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NATO BIODOSIMETRY STUDY

Comparison of Established and Emerging Biodosimetry Assays

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Rapid biodosimetry tools are required to assist with triage in the case of a large-scale radiation incident. Here, we aimed to determine the dose-assessment accuracy of the well-established dicentric chromosome assay (DCA) and cytokinesis-block micronucleus assay (CBMN) in comparison to the emerging γ-H2AX foci and gene expression assays for triage mode biodosimetry and radiation injury assessment. Coded blood samples exposed to 10 X-ray doses (240 kVp, 1 Gy/min) of up to 6.4 Gy were sent to participants for dose estimation. Report times were documented for each laboratory and assay. The mean absolute difference (MAD) of estimated doses relative to the true doses was calculated. We also merged doses into binary dose categories of clinical relevance and examined accuracy, sensitivity and specificity of the assays. Dose estimates were reported by the first laboratories within 0.3–0.4 days of receipt of samples for the γ-H2AX and gene expression assays compared to 2.4 and 4 days for the DCA and CBMN assays, respectively. Irrespective of the assay we found a 2.5–4-fold variation of interlaboratory accuracy per assay and lowest MAD values for the DCA assay (0.16 Gy) followed by CBMN (0.34 Gy), gene expression (0.34 Gy) and γ-H2AX (0.45 Gy) foci assay. Binary categories of dose estimates could be discriminated with equal efficiency for all assays, but at doses ≥1.5 Gy a 10% decrease in efficiency was observed for the foci assay, which was still comparable to the CBMN assay. In conclusion, the DCA has been confirmed as the gold standard biodosimetry method, but in situations where speed and throughput are more important than ultimate accuracy, the emerging rapid molecular assays have the potential to become useful triage tools.

INTRODUCTION

Whenever a person may have been exposed to significant levels of ionizing radiation, it is important to estimate the dose received to determine any short- or long-term health implications and provide the evidence base for counseling. Such overexposure cases are typically rare and involve only one or a few potential casualties. The main focus for such isolated cases is to provide the most accurate dose estimate, taking into account exposure characteristics such as radiation type and quality as well as uniformity, duration and timing of the exposure (*1, 2*).

The dicentric chromosome assay (DCA) and the cytokinesis-block micronucleus assay (CBMN) have been established as the main biodosimetry tests for ionizing radiation exposure (*3*). These two cytogenetic methods combine high (DCA) or reasonable (CBMN) specificity, sensitivity of the order of 100 mGy and persistence of the signal for several months. Dozens of laboratories around the world have established calibration curves that enable chromosome aberration yields to be converted to dose estimates, and the quantitative impact of the specific exposure characteristics listed above on aberration yields and distributions has

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been assessed in great detail and implemented in the methodology for radiation dose estimation. Sample preparation and data analysis procedures have been harmonized (*3*) and standardized for the DCA (*4*), ensuring consistency between laboratories.

In contrast to the typical scenario of a radiation incident that affects only a few individuals, rapid tools are required in the case of large-scale accidental exposure or deliberate radiation exposure to (1) help identify the few severely exposed individuals who may require clinical monitoring and treatment and (2) reassure the many ''worried-well'', to prevent them from overwhelming emergency responders and healthcare infrastructure (*5, 6*). Therefore, the focus shifts with increasing number of potential casualties from utmost accuracy, sensitivity and specificity to large capacity and rapid delivery of test results. Consequently, the characteristics of the ideal biodosimeter for rapid triage differ significantly from those for individual dose assessment and pose major challenges for the cytogenetic assays with their slow turn-around times and low throughput.

Several different approaches have been or are currently being implemented to address the capacity gap: triage mode scoring for the dicentric assay (*7, 8*), ''QuickScan'' dicentric chromosome analysis (*9*), networking between biodosimetry laboratories (*10–16*), telescoring (*17*), automation (*18, 19*) and the development of novel assays that have the potential for quicker reporting times and higher throughput than the established cytogenetic methods (*1, 2*). These novel assays include gene expression analysis (e.g., *20, 21*) and the γ-H2AX foci assay (*22*), two methods that enable the analysis of interphase cells and thus avoid the two- to three-day incubation steps necessary for the DCA and CBMN assays. Furthermore, sample preparation and analysis for these molecular endpoints are more amenable to parallel processing and automation, enabling larger capacity. However, while the cytogenetic assays quantify the end products of DNA double-strand break misrepair (*23*), which persist in a nondividing lymphocyte, the gene expression and foci assays measure intermediate signals formed in irradiated cells which change rapidly over time: transcriptional responses to radiation damage and DNA double-strand breaks, most of which are repaired within a day after exposure. These characteristics may cause larger signal variability (and therefore dose uncertainty) and likely restrict the window of opportunity for these assays to a few days post exposure. Given the emphasis on rapid triage in a large-scale incident, these disadvantages may still be acceptable, especially when considering a two-tiered system where initial triage using one of these molecular assays is followed by more accurate chromosome dosimetry for those identified as highly exposed.

Intercomparison exercises using *ex vivo* irradiated coded samples are an important tool for validating the performance of assays as well as laboratories. They have gained even more relevance with the increasing role of international networking and the search for new rapid biomarkers for radiation exposure. Most intercomparisons reported so far have tested the proficiency of participating laboratories in using one specific well established assay for estimating doses for a small number of samples with no time pressure (e.g., *13–16*). Here, we have added several dimensions by (1) comparing established (DCA, CBMN) as well emerging assays (gene expression, γ-H2AX foci) and one assay (H-module) dealing with estimates of hematological damage and not exposure (dose estimate), (2) allowing for both manual and automated scoring, (3) testing how the number of scored cells affects dose estimates and (4) by timing the delivery of dose estimates. In addition to the intra-assay (interlaboratory) comparisons for individual biodosimetry assays described in our four companion articles. This design has allowed us to directly compare the two most important characteristics relevant for decision making regarding diagnosis and therapy in a large-scale incident, accuracy and speed, for the four different assays (inter-assay comparison). The findings of this comparison are presented in this article, together with an assessment of the assays' ability to discriminate between binary dose categories representing clinically relevant treatment groups of potentially overexposed individuals. In particular this approach was introduced to attempt to bridge the gap between dose estimates and medical decision making.

This NATO exercise was organized under the umbrella of the NATO Research Task Group RTG-033 ''Radiation Bioeffects and Countermeasures'' (*24*). It was limited to *ex vivo* uniformly irradiated blood to simulate acute whole body exposure. Given the low level of ''technological readiness'' of the emerging assays we decided not to enforce harmonization of assay procedures across participating laboratories but instead invited partners to use each assay in the format it was established in the individual laboratory. The level of proficiency of the assay format and laboratory would be reflected in the accuracy and reporting time of dose or radiation injury estimates for the unknown samples.

MATERIALS AND METHODS

Blood Sampling, Radiation Exposure and Distribution to Participants

Peripheral blood was drawn from one healthy human volunteer (29-year-old male) and aliquots of 2–3 ml whole blood filled into heparinized vials using a vacutainer system (Becton Dickinson, Germany). Blood was taken with informed consent and the approval of a local ethics committee. Blood samples exposed to known radiation doses were initially provided to participants for the optional generation of calibration data in the respective laboratory using the same radiation quality and irradiation conditions as for the blind samples. Two months later, ten coded blood samples were distributed to participating laboratories for rapid biodosimetry. Fresh blood samples were irradiated at 37°C using single doses of X rays with a mean photon energy of 100 keV (240 kVp; X-ray tube type MB 350/1 in Isovolt 320/10 protection box; Agfa NDT Pantak Seifert GmbH & Co.KG, Ahrensburg, Germany) filtered with 7.0 mm beryllium and 2.0 mm aluminum. The absorbed dose was measured using a duplex

TABLE 1
Participating Institutions and Their Contributions to the Exercise

Institution	Dicentric assay	CB micronucleus assay	γ-H2AX foci	Gene expression	Blood cell counts/ARS severity
Ghent University, Department of Basic Medical Sciences, Research group: 'Radiation and DNA repair', Ghent, Belgium		X			
Institut de Recherche Biomédical des Armées/ CRSSA, Grenoble, France	X			X	
Life Technologies, Company, Frankfurt, Germany				X	
Bundeswehr Institute of Radiobiology, Munich, Germany	X	X	X	X	X
Bundesamt für Strahlenschutz, Munich, Germany	X	X	X		
Qiagen, Company, Hilden				X	
Health Protection Agency, Centre for Radiation, Chemical and Environmental Hazards, Chilton, Didcot, Oxon, UK	X	X	X	X	
Basic Medical Sciences, Center for Applied Nanobioscience and Medicine, College of Medicine Phoenix, University of Arizona, Phoenix, AZ				X	
DxTerity Diagnostics, Company, Rancho Dominguez, CA				X	
Sezione di Istologia e Biologia e Molecolare, Centro Studie Ricerche di Sanita'e Veterinaria, Roma, Italy	X	X	X	X	
Defence Research and Development, Ottawa ON, Canada	X	X			
	6	6	4	8	1

dosimeter (PTW, Freiburg, Germany). The dose-rate was approximately 1.0 Gy min^{-1} at 13 mA. Applied doses for calibration curve production ranged from 0.25–5 Gy, whereas doses for blind samples ranged from 0.1–6.4 Gy. After irradiation, samples were incubated at 37°C for a certain period of time and shipped by overnight courier service under defined conditions according to United Nation Regulation 650. Temperature profiles and potential radiation exposures were monitored by adding temperature loggers (TL30, 3M, Neuss, Germany) and film badges (Helmholtz Zentrum Munich, Germany) to the packages.

Sample Processing and Analysis for the Different Biodosimetry Assays

Protocols for sample preparations and analysis for the four biodosimetry assays are described in the four companion articles of this series.

Collection of Biodosimetry Data and Survey Information

Two data sheets were provided, one to rapidly report the triage dose estimates of blind samples and a second one to provide the complete data including calibration data, and details concerning the technical performance. The time between the arrival of the samples at the participating laboratory (courier report) and the return of the dose estimates of blind samples to the organizer by e-mail was documented. Further information about each laboratory was collected as follows using a questionnaire: (a) number of exercises the laboratory had participated in prior to the NATO exercise; (b) a self assessment of the laboratory's proficiency level for each assay; (c) how long each assay had been established; and (d) used for biodosimetry; (e) level of priority given to the analysis of the NATO samples during daily business.

Statistical Methods

The accuracy of reported dose estimates was measured by calculating the mean of the absolute differences (MAD) of estimated doses to their corresponding true doses. We then examined whether information of the questionnaire might contribute to the MAD values using the Spearman rank correlation test and the Wilcoxon test for a two-group comparison. Finally, we merged doses into binary categories reflecting clinically/diagnostically/epidemiologically relevant aspects and assessed the agreement between the true doses and the reported dose estimates among the binary categories (sensitivity, specificity, overall accuracy). Those categories were:

- never versus ever single radiation exposure (0 Gy/$\geq$0.1 Gy) to avoid clinical resources being occupied by the ''worried-well'' – unexposed individuals who are concerned they may have been exposed,
- marginal versus higher single radiation exposures ($\geq$0.1 Gy/$>$0.1 Gy) to distinguish groups such as those who do not need clinical support from others where deterministic or stochastic effects in adults might occur or become detectable using epidemiological methods,
- lower versus medium-high single radiation exposure ($\leq$1.5 Gy/ $>$1.5 Gy) to select the group of patients who will probably suffer from the acute radiation syndrome (ARS) several days after radiation exposure,
- medium versus high-single radiation exposure (2–4 Gy/$\geq$4 Gy) for triage purposes in the case of limited clinical resources.

Sensitivity, specificity and accuracy were assessed on the basis of the estimated doses and a 2×2 table of true positive = TP, true negative = TN, false positive = FP and false negative = FN was applied. The corresponding percentages were calculated for accuracy

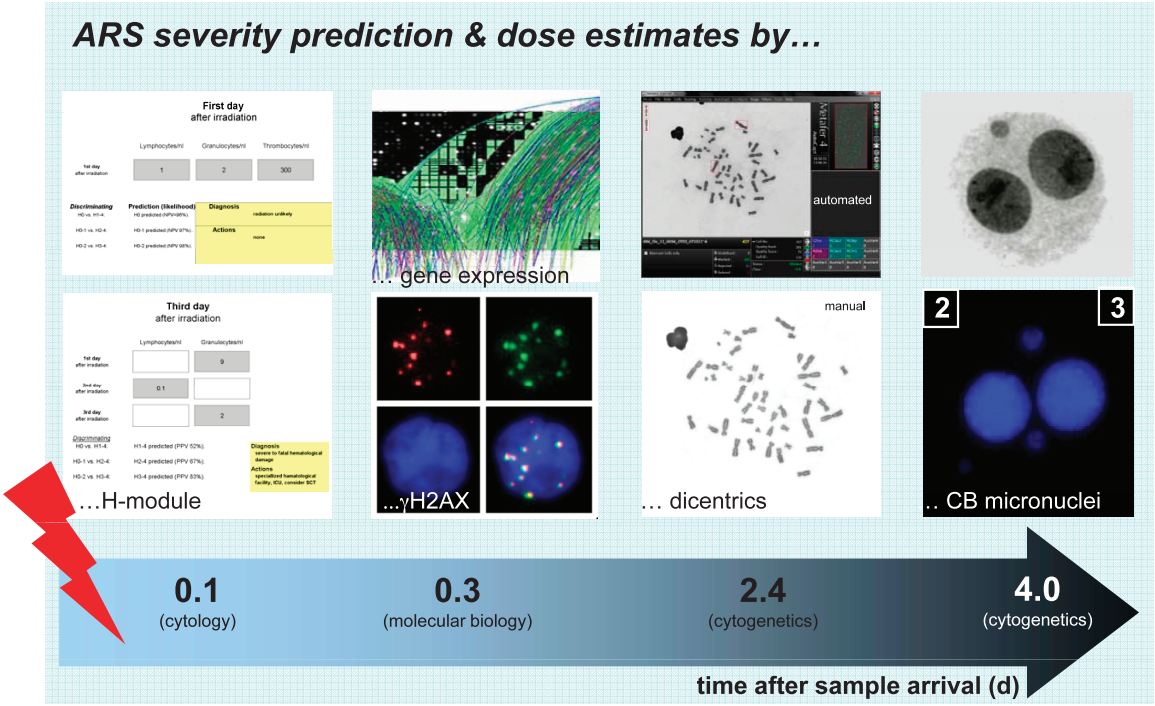

FIG. 1. Earliest report times for ARS severity score prediction (H-module) or dose estimations using molecular (gene expression or γ-H2AX) or cytogenetic assays (dicentric chromosomal assay and cytokinesis block micronucleus assay).

= TP + TN × 100/(total), sensitivity = TP × 100/(TP + FN) and specificity = TN × 100/(TN+ FP).

To assess the accuracy of dose estimates based on 20, 30, 40 or 50 metaphase spreads for the DCA and the γ-H2AX assays we employed Spearman's rank correlation test.

RESULTS

Temperature Profiles

Blood samples were sent to 12 institutions, 11 of which returned dose estimates (Table 1). Six laboratories performed the DCA and CBMN, four the γ-H2AX and eight the gene expression assay. For the calibration samples (sent in July) temperatures ranged from 10–18°C when using ice (i.e., gene expression or γ-H2AX assays) and were approximately 20°C when sending samples at ambient temperature (i.e., DCA or CBMN assays). For the blind samples (sent in September) temperatures remained below 10°C when using ice and 18–20°C for the transport at ambient temperature. Film badges recorded no additional radiation exposure during transport.

Transit and Reporting Times

Transit times of samples were approximately one day within Europe (The Netherlands: 19.5 h, Belgium/France/UK: 21 h and Italy: 25 h). We recorded about 1.5 days transit time for samples sent to the U.S. (west coast: 29–31 h) and Canada (32 h). The reports of all ten dose estimates were received 7–8 h after arrival of blood samples at the participants' laboratories for the γ-H2AX and gene expression assays and after 2.4 and 4 days for the DCA and CBMN assays, respectively (Fig. 1).

Accuracy of Dose Estimations

Comparison of MAD values per assay revealed that the DCA delivered the most accurate dose estimates (Fig. 2 and Table 2). Significant 2–3-fold lower accuracy of the emerging assays was observed when using the DCA as the reference assay (Fig. 2 and Table 3). Differences were less significant when using the CBMN as the reference assay. However, all assays showed considerable variance in the accuracy of dose estimates reported by different laboratories, with approximately a threefold difference between minimal and maximal MAD values for all assays (Table 2) which decreased when focusing on the 50th or 25th percentile of contributions, reaching values of 1.2–1.5 for all assays for the 25th percentile. Exclusion of dose estimates for the 6.4 Gy sample (for which the highest MAD was recorded) did not significantly change these results. Importantly, we observed the lowest MAD values (first number in parenthesis) and lowest numbers of dose estimates outside the 0.5 Gy interval as recommended for triage dosimetry (second entry in parenthesis) for DCA (0.16 Gy/0–6 false dose estimates), followed by CBMN

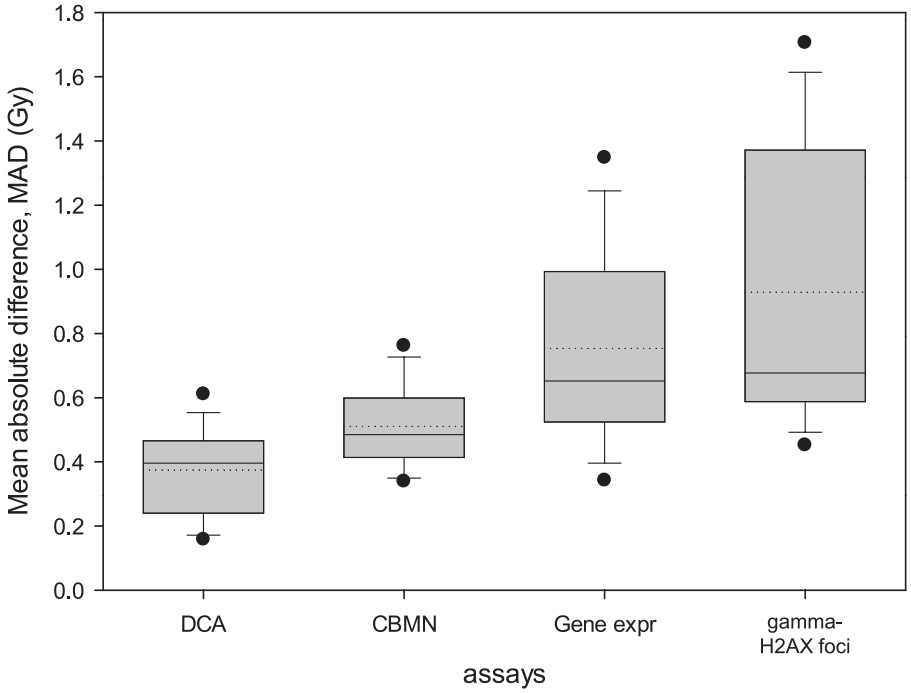

FIG. 2. Distributions of mean absolute differences (MAD) in dose estimations reported by each laboratory are shown for each assay separately. Dotted lines refer to the mean value and straight lines to the median.

TABLE 2
Descriptive Statistics of MAD Values Generated for All Performers, the 50th and 25th Performance Percentile per Assay

	Including 6.4 Gy sample				Excluding 6.4 Gy sample			
	DCA	CBMN	Gene expression	γ-H2AX foci	DCA	CBMN	Gene expression	γ-H2AX foci
MAD, all performers								
n	10	11	8	8	10	11	8	8
Minimum	0.16	0.34	0.34	0.45	0.16	0.20	0.27	0.41
Maximum	0.61	0.76	1.35	1.71	0.52	0.50	1.08	1.26
Mean	0.37	0.51	0.75	0.93	0.32	0.34	0.59	0.77
Median	0.40	0.49	0.65	0.68	0.34	0.33	0.55	0.62
Fold-change	3.9	2.2	3.9	3.8	3.2	2.5	4.0	3.1
Number of dose estimates outside 0.5 Gy	0–6	1–6	1–8	2–8	0–5	0–5	0–7	1–7
MAD, 50th percentile								
n	5	6	4	4	5	6	4	4
Minimum	0.16	0.34	0.34	0.45	0.16	0.20	0.27	0.41
Maximum	0.39	0.49	0.54	0.63	0.34	0.37	0.56	0.64
Mean	0.27	0.41	0.48	0.56	0.23	0.26	0.41	0.51
Median	0.24	0.42	0.52	0.59	0.20	0.24	0.40	0.50
Fold-change	2.5	1.4	1.6	1.4	2.1	1.8	2.1	1.5
Number of dose estimates outside 0.5 Gy	0–3	1–3	1–4	2–6	0–2	0–2	0–3	1–5
MAD, 25th percentile								
n	3	4	2	2	3	4	2	2
Minimum	0.16	0.34	0.34	0.45	0.16	0.20	0.27	0.41
Maximum	0.24	0.43	0.52	0.59	0.20	0.33	0.36	0.54
Mean	0.19	0.38	0.43	0.52	0.18	0.25	0.31	0.50
Median	0.19	0.38	0.43	0.52	0.17	0.24	0.31	0.50
Fold-change	1.5	1.3	1.5	1.3	1.2	1.6	1.3	1.3
Number of dose estimates outside 0.5 Gy	0–1	1–3	1–3	2–3	0–1	0–2	0–2	1–3

Notes. MAD values are shown with and without the 6.4 Gy sample which lay outside the calibrated range of all assays. The fold-change refers to the ratio between the maximum relative to the minimum MAD value per assay. For each assay we provide ranges in the number of reported dose estimates lying outside the recommended 0.5 Gy interval for triage dosimetry.

TABLE 3
Statistical Analysis of MAD Values

Reference, DCA	CBMN	Gene expression	γ-H2AX foci	Reference, CBMN	DCA	Gene expression	γ-H2AX foci
All performers				**All performers**			
(reference: DCA, n = 10, mean = 0.38, median = 0.40)				(reference: CBMN, n = 11, mean = 0.49 median = 0.51)			
n	11	8	8	n	10	8	8
Test	*t* test	Mann Whitney	*t* test	Test	*t* test	Mann Whitney	*t* test
Median/mean	0.51	0.65	0.93	Median/mean	0.38	0.65	0.93
Ratio to reference	1.3	1.7	2.5	Ratio to reference	0.7	1.3	1.8
P value	0.04	0.009	0.003	*P* value	0.04	0.09	0.01
50th percentile				**50th percentile**			
(reference: DCA, n = 5, mean = 0.27, median = 0.24)				(reference: CBMN, n = 6, Mean = 0.41, median = 0.42)			
n	6	4	4	n	5	4	4
Test	Mann Whitney	*t* test	*t* test	Test	Mann Whitney	*t* test	*t* test
Median/mean	0.42	0.48	0.56	Median/mean	0.24	0.48	0.56
Ratio to reference	1.7	1.8	2.1	Ratio to reference	0.6	1.2	1.4
P value	0.03	0.01	0.002	*P* value	0.03	0.18	0.008
25th percentile				**25th percentile**			
(reference: DCA, n = 3, mean = 0.19)				(reference: CBMN, n = 4, Mean = 0.38, median = 0.38)			
n	4	2	2	n	3	2	2
Test	*t* test	*t* test	*t* test	Test	*t* test	Mann Whitney	*t* test
Median/mean	0.38	0.43	0.52	Median/mean	0.19	0.43	0.52
Ratio to reference	2.0	2.2	2.7	Ratio to reference	0.5	1.1	1.4
P value	0.002	0.049	0.012	*P* value	0.002	0.8	0.1

Notes. MAD values derived from reported dose estimates using DCA or CBMN assays as reference and examining all performers or 50th and 25th percentiles of the other assays. Depending on equality of normal distribution or variance either the *t* test or the Mann Whitney rank sum test were applied.

(0.34 Gy/1–6 false dose estimates), gene expression (0.34 Gy/1–8 false dose estimates) and γ-H2AX assays (0.45 Gy/2–8 false dose estimates) (Table 2). Both the MAD values and the number of false dose estimates decreased when the 6.4 Gy sample was excluded from the analysis and/or contributions were restricted to the 50th or the 25th percentile.

Assay Performance in a Triage Setting

The main objective of triage in a large-scale incident is to classify patients depending on their need for acute clinical intervention, more detailed diagnostic tests or long-term epidemiological follow-up. To test the performance of the assays within such a framework, we aggregated dose estimates into binary categories using corresponding threshold doses of 4 Gy, 1.5 Gy and 0.1 Gy and determined the accuracy, sensitivity and specificity of the classifications performed with the four assays (Table 4). We restricted this comparison to laboratory contributions with low MAD values (gene expression and γ-H2AX) and manual scoring procedures (DCA and CBMN) to reflect assay rather than laboratory performance characteristics. Gene expression and γ-H2AX assays delivered similar results to the CBMN assay for all binary categories. In comparison to the DCA assay we observed about 10% lower accuracy and sensitivity for the higher cut-off levels at 1.5 (γ-H2AX) and 4 Gy (γ-H2AX and gene expression).

DISCUSSION

It was the goal of the NATO exercise to compare laboratories performance in dose assessments using established (DCA and CBMN) and emerging assays (gene expression and γ-H2AX) for triage mode biodosimetry. Surprisingly, all assays showed a similar ~3-fold inter-laboratory variation in the accuracy of dose estimates. This finding demonstrates that, in addition to the fixed intrinsic accuracy of an assay, each assay's ''real world'' performance also depends very much on the expertise of the laboratory performing the assay, which may vary significantly over time, depending on e.g., staffing and the focus

TABLE 4
Accuracy, Sensitivity and Specificity of Triage Classifications are Shown for Each Assay after Aggregation of Dose Estimates into Binary Categories

	Overall		
Assay	Accuracy	Sensitivity	Specificity
Never/ever radiation exposure			
DCA	91.5%	94.3%	66.7%
CBMN	90.0%	97.2%	25.0%
Gene expression	92.5%	94.4%	75.0%
γ-H2AX foci	94.0%	100.0%	40.0%
≤0.1 vs. >0.1 Gy radiation exposure			
DCA	91.5%	100.0%	58.3%
CBMN	92.5%	100.0%	62.5%
Gene expression	97.5%	100.0%	87.5.0%
γ-H2AX foci	88.0%	100.0%	40.0%
<1.5 vs. ≥1.5 Gy radiation exposure			
DCA	94.9%	100.0%	87.5%
CBMN	95.0%	100.0%	87.5%
Gene expression	95.0%	100.0%	87.5%
γ-H2AX foci	86.0%	96.7%	70.0%
2–4 vs. ≥4 Gy radiation exposure			
DCA	97.1%	90.9%	100.0%
CBMN	83.3%	50.0%	100.0%
Gene expression	83.3%	75.0%	87.5%
γ-H2AX foci	86.7%	60.0%	100.0%

Note. This comparison focuses on laboratory contributions with low-MAD values (gene expression and γ-H2AX) and manual scoring procedures (DCA and CBMN) to reflect the intrinsic assay characteristics and to a lesser extent performance specific differences.

of the laboratory. In this exercise, the variance in dose estimate accuracy between laboratories using the same assay was at least equal or even greater than the variance between the assays themselves when used by the best performing laboratories. Results of the questionnaire confirmed that the variance in accuracy was in part caused by differences in the experience of laboratories, as illustrated by the number of previous exercises or the period for which the assays had been established prior to this exercise (for details see the four companion articles of this series). It is well conceivable that it may take years to accurately perform either established or emerging assays and it is these skills which affect performance differences, together with the level of ''maturity'', i.e., harmonization and standardization of the assay.

Because of these performance differences we decided to compare not just all contributions but to also restrict our analysis to the 50th and 25th performance percentile of contributions per assay to obtain an unmasked picture of the intrinsic biological and methodological differences between established and emerging assays. Our data suggest a significant 2–3-fold higher accuracy of dose estimates generated by the DCA assay in comparison to the other assays including the CBMN assay. When using the CBMN assay as the reference this advantage in accuracy becomes insignificant for the gene expression assay and borderline significant for the γ-H2AX assay. Concomitantly, we observed fewer reported dose estimates outside the 0.5 Gy interval for the DCA assay compared to the other assays. However, this effect became less significant after restricting the analysis to the 25th performance percentile and excluding the 6.4 Gy sample (Table 2). Hence, even at this early stage, emerging assays under optimal conditions provide dose estimates with an accuracy as good as the CBMN assay, though they are 2–3 times less precise than the DCA assay.

All assays showed an upper limit of applicability below the highest blind dose of 6.4 Gy, which was systematically underestimated by all assays. While it is known that the established cytogenetic assays perform less well at very high doses, one can speculate that the lack of calibration data above 5 Gy is the primary reason for the poor performance of the assays. This finding therefore underlines the importance of relying on the assays only within the calibrated dose range, as extrapolation may not take into account deviations in the dose response for the assay. In the case of the cytogenetic methods such deviations may be associated with the prolonged cell cycle delay that is observed at high doses (*3*), whereas for gene expression and γ-H2AX assays saturation effects in gene transcription and foci scoring, respectively (*22, 25*), may be responsible for a leveling off towards high doses that would be unaccounted for in the calibration curve with its upper limit of 5 Gy. Yet, removing the 6.4 Gy point from the analysis had no impact on fold-change differences between assays, since it affected all assays similarly.

From the dosimetry point of view and for long-term epidemiological follow-up it is desirable to estimate doses as accurately as possible. From the clinical point of view dose ranges often provide sufficient information to address urgent clinical or diagnostic needs. This is why we divided the 10 samples into binary categories as already described. In this framework, the emerging assays performed as well as the CBMN assay and almost as well as the DCA assay, despite their comparatively low level of validation, harmonization and standardization.

Within this exercise one institution also employed a software tool for prediction of effects (hematological syndrome of the acute radiation syndrome, ARS), but not dose. Blood cell counts (BCC) from radiation accident victims were correlated with the severity of the clinical outcome using the database SEARCH (*S*ystem for *E*valuation and *A*rchiving of *R*adiation Accidents based on *C*ase *H*istories) (*26, 27*). With logistic regression we developed models to predict the ARS severity based on BCC. The models were converted into Excel and after entering BCCs by the user, these algorithms automatically generate predictions on diagnosis (ARS severity) and recommend treatment options. BCCs were chosen from real radiation accident cases and we compared the accuracy of ARS predictions based on BCCs taken on day 1 or on days 1 and

2 or on the first 3 consecutive days after radiation exposure. Irrespective of time we received correct ARS categories in 8 of our 10 BCCs. These 80% correctly predicted ARS categories were detected even within a few hours after exposure, the time it takes to generate the BCC using the widely available automated blood cell counters in hospitals. The accuracy of this early estimate taken shortly after exposure can be validated using a sequential diagnosis with BCC performed on the next 2–3 days after exposure. Hence, this tool adds early additional information for clinical diagnostic and therapeutic decision-making, but it certainly has to be combined with other assays to further reduce the false categorization.

This exercise provided additional experiences and insights, which could be covered under the title ''lessons learned''. These included one failed sample delivery because reception staff had not been informed; breakdown of the automated metaphase finder – DCA measurements were done manually instead; difficulties to find 50 scorable cells for the γ-H2AX assay when using only 1 ml blood; delays in reporting dose estimates caused by a lack of automation in dose calculations from raw data; delays caused by strict minimum staffing rules when performing assays; reduction of temperature variations during sample transport by additional wrapping in aluminum foil; successful exercise organization using email only.

The exposure scenario used for this exercise has a number of limitations. We deliberately restricted all measurements to blood samples taken from only one individual to focus on methodological variance and to exclude interindividual variance which has been reported for several of the assays (*20, 28*). For the same reason we varied only the dose and did not simulate nonuniform radiation exposures. It is important to note that results may well differ considerably in the case of nonuniform exposures. Only the DCA and γ-H2AX assay but not the other methods have been formally shown to be able to detect and quantify nonuniform exposures in certain scenarios, based on the distribution of dicentrics or foci, respectively, among analyzed cells (*3, 25, 29, 30*). Even then, the low number of cells scored in triage mode may pose difficulties in the case of highly nonuniform exposures (*31*). More data and better statistical methods are needed to fully understand the impact of nonuniform exposures on biological dose estimates and associated uncertainties (*32*).

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