2012/01/26 CW-121110-CONF-017
UNRESTRICTED

Paper

VALIDATION OF QUICKSCAN DICENTRIC CHROMOSOME ANALYSIS FOR HIGH THROUGHPUT RADIATION BIOLOGICAL DOSIMETRY

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Abstract—**Currently, the dicentric chromosome assay (DCA) is used to estimate radiation doses to individuals following accidental radiological and nuclear overexposures when traditional dosimetry methods are not available. While being an exceptionally sensitive method for estimating doses by radiation, conventional DCA is time-intensive and requires highly trained expertise for analysis. For this reason, in a mass casualty situation, triage-quality conventional DCA struggles to provide dose estimations in a timely manner for triage purposes. In Canada, a new scoring technique, termed DCA QuickScan, has been devised to increase the throughput of this assay. DCA QuickScan uses traditional DCA sample preparation methods while adapting a rapid scoring approach. In this study, both conventional and QuickScan methods of scoring the DCA assay were compared for accuracy and sensitivity. Dose response curves were completed on four different donors based on the analysis of 1,000 metaphases or 200 events at eight to nine dose points by eight different scorers across two laboratories. Statistical analysis was performed on the data to compare the two methods within and across the laboratories and to test their respective sensitivities for dose estimation. This study demonstrated that QuickScan is statistically similar to conventional DCA analysis and is capable of producing dose estimates as low as 0.1 Gy but up to six times faster. Therefore, DCA QuickScan analysis can be used as a sensitive and accurate method for scoring samples for radiological biodosimetry in mass casualty situations or where faster dose assessment is required.**
Health Phys. 102(2):143–153; 2012

Key words: chromosome aberration; cytogenetics; dose assessment; emergencies, radiological

INTRODUCTION

Biological dosimetry is a method for determining the radiation dose received by an individual when physical dosimetry is missing or in dispute. Over the past 60 y, much work has been conducted developing and standardizing the dicentric chromosome assay (DCA), resulting in a robust method that has become the "gold standard" for biological dosimetry. One advantage of this assay is that it is specific to ionizing radiation, since dicentric chromosomes are mainly induced by ionizing radiation and only a very few radiation mimetic drugs. Secondly, due to the low background levels of dicentric chromosomes found in human lymphocytes, it is a very sensitive assay, allowing dose estimates to be made down to 0.1–0.2 Gy. As a result of its widespread acceptance, in 2004 the International Organization for Standardization (ISO) selected the DCA for International Standardization and published guidelines for service laboratories performing radiation biological dosimetry by cytogenetics (ISO 2004).

Although the DCA is specific and sensitive to ionizing radiation, its labor-intensive nature limits it for use in large-scale radiological or nuclear emergency situations. The most time consuming aspect of the assay is the scoring of slides, which traditionally involves analyses of 500 to 1,000 metaphase spreads for each sample in order to provide biological dose estimates with high accuracy and sensitivity. This requires many hours of microscope scoring for each sample and is, therefore, not feasible for large-scale events where potentially thousands of individuals may require biologically based dose estimates. In this case, however, accuracy and sensitivity are of less importance, as only those individuals receiving a whole body equivalent dose of more than 1.5 Gy would require any medical intervention (Alexander et al. 2007). It has, therefore, been suggested by Lloyd et al. to decrease the number of metaphase spreads analyzed per sample so that clinically relevant doses could be detected while increasing the throughput of the scoring. This level of sensitivity can be achieved by scoring only 50 metaphase spreads (or 30 dicentrics) and would provide biological dose estimates with an accuracy of 0.5 Gy (Lloyd et al. 2000). In support of this

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The authors declare no conflict of interest.
(*Manuscript accepted* 30 *July* 2011)
0017-9078/11/0

DOI: 10.1097/HP.0b013e3182307758

strategy, the ISO published a standard for laboratories performing cytogenetic triage for assessment of mass casualties in radiological or nuclear events (ISO 2008).

In order to increase capacity for biological dosimetry in Canada, a National Biological Dosimetry Response Plan (NBDRP) has been developed through funding provided by the Chemical, Biological, Radiological-Nuclear and Explosives (CBRNE) Research and Technology Initiative (CRTI). One strategy of the NBDRP was to increase capacity through the development of a Canadian Emergency Network (CEN) currently comprised of four core laboratories [Health Canada-Ottawa, ON (HC), Atomic Energy of Canada Limited-Chalk River Laboratories, ON (AECL), Defence Research and Development Canada-Ottawa, ON (DRDC) and McMaster University-Hamilton, ON (MU)] that are capable of providing radiation biological dose estimates using the conventional DCA (Miller et al. 2007).

A second strategy of the NBDRP was to take triage-based scoring to a new level by introducing a novel scoring technique, termed "DCA QuickScan." In a previous pilot study, this scoring strategy was investigated, and it was determined that scoring throughput could be increased without losing accuracy in the dose estimate (Flegal et al. 2010). In this study, as follow-up and validation of this scoring strategy, triage-quality conventional DCA and QuickScan have been compared by generating full dose response curves using four donors with 1,000 metaphases or 200 events scored at each of eight to nine dose points to determine whether this scoring strategy could be used as a replacement for conventional scoring in emergency biodosimetry situations without having to generate new QuickScan calibration curves.

MATERIALS AND METHODS

Blood collection and irradiation

All blood donors were volunteers who willingly responded to an advertising call for participation in a research proposal approved by either Health Canada's or AECL's Research Ethics Boards, and none of the donors had a recent history of ionizing radiation exposure. Blood samples from four donors [two male (AECL) and two female (HC), one of each being in the age groups 25–30 y and 55–60 y] were collected with informed consent by venipuncture into 8-mL sodium heparinized (AECL) or 6-mL lithium heparinized (Health Canada) Vacutainer tubes (Becton Dickenson, 2771 Bristol Circle, Oakville, ON, Canada). The blood samples from AECL were then transported to Health Canada by truck to complete irradiations. The samples were packaged according to TDG guidelines with a temperature logger and OSL chip to record any external radiation dose to which the samples might be exposed during transit.

Irradiation of all blood samples was completed ex vivo in the Vacutainer collection tubes at room temperature using a ^{137}Cs Gammacell40 (Atomic Energy of Canada Ltd., Ottawa, ON, Canada) at a dose rate of 0.801 Gy min^{-1}. The Gammacell40 was calibrated by Fricke dosimetry (MDS Nordion, 447 March Rd., Ottawa, Canada). Irradiations at nine dose points were completed for the AECL samples (0, 0.1, 0.25, 0.5, 0.75, 1.0, 2.0, 3.0 and 4.0 Gy), and eight dose points (no 0.1 Gy treatment) were completed for the Health Canada samples. The AECL samples were then returned to their lab via the same truck used to bring them to Health Canada for irradiation, travelling a total of approximately 400 km for 4–5 h.

Cell culture and harvest

Where reagent sources varied between the laboratories, the source is not listed. Upon return of the irradiated blood tubes to their respective laboratories, the samples were cultured by each laboratory using its own protocols that follow the recommended methods provided by IAEA (2001) and ISO (2008). Briefly, whole blood was diluted 1:9 with complete RPMI 1640 medium, supplemented with 15% fetal bovine serum (FBS), 2 mM L-glutamine, 15–20 μM bromodeoxyuridine (BrdU), 100 units mL^{-1} penicillin, 100 μg mL^{-1} streptomycin, and 1–2% phytoheamagglutinin (PHA). Cultures were incubated in a humidified atmosphere at 37°C with 5% CO_2 for 48 h, and colcemid (0.1 μg mL^{-1} final concentration) was added to the cultures for the final 4 h of incubation. Cells were then harvested by centrifugation and treated with 10 mL of 75 mM KCl, "soft-fixed" by either adding 1 mL fresh Carnoy's Fixative (3 methanol: 1 glacial acetic acid) directly to the cells incubating in hypotonic solution (Health Canada) or by a 5-min incubation in 5% acetic acid (v/v in water) post hypotonic treatment (AECL). For both labs, cells were then washed three times with Carnoy's Fixative, and harvested cells were stored at −20°C in fixative until ready for slide preparation. Slides were prepared using a Hanabi-HS Metaphase Spreader (Transition Technologies Inc., 257 Norseman St., Toronto, ON, Canada), and then slides were stained with 20 μg mL^{-1} Hoeschst 33258 (Bisbenzimide H 33258) for 2 min, exposed to a 365 nm UV light source for 4 (AECL) or 8 min (Health Canada), rinsed in three successive washes of water, and then stained for at least 10 min in 10% Giemsa Gurr solution (one part Giemsa stain to nine parts Gurr buffer, Invitrogen, Canada) to achieve fluorescence plus Giemsa (FPG) staining, which allowed the differentiation between first and second metaphase cells. The slides were

allowed to dry and then mounted and sealed under glass coverslips with Permount.

Slide scoring

In each laboratory, sufficient slides were prepared such that no more than 100 spreads were examined per slide and a total of 1,000 spreads (or 200 events when reached before 1,000 spreads) were analyzed for each dose and donor treatment with only the first metaphase spreads being scored. All slides were blinded, and scoring data were controlled by an individual familiar with the scoring process but not participating in the scoring exercise. Therefore, once 200 events were reached for the upper doses, the remaining slides in that treatment could be removed while keeping the remaining slides blind to the scorers. Standard reference curves were constructed for the two donors using one method of scoring (either conventional DCA or QuickScan). Then slides were re-blinded and scoring was completed using the alternate method. Conventional DCA scoring followed the recommendations of ISO (ISO 2004), and the method for QuickScan scoring was as described by Flegal et al. (2010) except for the stipulation that examination stopped if five dicentrics were seen in less than 20 metaphases. The results were not decoded until all samples were analyzed by the laboratory.

Statistical Analysis

Traditionally, the quadratic Poisson regression model with identity link (QPRM-I) is used to relate the rate of dicentrics (y) scored to dose (x),

$$y = C + \alpha x + \beta x^2, \quad (1)$$

(Merkle 1983). The intercept C corresponds to the background rate of dicentrics, α is the slope of the dose response curve, and β is the curvature of the dose response model. A value of $\alpha = 0$ corresponds to a no-dose effect, whereas $\alpha > 0$ reflects an increasing trend in the rate of dicentrics scored with increasing dose. A value of $\beta = 0$ corresponds to a linear relationship, whereas $\beta > 0$ reflects a concave quadratic relationship. The parameters C, α, and β are unknown positive fixed effects.

Each of the two laboratories involved in the study had different scorers and donors. Further, not all scorers were available to score each donor's blood sample at each dose group. Both scorers and donors were considered to be random effects in the study design that could potentially explain variability within and between labs. The significance of these random effects was further investigated using generalized linear mixed models (GLMMs) with two random effects; scorers (s_i, $i = 1, 2, \ldots, I$) and donors (d_j, $j = 1, 2, \ldots, J$). The random effects s_i and d_j are assumed to be mutually independent and normally distributed variates with mean zero and variances σs^2 and σd^2, respectively (Drum and McCullagh 1993). The variance component σs^2 measured the degree of heterogeneity among scorers, and σd^2 measured the degree of heterogeneity among donors. The Restricted Maximum Likelihood method (REML) was used to estimate the variance components for the random effects, and the Maximum Likelihood method (ML) was used to estimate fixed effect parameters C, α, and β (Wolfinger and O'Connell 1993).

The saturated model with both of these random effects was compared to a reduced model with no random effects [simply a generalized linear model (eqn 1)] using the likelihood ratio test (LRT). In the case where at least one random effect was significant, then a reduced GLMM with only one random effect at a time was compared to the saturated GLMM, again using the LRT. The two scoring methods (conventional and QuickScan) were analyzed separately in order to characterize the random variability due to scorers and donors within and between laboratories.

Comparison of donors within and between laboratories by scoring method

To compare donors within a laboratory for each method of scoring, simultaneous QPRM-I were fit to the data. In this setting, donors were treated as a fixed effect since the objective was to allow a different dose response curve for each donor and then compare them. The random effect of scorer (s_i) was kept in the model in order to account for the variability due to scorers. The model was

$$y = C + \alpha x + \beta x^2 + C_i + \alpha_i x + \beta_i x^2 + s_i, \quad (2)$$

where $y = C + \alpha x + \beta x^2$ is the base QPRM-I for both donors. The parameters C_j, α_j, β_j ($j = 1, 2$) are the background rate, slope and curvature, respectively, of the QPRM-I for each donor j different from the base model. If the donors within a laboratory have the same background rate, then the F-test for the null hypothesis (H_0: $C_j = 0$, $j = 1, 2$) would be insignificant; i.e., $p > 0.05$. A similar approach was used when assessing the slope and curvature parameters of the model.

When comparing donors between laboratories, the QPRM-I model (eqn 2) was fit to all donors between the two labs, where the subscript $j = 1, 2, 3, 4$. If the donors have the same background rate, then the F-test for the null hypothesis (H_0: $C_j = 0$, $j = 1, 2, 3, 4$) would be insignificant; i.e., $p > 0.05$. Conversely, if the null hypothesis is rejected in favor of the alternative (H_a: *at least one donor has a different background rate from the rest*), then multiple comparison tests were carried out to compare all possible pairs of donors. Bonferroni corrections were used to ensure that the overall Type I error rate was less than 0.05. A

similar approach was used when assessing the slope and curvature parameters of the model.

Comparison of laboratories by scoring method

A similar methodology as described in the previous section was used to compare laboratories by scoring method. Again simultaneous QPRM-I were fit to the data, where each laboratory was allowed its own dose response curve. The random effects of scorers and donors were included in the model in order to account for the variability due to scorers and donors in each laboratory. The following simultaneous QPRM-I was fit to the data for both laboratories simultaneously:

$$y = C + \alpha x + \beta x^2 + C_l + \alpha_l x + \beta_l x^2 + s_i + d_i. \quad (3)$$

Again, $y = C + \alpha x + \beta x^2$ is the usual QPRM-I across laboratories and the parameters C_l, α_l, and β_l ($l = 1, 2$) are the background rate, slope, and curvature, respectively, of the QPRM-I for each laboratory l. If laboratories have similar background rates, then the F-test for the null hypothesis H_0: $C_l = 0$, $l = 1, 2$ would be insignificant; i.e., $p > 0.05$. A similar approach was used when assessing the slope and curvature parameters of the model.

Comparison of scoring method within each donor and within each lab

Simultaneous QPRM-I were used to compare scoring methodologies within each donor, where each scoring method was allowed its own dose response curve. The random effect of scorers was included in the model in order to account for the variability due to scorers. The following simultaneous QPRM-I was fit to the data for each donor:

$$y = C + \alpha x + \beta x^2 + C_k + \alpha_k x + \beta_k x^2 + s_i. \quad (4)$$

Again, $y = C + \alpha x + \beta x^2$ is the usual QPRM-I for the donor being analyzed, and the parameters C_k, α_k, and β_k ($k = 1, 2$) are the background rate, slope, and curvature, respectively, of the QPRM-I for each scoring method k. If the two scoring methods have similar background rates, then the F-test for the null hypothesis H_0: $C_k = 0$, $k = 1, 2$ would be insignificant; i.e., $p > 0.05$. A similar approach was used when assessing the slope and curvature parameters of the model.

If the donors within a laboratory and for each scoring method were similar, then the data for the two donors was pooled together, and the two scoring methods were compared within each laboratory. A similar model to that stated in eqn (4) was used with the inclusion of the random variate donors (d_j).

Sensitivity analysis

The second objective of this study was to determine the sensitivity of the DCA assay using both the conventional method and QuickScan method. Traditionally Poisson regression models are used for the sensitivity analysis (similar to analysis of variance for continuous data). The average dicentrics rate was compared among the nine dose groups (τ_k, $k = 0$, 0.1, 0.25, 0.5, 0.75, 1, 2, 3, and 4 Gy). The objective was to determine the smallest dose significantly different from background for each laboratory and scoring method. Donors and scorers were kept in the model as random blocking effects. The Poisson regression model for the sensitivity analysis was:

$$\log(y_{ijk}) = \log(n_{ijk}) + \mu + \tau_k + s_i + d_j + \varepsilon_{ijk}, \quad (5)$$

where $\log(y_{ijk}/n_{ijk})$ is the natural log rate of dicentrics per n cells scored in the k^{th} dose level from the i^{th} scorer (s_i, $i = 1, 2, .., I$) of the j^{th} donor (d_j, $j = 1, 2$) for each laboratory and scoring method. The overall average rate of dicentrics in the laboratory is indicated by the parameter μ, and ε_{ijk} is the error term of the model and is assumed to follow the Poisson distribution. The models were corrected for over/underdispersion using the Pearson deviance scale. If the overall effect of dose was significant (i.e., $p < 0.05$), then multiple comparison tests were carried out to compare each dose group to the control group. Bonferroni corrections were used to ensure the overall Type I error rate was less than 0.05.

RESULTS

One of the issues of model fitting data arising from DCA is that the background rate is sometimes estimated to be negative, which is biologically impossible. There are three options that have been suggested as to how to deal with this problem: 1) increase the number of observations in the background dose group; 2) force the model through the origin; or 3) increase the background rate in the model to be a small number close to zero (e.g., 0.0005–0.001).* In the analysis presented in this paper, all three options were assessed. The first option was limiting due to the small amounts of background data that were available from the laboratories. Furthermore, the background data that were available were from various donors who were not used in the other dose groups, thereby making it difficult to carry out simultaneous regressions to compare donors and scoring methods. Options two and three were compared graphically. The fits were comparable, although

*IAEA. Cytogenetic analysis for radiation dose assessment: a manual. Vienna: IAEA; 2011 (in press).

forcing the model through the origin gave a much closer fit to that of the original model. This option was applied only in cases where the intercept was negative, in which case the intercept was set to zero and the model was refit to the data.

The significance of the random effects for scorers and donors was assessed in each laboratory for each scoring method (results not shown). In the HC laboratory, a significant amount of variability was explained by donors ($\chi^2_1 = 63.37$, $p < 0.0001$) and scorers ($\chi^2_1 = 10.13$, $p = 0.0015$) using the QuickScan method, whereas there was a significant amount of variability due to scorers ($\chi^2_1 = 51.03$, $p < 0.0001$) in the AECL laboratory, again using the QuickScan method. However, when assessing the significance of these random effects between the two laboratories for the QuickScan method, there was no significant amount of random variability due to either scorers or donors.

In each of the two laboratories, for the conventional counting method, the random effects for scorers and donors did not add a significant amount of variability to the model. However, both random effects were significant between the laboratories (scorers $\chi^2_1 = 382.63$, $p < 0.0001$; donors $\chi^2_1 = 122.65$, $p < 0.0001$), indicating a difference between both scorers and donors between the two laboratories.

Comparison of donors within and between laboratories, QuickScan scoring method

The QPRM-I presented in eqn (2) was fit to the data from each laboratory in order to compare the donors within a laboratory. Recall that in this setting, the objective was to compare donors and not characterize the within-laboratory variability due to donors. In order to compare donors, each donor was allowed its own dose response model, and then the models were compared (simultaneous Poisson regression models). Table 1a presents the results from the overall F-test to compare the background rate, slope, and curvature from each donor within a laboratory using the QuickScan scoring method.

In both the HC and AECL laboratories, the background rate, slope, and curvature were statistically similar for both donors in their own respective laboratories. The overall F-tests for the background rate, slope, and curvature parameter estimates were not significant ($p > 0.05$ in all cases) within these two laboratories for the QuickScan scoring method. Fig. 1 presents the dose response models for each donor in the AECL laboratory and HC laboratory.

Next the donors between the two laboratories were compared (eqn 2). The models for each donor were considered similar with respect to background rate [$F_{(3,264)} = 1.81$, $p = 0.1463$, Table 1a]. The slope [$F_{(3,264)} = 5.17$, $p = 0.0017$], and curvature [$F_{(3,264)} = 11.73$, $p < 0.0001$] parameters were significantly different for at least one of the donors between the two laboratories. Further comparisons between donors in the two laboratories determined that the slope (or increase of dicentrics with respect to dose) was slightly greater in donor B from the AECL laboratory compared to donor D from the HC laboratory [$F_{(1,154)} = 10.14$, $p = 0.0072$] (Fig. 2). The curvature of the models for each set of donors in the two laboratories was also significantly different ($p < 0.05$) (Table 1a and Fig. 2). Although Donor C appears to be more different than Donor B, higher variability in the data makes the test insensitive to detecting differences.

Table 1a. Overall *F*-test based on simultaneous Poisson regression models to compare donors within and between laboratories; scoring method = QuickScan; scorers are considered a random variable.

	C_i $F(p\text{-value})$	α_i $F(p\text{-value})$	β_i $F(p\text{-value})$
Comparing donors within laboratory			
AECL $F_{(1,109)}$	2.31 (0.1318)	0.42 (0.5203)	0.82 (0.3668)
HC $F_{(1,130)}$	1.76 (0.1868)	3.68 (0.0574)	1.77 (0.1863)
Comparing donors between laboratories			
Both Labs $F_{(3,264)}$	1.81 (0.1463)	5.17 (0.0017)	11.73 (<0.0001)
A vs. C[a] $F_{(1,105)}$		0.85 (1.0000)	10.17 (0.0076)
A vs. D[a] $F_{(1,155)}$		3.89 (0.2012)	6.28 (0.0528)
B vs. C[a] $F_{(1,115)}$		1.10 (1.0000)	20.96 (<0.0001)
B vs. D[a] $F_{(1,154)}$		10.14 (0.0072)	26.26 (<0.0001)

[a] Multiple comparison tests have been corrected using a Bonferroni correction.

Table 1b. Overall *F*-test based on simultaneous Poisson regression models to compare donors within and between laboratories, scoring method = Conventional, scorers are considered a random variable.

	C_i $F(p\text{-value})$	α_i $F(p\text{-value})$	β_i $F(p\text{-value})$
Within laboratory			
AECL $F_{(1,106)}$	0.47 (0.4949)	0.96 (0.3286)	0.63 (0.4276)
HC $F_{(1,120)}$	3.47 (0.0649)	3.67 (0.0578)	0.03 (0.8624)
Between laboratory			
Both labs $F_{(3,226)}$	4.10 (0.0074)	5.09 (0.0020)	3.95 (0.0090)
A vs C $F_{(1,99)}$	0.87 (1.0000)	0.00 (1.0000)	4.37 (0.1568)
A vs D $F_{(1,122)}$	4.80 (0.0909)	8.73 (0.0152)	7.58 (0.0272)
B vs C $F_{(1,101)}$	3.47 (0.1959)	1.00 (1.0000)	0.80 (1.0000)
B vs D $F_{(1,128)}$	0.00 (1.0000)	5.40 (0.0868)	1.98 (0.5688)

Comparison of donors within and between laboratories, conventional scoring method

Table 1b presents the results from the overall F-test to compare the background rate, slope, and curvature from each donor within a laboratory for the conventional scoring method. In both the HC and AECL laboratories, the background rate, slope, and curvature were statistically similar for both donors in their own respective laboratories

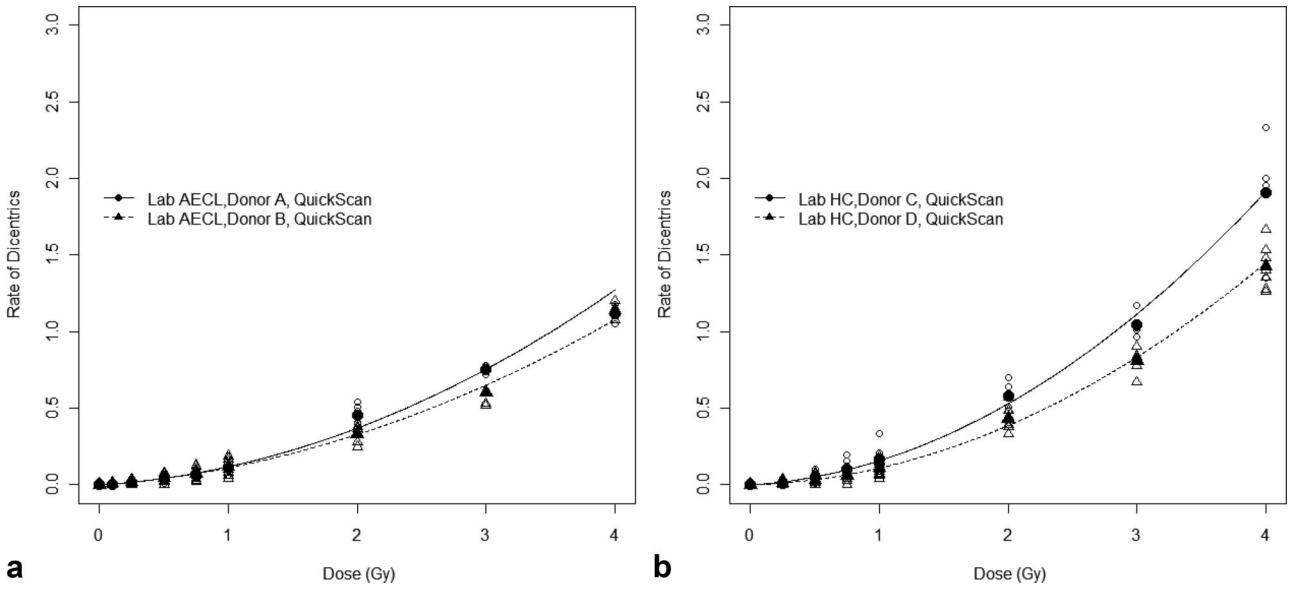

Fig. 1. The dose response models for QuickScan scoring for each donor in (a) the AECL laboratory and (b) HC laboratory. Open symbols represent individual slides scored at each dose. Closed symbols represent the average frequency of all slides scored for each donor.

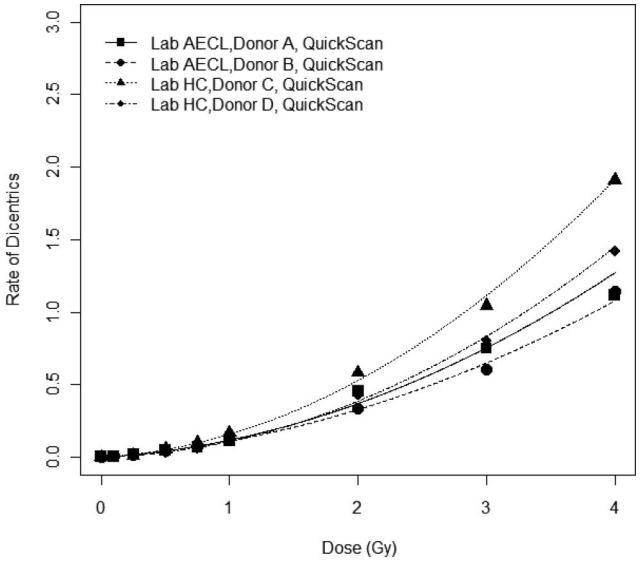

Fig. 2. A comparison of the dose response models for QuickScan scoring for all donors.

($p > 0.05$ in all cases) using the conventional scoring method. Fig. 3 presents the dose response models for each donor in the AECL laboratory and HC laboratory.

Next the donors between the two laboratories were compared (eqn 2). At least one of the donors was considered significantly different from the others with respect to background rate [$F_{(3,226)} = 4.10$, $p = 0.0074$], slope [$F_{(3,226)} = 5.09$, $p = 0.0020$], and curvature [$F_{(3,226)} = 3.95$, $p = 0.0090$]. Multiple comparison tests between donors in the two laboratories determined that the background rate was similar between donors in the two laboratories ($p > 0.05$, Table 1b). The slope (or increase of dicentrics with respect to dose) was slightly greater in donor A of the AECL laboratory compared to donor D in the HC laboratory [$F_{(1,122)} = 8.73$, $p = 0.0152$] (Fig. 4). Also the curvatures for these two donors were significantly different from one another [$F_{(1,122)} = 7.58$, $p = 0.0272$) (Table 1b and Fig. 4), Again, Donor C appears to be different from Donor A due to the variability in the data.

Comparison of laboratories by scoring method

The Simultaneous Poisson regression model (3) was fit to the data from both laboratories in order to determine if the dose response model from the AECL and HC laboratories were similar for each scoring method. Table 2 presents the results from the overall F test to compare the background rate, slope and curvature parameters from both laboratories, and Fig. 5a presents the average dose response curve from each laboratory using the QuickScan scoring method. The background rate [$F_{(1,242)} = 2.16$, $p = 0.1433$] and slope [$F_{(1,242)} = = 2.95$, $p = 0.0873$] from both laboratories were statistically similar. The curvature parameter was significantly different for the two laboratories [$F_{(1,242)} = 5.89$, $p = 0.0159$].

Similarly, the simultaneous Poisson regression model (3) was fit to the data from both laboratories in order to determine if the dose response model from the AECL and HC laboratories were similar for the conventional scoring method. Table 2 presents the results from the overall F-test to compare the background rate, slope,

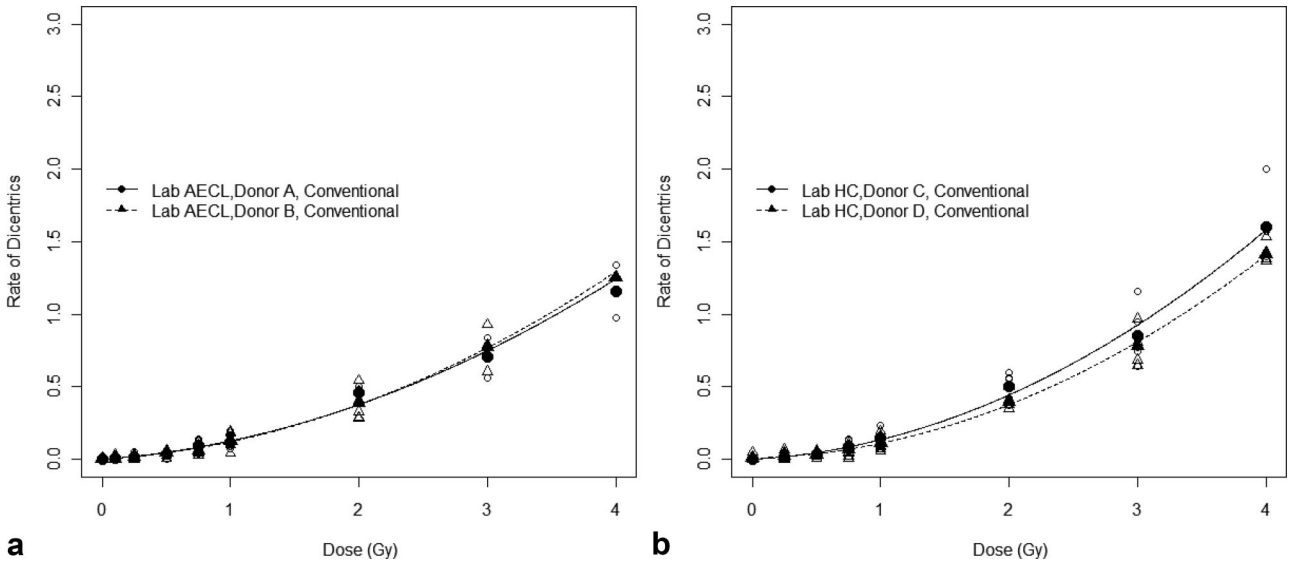

Fig. 3. The dose response models for conventional scoring for each donor in (a) the AECL laboratory and (b) HC laboratory. Open symbols represent individual slides scored at each dose. Closed symbols represent the average value of all slides scored for each donor.

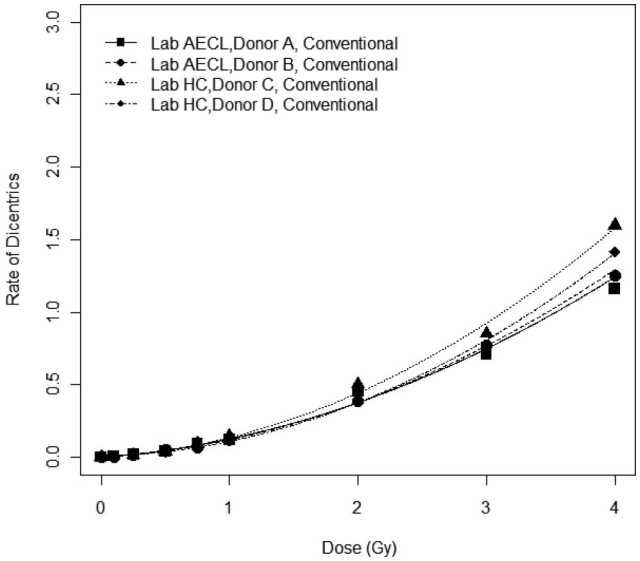

Fig. 4. A comparison of the dose response models for conventional scoring for all donors.

Table 2. Overall *F*-test based on simultaneous Poisson regression models to compare laboratory, by scoring method. Scorers and donors are considered random variables.

Scoring method	C_i $F(p$-value)	α_i $F(p$-value)	β_i $F(p$-value)
Quick Scan $F_{(1,242)}$	2.16 (0.1433)	2.95 (0.0873)	5.89 (0.0159)
Conventional $F_{(1,245)}$	3.30 (0.0704)	6.51 (0.0113)	12.72 (0.0004)

and curvature parameters from both laboratories, and Fig. 5b presents the dose response curve from each laboratory using the conventional scoring method. The background rate [$F_{(1,245)} = 3.30$, $p = 0.0704$] from both laboratories was statistically similar. The slope or rate of increase in dicentrics was greater in the AECL laboratory compared to the HC laboratory [$F_{(1,245)} = 6.51$, $p = 0.0113$]. The curvature parameter was significantly different for the two laboratories [$F_{(1,245)} = 12.72$, $p = 0.0004$].

Comparison of scoring method within each donor and within each lab

The simultaneous Poisson regression model (4) was fit to the data for each donor in each laboratory in order to determine if the dose response models using the two scoring methods (QuickScan and conventional) were similar. Table 3 presents the results from the overall F-test to compare the background rate, slope and curvature parameters from each scoring method, for each donor. Fig. 6 presents the dose response curves from each scoring method for each donor.

As can be observed in both Table 3 and Figs. 6, the two scoring methods are statistically similar in background rate, slope and curvature for each donor in each of the two laboratories ($p > 0.05$ in all cases).

The next item of interest was to determine if the two scoring methods were similar within each laboratory. Since the donors within each laboratory were similar, the data from both donors were pooled within a laboratory to determine if the two scoring methods within each laboratory were similar. The simultaneous Poisson regression model (4), with the addition of the donor random variable, was fit to the data in each laboratory to determine if the two scoring methods were similar. Table 4 presents the results from the overall F-test to compare the background rate, slope, and curvature parameters from each scoring method in each

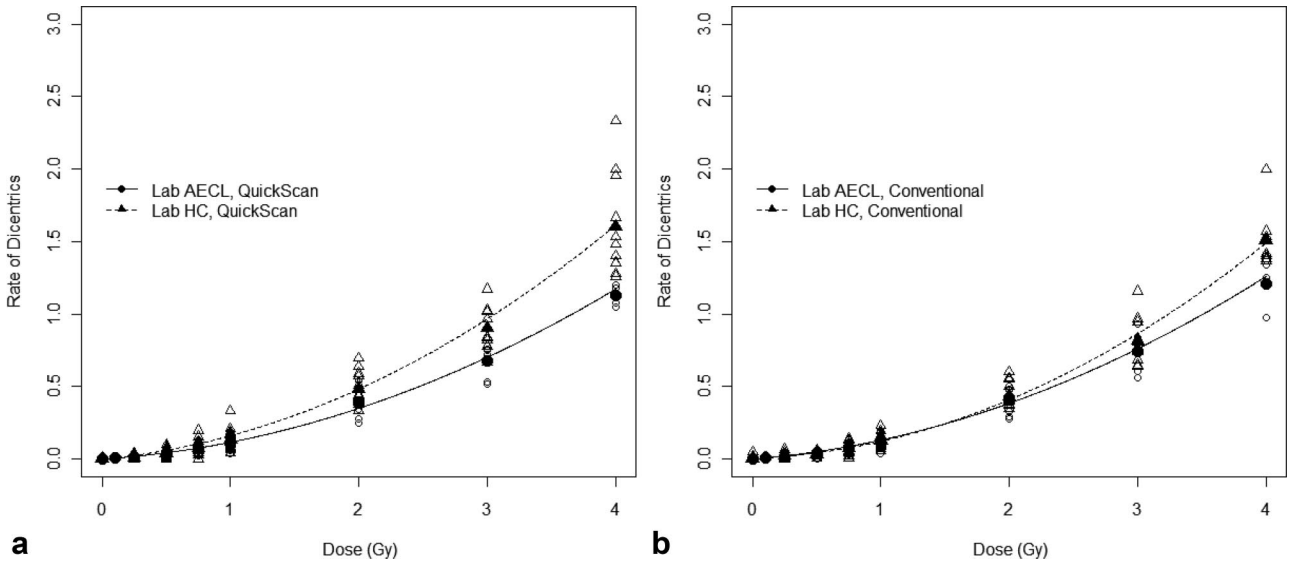

Fig. 5. The laboratory averaged dose response models for each laboratory using (a) QuickScan scoring and (b) conventional scoring. Open symbols represent individual slides scored at each dose. Closed symbols represent the average value of all slides scored.

Table 3. Overall *F*-test based on simultaneous Poisson regression models to compare scoring methods for each donor within a laboratory. Scorers are considered a random variable.

	C_i $F(p\text{-value})$	α_i $F(p\text{-value})$	β_i $F(p\text{-value})$
AECL, A $F_{(1,102)}$	0.01 (0.9154)	0.68 (0.4132)	0.40 (0.5262)
AECL, B $F_{(1,112)}$	2.99 (0.0863)	0.27 (0.6039)	1.68 (0.1975)
HC, C $F_{(1,99)}$	0.30 (0.5822)	0.26 (0.6126)	1.62 (0.2067)
HC, D $F_{(1,151)}$	2.16 (0.1441)	0.32 (0.5734)	0.00 (0.9851)

laboratory. Fig. 7 presents the dose response curve from each scoring method in each laboratory.

Again, it can be seen in both Table 4 and Fig. 7 that the two scoring methods are statistically similar in background rate, slope, and curvature for each laboratory ($p > 0.05$ in all cases).

Sensitivity analysis

As previously mentioned, the objective in the sensitivity analysis was to determine the smallest dose significantly different from background for each laboratory and scoring method. For multiple comparisons, p-values between 0.01 and 0.05 were considered to be marginal, and possibly due to a low power of the test; for this reason, the next highest dose group with $p < 0.01$ was also reported.

The Poisson regression model (5) was fit to the rate of dicentrics. The overall effect of dose was highly significant for both laboratories, as well as for both methods ($p < 0.0001$, results not presented). Table 5 reports the results from the sensitivity analysis for each laboratory and method combination. In the AECL laboratory using the QuickScan method, the smallest dose significantly different from background was 0.1 Gy ($p = 0.0110$). The *p*-value for this dose group was marginal, and therefore the next group was also reported. Dose group 0.25 Gy ($p = 0.0003$) was significantly different from background. Similar results were noted for the AECL laboratory using the conventional method, where the smallest dose significantly different from background was 0.1 Gy ($p = 0.0199$). Again the p-value for this dose group was marginal, and therefore the next group was also reported. Dose group 0.25 Gy ($p = 0.0016$) was significantly different from background.

In the HC laboratory, dose group 0.1 Gy was not included in the experimental design. The smallest dose that was significantly different from background for the QuickScan method was 0.25 Gy ($p = 0.0012$), and for the conventional method it was also 0.25 Gy ($p = 0.0001$).

Parameter estimates, along with their standard errors for the fixed effects and random effects (i.e., covariance components) and underdispersion scale for each model, are listed in Table 6. Fig. 8 is a graphical representation of the dose response model for each laboratory and scoring method combination.

DISCUSSION

In a mass casualty event, it is essential to determine dose estimates for casualties as quickly as possible in order to provide information for their medical management. Biological dosimetry plays an important role in the determination of these exposure levels and is most commonly conducted using the DCA. It is, however, widely understood that although the DCA is specific and

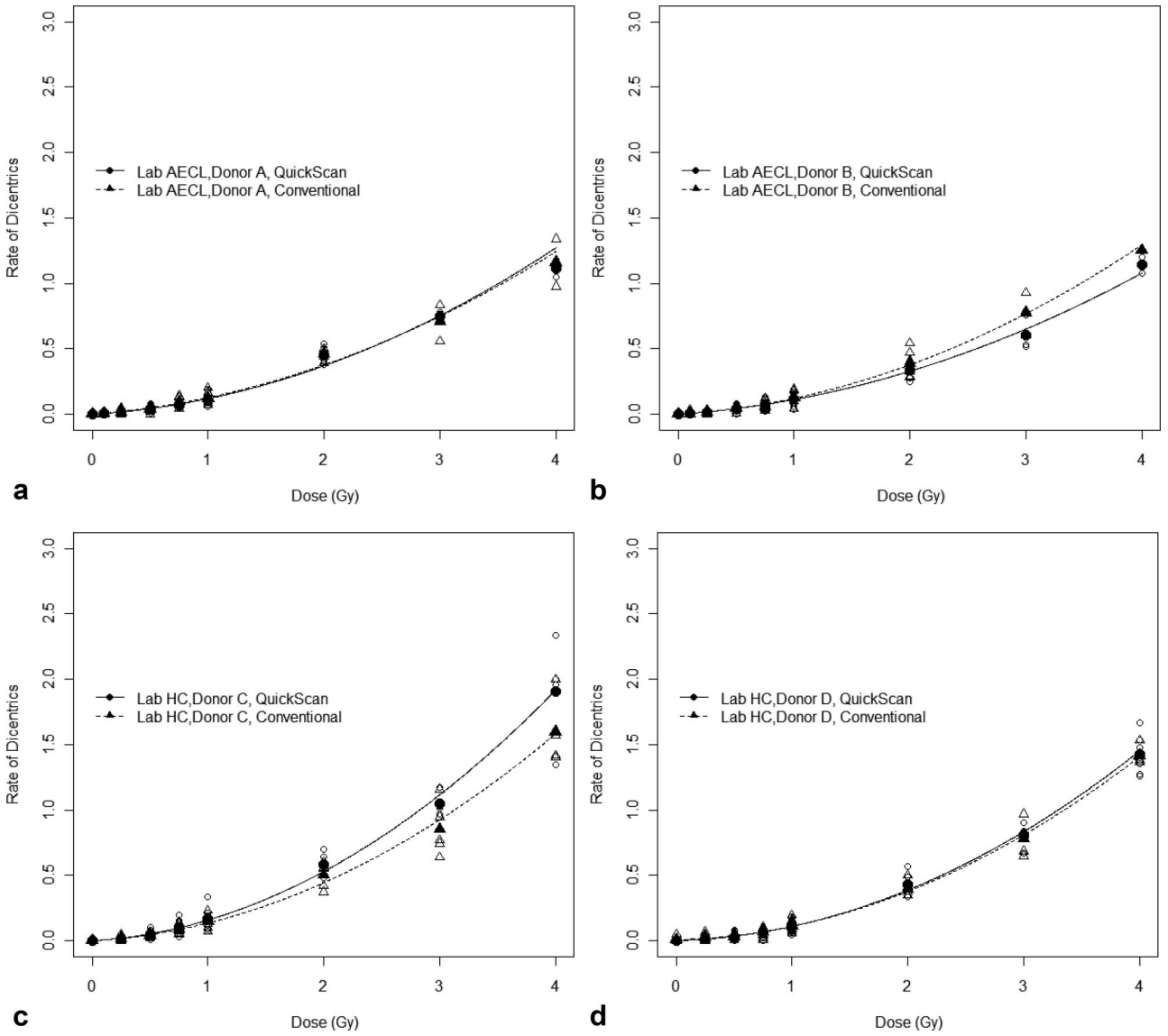

Fig. 6. Comparison of QuickScan and conventional scoring methods in (a) AECL laboratory, donor A, (b) AECL laboratory, donor B, (c) HC laboratory, donor C, and (d) HC laboratory, donor D.

Table 4. Overall *F*-test based on simultaneous Poisson regression models to compare scoring methods within each laboratory. Donors and scorers are considered random variables. (This analysis is possible since the methods were similar within each donor/lab combination).

	C_i $F(p\text{-value})$	α_i $F(p\text{-value})$	β_i $F(p\text{-value})$
AECL $F_{(1,251)}$	2.96 (0.0868)	1.15 (0.2851)	0.23 (0.6340)
HC $F_{(1,268)}$	3.84 (0.0512)	1.43 (0.2330)	0.09 (0.7594)

sensitive to ionizing radiation, it is time consuming and labor intensive.

Previous studies have demonstrated strategies for increasing the throughput of the DCA. For example, Lloyd et al. introduced the concept of triage scoring, in which doses were estimated after scoring only 50 metaphase spreads (Lloyd et al. 2000). Other strategies for increasing capacity and throughput for DCA scoring have included the development of networks (Miller et al. 2007; Yoshida et al. 2007; Wilkins et al. 2008; Romm et al. 2011), automation of processing and scoring (Martin et al. 2007; Vaurijoux et al. 2009), and the use of web-based scoring (Livingston et al. 2011).

The aim of this study was to validate another strategy for increasing the throughput of the DCA by use of the DCA QuickScan scoring technique. Through the generation of calibration curves using both conventional and QuickScan scoring, the two methods were compared for accuracy and sensitivity.

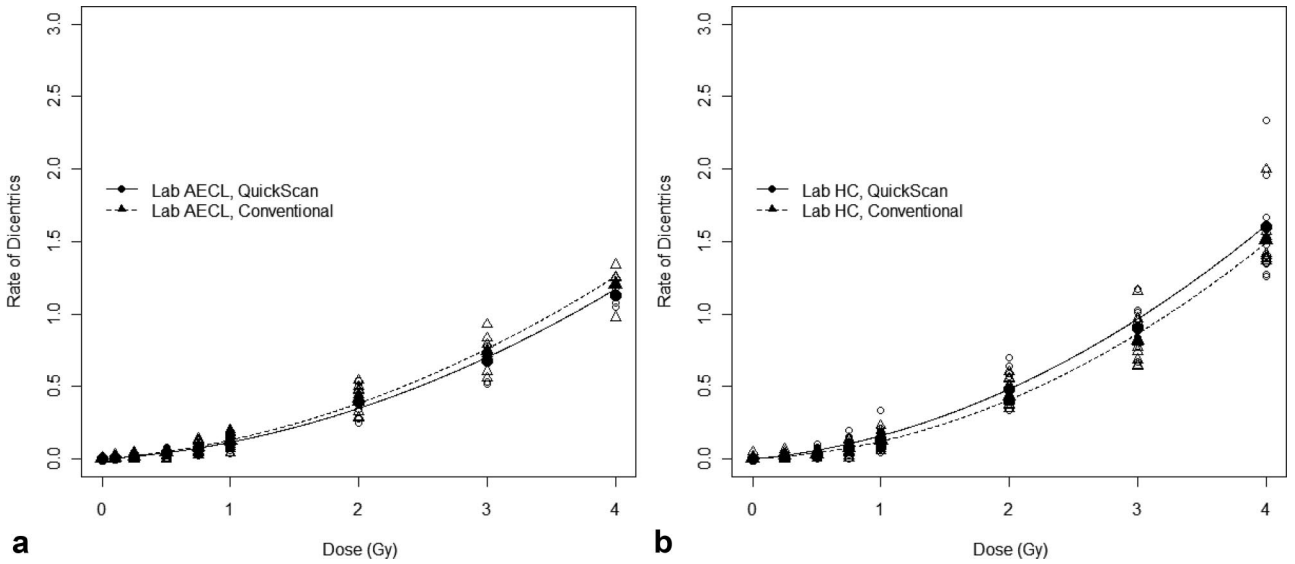

Fig. 7. Comparison of QuickScan vs. conventional scoring methods in (a) AECL laboratory and (b) HC laboratory.

Table 5. Sensitivity analysis: Lowest dose (Gy) significantly different from background for each laboratory and method of scoring.

Lab	Method	Lowest dose significantly different over background	*p*-value
AECL	QuickScan	0.1/0.25	0.0110/0.0003
	Conventional	0.1/0.25	0.0199/0.0016
HC	QuickScan	0.25[a]	0.0012
	Conventional	0.25[a]	0.0001

[a] The smallest dose over background at the HC lab was 0.25 Gy; there was no dose group 0.1 Gy at the HC lab.

To begin with, the significance of the random effects of scorers and donors was assessed within and between laboratories. It was found in the HC laboratory that there was a significant amount of variability that was present due to donors and scorers in the QuickScan method, although there was no significant amount of variability from scorers or donors in the conventional method. In the AECL laboratory, the only explainable variability was due to scorers in the QuickScan method and, again, no variability due to scorers or donors was present in the conventional method. Pooling the data together between laboratories for each method yields a significant amount of variability due to both scorers and donors in the conventional method; however, scorers and donors are not a significant source of variability in the QuickScan method.

Comparison of dose response curves for each donor within a laboratory and for each scoring method indicated that the donors were similar; however, comparisons of donors between laboratories by scoring method were not always statistically similar. When the dose response curves for each laboratory were compared by scoring method, they were significantly different from one another for both scoring methods.

The main objective of this study was to determine if the two scoring methods, QuickScan and conventional, were comparable. Within each donor in each laboratory, the results from both the QuickScan and conventional methods were statistically similar. Also, within each laboratory across donors, the two methods were statistically similar. In addition, it has previously been demonstrated that the QuickScan scoring method reduced the scoring time by a factor of about six (Flegal et al. 2010). One important result of this study, however, was that there were, in fact, differences between laboratories within each scoring method. This supports the ISO 2004 standard, which states that laboratories should have their own dose response curve (ISO 2004).

The second objective of the study was to determine the lowest dose exposure significantly different from background levels for both scoring methods in each laboratory. In both laboratories and for each scoring method, the smallest dose significantly different from background was 0.25 Gy. The AECL laboratory did include a dose group 0.1 Gy, and in both methods (QuickScan and conventional) this dose group was marginally significantly different from background rates.

CONCLUSION

It has been clearly demonstrated though this comparison that the QuickScan method provides dose estimates equivalent to those determined using conventional DCA scoring criteria. As this method has also been demonstrated to vastly decrease the scoring time, increasing the sample

Table 6. Final parameter estimates for the dose response models of each laboratory by method.

	C (s.e.)	α (s.e.)	β (s.e.)	σ_s^2 (s.e.)	σ_d^2 (s.e.)	Φ
AECL: QuickScan	0	0.057 (0.0069)	0.059 (0.0037)	6.9e-06 (8.5e-06)		0.0117
AECL: Conventional	0	0.070 (0.0088)	0.061 (0.0043)	7.5e-05 (8.8e-05)		0.0128
HC: QuickScan	0	0.080 (0.0142)	0.081 (0.0051)	4.9e-05 (9.6e-05)	6.1e-04 (7.2e-04)	0.0225
HC: Conventional	0.004 (0.0018)	0.033 (0.0087)	0.085 (0.0042)			0.0191

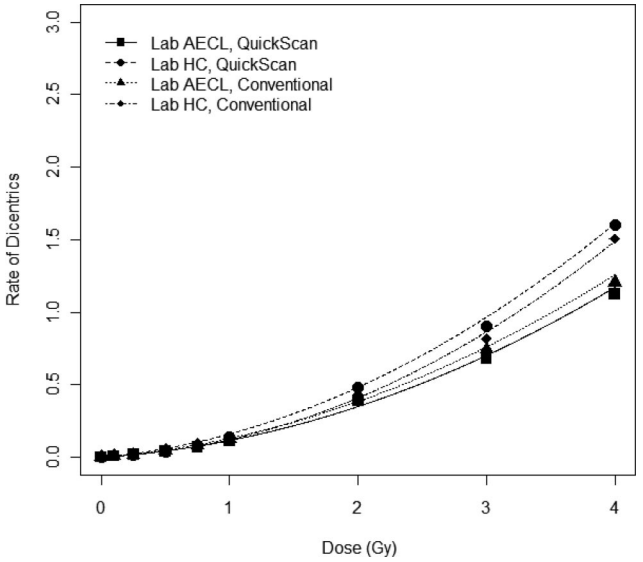

Fig. 8. Overall dose response models for each laboratory and scoring method.

throughput by a factor of approximately six, the combination of QuickScan and triage scoring would significantly decrease biological dosimetry response times. Furthermore, integrating these strategies with networking and/or web-based scoring would result in a biological dosimetry community with the ability to provide accurate and timely dose estimates in a mass casualty event involving radiological or nuclear material.

Acknowledgments—The authors wish to thank the Chemical, Biological, Radiological-Nuclear and Explosive Research and Technology Initiative (CRTI) for the funding to carry out this work. We would also like to thank all those who contributed to this study by diligently scoring many metaphase spreads. From Health Canada: Catherine Ferrarotto, Barbara Kutzner, Sylvie Lachapelle, and Nancy Ringuette; from Atomic Energy of Canada Ltd: Dale Buchanan, Laura Paterson, and Melinda Blimkie.

REFERENCES

Alexander GA, Swartz HM, Amundson SA, Blakely WF, Buddemeier B, Gallez B, Dainiak N, Goans RE, Hayes RB, Lowry PC, Noska MA, Okunieff P, Salner AL, Schauer DA, Trompier F, Turteltaub KW, Voisin P, Wiley Jr AL, Wilkins R. Biodosepr-2006 meeting: acute dosimetry consensus committee recommendations on biodosimetry applications in events involving uses of radiation by terrorists and radiation accidents. Radiat Meas 42:972–996; 2007.

Drum M, McCullagh P. REML estimation with exact covariance in the logistic mixed model. Biometrics 49:677–689; 1993.

Flegal FN, Devantier Y, McNamee JP, Wilkins RC. Quickscan dicentric chromosome analysis for radiation biodosimetry. Health Phys 98:276–281; 2010.

IAEA. Cytogenetic analysis for radiation dose assessment: a manual. Vienna: IAEA; 2001.

ISO. Radiation protection—perfomance criteria for service laboratories performing biological dosimetry by cytogenetics: ISO 19238. Geneva: International Organization for Standardization; 2004.

ISO. Radiation protection—performance criteria for laboratories performing cytogenetic triage for assessment of mass casualties in radiological or nuclear emergencies—general principles and application to dicentric assay: ISO 21243. Geneva: International Organization for Standardization; 2008.

Livingston GK, Wilkins RC, Ainsbury EA. Pilot website to support international collaboration for dose assessments in a radiation emergency. Radiation Measurements 46:912–915; 2011.

Lloyd DC, Edwards AA, Moquet JE, Guerrero-Carbajal YC. The role of cytogenetics in early triage of radiation casualties. Appl Radiat Isot 52:1107–1112; 2000.

Martin PR, Berdychevski RE, Subramanian U, Blakely WF, Prasanna PGS. Sample tracking in an automated cytogenetic biodosimetry laboratory for radiation mass casualties. Radiat Meas 42:1119–1124; 2007.

Merkle W. Statistical methods in regression and calibration analysis of chromosome aberration data. Radiat Environ Biophys 21:217–233; 1983.

Miller SM, Ferrarotto CL, Vlahovich S, Wilkins RC, Boreham DR, Dolling JA. Canadian cytogenetic emergency network (cen) for biological dosimetry following radiological/nuclear accidents. Int J Radiat Biol 83:471–477; 2007.

Romm H, Wilkins RC, Coleman CN, Lillis-Hearne PK, Pellmar TC, Livingston GK, Awa AA, Jenkins MS, Yoshida MA, Oestreicher U, Prasanna PG. Biological dosimetry by the triage dicentric chromosome assay: potential implications for treatment of acute radiation syndrome in radiological mass casualties. Radiat Res 175:397–404; 2011.

Vaurijoux A, Gruel G, Pouzoulet F, Gregoire E, Martin C, Roch-Lefevre S, Voisin P, Roy L. Strategy for population triage based on dicentric analysis. Radiat Res 171:541–548; 2009.

Wilkins RC, Romm H, Kao TC, Awa AA, Yoshida MA, Livingston GK, Jenkins MS, Oestreicher U, Pellmar TC, Prasanna PG. Interlaboratory comparison of the dicentric chromosome assay for radiation biodosimetry in mass casualty events. Radiat Res 169:551–560; 2008.

Wolfinger R, O'Connell M. Generalized linear mixed models: a pseudo-likelihood approach. J Statistical Computation Simulation 48:233–243; 1993.

Yoshida MA, Hayata I, Tateno H, Tanaka K, Sonta S, Kodama S, Kodama Y, Sasaki MS. The chromosome network for biodosimetry in Japan. Radiat Meas 42:1125–1127; 2007.

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