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High-throughput sample processing and sample management; the functional evolution of classical cytogenetic assay towards automation.

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ABSTRACT

High-throughput individual diagnostic dose assessment is essential for medical management of radiation-exposed subjects after a mass casualty. Cytogenetic assays such as the Dicentric Chromosome Assay (DCA) are recognized as the gold standard by international regulatory authorities. DCA is a multi-step and multi-day bioassay. DCA, as described in the IAEA manual, can be used to assess dose up to 4–6 weeks post-exposure quite accurately but throughput is still a major issue and automation is very essential. The throughput is limited, both in terms of sample preparation as well as analysis of chromosome aberrations. Thus, there is a need to design and develop novel solutions that could utilize extensive laboratory automation for sample preparation, and bioinformatics approaches for chromosome-aberration analysis to overcome throughput issues. We have transitioned the bench-based cytogenetic DCA to a coherent process performing high-throughput automated biodosimetry for individual dose assessment ensuring quality control (QC) and quality assurance (QA) aspects in accordance with international harmonized protocols. A Laboratory Information Management System (LIMS) is designed, implemented and adapted to manage increased sample processing capacity, develop and maintain standard operating procedures (SOP) for robotic instruments, avoid data transcription errors during processing, and automate analysis of chromosome-aberrations using an image analysis platform. Our efforts described in this paper intend to bridge the current technological gaps and enhance the potential application of DCA for a dose-based stratification of subjects following a mass casualty. This paper describes one such potential integrated automated laboratory system and functional evolution of the classical DCA towards increasing critically needed throughput.

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1. Background

The advent of nuclear technology and its extensive use in almost every field has brought in a set of its own risks and benefits. Use of radioisotopes and radiation generating units has become very common in medical industries. Accidental occupational overexposure due to a lack of industrial precautionary safety or radiation mass casualties driven by acts of terrorism or natural calamity such as the recent Fukushima incident, can potentially expose thousands of individuals, cause disruption to normalcy and result in dire economic and health management crises. There are plausible circumstances where populations are potentially exposed to doses of ionizing radiation (IR) that could cause direct clinical effects within days or weeks. It is also likely that many individuals may not have been exposed to clinically significant doses of radiation, while others may have been exposed to potentially life-threatening doses. Therefore, it is important to estimate radiation dose to victims for treatment of imminent acute radiation syndrome (ARS). Exposure to IR with a dose range of 0.5 to 30 Gy (photons) causes a variety of symptoms including nausea, vomiting, diarrhea, and peripheral blood lymphocyte (PBL) depletion [1]. The median lethal dose of radiation, LD50/60 is estimated to be 4.14 Gy [2]. The duration of initial or prodromal symptoms and the latent phase of ARS is anywhere from 1 h to 2 weeks. Thousands of lives can be saved by timely medical intervention and supportive care aided by indi-

Abbreviations: DCA, dicentric chromosome assay; LIMS, laboratory information management system; SOP, standard operating procedures; IAEA, international atomic energy agency; IR, ionizing radiation.

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vidualized dose estimates; ideally estimated before the onset of ARS.

Cytogenetic techniques are often used for triage and definitive radiation biodosimetry. Dicentric Chromosome Assay (DCA) is considered as the 'gold standard' biodosimetry method. DCA is highly radiation-specific, shows low background levels (about 1 dicentric in 2000 cells) and low inter-individual variations in its frequency, high sensitivity (a threshold detection dose of 0.05 Gy), and known dose dependency up to 5 Gy for photons [3]. The assay can be used to accurately assess a dose up to 4–6 weeks after radiation exposure and possibly assess dose up to 2–3 years post-exposure with appropriate corrections accounting for lymphocyte lifespan in circulating blood. Typically, about 500 metaphases per subject are scored for dose estimation. However, for triage purposes this number may be reduced to 50–100 metaphases per subject. Lloyd et al., showed that an analysis of 20 to 50 metaphase spreads is adequate for risk-based stratification of exposed subjects for treatment of ARS [4,5]. DCA has been historically used to assess radiation dose in accidents such as Chernobyl [6], Goiania [7], and Tokaimura [8]. Estimated doses correlated well with the severity of ARS [6]. In the Chernobyl accident, a rapid preliminary examination of 50 metaphase spreads per subject provided an approximate dose estimate for several individuals [9].

Several authors such as Lloyd et al. [4], Mettler and Voelz [10], Goans and Waselenko [11], Pellmar and Rockwell [12], and Coleman et al. [13], have reviewed in detail the need for cytogenetic biodosimetry to aid medical management of exposed subjects. Several others, Weber et al. [14], Schunk et al. [15], Martin et al. [16], Romm et al. [17], Garty et al. [18] and Fenech et al. [19], have described the need for automation of cytogenetic assays, such as DCA and/or CBMN (Cytokinesis-blocked micronucleus assay), in order to support medical management of accidentally exposed individuals. Here in this paper, we describe our progress in the automation of sample preparation and chromosome-aberration analysis and highlight important limitations and potential remedies for improving a laboratory's throughput to overcome important barriers in the potential application of cytognetic assays in radiation mass casualties for diagnostic dose assessment of ARS.

2. Description of systems

2.1. High throughput laboratory automation

The concept of laboratory automation for the DCA is shown in Fig. 1. A laboratory information management system (LIMS) is central to the automation of the workflow in a cytogenetic biodosimetry laboratory, which involves automation of various modules: sample acquisition, sample processing, image acquisition, chromosome aberration analyses, dose estimation and reporting. Our initial efforts on development and implementation of a LIMS to eliminate data transcription errors, increase efficiency, and maintain positive chain-of-custody of samples by sample tracking during sample processing and chromosome-aberration analysis has been previously described [16]. For automated processing of blood samples in the sample-processing module, 15ml-conical falcon tubes (Beckton-Dickinson, USA) were barcoded. Similarly, slides in the analysis module are tracked by barcoding high temperature glass slides as described previously [16].

Sample acquisition module provides functionalities to support peripheral blood drawn from human subjects into vacutainers containing sodium heparin as an anticoagulant (BD Biosciences, USA). This module keeps records of informed consents obtained on a form, which is approved by the Institutional Review Board (IRB)

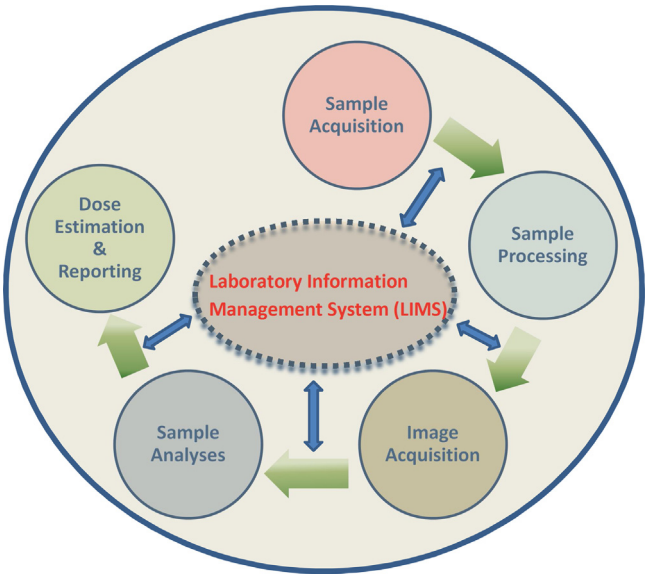

Fig.1. High-throughput cytogenetic automation platform: sample acquisition, sample processing, image acquisition, sample analyses and dose estimation and reporting modules, integrated with the core LIMS engine, for positive chain-of-custody and efficient sample management.

along with details of dosimetry and irradiations. For example, all details associated with a given experiment or a set of individual experiments for constructing dose-response calibration curves or assessment of dose to an individual are recorded and archived.

Process simulation experiments are performed (when necessary), to optimize and test the liquid handling robot (Tecan Freedom Evo, USA). Random tests are conducted for checking any potential of sample cross contamination and to check maintenance of sterility during high throughput handling of blood samples. In order to rule out any sample cross contamination un-diluted FD&C Brilliant Blue 1 dye is aliquoted using the automated liquid handler. Pipette tips are washed by aspirating and ejecting 30 ml of water, two times, before they are used to aliquot eight 100 micro-liter aliquots of PBS into 96-well plates. The procedure is repeated about 8 times. Sample cross-contamination is determined by spectroscopic reading at 630 nm (max absorbance of the dye).

The maintenance of sterility of lymphocyte cultures is ensured during high throughput sample processing by ruling out mycoplasma contamination. Fifteen 1-ml aliquots of sterile MarrowMax bone marrow medium in Falcon tubes is distributed by the liquid handler and aliquots are incubated at 37 °C/5% CO_2 for one week. Mycoplasma detection PCR kit 16s rRNA gene (Maximbio, USA) that detects segments of 16s rRNA gene of mycoplasma by PCR amplification is used to rule out mycoplasma contamination in cultures.

An automated metaphase harvester (Hanabi PII, Japan) is used to harvest metaphase spreads 48 h after culture initiation where cells are treated with hypotonic solution (75 mM KCl) and fixative (ice-cold solution of 1:3 acetic acid: methanol). An automated metaphase spreader (HANABI P1 Metaphase Spreader, Japan) is used to prepare metaphase spreads by dropping 15-μl of cell suspension (consisting of fixed and swollen cells) onto methanol-cleaned barcoded glass slides. Metaphase spreader provides controlled environmental conditions (37 °C and 55% humidity) for drying to accomplish optimal metaphase spreading. An autostainer (Thermo Scientific, USA) is used to stain slides with Giemsa.

Metaphase-spread slides from the previously described inter-laboratory comparison studies [20] were used for validating the

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