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Biological Dosimetry by the Triage Dicentric Chromosome Assay – Further validation of International Networking

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Abstract

Biological dosimetry is an essential tool for estimating radiation doses received to personnel when physical dosimetry is not available or inadequate. The current preferred biodosimetry method is based on the measurement of radiation-specific dicentric chromosomes in exposed individuals' peripheral blood lymphocytes. However, this method is labour-, time- and expertise-demanding. Consequently, for mass casualty applications, strategies have been developed to increase its throughput. One such strategy is to develop validated cytogenetic biodosimetry laboratory networks, both national and international. In a previous study, the dicentric chromosome assay (DCA) was validated in our cytogenetic biodosimetry network involving five geographically dispersed laboratories. A complementary strategy to further enhance the throughput of the DCA among inter-laboratory networks is to use a triage DCA where dose assessments are made by truncating the labour-demanding and time-consuming metaphase-spread analysis to 20 to 50 metaphase spreads instead of routine 500 to 1000 metaphase spread analysis. Our laboratory network also validated this triage DCA, however, these dose estimates were made using calibration curves generated in each laboratory from the blood samples irradiated in a single laboratory. In an emergency situation, dose estimates made using pre-existing calibration curves which may vary according to radiation type and dose rate and therefore influence the assessed dose. Here, we analyze the effect of using a pre-existing calibration curve on assessed dose among our network laboratories. The dose estimates were made by analyzing 1000 metaphase spreads as well as triage quality scoring and compared to actual physical doses applied to the samples for validation. The dose estimates in the laboratory partners were in good agreement with the applied physical doses and determined to be adequate for guidance in the treatment of acute radiation syndrome.

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1. Introduction

Accurate dose estimates can be made by biological dosimetry to predict acute radiation syndrome (ARS) within days after a radiation accident or a malicious act involving radiation. Timely information on dose is quintessential for the medical management of acutely irradiated personnel (IAEA, 2001; Waselenko et al, 2004; Coleman et al, 2009; Weinstock et al 2008).

The dicentric chromosome assay (DCA) provides dose estimates to acutely irradiated personnel based on the frequency of radiation-specific dicentric chromosomes in irradiated persons' peripheral blood lymphocytes. DCA is very sensitive due to a low and stable background dicentric frequency (1–2 per 1000 metaphase spreads). Laboratory protocols have been standardized by the International Organization for Standardization (Voisin, 2002; ISO, 2004) and dose levels as low as 0.1 to 0.2 Gy can be detected, when 500 to 1000 metaphase spreads are analyzed (IAEA, 2001).

Analysis of 500 to 1000 metaphase spreads per irradiated subject, however, is neither practical as it is labor-intensive, nor essential in a radiation mass casualty event, where acute risk of ARS development needs to be assessed for potentially a large number of individuals for making treatment decisions. Therefore, in these situations, the precision on estimated doses may be decreased to improve throughput by reducing the number of metaphases analyzed. Analyzing only 50 metaphase spreads, contrary to the routine analysis of 500 to 1000 metaphases, increases the threshold level of detection to 1–2 Gy, which is still adequate to guide treatment of ARS (Lloyd, 1997; Lloyd et al, 2000; Voisin et al, 2001; ISO, 2008), while vastly increasing the speed of analysis and hence dose estimations (Lloyd, 2000). A recently published International Organization for Standardization (ISO) standard specifically addresses the use of the DCA for triage dose estimation applications for radiological mass casualties (ISO, 2008).

Automating sample preparation (Martin et al, 2007) and establishing inter-laboratory networks can complement truncated metaphase spread analysis to further improve throughput of dose estimation. Several networks have already been established or are being established to improve dose estimation throughput, such as Canada (Miller et al, 2007), Latin America (Garcia et al, 1995), Japan (Yoshida et al, 2007) and Europe (Romm et al, 2008; Wojcik et al, 2010). Our recent double-blinded study determined DCA's accuracy and validity among five laboratories following ISO guidelines and demonstrated that an international network even when geographically dispersed could provide accurate dose estimates for dose blinded samples thus validating the DCA for applications in mass casualties (Wilkins et al, 2008). As follow-up, we further analyzed the data obtained in this study and demonstrated that the triage dose estimates were in good agreement among laboratory network partners. While the accuracy of assessed doses decreased with lower numbers of metaphase spreads analyzed, as expected, but it was determined to be still adequate to guide treatment decisions for ARS (Romm, 2010). Encouraging results from these and several similar studies (Miller et al, 2007; Garcia et al, 1995; Yoshida et al, 2007) have led the United Nations (UN) agencies, WHO and IAEA, to facilitate international cooperation among biological dosimetry laboratories and formalizing networks involving several countries around the globe (Blakely et al, 2009).

In the above-mentioned intercomparison study, calibration curves were generated from blood samples collected and irradiated in one central location and shipped to each of the participating laboratories for processing and analysis. During a mass casualty event, however, dose estimates will have to be made using a pre-existing calibration curve that best matches the exposure scenario for radiation type and dose rate. To study the influence of

using a pre-existing calibration curve on assessed dose, the data from the above described study were re-analysed to predict doses to the dose-blinded samples and compared among the laboratory network partners. These estimates were made using full 1000 metaphase spread scoring as well as triage quality scoring and compared to actual doses applied to the samples.

2.0 Materials and Methods

The results presented in this paper were based on an analysis of data obtained in the previous study, where detailed descriptions of materials and methods including blood collection after obtaining informed consent, dosimetry and irradiation procedures, general study design, blood shipment and sample processing procedures, analysis of chromosome aberrations, and statistical analysis can be found (Wilkins et al, 2008).

2.1 Laboratories

This analysis was performed on data from four of the five established radiation cytogenetic biological dosimetry laboratories used in the international intercomparison study (Wilkins et al, 2008): (i) Armed Forces Radiobiology Research Institute (AFRRI), Bethesda, MD, USA; (ii) Bundesamt fuer Strahlenschutz, Oberschleissheim, Germany; (iii) Consumer and Clinical Radiation Protection Bureau, Health Canada, Ottawa, ON, Canada; and (iv) The Research Center for Radiation Emergency Medicine, the National Institute of Radiological Sciences, Chiba, Japan. The Radiation Emergency Assistance Center/Training Site (REAC/TS), Oak Ridge, TN, USA laboratory was not included since, at the time of the original study, they did not have a calibration curve generated in their own laboratory.

2.2 Study Design

2.2.1 Calibration curves—The study design of the original inter-laboratory comparison has been described in detail along with the coefficients of the calibration curves (Wilkins et al, 2008). The parameters for the pre-existing calibration curves including radiation type, dose-rate, dose range, coefficients and standard deviation of the coefficients of the fitted curves for each laboratory is provided in Table 1.

2.2.2 Statistical analysis of calibration curves—Existing calibration curves from each laboratory (lab curves) for Labs B-E were compared to their respective calibration curves as determined by samples sent to them by AFRRI (AFRRI curves). In order to compare if each lab curve was statistically similar to their AFRRI curve two sample tests were used to compare each of the coefficients in the Poisson quadratic regression model. The model includes three parameters (background rate or intercept b_0 , rate of dicentrics or slope b_1 , and curvature of model b_2), therefore Bonferroni corrections were applied (by a factor of 3) to control the overall type I error rate to be less than or equal to 0.05.

The form of the two sample test to assess the null hypothesis of no difference between the parameters in the two models (pre-curve and AFRRI curve) ($H_0: b_{\text{lab curve},i} = b_{\text{AFRRI},i}$, $i=0,1,2$) is as follows:

$$z = \frac{\hat{b}_{\text{pre-curve},i} - \hat{b}_{\text{AFRRI},i}}{\sqrt{\text{var}(\hat{b}_{\text{pre-curve},i} - \hat{b}_{\text{AFRRI},i})}}, \quad (1)$$

which simplifies to:

$$z = \frac{\widehat{b}_{\text{pre-curve},i} - \widehat{b}_{\text{AFRRI},i}}{\sqrt{\text{var}(\widehat{b}_{\text{pre-curve},i}) + \text{var}(\widehat{b}_{\text{AFRRI},i})}}. \quad (2)$$

Where $\widehat{b}_i$, $i=0,1,2$ are the parameter estimates in the two models representing the same coefficient, i.e., both intercepts, or both slopes or both quadratic parameter estimate, and $\text{var}(\cdot)$ is the variance of the component in brackets. The denominator in Eq. 1 simplifies to that in Eq. 2, since we know that the parameter estimates arise from independent data sets and therefore are independent of one another, i.e., there is no covariance between the two parameters.

2.2.3 Dose assessments to dose-blinded samples—Blood samples irradiated with 0.75, 1.5, 3.0 and 4.5 Gy ^{60}Co rays were shipped by air to each of the participating laboratories from AFRRI by FedEx as previously described. All laboratories were blinded with respect to radiation doses to the samples. Radiation doses to unknown doses were estimated by a comparison with each lab curve. For each dose-blinded sample, the dose was predicted by comparison with the pre-existing calibration curves in each laboratory and the calibration curves generated from the samples shipped from AFRRI (AFRRI curves) in the inter-laboratory comparison study (Wilkins et al, 2008). The corresponding 95% Confidence Intervals (CI) were calculated in all cases assuming a Poisson distribution, using the CABAS program (Deperas et al, 2007). Furthermore, the deviation between the estimated dose and the applied dose (% Error) was determined.

3.0 Results

Dose estimates based on the AFRRI curves have been published (Wilkins et al, 2008; Romm et al, 2010). For comparison these previously constructed calibration curves are shown with each laboratory's preexisting curves, albeit radiation types and dose rates are often different among laboratories (Figure 1). Table 2 presents the results from parameter estimates in lab curve compared to those from AFRRI curve in each respective lab. Lab curves in Labs B and D were fairly similar to their respective AFRRI curves, although they differed marginally in the curvature of the model. The lab curve in Lab C was statistically similar to its AFRRI curve with respect to all three parameters. The rate of dicentrics (slope) of the lab curve in Lab E was statistically different from that in their AFRRI curve. Figure 2 shows predicted doses to dose unknowns based on the lab curves from the four laboratories at 20, 30, and 50 metaphase spread analysis levels with all analyzed metaphases. The data from this figure along with the % Error are shown in table 3. It is clear from this figure that increasing the number of metaphases analyzed reduces the confidence interval for the estimated dose. All laboratories generally were able confirm the irradiation dose with only 50 metaphases analyzed, often at 20 metaphase spread level.

As a measure of the accuracy of predicted doses to dose unknowns by the rapid triage DCA as required for radiation mass casualties, table 4 demonstrates whether the error between the estimated and applied dose is greater than 20%. At high clinically relevant doses for the management of ARS, dose prediction was very reliable even with only a few metaphases analyzed. For example, at the 3.0 and 4.5 Gy doses, all samples were within the 20% range for the AFRRI curve results, regardless of the number of metaphases analyzed. For the lab curve results, this was true for the 4.5 Gy samples but less so for the 3.0 Gy samples. At lower doses, fewer dicentrics are expected in 20 or 30 metaphases, which leads to a higher error rate regardless of the calibration curve used.

The dose calculations on the basis of 30 dicentrics were only performed at 4.5 Gy, because at lower doses, 50 metaphases were scored before 30 dicentrics were observed. On the basis of 30 dicentrics at 4.5 Gy, all samples described the dose correctly and the dose estimations were all within 20% of the physical dose applied regardless of which calibration curve was used.

4.0 Discussion

During a mass casualty event involving radiological or nuclear material, there is the potential for hundreds or thousands of individuals to be exposed to ionizing radiation. In such a scenario, the local capacity for biological dosimetry would quickly become overwhelmed and require assistance of other laboratories.

Previous studies established the feasibility of using geographically dispersed laboratories to provide accurate dose estimates from samples originating in one location and being shipped around the world for processing and analysis using the dicentric DCA (Wilkins et al, 2008; Romm et al, 2010). In addition, it was shown that analyzing 50 metaphases gives very reliable and accurate individual dose estimations over the whole dose range of 0.75 to 4.5 Gy and most of these dose estimations are within 20% of the range of the applied doses. Even dose estimations based on analysis of only 30 metaphases and even 20 metaphases allowed an accurate evaluation, however more so at the higher dose ranges.

In the above studies, dose estimates were based on calibration curves samples generated by irradiation with the same exposure system at AFRRI and shipped to each laboratory for analysis. In an emergency situation, however, dose estimates will be made using calibration curves already existing in each laboratory that best match the exposure scenario and therefore, laboratories within one network will have calibration curves produced from different radiation sources and different dose rates. This study strove to determine if dose estimates made from pre-existing curves in the laboratory were accurate enough to provide useful information to the medical community.

A comparison of the AFRRI and lab curves demonstrated that in some laboratories, there was a significant difference between the coefficients of the curves. The differences in dose rate could explain this to some extent as the pre-curve from Lab E was made from radiation with the highest dose rate and had the largest difference between the two curves. However, dose rate only partially accounts for these differences, as demonstrated by lab curve from Lab C being statistically closest to its respective AFRRI curve although it did not have the same dose rate. From the original interlaboratory comparison, it is clear that calibration curves made in different laboratories can differ even when the same samples are used to generate the curve. These variations can be attributed to factors such as culture conditions, slide preparation, metaphase cell selection and scoring, all of which can potentially outweigh differences in dose rate as long as the irradiation for each dose is delivered within the recommended 15 minutes. (IAEA, 2001)

This current evaluation has demonstrated that, although dose estimates made with preexisting calibration curves from each laboratory shows a slight reduction in the accuracy of the dose estimates, results are still adequate to provide useful input to the medical community for ARS treatment. This is particularly true in the high dose range, where identification of individuals in need of medical support is paramount. These finding fully support the development of international networks where each laboratory provides dose estimates based on their own calibration curves.

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Figure 1.

Dose-response curves for gamma rays from each participating laboratory. The dotted curves are from samples irradiated and shipped from AFRRI, The solid curves are the pre-existing gamma curve in each laboratory.

Figure 2.

Illustration of effect of truncated analysis of metaphases at different numbers on 95% upper (U) and lower (L) confidence intervals in laboratories B through E. Each laboratory's own calibration curve was used for dose prediction calculations.

Table 1

Radiation quality and dose rates for pre-existing calibration curves in each laboratory.

Lab	Radiation Type	Dose Range	Dose Rate (Gy/min)	Coefficients of Calibration Curves
B	Cs-137	0.24–4.0	0.9	$Y = (0.0019 \pm 0.002) + (0.03 \pm 0.01) D + (0.071 \pm 0.006) D^1$
C	Cs-137	0.10–4.0	0.4	$Y = (0.0002 \pm 0.0001) + (0.019 \pm 0.005) D + (0.053 \pm 0.004) D^2$
D	Cs-137	0.50–5.0	0.6	$Y = (0.039 \pm 0.001) D + (0.056 \pm 0.004) D^2$
E	Co-60	0.25–5.0	1.0	$Y = (0.059 \pm 0.014) D + (0.029 \pm 0.0046) D^2$

Table 2

Comparison of parameter estimates from lab curve to AFRRRI curve in Labs B-E

Lab	Intercept (b_0) z (p -value [*])	Slope (b_1) z (p -value [*])	Curvature (b_2) z (p -value [*])
B	0.95 (0.51)	0.42 (1.0)	2.43 (0.023)
C	2.00 (0.068)	−0.56 (0.86)	0.32 (1.0)
D	−1.33 (0.27)	−0.23 (1.0)	−2.12 (0.040)
E	−2.00 (0.068)	3.07 (0.0032)	0.73 (0.70)

*
 p -values are Bonferroni adjusted for 3 comparisons in each Lab

Table 3

Estimated doses with 95% lower (L) and upper (U) confidence intervals (CI) based on calibration curves for each laboratory when different numbers of metaphases analysed.

Physical Dose (Gy)	Laboratory	Ncell	Ndicent	Estimated dose (Gy)	95% CI L	95% CI U	Error (%)
0.75	B	20	1	0.64	0.00	1.78	-15.1
		30	1	0.49	0.00	1.41	-35.3
		50	2	0.55	0.08	1.22	-26.7
		1000	45	0.59	0.48	0.72	-20.8
	C	20	1	0.81	0.05	2.12	8.1
		30	1	0.64	0.03	1.71	-15.3
		50	3	0.90	0.34	1.65	20.3
		1000	51	0.82	0.69	0.96	9.4
	D	20	1	0.64	0.00	1.89	-14.3
		30	3	1.02	0.33	1.95	35.7
		50	4	0.88	0.35	1.58	17.5
		1000	82	0.90	0.77	1.03	19.5
1.5	E	20	0	0.00	0.00	1.70	-100.0
		30	0	0.00	0.00	1.28	-100.0
		50	1	0.30	0.01	1.19	-60.0
		1000	38	0.52	0.38	0.67	-31.3
	B	20	4	1.47	0.67	2.48	-2.0
		30	7	1.60	0.95	2.40	6.7
		50	9	1.39	0.87	1.99	-7.3
		810	100	1.11	0.99	1.25	-26.0
	C	20	3	1.52	0.61	2.71	1.2
		30	5	1.61	0.85	2.54	7.2
		50	10	1.78	1.18	2.47	18.5
		573	103	1.68	1.50	1.86	11.8
	D	20	4	1.56	0.68	2.68	3.9

Physical Dose (Gy)	Laboratory	Ncell	Ndicent	Estimated dose (Gy)	95% CIL	95% CIU	Error (%)
3.0		30	7	1.71	0.98	2.58	13.8
		50	9	1.46	0.90	2.13	-2.4
		593	100	1.41	1.24	1.58	-6.2
	E	20	7	2.60	1.41	4.06	73.3
		30	7	1.99	1.05	3.17	32.7
		50	11	1.92	1.18	2.80	28.0
		624	101	1.55	1.34	1.78	3.3
	B	20	13	2.82	2.00	3.75	-6.0
		30	17	2.62	1.95	3.37	-12.7
		50	31	2.75	2.23	3.31	-8.3
		159	100	2.77	2.48	3.08	-7.7
	C	20	9	2.75	1.81	3.85	-8.4
		30	13	2.69	1.92	3.56	-10.2
		50	21	2.65	2.05	3.32	-11.6
		239	112	2.81	2.53	3.10	-6.4
	D	20	19	3.76	2.85	4.78	25.4
		30	29	3.80	3.05	4.62	26.6
		50	43	3.56	2.98	4.19	18.8
		129	100	3.37	3.01	3.75	12.3
	E	20	11	3.45	2.22	4.88	15.0
		30	15	3.25	2.25	4.40	8.3
		50	29	3.56	2.78	4.43	18.7
4.5	B	239	129	3.41	3.05	3.79	13.7
		20	29	4.32	3.50	5.22	-4.0
		30	37	3.96	3.29	4.69	-12.0
		50	59	3.87	3.35	4.43	-14.0
		85	100	3.77	3.38	4.19	-16.2
		22	31	4.25	3.46	5.10	-5.6

Physical Dose (Gy)	Laboratory	Ncell	Ndicent	Estimated dose (Gy)	95% CIL	95% CIU	Error (%)
c		20	24	4.60	3.65	5.65	2.2
		30	31	4.25	3.48	5.10	-5.5
		50	62	4.68	4.07	5.32	3.9
		101	106	4.29	3.86	4.73	-4.7
		25	30	4.60	3.75	5.53	2.2
D		20	28	4.64	3.72	5.64	3.1
		30	47	4.93	4.18	5.73	9.5
		50	70	4.64	4.06	5.25	3.0
		73	100	4.58	4.10	5.09	1.9
		21	30	4.69	3.79	5.67	4.2
E		20	21	5.07	3.82	6.47	12.7
		30	29	4.83	3.81	5.96	7.3
		50	55	5.21	4.41	6.06	15.8
		101	107	5.09	4.54	5.69	13.1
		31	30	4.84	3.83	5.94	0.1
		20	29	4.32	3.50	5.22	-4.0

Table 4

Number of dose estimates within $\pm 20\%$ of the applied dose at 4 different doses when different numbers of metaphases analysed based data from 4 laboratories using AFRI curves. The usefulness of scoring a maximum of 30 dicentric was only tested for the 4.5 Gy sample.

	Cells	0.75 Gy	1.5 Gy	3.0 Gy	4.5 Gy
AFRI curves	20	2	3	4	4
	30	1	2	4	4
	50	3	3	4	4
	All cells	4	4	4	4
	30 dic				4
Lab curves	20	3	3	3	4
	30	1	3	3	4
	50	1	3	4	4
	All cells	2	3	4	4
	30 dic				4