Sample collection for high throughput radiation biodosimetry

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Abstract

The **R**apid **A**utomated **BI**odosimetry **T**ool (RABIT) is a fully automated, ultra-high throughput robotically-based biodosimetry workstation. It screens fingerstick-derived blood samples, either to estimate past radiation dose, or to sort individuals exposed above or below a cutoff dose. The throughput of the RABIT is 6,000 samples per day per machine with future upgrade to 30,000 samples per day per machine. One of the major challenges encountered in the design of high throughput screening platforms, such as the RABIT, is that of sample collection. Due to the necessary interaction with people, this is the only part of the RABIT that cannot be automated. We present here the requirements for sample collection, our operational concept for sample collection, as well as first tests of its reliability.

Introduction

After a large-scale radiological event, there will be a major need to assess, within a few days, the radiation doses received by tens or hundreds of thousands of individuals, both in order to assess radiation risk in what is sure to be a resource-limited scenario [1, 2], as well as to reduce panic by reassuring those who were not significantly exposed. As the general population would not be carrying physical dosimeters, a very high throughput means of assessing the radiation exposure, based on biological endpoints will be needed. Current "high throughput" biodosimetry can, at best, assess ~100 individuals per day [3, 4].

In recent years, we have developed the **R**apid **A**utomated **BI**odosimetry **T**ool (RABIT) [5-8], a fully automated, ultra-high throughput (6,000-30,000 samples per day per machine) robotically-based biodosimetry workstation. The RABIT is based on complete automation of two well-characterized biodosimetric assays, the γ-H2AX assay [9] and the micronucleus assay [10, 11]. Both assays, as implemented manually, are in current use in radiation biodosimetry, and are highly radiation-specific [11-15].

The γ-H2AX assay [9, 16] directly measures DNA double strand breaks (DSB), which have a highly linear relationship with dose, by immuno-staining the phosphorylated H2AX histone which localizes to them. This assay gives a same day result, but requires that the blood samples are available within about 36 hours of irradiation.

The micronucleus assay [10, 11] quantifies radiation-induced chromosome damage expressed as post-mitotic micronuclei. Lymphocytes are cultured to division but cytokinesis is blocked, preventing separation of the two new cells. Healthy lymphocytes form binucleate cells, while those with chromosome damage can form an additional micronucleus containing chromosomal fragments, with the yield of micronuclei per

binucleated cell increasing monotonically with dose. A key advantage of the micronucleus assay is that the signal is stable for years post-exposure, so the need for early acquisition of blood samples is removed. Due to the required culture time, analysis time for this assay is 3 days.

One of the major challenges of ultra-high throughput screening is that of sample collection. Due to the necessary interaction with people, this is the only part of the RABIT that cannot be automated. In this context, one of the important design improvements of the RABIT is that it has been designed to use fingerstick blood-samples as opposed to larger, venipuncture-based, samples required for manual processing.

Since public health agencies are not likely to have enough trained staff or volunteers to effectively respond to such an event [17], we have designed a sample collection procedure and kit based on collecting fingersticks of blood using a lancet, which allows obtaining blood samples, compatible with processing in the RABIT, by a minimally trained sample collector.

We present here our operational concept for sample collection, as well as the first tests of the procedure's reliability.

Requirements

- To achieve high throughput, sample collection must be **minimally invasive** and should not require highly trained personnel.
- Samples will be collected in the field and will need to be transported to the RABIT with **no spillage** and **no cross contamination**.
- The RABIT is designed to isolate lymphocytes, by centrifugation, from small volumes of whole blood, in heparin-coated capillaries. To ensure separation of lymphocytes out of whole blood samples, the blood needs to be **layered** above separation medium with **no mixing**.
- The lymphocytes in the collected blood need to be kept **viable** as the micronucleus assay requires them to be cultured to division.
- During transport, the blood needs to be kept **chilled** to prevent γ-H2AX foci decay (see figure 4a in [18]).
- Patient information needs to be **tracked** and **correlated** with the samples.

Collection sites

Over the last few years local and federal agencies have developed plans for rapid distribution of countermeasures in response to an outbreak of infectious disease. At the center of this planning is the POD (Point of Dispensing) concept [19-21]. The original purpose of a POD is to dispense countermeasures, educational materials and emotional support, primarily to asymptomatic individuals during an epidemic. The layout and workflow [19] of a POD is optimized for an orderly flow of people from the entrance to the point of dispensing, and finally to the exit of the POD, with symptomatic individuals directed to hospitals.

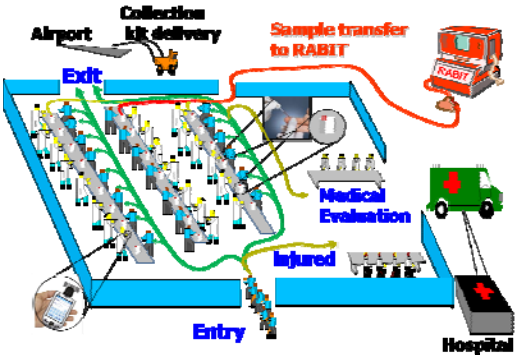

Figure 1: Possible scheme of RABIT sample collection site.

As an example, during the World Trade Center disaster [22], multiple triage sites were set up by local authorities at church halls, schools and hospitals in the immediate vicinity of ground zero. Injured individuals

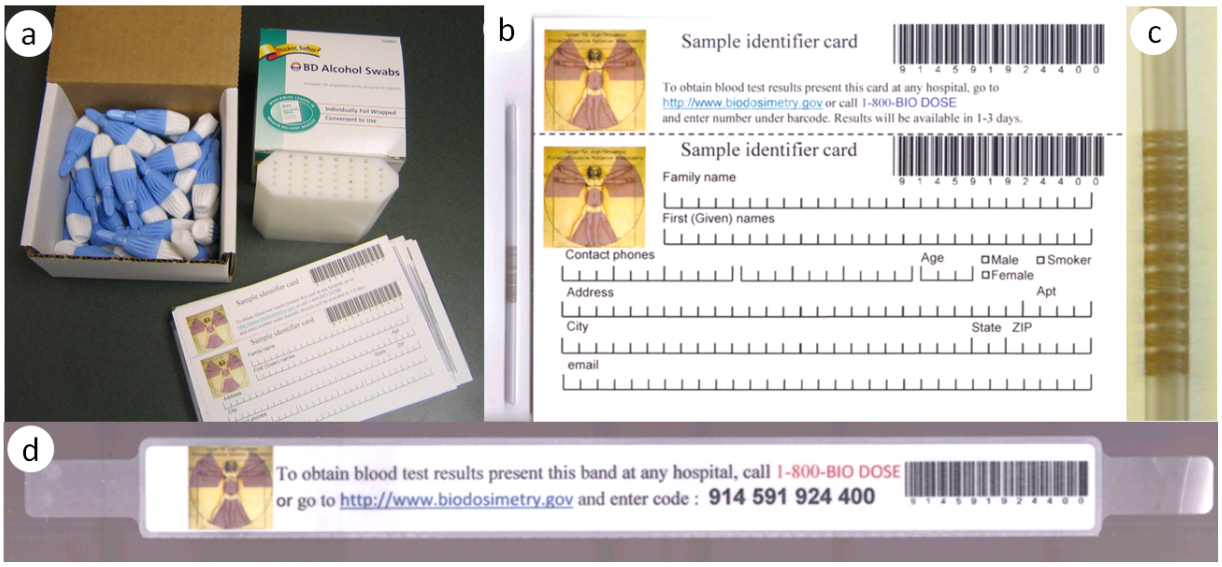

Figure 2: a) sample collection kit, b) data collection card with capillary, c) close-up of bar-coded capillary d) wristband.

were evaluated at these locations and then evacuated out of the area. It is expected that a similar scenario will occur following a radiological event. Sample collection for the RABIT, as described below can be easily merged into this emergency response scenario (Figure 1):

Each site will be augmented by a number of sample collectors, who will draw the blood and verify the contact information. Individuals with other injuries (e.g. trauma) will be triaged by a medical professional and can be evacuated to a hospital. Those who appear healthy, other than the possible radiation exposure, will be sent home after the sample collection. Samples will then be packed and transported to the RABIT (across the hall or in a different state).

Sample collection kit

In order to facilitate blood collection we have developed a sample collection kit (Figure 2a), consisting of lancets, bar-coded, heparin coated, capillary tubes with matched personal data cards and patient tracking wristbands, alcohol wipes and sample holders for filled capillaries. The kit is designed to match the 32 samples that can be collected over a 2-3 hour collection period by one sampler. We envision a few hundred such collection kits would be kept at local emergency response stores, as part of the POD Go-kit [23] and would be ready to be used immediately. A much larger number of kits can then be stored at the Strategic National Stockpile (SNS), as part of the "12-hour push package" [24], and will arrive at the POD within 12 hours of a request by local authorities.

Data collection card

Similar to the situation for foreign nationals entering the US, individuals will be handed a data collection card (Figure 2b), where they are to enter personal and contact information, on entering the POD. In addition to the contact details, processing in the RABIT may require knowing the age, gender and smoking status so that this information is also included.

The card has a printed barcode which is matched to the barcode etched on a heparinized PVC capillary (Figure 2b, c), attached to the card, and a detachable human readable version of the same code, with instructions on how to obtain the results of the blood test, is also provided.

Alternatively, the card can contain an integrated self laminating wristband (Figure 2d, Laserband, St Louis, MO) which is detached and applied to the individual. The wristband contains information allowing the individual, or their medical caregiver to obtain the results of the blood test 1-3 days following the sample collection.

Lancet

Since the RABIT requires 30 µl of blood and since multiple fingersticks would reduce the speed of triage, we have tested a variety of lancets, offering wide range of blade depths and needle gauges to optimize blood flow with minimal pain. The Microtainer® Contact-Activated Genie™ Lancets from BD Diagnostic Systems have proven to be the most reliable in providing a 30 µl finger drop of blood that is needed for blood collection into capillaries. Indeed, Fruhstorfer [25] has demonstrated that these lancets result in >50µl of blood for 84% of the individuals tested.

Capillary

Each individual sample is collected into a 100 µl barcoded PVC capillary. The advantage of plastic capillary tubes is that they are safer to handle than glass and are easily laser etched, allowing bar-coding and rapid cutting, both of which are required for use in the RABIT.

The 10-digit bar code on the capillary, Figure 2b, c was etched by an ultraviolet laser marking system (Quantronix, Osprey-355-1-0) with a wavelength of 355 nm and using a power of 0.5 W. Each etching covers about a third of the circumference of the capillary. Three laser etchings are used to assemble a bar code around the entire circumference of the capillary.

The success of reading these bar codes by the high-precision bar code reader (Siemens, Hawkeye 1525HD) installed in the RABIT is currently 95%, due to a slight overlap of the three etchings performed. This will be rectified by rotating the capillary during barcode reading.

The lymphocytes are isolated from whole blood by centrifugation of the capillaries containing whole blood, followed by laser-cutting the capillaries in between the isolated lymphocyte band and the red blood cell pellet. The Same UV laser, with 1-W output power, 3.5-sec cutting time, and 40-rpm capillary rotation speed, has achieved better than 98% success rate in cutting these capillaries.

Capillary holder

A capillary holder, Figure 2a, 3 was designed to serve the dual purpose of facilitating sample collection and shipping as well as an insert for loading multiple capillaries into the RABIT.

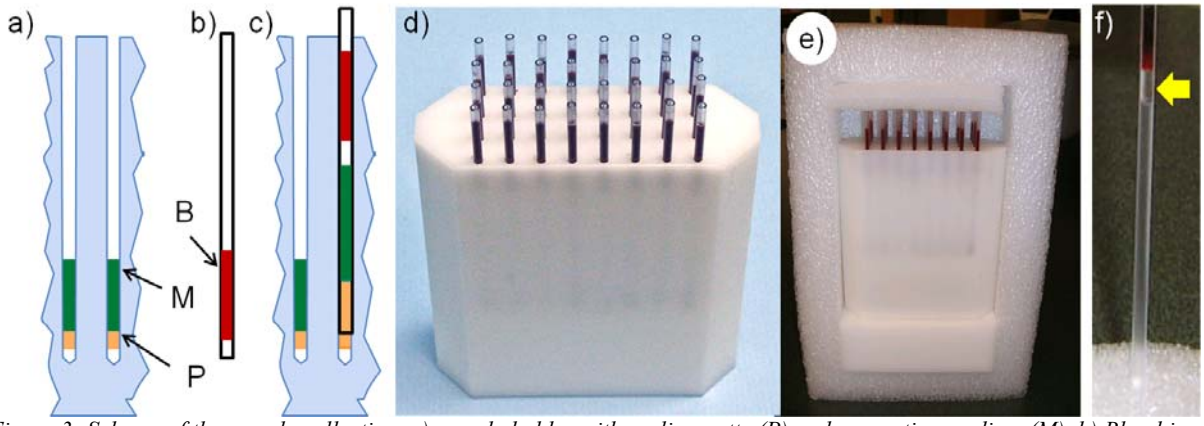

Figure 3: Scheme of the sample collection. a) sample holder with sealing putty (P) and separation medium (M), b) Blood in a capillary (B), c) capillary loaded into sample holder layering blood above the separation medium without mixing. d) Photo of filled capillary holder. e) Samples packaged for shipping. f) Blood-filled capillary removed from holder after shipping from ASU. Note that the air gap between the separation medium and blood is intact.

Design of the holder was driven by the size and weight limitations of the centrifuge, and the 7.5 mm pitch between capillaries required for robotic access. Each holder (length 83.9 mm, width 27.90 mm, height 95.5 mm) contains 32 capillaries and 3 holders fit snugly into each centrifuge bucket (400 ml rectangular bucket, Eppendorf, Hauppauge, NY), leading to a total centrifuge capacity of 384 capillaries.

A schematic cross section of the capillary holder, prefilled with sealing putty (Fisherbrand Hemato-Seal Capillary Tube Sealant, Fisher Scientific) and separation medium (Histopaque-1083, Sigma Aldrich) is shown in Figure 3a. The dimension of the holes is 2.2 mm in diameter and 55.0 mm in depth.

Sampling procedure

After loading about 30 μl of blood into the capillary, the sample collector then seals the top of the capillary with their (gloved) thumb, begins inserting it into the holder, while releasing their thumb to allow trapped air to escape from the capillary.

As the blood in the capillary (Figure 3b) does not reach its edge, when the capillary is inserted into the holder, an air bubble is trapped between the blood and separation medium, preventing their mixing during shipping (up to 24 hours).

The sealing putty is compressed into and around the capillary ensuring a seal (Figure 3c), requiring 0.2-0.8 N of force to extract the capillary from the holder. This prevents the capillary from falling out even if the holder is inverted and vigorously shaken, but still allows the RABIT robotics to extract the capillary from the holder (better than 99% reliability). As the bottom of the capillary is sealed, the blood and separation medium cannot leak out. This procedure allows the sample to be collected by an individual with minimal training, while maintaining the required layering of the blood and separation medium and preventing contaminations. We have seen that the technique for this can be learned in a few minutes.

Shipping

After the capillary holder is filled with 96 capillaries (figure 3d), the top of the capillaries is sealed with a foam rubber mat, to prevent contamination of the samples, as shown in figure 3e, and the capillary holder can be wrapped and shipped to the RABIT.

As the γ-H2AX assay, which does not require culturing the lymphocytes, provides a much faster processing (a few hours compared to 3 days for the micronucleus assay), it is the assay of choice for rapid Triage. To reduce γ-H2AX signal decay during shipping, the samples need to be chilled to 4-10 °C. This can be done by adding ice packs in with the samples for shipping (see for example [26]).

No such cooling needs to be done for the micronucleus assay. Indeed, we have verified that capillaries stored at room temperature for 24 to 48 hours, still contain viable lymphocytes, which undergo mitosis when stimulated in the RABIT.

Testing

Micronucleus assay

In initial field tests, irradiated (0 and 2 Gy γ-rays) blood samples were collected at Arizona State University, loaded into capillary holders and shipped to Columbia University in the city of New York, using a commercial carrier. All capillaries arrived without spillage and without the disruption of the blood and lymphocyte separation medium layering (Figure 3f).

To test the viability of the cells following this procedure, the lymphocytes were isolated as described previously and then

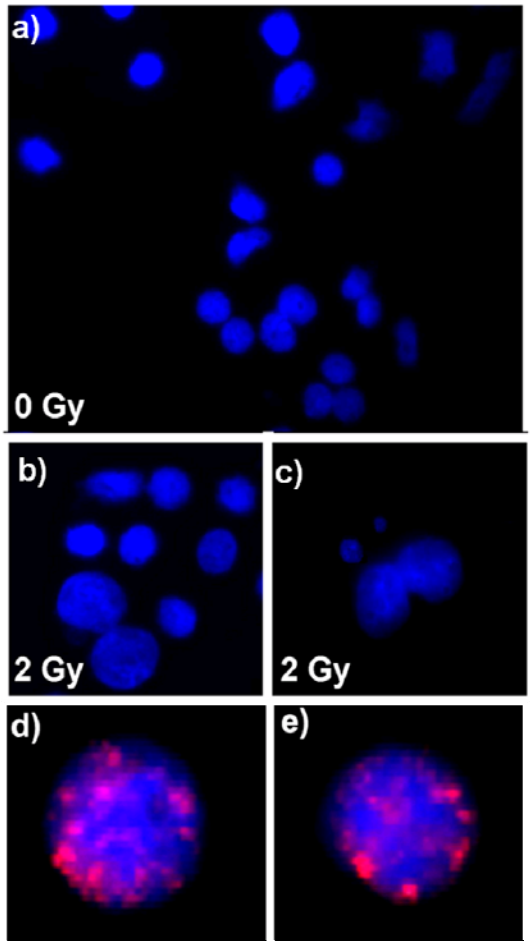

Figure 4: a-c) CBMN assay performed on blood samples collected at ASU and analyzed in NYC. Note: The formation of healthy-looking bunucleate cells indicate that the lymphocytes remained viable and underwent mitosi following 24 h transportation. a) unirradiated lymphocytes b) 2Gy, c) Close up of binucleated lymphocyte with two micronuclei. d-e) γ-H2AX Foci in lymphocytes irradiated with 2 Gy: d) 30 min post exposure, d) after an additional 24 h at 4°C.

processed, in multi-well plates using the cytokinesis-blocked micronucleus assay [27]. Figure 4a shows images of the DAPI-stained lymphocytes after the first division. An appropriate fraction of binucleated cells is visible at both 0 and 2Gy. In addition, at 2 Gy we observed micronuclei in some of the lymphocytes (figure 4c). This demonstrates that the cells maintained their viability in the capillary tubes.

γ-H2AX assay

To verify that it is feasible to delay the γ-H2AX decay by chilling the blood samples, blood-filled capillaries, irradiated with 2 Gy γ-rays were prepared and stored at 4°C. Preliminary results showed that 24 hours post-irradiation the γ-H2AX foci yields (figure 4d) remained close to the yields observed 30 min post irradiation (figure 4e), and were significantly higher than those of blood samples stored at room temperature or 37°C (Manuscript in preparation).

Conclusions

Following a mass radiological event, there will be an immediate need to triage large numbers of individuals for radiation exposure. Over the past few years, we have developed an automated workstation for retrospective biodosimetry in a mass casualty scenario. We present here our concept of sample collection and shipping, compatible with robotic processing and demonstrate its use.

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