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

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19 Abstract

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

41 Abbreviations

42 DCA, Dicentric Chromosome Assay; LIMS, Laboratory Information Management System; SOP,
43 Standard Operating Procedures; IAEA, International Atomic Energy Agency; IR, Ionizing
44 Radiation.

45 Key words

46 cytogenetic laboratory, automation, laboratory information system, dicentric assay, radiation
47 exposure assessment, biodosimetry, radiation mass casualty triage

48 1. Background

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

107 USA). This module keeps records of informed consents obtained on a form, which is approved
108 by the Institutional Review Board (IRB) along with details of dosimetry and irradiations. For
109 example, all details associated with a given experiment or a set of individual experiments for
110 constructing dose-response calibration curves or assessment of dose to an individual are recorded
111 and archived.

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

121 The maintenance of sterility of lymphocyte cultures is ensured during high throughput sample
122 processing by ruling out mycoplasma contamination. Fifteen 1-ml aliquots of sterile MarrowMax
123 bone marrow medium in Falcon tubes is distributed by the liquid handler and aliquots are
124 incubated at 37°C/5% CO₂ for one week. Mycoplasma detection PCR kit 16s rRNA gene
125 (Maximbio, USA) that detects segments of 16s rRNA gene of mycoplasma by PCR amplification
126 is used to rule out mycoplasma contamination in cultures.

127 An automated metaphase harvester (Hanabi PII, Japan) is used to harvest metaphase spreads 48 h
128 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- μ l 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 dose estimator module of the Cytogenetic Laboratory Automated Scoring Platform (CLASP).

2.2 Cytogenetic Laboratory Automated Scoring Platform (CLASP)

CLASP is an automated cytogenetic analysis suite with integrated hardware and software tools to facilitate information management, module development, prototype testing, application deployment, analysis and reporting of dicentrics analysis. It consists of a high-performance UNIX network (a widely used multiuser computer operating system), standalone cluster or Oracle GRID engine (a computer cluster software system), which is fully integrated with the CytoTrack Image SQL (Structured Query Language) server via software interfaces and a self-contained LIMS Network. LIMS network is hosted on a Windows 2003 server and administered locally. CLASP establishes its network cluster using the ROCKS ganglia (a graphical user interface to monitor each cluster) and NUMA (non-uniform memory access, is the phenomenon that memory at various points in the address space of a processor has different performance characteristics to enhance processing) toolkit was used to measure system performance. The GRID (Interconnected computer systems where the machines utilize the same resources

collectively) runs an x64 UNIX OS (Operating System) for all number crunching and processing operations. Most image processing operations and spooling is done using a TESLA (a brand name for NVIDIA products targeting stream processing) GPU (Graphic Processing Unit). The unit is integrated with the GRID engine. CIT (Cyto Image Track) server, which is a part of the CLASP that has a dedicated TESLA GPU server consisting of 2 dual Quad-Core Intel® Xeon® processor 5600/5500 series, with QPI (quick path interconnect) up to 6.4 GT/s. (Giga transfers/second). Each core is banked with 48 GB DDR RAM (Giga Bytes Double Data Rate Random Access Memory) 1366 MHz RAM giving about 96 GB of dedicated memory for image processing. The TESLA is backed on a RAID 5 (Redundant Array of Independent Disks, a data storage technology) with the central LIMS and each TESLA has 1 Terabyte Ultra SCSI (Small Computer System Interface, 1000/RPM) HDD (Hard Disk Drive). TESLA provides ultra-performance in processing images and also reduces I/O (input/output) operations enhancing transfer bandwidth. Most image processing is performed on the GPU. Each unit has 2x Nvidia TESLA M1060 GPU Cards with Intel SATA 3.0 Gbps Controller (a computer bus interface to connect host computer to mass storage device). The LIMS core is made up of 96 Xeon CPU (Central Processing Unit). Each unit is banked with 24 GB of RAM providing a total server memory of 216 GB. A BLADE server (a server chassis) with 10 Hot blades with each blade configured with Intel® Xeon® processor 5600/5500 series, with QPI up to 6.4 GT/s and 24 GB RAM on each blade is dedicated for all LIMS core processing.

3. Recent Advances

DCA is a multi-day laboratory assay and demands cytogenetic expertise for scoring dicentrics in metaphase spreads to estimate radiation dose. When performed manually, the assay suffers from

major practical limitations due to overall sample processing delay, laborious and repetitious procedures resulting in less than optimal throughput for dose assessment for a large number of individuals. The assay involves collection of blood samples, culturing, harvesting of cells, slide preparation, staining of cells on standard microscopy slides, mounting of these slides, image acquisition or manual scoring of cells. Most of these steps are repetitive, highly labor-intensive and demand skilled cytogeneticists or trained expertise, which is becoming increasingly scarce. For the DCA to be applicable and useful in a triage dose estimation setting for radiation accidents or in conditions where higher throughput is necessary each and every step in the assay process has to be automated. Below we review our own efforts over the last decade to automate both sample preparation and chromosome-aberration analysis.

3.1 High throughput sample processing

High throughput cytogenetic sample processing laboratories can make use of commercially available off-the-shelf laboratory automation instruments, which can be customized and integrated to suit any laboratory's needs. Key aspects while selecting the instruments are; scalability, upgradability and integration, particularly, if already a set of usable equipment exists. Throughput can be increased by using robotic instruments such as liquid-handling devices with barcode scanning capabilities, metaphase harvesters and spreaders, large-scale slide staining equipment, and high-throughput metaphase finders with multiple satellite chromosome-aberration analysis stations, as described here. Alternatively, cost-effective, simple automated solutions could also be implemented to eliminate most important bottlenecks in the workflow. For example, in the sample-processing module, multi-channel pipettes can substitute for automated liquid handlers, when cost is an issue. Sample barcode cross-referencing and tracking via LIMS may be included: to eliminate transcription error, ensure positive chain of custody

through the multiple steps in the workflow, monitor progress and for quality control and quality assurance.

3.2 CLASP, its need and architecture

CLASP forms the breadboard system for all application development, analysis and encompasses the LIMS SQL servers. CLASP is based on existing commercially available hardware (such as TECAN EVO 200 from TECAN, Hanabi Harvesters and Spreaders from ADSTEC etc.) and software components and is customized for specific (cytogenetic) applications. The CLASP system significantly reduces human error in performing repetitive tasks, increases productivity, frees lab personnel for other tasks, and helps provide more scientifically reliable data obtained using good laboratory practice (GLP) guidelines. CLASP architecture and functional design is shown in Fig. 2.

3.3 LIMS, its need and architecture

Laboratory information management systems (LIMS) are widely used in high-throughput and GLP based laboratory practices and clinical laboratories. It subsidizes the repetitive processes and ensures a positive chain of custody of samples and data. LIMS software is commercially available off-the-shelf and various types of LIMS are available and for our cytogenetic needs, we used ATL LIMS from ATLABS (West End, NC, USA) and customized it completely to meet specific requirements. LIMS is a central SQL server of CLASP. LIMS archives all processed samples, maintains positive chain-of-custody, facilitates managing cytogenetic experiments, helps in generation of reports and keeps track of all client stations and analyses processes at all times. The LIMS system is capable of tracking each and every stage of the Cytogenetic Biodosimetry workflow; from sending out a Blood Collection Kit (BCK) to the recording of

scoring details for all samples. The data are stored using normalized entities on a secure and high-performance SQL server database. The entity-relation design of the stored data provides a sophisticated query platform for performing advanced statistical analysis. The LIMS architecture in relation to CLASP is shown in Fig. 3. The CLASP LIMS contains several modules to facilitate management of data generated in the laboratory via interaction with various instruments and storage locations. Various LIMS modules and their associated functionality are listed in Table 1.

3.4 Sample acquisition and processing

Sample acquisition is the first step in the workflow in the cytogenetic lab. Samples can be acquired in two ways in CLASP: (i) Blood collection kit (barcoded) is sent out to the collection site are automatically accepted in CLASP (ii) alternatively, CLASP can accept physical or digital slides of metaphase-spreads for analysis, if these are already prepared in another laboratory. In the latter case, if it is a slide with no barcode, then a barcode can be generated and a corresponding barcoded form approved by an Institutional Review Board (IRB) is also generated with annotated information on samples. As all samples have unique barcodes, at any given time during the workflow, querying the sample status provides all relevant information associated with the sample. SOPs for blood collection and blood processing through automated systems are generated and validated before deploying the protocol in CLASP. Blood is collected following specific procedures [21] and the temperature inside the BCK is monitored with a temperature data logger. Upon arrival of samples and based on preliminary prodromal symptoms if reported on the forms, density of viable cells is measured using a blood cell viability analyzer (Celldex, Germany) and culture conditions are adjusted accordingly to ensure presence of a sufficient number of spreads for analysis. All the liquid handling is done automatically with minimum user interaction to increase throughput and limit exposure to biohazards. Samples are

then sequentially processed, logged in and out from each workstation and monitored in real-time through the LIMS to ensure quality control.

Once the samples are logged in and accepted in the CLASP along with all associated consent forms, the samples are processed through a series of robotic instruments. All the SOPs for these robotic instruments are developed prior and loaded. Equipment calibrations and quality control (QC) procedures are periodically and routinely performed and associated data is maintained for audit. The first step in sample processing is through the TECAN liquid handling robot.

3.5 TECAN liquid handling robot

Use of an automated liquid handling robot such as TECAN EVO 200 for blood culturing significantly improves throughput and also reduces any possible sample cross contamination or a potential situation that could compromise sterility. We experimentally addressed these two critical detriments. The FD&C brilliant blue dye test indicated there is no cross contamination as determined by the spectroscopic reading at 630 nm and sufficient washing of the pipette tips is accomplished with repeated aspiration and ejecting of water. Similarly, examination of blood cultures for mycoplasma after processing with the automated blood handler indicated no mycoplasma contamination ruling out such concerns, Fig. 4.

The design of the customized and integrated TECAN Freedom EVO liquid handler is shown in Fig. 5. The liquid handling robot performs all liquid handling needs such as dispensing, robotic aspiration, source-to-destination sample transfer as well as various other processes including barcodes scanning for maintenance of positive chain-of-custody of samples. The robot's use for high-throughput processing of blood samples and isolation of lymphocytes from peripheral whole blood eliminates an important rate-limiting bottleneck in sample processing. The

commercial sector had developed liquid-handling robots capable of dispensing, diluting and aspirating blood samples, specifically for blood banking applications [22]. These systems are precise, accurate, and do not cross-contaminate specimens [23] The robot in our laboratory is customized for liquid handling for cytogenetic sample preparations and is integrated with both a cell viability analyzer (Cedex Cell Viability Analyzer, Germany) and a swinging bucket-automated centrifuge (Hettich AG, Switzerland). All instruments are enclosed in an engineered Biosafety Level 2 environment (JT Baker, USA) to ensure sterility of the samples and occupational safety of laboratory personnel. The system provides a positive chain-of-custody via barcode identification of samples and destination tubes matching.

3.6 SOP's for blood processing and culturing

Two separate SOPs are developed for culturing whole blood and isolated lymphocytes using the robotic blood handler. Since there is no significant difference in radiation-induced dicentrics' yield between whole-blood and isolated lymphocyte cultures [24], whole blood culture method may be preferred for stratifying radiation-exposed individuals in mass casualties. This process is much simpler compared to culturing isolated lymphocytes and offers an estimated 4-fold increase in process throughput (data not shown here). However, isolated lymphocyte cultures provide better quality and higher yields of metaphase spreads and also allow optimization of culturing conditions when cell viability is likely to be low after high dose exposures. Isolated lymphocyte culture may be preferred when precise dose estimations are required for occupational overexposure assessments or after high dose exposures. Robotic blood handling for whole blood cultures for radiation mass casualty applications provides an estimated 40-fold increase (data not shown) in sample throughput when compared with the manual procedure. Depending on the chosen SOP, all robotic assay modules and internal SOPs with their

calibrations and references are loaded automatically and the system starts a self-check procedure. During this 'self-check' procedure, the system self-calibrates by moving its robotic arm from the start position to the end and to the resting position. It will perform a material and reagent inventory to ensure availability of all reagents and materials necessary for processing a batch of samples prior to the actual processing. If the reagent inventory is low, the system is programmed to generate user warnings before processing of samples in order to avoid any mid-run failures.

3.7 Metaphase harvesters and spreaders

To obtain reliably high quality metaphase spreads either from whole blood or from isolated lymphocyte cultures, our laboratory uses commercially available metaphase harvesters, Hanabi PII and PIII harvesters (Adstec Technologies, Japan), which are customized and integrated to the LIMS. The capacities of these two units are 24 and 64 samples, respectively. The main components of these metaphase harvesters are a heated centrifuge and several aliquoting/vortexing devices that inject and aspirate hypotonic or fixative solutions as needed. These metaphase-spread harvesting devices eliminate the labor-intensive process of manually harvesting metaphase spreads. More importantly, since there are no user manipulations of the process, the quality of metaphase spreads obtained is always reliable and consistent. These systems perform repetitious tasks such as centrifugation of cell suspensions, aspiration and safe disposal of supernatant, treatment with hypotonic solution and fixation of cells with an acetic acid: methanol fixative involved in harvesting of metaphase spreads from cultures under controlled environmental conditions in a one-step protocol with very limited user interaction. The harvesters are also integrated with temperature probes connected to a database repository to ensure accurate temperature during processing. In order to accommodate 15-ml centrifuge tubes centrifuge rotors of the PII and PIII harvesters are customized and equipped with specially

designed rotor inserts. Customization also involved adjustment of the position of the vortex unit relative to the centrifuge buckets so that centrifuge tubes are vortexed precisely. Similarly, the aspiration tubes have also been customized and designed to avoid any clogging. Actual temperature readings were found reproducible over independent runs. Incubation time during fixation and swelling were precise and sample test runs are repeated for calibrations.

The Hanabi metaphase spreader provides a chamber for the spreading of cytogenetic specimens (ex: chromosome spreads) with controlled environmental conditions with optimized temperature and humidity. The spreader is equipped with temperature and humidity probes connected to the LIMS repository. It is well known that metaphase spreading on glass slides is influenced by temperature and humidity [25]. We have tested both humidity and temperature conditions during several runs and its consequence on the quality of metaphase spreads. Spread level, base temperature, drop volume and angle of slides are optimized to achieve optimal quality metaphase spreads, which is crucial for automated chromosome-aberration analysis. Slide cleaning by methanol wiping and hydrophilic surface treatment by alkali were tried to further optimize the spread levels. Drop volume of 15 μ l was found to be optimum to achieve desired and consistent spread quality.

3.8 Autostainer

The Thermo Shandon Varistain autostainer (Thermo Scientific, USA) provides a rapid and consistent method of staining slides with Giemsa with minimal human involvement. Shandon Varistain Gemini is a software-driven automated stainer, which moves slide holders among pre-selected bins. It is used for automated staining of metaphase spreads. We have developed a protocol for staining the spreads and a SOP has been established for use of this equipment in a

high throughput laboratory and integrated with the LIMS. The instrument is calibrated for its movement, processing time, stain time and staining quality over several runs. The unit has been programmed and optimized for commercially available Giemsa stain (Merck, USA).

3.9 Cytogenetic image acquisition

Cytogenetic assays are time demanding, even for automated systems, as they require: (a) extensive scanning of microscope slides under low-resolution to identify chromosome plates, and (b) imaging of chromosome plates under high-resolution for detecting and enumerating dicentrics. The latter presents a tradeoff between field-of-view (FOV) and resolution. For single-objective-lens microscope systems, a slide can be imaged under low magnification or in contrast, a small area can be imaged under high resolution but not both. To overcome these hurdles and automate the repetitious processes of scanning and analyses [27] under high resolution, researchers have adopted 3 principal approaches: **(A) Tiled imaging**, which is based on acquiring a number of 2-D image tiles and then stitching them to develop a composite image using an appropriate algorithm. This approach is susceptible to mechanical inaccuracies of the scanning process. Improved focusing requires more focus points across the whole slide or periodic focus checks, both of which decrease throughput. At high resolution, i.e., 250 nm/pixel sampling distance, the number of tiles required to image an entire assay slide rises into the tens of thousands making it very difficult for algorithmic operations and usage. **(B) Pushbroom scanning or Line scanning** that combines a linear sensor with a continuous motion of the stage to produce parallel image strips. This approach is also subject to errors because of non-repeatable stage performance, leading to strip discontinuities and confounding image analysis. Improved focusing requires more focus points across the whole slide, thereby slowing down throughput. Focus points need to be carefully registered and focus discontinuities between adjacent strips are

a likely defect. **(C) Flying spot approach**, in which a single detector is used to accumulate a full image within the FOV. The flying spot approach for whole slide imaging is by far the slowest among the three options (A), (B) and (C).

The above conventional paradigm between high-resolution imaging and FOV [27, 28] can be overcome by the use of *array lens*, where a compound objective consisting of many small objective lenses can image a large area such as an entire slide in a single swipe along the slide. Weinstein *et al* [29] have illustrated the capabilities and advancement of imaging techniques that was later adapted in DMetrix EX 40, a digital pathology platform. This system has been customized (under a co-operative development agreement with Dmetrix, Inc., USA) for digitizing the glass slides with metaphase-spreads to high-resolution digital images for subsequent analyses. Transport of slides from the slide magazine to the microscope stage is accomplished by the Slipstream® system, in which each slide is lifted on a cushion of air, for transport. The slide is then gently lowered onto a precision stage for identification and imaging. Once imaging is completed and digitized the slide is returned to the magazine by the same airlift.

Image acquisition is done by CLASP, which uses DMetrix DX 40 for image acquisition or conversion of physical slides into high-resolution digitized *eSlides* without any image manipulations. Raw images acquired using this system are shown in Fig. 6. DX 40 uses 80 lenses in a single objective and scans the whole-slide on an average of about 1.5~2.5 minutes/slides at 20x optical magnifications or at 40x digital lens producing a 4-12 GB image file converting the physical slide into an *eSlide*. The slides once mounted and cover-slipped can be loaded into the DMetrix cassette, which can hold up to 50 slides in each cassette. A multi-cassette deck can be included when more glass slides need to be analyzed at one time. The image files of *eSlides* are ported to a network computer and are transferred on a gigabit port to the LIMS for image

archive. There are several well-developed ePathology platforms such as MetaSystems GmbH, Germany, Ventana Medical Imaging Inc, Tucson USA and Leica Biosystems, Illinois, USA that could be used besides DMetrix EX 40. Any of these ePathology platforms can be further customized and integrated to a LIMS to improve throughput. However, such an effort may require further validation of sensitivity and validation of analysis.

3.10 Image analyses algorithm and data reporting

Automation of DCA and other cytogenetic assays involves a significant amount of imaging, image feature extraction and image interpretation for analysis. Once the eSlides are created from the glass slides, image analysis is performed to detect metaphase spreads and then detect and enumerate dicentrics. We have developed an Artificial Intelligence (AI) based self-training algorithm called “Supervised Spread Detection Algorithm” (SSDA), which is illustrated in Fig. 7. SSDA uses a neural network based model for scanning all digitized images at low magnification. SSDA algorithm uses a Laplace transform¹ to compute similarity of a metaphase spread against a database of good spreads and self trains for detecting metaphase spreads. Once the spreads are correctly picked, the process becomes a highly iterative machine learning and neural network process, and searches for patterns of dicentrics against the CIT SQL server. Then it scores them accordingly by matching the signal intensity using a cross checking algorithm. If the samples do not conform to the standards set by the first filter then they are discarded after 3 iterations and stored on the LIMS server for evaluation purposes and log. The second filter checks for the spreads and dicentrics and within the spreads by enumerating the number of chromosomes in each spread of the digitized slide. The programming algorithm takes a high

¹ The **Laplace transform** is a widely used integral transform in mathematics and electrical engineering that transforms a function of time into a function of complex frequency. It is named after Pierre-Simon Laplace.

398 resolution digitized image of a metaphase spread from a slide, counts the number of
399 chromosomes in that image and determines if the chromosomes are dicentrics, tracentrics,
400 acentrics, or others. Each spread is checked for the number of chromosomes and subsequently
401 for dicentrics, if any. The scored images are cross-evaluated against a virtual digital image
402 library before the predictions are made and position weight metric scoring takes place. Bio-
403 statistical AI algorithm is applied on the selected or on the scored dicentrics to validate their
404 specificity (SP) and sensitivity (SN). Using the metaphase spreads from the inter-laboratory
405 comparison study [20] we validated the dose estimator module. Table 2 shows data on validation
406 of the dose estimator module of CLASP by a comparison against manual scores in the relevant
407 dose range. This represents analysis of blinded samples during a simulation exercise that were
408 randomly selected from the LIMS CIT and validated against manual scoring. The automated
409 scoring was performed using a contextual position weight matrix (CPWM). The dose estimator
410 module of CLASP is tested and validated against scores analyzed, to provide a dose range to the
411 user as shown in the Table 2.

412 CLASP uses an algorithm prototype developed in-house that can make use of various image
413 attributes of metaphase spreads and dicentrics, parameterize them on a weighing scheme using a
414 CPWM, and use that information for building a suitable Markov order chain. This process is
415 developed using human annotation, which is later transformed using a weighing vote scheme in
416 to a Markov Model (MM) chain of ' n^{th} ' order. The process is automated subsequently. The
417 developed prototype features a module that helps in re-adjusting the precision of result-oriented
418 excision, which can be increased with higher order of the chains based on the real time data
419 (hence the " n^{th} " order MM chain). The benefit of such modeling relies on the use of the real time
420 sample data that can be used in training before automating the algorithm. The image analyses is

421 done using highly customized code scripts developed in Definiens Developer XD (Definiens
422 AG, Germany) using contextual image analyses base code.

423 Conclusion

424 An accurate and timely biological radiation dose assessment is critical to triage a large
425 number of people who are exposed to radiation and predict ARS, determine partial-body
426 exposures to guide treatments as well as for assessing late health risks after a radiological
427 mass casualty. The widely accepted gold standard cytogenetic assay, DCA, in the present
428 form is of limited use to assess radiation dose to individuals after mass casualty, because
429 of throughput issues. However, laboratory automation can help improve throughput and
430 enhance the value of this biodosimetry method. Automation will also improve the quality
431 of data. Cytogenetic laboratory automation requires automated processing of a large
432 number of peripheral blood samples obtained from individuals for preparing cytogenetic
433 slides and then analyzing metaphase spreads or micronuclei by expert scorers according to
434 recently standardized protocols. In this paper, we have demonstrated such laboratory
435 automation using commercially available, off-the-shelf technologies by customized
436 technology integration and implementation of a LIMS for data management.

437 Alternatively, increased workflow in a cytogenetic biodosimetry laboratory may also be
438 accomplished by the combined use of microplates and tube racks of standardized formats
439 at all stages of the biodosimetry assay protocol, which may help overcome some critical
440 bottlenecks, if not all. Substantial progress has also occurred over the years in the
441 development and commercialization of instruments that are capable of automatically
442 analyzing and scoring cytogenetic aberrations with or without scorer intervention. Future
443 work should focus on standardization and validation of these automated systems across

444 different laboratories. Also use of the internet for analyzing images of cytogenetic
445 specimen by scorers in established cytogenetic networks to overcome labor intensive
446 burden by increasing the number of skilled personnel across the globe, is also a step
447 forward.

448 Conflict of interest statement

449 There are no conflicts of interest to report.

450 Acknowledgement and Disclaimer

451 This research was primarily supported by the Armed Forces Radiobiology Research
452 Institute (AFRRI) and inter-agency agreement, Y1-AI-5045-04, between the National
453 Institute of Allergy and Infectious Diseases (NIAID), NIH, AFRRI's Intramural grant
454 RBB42167 and Defense Medical Research and Development Program (DMRDP) grant
455 D61_I_10_J7_39. Preparation of manuscript is in part is supported by the National Cancer
456 Institute's (NCI) Radiation Research Program. The content of this article represents the
457 personal views of the authors and does not necessarily express the opinion or policy of the
458 US Department of Defense, U.S. Department of Health and Human Services or its
459 components. No statement in this article should be construed as an official position of the
460 U.S. Department of Defense or Department of Health and Human Services or its
461 components. We profusely thank Mr. Hiten Pandya for his very valuable computational
462 simulations and automation network support. We thank CAPT David R. Lesser and LTC
463 Mitchell Woodberry for very useful discussions and support during the course of
464 manuscript preparation. We thank Dr. Nagesh Bhat for cytogenetic validation and scoring.
465 We thank Col Andrew Huff, COL Patricia Lillis-Hearne, COL Mark Melanson, Dr. Bert

466 Maidment, Dr. Richard Hatchet, Dr. Narayani Ramakrishnan and Dr. Norman Coleman
467 for their support.

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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.

CLASP ARCHITECTURE

Fig.2. Overview of cytogenetic laboratory automated scoring platform (CLASP) architecture and its various modules. CLASP is an automated cytogenetic analysis suite with integrated hardware and software tools to facilitate information management, module development, prototype testing, application deployment, analysis and reporting of dicentric analysis.

Fig.3. A component overview of the cytogenetic laboratory automated scoring platform (CLASP) and the laboratory information management system (LIMS) architecture and its cross communication with sample processing equipment and environmental monitoring systems.

Fig.4. Sterility of cell culture medium aliquoted using the Tecan EVO 200. Agarose gel showing PCR products using primers for Mycoplasma genomic DNA. No mycoplasma was detected in any of the samples. Lane 1 and 20: 100 bp ladder; lane 2: positive control; lane 3: negative control; lane 4-19 MarrowMax medium dispensed with Tecan Evo 250 and maintained in a cell culture incubator for one week. No mycoplasma or bacteria/mold contamination was detected in the samples handled by the Tecan.

Fig.5. Schematic illustration of integrated robotic blood handler (A) syringe dilutors (B) vacutainer piercing syringes, (C) centrifuge access arm, (D) tube manipulation arm, (E) automated indexed centrifuge, (F) cell viability analyzer, (G) centrifuge access door, (H) cell viability analyzer sample access port, (I) system waste containers, (J) original vacutainer holders, (K) sample mixing centrifuge tubes, (L) reagent carriers, (M) integrated positive identification station. (Acknowledgement; Courtesy of Emily Coren, digital artist, ex employee AFRRI)

Fig.6. Digitized image from a slide using DMterix DX 40 (DMetrix Inc., USA) where as shown in the arrow, a small area of slide magnified.

Fig. 7. Neural network and CPWM supported image diaphragm capture in high-throughput enumerating the spreads in about ~15 seconds per image per GRID CPU.