The dicentric assay in triage mode as a reliable biodosimetric scoring strategy for population triage in large scale radiation accidents

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Abstract

Introduction: Mass casualty scenarios of radiation accidents require high throughput techniques of biological dosimetry for population triage to identify individuals for whom clinical treatment is indicated. To this end the dicentric assay in a triage mode is a very suitable technique. Within the MULTIBIODOSE EU FP7 project a network of eight biodosimetry laboratories has been established with expertise in dose estimations based on the dicentric assay.

Results: In the first task the conventional dicentric assay was tested in the triage mode. Three types of irradiation scenarios were included: acute whole body, partial body and protracted exposure. Blood samples from 33 healthy donors (> 10 donors / scenario) were irradiated in vitro with gamma rays, simulating the 3 different types of exposure and the 3 different doses. All the blood samples were irradiated at the University of Gent, Belgium, and then shipped to the participating laboratories. The dose estimates of acute whole body exposure show a good agreement with actual radiation doses (0.5, 2.0 and 4.0 Gy) for all labs. Most labs could identify correctly the partial body doses at 4 and 6 Gy, but this was not possible at 2 Gy and indicates a need for more cells to be analysed. After protracted exposure, all labs performed these dose estimations well and attained good results at 1.0 and 2.0 Gy.

Conclusions: The results obtained up to now within the MULTIBIODOSE project are very promising for the application of the dicentric assay in triage mode as a high throughput scoring strategy for biodosimetry in case of large scale accidents by a network of eight collaborating laboratories throughout Europe.

1. Introduction

The dicentric assay has been validated as a reliable biosimetric tool in small radiation accidents involving a low number of casualties (<100) (Stephan et al 2007, Romm et al 2009). To be prepared for a large scale radiation accident with a large number of potentially exposed persons, there is a need for a high throughput of samples. There have been several strategies developed to cope with this by establishing networks of biodosimetry labs giving mutual assistance (Blakely et al 2009, Di Giorgio et al 2011, RENEB 2012), by developing new scoring strategies (Flegal et al 2010, 2012), by improving the methods with automation (Martin et al 2007, Vaurijoux et al 2009, Jaworska et al 2011) and web based scoring (Livingston et al 2011). To increase the throughput for triage with the dicentric assay a reduction of the number of cells to be scored has been suggested (Lloyd et al 2000). The potential and limits of the new strategy of dicentric scoring in triage mode by a network needs to be examined. A new perspective regarding cooperation between trained laboratories was opened and validated for different radiation exposure conditions.

To explore the homogeneity of dose estimations between 8 European biodosimetry laboratories in the framework of the EU project MULTIBIODOSE it was planned to send blind coded irradiated blood samples and to compare the resulting dose estimations.

The major steps in this interlaboratory comparison can be described as follows: Blood from healthy donors was irradiated in vitro with gamma rays in one laboratory (University of Ghent) and blood samples were sent to the participants. Three different exposure scenarios were simulated, whole and partial body irradiation after acute exposure and low dose protracted exposure, using three different doses per exposure scenario and three repeats per dose point. Scoring was done with two slides of each blood sample in order to obtain information regarding inter- and intra-individual variations. Furthermore, the dicentrics scored from 50 cells in total were to be compared with the results of the first 20 and 30 scored cells of the same slide to get more information about the uncertainties associated with the scoring in triage mode. The aim was to show whether the resulting dose estimations of the participating laboratories were in good agreement or if there was a need for further training and harmonisation of the methods.

2. Material and Methods

In total 33 blood samples from 33 healthy donors were irradiated at the University of Ghent, simulating three different types of exposure scenarios. The blood samples were shipped coded as blind samples in six shipments to the eight participating laboratories and always included one unirradiated shame control. Furthermore each batch of blood samples contained a temperature logger and TLD dosemeter to monitor the temperature and to measure any dose received by the samples during transport. Each shipment contained samples of a high dose rate, a low dose rate and a partial body exposure, thereby giving a total of 27 irradiated samples.

The blood samples were irradiated in vitro in a water bath at 37˚C in a ^{60}Co γ-beam at a dose rate of 0.27 Gy / min for acute whole body (0.5, 2.0 and 4.0 Gy) or partial body exposure (2.0, 4.0 and 6.0 Gy). To simulate partial body irradiations, irradiated blood samples were mixed with sham irradiated controls in a ratio of 1:1. For the protracted exposures the dose rate was 0.0015 Gy / min at 1 Gy (irradiation time 10 h 52 min 14 sec), 0.002 Gy/min at 2 Gy (16 h 05 min 49 sec) and 0.004 Gy / min at 4 Gy (16 h 35 min 41 sec).

Each laboratory set up cultures according to the ISO and IAEA standards (ISO 2004, 2008, IAEA 2011), determined the frequency of dicentrics in the first 20, 30 and 50 cells in two parallel slides (a & b) per sample and then estimated the dose. To minimize uncertainties in dose assessment, each lab used his own calibration curve (Wilkins et al 2009). Therefore, the method of cell cycle control (only complete metaphases in ^{1st} mitoses were scored) depended on the standard protocol already established in each participating lab.

To identify the samples after partial body exposures, the distribution of dicentrics was analysed for deviation from Poisson with the u-test. If the u-test was significant, a partial body dose was estimated together with the irradiated volume of the body using the Dolphin method (IAEA 2011) and assuming a mean lethal dose of 3.5 Gy.

For the dose estimations of the protracted exposures, the G function (IAEA, 2011) was applied, taking the duration of exposure into account and assuming a mean lifetime of breaks of 2 hours.

The dose estimations were performed with the free software CABAS V2.0 (Deperas et al 2007) or Dose-Estimate V4.0 (Ainsbury and Lloyd, 2010). The data were analysed using the chi-squared test for homogeneity to compare the variability of samples within and between groups (sample repeats, doses, irradiation types, labs), the z-test for interlaboratory variability, and General Linear Model Analysis of Variance (ANOVA) with post hoc testing (including Dunnett's test for comparison of doses with controls and Tukey's simultaneous tests for pair-wise comparisons of irradiation types and between laboratories) to investigate the combined dose results from all laboratories. In addition, 2-sample (Youden) plots were prepared to compare the reliability of the dose response among all the labs for each dose compared to 0 Gy in each irradiation category and to allow visual interpretation of the origin of the uncertainties. For the purposes of this intercomparison, laboratories were told whether the samples had been exposed to acute or protracted radiation (as this information is usually available in real life biodosimetry cases), but were not told which or how many samples had been partially irradiated, nor were they given the % partial irradiation. The numbers of correctly or falsely identified samples and the calculated percentage were therefore also analysed for each lab.

3. Results and Discussion

The calibration curves used for dose estimation by each laboratory (Fig. 1, Tab. 1) were compared. There was a good agreement between the curves used and the individual calibration coefficients, with a mean of 0.0230 (range 0.0135 – 0.0375) for the alpha (linear) coefficient and a mean of 0.0588 (range 0.0527 – 0.0759) for the beta (quadratic) coefficient. The standard errors of the coefficients were low for beta, 7 ± 2 % across all labs, but slightly higher for alpha, at 28 ± 4 %. Overall, there were no significant differences between the calibration curves used at each of the laboratories for dose calculations (p all > 0.99).

Lab	C	SE	alpha	SE	beta	SE	radiation quality	dose rate (Gy / min)
Lab 1	0.0002	0.0001	0.0187	0.0047	0.0527	0.0039	Caesium 137	0.42
Lab 2	0.0007	0.0004	0.0375	0.0085	0.0531	0.0054	Cobalt 60	0.30
Lab 3	0.0005	0.0005	0.0142	0.0044	0.0759	0.0027	Cobalt 60	0.50
Lab 4	0.0011	0.0008	0.0228	0.0048	0.0460	0.0020	Cobalt 60	0.28
Lab 5	0.0010	0.0004	0.0338	0.0101	0.0536	0.0044	Cobalt 60	0.50
Lab 6	0.0006	0.0003	0.0135	0.0043	0.0544	0.0034	Cobalt 60	0.25
Lab 7	0.0072	0.0063	0.0538	0.0310	0.0716	0.0163	Caesium 137	0.40
Lab 8	0.0013	0.0005	0.0210	0.0052	0.0631	0.0040	Cobalt 60	1.07

Tab. 1: The coefficients α, β and C ($Y = C + \alpha D + \beta D^2$) of the γ-ray dose effect curves of the participants of the intercomparison, SE = standard error

The proportion of M1 and M2 cells at 3 h colcemid were compared and there was very little variation between laboratories, with an average of $95\% \pm 5\%$ in M1 being observed across the board. This is with the exception of lab 5, for which the proportion in M1 was found to be $74 \pm 22\%$, resulting from a slightly longer and variable culture time of 48 to 50 h.
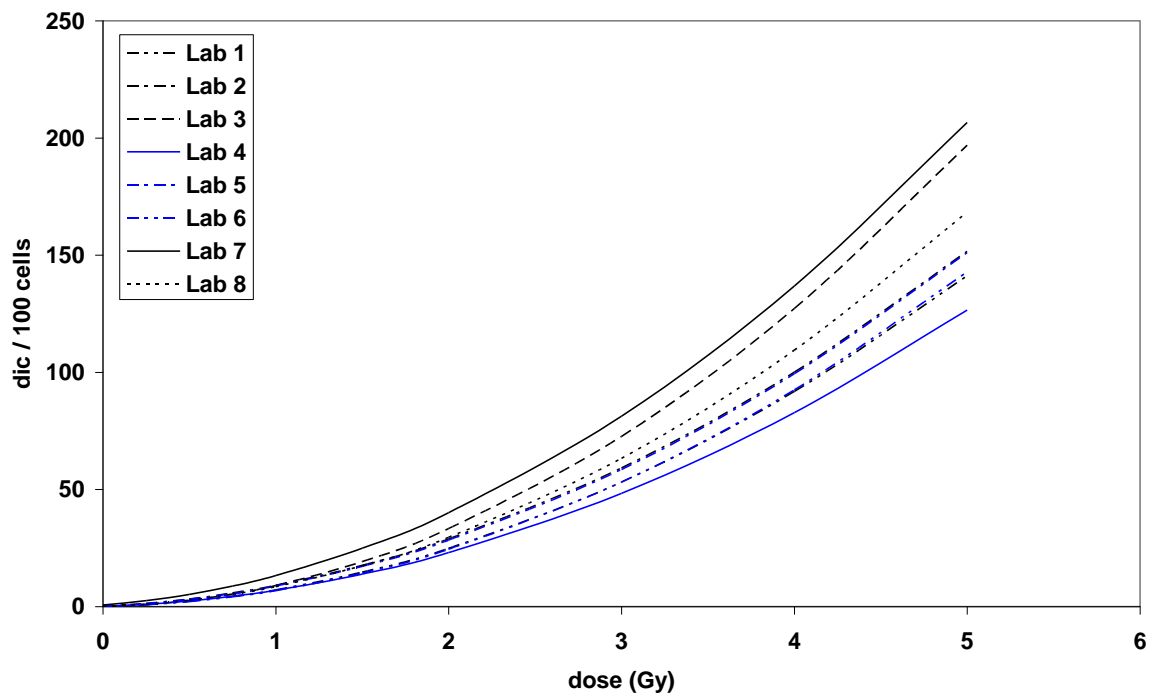
Fig. 1: The γ-ray dose effect curves of the participants of the intercomparison used for dose estimations

Dicentric data were analysed in each individual lab. The chi-squared test for homogeneity was used to demonstrate that, in general, there were no significant differences between parallel slides a and b for either 20, 30 or 50 cells scored, for each lab. There was also generally no significant inhomogeneity within dose categories for each radiation type, and all dose categories were significantly different (distinguishable) from the sham groups. There were exceptions to the above, notably for labs 1 and 2, the results for 0.5 Gy HDR were not statistically different from 0 Gy even for 50 cells ($p = 0.454$ and $p = 0.158$ respectively).

The protracted samples were irradiated on a Monday evening. The acute exposures were always performed on the Tuesday itself, i.e. the same day as when the shipment started. In most cases the blood samples arrived after 24 hours on Wednesday. A strike at Brussels´ airport caused the blood samples from the first shipment (6 samples) to take 48 to 72 h to arrive at their destinations. In the recent literature the impact of transportation time on the yield of dicentrics is under discussion (Moroni et al 2008). To ensure that this did not influence the results of the intercomparison in any way, the numbers of dicentrics scored on these slides were specifically compared to the others in the same irradiation/dose group. The result of this analysis was that there was no evidence of inhomogeneity between these samples, with all p values > 0.86. This indicates that the delay in shipment of these samples did not affect the assay performance.

General Linear Model Analysis of Variance (ANOVA) was carried out to compare the doses estimated for each sample by each laboratory. The different exposure patterns (acute, protracted and partial body), the numbers of cells scored and the sample repeats were also considered. As with the results from the individual labs, the results show that across all labs, there was no significant difference between sample repeats ($p = 0.40$). There was also no significant difference between the dose estimation performed with 20, 30 or 50 cells ($p = 0.07$). Therefore it may be possible to use a number of cells as low as 20 to give a rough indication of absorbed dose. However, the p values varied for individual doses, and on the whole (as expected), dose estimates proved most accurate when 50 cells were used.

The doses estimates for acute whole body exposures show good agreement with the actual doses for all labs (Fig 2). The mean dose estimations were already in agreement for 20 cells and did not change much with increasing cell number (20-30-50cells) at 0.5 Gy: 0.51 Gy, 0.57 Gy and 0.60 Gy ($p = 0.74$), at 2 Gy: 2.21 Gy, 2.20 Gy and 2.22 Gy ($p = 0.64$) and at 4 Gy: 4.26 Gy, 4.33 Gy and 4.34 Gy ($p = 0.53$).
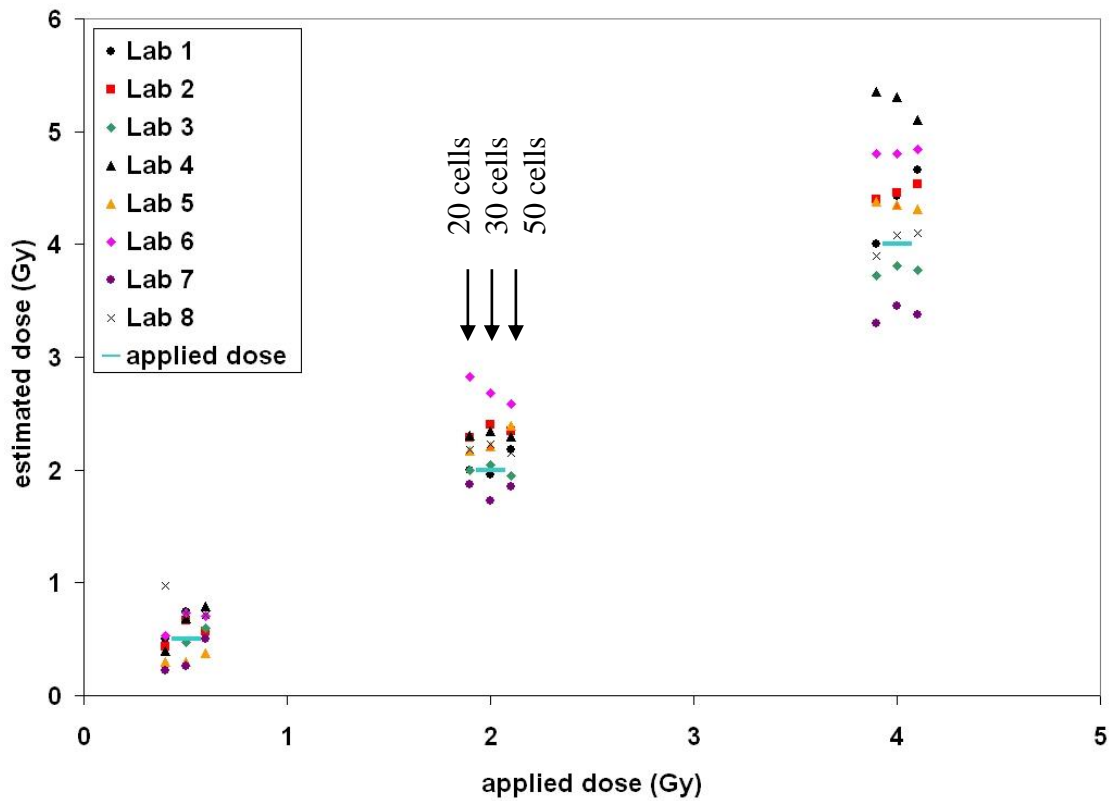
Fig. 2: Dose estimates of 8 laboratories based on 20, 30 and 50 cells (displayed as parallel columns, as indicated at 2 Gy) after simulated acute whole body exposure (HDR) with 0.5, 2.0 and 4.0 Gy
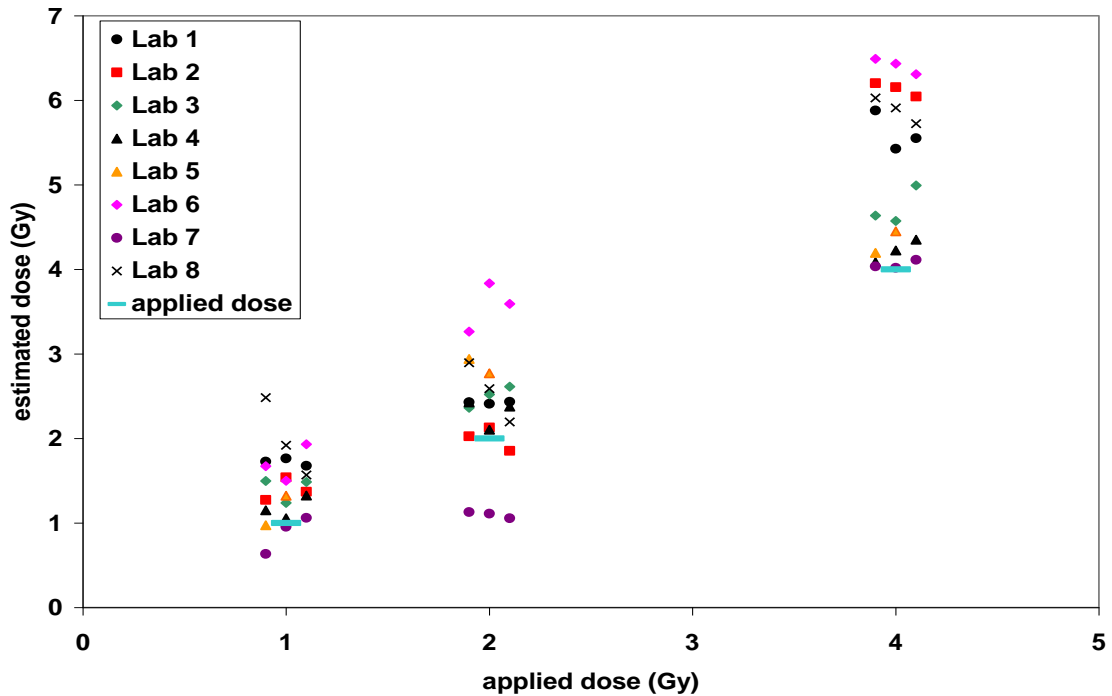

Fig. 3: Dose estimates of 8 laboratories based on 20, 30 and 50 cells (displayed as parallel columns) after simulated protracted whole body exposure (LDR) with 1.0, 2.0 and 4.0 Gy

For this exercise, participants were told which samples were exposed to protracted radiation (LDR), which is similar to real exposure scenarios. Furthermore, it was known, for how many hours the blood had been irradiated, to have a chance to apply the G-function in a correct manner for dose estimation. In general, all labs performed these dose estimations well and received good results for dose

estimations of 1.0 and 2.0 Gy. Only the mean (5.3 Gy) for 4 Gy LDR was significantly different from the actual dose (p = 0.02). There is a trend of decreasing dispersion with increasing cell numbers (Fig. 3), although the estimates themselves do not really change much with increasing cells numbers (20-30-50); at 1 Gy: 1.37 (± 1.43), 1.41 (± 0.33) and 1.45 (± 0.28) (p = 0.24), at 2 Gy: 2.43 (± 0.66); 2.43 (± 0.76) and 2.28 (± 0.71) (p = 0.48), and at 4 Gy: 5.19 (± 1.05), 5.15 (± 0.95) and 5.26 (± 0.78) (p = 0.02).

The general dose over-estimation may indicate that 50 cells are not sufficient to correctly identify this type of exposure when a protracted dose of 4 Gy of low LET radiation is received. Possibly further low dose-rate experiments are needed to explore the uncertainties associated with the model of the G-function and the reliability of the used alpha terms.
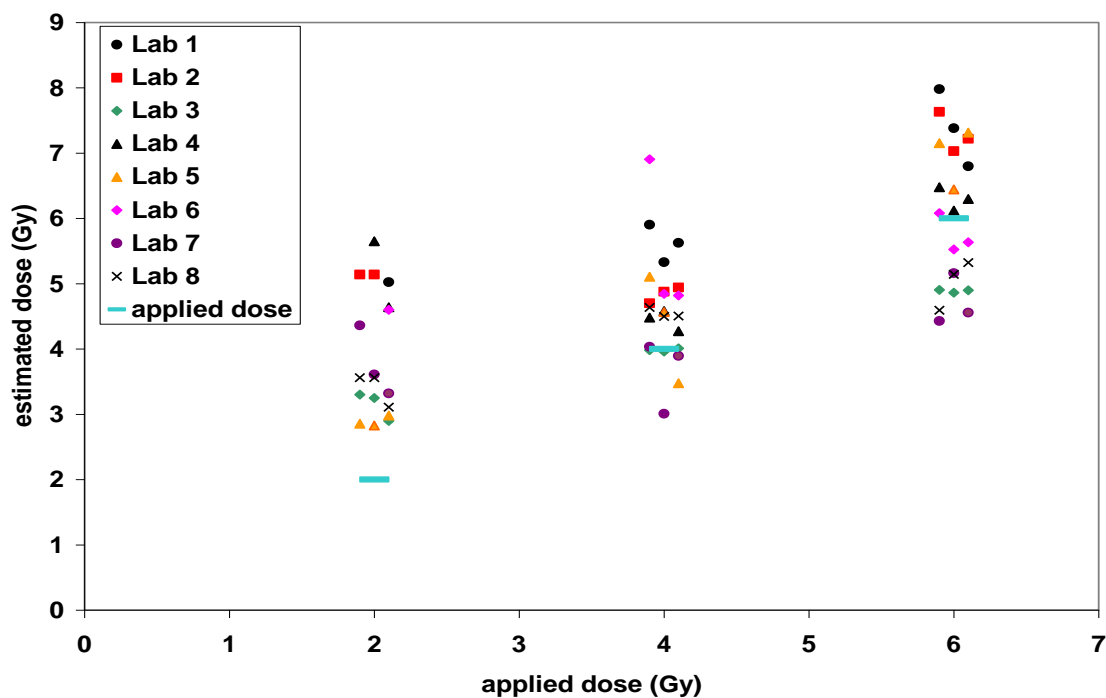

Fig. 4: Dose estimates of 8 laboratories based on 20, 30 and 50 cells (displayed as parallel columns) after simulated partial body exposure (PAR) with 2.0, 4.0 and 6.0 Gy and 50 % irradiated volume.

For simulated 50% partial body exposures to 2.0, 4.0 and 6.0 Gy the results show some variation. Participants were not told which samples were partially exposed – it was left to the individual laboratories to identify these using the u-test and/or variance/mean ratio (Fig. 4). All labs correctly identified all 6 partially exposed samples at 6 Gy when 50 cells were scored; at 4 Gy, the mean number of correctly identified samples was 5.5 out of 6 (range 4 - 6) at 2 Gy, the mean number of correctly identified samples was only 1.75 out of 6 (range 0 - 4). These numbers are very similar but slightly decreased in turn for 30 and 20 cells. The mean dose estimates of all labs together are with increasing cell number (20-30-50 cells) at 2.0 Gy: 1.67 (± 0.62), 1.36 (± 0.61), 2.33 (± 0.20) (p = 0.02), at 4 Gy: 3.71 (± 0.87), 3.63 (± 0.90), 4.18 (± 0.72) (p = 0.54) and at 6 Gy: 5.16 (± 1.44), 5.78 (± 1.04), and 5.88 (± 0.90) (p = 0.84).

No individual lab identified a percentage significantly different from 50%, and, for the data as a whole, there was no significant variation in the irradiated volume calculated for each dose (2, 4 or 6 Gy, p all >0.05). The estimated volumes became more accurate with increasing dose (Fig. 5).

An important conclusion is therefore that while most labs can correctly identify the partial body exposures at 6 Gy and 4 Gy, this was not always possible at 2 Gy when scoring 50 cells. There is a large amount of variability between labs in the numbers of falsely identified partially exposed samples. Therefore calculations based on the three different methods of dose estimation were carried out to estimate the number of cells that would need to be scored in order to correctly identify a dose of 2 Gy in the case of a partial body exposure. Based on the results obtained in this intercomparison, approximately 150 cells would have to be scored in order to identify 50% partial body exposures at 2 Gy with an accuracy of ± 0.5 Gy.
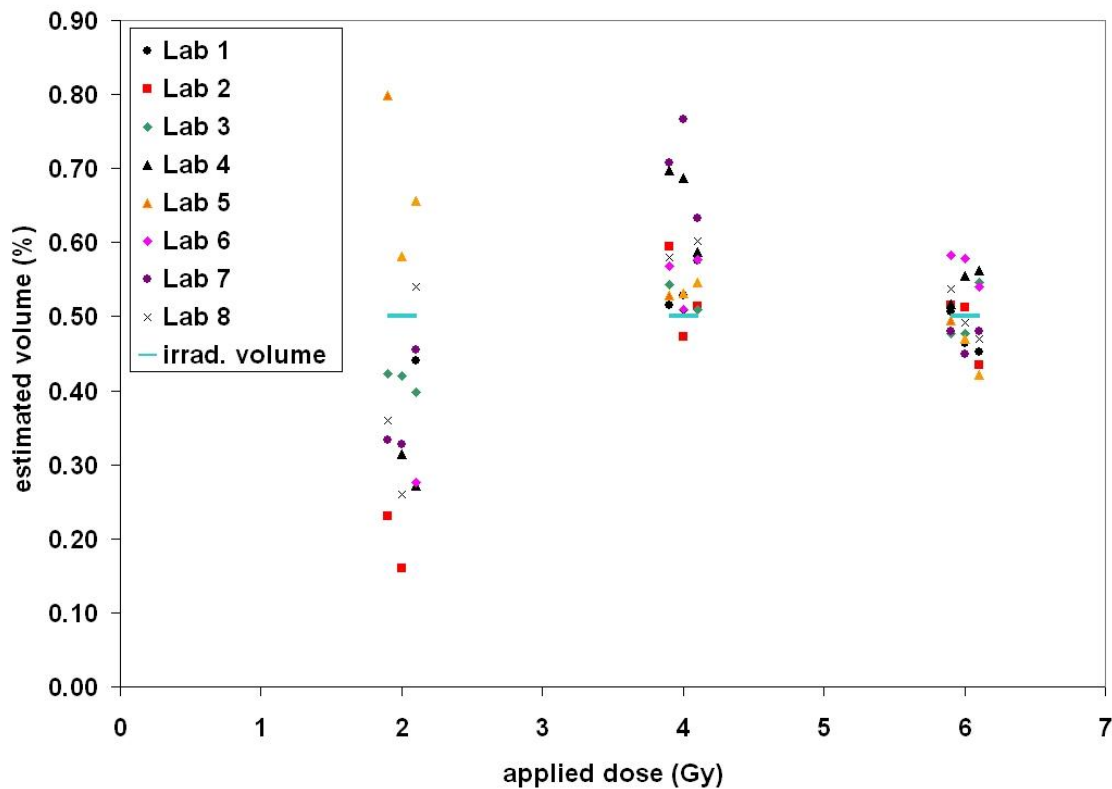
Fig. 5: Estimates of irradiated body volumes of 8 laboratories based on 20, 30 and 50 cells (displayed as parallel columns) after simulated partial body exposure (PAR) with 2.0, 4.0 and 6.0 Gy and 50 % irradiated volume.

Figure 6 shows three-dimensional versions of a Youden plot, comparing z-scores for all of the labs, for 20, 30 and 50 cells. Basically, Youden plots indicates where the uncertainties come from – i.e. whether they reflect random or systematic errors. An ideal plot would be a nice straight surface, through identical points (e.g. (0,0,0)), with most of the values fairly close to zero. Values on the straight surface but far from the origin indicate large systematic errors, values far from the origin and not on the surface indicate large random errors. Figure 6 a) (HDR) compares actual and calculated doses by lab and by the number of cells scored, for the HDR samples. This figure nicely illustrates the spread of dose estimates across the labs, which increases with increasing dose, but decreases (though not to the same extent) with increasing numbers of cells scored. Figure 6 b) (LDR) is fairly flat, but the higher z-values are related to the systematic trend of dose over-estimation with increasing protracted dose. The increased z-values in figure 6 c) (PAR) indicate systematic errors and the bumps indicate random errors, highlighting the need to score more cells when assessing partial body exposures.

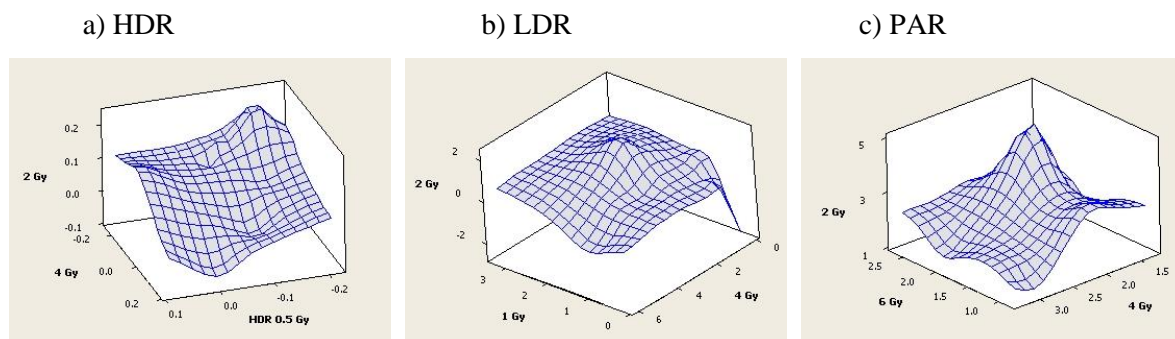a) HDR   b) LDR   c) PAR

Fig. 6: Youden plots showing the uncertainties caused by random and systematic errors for a) acute (HDR), b) protracted (LDR) and c) partial body (PAR) exposure scenarios.

4. Conclusions

In total, all labs were well trained during this exercise and showed their experience for the different scenarios by using the corresponding cytogenetic tools to provide reliable dose estimations.

While there were good results after acute exposure, there was some unexpected trend to dose overestimations after protracted exposure, which may be explained by uncertainties in the alpha term.

Furthermore, some limitations of scoring in triage mode after partial body exposure were observed, and therefore we recommend to increase the cell number in cases, where there are signs of potential inhomogeneous exposure in order to obtain more reliable data.

With respect to the homogeneity of the conventional scoring results, it can be concluded, that each lab was able to demonstrate great expertise in managing the biological dosimetry challenge of this exercise. In the frame of the MULTIBIODOSE project, further investigations with these samples will be performed in the near future to improve the automated scoring procedure and to validate new scoring strategies.

5. Acknowledgment

6. References

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