

Building Connecticut's clinical biodosimetry laboratory surge capacity to mitigate the health consequences of radiological and nuclear disasters: A collaborative approach between the state biodosimetry laboratory and Connecticut's medical infrastructure

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Abstract

Biodosimetry, based on the analysis of dicentric chromosomes in circulating mononuclear cells, is considered the “gold standard” for estimating radiation dose and is used to make informed decisions regarding the medical management of irradiated persons. This paper describes the development of biodosimetry laboratory surge capacity for the health consequences of radiological and nuclear disasters in Connecticut, including: (1) establishment of the Biodosimetry Laboratory for the timely assessment of radiation dosage in biodosimetry specimens; (2) identification of clinical laboratories qualified and willing to process biodosimetry specimens from a large number of victims; (3) training of clinical laboratorians in initial biodosimetry specimen processing; and (4) conducting a functional drill that evaluated the effectiveness of these elements. Descriptive information was obtained from: (1) personal observations; (2) a needs assessment of clinical laboratories in Connecticut; (3) records from a training program of clinical laboratorians in biodosimetry specimen processing that was developed and provided by the Yale New Haven Center for Emergency Preparedness and Disaster Response; and (4) records from a statewide functional drill in biodosimetry specimen processing that was developed and conducted by the State of Connecticut Biodosimetry Laboratory. A needs assessment of clinical laboratories in Connecticut identified 30 of 32 clinical laboratories qualified and willing to perform initial biodosimetry specimen processing. Currently, 79 clinical laboratorians in 19 of these qualified clinical laboratories have been trained in biodosimetry specimen processing. A functional exercise was conducted involving 37 of these trained clinical laboratorians in 18 qualified laboratories as well as the Biodosimetry Laboratory. The average turnaround time for biodosimetry specimen processing in this drill was 199 min. Exercise participants provided feedback which will be used to further optimize biodosimetry specimen processing protocols in Connecticut. Based on our findings, we conclude that clinical laboratory professionals are an important resource for assisting with the processing biodosimetry specimens that are used for triage of patients from accidental or terrorist-related mass-casualty radiological or nuclear catastrophies. The approach described in this paper to enroll and train clinical laboratorians in sample preparation for dicentric analysis forms the basis for the next step (namely, further training on harvesting cultured cells and preparing cytogenetic slides) in collaborative efforts between the State of Connecticut's Biodosimetry Laboratory and the state's medical infrastructure towards building laboratory surge capacity to estimate radiation dose in victims of a mass casualty event. Published by Elsevier Ltd.

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1. Introduction

The rising use of radiological materials and radiation equipment in academic, clinical and industrial settings as well as the potential for radiological terrorism has escalated the potential risk of accidental and intentional radiation exposure. Recent efforts of governmental and private sector organizations have focused on increasing awareness of the potential for a large-scale radiological casualty incident (NCRP Report No. 138, 2001; Centers for Disease Control and Prevention, 2003; NCRP Commentary No. 19, 2005). Recommendations for management of the medical consequences from such an event have been made as the health consequences are potentially devastating (Dainiak et al., 2003, 2006; Waselenko et al., 2004; Weisdorf et al., 2006).

It has been suggested that effective medical management of irradiated individuals is contingent upon prompt and definitive estimation of radiation dose (Moulder, 2004; Blakely et al., 2005; Weisdorf et al., 2006). Currently, dicentric analysis, based on the presence and frequency of dicentric chromosomes in metaphases from peripheral blood mononuclear cells, represents the gold standard for radiation dose assessment. The procedure originally described by Bender and Gooch (1996) typically involves isolation of mononuclear cells from whole blood samples, incubation of isolated cells for two days in a tissue culture incubator, preparation of metaphase slides and analysis of chromosomal aberrations (IAEA, 1986). While the dicentric assay is a robust and relatively sensitive (limits of detection ranging between 5 and 10 Rad) methodology for estimating radiation dose, the process is time-consuming, labor-intensive and restricted in sample throughput.

The State of Connecticut's Biodosimetry Laboratory, located at Bridgeport Hospital, was established in 2003 as a result of a collaborative effort between the Yale New Haven Center for Emergency Preparedness and Disaster Response and the Connecticut Department of Public Health. Its role in emergency preparedness is to provide timely radiation dose estimates, based on cytogenetic analysis, in the event of a radiological or nuclear incident, in order to predict the clinical severity, treatment and survivability of exposed individuals and triage those with minimal or no exposure (Waselenko et al., 2004). Large-scale events involving radiological or nuclear material will engender a large number of samples and quickly overwhelm the State of Connecticut Biodosimetry Laboratory's capacity to prepare and process the specimens in a timely fashion. Consequently, there is a need to identify and train qualified laboratory personnel who can assist the Biodosimetry Laboratory with biodosimetry sample preparation.

Here, we describe Connecticut's ongoing efforts to establish a laboratory surge capacity for the purpose of providing timely assessments of absorbed radiation dose in a mass-casualty radiological event by providing education and training to clinical laboratory professionals. We conclude that while the State of Connecticut's Biodosimetry Laboratory will have to handle the major portion of the work in processing and analyzing samples, hospital and commercial clinical laboratory professionals are a valuable resource for the purpose of ini-

tiating biodosimetry specimen preparation in the event of a large-scale radiological or nuclear disaster. Equally important is the observation that an awareness of the negligible health consequences of manipulating samples from radiation-exposed individuals significantly reduced apprehensions of working with these samples and prompted participants to volunteer for more advanced stages of sample preparation.

2. Methods

2.1. Needs assessment of clinical laboratories in Connecticut

A survey was developed and distributed to the laboratory directors of 32 acute care hospitals and one laboratory director of a commercial diagnostic laboratory in Connecticut. The survey focused on identifying qualified laboratories and clinical laboratory personnel for the purpose of initiating preparation of biodosimetric samples, i.e., isolation of peripheral blood mononuclear cells from samples of whole blood. A laboratory and its staff were deemed qualified if: (1) the laboratory was equipped with a table-top centrifuge, a biological cabinet (or laminar flow hood) in which to prepare samples aseptically and a tissue culture incubator with a 5% CO₂ atmosphere to store isolated mononuclear cells; and (2) laboratorians were willing to voluntarily assist the Biodosimetry Laboratory in preparation of samples.

2.2. Education and training of laboratory professionals

A 1.5-h hands-on training program was developed and delivered to each clinical laboratory that met the inclusion criteria. The program was designed to provide practical training for harvesting and culturing mononuclear blood cells from 4–6 ml of whole blood collected into BD vacutainer CPT cell preparation tubes (Cat No. 362753; Becton Dickinson, Franklin Lakes, NJ). The procedure was performed according to the manufacturer's instructions.

Prior to starting the hands-on training, the participants were provided with background information on basic radiation principles, separation of mononuclear cells from whole blood samples and dicentric analysis and its application to radiation dose assessment.

2.3. Biodosimetry functional exercise

Six months after the education and training program was delivered to clinical laboratory personnel, a notification letter was mailed to the directors of participating laboratories asking for their laboratory participation in a functional exercise. Participation in the exercise was not mandatory but recommended. The objectives of the exercise were to: (1) assess skills of trained participants to isolate and culture mononuclear cells from whole blood samples; (2) determine turnaround times; and (3) assess communication between participants and Biodosimetry Laboratory personnel.

On the day of the exercise, Biodosimetry Laboratory personnel delivered biodosimetry supplies and reagents, consisting

of CPT vacutainer tubes, phosphate-buffered saline solution, PB-Max karyotyping Medium (Cat No. 12557021; Invitrogen Corp., Carlsbad, CA), sterile transfer pipettes and T-25 tissue culture flasks, to participating laboratories. Each laboratorian was required to process, at minimum, two samples drawn from volunteers in their facility. Flasks were labeled with the facility's name and a sample number (e.g., sample 1, sample 2, etc.); the identity of sample donors and laboratory participants were not disclosed to the Biodosimetry Laboratory personnel. Turnaround time was documented as the time between receipt of the biodosimetry kit and the time when participants communicated (required to meet objective 3) to the Biodosimetry Laboratory personnel that samples had been processed and were ready for pickup. The samples were collected from the participating laboratories and delivered to the Biodosimetry Laboratory on the day of the exercise or the day after the exercise.

Samples were monitored for evidence of contamination by microscopic evaluation 48 h following incubation. At this time, cell viability was assessed by Trypan Blue dye exclusion and the samples discarded according to Bridgeport Hospital regulations for disposal of biohazardous materials.

3. Results

3.1. Identification of qualified laboratory professionals

Initial survey results revealed that 30 of 33 responding clinical laboratories were equipped with the necessary instruments, i.e., table-top centrifuge, laminar flow hood or biological cabinet and a tissue culture incubator with a 5% CO₂ atmosphere, to isolate mononuclear cells from whole blood samples. Of these, however, only two respondents indicated an interest in assisting the State of Connecticut's Department of Public Health (CT DPH) to prepare specimens for biodosimetric analysis. This was true despite the fact that 32 of these laboratories had volunteered their services to assist CT DPH in processing samples for biological and chemical events.

A follow-up questionnaire to determine the cause for the apparent lack of interest in assisting with radiological and nuclear events was mailed to laboratory directors. Approximately 85% of respondents (25/30) indicated that they were unable to assist with processing whole blood samples from potentially irradiated/contaminated patients because their "laboratory are not equipped to handle radioactive blood samples". Ten percent (3/30) indicated that their "laboratory personnel is not trained to isolate mononuclear cells from whole blood samples". Approximately 5% of respondents (2/30) indicated that their "laboratory could not spare the resources necessary to assist with processing of biodosimetry specimens."

3.2. Education and training of laboratory personnel

Results from the survey and questionnaire suggested that while most of the clinical laboratories throughout Connecticut are equipped with the required instrumentation to process samples for biodosimetric evaluation, there was a clear need to provide training regarding mononuclear cell isolation technique,

and moreso, to address safety concerns regarding the handling and processing of specimens from irradiated and/or contaminated individuals.

Accordingly, a 1.5-h hands-on training program (see Fig. 1) was developed and delivered to laboratory personnel of the 30 laboratories that had the required equipment to initiate processing samples for biodosimetric evaluation. Included in the training session was a 30-min presentation focusing on basic principles of radiation, the difference between irradiation and contamination (internal and external), routes of internal contamination and the basis of the dicentric assay. This presentation underscored the unlikelihood of significant levels of radioactive contamination in the blood of individuals contaminated with radioactive materials and the need to perform the isolation of mononuclear cells under sterile conditions.

Following the training, 22 out of 30 clinical laboratories volunteered their services to assist with the processing of samples for biodosimetry evaluation in the event of a large-scale radiological or nuclear disaster. To date, 79 clinical laboratory professionals have been trained to isolate and culture mononuclear cells from whole blood samples. These findings suggest that awareness level training regarding the collection and manipulations of whole blood specimens from potentially contaminated persons greatly reduced apprehension of working with such samples and highlight the importance of addressing safety concerns and providing information to alleviate the fear engendered by radiation or radioactive material to personnel with limited experience in working with biological samples collected from irradiated or contaminated patients.

3.3. Biodosimetry functional exercise

In order to assess the ability of trained laboratory professionals to isolate mononuclear cells and culture these cells under sterile conditions in a timely fashion, the Biodosimetry Laboratory personnel conducted a statewide functional exercise in which participants were required to harvest and culture mononuclear cells from whole blood samples (two samples/participant), as described in protocols provided by the Biodosimetry Laboratory during training sessions.

As shown in Table 1, forty seven percent (37 out of 79) of trained laboratory professionals in 18 of 19 clinical diagnostic laboratories participated in the functional exercise. The total number of samples returned to the Biodosimetry Laboratory was 76 (36 participants processed 2 samples; 1 participant processed 4 samples). The average turnaround time required to process whole blood samples was 199 min.

Following a two-day incubation period, viability assays revealed greater than 90% cell viability in all samples. No evidence of micro-organism contamination was apparent in these samples, as determined by microscopic evaluation.

4. Discussion

Although the dicentric assay is considered the gold standard as a measure of absorbed radiation dose, the methodology is arduous, time-consuming and affords limited sample throughput.

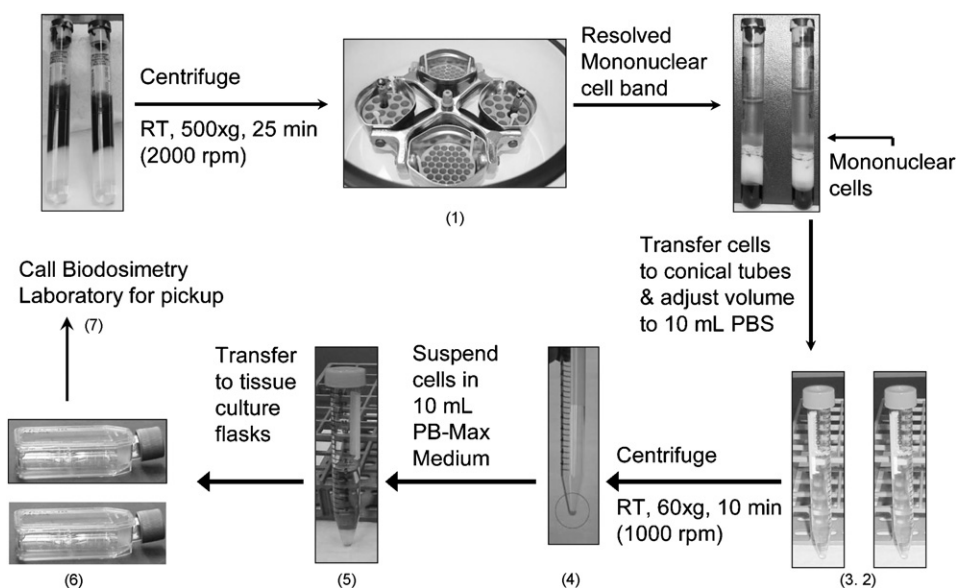


Fig. 1. Flow diagram of the hands-on training provided to volunteer clinical laboratory professionals. Volunteers in participating laboratories were trained to isolate mononuclear cells from whole blood samples collected in CPT vacutainer tubes as follows: Samples were centrifuged at room temperature (RT) (1), and the resolved mononuclear cell bands were collected and transferred to conical tubes (2); volume was adjusted to 10 ml with phosphate-buffered saline (PBS) (3), and the cell suspension was centrifuged at RT (4); cell pellet was resuspended in PB-Max Medium (5) and transferred into tissue culture flasks (6); participants were instructed to call the Biodosimetry Laboratory to request sample pickup (7). During the training sessions, the procedure was typically completed in 50–75 min.

Table 1
Summary results of biodosimetry functional exercise

Parameter tested	Result
No. of technologists participating in biodosimetry exercise (total number trained)	37 (79)
No. of labs participating in biodosimetry exercise (total number trained)	18 (19)
No. of samples returned to biodosimetry laboratory for analysis	76
Average turnaround time (time between kit delivery and call for sample pick-up)	199
No. of samples with > 90% lymphocyte viability	76
No. of contaminated samples	0

These characteristics restrict its applicability in a large-scale radiological scenario. In an effort to increase sample throughput, the State of Connecticut's Biodosimetry Laboratory recruited clinical diagnostic laboratory professionals from hospital and commercial diagnostic laboratories throughout Connecticut to assist with the initial preparation of samples for radiation dose determination. To date, 19 out of 22 laboratories which are qualified to assist with the preparation of samples for dicentric analysis and 79 clinical laboratory professionals have been trained to isolate mononuclear cells from whole blood samples.

Results of the recruitment process show that while delivery of technical training required for mononuclear cell isolation to potential laboratory volunteers is important, it is also critical to address safety concerns of the participants. In this case, emphasizing the improbability of significant radioactive contaminants in blood samples collected from potentially contaminated patients was sufficient to reassure volunteers.

Interestingly, prior to the training provided by the Biodosimetry Laboratory personnel, 90% of laboratorians (71/79) indi-

cated that they had limited experience with density gradient mononuclear cell isolation protocols. Findings that all samples processed during the functional exercise contained a significant fraction of viable cells (> 90% viability) and that none showed apparent contamination by micro-organisms indicate that clinical laboratory professionals possess the skills to become proficient in isolating mononuclear cells from whole blood samples.

Although mononuclear cell isolation using density gradient centrifugation, as described in the Methods section of the paper (also see Fig. 1), is not essential in order to perform the dicentric assay and can be performed by culturing whole blood (IAEA, 1986), in our experience, cultured mononuclear cells result in a higher quality of chromosome spreads (i.e., significantly less debris) than those prepared using whole blood cultures. An investigator related similar observations (i.e., personal communication with Dr. Gayle Littlefield, Radiation Emergency Assistance Center/Training Site, Oak Ridge, TN). In a catastrophic event, in the interest of expediting sample analysis throughput, however, isolation of mononuclear cells may not be

feasible. Under such circumstances, trained laboratory professionals should bypass density gradient centrifugation and culture whole blood or “buffy coat” specimens (IAEA, 1986; Au et al., 1995), prior to sending samples to the Biodosimetry Laboratory.

The strategy to manage a large-scale nuclear or radiological event presented in this paper requires that the State of Connecticut’s Biodosimetry Laboratory personnel harvest, prepare and stain metaphase chromosome slides and score dicentric chromosomes; thus, they still must perform the majority of the work in order to estimate radiation dose. However, the education and hands-on training program developed by the Biodosimetry Laboratory staff has significantly increased interest in volunteer clinical laboratory professionals in assisting with biodosimetry sample preparation, mainly as a result of diminished unfounded fears regarding the manipulation of blood specimens from contaminated/irradiated victims. In an effort to further build on biodosimetry laboratory surge capacity, the Biodosimetry Laboratory staff have developed a hands-on training program for educating clinical laboratory personnel on how to harvest, prepare and stain slides for dicentric analysis. Currently, as indicated by a preliminary survey of volunteer laboratory professionals who participated in the mononuclear cell isolation and tissue culture training, approximately 1 of 3 laboratorians is willing to undergo training for harvesting and preparing slides for dicentric analysis. Based on the aptitude displayed by laboratory professionals in isolating and culturing mononuclear cells (see Table 1), no critical issues are anticipated in training volunteer laboratorians to become proficient in metaphase slide preparation.

Accordingly, we conclude that clinical laboratory professionals represent an important resource in assisting with triaging patients from accidental or terrorist-related mass-casualty radiological or nuclear catastrophes by providing surge capacity for biodosimetry analysis.

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