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Development of electronic training and telescoring tools to increase the surge capacity of dicentric chromosome scorers for radiological/nuclear mass casualty incidents

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Abstract

Dicentric chromosome assay (DCA) is most frequently used for estimating the absorbed radiation dose in the peripheral blood lymphocytes of humans after occupational or incidental radiation exposure. DCA is considered to be the “gold standard” for estimating the absorbed radiation dose because the dicentric chromosome formation is fairly specific to ionizing radiation exposure and its baseline frequency is extremely low in non-exposed humans. However, performance of DCA for biodosimetry is labor intensive and time-consuming making its application impractical for radiological/nuclear mass casualty incidents. Realizing the critical need for rapid dose estimation particularly after radiological/nuclear disaster events, several laboratories have initiated efforts to automate some of the procedural steps involved in DCA. Although metaphase image capture and dicentric chromosome analysis have been automated using commercially available platforms, lack or an insufficient number of these platforms may pose a serious bottleneck when hundreds and thousands of samples need to be analyzed for rapid dose estimation. To circumvent this problem, a web-based approach for telescoring was initiated by our laboratory, which enabled the cytogeneticists around the globe to analyze and score digital images. To further increase the surge capacity of dicentric scorers, we recently initiated a dicentric training and scoring exercise involving a total of 50 volunteers at all academic levels without any prerequisite for experience in

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Conflict of interest

The authors declare no conflict of interest

radiation cytogenetics. Out of the 50 volunteers enrolled thus far, only one outlier was found who overestimated the absorbed radiation dose. Our approach of training the civilians in dicentric chromosome analysis holds great promise for increasing the surge capacity of dicentric scorers for a rapid biodosimetry in the case of mass casualty scenarios.

Keywords

ionizing radiation; dicentric chromosome assay; lymphocyte culture; radiation dose assessment; radiological triage; radiological/nuclear mass casualty incidents

Introduction

Ionizing radiation is a well-known carcinogen and ionizing radiation (IR) exposure results in the induction of a wide spectrum of DNA lesions involving DNA single-strand breaks, DNA double-strand breaks, base damage and DNA-protein crosslinks. Among these lesions, DNA double-strand break is considered to be the most lethal lesion which when mis-rejoined leads to structural chromosome alterations. Occurrence of such alterations in the germ cells can lead to deleterious heritable pathological conditions. Human exposure to IR has become inevitable and radiation exposure ranges from medical diagnostics/treatment to incidental and accidental radiological or nuclear disasters. Additionally, an ever increasing threat of radiological terrorism around the globe is a growing concern because it may result in the exposure of several hundreds and thousands of people. In the case of radiological or nuclear mass casualty incidents, efficient radiological triage methods are critical for risk stratification so that overly exposed people can get immediate medical attention.

In the absence of a personal physical dosimeter, which is predominantly worn only by occupational radiation workers, biological dosimeters are pivotal for estimating the absorbed radiation dose in civilians. The last decade has seen the development of a whole battery of biomarkers in the field of transcriptomics, metabolomics and proteomics but none of these biomarkers have been tested and validated for reliable dose estimation in radiation exposed humans. Further, most “omics” markers still remain as very good bio indicators of radiation exposure rather than biodosimeters owing to an extensive inter-individual variation in their baseline frequencies. The Dicentric Chromosome Assay (DCA), which was originally developed by Bender and Gooch in 1962 (Bender & Gooch, 1962), still remains as the “gold standard” for radiation dose assessment because of high specificity for radiation exposure and extremely low baseline frequency. Strikingly, the baseline frequency of dicentric chromosomes in healthy human subjects (1 in 1000 metaphase cells) is not largely influenced either by age or gender. The DCA has been successfully used for more than four decades for radiation dose assessment of people exposed in many well-known mass casualty incidents such as Chernobyl, Goiania, and Fukushima (Khvostunov, Snigiryova, Moiseenko, & Lloyd, 2015; Lee et al., 2012; Ramalho, Curado, & Natarajan, 1995; Suto, 2016; Suto et al., 2013).

Although DCA is considered to be the gold standard, its performance is time-consuming and laborious with an estimated turnaround time of 4–5 days for radiation dose estimation. These attributes make DCA impractical for its application to radiation mass casualty

incidents. Realizing the critical need for a rapid dose estimation, scoring strategy has been modified to increase the throughput for radiological triage by restricting the analysis to 50 metaphase cells or 30 dicentric chromosomes, whichever is achieved first (Lloyd, Edwards, Moquet, & Guerrero-Carbajal, 2000; Romm et al., 2011). Additionally, inter-laboratory scoring exercises were started through telescoring of digital images (Garcia et al., 2013; Romm, Ainsbury, Bajinskis, et al., 2014; Romm et al., 2013; Romm et al., 2011) which is essential not only for assessing the proficiency and accuracy but also for building the global network of cytogenetic laboratories to full the biodosimetry needs of radiation mass casualty incidents. A few earlier studies have successfully utilized Metafer (MetaSystems, Boston, MA, USA) assisted semi-automated scoring of dicentric chromosomes for considerably reducing the analysis time for dose estimation (De Amicis et al., 2014; Gruel et al., 2013; Romm et al., 2013; Romm, Ainsbury, Barnard, et al., 2014). Recently, Rogan and his colleagues (Rogan et al., 2014; Rogan, Li, Wilkins, Flegal, & Knoll, 2016) developed a prototype software system for automated dicentric chromosome detection with a high sensitivity and specificity, which will be useful for rapid image analysis.

Automated dicentric scoring is certainly advantageous to reduce the turnaround time for dose estimation but its accuracy depends on the quality of metaphase chromosome spreads. Since automated dicentric scoring invariably results in a few false positives and negatives, manual verification becomes inevitable for precise estimation of the absorbed radiation dose. Further, achieving a timely assessment of radiation dose in the case of large-scale radiological/nuclear incidents is likely to be hampered by the restricted number of Metafer microscopes and the limited number of experienced cytogenetic personnel. Therefore, increasing the surge capacity of dicentric scorers is a critical requirement for fulfilling the biodosimetry needs of any mass casualty incident. With this objective, a dicentric chromosome training/scoring program was initiated by us to teach and train the volunteers in dicentric chromosome analysis. The scorers were given instructions for analyzing the number of dicentric chromosomes in approximately 100 digital images of metaphase cells that contained varying numbers (0–3) of dicentric chromosomes. Additionally, we also developed a dicentric scoring module where analysis time was recorded to assess the efficiency and accuracy of dicentric chromosome scoring. Development of e-training tools and telescoring will substantially enhance the surge capacity of dicentric scorers thereby enabling an effective triage in the aftermath of radiological/nuclear mass casualty incidents.

Materials and Methods

Lymphocyte culture

Whole blood samples (~10 ml) were collected from healthy human donors and the blood collection was performed with the consent of donors in strict compliance with the Institutional Review Board (IRB) protocol (ORAU 000349). Blood samples from healthy human volunteers were collected in heparinized vacutainers in compliance with our IRB protocol. Aliquots (0.5 ml) of blood samples irradiated with different doses of γ -rays (0–5Gy) were cultured for 44 hrs in complete growth medium (RPMI 1640, 15% fetal bovine serum, 2% Phytohaemagglutinin, 10uM BrdU and antibiotics) followed by the addition of colcemid for an additional 4 hrs to arrest the cells at metaphase. Cells harvested at 48 hrs

after culture initiation were subjected to a hypotonic treatment (0.56% KCl) for 16–18 min at 37°C followed by fixation in acetic acid: methanol (1:3). The fixed cells were washed three times in fixative and the chromosomes were prepared by the air drying technique. The slides were briefly treated with RNase and subsequently stained with 2% Giemsa in phosphate buffered solution.

Metaphase Image capture

The slides were scanned for metaphase cells using the metaphase finder (Metafer; MetaSystems, Boston, MA, USA) first at 10X objective followed by 63X objective. For the dicentric scoring exercise, approximately 100 analyzable high-quality metaphase cells were chosen from unirradiated and irradiated samples. The digital images of metaphase cells were uploaded into SharePoint site for telescoring. Both grey scale and color images were loaded for dicentric scoring. For those volunteers who could not access the “SharePoint” site, a compact disc was provided with metaphase images and dicentric scoring instructions.

Recruitment of volunteers for dicentric chromosome scoring

A total of 50 volunteers at various academic levels (high school students, teachers, professional individuals with various academic degrees (BS, MS and Ph.D. degrees as well as post-doctoral students) were randomly chosen. With the exception of one, the participants in the scoring exercise did not have any prior experience either in basic cytogenetics or in radiation cytogenetics.

Evaluation of scoring efficiency

The efficiency of dicentric scoring was evaluated by estimating the radiation dose based on dicentric chromosome frequency using the standard calibration curve generated in our laboratory for Co-60. The dicentric scoring performed by the experienced cytogeneticist in our laboratory was considered to be the reference for evaluating the dicentric scoring efficiency of the participants. The lower (LCL) and upper (UCL) confidence limits were calculated at 95% confidence intervals using CABAS (Chromosomal ABerrations Calculation Software).

Development of e-training and scoring tools

In an effort to identify the efficient dicentric scorers based on speed and accuracy, a combination of training and scoring modules were developed. For the training module, a minimum of 10 digital images with differing quality of metaphase chromosome spreads was uploaded. This module was designed to teach the basic criteria for choosing the metaphase cells for dicentric chromosome analysis. The scoring module was designed with a timer to determine the accuracy and speed of the dicentric analysis. These modules will be useful for training and identifying the efficient dicentric scorers for radiological triage scenarios.

Statistical Analysis for Scorer Reliability

Grubbs' test in R software (version 3.5) was utilized to identify the outliers, if any, among the 50 volunteers. Intraclass Correlation Coefficient (ICC) was used to evaluate scorer reliability, i.e. level of agreement among the scorers in estimating the number of dicentric

chromosomes. ICC is a standard tool to assess measurement error (Koo and Li, 2016) and an ICC value of 1 (ICC=1) indicates a perfect reliability.

Results

To increase the surge capacity of dicentric scorers for mass casualty incidents, we had recruited 50 randomly chosen volunteers at various academic levels. For the dicentric scoring exercise, two sets of 107 and 102 images of high-quality metaphases, one in greyscale and one in color scale respectively were chosen with varying numbers of dicentric chromosomes (0–3). 50 volunteers analyzed the greyscale images and 33 out of these 50 volunteers also analyzed the color images. The representative metaphase images used for the dicentric scoring exercise are shown in Fig.1. The TIFF images were uploaded into the SharePoint site and the scorers were asked to register with a username and password for accessing the images for dicentric chromosome scoring. The scoring instructions including the criteria for selection of metaphases were also given in the form of powerpoint slides. Those scorers who could not access the website were provided with a compact disc containing either one (grey scale or color images) or both sets of images along with dicentric chromosome scoring instructions. The radiation dose estimated from the dicentric chromosome frequency scored by an experienced cytogeneticist in our laboratory was considered to be the reference for evaluating the scoring efficiency and accuracy of the scorers. Among the volunteers chosen, only one participant had a prior experience in clinical cytogenetics but not in radiation cytogenetics. The volunteers were asked to score the number of dicentric chromosomes with accompanying acentric fragments in metaphase cells with 46 centromeres. In the initial phase, a total of 50 volunteers were recruited and they analyzed the metaphase images in grey color. Among the 50 participants, only one outlier was found who overestimated the number of dicentric chromosomes and therefore the estimated dose. Chromosome ABerration cAlculation Software (CABAS) was used for the radiation dose estimation using our standard ^{60}Co calibration curve (Fig.2). The radiation dose estimated by CABAS using the dicentric frequency scored by the participants was within the range of 2.32 ± 0.3 Gy. A total of 33 volunteers who analyzed the dicentric frequency in 102 color images predicted the radiation dose fairly accurately and the estimated dose was within a very narrow range of 2.59–2.74 Gy. The results of these scorers on the estimated radiation dose with low and upper limits at 95% confidence interval are shown in Fig.2. Using the Grubb's test R software version (3.5), we found only one outlier among the 50 volunteers who analyzed the grey scale images. The outlier (scorer no.6) scored 68 dicentric chromosomes while the mean for the 50 volunteers was found to be 48.9 ± 6.2 (mean $\pm$ SD) and estimation of 68 dicentric chromosomes yielded a dose of 2.8 Gy while the mean dose was 2.34 ± 0.14 Gy. The Z value for the scorer 6 was 3.08 whereas the critical value was 3.13. In addition to scorer 6, three other scorers (no. 18, 42 and 44) underestimated the number of dicentric chromosomes (34–37 dicentrics relative to 48 by the reference) and this underestimation was not found to be statistically significant. Interestingly, no outlier was observed among the volunteers who analyzed the color scale images and in fact most of the volunteers (28 out of 33) preferred the color scale images to grey scale images for reliable scoring. Scorer 13 estimated 49 dicentric chromosomes in a total of 102 metaphase cells which was the farthest from the mean (57.9 ± 3.8) but this

deviation was not found to be statistically significant in terms of the estimated absorbed radiation dose. The Z value for the scorer 13 was 2.35 whereas the critical value was 2.95. As mentioned before, an Intraclass Correlation Coefficient was used to identify the outliers, if any, among the volunteers. As our goal was to assess the reliability among non-professional scorers, the reference scorer (experienced cytogeneticist) was excluded in this analysis. The ICC (2,1) version was used, which represents two-way random effects, absolute agreement, and single rater/measurement. This set of assumptions was found appropriate for our study because we planned to generalize the reliability results to any given scorer with the same characteristics as the selected scorers in the reliability study. Missing or N/A values in the data were converted to 0 to calculate ICC. An ICC value of 0.812 (95% CI: 0.769, 0.852) was observed for the volunteers who analyzed the grey scale images. Removal of outliers slightly increased the ICC value to 0.842 (95% CI 0.805, 0.878). These values indicate a good reliability among the scorers. The ICC value obtained for the volunteers, who analyzed the color images, was higher than that obtained for grey scale images [0.913 (95% CI: 0.889, 0.934)] indicating an excellent reliability among the scorers. One sample “t” test was next utilized to compare the efficiency of scoring between a professional scorer and non-professional scorers. The difference was not statistically significant and the *p* values were found to be 0.30 and 0.93 for the volunteers, who analyzed grey scale and color images respectively. The statistical analysis performed here assumes that the dicentric count is a continuous variable but in fact it is an integer. However, this assumption is unlikely to alter the conclusions because the dicentric counts were higher than 1 and their distribution was consistent with the normal distribution. Specifically, on grey scale image data, after removing the professional scorer 1 and the outliers, the number of total dicentric chromosomes was consistent with a Normal distribution: Shapiro-Wilk test *p*=0.12. On color file data, after removing professional scorer 1, the result was similar: Shapiro-Wilk test *p*=0.08.

In order to create the dicentric training tools, a number of components were considered: (I) creation of training session involving the loading of metaphase images with varying levels of complexity, (II) metaphase scoring instructions, (III) setting up of dicentric scorer groups and (IV) aggregation, data analysis and dose assessment using CABAS. A training module comprising dicentric chromosome scoring instructions, demo images, images for analysis and dose assessment was successfully created. Efforts were also made towards developing a scoring module for assessing the efficiency of dicentric scorers. To track the accuracy and efficiency of dicentric chromosome scoring, the module was designed to record the analysis time taken for each metaphase cell. Prior to scoring, reader will be given the choice of choosing or rejecting a cell based on chromosome number, morphology, size and spreading. Once chosen, reader is asked to enter the number of dicentrics found in a given cell and then move to the next cell for analysis. At the completion of analysis of each cell, analysis time will be recorded. Figures representing the development of dicentric chromosome scoring module are shown in Fig.3 (A–F). Typically, Fig.3A shows the name of the scorer, group, session number and difficulty level. When the scorer clicks on the green “Start” button, scoring time is recorded. Fig.3B is designed to assess the quality of metaphase for analysis by the scorer. The scorer can decide whether to choose or reject a metaphase by clicking “Yes” or “No” by judging the quality of metaphase chromosome spreads based on

chromosome morphology and spreading. The reader's decision is verified as shown in Fig. 3C and the reader can move to the next slide (Fig.3D) for analysis. The reader is required to enter the number of dicentrics found in the chosen metaphase cell and then click on the submit button (Fig.3E). The consolidated analysis result is shown in Fig.3F. The scoring module will be effective in tracking the training history as well as in assessing dicentric scoring efficiency. We intend to use this tool for identifying efficient scorers based on accuracy and analysis time.

Discussion

In the case of radiological or nuclear mass casualty incidents, several hundreds and thousands of people are likely to get exposed to ionizing radiation (IR). IR induces various adverse effects on humans including mortality from acute radiation syndrome with a LD50/60 of 3.5–4.5 Gy without therapy and higher than 6.0–7.0 Gy with appropriate therapy. IR also causes stochastic effects resulting in increased deaths from cancer and other tissue degenerative diseases. Therefore, timely assessment of absorbed radiation dose in exposed individuals is critical for making “lifesaving” clinical decision. Bender and Gooch (1962) first demonstrated that detection of a chromosomal anomaly known as dicentric chromosome in the peripheral blood lymphocytes can be effectively utilized for estimating the absorbed radiation dose in humans. Ever since, the dicentric chromosome assay (DCA) has become the most preferred method of choice for radiation biodosimetry and is considered to be the “gold standard” for radiation dose assessment owing to a number of unique features: (I) minimally invasive technique involving peripheral blood lymphocytes, (II) extremely low level of base line frequency in healthy individuals (1–2 dicentric chromosomes in 1000 metaphase cells), (III) minimal or no significant dependence on age, gender and lifestyle, (IV) frequency of formation is radiation dose dependent and (V) fairly long persistence of dicentric chromosome signal (up to 4 weeks). One distinct advantage of DCA over other “omics” biomarkers is the extremely low level of baseline frequency without any significant inter-individual variation.

Although DCA is considered to be the gold standard for absorbed radiation dose assessment, time consuming and labor intensive nature of this assay with a turnaround time of 3–4 days for dose assessment make it somewhat unsuitable for triage application. Considering the specificity and sensitivity of DCA to radiation exposure, a multitude of efforts has been taken recently by several cytogenetic biodosimetry laboratories worldwide to increase the utility/application of DCA for radiological or nuclear mass casualty incidents: (I) automation of chromosome preparatory steps using commercially available harvester and spreader (HANABI, USA), (II) development of high throughput robotic platforms such as RABiT for handling/processing large number of blood samples (Bian et al., 2016; Garty et al., 2016; Repin, Pampou, Karan, Brenner, & Garty, 2017), (III) automation of metaphase chromosome finder and image capture (Romm et al., 2013; Romm, Ainsbury, Barnard, et al., 2014), (IV) automated analysis using dicentric chromosome scoring algorithms such as DCScore by Metafer, USA and Automated Dicentric Chromosome Identifier (ADCI) by Cytognomix, Canada (Rogan et al., 2014; Rogan et al., 2016; Romm et al., 2013; Romm, Ainsbury, Barnard, et al., 2014), (V) Increasing the surge capacity of scorers by web-based telescoring (Romm, Ainsbury, Bajinskis, et al., 2014; Sugarman et al., 2014), (VI)

development of miniaturized version of DCA for high throughput sample processing (Balajee et al., 2018), (VII) radiological triage mode of scoring for dose assessment (Lloyd et al., 2000; Romm et al., 2013; Romm et al., 2011), (VIII) quick scan method (Flegal, Devantier, Marro, & Wilkins, 2012; Flegal, Devantier, McNamee, & Wilkins, 2010) and (IX) development of cytogenetic biodosimetry laboratory network(s) for increasing the synergy and harmony for assistance during radiological or nuclear emergencies (Flegal et al., 2012; Garcia et al., 2013; M'Kacher et al., 2015; Miller et al., 2007; Sugarman et al., 2014; Thierens et al., 1999).

In the case of mass casualty incidents, timely assessment of radiation dose assessment by DCA is likely to be constrained by the restricted number of both Metafer microscopes and trained cytogenetic personnel. Further, manual verification of automated dicentric chromosome analysis is often required for precise dose estimation after correcting false positives and negatives. To circumvent some of these issues, a web-based telescoring approach was initiated by us (Sugarman et al., 2014) to determine the feasibility of enhancing the biodosimetric response in the case of a large scale radiological event. In this study, a mock exercise scenario was presented involving an acutely exposed individual to a gamma source (^{60}Co) and a total 6 laboratories from North and South America, Europe and Asia were asked to estimate the absorbed radiation dose for the exposed individual from a gallery of 810 digital images that were shared using Microsoft SharePoint Software by creating an external SharePoint internet site at Oak Ridge Associated Universities, Oak Ridge, Tennessee, USA. The mean of the absorbed radiation dose (1.89 Gy) estimated by the participating laboratories was in conformity with the actual physical dose of 2 Gy. In the current study, we utilized the same SharePoint site to further increase the surge capacity of dicentric scorers for radiological emergencies. The current study suggests that the dicentric training/ scoring program is very effective in increasing the surge capacity of dicentric scorers. Our study is also unique from some of the earlier inter-laboratory comparison studies where several trained cytogeneticists participated in web-based dicentric scoring for verifying the proficiency and accuracy of radiation dose assessment (Oestreicher et al., 2017; Romm, Ainsbury, Bajinskis, et al., 2014; Sugarman et al., 2014). The current approach of training the civilians interested in dicentric chromosome scoring has immense potential for meeting the biodosimetry needs of radiological mass casualty situations. To monitor and identify efficient scorers based on both accuracy and speed of analysis, a scoring module was developed. This module, once fully developed, will be useful for tracking and selecting efficient scorers. We plan to expand our training and scoring program by incorporating metaphase images with varying levels of complexity (in terms of dicentric chromosome frequency and chromosome quality) so that volunteers will be sufficiently trained to perform the dicentric chromosome analysis. Development of an emergency network involving currently available Cytogenetic Biodosimetry Laboratories (CBLs) in the USA is crucial to meet the biodosimetry needs of any radiological/nuclear mass casualty incident(s). Towards this end, our laboratory has already initiated efforts to leverage the facilities of CBL at Yale University School of Medicine, New Haven CT so that samples can be shared among the CBLs and processed for rapid biodosimetry. Efforts will be initiated to include some of the commercial clinical cytogenetic laboratories in the network as these labs are well equipped for carrying out routine chromosome analysis. To better prepare for any

radiological/nuclear emergencies, a centralized database will be developed with galleries of metaphase images obtained from various donor samples for analysis. The trained scorers with access to the database can rapidly analyze either manually or verify the automated Metafer scoring for a timely assessment of radiation dose. Our approach of utilizing a web-based training and dicentric chromosome-scoring program for increasing the surge capacity of scorers will be certainly beneficial to adequately address the biodosimetry needs of mass casualty incidents.

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Fig.1.
Representative grey scale and color images of metaphase cells used for the dicentric scoring exercise for the volunteers.

Fig.2.

Results of the dicentric chromosome scoring analysis performed by the volunteers using grey sclae (A) and color (B) images. Radiation doses were estimated using the CABAS software with lower (LCL) and upper (UCL) limits at 95% confidence interval (CI). Scorer 1, an experienced cytogeneticist/biodosimetrist, was the reference for determining the accuracy and efficiency of dicentric scoring among the volunteers.

Fig.3.

Development of the dicentric chromosome scoring tool for monitoring the efficiency of dicentric chromosome scoring. Typically, Fig.3A shows the name of the scorer, group, session number and difficulty level. When the scorer clicks on the green “Start” button, scoring time is recorded. Fig.3B is designed to assess the quality of metaphase for analysis by the scorer. The scorer can decide whether to choose or reject a metaphase by clicking “Yes” or “No” by judging the quality of metaphase chromosome spreads based on chromosome morphology and spreading. The reader’s decision is verified as shown in Fig.3C and the reader can move to the next slide (Fig.3D) for analysis. The reader is required to enter the number of dicentrics found in the chosen metaphase cell and then click on the submit button (Fig.3E). The consolidated analysis result is shown in Fig.3F.

Table 1.

Dicentric chromosome scoring analysis of volunteers using grey scale images of metaphase cells

	<u>Cells scored</u>	<u>No. Dicentrics</u>	<u>LCL 95% CI</u>	<u>Estd.Dose</u>	<u>UCL 95% CI</u>
Scorer 1	107	48	1.96	2.32	2.7
Scorer 2	107	46	1.9	2.26	2.65
Scorer 3	107	45	1.87	2.24	2.62
Scorer 4	107	41	1.76	2.12	2.51
Scorer 5	107	47	1.93	2.29	2.68
Scorer 6	107	68	2.44	2.8	3.19
Scorer 7	107	51	2.03	2.4	2.78
Scorer 8	107	54	2.11	2.47	2.86
Scorer 9	107	45	1.87	2.24	2.62
Scorer 10	107	40	1.73	2.09	2.48
Scorer 11	107	48	1.96	2.32	2.7
Scorer 12	107	46	1.9	2.26	2.65
Scorer 13	107	47	1.93	2.29	2.68
Scorer 14	107	45	1.87	2.24	2.62
Scorer 15	107	49	1.99	2.35	2.73
Scorer 16	107	48	1.96	2.32	2.71
Scorer 17	107	50	2.01	2.37	2.76
Scorer 18	107	37	1.64	2.01	2.4
Scorer 19	107	51	2.04	2.4	2.79
Scorer 20	107	48	1.96	2.32	2.7
Scorer 21	107	46	1.91	2.27	2.65
Scorer 22	107	51	2.04	2.4	2.79
Scorer 23	107	50	2.01	2.37	2.76
Scorer 24	107	50	2.01	2.37	2.76
Scorer 25	107	50	2.01	2.37	2.76
Scorer 26	107	52	2.06	2.42	2.81
Scorer 27	107	50	2.01	2.37	2.76
Scorer 28	107	48	1.96	2.32	2.71
Scorer 29	107	49	1.99	2.35	2.73
Scorer 30	107	40	1.99	2.35	2.73
Scorer 31	107	45	1.88	2.32	2.63
Scorer 32	107	62	2.31	2.67	3.1
Scorer 33	107	43	1.82	2.2	2.57
Scorer 34	107	53	2.09	2.45	2.84
Scorer 35	107	50	2.01	2.37	2.76
Scorer 36	107	48	1.96	2.32	2.71
Scorer 37	107	51	2.04	2.4	2.79
Scorer 38	107	50	2.01	2.37	2.76
Scorer 39	107	58	2.21	2.58	2.96

	<u>Cells scored</u>	<u>No. Dicentrics</u>	<u>LCL 95% CI</u>	<u>Estd.Dose</u>	<u>UCL 95% CI</u>
Scorer 40	107	52	2.06	2.43	2.81
Scorer 41	107	46	1.91	2.27	2.67
Scorer 42	107	37	1.65	2.01	2.4
Scorer 43	107	55	2.14	2.5	2.89
Scorer 44	107	34	1.55	1.92	2.31
Scorer 45	107	47	1.93	2.29	2.68
Scorer 46	107	54	2.12	2.48	2.86
Scorer 47	107	48	1.96	2.32	2.71
Scorer 48	107	60	2.26	2.62	3.01
Scorer 49	107	55	2.14	2.5	2.89
Scorer 50	107	58	2.21	2.58	2.96

Table 2.

Dicentric chromosome scoring analysis of volunteers using color images of metaphase cells

	<u>Cells scored</u>	<u>No. Dicentrics</u>	<u>LCL 95% CI</u>	<u>Estd.Dose</u>	<u>UCL 95% CI</u>
Scorer 1	102	58	2.27	2.64	3.03
Scorer 2	102	56	2.22	2.59	2.99
Scorer 3	102	57	2.25	2.62	3.01
Scorer 4	102	59	2.3	2.67	3.06
Scorer 5	102	52	2.12	2.49	2.87
Scorer 6	102	56	2.22	2.59	2.99
Scorer 7	102	59	2.3	2.67	3.06
Scorer 8	102	63	2.39	2.76	3.16
Scorer 9	102	60	2.32	2.69	3.09
Scorer 10	102	57	2.25	2.62	3.01
Scorer 11	102	58	2.27	2.64	3.03
Scorer 12	102	56	2.22	2.59	2.99
Scorer 13	102	49	2.04	2.41	2.81
Scorer 14	102	56	2.22	2.59	2.99
Scorer 15	102	59	2.3	2.67	3.06
Scorer 16	102	63	2.39	2.76	3.16
Scorer 17	102	57	2.25	2.62	3.01
Scorer 18	102	52	2.12	2.49	2.87
Scorer 19	102	58	2.27	2.64	3.03
Scorer 20	102	59	2.3	2.67	3.06
Scorer 21	102	61	2.35	2.72	3.11
Scorer 22	102	58	2.27	2.64	3.04
Scorer 23	102	56	2.22	2.59	2.99
Scorer 24	102	57	2.25	2.62	3.01
Scorer 25	102	61	2.35	2.71	3.11
Scorer 26	102	57	2.25	2.62	3.01
Scorer 27	102	50	2.07	2.44	2.83
Scorer 28	102	57	2.25	2.62	3.01
Scorer 29	102	60	2.32	2.69	3.09
Scorer 30	102	57	2.25	2.62	3.01
Scorer 31	102	65	2.44	2.81	3.2
Scorer 32	102	63	2.39	2.76	3.16
Scorer 33	102	66	2.47	2.84	3.23