Published in final edited form as:

Radiat Meas. 2007 July ; 42(6-7): 1119–1124.

Sample Tracking in an Automated Cytogenetic Biodosimetry Laboratory for Radiation Mass Casualties

P.R. Martin, R.E. Berdychevski, U. Subramanian, W.F. Blakely, and P.G.S. Prasanna*
*Uniformed Services University of Health Sciences, Armed Forces Radiobiology Research Institute,
8901 Wisconsin Avenue. Bethesda, MD 20889-5603, USA.*

Abstract

Chromosome aberration-based dicentric assay is expected to be used after mass casualty life-threatening radiation exposures to assess radiation dose to individuals. This will require processing of a large number of samples for individual dose assessment and clinical triage to aid treatment decisions. We have established an automated, high-throughput, cytogenetic biodosimetry laboratory to process a large number of samples for conducting the dicentric assay using peripheral blood from exposed individuals according to internationally accepted laboratory protocols (i.e., within days following radiation exposures). The components of an automated cytogenetic biodosimetry laboratory include blood collection kits for sample shipment, a cell viability analyzer, a robotic liquid handler, an automated metaphase harvester, a metaphase spreader, high-throughput slide stainer and coverslipper, a high-throughput metaphase finder, multiple satellite chromosome-aberration analysis systems, and a computerized sample tracking system. Laboratory automation using commercially available, off-the-shelf technologies, customized technology integration, and implementation of a laboratory information management system (LIMS) for cytogenetic analysis will significantly increase throughput.

This paper focuses on our efforts to eliminate data transcription errors, increase efficiency, and maintain samples' positive chain-of-custody by sample tracking during sample processing and data analysis. This sample tracking system represents a "beta" version, which can be modeled elsewhere in a cytogenetic biodosimetry laboratory, and includes a customized LIMS with a central server, personal computer workstations, barcode printers, fixed station and wireless hand-held devices to scan barcodes at various critical steps, and data transmission over a private intra-laboratory computer network. Our studies will improve diagnostic biodosimetry response, aid confirmation of clinical triage, and medical management of radiation exposed individuals.

1. INTRODUCTION

Ionizing radiation exposure induces many types of chromosomal aberrations in an exposed individual's peripheral blood lymphocytes. Dicentrics, a type of chromosomal aberration, are considered relatively radiation specific; only a few chemicals are known to interfere with the assay's results. Low background levels (about 1 dicentric in 2000 cells), high sensitivity, and known dose dependency of up to 5 Gy (for acute photon exposures), make this assay robust and a "gold standard" biodosimetry method and diagnostic dose indicator (IAEA, 2001).

Corresponding author: *Pataje G.S. Prasanna, Ph.D., prasanna@afrii.usuhs.mil.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The International Atomic Energy Agency (IAEA) published a technical manual containing a harmonized methodology for performing dicentric assay for assessing dose (IAEA, 2001). An International Standardization Organization (ISO) Work Group was established to standardize biological dosimetry by cytogenetics. Under the auspices of the ISO, regulatory compliance and validation standards have been developed (Voisin *et al.*, 2002).

Early dose estimates using cytogenetic methods correlate well with the severity of imminent acute radiation syndrome as demonstrated in the Chernobyl accident (Sevan'kaev, 2000). Current medical management guidelines treating radiation exposed individuals encourage early administration of cytokines, which requires timely identification and stratification of patient cohorts who will benefit from therapy (Waselenko *et al.*, 2004; Weisdorf *et al.*, 2006).

The utility of cytogenetic assays for assessing dose in radiation mass casualties and guiding treatment decisions was previously demonstrated; for example, Chernobyl (Pyatkin *et al.*, 1989) Goiania, Brazil (Ramalho and Nascimento, 1991) and Tokaimura, Japan (Hayata *et al.*, 2001; Kanda *et al.*, 2002). The dicentric assay can be adopted quickly to assess high- and midrange radiation doses to individuals after a mass casualty incident, with aggressive operational planning and minimal further assay development (Lloyd *et al.*, 2000; Voisin *et al.*, 2001; Prasanna *et al.*, 2003). Lloyd (1997), after studying lymphocyte chromosome damage in 10 of the 13 severely irradiated Chernobyl victims, demonstrated that the frequency of metaphase spreads without dicentrics can be used to identify patients suitable for cytokine therapy.

Under emergency situations for triage purposes only, abbreviated cytogenetic protocols could be implemented to guide medical management of patients. Dose-based triage can be made by scoring as few as 20 to 50 metaphase spreads per subject, compared to the typical 500 to 1000 spreads scored for routine analyses for estimating dose (Lloyd *et al.*, 2000; Voisin *et al.*, 2001; Prasanna *et al.*, 2003). Based on *in vitro* dose response data, stratification of radiation-exposed individuals between acute radiation syndrome (ARS) treatment (>2-Gy) vs. long-term surveillance categories (>1 Gy) is possible.

The ISO working group on biological dosimetry is now focused on developing the standard titled "Radiation Protection—Performance Criteria for Service Laboratories Performing Cytogenetic Triage for Assessment of Mass Casualties in Radiological and Nuclear Emergencies." This standard will define quality control and assurance standards for using cytogenetic methods for triage, and information that will supplement the early clinical categorization of casualties.

Improved efficiency of cytogenetic biodosimetry assays is required to provide results rapidly; enable timely, effective triage; and guide treatment. Increase in sample processing throughput is critical. We have previously shown that a cytogenetic laboratory's sample throughput can be increased significantly by use of automated equipment—robotic instruments, metaphase harvesters and spreaders (Prasanna *et al.*, submitted). This article focuses on sample tracking prioritization and reprioritization of samples, and resource and data management in an automated cytogenetic biodosimetry laboratory. Our efforts to increase efficiency, eliminate data transcription errors, data and resource management, and maintenance of samples' positive chain-of-custody during sample processing and data analysis with the aid of customized Laboratory Information Management System (LIMS) are presented.

2. SYSTEM IMPLEMENTATION

2.1. Intra-laboratory private network

Figure 1 illustrates a flexible and scalable private intra-laboratory communication network connecting various functional workstations and the database in our automated cytogenetic biodosimetry laboratory for sample tracking. This computer network consists of a combination of wired and wireless utilities. The wireless access points, supporting 802.11 b and g, encrypt and transfer data at speeds up to 56 megabits per second and are strategically located throughout the laboratory. The wireless access points are integrated into a wired, intra-laboratory, high-speed (1 gigabit per second) Ethernet network that connects various cytogenetic laboratory equipment, computers, workstations, and robots.

2.2. Hardware

2.2.1. Server and workstations—Our sample tracking system uses a Windows 2003-based central server with quad processors, multiple, redundant, high-speed hard drives, and an external data backup system (1 terabyte). Several Pentium 4-class Windows XP Professional-based personal computers with 512 megabytes of memory serve as sample-tracking stationary workstations. The server has two high-speed (1 gigabit per second) wired Ethernet connections to multiple stationary workstations; a gateway PC for routing LIMS traffic; and a secured, wireless 802.11 connection to hand-held PDA computers equipped with barcode scanners. Several laboratory automation robots, a laser document printer, a thermal transfer barcode printer, a microscope slide barcode laser printer, an alphanumeric messaging display, and various environmental monitors comprise the remainder of the network. The LIMS database runs on top of Microsoft's SQL Server technology, providing an established, flexible, and scalable foundation for further development. Data from workstations and networked laboratory equipment and functional stations is communicated directly to the LIMS via Microsoft's ODBC protocol.

2.2.2. Palm SPT 1800 series PDA scanners—A Palm SPT1800 series wireless PDA scanner equipped with the Palm operating system is used in stations where hand-held barcode scanning is essential. The PDA scanner provides ruggedness, mobility, integrated scanning, wireless connectivity to the server, and powerful processing. Barcode capture and wireless communications enhance data transfer and increase productivity by sample tracking at stations such as blood sample shipping and receiving, prioritizing and reprioritizing of samples, reagent and chemical inventory recording, etc.

2.2.3. Barcode printers—The sample tracking system uses 3 different printer types to generate barcodes.

Printer for documents: An off-the-shelf commercially available (Hewlett Packard 2430, Hewlett-Packard Co., Palo Alto, CA) 1200 dots-per-inch laser printer with 35 pages-per-minute output is used to print barcodes on paper documents generated by the LIMS, such as human use consent forms, test reports, etc.

Printer for lab-ware: A commercially available Zebra TLP 3842 printer (Zebra Technologies Corp., Vernon Hills, IL) is used to print barcodes on general lab-ware, such as centrifuge tubes and reagent bottles, etc. This printer uses a thermal transfer-based technology to quickly and crisply print long-lasting barcodes and human-readable text onto small labels. All barcodes are chemical, moisture, heat, and stain resistant.

Printer for glass microscope slides: The ViaLabel KV2S laboratory marker (ViaLabel, InfoSight Corp., Chillicothe, OH) is used to etch barcodes and human-readable text onto glass

cytogenetic microscope slides. Barcodes on slides (a) are well defined and high contrast, (b) survive harsh chemical solvents and stains, (c) present required information in a limited space, (d) enhance lab efficiency, (e) provide an ability to accurately trace specimens, (f) are cost effective, (g) are readable both by barcode scanners and lab personnel, (h) can be printed singly or in batches, and (i) read by barcode scanners and lab personnel after washes and staining cycles.

2.3. Work flow in automated cytogenetic laboratory

Figure 2 shows an industrial analysis of work flow at different stations in our automated cytogenetic biodosimetry laboratory illustrating each station's functions, maximum capacity per run, volume, rate or total time, and number of personnel required for unit throughput. For example, in the prelogin sample dispatch station, 96 blood vacutainers and human use consent forms are bar coded at a rate of 12 min/kit; each kit consisting of 20 samples.

Cell viability analyzer—Radiation induces lymphocyte depletion in peripheral blood. Optimal lymphocyte concentration is essential in cultures for obtaining metaphase spreads for estimating dicentric frequency. Therefore, an automated and regulatory-compliant, high sample throughput, cell viability analyzer (Cedex, Innovatis, Malvern, PA) that can be integrated with the current liquid handling robot is being procured and integrated. At this station, 20 blood vacutainers need to be tracked per run and the data transferred electronically to the LIMS data base to eliminate data transcription errors.

Integrated liquid handling station—A Tecan Freedom EVO 250 series liquid handling robot (Tecan Switzerland AG, Männedorf, Switzerland) performs all liquid handling for setting up blood cultures from up to 96 samples per run. Before each run begins, the robot automatically scans the barcode of each piece of lab-ware on the work-bed and tracks sample transfers between lab-ware. The robot's software, EVOware, generates an auditable file of all operations performed during the run. Scripting ensures that all lab-ware are loaded accurately and that samples are transferred properly in real time. This audit trail information is imported automatically into the LIMS and associated with relevant sample IDs.

Incubators—Samples are checked into and out of the incubator via hand-held PDA barcode readers on the LIMS or PC-attached barcode scanners. Two Forma Scientific 3110 CO₂ (Thermo Electron Corp, Waltham, MA) stackable incubators are monitored for temperature and humidity constantly by the LIMS server via a Sensatronics EM1 environmental monitor (Bow, NH).

Metaphase harvester—The Hanabi PII automated metaphase harvester (ADS-Tec Corp., Chiba, Japan) harvests metaphase spreads from short-term blood lymphocyte cultures automatically from up to 24 samples per run in less than 3 hours by sequentially performing centrifugation of cell suspension, aspiration and disposal of supernatant, disruption of cell pellets, treatment of cells with pre-warmed hypotonic solution, incubation at 37°C, centrifugation and disposal of hypotonic solution, and treatment with Carnoy's fixative as required and preparation of cell suspension for cytogenetic slides. Samples are checked into the machine using a PC-attached barcode scanner. Sensatronics environmental data from this workstation, along with robot's run parameters and results, are tagged to the files in the machine during processing for real-time monitoring of temperature and humidity conditions. Temperature and humidity conditions influence mitotic yield and quality of metaphase spreads (Yamada *et al.*, 1992).

Metaphase spreader—The cell suspension prepared using the metaphase harvester is dropped onto the pre-barcoded glass slides using ViaLabel KV2S laboratory marker as

described above. The samples are tracked in this station using a hand-held scanner. The Hanabi Metaphase Spreader (ADS-Tec Corp., Chiba, Japan) provides ideal temperature and humidity conditions for metaphase spreading. The capacity of this station is 5 slides per run with a rate of 5 min per run.

Autostainer and coverslipper—The Thermo Shandon Varistain Gemini slide stainer and Thermo Shandon Consul coverslipper (Thermo Electron Corp., Waltham, MA) are used to stain and coverslip up to 150 slides per run in less than 40 minutes with minimal user intervention. The slides are checked in and out of these stations using a hand-held scanner.

Metaphase finder—The metaphase finder (Loats Associates, Inc., Westminster, MD) is customized to scan barcodes on slides as it automatically locates chromosome spreads (up to 150 slides per run). Software customizations allow barcode information to be associated with data and image files as well as for centralized storage of case file data on the centralized server.

Satellite chromosome aberration analysis workstations—Customized networked satellite chromosome aberration analysis workstations allow access to case files from the centralized server, preventing other users from concurrently working on the same files. Enhancements to satellite station hardware permit the station to double check barcodes on slides loaded against the coded information in the loaded case file data. User scoring data is automatically recorded directly in the central server, eliminating data transcription errors.

Report generation—The frequency of dicentrics in individual samples is compared with an *in vitro*-generated calibration curve to assess individualized radiation dose. This data is used to automatically generate reports from a form letter in the LIMS. A formatted, customized and barcoded report can then be printed automatically from the LIMS system, or sent electronically to a physician.

3. DISCUSSION

Radiation exposures potentially resulting in mass casualties from an accident or terrorism will require individual, early, and definitive diagnostic radiation dose assessment within days of a catastrophe to help provide medical aid. A radiological catastrophe could result in proportions of victims ranging from none to as many as 40% of the exposed population (Coleman *et al.*, 2003). Medical management will require a capability to stratify victims rapidly into different dose categories in the dose range 0 to 5-Gy, to discriminate for acute radiation syndrome treatment vs. long-term surveillance within a clinically relevant time-span (NCRP, 2001). Therefore, in the event of a mass-casualty incident, the need to process large samples for diagnostic dose assessment is paramount (Weisdorf *et al.*, 2006).

Because measurement of chromosomal aberrations, particularly the dicentric assay, offers capabilities to stratify exposed population cohorts into dose categories supporting medical management within a clinically relevant time-span (Lloyd *et al.*, 2000; Prasanna *et al.*, 2003), and assess radiation dose both in whole-body and partial-body radiation exposure scenarios (IAEA, 2001), a network of biodosimetry laboratories is needed to establish rapid sample analysis. Our efforts focus on developing a flexible, scalable, and high-sample-throughput laboratory via automation (Prasanna *et al.*, 2005; Prasanna *et al.*, submitted), implementing rigorous quality control and quality assurance standards for cytogenetic methods (Voisin *et al.*, 2002) with harmonization of laboratory protocols (IAEA, 2001), and establishing a network of cytogenetic biodosimetry laboratories and technology transfer for rapid dose assessments.

Our work on laboratory automation to increase sample throughput focused on concept feasibility, workflow analysis, possible process reengineering, bottleneck elimination in

manual processing, and proof-in-principle experiments (Prasanna *et al.*, 2005, Prasanna *et al.*, submitted). With automation, we previously estimated that up to 500 samples per week can be processed in triage mode in which chromosome aberration analysis is restricted to 20 metaphase spreads per sample compared with the conventional approach of 500 to 1000 spreads (Prasanna *et al.*, 2005; Prasanna *et al.*, submitted). The estimated number of samples was based on a summation of system components and available personnel resources. Figure 2 shows an industrial analysis of work flow in our automated cytogenetic biodosimetry laboratory illustrating functions at various stations, projected maximum capacity per run, volume, rate or total time, and number of personnel required for unit throughput. This industrial analysis helps to understand rate limiting steps, critical factors, competitive demands for cost reduction, and improved turnaround time. A radiation mass-casualty incident requiring processing of large number of samples is expected to challenge existing sample-tracking procedures, from sample receipt to reporting, because of high volume and turnover rate in an automated cytogenetic laboratory.

In our laboratory, tracking begins upon sample receipt by bar-coding the vacutainers and consent forms. The vacutainers are processed in a liquid-handling robot where barcodes are scanned and cell viability is analyzed (a blood-cell viability counter is being procured and integrated with the existing robot for this purpose). Samples are checked into each successive phase of processing via a handheld wireless PDA barcode scanner or a barcode scanner attached to a PC. Other sample processing stations include CO₂ incubators, an automated metaphase cell harvester, metaphase spreader, slide stainer and coverslipper, respectively, (Figure 2). For example, the metaphase finder automatically scans the barcode on each slide before locating metaphase spreads, and uses this information to separate data and images from related slides into separate case files instantaneously, thereby preventing data-transcription errors between patients. Case files and related images are stored on the server and are accessed from networked workstations for scoring. Metaphase-finder case files can be “checked out” from the server by users at workstations for analysis. This prevents other users from concurrently using the file, avoiding redundant efforts. In addition, bar-coding of samples also provides an ability to reprioritize the processing and analysis of samples at different stations.

Rigorous quality control and assurance should be integral in a dose assessment plan for stratifying a population into treatment categories (IAEA, 2001; Voisin *et al.*, 2002). ISO’s new standard will define the process and identify rigorous quality standards for using cytogenetic methods to rapidly assess radiation dose. Large volumes of samples plus the required record keeping using laboratory notebooks invariably pose quality control and assurance challenges. In contrast, electronic sample tracking and data management using barcodes provide an unbroken chain-of-custody of information for every sample, thereby increasing efficiency, confidence, speed, and throughput while minimizing data-transcription errors. This sample tracking system represents a “beta” version and may be modeled elsewhere in a high-throughput radiation cytogenetic biodosimetry laboratory.

Acknowledgements

AFRRI and the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, supported this research under work unit RAB4AF and research grants Y1-AI-3823-01 and Y1-AI-5045-01, respectively. Discussions with Dr. N.C. Coleman, (Office of Public Health Preparedness, HHS, Washington, DC) on industrial analysis of work flow in our automated cytogenetic laboratory are very helpful. We also appreciate the research assistance of the technical staff, Mr. R. Rivas, Ms. K. Krasnopolsky, HM1. M.A. Martinez, and our summer intern, Mr. B. Jois. We would like to thank Mr. M. Behme for assistance with illustrations, and Mr. F. Duffy for edits. Similarly, Mr. I. Levine, J. Burke, G.L. Robey, and S. Davis assisted in the development of the sample tracking system.

References

- Coleman CN, Blakely WF, Fike JR, MacVittie TJ, Metting NF, Mitchell JB, Moulder JE, Preston RJ, Seed TM, Stone HB, Tofilon PJ, Wong RSL. Molecular and cellular biology of moderate-dose (1–10 Gy) radiation and potential mechanisms of radiation protection: Report of a workshop at Bethesda, MD, December 17–18, 2001. *Radiat Res* 2003;159:812–834. [PubMed: 12751965]
- Hayata I, Kanda R, Minamihisamatsu M, Furukawa M, Sasaki MS. Cytogenetic dose estimation for 3 severely exposed patients in the JCO criticality accident in Tokaimura. *J Radiat Res* 2001;42:S149–155. [PubMed: 11791748]
- IAEA. Cytogenetic analysis for radiation dose assessment: A manual. Vienna, Austria: International Atomic Energy Agency; 2001.
- Kanda R, Minamihisamatsu M, Hayata I. Dynamic analysis of chromosome aberrations in three victims of the Tokaimura criticality accident. *Int J of Radiat Biol* 2002;78:857–862. [PubMed: 12428926]
- Lloyd DC. Chromosomal analysis to assess radiation dose. *Stem Cells* 1997;15(2):195–201. [PubMed: 9368304]
- Lloyd DC, Edwards AA, Moquet JE, Guerreiro-Carbajal YC. The role of cytogenetics in early triage of radiation casualties. *Applied Radiation Isotopes* 2000;52:1107–1112.
- NCRP. NCRP Report No. 138. NCRP; Bethesda, MD: 2001. Management of terrorist events involving radioactive material.
- Prasanna PGS, Martin PR, Subramanian U, Berdechevsky RE, Manglapus GL, Landauer MR, Srinivasan V, Blakely WF. Cytogenetic laboratory automation and laboratory information management system for radiation dose assessment. *Mut Res Reviews*. submitted
- Prasanna, PGS.; Martin, PR.; Subramanian, U.; Berdychevski, R.; Krasnopolsky, K.; Duffy, KL.; Manglapus, GL.; Landauer, MR.; Srinivasan, V.; Boreham, D.; Hagan, MP.; Jinaratana, V.; Blakely, WF. Bethesda, MD, USA: NATO; 2005. Cytogenetic biodosimetry for radiation disasters: Recent advances. NATO RTG-099
- Prasanna, PGS.; Subramanian, U.; Greenhill, RG.; Jacocks, JM.; Jackson, WE.; Blakely, WF. Radiation Safety Aspects of Homeland Security and Emergency Response. San Antonio, TX: Health Physics Society; 2003. Cytogenetic biodosimetry strategy for potential radiation mass casualties.
- Pyatkin EK, Nugis VY, Chrikov AA. Absorbed dose estimation according to the results of cytogenetic investigations of lymphocyte cultures of persons who suffered in the accident at Chernobyl atomic power station. *Radiation Medicine* 1989;4:52.
- Ramalho AT, Nascimento AC. The fate of chromosomal aberrations in ¹³⁷Cs-exposed individuals in the Goiania radiation accident. *Health Phys* 1991;60:67–70. [PubMed: 1983985]
- Sevan'kaev AV. Results of cytogenetic studies of the consequences of the Chernobyl accident. *Radiats Biol Radioecol* 2000;40(5):589–95. [PubMed: 11252234]
- Voisin P, Barquinero F, Blakely WF, Lindholm C, Lloyd DC, Luccioni C, Miller S, Palitti F, Prasanna PGS, Stephan G, Thierens H, Turai I, Wilkinson D, Wojcik A. Towards a standardization of biological dosimetry by cytogenetics. *Cell Mol Biol* 2002;48:501–4. [PubMed: 12146703]
- Waselenko JK, MacVittie TJ, Blakely WF, Pesik N, Wiley AL, Dickerson WE, Tsu H, Confer DL, Coleman NC, Seed T, Lowry P, Armitage JO, Dainiak N. Medical management of the acute radiation syndrome: Recommendations of the Strategic National Stockpile Radiation Working Group. *Annals of Internal Medicine* 2004;15:1037–1051. [PubMed: 15197022]
- Weisdorf D, Chao N, Waselenko JK, Dainiak N, Armitage JO, McNiece I, Confer D. Acute radiation injury: Contingency planning for triage, supportive care, and transplantation. *Biology of Blood and Marrow Transplantation* 2006;12:672–682. [PubMed: 16737941]
- Yamada K, Kakinuma K, Tateya H, Miyasaka C. Development of an instrument for chromosome slide reparation. *J Radiat Res* 1992;33 (Supplement):242–249. [PubMed: 1507174]

Figure 1.
CytoTrack component and data flow

Figure 2.
Industrial analysis of work flow in AFRI's automated cytogenetic biodosimetry laboratory.