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NATO BIODOSIMETRY STUDY

Laboratory Intercomparison of the Cytokinesis-Block Micronucleus Assay

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The focus of the study is an intercomparison of laboratories' dose-assessment performances using the cytokinesis-block micronucleus (CBMN) assay 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. The CBMN assay was performed according to protocols individually established and varying among participating laboratories. 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 4 days after sample arrival. The CBMN dose estimates were reported with high accuracy (MAD values of 0.20–0.50 Gy at doses below 6.4 Gy for both manual and automated scoring procedures), but showed a limitation of the assay at the dose point of 6.4 Gy, which resulted in a clear dose underestimation in all cases. The MAD values (without 6.4 Gy) differed significantly ($P = 0.03$) between manual (0.25 Gy, SEM = 0.06, n = 4) or automated scoring procedures (0.37 Gy, SEM = 0.08, n = 5), but lowest MAD were equal (0.2 Gy) for both scoring procedures. Likewise, both scoring procedures led to the same allocation of dose estimates to triage categories of clinical significance (about 83% accuracy and up to 100% specificity).

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INTRODUCTION

In cases of unclear radiation exposures, biological dosimetry can be a useful tool to confirm whether an individual was actually exposed to ionizing radiation and, if necessary, to provide information concerning the dose range and homogeneity of exposure. A great deal of experience has been gained with biological dosimetry in different radiological emergencies during the last decades. In the last 10 years there has been much effort to prepare for a large-scale accident. One major problem after a large-scale event is the large number of samples that would have to be analyzed in a fast and reliable manner. Therefore, several strategies (networking, new scoring strategies, automation and method improvement) have been developed to achieve a higher sample throughput. For biodosimetry purposes, several well established cytogenetic assays exist to cover the different exposure scenarios and new molecular methods are emerging (*1–3*). These assays will all be performed in parallel, as a single biodosimetry technique cannot fully address the biodosimetry requirements in complex exposure scenarios (*4*). In the case of a large-scale radiation accident, a single cytogenetic laboratory would be quickly overwhelmed by the large number of samples. Therefore, several biodosimetry networks have been established on a national (*5, 6*), international (*7–9*) and global level (*10–12*) to be better prepared to manage a high sample throughput. One important lesson gained from international intercomparison studies with the most validated biodosimetry method, the dicentric chromosome assay, is the need to harmonize and standardize the method among different laboratories to get comparable data. In addition, it remains necessary for each laboratory to generate its own calibration curves to be able to provide reliable dose estimations by biological dosimetry (*8*).

The lymphocyte cytokinesis-block micronucleus (CBMN) assay was developed in 1985 (*13*) and is now a standard method that is well established in the field of *in vitro* genetic toxicology testing (*14*), population monitoring

and radiation biodosimetry (*15*). Micronuclei (MN) may arise from acentric fragments or whole chromosomes. They are small spherical objects with the same staining property and morphology as the two nuclei within the plasma of the binucleated cell (*15*). An international collaborative network on micronucleus frequency in human populations called ''HUMN'' (HUman MicroNucleus) was launched in 1997 and counts more than 40 participating laboratories from all over the world (*16*). Within this project detailed scoring criteria of the CBMN assay were defined and standardized (*17*). The various standard protocols for culturing and scoring in the different laboratories were summarized and compared and the impact of age, gender, diet and lifestyle factors on this biomarker has been well documented (*18*).

The CBMN assay has also been recommended by the International Atomic Energy Agency as a biodosimeter for the exposure to ionizing radiation (*15*). Many studies have shown that the frequency of micronuclei in binucleated cells shows clear reproducible dose-effect curves for different radiation qualities (*19–21*). Today, the CBMN assay is a well established and thoroughly validated method in biological dosimetry. The CBMN and dicentric assays are both robust biomarkers which allow investigations weeks or months after an assumed *in vivo* radiation exposure (*22, 23*), but the CBMN assay is much easier to perform and faster. Both methods are suited for automation and are under investigation within EU project ''Multibiodose'' (*24*) whose goal it is to make available rapid emergency biodosimetry in case of a large-scale radiation accident. The results achieved so far in terms of automation of these techniques are very promising (*25, 26*).

This NATO exercise was organized under the umbrella of the NATO Research Task Group HFM-099 RTG-033 ''Radiation Bioeffects and Countermeasures'' to compare the performance and properties of established cytogenetic dosimetry tools, such as the CBMN assay and dicentric analysis with novel emerging molecular dosimetry methods (e.g. γ-H2AX assay and gene expression). This article presents the findings and discusses the outcome of the laboratory intercomparsions of performance for dose assessment using the CBMN assay as a diagnostic tool for rapid emergency biodosimetry. It focuses on manual scoring results in comparison to automated scoring results with regard to the time needed to provide dose estimations, the reliability of dose estimates and their discriminatory power regarding binary dose categories representing clinically relevant treatment groups of e.g., potentially overexposed individuals.

MATERIALS AND METHODS

Procedures Common to All Assays

Blood sampling (2–3 ml whole blood from one healthy male individual filled in heparinized tubes), radiation exposure (X-ray source, 240 kVp, 1 Gy/min), incubation at 37°C for 2 h (repair time), distribution of calibration (optional, 0.25–5 Gy) and blind samples (0.1–6.4 Gy) to participating laboratories as well as collection of data (harmonized data sheets), requested information from our participants (questionnaire) and statistical analysis (e.g., MAD calculations, impact of questionnaire information on MAD and binary categories of clinical significance) represent procedures employed for all assays. To compare absolute deviations (AD) between pairs of different subgroups the Wilcoxon signed rank test was used, which takes singles doses into account pairwise. To assess the quality of binary dose assignments, the factors for sensitivity, specificity and accuracy were used, but standard deviations were not calculated because of the low numbers. Comparison between groups of participants was done descriptively with these factors. A detailed description of the inter-assay comparison is the lead article in a series of companion articles (NATO Biodosimetry Study, *Radiat. Res*. 2013; 180:111–19).

Cell Culture and Scoring Procedure

Culture setup and scoring was performed in the laboratories according to their own standard protocols. Each laboratory established whole blood cultures for the CBMN assay, with the majority using RPMI 1640 medium (one laboratory used MEM) and 10% FCS (in one case 20%; Table 1). The culture time varied between 70–72 h. Cytochalasin B was added 23–44 h after culture initiation. For the manual scoring the slides were stained with acridine orange or giemsa, the automated scoring was done with DAPI stained slides. The automated scoring was performed with the software module MNScore (MetaSystems, Altlussheim, Germany). Automated scoring means that the yield and distribution of micronuclei (MN) in binucleated cells (BN) has been measured with a fully automated scoring system without intervention by a human scorer. Cells with more than 4 MN/cell were excluded to avoid a staining bias. Semiautomated scoring means that a trained human scorer evaluated all machine detected MN in a second step. Furthermore, one laboratory evaluated the remaining cells without detected MN, which was accepted here as equivalent to conventional manual scoring as all detected binucleated cells were completely reanalyzed by a human scorer.

Each laboratory used its own calibration curve for dose estimation. In general the dose effect curves were following the linear-quadratic curve model: $Y = C + \alpha^* D + \beta^* D^2$. The observed frequency of micronulei in binucleated cells = Y depends on the spontaneous frequency C and the dose D, which has a linear (α) and quadratic (β) component (Table 2). The calibration curves and the dose were done with estimations standard software programmes (e.g., CABAS or DoseEstimate).

RESULTS

Six institutions participated and provided dose estimates for each of the 10 blind samples (laboratories 1–6). Several participants have sent more than one contribution (''laboratory contribution'': one set of 10 dose estimates corresponding to the 10 blind samples), e.g., automated, semiautomated or manual scores, calculated based on their own specific standard calibration curves. In total, 11 contributions by all participants provided 110 dose estimates for data analysis.

General information of the participating institutions such as answers to the questionnaire about their experience with the assay, methodological details provided by all participants and the documented time required to report dose estimates are given in Table 1.

The earliest reports on dose estimates were provided 4 days after sample arrival at the respective laboratory when using automated scoring procedures and 7.1 days thereafter

TABLE 1
Experience of Participating Institutions, Technical Characteristics and Variables in the Standard Protocols (Answers to the Questionnaire) Related to the CBMN Assay

Institution	Laboratories specialized in biodosimetry	Previous excercises	Method established since... (month)	Medium	FCS (%)	Culture time	Time, CB was added to culture	Fixtion methanol: acetic acid	Staining
Laboratory 1	Yes	1	72	RPMI 1640	10%	72 h	24 h	4:1	DAPI
Laboratory 2	Yes	1	12	RPMI 1640	20%	70 h	23 h	4:1	DAPI
Laboratory 3	Yes	4	48	RPMI 1640	10%	72 h	44 h	5:1	Acridine Orange
Laboratory 4	Yes	0	360	MEM	10%	72 hr	24 h	3:1	DAPI
Laboratory 5	Yes	0	72	RPMI 1640	10%	72 h	44 h	a) 5:1 b) 3:5	Giemsa
Laboratory 6	Yes	4	240	RPMI 1640	10%	70 h	24 h	4:1	DAPI

when using a manual scoring procedure. In total, the report time ranged between 4–17 days after sample receipt. One laboratory was not able to give first priority to this exercise and therefore needed more time than the others.

Temperature logs indicated an almost constant temperature of 20°C (±2°C) for the transport of the calibration samples sent in July and the blind samples sent in September. Film badges did not detect any undesired additional radiation exposure to the samples during the transport.

All participating institutions used their own CBMN calibration curves (see Table 2 and Fig. 1) for dose estimations of the blind samples. Five laboratories used the provided blood samples in the first step of the exercise for calibration purposes. Laboratories 1 and 4 generated new calibration curves based on automated micronucleus scoring and laboratory 5 established a curve by manual scoring. Laboratory 2 used the samples to increase the number of donors (and cells) of their calibration curve. And laboratory 3 used the samples for cross checking the reference samples against their own calibration curve to ensure that the existing curve could be used instead of generating a new curve. In total 10 different dose effect curves were used during the exercise.

The number of analyzed cells per dose point varied significantly (140 to 4,000 cells, Table 3), when complete slides were scanned by the automated procedures. There were 4 ''laboratory contributions'' based on manual scoring provided by laboratories 1, 3 and 5. Laboratory 5 analyzed 200 and 2,000 cells per sample manually. Furthermore there were 5 contributions by 4 laboratories based on automated scoring, which were supplemented by 2 contributions, where semiautomated analysis was performed. Concerning the radiation quality, 5 contributions were based on calibration curves for X rays and 6 on those for γ rays. In total, the 6 participating institutions delivered 11 different contributions of dose estimations (Table 4).

In Fig. 2, the reported dose estimates of the 10 blind samples are shown relative to the true absorbed doses, sorted with increasing order. The different scoring procedures can be compared directly and appear to give quite comparable results. Only the analysis of the 6.4 Gy sample demonstrated the limit of the assay at doses above 4.5 Gy and shows here a clear dose underestimation in all cases. A dose of 6.4 Gy is not included in the dose effects curves (dose range up to 4.0 Gy with X rays) shown here. A test to determine if this 6.4 Gy dose point could be included in the assay was negative ($P < 0.001$, Wilcoxon rank test).

For the interlaboratory comparison laboratory contributions were stratified based on the scoring procedure and the calibration curve applied for dose reconstruction (Table 4). MAD values per irradiated samples also indicate an upper limit in dose estimates at 6.4 Gy, since true doses were underestimated by all laboratories with a total MAD of 2.08 Gy, which corresponds to an unacceptably high mean difference of about 33% from the true dose. At doses below 2.5 Gy, the MAD values are small (0.14 to 0.38 Gy), which fit the 0.5 Gy uncertainty interval introduced for triage dosimetry of chromosomal aberrations (*27*). At doses of 2.6, 3.0 and 4.2 Gy, the MAD increases in the range of 0.6, 0.4 and 0.52 Gy, which is still below or near 20% of the actual dose, and therefore still in good agreement. The number of dose estimates which exceeds 20% of the true dose increases at 2.6 Gy (6 dose estimates) and peaks at 6.4 Gy [10 estimates: >20% difference from actual dose, all estimates greater than the 0.5 Gy uncertainty interval accepted for triage biodosimetry (28)].

Because of the strong misleading impact of the highest dose point on all MAD values, for further analysis, the highest 6.4 Gy dose point was excluded. Analysis of contributions based on conventional manual scoring resulted in the lowest MAD values (0.20 and 0.20 Gy, Table 4) when a calibration curve based on the same radiation quality like the blind samples were exposed to was applied. The MAD increased significantly using a calibra-

TABLE 1
Extended.

Scoring procedure (contributions)	NATO samples processed with	Time required to report dose estimates (days)
Manual Semiautomated Automatic (4)	priority	4
Semiautomated Automated (2)	priority	4
Manual (1)	priority	7
Automated (1)	priority	4
Manual (2)	priority	8
Automated (1)	when appropriate	17

tion curve derived after γ-ray exposure (0.26–0.33 Gy, Wilcoxon signed rank test, $P = 0.021$). Semiautomated scoring (0.37 and 0.46 Gy) showed significantly higher MAD values than manual scoring (Wilcoxon signed rank, $P = 0.02$). The automated scoring procedure received a good performance for X rays (0.21–0.36 Gy) and increased when estimates were based on γ-ray curves (0.46–0.50 Gy, Wilcoxon signed rank, $P = 0.020$). Furthermore, accuracy of manual scoring appeared significantly higher compared to the automated scoring procedure ($P = 0.03$), but only when considering all laboratory contributions. However, comparable accuracy was found for both scoring procedures when considering only laboratory contributions with lowest MAD values of 0.2 (Table 4).

Furthermore, is an increased number of measurements outside the 0.5 Gy uncertainty interval was observed relative to the true dose when using semiautomated instead of manual scoring (3 vs. 1–2 false dose estimates) or employing a calibration curve generated with gamma instead of X rays (0–2 vs. 4–5 false).

One laboratory analyzed 200 and 2,000 cells per dose point by conventional manual scoring. Remarkably, the MAD values (0.20 and 0.21 Gy) are nearly the same and show no improved accuracy with higher cell number. In total, all contributions show MAD values of the dose estimations $\leq$0.5 Gy, which corresponds well with the uncertainty interval (Table 4).

To elucidate the reason for variability of MAD values we examined the impact of answers from our questionnaires as well as the impact of the scoring procedure and the calibration source used. No association of MAD was found with any of the factors of the questionnaire (Spearman's rank correlation test, $P > 0.10$). Also no significant correlation was found with the scoring procedure when using the Kruskal Wallis rank test between all three procedures, $P = 0.15$ or the Wilcoxon rank test between ''auto'' and ''manual'', $P = 0.063$) and the type of calibration curve used (Wilcoxon rank test, $P = 0.25$). However, these values approached significance using the Wilcoxon signed rank test (pairwise dose dependencies), which implies that differences are small. This is why we compared the lowest MAD among different laboratories using different scoring procedures as well. MAD of 0.2 Gy were found for manual and automated scoring procedures, which might imply comparable performance inherent to both scoring procedures. Of course, this result has to be validated in future studies comparing many more laboratories. To reflect clinically/diagnostically/epidemiologically relevant aspects we also aggregated CBMN based dose estimates within binary categories. Irrespective of the scoring procedure specificity increased for binary categories when discriminating higher exposure groups (Table 5) and revealed comparable values for both the manual and the automated scoring procedure. For the first three binary categories we found comparable values in accuracy (85–95%) and sensitivity (97–100%) for manual and automated scoring. These values slightly decreased for the binary group comprising 2–4 Gy vs. >4 Gy radiation exposure, but remained comparable for both scoring procedures in accuracy (83.3%) and specificity (100%).

DISCUSSION

The CBMN assay is a well established cytogenetic dosimetry method. In an emergency situation radiation dose estimates should be provided as soon as possible to

TABLE 2
Coefficients and Characteristics of Dose Effect Curves Applied in this Study

Institution	C (±SE)	α (±SE)	β (±SE)	Radiation quality, scoring mode, scored BN cells
Laboratory 1	0.0318 (±0.0043)	0.0370 (±0.0114)	0.0360 (±0.0044)	240 kVp X ray, automated, 38,675 BN cells
Laboratory 1	0.0274 (±0.0020)	0.0614 (±0.0111)	0.0163 (±0.0042)	^{60}Co γ ray, automated, 94,858 BN cells
Laboratory 1	0.0133 (±0.0012)	0.0456 (±0.0090)	0.0322 (±0.0037)	^{60}Co γ ray, semiautomated, 94,820 BN cells
Laboratory 1	0.0275 (±0.0027)	0.0767 (±0.0180)	0.0418 (±0.0073)	^{60}Co γ ray, manual, 94,346 BN cells
Laboratory 2	0.0095 (±0.0076)	0.0547 (±0.0096)	0.0166 (±0.0002)	240 kVp X ray, semiautomated, 184,937 BN cells
Laboratory 2	0.0394 (±0.0239)	0.0535 (±0.0066)	0.0154 (±0.0012)	240 kVp X ray, automated, 188,436 BN cells
Laboratory 3	0.0277 (±0.0170)	0.0718 (±0.0407)	0.0473 (±0.0963)	^{137}Cs γ ray, manual, 80,000 BN cells
Laboratory 4	0.0255 (±0.0084)	0.0589 (±0.0116)		240 kVp X ray, automated, 22,381 BN cells
Laboratory 5	0.0039 (±0.0031)	0.0923 (±0.0159)	0.0601 (±0.0058)	240 kVp X ray, manual, 32,000 BN cells
Laboratory 6	0.0202 (±0.0003)	0.01842 (±0.0013)	0.0267 (±0.0007)	^{60}Co γ ray, automated, 90,000 BN cells

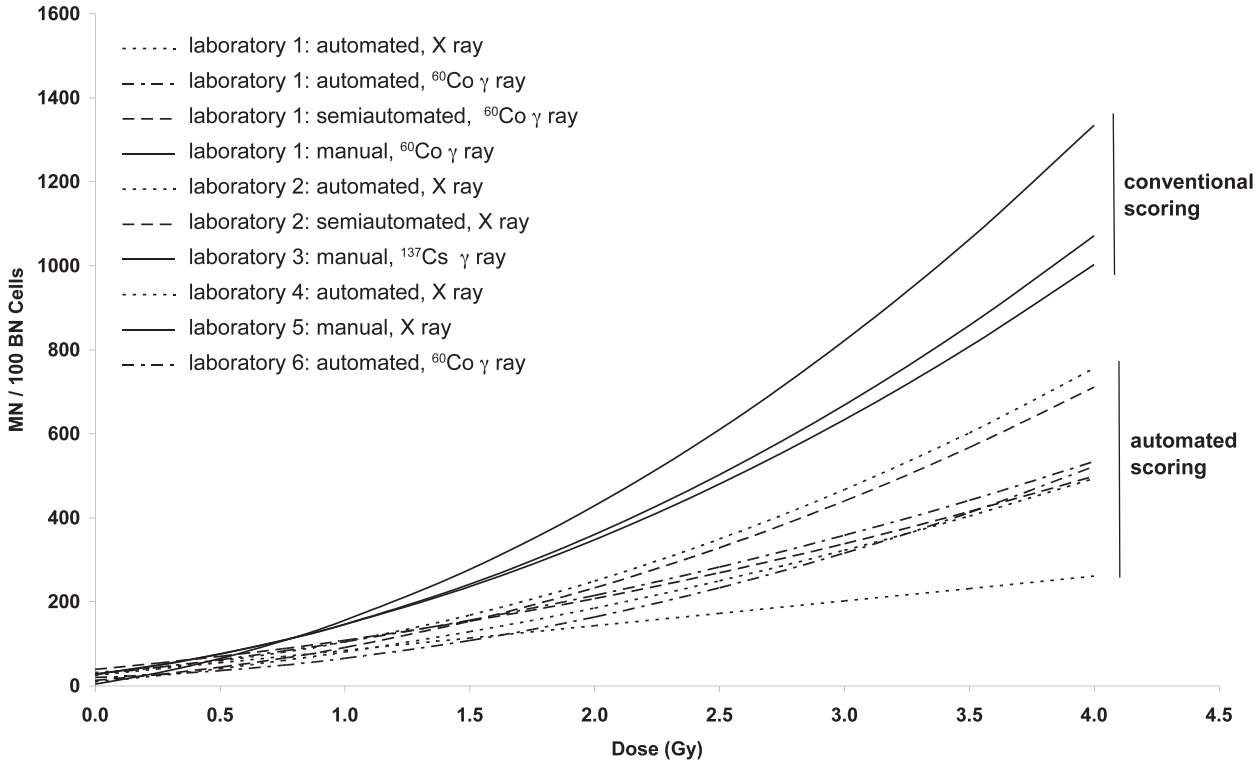

FIG. 1. CBMN dose effect curves of the participating institutions established for X and γ rays (^{60}Co or ^{137}Cs) after conventional, automated or semiautomated scoring.

support clinical decision making. According to the data of this exercise dose estimates based on automated scoring could be provided 4 days after sample arrival at the laboratory, but for the manual scoring procedure, 7 days was required. A very important time factor here is the 3-day culture time necessary to perform the assay.

One strategy to accelerate the method is to reduce the cell numbers to be analyzed. Currently there is a recommendation to manually analyze 200 cells with the CBMN assay in a triage mode (*15, 29*) comparable to the triage mode of the dicentric assay (*30*). A corresponding ISO standard for the CBMN assay is in preparation, which will probably recommend the scoring of 200 cells per sample in a large-scale accident. In this exercise, one laboratory separately analyzed 200 and 2,000 cells per dose point and the MAD values (0.20 and 0.21 Gy) did not show any improvement of accuracy with the higher cell number.

Another improvement of the method can be achieved by automation of scoring, which can increase the throughput of the samples as was demonstrated by the number of cells analyzed in this study (Table 3). Several automated systems are available on the market and have been tested by human monitoring (*28, 31–34*). In our study the scoring system of MetaSystems (Altlussheim, Germany) was utilized. MetaSystems can analyze a whole slide with $>$2,000 cells completely automatically in less than 8 min (*35*). This is 2 times faster than the manual scoring of 200 binucleated cells which takes approximately 15 min (*15*). The resulting calibration curves show a good regression, but in general curves of manual scoring were steeper by a factor of about 2 in relationship to the curves generated by automated scoring procedures, which can probably be explained by the better detection efficiency of human scorers (*25, 28, 31*).

Comparing the accuracy of different scoring procedures (manual, semiautomated and automated) performed by the same laboratory (Table 4 and laboratory 1), we find comparable MAD values for the different scoring procedures. If we focus on laboratories with similar quality calibration curves, the blind samples show MAD values $<$0.4 Gy irrespectively of the scoring procedure and the lowest MAD (0.2) were found for manual as well as automated scoring procedures supporting the applicability of the faster automated scoring for biodosimetry (Table 4). In addition, the numbers of dose estimates lying outside the 0.5 Gy uncertainty interval accepted for triage dosimetry based on dicentric analysis (*27*) (or within 20% of the true dose) were similar for the manual and the automated scoring procedures. These comparisons are currently limited by the sample size and number of laboratory contributions available within this study and may be biased by other factors such as interlaboratory performance differences and exposure differences between radiation quality of calibration curves and blind samples. Therefore, these interpretations have to be taken with caution.

TABLE 3
Number of Scored Cells and Observed Micronucleus Frequency [Micronuclei (MN)/1,000 Binucleated cells (BN)]

	Dose (Gy)									
	0		0.1		0.7		1.4		2.0	
Institution	BN	MN/1000 BN	BN	MN/1000 BN	BN	MN/1000 BN	BN	MN/1000 BN	BN	MN/1000 BN
Laboratory 1: automated, X ray/^{60}Co γ ray	3858	53	3955	39	2522	86	3960	213	2314	271
Laboratory 1: semiautomated, ^{60}Co γ rays	3858	26	3955	24	2521	69	3960	214	2311	299
Laboratory 1: manual, ^{60}Co γ ray	3858	45	3954	39	2520	111	3960	292	2311	435
Laboratory 2: automated, X ray	2391	28	3221	48	2767	62	2698	176	2243	277
Laboratory 2: semiautomated, X ray	2391	11	3221	20	2767	61	2698	179	2243	267
Laboratory 3: manual, ^{137}Cs γ ray	200	5	200	65	200	145	200	240	200	555
Laboratory 4: automated, X ray	1154	9	1435	20	1370	160	3842	160	1097	201
Laboratory 5: manual (200 cells), X ray	200	5	200	0	200	80	200	335	200	490
Laboratory 5: manual (2,000 cells), X ray	2000	5	2000	9	2000	77	2000	223	2000	414
Laboratory 6: automated, ^{60}Co γ rays	3986	27	3974	27	3427	69	2953	171	1576	279

	Dose (Gy)									
	2.2		2.6		3.0		4.2		6.4	
Institution	BN	MN/1000 BN	BN	MN/1000 BN	BN	MN/1000 BN	BN	MN/1000 BN	BN	MN/1000 BN
Laboratory 1: automated, X ray/^{60}Co γ ray	2098	294	1960	413	2035	509	1146	700	250	628
Laboratory 1: semiautomated, ^{60}Co γ ray	2097	324	1960	492	2033	612	1146	945	250	1008
Laboratory 1: manual, ^{60}Co γ ray	2096	478	1960	627	2031	779	1146	1212	249	1301
Laboratory 2: automated, X ray	2055	273	2127	402	2830	388	1294	576	661	708
Laboratory 2: semiautomated, X ray	2057	264	2127	441	2830	380	1294	695	660	724
Laboratory 3: manual, ^{137}Cs γ ray	200	390	200	420	200	775	200	790	200	940
Laboratory 4: automated, X ray	841	157	576	222	524	176	365	279	140	71
Laboratory 5: manual (200 cells), X ray	200	360	200	520	200	690	200	1250	200	1905
Laboratory 5: manual (2,000 cells), X ray	2000	442	2000	496	2000	714	2000	1125	2000	1740
Laboratory 6: automated, ^{60}Co γ ray	2022	295	1256	367	1534	422	633	671	290	372

Note. Automated scoring: cut of cells with >4 MN/cell.

One limitation of our MAD approach is that for arbitrary exposure conditions and groups of exposed victims is MAD may not be applicable and maybe valid only under the fixed specified experimental design of this investigation. They do however reflect the overall accuracy of dose estimates per laboratory contribution.

It is well known, that the CBMN assay is less sensitive for very low doses because of the relatively high background frequencies of MN, ranging from 2–36 MN per 1,000 BN cells (*36*) resulting from individual variations depending on age and gender (*18*). Due to this individual variation, the sensitivity of the CBMN assay is in general restricted to doses of not less than 0.2–0.3 Gy (*1, 14, 15*). In this exercise, it was possible to distinguish the unexposed sample (3 correct estimates) and the very low dose of 0.1 Gy (9 correct estimates) with an accuracy of 85–92% from doses >0.1 Gy (Table 5). Furthermore, the results also confirm an upper limit in applicability of the CBMN method above >4 Gy; which has been reported by other studies (*1, 15, 35*).

From the biological dosimetry point of view it is desirable to perform dose estimates as accurately as possible. From the clinical point of view dose ranges often provide sufficient accuracy in order to meet urgent clinical or diagnostic needs. Therefore, the 10 blind samples were divided into binary categories as already described. Again,

TABLE 4
Reported Dose Estimates from Contributing Laboratories are Shown for Each Sample Irradiated with a Certain (True) Dose and Stratified by Scoring Procedure

	True doses (Gy)										With* 6.4 Gy		Without* 6.4 Gy	
	0	0.1	0.7	1.4	2	2.2	2.6	3	4.2	6.4				
Scoring procedure/Institution	estimated doses (Gy)										MAD (Gy)	MAD (SEM)	MAD (Gy)	MAD (SEM)
Manual scoring														
Laboratory 1: manual, ^{60}Co γ ray	0.2	0.1	0.8	1.8	2.3	2.5	3.0	3.4	4.5	4.7	*0.4*	*0.2*	*0.3*	*0.0*
Laboratory 3: manual, ^{137}Cs γ ray	0.0	0.4	1.0	1.5	2.7	2.1	2.2	3.3	3.3	3.7	*0.6*	*0.3*	*0.3*	*0.1*
Laboratory 5: manual (2,000 cells), X ray	0.0	0.1	0.6	1.2	1.9	2.0	2.2	2.8	3.6	4.7	*0.4*	*0.2*	*0.2*	*0.1*
Laboratory 5: manual (200 cells), X ray	0.0	0.0	0.6	1.7	2.2	1.9	2.3	2.7	3.9	4.9	*0.3*	*0.1*	*0.2*	*0.0*
MAD (Gy)	**0.1**	**0.1**	**0.2**	**0.2**	**0.3**	**0.2**	**0.4**	**0.3**	**0.5**	**1.9**				
MAD (SEM)	**0.0**	**0.1**	**0.0**	**0.1**	**0.1**	**0.0**	**0.0**	**0.0**	**0.1**	**0.3**				
Semiautomated scoring														
Laboratory 1: semiautomated, ^{60}Co γ ray	0.3	0.2	0.8	1.9	2.4	2.5	3.2	3.7	4.7	4.9	*0.5*	*0.1*	*0.4*	*0.1*
Laboratory 2: semiautomated, X ray	0.0	0.2	0.8	2.0	2.6	2.6	3.7	3.4	5.1	5.6	*0.5*	*0.1*	*0.5*	*0.1*
MAD (Gy)	**0.1**	**0.1**	**0.1**	**0.5**	**0.5**	**0.3**	**0.9**	**0.5**	**0.7**	**1.2**				
MAD (SEM)	**0.1**	**0.0**	**0.0**	**0.0**	**0.1**	**0.1**	**0.3**	**0.2**	**0.2**	**0.3**				
Automated scoring														
Laboratory 1: automated, X ray	0.4	0.2	0.8	1.8	2.1	2.2	2.8	3.2	3.8	3.6	*0.5*	*0.3*	*0.2*	*0.0*
Laboratory 1: automated, ^{60}Co γ ray	0.4	0.2	0.8	2.0	2.4	2.6	3.3	3.9	4.8	4.5	*0.6*	*0.2*	*0.5*	*0.1*
Laboratory 2: automated, X ray	0.0	0.2	0.4	1.7	2.6	2.5	3.4	3.3	4.4	5.1	*0.4*	*0.1*	*0.3*	*0.1*
Laboratory 4: automated, X ray	0.0	0.0	0.2	1.1	2.1	2.7	1.7	3.2	3.6	2.6	*0.7*	*0.4*	*0.4*	*0.1*
Laboratory 6: automated, ^{60}Co γ ray	0.3	0.3	1.1	2.1	2.8	2.9	3.3	3.6	4.6	3.3	*0.8*	*0.3*	*0.5*	*0.1*
MAD (Gy)	**0.2**	**0.1**	**0.3**	**0.4**	**0.4**	**0.4**	**0.7**	**0.4**	**0.4**	**2.6**				
MAD (SEM)	**0.1**	**0.0**	**0.1**	**0.1**	**0.1**	**0.1**	**0.1**	**0.1**	**0.1**	**0.4**				
MAD all performer (Gy)	**0.1**	**0.1**	**0.2**	**0.4**	**0.4**	**0.3**	**0.6**	**0.4**	**0.5**	**2.1**				
SEM	**0.0**	**0.0**	**0.0**	**0.1**	**0.1**	**0.1**	**0.1**	**0.1**	**0.1**	**0.3**				

Notes. Dose estimations were based on calibration curves for X rays or γ rays after various scoring procedures. MAD = mean absolute difference (Gy) values were calculated for the reported dose estimates, (1) *per laboratory contribution (*italicized* numbers, including and excluding the 6.4 Gy sample) and (2) **bolded** numbers are per sample.

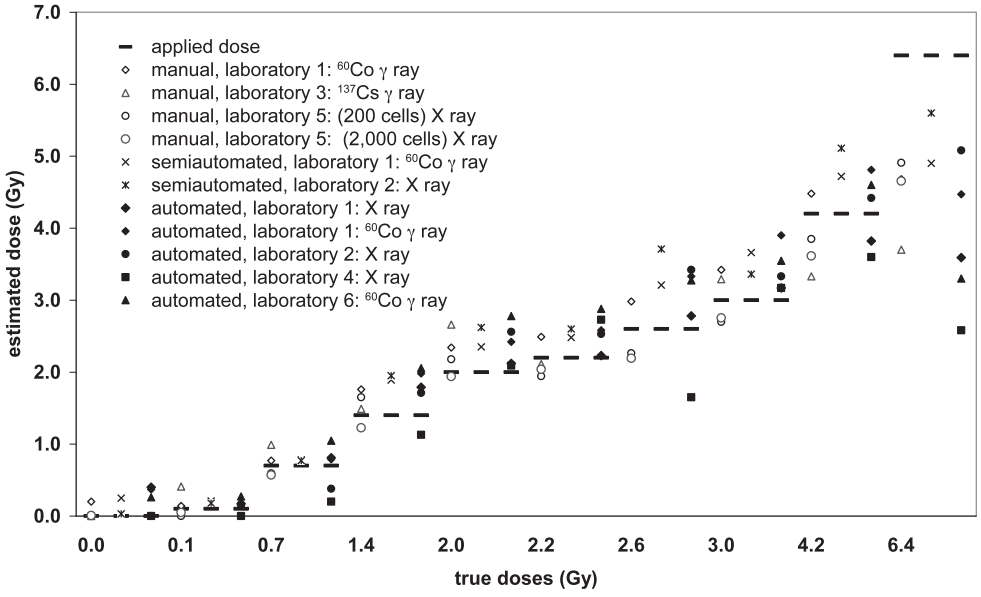

FIG. 2. Reported dose estimates are shown relative to the true absorbed doses per sample (black bar) for manual (open form), semiautomated (asterix) and fully automated scoring (solid form). The 10 samples irradiated with known doses (applied dose) are shown on the x-axis with increasing order.

TABLE 5
True Dose Values were Summarized into Four Binary Categories of Clinical/Diagnostic/Epidemiological Significance

	Totals	Totals per dose	True doses (Gy)[a] 0	0.1	0.7	1.4	2.0	2.2	2.6	3.0	4.2	6.4	Percentage overall[b] Accuracy	Sensitivity	Specificity
Never/ever radiation exposure															
all performer	99	11	3	9	11	11	11	11	11	11	11	11	90.9%	98.0%	27.3%
manual score	36	4	1	3	4	4	4	4	4	4	4	4	90.0%	97.2%	25.0%
semiautomated score	18	2	0	2	2	2	2	2	2	2	2	2	90.0%	100.0%	0.0%
automated score	45	5	2	4	5	5	5	5	5	5	5	5	92.0%	97.8%	40.0%
$\leq$0.1 Gy vs. >0.1 Gy radiation exposure															
all performer	99	11	6	3	11	11	11	11	11	11	11	11	88.2%	100.0%	40.9%
manual score	36	4	3	2	4	4	4	4	4	4	4	4	92.5%	100.0%	62.5%
semiautomated score	18	2	1	0	2	2	2	2	2	2	2	2	85.0%	100.0%	25.0%
automated score	45	5	2	1	5	5	5	5	5	5	5	5	86.0%	100.0%	30.0%
<1.5 Gy vs. $\geq$1.5 Gy radiation exposure															
all performer	99	11	11	11	11	3	11	11	11	11	11	11	92.7%	100.0%	81.8%
manual score	36	4	4	4	4	2	4	4	4	4	4	4	95.0%	100.0%	87.5%
semiautomated score	18	2	2	2	2	0	2	2	2	2	2	2	90.0%	100.0%	75.0%
automated score	45	5	5	5	5	1	5	5	5	5	5	5	92.0%	100.0%	80.0%
2–4 Gy vs. $\geq$4 Gy radiation exposure															
all performer	66	11					11	11	11	11	6	7	86.4%	59.1%	100.0%
manual score	24	4					4	4	4	4	1	3	83.3%	50.0%	100.0%
semiautomated score	12	2					2	2	2	2	2	2	100.0%	100.0%	100.0%
automated score	30	5					5	5	5	5	3	2	83.3%	50.0%	100.0%

Notes. 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 (left part of the table) for all performers and separately for the manual, semiautomated and automated scoring procedures. The accuracy, sensitivity and specificity are calculated (right part of the table).

[a]Related to the totals per dose.

[b]Averages from the true doses, related to the totals.

the data suggest comparable accuracy, sensitivity and specificity for both the manual and the automated scoring procedures.

As stated above, our analysis is limited by the number of contributing laboratories. We restricted our investigations to blood samples taken from a single individual to focus on methodological variance and exclude interindividual variance. For the same reason we varied only the dose and did not simulate partial body radiation exposures. In general the purpose of this exercise was to demonstrate the comparability of established and new available methods under standardized conditions. It is important to note that a real emergency situation, when the blood samples will arrive in the laboratories, the time delay between exposure and sampling will have an important influence on the results, as there are different dynamics in the persistence of the biomarker signals used here.

CONCLUSION

Taken together, CBMN dose estimates based on automated and manual scoring were reported after 4 and 7.1 days, respectively, with a slightly better accuracy for the manual scoring procedure when considering all laboratory contributions and comparable accuracy when only considering laboratory contributions with the lowest MAD values. Binary dose categories of clinical significance could be discriminated with equal efficiency for both scoring procedures and supports the applicability of the automated CBMN assay for triage mode biodosimetry purposes below 6.4 Gy.

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