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NATO DOSIMETRY STUDY

Laboratory Intercomparison of the Dicentric Chromosome Analysis Assay

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The study design and obtained results represent an intercomparison of various laboratories performing dose assessment using the dicentric chromosome analysis (DCA) as a diagnostic triage tool for individual radiation dose assessment. Homogenously X-irradiated (240 kVp, 1 Gy/min) blood samples for establishing calibration data (0.25–5 Gy) as well as blind samples (0.1–6.4 Gy) were sent to the participants. DCA was performed according to established protocols. The time taken to report dose estimates was documented for each laboratory. Additional information concerning laboratory organization/characteristics as well as assay performance was collected. The mean absolute difference (MAD) was calculated and radiation doses were merged into four triage categories reflecting clinical aspects to calculate accuracy, sensitivity and specificity. The earliest report time was 2.4 days after sample arrival. DCA dose estimates were reported with high and comparable accuracy, with MAD values ranging between 0.16–0.5 Gy for both manual and automated scoring. No significant differences were found for dose estimates based either on 20, 30, 40 or 50 cells, suggesting that the scored number of cells can be reduced from 50 to 20 without loss of precision of triage dose estimates, at least for homogenous exposure scenarios. Triage categories of clinical significance could be discriminated efficiently using both scoring procedures.

INTRODUCTION

Incidents involving human exposure to ionizing radiation can assume huge dimensions and may require extensive medical resources including personnel, patient care management and adequate health care facilities. Individuals with little or no exposure, not facing acute health impairments, have to be distinguished from those with mild, moderate or severe exposures to ensure the best possible use of medical resources (*1*). Effects of high doses of whole-body or significant partial-body exposures delivered at a high-dose rate range from skin damage and blood cell depletion to the risk of developing acute radiation syndrome (ARS). The rapid and accurate diagnosis of ARS is a critical part of health care, since, depending on the severity of damage, affected individuals require early, intensive and multidisciplinary treatment. Particularly in large-scale events rapid categorization of potentially overexposed victims into clinically relevant treatment groups is of prime importance.

For this purpose, clinical signs and symptoms and biodosimetry methods are the two main approaches for assessing radiation exposure in situations where no dosimetry badge allowing physical dosimetry was worn. For biodosimetry a number of various cytogenetic and molecular dosimetry techniques with different characteristics are potentially available (*2*). Projects throughout Europe aim at the harmonization and validation of selected biodosimetry methods and their adaption to large-scale scenarios to finally establish a functional network of cooperating laboratories for biodosimetry based diagnostics enabling mutual assistance (*3, 4*). The ultimate goal is the increase of the actually limited capacity of cytogenetic triage to an extremely high chromosome analysis capacity, especially needed in the case of a mass casualty event. Defined strategies towards this goal include automation of current available technologies, establishing new fast methodologies and scoring protocols and enabling mutual

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TABLE 1
Summary of DCA Performance Characteristics (blind samples)

Institution	Set of dose estimates[1]	Lymphocyte isolation	Culture medium	Culture time	Colcemid incubation	Colcemid concentration (final)	Cyt B concentration
A	1	no	RPMI/20% FCS	48 h	3 h	0.15 μg/ml	
B	1	no	RPMI/10 % FCS	48 h	curve: 3 h blind: 24 h	0.1 μg/ml	
	2	no	RPMI/10 % FCS	48 h	24 h	0.04 μg/ml	
C	1	no	RPMI/10% FCS	48 h	3 h	0.2 μg/ml	
	2	no	RPMI/10% FCS	48 h	3 h	0.2 μg/ml	
D	1	no	MEM/10 % FCS	48 h	3 h	0.5 μg/ml	
E	1	no	RPMI/10% FCS	48 h	24 h	0.25 mg/ml	
	2	no	RPMI/10% FCS	48 h	24 h	0.25 mg/ml	
	3	no	RPMI/10% FCS	48 h	24 h	0.25 mg/ml	
F	1	no	RPMI/ 15% FCS (no BrdU)	52 h[2]	4 h	0.1 μg/ml	2 μg/ml

Note. ''Laboratory contribution'': set of ten dose estimates corresponding to ten coded samples, generated by a particular evaluation method (manual or automated scoring and subsequent dose calculation based on a definite standard calibration curve).

[1] First set of dose estimates: triage dose estimates for documentation of time required; second/third set: additional dose estimates (''laboratory contributions'') performed optionally.

[2] Addition of Cytochalasin B after 24 h culture time (CytB method, IAEA 2011).

[3] Dose estimate software [12], CABAS software [13].

assistance within a network of cooperating biodosimetry laboratories.

The dicentric chromosome assay (DCA), a highly standardized and harmonized technique for individual dose assessment after acute whole-body or significantly partial-body radiation overexposure, is still the ''gold standard'' biodosimetry method. The technical performance has been described in detail by the International Atomic Energy Agency (*5*), whereas ISO standards provide performance criteria for cytogenetic service laboratories conducting the DCA in its routine or triage mode ensuring reproducibility and accuracy (*6, 7*). At present, efforts are being made to establish the software-based automation of dicentric aberration scoring for individual dose assessment in triage situations (*3, 8, 9*) and to explore new paths for laboratory networking such as telescoring (*3, 10*). Promising results have already been shown within the Multibiodose project with regard to the application of the dicentric assay in triage mode as a high throughput scoring strategy for biodosimetry in cases of large-scale accidents by a network of eight collaborating laboratories throughout Europe. This applies for triage mode scoring after acute, partial body as well as protracted exposures (*11*).

This NATO exercise was organized under the umbrella of the NATO Research Task Group RTG-033 ''Radiation Bioeffects and Countermeasures'' to compare the performance and properties of the two most established cytogenetic dosimetry tools, DCA and cytokinesis block micronucleus assay, to the novel emerging dosimetry methods, γ-H2AX foci assay and gene expression analysis. These methods were analyzed with regard to the time needed to provide dosimetric results, the reliability of dose estimates and their discriminatory power regarding binary dose categories representing clinically relevant treatment groups of potentially overexposed individuals. Groups in this regard comprise of e.g., unexposed versus exposed individuals to identify individuals who were actually exposed versus those who are not. This will save hospital resources for patients who most likely will suffer from severe acute radiation injury after high-dose irradiation. The study represents an intercomparison of various laboratories with regard to dose assessment using the DCA as a diagnostic tool for rapid emergency biodosimetry within the medical management of radiation accidents. In particular, manual scoring results were compared with automated scoring results, the precision of triage dose estimates based on either 20, 30, 40 or 50 scored cells has been analyzed and the discrimination ability of the DCA with regard to clinically relevant subgroups has been examined.

MATERIALS AND METHODS

Procedures Common for All Assays

Blood sampling (2–3 ml whole blood from one healthy male individual placed in heparinized tubes), radiation exposure (X-ray source, 240 kVp, 1 Gy/min), incubation for 2 h at 37°C (repair time), distribution of calibration (optional, 0.25–5 Gy) and blind samples (0.1–6.4 Gy) to participating laboratories (room temperature) as well as collection of data (harmonized data sheets), requested information from our participants (questionnaire) and statistical analysis [e.g., mean absolute difference (MAD), calculations, impact of questionnaire information and others on MAD and binary categories of clinical significance] represent procedures employed for all assays. To compare mean absolute deviations per dose between manual and automated scoring, Wilcoxon signed rank test was used, which takes MADs at single doses into account pairwise. To assess the quality of binary dose

TABLE 1
Extended.

Fixation procedure	Staining	Automated metaphase finding system	Scoring of blind samples	Criteria for the selection of metaphases to be scored	Software[3] used for dose estimation
automated	FpG	yes	manual	well spreaded complete cells (46 centromeres)	dose estimate
manual	curve: FpG blind: Giemsa	yes	manual	well spreaded complete cells (46 centromeres)	dose estimate
manual	Giemsa	yes	automated	selected by software classifier	dose estimate
manual	Giemsa	no	manual	well spreaded complete cells (46 centromeres)	dose estimate
manual	Giemsa	yes	automated	selected by software classifier	dose estimate
manual	Giemsa (FpG check done the following day)	automated and manual	manual	good morphology, few overlapping chromosomes, 46 centromeres, dicentrics and centric rings must have an acentric	dose estimate
manual	Giemsa	yes	manual	complete cells (46 centromeres)	CABAS
manual	Giemsa	yes	automated	selected by software classifier	CABAS
manual	Giemsa	yes	manual	complete cells (46 centromeres)	CABAS
manual	Giemsa	yes	manual	selected by software (Cytovison 3.92)	CABAS

assignments, the factors for sensitivity, specificity and accuracy were used. Comparison between groups of participants was done descriptively with these factors. A detailed description of the inter-assay comparison is the first in a series of five published companion articles (NATO Biodosimetry Study. *Radiat. Res*. 2013; 180:111–19).

Triage Biodosimetry Based on Dicentric Analysis

Participants were requested to perform DCA according to the protocol established in the respective laboratory, without explicit arrangements concerning the details of the method. It was up to each laboratory, whether manual or automated scoring was performed and which calibration curve was applied. Details describing the methodology used by the various laboratories are described in Table 1. Participants were asked to provide interim results of the 50 cells scored during the triage mode to find out about a potential gain of precision when reconstructing dose estimates based on 20, 30, 40 or 50 scored cells. For dose assessment based on measured dicentric yields each participant had to choose an appropriate *in vitro* calibration curve and calculation method. Details on the chosen calibration curves and the methods/software packages applied for dose estimations are summarized in Table 2. All calibration curves were generated by fitting calibration data to the linear-quadratic dose-response relationship. This is a common radiobiological model used to model low-LET induced dicentric frequencies (Y) as a linear-quadratic function of dose D: $Y = \alpha D + \beta D^2$ [5].

RESULTS

Shipping of Samples and Return of Data

Film badges included with the samples did not indicate any undesired radiation exposure and temperature logs showed a mean temperature of 20°C with a range of 18–24°C during transport. Transportation time ranged from minutes (organizing laboratory) to 26 h. The six participants provided triage dose estimates within 2.4–6.1 days after sample receipt, whereas the remaining data and completed questionnaires were supplied shortly there after. The requested information on the methods used for the DCA performance in each laboratory with regard to blind sample processing (triage mode and additionally provided dose estimates) is summarized in Table 1, whereas Table 2 specifies the requested details of the calibration curves used to estimate radiation doses for blind samples. Results of the questionnaire and the documented times required to provide dose estimates by participants (referred to as institutions A–F) are shown in Table 3.

Standard Calibration Curves

All participating institutions applied pre-existing standard calibration curves for triage biodosimetry of blind samples. All calibration curves were based on manual dicentric scoring. For comparison purposes, three laboratories generated new calibration curves based on manual or automated dicentric scoring of the calibration samples and reported additional dose estimates based on manual or automated scoring, respectively. Coefficients (α, β, c) of applied standard calibration curves are listed in Table 2 and the corresponding curves are shown in Fig. 1.

Reported Dose Estimates by Laboratory, Scoring Procedure and Irradiated Sample

Comparison of the reported dose estimates per laboratory showed a 2.8-fold difference in MAD for manual scoring (range, 0.18–0.5) and 2.5-fold MAD changes for automated scoring (range, 0.16–0.41) (Table 4). This difference was statistically significant (Wilcoxon signed rank test, $P = 0.0098$). The number of measurements lying outside a ± 0.5 Gy interval of the reported doses versus corresponding actual dose was also comparable between manual and automated scoring procedures (manual: up to 5 estimates; automated: up to 3 estimates). We also observed an increased MAD with increasing absorbed dose per sample, which appeared to be independent of the scoring procedure ($P < 0.001$). A MAD of 0.8 Gy on the sample irradiated with 6.4 Gy was the highest, but almost of the same magnitude as the MAD (0.6 Gy) for the 4.2 Gy irradiated sample when comparing MAD after manual scoring. For the automated

TABLE 2
Fitted Coefficients [α, β, c; with Standard Errors (SE)] to Standard Calibration Curves

Institution	Set of dose estimate for which the calibration curve has been applied	Standard calibration curve			
		c ± SE	α ± Σε	β ± Σε	Origin, radiation quality*, scoring mode
A	1	0.0007 (±0.0002)	0.0432 (±0.0059)	0.0630 (±0.0039)	own, 240 kVp X ray, manual
B	1	0.0000 (±0.0000)	0.0301 (±0.0068)	0.0480 (±0.0036)	own, ^{60}Co γ ray, manual
	2	0.0019 (±0.0010)	0.0306 (± 0.0056)	0.0206 (±0.0028)	own, based on NATO samples, 240 kVp X ray, automated
C	1	0.0000 (±0.0000)	0.0185 (±0.0060)	0.0550 (±0.0031)	own, 200 kVp X ray, manual
	2	0.0008 (±0.0004)	0.0221 (±0.0041)	0.0217 (±0.0022)	own, based on NATO samples, 240 kVp X ray, automated
D	1	0.0005 (±0.0005)	0.046 (±0.005)	0.065 (±0.003)	own, 250 kVp X ray, manual
E	1	0.0004 (±0.0023)	0.0374 (±0.0083)	0.0549 (±0.0034)	from literature, ^{60}Co γ ray, manual (Voisin *et al*. 2000)
	2**	0.0000	0.0272	0.0150	own, based on NATO samples, 240 kVp X ray, automated
	3**	0.0052	0.0522	0.0708	own, based on NATO samples, 240 kVp X ray, manual
F	1	0.0093 (±0.0018)	0.0377 (±0.0097)	0.0682 (±0.0045)	own, 200 kVp X ray, manual

* kVp: kV potential.
** SE not determined.
[1] Dose estimate software [12] has been used for iteratively re-weighted least squares-based curve fitting to a linear-quadratic dose response model and CABAS software [13] has been used for maximum likelihood methods-based curve fitting to a linear quadratic dose response model.

scoring procedure MAD values for samples irradiated with up to 4.2 Gy were of comparable size (0.1–0.4), but increased 2.7-fold for the samples irradiated with 6.4 Gy.

To elucidate the reason for the discrepancy of MAD measurements we examined the impact of answers from our questionnaires (Table 3) on the MAD values for all laboratories (ten dose estimates reported per laboratory) and the scoring procedure. No significant correlations were found for any of these parameters.

Reported Dose Estimates Based on 20, 30, 40 and 50 Metaphase Spreads

Interim results of dose estimates based on either 20, 30, 40 and 50 metaphase spreads did not significantly differ from each other (Spearman's rank correlation test, $P > 0.05$), a finding which held true irrespective of the actual absorbed dose or the laboratories which performed the analysis (Table 5). Dose estimates of the 3.0, 4.2 and 6.4 Gy samples assessed by laboratory B are based on the observation of 30 dicentrics and, thus, on fewer cells than the indicated number (Table 5). Corresponding dose estimates were 3.7, 5.0 and 7.4 Gy, based on 38 cells, 23 cells and even 11 cells, respectively.

Reported Dose Estimates Aggregated into Binary Categories of Clinical Significance

To reflect clinical, diagnostic or epidemiological relevant aspects we aggregated DCA based dose estimates within binary categories and compared sensitivity, specificity and accuracy depending on the scoring procedure (Table 6). In

TABLE 3
Details on Experience and Exercise Performance of the Different Participants; Time Required by Participants to Report Triage Dose Estimates

Institution	No. previous exercises	Laboratory specialized in biodosimetry	Method established since (month)	Method established for biodosimetry purposes since (month)	NATO samples processed with	Time required to report dose estimates (days)
A	2	yes	60	60	priority	5.3
B	5	yes	360	360	priority	4
C	0	yes	18	36	priority	4
D	6	yes	480	480	priority	2.4
E	0	yes	30	30	priority	2.6
F	9	yes	120	96	priority	6.1

TABLE 2
Extended.

Standard calibration curve		
Dose rate	Software[1]/method for curve fitting	No. of cells scored in total for ''new'' calibration curves based on NATO samples
1 Gy/min	Dose estimate	
0.64 Gy/min	Dose estimate	
1 Gy/min	Dose estimate	14,723
3 Gy/min	Dose estimate	
1 Gy/min	Dose estimate	9,849
1 Gy/min	Iteratively reweighted least squares	
0.5 Gy/min	Maximum likelihood	
1 Gy/min	CABAS	$<$ 2 Gy: average of 681 cells /dose, $>$2 Gy: average of 300 cells/ dose
1 Gy/min	CABAS	$>$ 250 cells/dose
0.13 Gy/min	CABAS	

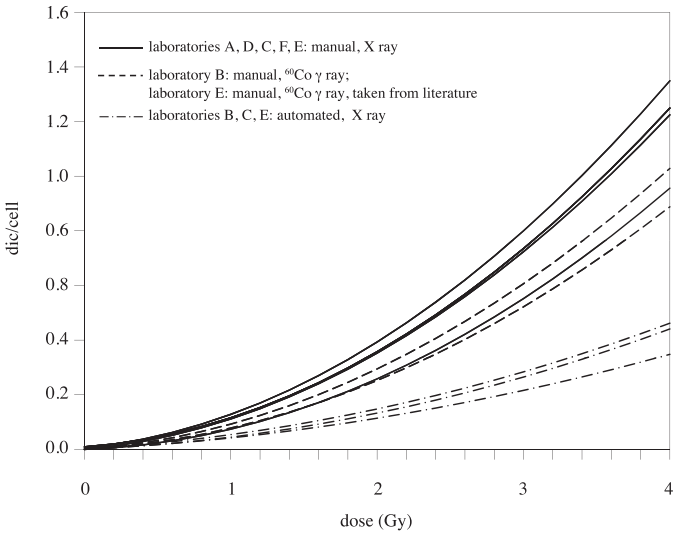

FIG. 1. Comparison of dose-response calibration curves used for estimating doses by manual and automated scoring. Calibration curves from laboratories A and F are overlapping.

all performers specificity varied between 55.6% and 100%, sensitivity between 88.2% and 100% and accuracy from 91% to 96.6%. Accuracy and sensitivity were comparable between the manual and the automated scoring procedure, but specificity appeared higher for automated over manual scoring procedures, an effect which became negligible for binary categories discriminating 2–4 Gy vs. $>$4 Gy exposed samples.

DISCUSSION

The dicentric chromosome analysis (DCA) is considered to represent the gold standard for diagnostic biodosimetry,

TABLE 4
Comparison of MAD between "Laboratories Contributions" and between Different Samples

Laboratories contribution	Actual dose for each sample (Gy)										MAD (Gy)	MAD (SEM)	No. measurements out of ±0.5 Gy
	0	0.1	0.7	1.4	2	2.2	2.6	3	4.2	6.4			
Triage mode only	Estimated doses*												
Institution F: dicentric 50 cell, manual, X ray	0.0	0.6	0.9	1.6	1.9	2.3	3.0	3.3	4.2	6.4	0.18	0.1	0
Institution A: dicentric 50 cell, manual, X ray	0.3	0.5	1.1	1.3	2.3	2.8	3.0	3.5	4.3	5.9	0.36	0.1	1
Institution B: dicentric 50 cell, manual, ^{60}Co γ ray	0.0	0.0	0.9	1.5	1.9	2.6	3.2	3.8	5.0	7.4	0.41	0.1	4
Institution C: dicentric 50 cell, manual X ray	0.0	0.0	1.3	1.8	3.0	2.8	3.0	3.1	5.2	7.3	0.47	0.1	5
Institution D: dicentric 50 cell, manual, X ray	0.5	0.7	1.2	0.9	2.3	2.1	2.9	2.5	4.5	5.3	0.47	0.1	2
Institution E: dicentric 50 cell, manual, ^{60}Co γ ray	0.0	0.0	0.8	0.9	1.6	2.6	2.1	2.5	3.0	5.1	0.50	0.1	2
Additional contributions													
Institution E: dicentric 50 cell, manual, X ray	0.0	0.0	0.6	0.7	1.4	2.4	1.9	2.3	2.8	5.0	0.59	0.2	6
Institution B: dicentric 200 cell, automated, X ray	0.0	0.1	0.9	1.4	1.9	2.4	3.2	2.9	4.5	6.5	0.16	0.1	1
Institution C: dicentric variable cell, automated, X ray	0.2	0.1	0.9	1.3	2.3	2.0	2.7	2.6	4.1	5.6	0.24	0.1	1
Institution E: dicentric variable cell, automated, X ray	0.0	0.0	0.8	1.3	2.1	2.8	2.1	2.6	3.6	4.8	0.41	0.2	3
Triage mode (manual scoring)													
MAD (Gy)	0.1	0.3	0.3	0.3	0.4	0.4	0.4	0.5	0.6	0.8			
MAD (SEM)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2			
Additional contributions (automated scoring)													
MAD (Gy)	0.1	0.0	0.2	0.1	0.2	0.3	0.4	0.3	0.3	0.8			
MAD (SEM)	0.1	0.0	0.0	0.0	0.1	0.1	0.2	0.1	0.1	0.4			

Notes. Triage dose estimates of manual scoring of 50 metaphase spreads or 30 dicentrics are shown on top of the table and results from automated dicentric scoring procedures are shown on the bottom. Dose estimates not falling into the ±0.5 Gy uncertainty interval accepted for triage are underlined (12).

* Triage dose estimates. MAD: Mean absolute deviation of estimated doses compared to actual doses.

TABLE 5
Triage Dose Estimates Based on Manual Scoring of 20, 30, 40 or 50 Metaphase Spreads are Shown for each Laboratory and Actual Dose

Actual dose (Gy)		No. cell counts 20	30	40	50	Actual dose (Gy)		No. cell counts 20	30	40	50
		Estimated dose (Gy)						Estimated dose (Gy)			
0.0	Laboratory A: manual, X ray	0.0	0.0	0.0	0.3	2.2	Laboratory A: manual, X ray	2.4	2.6	2.7	2.8
	Laboratory B: manual, ^{60}Co γ ray	0.0	0.0	0.0	0.0		Laboratory B: manual, ^{60}Co γ ray	2.6	2.6	2.5	2.6
	Laboratory C: manual, X ray	0.0	0.0	0.0	0.0		Laboratory C: manual, X ray	2.4	2.5	2.9	2.8
	Laboratory D: manual, X ray	0.0	0.0	0.0	0.5		Laboratory D: manual, X ray	2.3	2.4	2.2	2.1
	Laboratory E: manual, ^{60}Co γ ray	0.0	0.0	0.0	0.0		Laboratory E: manual, ^{60}Co γ ray	2.1	3.0	2.8	2.6
	Laboratory F: manual, X ray	0.0	0.0	0.0	0.0		Laboratory F: manual, X ray	1.8	2.0	1.9	2.3
0.1	Laboratory A: manual, X ray	0.0	0.0	0.6	0.5	2.6	Laboratory A: manual, X ray	3.0	2.9	3.2	3.0
	Laboratory B: manual, ^{60}Co γ ray	0.0	0.0	0.0	0.0		Laboratory B: manual, ^{60}Co γ ray	3.1	3.2	3.2	3.2
	Laboratory C: manual, X ray	0.0	0.0	0.0	0.0		Laboratory C: manual, X ray	3.4	3.1	3.1	3.0
	Laboratory D: manual, X ray	0.6	0.7	0.8	0.7		Laboratory D: manual, X ray	3.2	2.9	2.9	2.9
	Laboratory E: manual, ^{60}Co γ ray	0.0	0.0	0.0	0.0		Laboratory E: manual, ^{60}Co γ ray	1.1	2.0	1.8	2.1
	Laboratory F: manual, X ray	0.5	0.7	0.7	0.6		Laboratory F: manual, X ray	2.8	3.1	3.0	3.0
0.7	Laboratory A: manual, X ray	1.5	1.2	1.1	1.1	3.0	Laboratory A: manual, X ray	3.0	3.0	3.3	3.5
	Laboratory B: manual, ^{60}Co γ ray	0.0	0.6	0.5	0.9		Laboratory B: manual, ^{60}Co γ ray	3.4	3.7	*	*
	Laboratory C: manual, X ray	1.5	1.2	1.3	1.3		Laboratory C: manual, X ray	3.4	3.4	3.2	3.1
	Laboratory D: manual, X ray	0.9	1.3	1.1	1.2		Laboratory D: manual, X ray	3.1	2.7	2.6	2.5
	Laboratory E: manual, ^{60}Co γ ray	0.0	1.1	0.9	0.8		Laboratory E: manual, ^{60}Co γ ray	1.3	2.6	2.2	2.5
	Laboratory F: manual, X ray	0.5	1.3	1.1	0.9		Laboratory F: manual, X ray	3.4	3.4	3.4	3.3
1.4	Laboratory A: manual, X ray	0.6	0.7	1.4	1.3	4.2	Laboratory A: manual, X ray	4.9	4.5	4.3	4.3
	Laboratory B: manual, ^{60}Co γ ray	2.0	1.6	1.5	1.5		Laboratory B: manual, ^{60}Co γ ray	5.0	*	*	*
	Laboratory C: manual, X ray	2.0	2.0	2.0	1.8		Laboratory C: manual, X ray	5.4	5.4	5.5	5.2
	Laboratory D: manual, X ray	0.0	0.4	0.8	0.9		Laboratory D: manual, X ray	4.7	4.5	4.5	4.5
	Laboratory E: manual, ^{60}Co γ ray	0.2	0.8	0.9	0.9		Laboratory E: manual, ^{60}Co γ ray	3.0	3.8	3.2	3.0
	Laboratory F: manual, X ray	1.6	2.0	1.7	1.6		Laboratory F: manual, X ray	4.1	4.5	4.5	4.2
2.0	Laboratory A: manual, X ray	2.4	2.4	2.4	2.3	6.4	Laboratory A: manual, X ray	5.2	5.6	5.8	5.9
	Laboratory B: manual, ^{60}Co γ ray	1.8	1.9	2.0	1.9		Laboratory B: manual, ^{60}Co γ ray	*	*	*	*
	Laboratory C: manual, X ray	3.0	3.2	3.1	3.0		Laboratory C: manual, X ray	7.3	7.3	*	*
	Laboratory D: manual, X ray	2.7	2.6	2.4	2.3		Laboratory D: manual, X ray	5.6	5.3	5.0	5.3
	Laboratory E: manual, ^{60}Co γ ray	0.4	1.1	1.2	1.6		Laboratory E: manual, ^{60}Co γ ray	4.3	5.9	5.2	5.1
	Laboratory F: manual, X ray	1.6	1.9	1.9	1.9		Laboratory F: manual, X ray	6.5	6.6	6.7	6.4

* Estimates based on scoring of 30 dicentrics, but not on the indicated cell count.

which will be used as a reference method to validate new potential diagnostic tools. Dose estimates need to be provided as soon as possible to support clinical decision making. Therefore, a triage mode for manual scoring and recently, automated scoring procedures, have been introduced.

The focus of our study is an intercomparison of various cytogenetic dosimetry laboratories performing individual radiation dose-assessment based on the DCA. We did so by determining the accuracy of radiation dose prediction taking into account a variety of variables such as experience, specialization for biodosimetry and protocol characteristics. As DCA forms a common methodological platform for national, regional and global biodosimetry networks to enhance the response capacity in case of a large-scale radiological incident (*5, 14*), this study also contributes to the further validation of the DCA for network biodosimetry applied in large-scale radiological incidents. To maintain such an assistance network, periodically organized ring trials between biodosimetry service laboratories are recommended to ensure the accuracy and reliability of their results (*6, 15, 16*).

Notably, for this study no specified agreements concerning DCA performance were made to allow each laboratory to conduct the assay according to its established protocols. Interestingly, all triage dose estimates for time documentation were based on manual scoring of 50 metaphase spreads or the observation of 30 dicentrics, so demonstrating that manual scoring is still favored for reliable dose assessment. Only three laboratories used the provided samples to generate calibration data for automated dicentric scoring with the DCScore software module (Metasystems, Altlussheim, Germany), which allowed comparison of estimated doses with the conventional manual scoring of dicentrics. Furthermore, the time of specialization for biodosimetry as well as the practical experience of participating laboratories (prior ring trial participation) ranged from 2.5 to 40 years and from 0 to 9 years, respectively.

For triage dose estimation pre-existing calibration curves (laboratories A, B, C, D and F) or a calibration curve

TABLE 6
Comparison on Discrimination Ability of the DCA with Regard to Dose Estimates Aggregated into Binary Categories of Clinical Significance

Radiation exposure	Totals	Totals per dose[a]	Actual doses (Gy)[b]										Percentage overall[c]		
			0	0.1	0.7	1.4	2.0	2.2	2.6	3.0	4.2	6.4	Accuracy	Sensitivity	Specificity
Never/ever															
all performer	89	9	5	5	9	9	9	9	9	9	9	8	91.0%	95.0%	55.6%
manual scoring	59	6	4	3	6	6	6	6	6	6	6	5	91.5%	94.3%	66.7%
automated scoring	30	3	1	2	3	3	3	3	3	3	3	3	90.0%	96.3%	na
<0.1 Gy vs. >0.1 Gy															
all performer	89	9	6	6	9	9	9	9	9	9	9	8	93.3%	100.0%	66.7%
manual scoring	59	6	4	3	6	6	6	6	6	6	6	5	91.5%	100.0%	58.3%
automated scoring	30	3	2	3	3	3	3	3	3	3	3	3	96.7%	100.0%	83.3%
<1.5 Gy vs. >1.5 Gy															
all performer	89	9	9	9	9	6	9	9	9	9	9	8	96.6%	100.0%	91.7%
manual scoring	59	6	6	6	6	3	6	6	6	6	6	5	94.9%	100.0%	87.5%
automated scoring	30	3	3	3	3	3	3	3	3	3	3	3	100.0%	100.0%	100.0%
2–4 Gy vs. >4 Gy															
all performer	89	9					9	9	9	9	7	8	96.2%	88.2%	100.0%
manual scoring	59	6					6	6	6	6	5	5	97.1%	90.9%	100.0%
automated scoring	30	3					3	3	3	3	2	3	94.4%	83.3%	100.0%

Notes. The column ''Totals'' refers to the total number of reported assignments and the column ''Totals per dose'' describes the total number of reported assignments per dose. Numbers of correctly reported assignments (left of the respective critical dose true negatives, right of it true positives) to the groups are shown for each irradiated sample for all performers and for manual and automated scoring. Accuracy, sensitivity and specificity columns show overall agreement.

[a] Eight for 6.4 Gy.

[b] Related to the totals per dose.

[c] Averages from the reported dose estimates, related to totals.

produced elsewhere and published in the literature (laboratory E) were applied. Two of these six calibration curves were not generated with X rays, but with ^{60}Co γ radiation. The other four were based on X rays of slightly different accelerating potential (200–250 kVp) and filtration characteristics (with or without a half-value layer of copper). The values of the coefficients α, β and c, and thus the slopes of the curves differed between laboratories as shown in Fig. 1 and Table 2. These differences had been expected due to laboratory variation in pre-established calibration data (type of radiation, number of evaluated dose points and scored cells per dose point) and DCA performance (reagents, equipment, method, scoring). Figure 1 clearly shows a systematic difference between automated and manual calibration curves, reflecting a ~50% dicentric detection rate of DCScore relative to manual scoring, which is consistent with published data (*17, 18*).

Our data indicate that dose estimates can be provided as soon as 2.4 days after arrival of the samples at the laboratories using manual triage mode. Scoring of 50 metaphase spreads is normally recommended for SCA analysis, but our data indicate no loss of precision in obtaining triage dose estimates by only scoring 20 metaphase spreads at least for these homogenous exposure scenarios. However, in the case of partial body exposure scoring of only 20 metaphase spreads might be insufficient as previously shown by Lloyd *et al*. (*19*) and has to be considered if the homogeneity of radiation exposure is uncertain. This is of particular importance at higher doses where the number of metaphase spreads available to score decreases. Interestingly, when we compared manual scoring results using the triage mode with automated

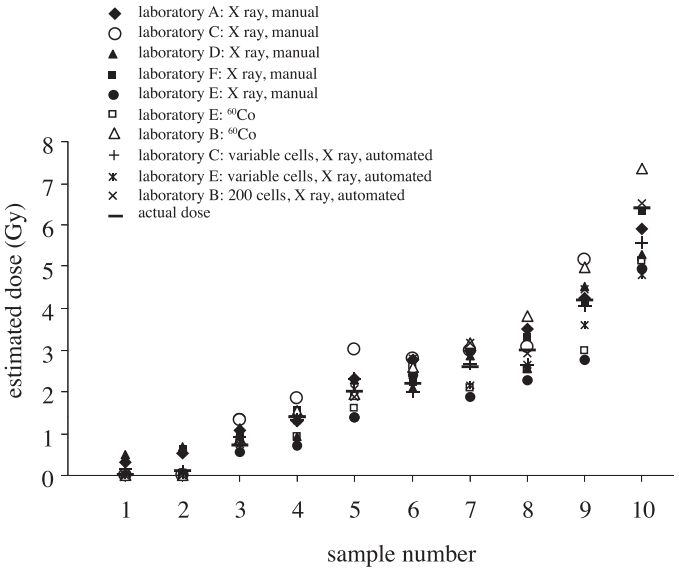

FIG. 2. Triage dose estimates of blind samples based on manual scoring of 50 metaphase spreads/30 dic, additional dose estimates based on automated dicentric scoring of a variable cell number and the actual doses.

scoring results, we did find the MAD values as well as the number of measurements lying outside the recommended 0.5 Gy interval to be comparable and even slightly better for the automatic scoring procedure ($P = 0.0098$). This finding points to the potential of the automated scoring algorithm. For arbitrary exposure conditions and groups of exposed victims the MAD may show incorrect values. Therefore these MADs are valid only for the presented study under the fixed specified experimental design, which was identical for all participants and reflect the overall accuracy of dose estimates per laboratory contribution.

From the dosimetry point of view it is highly desirable to get dose estimates as accurate as possible, but from a clinical viewpoint, dose ranges often provide sufficient precision to facilitate urgent clinical or diagnostic needs. This is why we divided our ten samples into binary categories as already described. Again, both scoring procedures performed with comparable accuracy, sensitivity and specificity, although the automated scoring procedure appeared to be slightly better. Again, this underlines the potential of the automated scoring algorithm due to time savings during aberration scoring.

Our current analysis is limited by the number of measurements. We purposely restricted our measurements to blood samples taken from one individual only, to focus on methodological variance and exclude interindividual variance. For the same reason we changed only the dose and did not simulate partial body radiation exposures. We recognize the importance of distinguishing partial body exposure doses from their homogenous exposure equivalents since they call for a different medical management. However, this was not investigated here as partial body exposures require more than 50 metaphases scored and probably additional endpoints to complement DCA data (*20*).

CONCLUSION

Taken together, ten DCA based dose estimates could be completed by the fastest operating laboratory in 2.4 days after sample arrival based on manual scoring. Both the precision of dose estimates as well the discrimination ability of binary dose categories of clinical significance emphasize the applicability of either manual or automated scoring procedures for biodosimetry. For the triage mode, our data indicate manual scoring results in no loss of precision in obtaining triage dose estimates even when only 20 metaphase spreads are scored compared to the usual 50, at least for the dose range and uniform exposures used in this exercise. Due to the decisive time savings in aberration scoring we propose that the automated scoring should be the method of choice for the future application of DCA in rapid triage biodosimetry, although the number of cells to be scored still has to be determined.

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