Applications of the Cytokinesis-Block Micronucleus Cytome Assay on Human Lymphocytes: Construction of a Calibration Curve for use in the Rapid Automated Biodosimetry Tool

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Abstract

An _ex vivo_ study of the dose responses of isolated human lymphocytes is conducted with the aim of creating calibrated dose response curves for biodosimetry measuring up to 10 Gy of radiation. These curves are created for future use in the Rapid Automated Biodosimetric Tool (RABIT), a robotic system used to quickly assess the received radiation of many blood samples in the case of a radiological event. The cytokinesis-block micronucleus cytome (CBMN Cyt) assay was employed to count the frequencies of micronuclei and mononucleated cells per binucleated cell in blood samples from 10 healthy donors, using manual scoring to collect micronuclei data and an automated program to collect mononucleated cell data. The whole blood samples studied received doses from 0 to 10 Gy of radiation. These samples were stored at 37°C and were scored at three time points (0, 24, and 48 hours) post-irradiation. It is shown that micronucleus frequency can be used for biodosimetry up to 5 Gy of radiation and mononucleated cell frequency can be used from 5 to 10 Gy of radiation for all time points. A calibration curve combining the average dose responses of both cell counts at 5 Gy for each time point is presented. It is concluded that biodosimetry up to 10 Gy can be achieved using the CBMN Cyt assay if both micronuclei and mononucleated cell frequencies are considered.


**Introduction**

*The RABIT*

If a radiological dispersion device were detonated or an industrial accident occurred in a populous area, immediate large-scale screening would be needed to evaluate the radiation doses received by thousands of people. Medical facilities would be overloaded with civilians seeking assessment, including both those exposed to damaging amounts of radiation as well as those who received little or non-harmful doses (Coleman and Parker, 2009). A fast and reliable automated method of radiation biodosimetry, the calculation of the absorbed dose of ionizing radiation, is required for triage and treatment in this critical scenario (Pellmar et al, 2005).

The Center for High-Throughput Minimally-Invasive Radiation Biodosimetry at Columbia University has developed a robotically-based Rapid Automated BIodosimetry Tool (RABIT) capable of handling large samples of human material for biological processing and imaging for biodosimetric purposes (Chen et al., 2010; Garty et al., 2010). Fingersticks of blood that can be easily and minimally-invasively obtained are loaded into the system, which stores the samples, cultures peripheral blood lymphocytes, prepares and images the samples, and estimates the received dose based on calibration curves. Two established biodosimetric assays, the \(\gamma\)-H2AX (Nakamura et al., 2006) and the cytokinesis-blocked micronucleus cytome (CBMN Cyt) assay (Fenech et al., 2007), are utilized by the RABIT. Beyond sample collection, the system is fully automated and self-contained in a compact workstation (Figure 1). To our knowledge, unlike other automated biological systems which have been proposed or produced (Prasanna et al., 2005; Schunck et al., 2004), the RABIT completes both the full biological protocol and data analysis without human intervention while achieving high sample throughput. High-throughput refers to the assessment of many samples at a time; the RABIT can presently process 6,000 samples per day and is under development to reach the ultimate objective of 30,000 samples per day, far above current biodosimetric standard capacities (Martin et al., 2007). The commercial production of this apparatus would therefore aid in the event of a radiological disaster in a highly populous area such as New York, where tens of thousands of people would need rapid assessment.

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*Figure 1: The RABIT workstation prototype.*
The CBMN Cyt Assay

While the RABIT prototype has been successfully constructed, improvements to the system for future production are under current development. Both the γ-H2AX assay, which has a short processing period and must be implemented within 36 hours of radiation exposure, and the CBMN Cyt assay, which has a 72-hour processing period but can be used beyond 36 hours, are being examined to create reliable dose response curves for specific use in the RABIT. Although other dose response curves utilizing these assays exist (Acharya et al. 2009; Thierens et al., 1991), it is necessary to determine device-specific calibration curves because scoring cells for data collection can differ even among labs using the same criteria and protocols (Fenech et al., 2003). This paper focuses on the construction of a calibration curve for the RABIT based on the CBMN Cyt assay.

The CBMN Cyt assay is a comprehensive technique for measuring chromosomal damage by stimulating cells to mitosis and scoring the occurrence of micronuclei (MNi) and other features (e.g. nucleoplasmic bridges and nuclear buds) in them (Fenech, 2007). Lymphocytes are cultured to division but cytokinesis is blocked, preserving the daughter nuclei within the original cytoplasm. Healthy lymphocytes form binucleated (BN) cells with two daughter nuclei, while fragments of chromosomes damaged by radiation that are not incorporated into the main nuclei during cell division form MNi in addition to the daughter nuclei. MNi, which are morphologically identical to the main nuclei, are differentiated by their size, between 1/16 and 1/3 the diameter (Fenech 2007). As radiation dose increases, the expression of MNi in BN cells increases monotonically as a linear-quadratic dose response curve (Thierens et al., 1991).

The CBMN Cyt assay provides a simple scoring procedure which is relatively quick in comparison to other assays, requires less specialized expertise to implement, and allows for repeatability and straightforward automation. These attributes make the CBMN Cyt assay a particularly good candidate for large-scale screening (Amundson et al., 2001). Moreover, these advantages have contributed to the increased use of the CBMN assay as a biomonitoring tool within the last decades since MNi were first suggested as chromosomal biomarkers in the seventies (Heddle, 1973) and the CBMN assay was formally introduced by Michael Fenech and Alexander Morley in the eighties (Fenech and Morley, 1985).

Although the CBMN assay has proven a reliable biodosimetric tool (Amundson et al., 2001; Bonassi et al., 2007; Sari-Minodier et al., 2007), Müller and Rode have shown that MNi frequency alone cannot be used to estimate high radiation doses, above 5-7 Gy (2002). When heavily damaged by radiation, cells are unable to function normally and fewer enter into mitosis, required for the expression of MNi. Our studies confirm this finding and explore the authors’ suggested use of mononucleated (MoN) cell frequency, an alternative feature in isolated lymphocytes that can be determined in the CBMN Cyt assay, as a complementary method at high doses. Because severe exposures to radiation have occurred in past radiation events such as Chernobyl, wherein individuals received doses above 10 Gy (UNSCEAR, 2000), it is necessary that the RABIT can detect doses into this high range.

We have worked to construct a calibration curve based on the CBMN Cyt assay with the aim of establishing its use for accurate biodosimetry up to 10 Gy of radiation. To evaluate the effectiveness of frequencies of MNi and MoN cells as biodosimetric markers, a systematic ex
**vivo** study of lymphocytes collected from ten healthy donors at varying levels of radiation (0-10 Gy) was conducted. The blood was stored at 37°C and studied at 0, 24, and 48 hours post-irradiation to simulate realistic conditions in a mass casualty event. Because our aim was to study the accurate biodosimetric range of MNi frequency as a radiation biomarker and affirm the efficacy of MoN cell frequency as a complementary biomarker outside of that range, preparation and scoring of the lymphocytes was performed by researchers rather than within the automated system. The resulting data will be used to improve the RABIT for future commercial production and operation.

**Materials and Methods**

**Blood Collection and Irradiation**

The RABIT is designed to use only a fingerstick sample (~100µl) of blood so that the procedure is minimally invasive and can be performed by a minimally trained collector (Garty et al., 2010). For our **ex vivo** irradiation studies, larger samples were collected to allow for testing of multiple radiation doses using the same sample. Peripheral blood (~ 12 ml) was collected by venipuncture from 10 healthy volunteers ranging from 25 to 50 years with informed consent. The volunteers had no radiation exposure within a year prior to collection. Gender, smoking status, and other confounding factors specific to the donors were recorded for future evaluation but not revealed to the researchers performing data collection and analysis for the purposes of this study.

Under sterile conditions, the blood was divided equally into 8 sterile centrifuge tubes (at least 1.5 ml each). A control dose was retained (0 Gy of radiation) while the remaining tubes were individually irradiated with gamma rays using a Gammacell 40 137Cs irradiator with different irradiation times to obtain samples of 1, 2, 3, 4, 5, 7.5, and 10 Gy doses.

**Lymphocyte Harvesting and Slide Preparation**

To facilitate study of the cells at multiple time points after radiation exposure (0, 24, and 48 hours), samples of the whole blood were incubated for varied periods. For the 0 hour time point, 500 µl whole blood samples were removed from each dose. The remaining whole blood samples were separated and stored in an incubator (37 °C, 5% CO2) for 24 or 48 hours to obtain samples of 1, 2, 3, 4, 5, 7.5, and 10 Gy doses.
for 28 hours to block cytokinesis and induce BN cells.

To prepare slides for examination, the lymphocytes were fixed with Carnoy’s solution, a mix of methanol and glacial acetic acid (ratio 3:1). A cell suspension from each sample was dropped onto a clean glass slide. The cells were mounted to the slides using Vectashield mounting medium with DAPI (Vector Laborators, Burlingame, CA), a fluorescent stain that binds strongly to DNA. Because most DNA resides in cell nuclei, DAPI enables direct visualization of the presence of both main nuclei and MNi. Finally, the slide was sealed with a cover slip.

**Microscopic Imaging of Cells**

Three fluorescence microscopes were used to view the DAPI-stained samples. For manual scoring of MNi in BN lymphocytes, a Zeiss Axio Imager.Z1 with a 63x Plan Neofluar oil objective and equipped with a DAPI SP100 filter set (Chroma Technology, Bellows Falls, VT) was primarily used. Additional manual scoring was performed on a Nikon Eclipse 50i epifluorescence microscope with a 50x LU Plan Fluor objective and a Nikon UV-2E/C fluorescent filter set.

For automated scoring of MoN and BN cells, images were first captured by a researcher using an Olympus IX70 Fluorescence Microscope with a 40x LWD Hoffman Modulation Contrast PLFL objective (Modulation Optics, Glen Cove, NY). The Olympus microscope is equipped with a Hamamatsu ORCA-ER camera and an Olympus U-MWU filter set. Image-Pro Plus (version 6.0) software (Media Cybernetics, Bethesda, MD) was used to capture images of the cells for entry in our automated scoring software.

**Scoring of Isolated Lymphocytes**

In the RABIT and the studies covered in this paper, CBMN Cyt protocol is only used to score MoN and BN cells and MNi among a range of other possible distinguishing features. For the purposes of this study, manual scoring of micronuclei and manual imaging with automated scoring of MoN and BN cells was performed. A fully automated imaging and scoring algorithm has been created for and proven effective in the RABIT (Chen et al. 2010; Lyulko et al. 2010), but because the automated imaging system is currently under development more efficient scoring could be achieved manually by the researchers.

**I. Manual Scoring of Micronuclei**

Based on the CBMN Cyt assay protocol developed by Michael Fenech (2007), the frequency of MNi occurrence in one-divided BN cells was scored as an indicator of radiological damage. Scoring only BN cells ensures that the cells have progressed to division, the stage in which MNi are expressed. A trained researcher scored the frequency of BN, BN with MNi, and the number of MNi in the BN cells, adhering to the following scoring criteria:

- Viable nuclei (MoN, BN, and MNi) should have intact nuclear membranes distinguishable from the nuclear boundaries of other nuclei and may touch but not
overlap other nuclei.
• The nuclei of an analyzable BN cell should be distinctly separated from the nuclei of adjacent cells, at least three radii lengths away. Because DAPI does not stain the cytoplasmic boundaries of cells, this criterion, which was accepted for the purpose of this study and is not part of the standard CBMN Cyt protocol, decreases the probability of scoring multinucleated cells (≥3 nuclei).
• The area of a MN is between $1/256^{\text{th}}$ and $1/9^{\text{th}}$ the area of a main nucleus.
• Viable nuclei (MoN, BN, and MNi) should be approximately circular in shape.
• The two nuclei of a viable BN cell should be approximately equal in size, staining pattern, and staining intensity.

Figure 2 shows examples of the described cell types. At least 250 BN cells per sample were scored. The resultant dose response data of MNi frequency in BN (MNi/BN) lymphocytes at each time point are summarized in figure 3.

![Figure 2: (Top row) Examples of viable cells scored in the CBMN Cyt assay. From left to right, a mononucleated cell, a binucleated cell, and a binucleated cell containing one micronucleus. (Bottom row) Examples of non-viable cells that would not be scored.](image)

**II. Automated Scoring of Mononucleated and Binucleated Cells**

Müller and Rode determined that up to 10 Gy of radiation exposure, there should be a monotonic increase in the fraction of MoN cells in a human lymphocyte sample (2002). Mononucleated lymphocytes represent the fraction of cells that were not able to divide, likely a result of poor cell function due to increased irradiation also responsible for the decreased numbers of MNi at high doses. The frequency of MoN per BN cell (MoN/BN) in each
Figure 3: Dose response curves exhibit the scoring results of the frequency of micronuclei per binucleated lymphocyte in all donors sampled at each time point studied. The same donor is represented by the same symbol in each plot.
sample was scored to evaluate its efficacy as a biodosimetric tool and to create a calibrated dose response curve.

Digital images of isolated lymphocyte nuclei and a background image (containing no biomaterial) were obtained from the microscope slides. The researchers observed that higher frequencies of MoN cells were found towards the edges of the slides. We conjecture that these lighter cells spread farther when the lymphocyte suspensions was dropped onto the slides during preparation. In an effort to reduce the errors associated with this non-uniformity of cell spreading, the researchers imaged multiple (3 or more) areas over the entirety of each slide.

Images were processed using analysis software designed for the RABIT (Lyulko et al., 2010). The software was written in C, using the Matrox Imaging Library (MIL 8.0; Matrox Imaging Systems Ltd., Dorval, Canada). The software successively processes each image; the procedure is described as follows with illustration of the steps shown in figure 4. Processing begins with background subtraction, in which the background image is subtracted from the nuclei image to remove systematic noise and variations in background illumination from microscopic imaging, followed by application of a median filter to remove random noise and smoothen the image. The image is then binarized, which renders pixels above a threshold level white and the remainder black. Rather than a fixed threshold level, the automatic threshold level in the MIL (the minimum value between the two principal peaks on the image intensity histogram) was used for these studies to account for intensity variations among images.

The analysis software applied the same criteria based on the CBMN Cyt assay protocol as used in manual scoring to identify viable BN and MoN cells and any MNi within them. Below are details of the automated implementation:

- A compactness parameter given by $p^2/(4\pi A)$ was used to describe how closely the binarized shapes were to a circle, where $p$ is the perimeter and $A$ is the area of the object. The parameter is equal to one for a circle and increases for less circular objects. The parameter limit was chosen as 1.5 for healthy lymphocytes; objects
with higher values that are not sufficiently circular (e.g. aggregations or clusters) were excluded from analysis.

- To segment individual nuclei from nearby or touching nuclei, the software first employed a distance transformation to assign peak values to object pixels and basin values to background pixels. A watershed transformation was then applied to build dividing lines between the minimas or maximas.
- A “distance-between-the-nuclei” parameter was introduced to analyze the distance from the center of each nucleus to the center of neighboring nuclei. If more than one other nucleus was within three radii of the nucleus being analyzed, the cell was identified as multinucleated and excluded from analysis.
- Because nuclear sizes can differ both between donors as well as within one donor sample, the size criterion for distinguishing main nuclei from MNi is applied to each cell individually. To identify nuclei as main or MNi, the area of the nuclei in a cell are calculated and sorted by size and the areas of all nuclei are calculated as fractions of the area of the biggest nucleus.

The information about the number of MoN and BN cells and the number of MNi in them was accumulated by the program until all available images were analyzed, at least 150 per sample. Only the MoN and BN cell counts were retained for analysis. The resultant dose response data of MoN/BN lymphocytes at each time point are summarized in figure 5.

Results and Discussion

Microsoft Excel 2003 (Microsoft Corporation, Redmont, WA) and Origin Pro 8 (OriginLab, Northampton, MA) were used for statistical analysis of the collected data.

Analysis of the Dose Response Data

First, the MNi data were studied to determine the use of MNi/BN cells as a biodosimetric tool in the 0-10 Gy range. As shown in figure 3, generally among all donors the MNi/BN cell dose response curves monotonically increased up to but not beyond 7.5 Gy, indicating that MNi frequency is not effective for biodosimetry at the highest doses as anticipated. To precisely evaluate the range in which the frequency of MNi per BN cells proves effective as a radiation biomarker, a paired t-test was applied among data from all of the donors between each consecutive dose to investigate the intervals in which the data were sufficiently statistically separated. Table 1 summarizes these results, which indicate that the frequency of MNi in BN cells is sufficient as a biodosimetric tool up to 5 Gy at all time points because the data are significantly separated (p < 5%) in that range. Above 5 Gy, the higher p-values reflect the discrepancy between donors at high doses. A one-sample Kolmogorov-Smirnov (K-S) test (College of Saint Benedict and Saint John’s University Physics Department, http://www.physics.csbsju.edu/stats/KS-test.html; Massey, 1951) was also applied to investigate the normality of the data, which is necessary for application of a t-test. The K-S test did not refute that the MNi data were drawn from a normal distribution and therefore verified that a t-test could be used to analyze the data.
Figure 5: Dose response curves exhibit the scoring results of the frequency of mononucleated per binucleated lymphocyte in all donors sampled at each time point studied. The same donor is represented by the same symbol in each plot.
Thus, the frequency of MNi in BN cells can be used for a calibration curve up to but not exceeding 5 Gy. These results signified that to attain our goal of using the CBMN Cyt assay for biodosimetry up to 10 Gy, another biomarker effective beyond 5 Gy needed to be identified. The ratio of MoN to BN cells was examined as a possible complementary method at high doses or, if also effective at low doses, as a replacement method.

The MoN cell data were studied similarly to the MNi data to determine the use of MoN/BN ratio as a biodosimetric tool in the 0-10 Gy range. As shown in figure 5, the MoN/BN dose response curves monotonically increased throughout the 0-10 Gy range generally among all donors, indicating that MoN/BN ratio has potential use for biodosimetry beyond the limits of MNi frequency as suggested by Müller and Rode (2002). A paired t-test was again applied among data from all of the donors between each consecutive dose to investigate the intervals in which the data were significantly statistically separated for use as a biodosimetric tool, the results of which are summarized in table 2. Similar to the MNi data, a one-sample K-S test applied to the MoN cell data confirmed the normality hypothesis needed for valid application of a t-test. As shown in table 2, the t-tests indicate sufficient separation (p < 5%) from 5 to 10 Gy at the 0 hour time point. The 24 hour time point shows sufficient separation between 7.5 and 10 Gy but not between 5 and 7.5 Gy, while the 48 hour time point shows sufficient separation between 5 and 7.5 Gy but not between 7.5 and 10 Gy.

The blood sample from one donor provided a clear outlier at 5 Gy. This outlier affected the distribution of the data and, thus, the t-test results. Removal of this point standardizes the dose response curve and indicates sufficient separation of the 24 hour data from 5 to 10 Gy (p = 7.44E-05) while preserving the consistency of the results among the remaining doses. Currently, the researchers are investigating the causes of this outlier to provide a future mechanism that would prevent such incidence. We postulate contamination of the slide, killing healthy cells that would otherwise divide as score-able BN cells, as a possible source of error. However, at the time this paper is written the causes for this error are unknown. At present, new samples from this donor are being prepared for study. The outlier is included in the remaining construction of the calibration curve until proof of error is concluded with the understanding that with removal of the outlier or added data to counteract such points it can

<table>
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<tr>
<th>Dose Comparison</th>
<th>0 Hours Post-Irradiation</th>
<th>24 Hours Post-Irradiation</th>
<th>48 Hours Post-Irradiation</th>
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Table 1: Resultant p-values from a paired t-test applied to MNi/BN cell data from all donors between consecutive doses at each time point. P-values below 5% are shown in red, while those below 10% are in orange.
be shown that the MoN/BN ratio is a viable radiation biomarker from 5 to 10 Gy at the 24 hour time point.

<table>
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<th>48 Hours Post-Irradiation</th>
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<td>0.032</td>
<td>0.059</td>
</tr>
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</table>

Table 2: Resultant p-values from a paired t-test applied to MoN/BN cell data from all donors between consecutive doses at each time point. P-values below 5% are shown in red, while those below 10% are in orange.

Müller and Rode found that the frequency of MoN cells per BN cells continued to increase monotonically up to doses of 15 Gy, but with decreasing slope (2002). The decreased separation of values from 5 and 7.5 Gy versus 7.5 and 10 Gy at the 48 hour time point are therefore consistent with their findings. However, with additional data that are currently being collected, we hypothesize that there will be a sufficient statistical separation between 7.5 and 10 Gy at all time points. Moreover, we note that any radiation damage above 7.5 Gy receives the same form of treatment if not lethal. Thus, a precise dose distinction above 7.5 Gy may not be essential for triage purposes, in contrast to lower doses which require quantity-specific treatment. Therefore, we continued analysis under the conclusion that MoN/BN ratio is a sufficient radiation biomarker from 5 to 10 Gy at all time points.

**Creation of a Biodosimetric Calibration Curve up to 10 Gy**

Based on the results of the t-tests, we concluded that for biodosimetry with the CBMN Cyt assay from 0 to 10 Gy, MNi frequency in BN cells could be used at low doses and the MoN/BN ratio at high doses, with a cutoff between the techniques at 5 Gy. We worked to create a calibration curve for each time point with both cell counts concatenated at this cutoff value.

At each time point, the mean value of MNi/BN or MoN/BN, obtained by averaging over the donor population, as well as the standard deviation among the individual donor values from the mean were derived for each administered dose to construct an average dose response curve for both cell counts. The error given at each dose is the standard error of the mean, the standard deviation divided by the square root of the sample size. A third-order polynomial fit was obtained by regression analysis for each average dose response curve. For the MoN/BN curves, an unweighted fit was applied to account for each dose equally. However, because of
the increased error at high doses, a fit weighted by the reciprocal value of the variance for the considered dose was applied to the MNi/BN frequency curves. Figure 6 summarizes these results.

The cutoff value for each time point was chosen as the value of the fit function for the MoN/BN ratio at 5 Gy. Using two axes to concatenate the MNi frequency fit with the MoN/BN fit at this point, a calibration curve for each time point was produced, shown in figure 7. For biodosimetric use, one first finds the MoN/BN value of their sample. This value can be used to determine a received dose above 5 Gy; if it falls below the cutoff value, a MNi/BN ratio would then be used to estimate the received dose.

**Figure 6:** (Left column) The average value and standard error of the frequency of micronuclei in binucleated cells in all donors at each time point sampled, with third-order polynomial regressions weighted by the inverse of the error at each dose. (Right Column) The average value and standard error of the ratio of mono- to binucleated cells in all donors at each time point sampled, with unweighted third-order polynomial regressions.
Figure 7:
Calibration curves combining fits of the average frequencies of micronuclei and mononucleated cells per binucleated cell for each time point.
Conclusions and Future Objectives

The creation of a calibration curve is essential for development of the RABIT. We have found that in the 0-10 Gy range the CBMN Cyt assay can only be applied for biodosimetry if both the MNi and MoN cell frequency per BN cells are considered for 0, 24, and 48 hour time points post-irradiation. The frequency of MNi per BN cells was confirmed to be an accurate radiation biomarker from 0 to 5 Gy of radiation, while the ratio of MoN to BN cells is accurate from 5 to 7.5, with reasonable accuracy up to 10 Gy. Both sets of data were analyzed separately for each time point. A calibration curve combining the MNi/BN and MoN/BN dose response curves for each time point has been constructed for biodosimetry from 0 to 10 Gy.

Currently, blood samples from additional donors are being collected for further study. These data will likely reduce the error and counteract outliers in our dose response curves and provide a more accurate calibration curve. Moreover, these additional data may substantiate our hypothesis that the frequency of MoN/BN lymphocytes can be used as an accurate radiation biomarker up to 10 Gy by increasing the statistical significance of the resulting data at 7.5 and 10 Gy.

As more data are acquired, we hope to improve our biodosimetry tool by accounting for various confounding factors, systematic inter-individual differences such as age, smoking status, and gender. These factors are known to affect the frequency of MNi in BN cells (Kirsch-Volders et al., 2006; Battershill et al., 2008) and perhaps also affect the ratio of MoN to BN cells. A study of the effect of these factors on the MoN/BN ratio will be conducted in the future using a much larger donor population, but was beyond the scope of this work. We envision the creation of calibration curves specific to individuals of one gender, age group, etc.

Another future improvement to our calibration curves will be the study of the effectiveness of this method beyond 48 hours post-irradiation. The CBMN assay in the RABIT is aimed for biodosimetry 36 hours or more after radiation exposure as a complementary method to the \( \gamma \)-H2AX assay, which is used more immediately after the event. The time points of this \textit{ex vivo} study were limited by the 48 hours in which lymphocytes can survive in culture. Current \textit{in vivo} mice studies and studies of radiotherapy patients may provide added information about MNi and MoN frequency in BN cells at times past 48 hours post-irradiation. Limited numbers of samples could be obtained immediately after a radiological event, rendering signal stability and knowledge of dose responses beyond 48 hours fundamental to production of the RABIT.

Our immediate goal of identifying methods that enable the CBMN assay to be used for biodosimetry up to 10 Gy was achieved as a first phase in our studies towards the ultimate goal of introducing our calibration curves into the RABIT for commercial production and use.

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References

Acharya, S. et al. (2009) The effect of electron and gamma irradiation on the induction of micronuclei in

Amundson, S.A. et al. (2001) Biological indicators for the identification of ionizing radiation exposure in

Battershill, J.M., Burnett, K., and Bull, S. (2008) Factors affecting the incidence of genotoxicity biomarkers in

Bonassi, S. et al. (2007) An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk


Coleman, C.N. and Parker, G.W. (2009) Radiation terrorism: what society needs from the radiobiology-


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bridges in binucleated human lymphocytes: Results of an international slide-scoring exercise by the HUMN


