| | |
|---|---|
| Bar-coding and tracking of reagents, sample processing, scoring, and analysis | Metaphase spreader |
| Automated blood lymphocyte isolation system | Automated slide stainer and cover-slipping |
| Automated blood lymphocyte culturing system | Automated microscope - metaphase finder |
| Automated metaphase harvester | Satellite microscope scoring system |
| Microscope slide washing device | Automated chromosome aberration scoring system |

# 3.0 APPLICATION OF MOLECULAR BIOMARKERS

Analyzing molecular biomarker responses to radiation exposure is a novel approach that can complement conventional chromosome-aberration assays and may significantly enhance biological dose assessments. Still in its infancy as a scientific discipline, radiation-responsive molecular biomarkers include proteins, gene expression, and DNA mutations. At AFRRI, we initiated studies to identify, evaluate, and validate molecular biomarkers that may provide diagnostic information for acute and prior radiation exposures. We are also exploring high-capacity, real-time detection technologies for nucleic acids or protein biomarkers. This technology would be useful in radiation accidents for forward-field dose assessment.

## 3.1 Radiation-Responsive Molecular Biomarkers

Recent technological advances in genomics and proteomics have contributed to the discovery of a plethora of radiation-responsive biomarkers. A few highly overexpressing sentinel radiation-responsive targets have been identified from an array of distinct gene expression profile responses [Amundson 2001]. Hofmann and colleagues [1990] reported radiation-induced increases of serum amylase in 41 patients, following either whole-body irradiation or irradiation of the head and neck regions. Becciolini and colleagues [2001] recently advocated the use of biochemical (e.g., serum amylase and tissue polypeptide antigen) dosimetry for prolonged spaceflights.

At AFRRI, using an *in vitro* model system of HPBL, we identified a candidate nucleic acid biomarker (i.e., gene expression target) responsive to ionizing radiation. A dose-dependent elevation in Ha*ras* gene expression levels was demonstrated using Northern blot analysis 17 h after exposure to 250-kVp x rays (25 to 100 cGy; 1 Gy/min) [Blakely 2002a, Miller 2002]. We also studied the effect of interindividual variation in Ha*ras* expression in healthy human donors. HPBL were given *in vitro* to 0- and 75-cGy $^{60}$Co gamma rays (25 cGy/min dose rate) and incubated at 37 °C for 17 h after irradiation. Among the 11 donors, the control levels of Ha*ras* expression, relative to β–actin levels, were consistently low relative to a nearly uniform ≅ six-fold increase after a 75-cGy dose [Blakely 2002b]. Similar proto-oncogene studies, using a murine model system [Blakely 2003a, Miller 2002], demonstrated radiation-responsive gene expression targets after *in vivo* exposure.

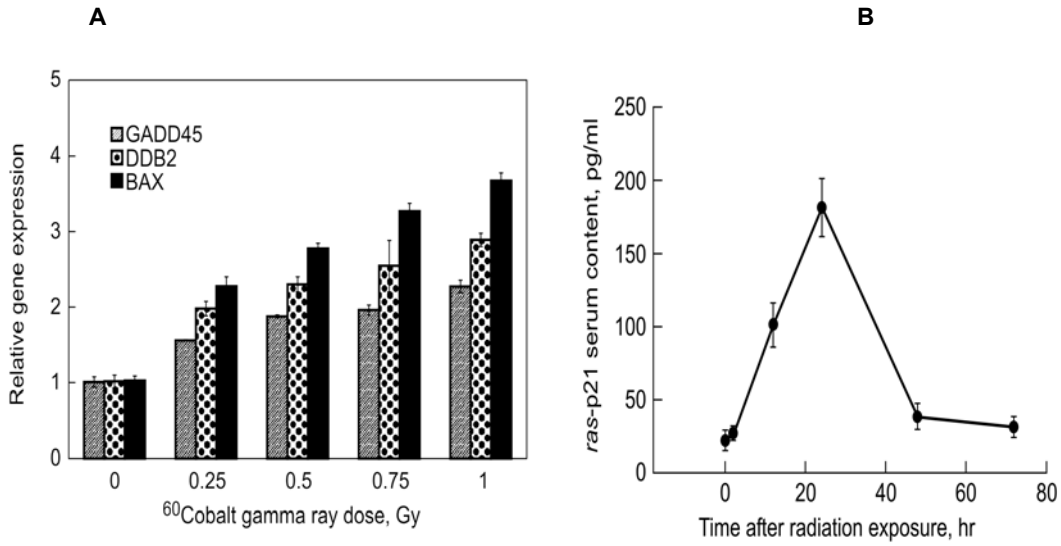
Use of a multiplex fluorogenic PCR analysis system is likely to improve the diagnostic utility for radiation dose assessment because the results are obtained rapidly. Protocols for detecting radiation-responsive gene expression, proto-oncogene Ha*ras* [Blakely 2002a; 2002b], and DNA repair gene GADD45 [Grace 2002,

Grace 2003] targets are developed using the real-time polymerase chain reaction (RT-PCR) assay. Identification and quantification of radiation-specific gene expression requires the measurement of multiple gene-expression targets. This will also increase sensitivity. Toward this goal, primers and Taqman probes are developed to evaluate additional candidate radiation-responsive targets (GADD45, DDB2, BAX). Figure 2, chart A shows the results of radiation response 24 h after 0 to 1 Gy [Blakely 2003a, Grace 2002].

An *in vivo* irradiation murine model system was further used to characterize candidate radiation-responsive *ras*-p21 proto-oncogene protein changes in blood. Similar to our *in vitro* data [Blakely 2003b], 25-cGy gamma rays resulted in detectable serum levels of *ras*-p21. An initial progressive increase and peak value of *ras*-p21 protein were observed at 24 h after irradiation, followed by a decline to control values at 48 and 72 h (Figure 2, chart B).

While nucleic acid and protein biomarkers appear to be of potential diagnostic utility, significant additional validation and assay optimization research are required. Still to be investigated are the effects of confounding factors such as the influence of radiation quality, the persistence of the endpoints after exposure, hyperthermia, and dose assessment methods in partial-body exposures.

**Figure 2. Illustration of the responses of various biological indicators following exposure to ionizing radiation. Panel A. Dose-responses of multiple gene expression targets (GADD45, DDB2, and BAX) by multiplex real-time RT-PCR assay. The x-axis illustrates the reported nominal doses ranging from 0 to 1 Gy (dose rate, 0.1 Gy/min). The data were obtained at 24 h after irradiation [Blakely 2003a, Grace 2002]. Panel B. Radiation–responsive changes in the expression of *ras*-p21 in blood serum of 25 cGy irradiated rodents [Blakely 2003a].**




## 3.2 Analytical Platforms for Measurement of Molecular Biomarkers

The ability to assess individual radiation exposures in a forward field will immensely help military operational and medical units. Recent rapid advances in nucleic acid biomarker analysis systems have made forward-field individual dose assessment possible. For example, new gene chip technology makes it possible to rapidly

monitor changes in both gene and protein expression. Similarly, real-time rapid fluorogenic PCR assay systems are commercially available as research and clinical laboratory systems. Military operations rely on molecular biology analysis platforms like those in reference laboratories for "reach-back" evaluations (e.g., samples transported from a forward field to a clinical, service, or research laboratory).

At AFRRI, we have initiated studies to optimize protocols and analytical systems for rapid measurement of radiation-responsive molecular biomarkers. A quadruplex and quantitative reverse transcriptase – PCR assay was developed using a 96-well, closed-plate format suitable for extracted RNA from whole blood [Grace 2003]. The simultaneous measurement of four amplicons in a single reaction using a closed-plate format provides significant cost and labor savings. Progress has also been made to rapidly detect blood serum protein biomarkers using a microsphere multi-analyte assay system, Luminex™-100 [Muderhwa 2003]. This technology is based on microscopic spherical polystyrol particles that serve as a solid phase for molecular detection reactions measured using a flow cytometer equipped with a 96-well microtiter plate platform. The system allows simultaneous, multiple detection reactions in very small sample volumes.

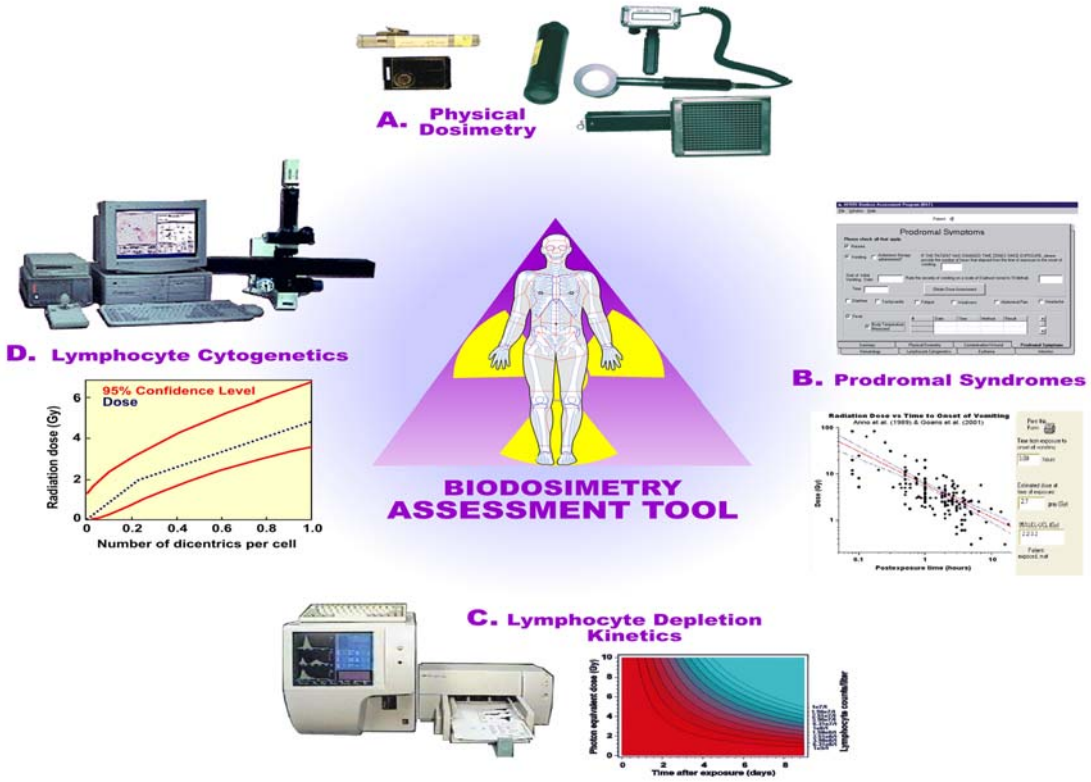
Deployable configurations of fluorogenic PCR assay systems have been used in field military operation units for pathogen detection [Belgrader 1998]. Significant efforts are under way to further miniaturize diagnostic equipment for nucleic acid sequence and antigen-based biosensor detection technologies [Blakely 2003b]. Dual-use applications of these and other diagnostic systems for radiation exposure assessment are essential to conserve the footprint required to equip forward deployable military laboratories and first responders.

## 4.0 BIODOSIMETRY ASSESSMENT TOOL SOFTWARE AND HEMATOLOGY

A U.S. Army-specific military requirement defines the need for a postexposure biodosimetry assessment tool to facilitate triage in the field, specifying that data collected should be in digital form for efficient transfer and availability to medical planners on the operational unit staff. The proposed STANAG 2474, NBC/MED of NATO, "***Determination and Recording of Ionizing Radiation Exposure for Medical Purposes,***" discusses the requirement for determining and recording of ionizing radiation exposure for medical purposes. The AFRRI Biological Dosimetry Team developed and released on the AFRRI website (www.afrri.usuhs.mil) the radiation casualty management software application BAT. This software is also suitable for civilian use [Sine 2001]. BAT is designed (a) to promote rapid collection of data for early use following a radiation exposure incident, (b) to provide diagnostic information and therapeutic guidance to manage radiation casualties, (c) to record related clinical information (e.g., extent of contamination, wounds, infection) necessary for proper medical care of radiation casualties, and (d) to archive collected data for later use. BAT, designed primarily for use within hours to days after a radiation disaster or exposure of personnel in a radiation environment, equips healthcare providers with diagnostic information, obtained via various modules, as illustrated in Fig. 3, that are germane to the management of human radiation casualties.

The guidance for collecting this diagnostic information is obtained primarily from preliminary physical dosimetry (Fig. 3 A) at a disaster site or after the discovery of the presence of personnel in a radiation environment and is secondarily aided by prodromal signs and symptoms (Fig. 3 B), serial lymphocyte counts (Fig. 3 C), and triage lymphocyte cytogenetics (Fig. 3 D). Detecting the presence of a radiological environment via physical dosimetry, where a population is exposed, may require a multiparametric approach and the use of several standard technologies. Examples of these include radiation detection meters that provide both dose and dose-rate information, portable meters that detect the presence of radioactive contamination, and self-reading personnel dosimeters. BAT provides structured templates to record this physical dosimetry information useful in the medical management of radiation casualties and in helping to rapidly triage individuals.

**Figure 3. Centrality of Biodosimetry Assessment Tool (BAT) for biological dose assessment in radiation disasters for casualty management.**




Similarly, BAT allows the recording of prodromal signs, symptoms, and erythema (Fig. 3B). Typical human symptoms in reaction to prompt total-body ionizing irradiation in the dose range of 0.5 to 30 Gy have been described by Anno [1989]. Recording of these radiation-induced signs and symptoms (e.g., nausea, vomiting, headache, and fever) before and during the course of medical management of radiation casualties will help triage and guide available treatment options.

Exposure to radiation causes a predictable depletion in lymphocytes in a time- and dose-dependent manner (Fig. 3 C). Deployable small foot-print point-of-care blood cell counters allow the medical professional to obtain on-site serial lymphocyte numbers and to determine lymphocyte depletion kinetics. A preliminary estimate of radiation dose in the region between 1 and 10 Gy (photon equivalent) can be obtained by this method. The contour of lymphocyte depletion kinetics following photon radiation exposure in human beings is shown in Fig. 3 C. Normal lymphocyte numbers in healthy individuals ranges between 1500/mm$^3$ and 3500/mm$^3$. Radiation doses as low as 0.2 Gy cause lymphocyte death in interphase, resulting in a severe depletion in the absolute count. For example, 1200 to 1500 lymphocytes/mm$^3$ at 24 h after irradiation may indicate a potentially lethal dose [Goans 1997]. However, due to the transient nature of radiation-induced lymphocyte depletion, the usefulness of this biodosimeter is limited to a few days (< 10) postexposure. Serial lymphocyte counts can be recorded in BAT program to convert them into dose predictions, using a lymphocyte depletion kinetic model based on previous dose responses in radiation accidents [Goans 1997].

The usefulness of lymphocyte cytogenetics and laboratory automation is discussed in section 2.3. In the triage dose prediction model for the dicentric assay, which used a calibration curve for $^{60}$Co gamma radiation, a predictable radiation dose along with 95% confidence intervals, obtained by analyzing 50 metaphase spreads, plotted as a function of varying numbers of dicentrics in an exposed individual's blood in Fig. 3 D. With this dose prediction model, one can triage a large number of potentially irradiated individuals for radiation doses. However, this definitive and diagnostic individual biodosimetry is performed in a "reach back" cytogenetic laboratory.

## 5.0 CONCLUSIONS

We are developing and validating simple, rapid, and automated biodosimetry solutions for radiation dose assessment for military and civilian applications. An integration of multifaceted physical, hematological, cytogenetic, and molecular biodosimetry solutions is quintessential to determining immediate health risks to military personnel as well as civilian populations after a radiation disaster or the discovery of the presence of personnel in a radiation environment. BAT is central to AFRRI's multifaceted, multiparametric, and integrated biodosimetry system that adequately and fully addresses the need for triage of radiation casualties and aids in effective clinical management of potentially or actually exposed individuals.

Toward those ends, AFRRI has addressed the following tasks.

- We have established a "reach-back" cytogenetic biodosimetry reference laboratory that uses conventional bioassays as well as dicentric and premature chromosome condensation assays for provide definitive analyses of biological samples.
- We are developing and validating forward-deployable, biological dosimetry assays for rapid radiation dose assessment, with an emphasis on the use of diagnostic platforms based on molecular biology.
- We are integrating the biodosimetry data in a suitable software platform (i.e., BAT) to assist in the medical management of radiation casualties.

## ACKNOWLEDGMENTS

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# SYMPOSIA DISCUSSION - PAPER 24

**Authors Name: Dr Prasanna (US)**

**Discussor's Name: Dr Rios-Tejada (SP)**

**Question:**
Is there relevance of biodosimetry in air crews who fly high and at high latitudes?

**Author's Reply:**
The application is evident and they are pursuing effort in that environment. (Relevant application to Commercial Aviation and Military Aviation in certain circumstances).