RADIATION RESEARCH **180**, 149–155 (2013)
0033-7587/13 $15.00

DOI: 10.1667/RR3238.1

NATO BIODOSIMETRY STUDY

Laboratory Intercomparison on the γ-H2AX Foci Assay

K. Rothkamm,[a] S. Horn,[a] H. Scherthan,[b] U. Rößler,[c] A. De Amicis,[d] S. Barnard,[a] Ulrike Kulka,[c] F. Lista,[d] V. Meineke,[b] H. Braselmann,[e] C. Beinke[b] and M. Abend[1,b]

[a] *Public Health England, Centre for Radiation, Chemical and Environmental Hazards, Chilton, Didcot, Oxon OX11 0RQ, United Kingdom;* [b] *Bundeswehr Institute of Radiobiology, Munich, Bavaria, 80937, Germany;* [c] *Bundesamt fuer Strahlenschutz, Germany;* [d] *Centro Studi e Ricerche di Sanita'e Veterinaria, Italy; and* [e] *Research Unit of Radiation Cytogenetics, Helmholtz Zentrum München, Germany*

Rothkamm, K., Horn, S., Scherthan, H., Rößler, U., De Amicis, A., Barnard, S., Kulka, U., Lista, F., Meineke, V., Braselmann, H., Beinke, C. and Abend, M. Laboratory Intercomparison on the γ-H2AX Foci Assay. *Radiat. Res*. 180, 149–155 (2013).

The focus of the study is an intercomparison of laboratories' dose-assessment performances using the γ-H2AX foci assay as a diagnostic triage tool for rapid individual radiation dose assessment. Homogenously X-irradiated (240 kVp, 1 Gy/min) blood samples for establishing calibration data (0.25–4 Gy) as well as blinded test samples (0.1–6.4 Gy) were incubated at 37°C for 2 and 24 h (repair time) and sent to the participants. The foci assay was performed according to protocols individually established in participating laboratories and therefore varied. 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) of estimated doses relative to the actual doses was calculated and radiation doses were merged into four triage categories reflecting clinical relevance to calculate accuracy, sensitivity and specificity. First γ-H2AX based dose estimates were reported 7 h after sample receipt. Estimates were similarly accurate for 2 and 24 h repair times, providing scope for its use in the early phase of a radiation exposure incident. Equal accuracy was achieved by scoring 20, 30, 40 or 50 cells per sample. However, MAD values of 0.5–0.7 Gy and 1.3–1.7 Gy divided the data sets into two groups, driven mainly by the considerable differences in foci yields between calibration and blind samples. Foci yields also varied dramatically between laboratories, highlighting reproducibility issues as an important caveat of the foci assay. Nonetheless, foci counts could distinguish high- and low-dose samples in all data sets and binary dose categories of clinical significance could be discriminated with satisfactory accuracy (mean 84%, ±0.03 SEM). Overall, the results suggest that the γ-H2AX assay is a useful tool for rapidly screening individuals for significant exposures that occurred up to at least 24 h earlier, and may help to prioritize cytogenetic dosimetry follow-up.

INTRODUCTION

The long-established dicentric assay (DCA) is the gold standard for accurate biological dose estimation following a suspected radiation overexposure (*1*) but suffers from: (1) long turn-around times (>2 days between sample receipt and dose estimate); (2) low throughput [current global capacity of ~3,000 tests per week in ''50 cell triage mode'' reported for the World Health Organization's BioDoseNet assistance network of 57 biodosimetry laboratories (*2*)]; and (3) the reliance on highly skilled cytogeneticists for dicentric scoring, complicating the development of surge capacity. These limitations do not pose any major difficulties in the routine management of isolated, small-scale radiation incidents. However, they may well become a major bottleneck in the event of a large-scale radiation accident, where rapid triage is of prime importance to identify the few severely exposed individuals who require acute clinical support and, to reassure the many ''worried-well'' who could overwhelm the local healthcare infrastructure. This unmet need continues to drive research into novel biomarkers for radiation exposure which may be less accurate than the DCA but enable rapid screening of hundreds, if not thousands, of potentially exposed individuals for radiation exposure levels of immediate clinical relevance.

The phosphorylated histone H2A variant γ-H2AX is a well established surrogate marker of ionizing-radiation-induced DNA double-strand breaks (DSBs) and a promising biomarker of radiation exposure (*3, 4*). γ-H2AX foci forms within minutes after exposure at the sites of DSBs and disappear with kinetics similar to those of DSB repair (*5*).

Editor's note. The online version of this article (DOI: 10.1667/RR3238.1) contains supplementary information that is available to all authorized users.

[1]Address for correspondence: Bundeswehr Institute of Radiobiology, affiliated to the University of Ulm, Neuherbergstraße 11, 80937 Munich, Germany; e-mail: michaelabend@bundeswehr.org.

They can be visualized in nucleated blood cells (*6–8*) and tissue sections (*9–11*) using immunofluorescence or immunohistochemical protocols that require only a few hours between sampling and analysis, in contrast to the 2–3 days required for conventional cytogenetic methods (*1*). The potential of the γ-H2AX assay to accurately estimate radiation dose has already been demonstrated after experimental human *ex vivo* (*7, 12–14*), nonhuman primate *in vivo* (*15*) and diagnostic (*6*) or therapeutic (*16*) human *in vivo* exposure. These studies demonstrate excellent sensitivity down to a few milligray (at least under well-controlled conditions), detection of recent partial body exposure at both high and very low doses, and persistence of foci for several days after high-dose exposure.

Here we determined the performance of the γ-H2AX assay in four laboratories for dose assessment and its use as a rapid dose assessment tool in an international intercomparison exercise which was organized under the umbrella of the NATO Research Task Group RTG-033 ''Radiation Bioeffects and Countermeasures'' we compared the performance and properties of the two most established cytogenetic dosimetry tools, DCA and cytokinesis block micronucleus assay with two novel emerging molecular dosimetry methods, γ-H2AX foci assay and gene expression analysis. The focus of the article is a comparison of four laboratories' ''performance for dose assessment'' using the γ-H2AX foci assay as a diagnostic tool for rapid emergency biodosimetry. All assays 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. For the γ-H2AX foci assay we also compared dose estimates and MAD values after a repair time of 2 and 24 h based on either 20, 30, 40 or 50 scored cells.

MATERIALS AND METHODS

Procedures Common for All Assays

Blood samples of 2–3 ml whole blood from one healthy male individual filled in heparinized tubes were taken and exposed to 240 kVp X rays at 1 Gy/min. After irradiation, calibration (0.25 to 4 Gy) and blinded tested samples (0.1 to 6.4 Gy) were distributed to participating laboratories along with standardized data sheets and questionnaire's pertaining to statistical analysis MAD calculations, impact of questionnaire information on MAD and binary categories of clinical significance, which represented procedures employed for all assays. To assess the quality of binary dose assignments, the factors for sensitivity, specificity and accuracy were used. Comparison between groups of participants was done descriptively with help of these factors. A detailed description of the inter-assay comparison is the lead article in this series of companion articles (NATO Biodosimetry Study, *Radiat. Res*. 2013; 180:111–19).

γ-H2AX Analysis

A standard protocol for the γ-H2AX foci assay was selected for this exercise [based on ref. (*14*)] to which all laboratories adhered for at least the main experimental steps (see Supplementary Material; http://dx.doi.org/10.1667/RR3238.1.S1). After initial attempts to use automated scoring in pilot samples produced inconsistent results (data not shown), manual scoring of 50 cells per sample was performed in all laboratories. Participants were also asked for interim results to determine any change in accuracy when estimating doses using 20, 30, 40 or 50 scored cells. Calibration curves were fitted individually by each laboratory for each time point (2 and 24 h) using a linear function. For one data set (laboratory 1, 2 h data), two separate linear functions were fitted for doses up to and above 0.5 Gy, respectively. Microsoft Excel and DoseEstimate software packages were used for curve fitting. Calculations of estimated blinded test doses were performed with the same software packages by converting foci counts into dose estimates using the established linear functions.

RESULTS

Participating Laboratories, Sample Shipments and Reporting Times

Four institutions participated and used the initially shipped calibration samples to produce reference data prior to the shipment of blinded test samples. All provided dose estimates for each of the 10 blinded test samples after a repair time of 2 and 24 h so that 80 dose estimates were available for data analysis. All laboratories completed the questionnaire. Two laboratories had used the γ-H2AX assay for biodosimetry for several years, the other two laboratories for only 5 months prior to the exercise (Table 1). Two of the laboratories were within walking distance of the blood sampling and irradiation location, and samples could therefore be processed without any shipment delay. The other two laboratories received the samples after a transit period of 21–25 h. Temperature profiles for calibration sample shipments (during a hot summer week) ranged between 10–18°C; for blinded test samples the range was only 4–10°C. Film badges recorded no additional radiation exposure during transport. Three laboratories treated the exercise with priority, and reporting times were between just under 7 h to two days. All laboratories followed the same standard protocol for γ-H2AX processing (see Supplementary Material; http://dx.doi.org/10.1667/RR3238.1.S1), except for laboratories 1 and 4 who spun cells onto slides, instead of manual spreading and another laboratory who used OptiPrep™ rather than Ficoll for lymphocyte isolation.

Calibration Data

Foci counts reported for calibration data were closely correlated with dose, with Pearson coefficients of 0.95–0.99 for six curves (laboratory 1: 24 h, 0.74; laboratory 3: 2 h, 0.87), and were on average higher for 2 h compared to 24 h, consistent with the loss of foci expected during repair incubation (Fig. 1). However, counts varied by more than fourfold between laboratories and, at least at 2 h tended to level off towards higher doses. Participants were free to choose their own strategy for fitting reference yield curves. Laboratory 1 fitted separate linear curves for 0–0.5 Gy ($y = 15D$) and 0.75–4 Gy ($y = 3.35D + 6.37$) for 2 h and one

TABLE 1
Contributing Institutions, Their Experience Related to the γ-H2AX Assay and Reporting Times for Dose Estimates

Laboratory	Institution	No. previous exercises	Biodosimetry laboratory	Method established since... (months)	Method used for biodosimetry since... (months)	NATO samples processed with	Reporting time for dose estimates (days)	
							2 h repair samples	24 h repair samples
ID 1	1	0	no	36	4	priority	1.1	1.1
ID 2	2	6	yes	120	96	priority	0.4	0.3
ID 3	3	1	yes	60	60	others	1.1	1.2
ID 4	4	0	yes	5	5	priority	2.1	1.9

linear curve for 24 h (y = 2.14D). Laboratory 2 modified its existing 250 kVp X-ray calibration function (*14*) to y = 3.36D (2 h) and y = 1.64D (24 h) to take into account the effects of overnight shipment. Laboratory 3 used only the 0–1 Gy data range to fit the function y = 4.05D–0.22 for 2 h and the complete data set for 24 h with y = 0.9D–0.06. Laboratory 4 used the function y = 1.85D + 0.55 (2 h) and y = 0.32D + 1.1 (24 h) to fit their data.

Dose Estimates for Blind Samples

Foci counts for blind samples (Table 2, increasing gray shades) sorted by true dose confirm the close correlation already reported above for calibration samples, with only one data set showing a correlation coefficient of less than 0.85. This demonstrated the potential of the γ-H2AX assay to rank exposed individuals according to dose, even in the absence of a calibration curve. However, actual dose estimates varied considerably, especially for samples exposed to moderate to high doses (Fig. 2). Laboratory 4 systematically underestimated exposures for both 2 and 24 h samples, resulting in several negative dose estimates. This was most likely caused by staining artifacts in their calibration curves, which resulted in very high baseline levels. Interestingly, the interim results of dose estimates based on 20, 30, 40 and 50 cells (individual data points in Fig. 2) were in good agreement with each other and independent of dose, time point or laboratory. Dose estimates reported after 2 h repair time were not statistically significant from the 24 h repair time (Wilcoxon rank sum test, $P = 0.69$).

MAD values for each laboratory showed a 3.4-fold difference in accuracy, with comparable ranges of 0.5–1.7 Gy and 0.6–1.4 Gy for repair times of 2 and 24 h, respectively (Table 3). The number of measurements falling outside a 0.5 Gy interval around the true dose varied similarly at 2 (3–7 measurements) and 24 h (2–8 measurements). Based on these measurements we divided the laboratory contributions into two groups characterized by low- (0.5–0.7 Gy) and high-MAD values (1.3–1.7 Gy).

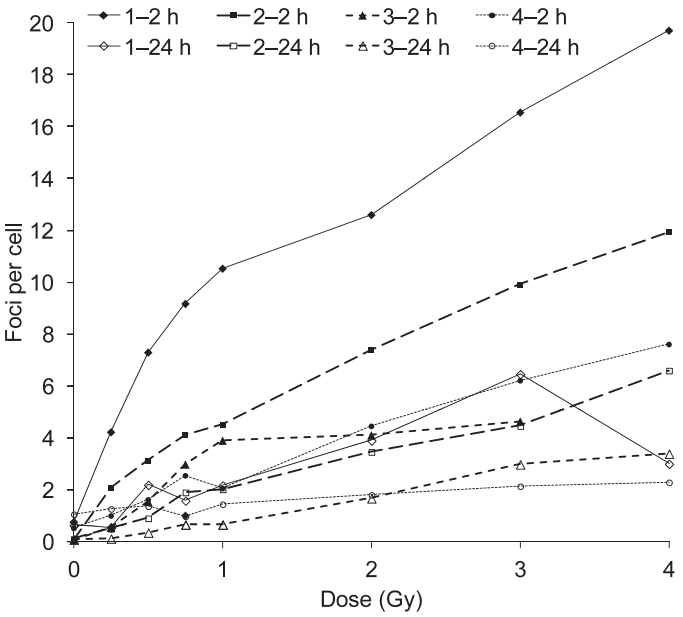

FIG. 1. Foci counts as a function of radiation dose obtained by four laboratories in calibration samples after 2 and 24 h repair time. These were used to obtain calibration curves for estimating doses given to blind samples. Symbols represent average values for 50 scored cells per data point. Solid (2 h) and dashed lines (24 h) show fitted linear functions.

TABLE 2
Foci Counts for Blind Samples (with increasing Gray Shades) Sorted by True Dose

True dose (Gy)	Foci yields at 2h				Foci yields at 24h			
	Lab 1	Lab 2	Lab 3	Lab 4	Lab 1	Lab 2	Lab 3	Lab 4
0	0	0.02	0.02	0.5	0.24	0.06	0.2	0.62
0.1	1.7	0.68	1.54	0.82	0.64	0.45	0.28	0.6
0.7	4.42	2.9	6.2	1.44	1.78	2.1	1.32	1.16
1.4	7.34	5	8.84	0.56	2.36	4.4	1.74	1.2
2	1.7	8.82	12.1	1.62	3.8	2.85	2.1	1.18
2.2	6.62	11.28	11.64	1.86	3.58	3.85	3.32	0.84
2.6	10.8	7.78	12.76	2.96	4.78	3.05	3.32	1.96
3	10.86	11.76	12.92	1.18	2.98	3.8	2.32	1.6
4.2	14.7	10.36	17.34	3.06	8.4	4.45	3.44	2.4
6.4	15	19.9	21.76	1.88	9	7.45	6.2	2.18
ρ[b]	0.89	0.95	0.96	0.60	0.95	0.91	0.95	0.86

[b] Pearson correlation coefficient

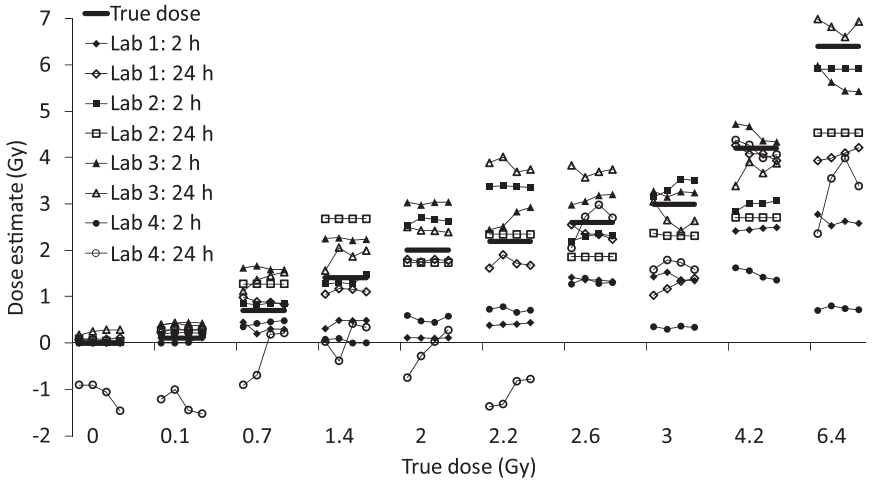

FIG. 2. Reported dose estimates are shown relative to the true absorbed doses per sample. Each chain of symbols shows dose estimates after scoring 20, 30, 40 and 50 cells (from left to right).

MAD values per irradiated sample indicate an upper limit in dose estimates to occur at ≥4.2 Gy for the 2 h time point and ≥3 Gy for 24 h time point and therefore true doses were underestimated by almost all laboratories for these absorbed doses (Fig. 2). To elucidate the reason for the discrepancy of MAD values between laboratories we examined any links to the information given in the questionnaires. No significant correlation was found with the number of previous exercises and the period for which the method had been established (Spearman's rank correlation test). However, the three data sets with high MAD values of 1.3–1.7 Gy were all associated with systematic underestimation of doses (1.7–6.8 fold lower linear coefficients for foci yields in blind data sets with high MAD vs. 0.9-1.3 fold lower coefficients in those with low MAD values; Pearson correlation coefficient 0.85). Therefore, a systematic difference in foci yields per unit dose between calibration and blinded test sample data appears to be the main reason for the high MAD values seen in three data sets.

TABLE 3
Aggregation of Foci-Based Dose Estimates into Binary Categories of Clinical Significance

	True dose for each sample (Gy)										MAD	MAD	No. estimates outside
	0	0.1	0.7	1.4	2	2.2	2.6	3	4.2	6.4	(Gy)	(SEM)	± 0.5 Gy
	Estimated doses for each sample (Gy)												
2 h repair													
laboratory ID 1	**0.0**	**0.1**	**0.3**	***0.5***	***0.1***	***0.4***	***1.3***	***1.3***	***2.5***	***2.6***	1.34	0.4	7
laboratory ID 2	**0.0**	**0.2**	**0.9**	**1.5**	***2.6***	***3.4***	**2.3**	**3.5**	***3.1***	**5.9**	0.45	0.1	3
laboratory ID 3	**0.1**	**0.4**	***1.6***	***2.2***	***3.0***	***2.9***	***3.2***	**3.2**	**4.3**	***5.4***	0.59	0.1	6
laboratory ID 4	**0.0**	**0.1**	**0.5**	***0.0***	***0.6***	***0.7***	***1.3***	***0.3***	***1.4***	***0.7***	1.71	0.5	7
MAD (Gy)	0.0	0.1	0.4	0.8	1.2	1.3	0.9	1.3	1.5	2.7			
MAD (SEM)	0.0	0.1	0.3	0.5	0.5	0.4	0.5	1.1	1.1	2.5			
No. measurements out of ± 0.5 Gy	0	0	1	3	4	4	3	2	3	3			
24 h repair													
laboratory ID 1	**0.1**	**0.3**	**0.8**	**1.1**	**1.8**	**1.7**	**2.2**	***1.4***	**3.9**	***4.2***	0.59	0.2	2
laboratory ID 2	**0.0**	**0.3**	***1.3***	***2.7***	**1.7**	**2.3**	***1.9***	***2.3***	**2.7**	***4.5***	0.73	0.2	6
laboratory ID 3	**0.3**	**0.4**	***1.5***	***2.0***	**2.4**	***3.7***	***3.7***	**2.6**	**3.9**	***6.9***	0.63	0.1	4
laboratory ID 4	***–1.5***	***–1.5***	***0.2***	***0.3***	***0.3***	***–0.8***	**2.7**	***1.6***	**4.1**	***3.4***	1.40	0.3	8
MAD[a] (Gy)	0.5	0.6	0.5	0.8	0.6	1.3	0.6	1.0	0.6	1.9			
MAD[a] (SEM)	0.7	0.7	0.3	0.4	0.7	1.3	0.5	0.6	0.6	1.0			
No. estimates outside ± 0.5 Gy[a]	1	1	3	3	1	2	2	3	1	3			
MAD[b] (Gy)	0.1	0.2	0.5	0.7	0.3	0.7	0.7	0.9	0.7	1.5			
MAD[b] (SEM)	0.1	0.1	0.4	0.5	0.1	0.7	0.4	0.6	0.7	0.9			
No. estimates outside ± 0.5 Gy[b]	0	0	2	2	0	1	2	2	1	2			

Notes. Reported 50-cell dose estimates (bolded data), MAD values *per lab contribution* (right column) and *per sample* (bottom rows) and the number of estimates lying outside a 0.5 Gy interval around the true dose (italicized) are shown for 2 and 24 h repair times. [a]MAD refers to calculations including all four measurements of the 24 h repair time, while [b]MAD refers to calculations based on laboratory ID 1–3 only.

TABLE 4
Total Number of Reported Assignments per Dose

Radiation exposure	Totals	Total per dose	True doses (Gy)[a]										Percentage overall[b]...		
			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	80	8	5	7	8	7	8	7	8	8	8	8	92.5%	95.8%	62.5%
low MAD	50	5	2	5	5	5	5	5	5	5	5	5	94.0%	100.0%	40.0%
high MAD	30	3	3	2	3	2	3	2	3	3	3	3	90.0%	88.9%	100.0%
2 h repair	40	4	3	4	4	3	4	4	4	4	4	4	95.0%	97.2%	75.0%
24 h repair	40	4	2	3	4	4	4	3	4	4	4	4	90.0%	94.4%	50.0%
< 0.1 Gy vs. > 0.1 Gy															
all	80	8	7	3	8	7	7	7	8	8	8	8	88.8%	95.3%	62.5%
low MAD	50	5	4	0	5	5	5	5	5	5	5	5	88.0%	100.0%	40.0%
high MAD	30	3	3	3	3	2	2	2	3	3	3	3	90.0%	87.5%	100.0%
2 h repair	40	4	4	2	4	3	3	4	4	4	4	4	90.0%	93.8%	75.0%
24 h repair	40	4	3	1	4	4	4	3	4	4	4	4	87.5%	96.9%	50.0%
< 1.5 Gy vs. > 1.5 Gy															
all	80	8	8	8	6	4	5	5	6	5	7	7	76.3%	72.9%	81.3%
low MAD	50	5	5	5	3	1	5	5	5	4	5	5	86.0%	96.7%	70.0%
high MAD	30	3	3	3	3	3	0	0	1	1	2	2	60.0%	33.3%	100.0%
2 h repair	40	4	4	4	3	2	2	2	2	2	3	3	67.5%	58.3%	81.3%
24 h repair	40	4	4	4	3	2	3	3	4	3	4	4	85.0%	87.5%	81.3%
2–4 Gy vs. > 4 Gy															
all	48	8					8	8	8	8	2	5	81.3%	43.8%	100.0%
low MAD	30	5					5	5	5	5	1	5	86.7%	60.0%	100.0%
high MAD	18	3					3	3	3	3	1	0	72.2%	16.7%	100.0%
2 h repair	24	4					4	4	4	4	1	2	79.2%	37.5%	100.0%
24 h repair	24	4					4	4	4	4	1	3	83.3%	50.0%	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 as well as after a 2 h and 24 h repair time. Columns on the right show the overall agreement on accuracy, sensitivity and specificity.

[a] Related to the totals per dose.

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

Binary Exposure Categories

While accurate dose assessment is important for estimating long-term risk, the most urgent requirement in a large-scale incident is the rapid triage of potentially exposed individuals to identify those who may need clinical monitoring and to reassure the less critically exposed. Foci-based dose estimates were therefore also aggregated into binary categories (Table 4). Accuracy (86–94% vs. 60–90%) and sensitivity (60–100% vs. 17–89%) appeared better for the data sets with low-MAD values, but specificity appeared better for those with high-MAD values, which achieved 100% in all four categories. This finding can likely be explained by the systematic underestimation of higher doses in these data sets, as explained above. The relatively low sensitivity observed for the highest dose category (2–4 Gy vs. ≥4 Gy) may in part have been caused by the lack of calibration data above 4 Gy which forced the laboratories to extrapolate, resulting in underestimation because of the flattening of foci yields towards very high doses which had not been fully taken into account. Performance of the same comparisons for the dose estimates reported after 2 and 24 h repair time were not different. Overall, these results demonstrate the usefulness of the γ-H2AX assay in a triage setting where rapid identification of significant exposures is more important than utmost accuracy in estimating doses. It also provides some indications for use of the assay even 24 h after exposure, which from a practical point of view would be preferable over just a 2 h window of opportunity to perform these analyses.

DISCUSSION

After a radiation accident, rough dose estimates need to be provided as soon as possible to support clinical decision making and help manage concerns among the potentially exposed. The fast turnaround time of less than 7 h for 10 dose estimates achieved in this exercise demonstrates that the γ-H2AX assay can enable rapid screening for significant exposures at a much higher throughput than that achievable with other cytogenetic methods. As the presented results suggest that dose estimates are equally accurate when based on 20 instead of 50 cells, it may be possible to reduce the turnaround time further. Also, the burden of manual scoring will be decreased further and may reduce the need or

desirability of automated foci scoring and all its associated complications (*4*). However, it is important to point out that in this exercise uniformly irradiated samples were used. In the case of nonuniform exposures, more cells would need to be scored to capture the full spectrum of damaged cells and avoid mistakes in estimating doses. In addition, guideline recommend testing of the raw foci data for overdispersion, which indicates a nonuniform exposure may have occurred (*6, 14, 17*).

Interestingly, when comparing results after 2 and 24 h repair time, the accuracy (MAD values) as well as the number of measurements lying outside the recommended 0.5 Gy uncertainty interval were quite similar, despite the significantly different foci yields observed at these two time points. Having at least a 24 h time window after irradiation is of great practical importance; this could ideally be extended to several days at least for higher doses (*14, 15*), but will require additional calibration to account for the continuing foci loss with time. However, it is important to remember that for arbitrary exposure conditions and groups of exposed victims the MAD values may be different than presented here and are valid only for this study with study specified experimental design, which was identical for all participants. There, these MAD values reflect the overall accuracy of dose estimates for each laboratory.

The huge differences in foci yields obtained for the same samples by the different laboratories and the systematic underestimation of doses seen in several data sets point to a considerable variability in foci detection. This may be explained either by variations in foci loss during shipment of blood samples or by variations in immunofluorescence staining quality from experiment to experiment (*4*). Given that these differences occurred even in a laboratory that could collect samples locally, the main factor seems to be the variability of the foci staining process. This is consistent with recent results from the EC Multibiodose project (Rothkamm *et al,* unpublished results). As a consequence it may be advisable to always add a negative and a positive (i.e., irradiated with a known dose) control sample as a reference, so that baseline dose estimates can be adjusted if necessary. Similarly, one should not regard any calibration curves for this assay as set in stone as it is the case for the dicentric and micronucleus assays. Instead, the foci assay should be frequently recalibrated to take into account any drift in foci yields, and protocols should be optimized to reduce variability as much as possible. It is clear, however, that γ-H2AX based dose estimates in radiation emergencies are unlikely to ever reach the high sensitivity and reproducibility that can be achieved with the DCA assay for well controlled and planned medical or experimental radiation exposures.

From the dosimetry point of view it is desired to perform dose estimates as accurately as possible. From the clinical point of view dose ranges often provide sufficient accuracy to meet urgent clinical or diagnostic needs. To address this, we divided the 10 samples into binary categories. Laboratory contributions with low-MAD values resulted in up to 40% improved accuracy and sensitivity. Importantly, however, high exposures could be distinguished from no or low exposures in all data sets by ranking primary foci data (Table 2). This demonstrates that, even in the absence of a suitable calibration curve, the foci assay can potentially be used to initially identify those few among a large cohort who may require clinical support and who should be analyzed first for radiation exposure using the gold standard DCA assay. Such a two-tier approach may provide a relatively robust and practical strategy for managing biodosimetry support in radiation emergencies, whether small or large in scale. In fact, one of the participating laboratories in this study used the γ-H2AX assay exactly for this purpose in a 2011 radiation accident and provided foci-based estimates of upper dose limits for 10 potentially exposed individuals within 24 h of the accident that helped stratify the order in which samples were analyzed by DCA (Rothkamm *et al.,* unpublished).

Within our current study we observed a 3.4-fold discrepancy in the performance of the γ-H2AX assay. This may well have been caused by the limited experience of two of the contributing laboratories in using the assay for biodosimetry, although these associations appeared insignificant. While it could be argued that, a certain level of expertise and experience is needed to run the γ-H2AX assay consistently and to achieve maximum possible performance inherent to this assay it was encouraging to see that all participants obtained good correlations between foci scores and actual dose, albeit with a systematic shift between calibration and blind samples in some cases. The power of this current study is limited by the number of measurements and interpretations and therefore should be taken cautiously. Furthermore, we intentionally restricted our measurements to blood samples taken from one individual only, in order to focus on methodological variance and exclude interindividual variance. For the same reasons we also changed only the total dose and did not simulate partial or protracted radiation exposures.

Future efforts for fully establishing the γ-H2AX assay as a rapid, yet robust, triage tool will focus on improving the sample processing in terms of throughput and consistency. Exciting technical developments have recently been reported in this regard (*18*). Further work is also needed to fully characterize the γ-H2AX response after exposure to different radiation types such as γ rays and neutrons which may be more relevant for large scale dirty bomb emergencies (*19–21*).

ACKNOWLEDGMENTS

We are very grateful for the extremely efficient and thoughtful technical and organizational work performed by Cornelia Grothe and Sven Senf (radiation exposure, shipment) and Julia Hartmann (venipuncture). This work was supported by the German Ministry of Defense. Parts of this study were funded by the NIHR Centre for Research in Health Protection. The funding agencies had no role in study design, data collection and

analysis, decision to publish, or preparation of the manuscript. The views expressed in this publication are those of the authors and not necessarily those of the funding bodies.

Received: October 24, 2012; accepted: May 3, 2013; published online: July 24, 2013

REFERENCES

1. International Atomic Energy Agency. Cytogenetic dosimetry: applications in radiation emergencies. A manual. Vienna: International Atomic Energy Agency; 2011.
2. Maznyk NA, Wilkins RC, Carr Z, Lloyd DC. The capacity, capabilities and needs of the WHO Biodosenet member laboratories. Radiat Prot Dosimetry 2012; 151:611–20.
3. Rogakou EP, Pilch DR, Orr AH, Ivanova VS, Bonner WM. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. J Biol Chem 1998; 273:5858–68.
4. Rothkamm K, Horn S. gamma-H2AX as protein biomarker for radiation exposure. Ann Ist Super Sanita 2009; 45:265–71.
5. Rothkamm K, Löbrich M. Evidence for a lack of DNA double-strand break repair in human cells exposed to very low x-ray doses. Proc Natl Acad Sci USA 2003; 100:5057–62.
6. Rothkamm K, Balroop S, Shekhdar J, Fernie P, Goh V. Leukocyte DNA damage after multi-detector row CT: a quantitative biomarker of low-level radiation exposure. Radiology 2007; 242:244–51.
7. Roch-Lefevre S, Mandina T, Voisin P, Gaetan G, Mesa JE et al. Quantification of gamma-H2AX foci in human lymphocytes: a method for biological dosimetry after ionizing radiation exposure. Radiat Res 2010; 174:185–94.
8. Beels L, Werbrouck J, Thierens H. Dose response and repair kinetics of gamma-H2AX foci induced by in vitro irradiation of whole blood and T-lymphocytes with X- and gamma-radiation. Int J Radiat Biol 2010; 86:760–68.
9. Qvarnström OF, Simonsson M, Johansson KA, Nyman J, Turesson I. DNA double strand break quantification in skin biopsies. Radiother Oncol 2004; 72:311–7.
10. Chua M, Somaiah N, Bourne S, Daley F, A'Hern R, Nuta O, et al. Inter-individual and inter-cell type variation in residual DNA damage after in vivo irradiation of human skin. Radiother Oncol 2011; 99:225–30.
11. Rothkamm K, Crosbie JC, Daley F, Bourne S, Barber PR, Vojnovic B, et al. In situ biological dose mapping estimates the radiation burden delivered to 'spared' tissue between synchrotron X-ray microbeam radiotherapy tracks. PLoS One 2012; 7:e29853.
12. Golfier S, Jost G, Pietsch H, Lengsfeld P, Eckardt-Schupp F, et al. Dicentric chromosomes and gamma-H2AX foci formation in lymphocytes of human blood samples exposed to a CT scanner: a direct comparison of dose response relationships. Radiat Prot Dosimetry 2009; 134:55–61.
13. Horn S, Rothkamm K. Candidate protein biomarkers as rapid indicators of radiation exposure. Radiat Meas 2011; 46:903–6.
14. Horn S, Barnard S, Rothkamm K. Gamma-H2AX-based dose estimation for whole and partial body radiation exposure. PLoS One 2011; 6:e25113.
15. Redon CE, Nakamura AJ, Gouliaeva K, Rahman A, Blakely WF, et al. The use of gamma-H2AX as a biodosimeter for total-body radiation exposure in non-human primates. PLoS One 2010; 5:e15544.
16. Sak A, Grehl S, Erichsen P, Engelhard M, Grannass A, et al. gamma-H2AX foci formation in peripheral blood lymphocytes of tumor patients after local radiotherapy to different sites of the body: dependence on the dose-distribution, irradiated site and time from start of treatment. Int J Radiat Biol 2007; 83:639–52.
17. Redon CE, Nakamura AJ, Gouliaeva K, Rahman A, Blakely WF, Bonner WM. Q[gamma]-H2AX, an analysis method for partial-body radiation exposure using [gamma]-H2AX in non-human primate lymphocytes. Radiation Measurements 2011; 46:877–81.
18. Turner HC, Brenner DJ, Chen Y, Bertucci A, Zhang J, Wang H, et al. Adapting the γ-H2AX assay for automated processing in human lymphocytes. 1. Technological aspects. Radiat Res 2011; 175:282–90.
19. Andrievski A and Wilkins RC. The response of gamma-H2AX in human lymphocytes and lymphocytes subsets measured in whole blood cultures. Int J Radiat Biol 2009; 85:369–76.
20. Bourton EC, Plowman PN, Smith D, Arlett CF, Parris CN. Prolonged expression of the γ-H2AX DNA repair biomarker correlates with excess acute and chronic toxicity from radiotherapy treatment. Int J Cancer 2011; 129:2928–34.
21. Chua MLK, Somaiah N, A'hern R, Davies S, Gothard L, Yarnold J, et al.. Residual DNA and chromosomal damage in ex vivo irradiated blood lymphocytes correlated with late normal tissue response to breast radiotherapy. Radiother Oncol 2011; 99:362–6.