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RENEB Inter-Laboratory Comparison 2021: The Gamma-H2AX Foci Assay

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The Running the European Network of biological and retrospective dosimetry (RENEB) network of laboratories has a range of biological and physical dosimetry assays that can be deployed in the event of a radiation incident to provide exposure assessment. To maintain operational capability and provide training, RENEB runs regular inter-laboratory comparison (ILC) exercises. The RENEB ILC2021 was carried out with all the biological and physical dosimetry assays employed in the network. The focus of this paper is to evaluate the results from 6 laboratories that took part using the gamma-H2AX radiation-induced foci assay. For two laboratories this was their first RENEB ILC. Blood samples were homogenously exposed to 240 kVp X rays (1 Gy/min) to provide calibration data, (0–4 Gy), and a few weeks later three blind coded test samples, (0, 1.2 and 3.5 Gy) were prepared. All samples were allowed a 2 h repair time at 37°C before being transported, on ice packs, to the participating laboratories. On arrival, the samples were processed, scored either manually or automatically for gamma-H2AX foci and dose estimates for the 3 blind coded samples sent to the organizing laboratory. The temperature of samples during transit and the time taken to report the dose estimates were recorded. Subsequent examination of the data from each laboratory used the doses estimates to assign triage categories to the samples. After receipt of the samples, the quickest report of dose estimates was 4.6 h. Analysis of variance revealed that the laboratory carrying out the assay had a significant effect on the foci yield ($P < 0.001$) for the calibration data, but not on the dose estimates of the blind coded samples ($P = 0.101$). All laboratories correctly identified the unirradiated and irradiated samples, although the dose estimates for the latter tended to under-estimate the dose. Two participants seriously under-estimated the dose for the highly exposed sample, which resulted in the sample being placed in the lowest triage category not the highest. However, this under-estimation resulted from the samples not remaining cold during shipment, due to a delay in transit and was not related to the experience of the participating laboratory. Overall, the RENEB network laboratories have demonstrated it is possible to quickly identify a recent whole-body acute exposure using the gamma-H2AX assay within the conditions of the ILC. In addition, an ILC provides a useful training and harmonization exercise for laboratories.

INTRODUCTION

After a large-scale radiological event there will be a need to quickly determine individual dose estimates or provide dose categorization to support clinical decision making (*1*). This can be achieved by using high throughput automated systems, (*2, 3*), or adaptation of protocols (*4–6*), or laboratories combining their efforts within a network (*7–9*). Standardization and validation of exposure biomarkers/physical dosimetry within a network are essential to ensure that dose assessment from different laboratories is consistent and all networks carry out regular inter-comparisons to evaluate their performance (e.g., *10–16*).

Within Europe, the Running the European Network of biological and retrospective dosimetry network (RENEB; https://www.reneb.net/), was fully established as a legal entity in 2016. The RENEB network of laboratories has expertise in dicentric (*17*), micronucleus (*18*), FISH-translocation (*19*), premature chromosome condensation (*20*), gamma-H2AX foci (*21*) and gene expression (*22*) biological dosimetry assays, as well as electron paramagnetic resonance and optically stimulated luminescence physical dosimetry (*23*). Regular inter-comparisons for each assay have been held by the RENEB network for

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harmonization, training and to maintain the networks readiness to respond to emergency response situations (*14, 16*). The 2021 RENEB inter-laboratory comparison (ILC) was performed with all the biological and physical dosimetry assays employed in the network (*24*) and this paper reports the results of the gamma-H2AX assay.

The gamma-H2AX foci assay is a sensitive measure of radiation-induced DNA double-strand breaks in human lymphocytes (*25*) and is widely used to detect radiation exposure in patients after diagnostic and therapeutic medical procedures (*26–30*). Providing blood samples can be obtained within a few hours or days of exposure (*31*) and are kept cold during transport to the laboratory, to slow DNA repair (*32*), the assay can be useful for triage biological dosimetry in an accident scenario (*33*). In Europe, the development and validation of the gamma-H2AX assay for biological dosimetry was made in the multi-disciplinary biodosimetric tools to manage high scale radiological casualties (MULTIBIODOSE; http://cordis.europa.eu/project/id/241536) and the Realizing the European Network of Biodosimetry (REBEB; http://cordis.europa.eu/project/id/295513) projects (*21, 32, 34, 35*).

The aim of the RENEB 2021 ILC was to simulate in real time, as far as possible, a realistic emergency scenario using exposures that corresponded to clinically relevant groups i.e., unexposed, lower, and highly exposed individuals (*24*). The performance of the gamma-H2AX assay was assessed in terms of response time, dose estimates and identification of triage categories. The gamma-H2AX data could then be incorporated into the assessment of all the assays used in the ILC. In addition, the ILC provided training for laboratories new to using gamma-H2AX for biological dosimetry.

METHODS

Blood Sampling, Irradiation and Shipment

Blood sampling with ethical approval and informed consent, irradiation and shipment was carried out at the Bundeswehr Institute of Radiobiology (BIR) and is described in this issue (*24*). In brief, blood was diluted 1:1 with RPMI medium (Gibco-BRL, Karlsruhe, Germany), irradiated at room temperature with 240 kVp X rays at 1.0 Gy/min, incubated at 37°C for 2 h to simulate in vivo repair, cooled to 4°C, then aliquoted into Falcon® tubes (6 ml per dose point). To ensure all laboratories had a dose effect curve that met the exercise conditions, i.e., a 240 kVp X-ray exposure followed by a 2 h repair time and cold shipment, calibration samples were sent 7 weeks prior to the delivery of the blind coded samples. All samples were shipped on frozen cold packs to the 6 participating laboratories. Included in the package were a dosimeter and a temperature logger and these were returned to BIR for analysis. The doses used for the calibration samples were 0, 0.25, 0.5, 1, 2, 3 and 4 Gy. Blind coded inter-comparison samples no.1, no.2 and no.3 were exposed to 0, 1.2 and 3.5 Gy, respectively.

Gamma-H2AX Immunofluorescence Staining and Microscope Analysis

On receipt, each laboratory processed the samples following their own protocol. There is no standard protocol for the gamma-H2AX assay used in biodosimetry as reagents are purchased from different suppliers and laboratories must modify the protocols supplied by companies to obtain good results (*36*). Within RENEB a common protocol based on the methods described elsewhere (*32*), have been used as a basis to optimize the technique in each laboratory using the following steps:

The diluted blood was layered onto histopaque-1077 (e.g., Merck Life Sciences UK Limited, Dorset, UK) to isolate the lymphocytes and the resulting cell suspensions were spotted onto adherent microscope slides. Cells were then fixed in formaldehyde (e.g. Polysciences Incorporated, Warrington, PA), extracted and permeabilized with triton X (e.g. Merck Life Sciences UK, Dorset, UK), blocked in bovine serum albumin (e.g. Fisher Scientific UK Limited, Loughborough, UK) and immunostained using an anti-gamma-H2AX antibody (e.g., mouse monoclonal to gamma-H2AX, Abcam, Cambridge, UK) and a fluorophore-conjugated secondary antibody (e.g. AlexaFluor 488 goat anti-mouse, Fisher Scientific UK Limited, Loughborough, UK). Table 1 shows the differences / similarities in the reagents used by the laboratories.

Foci were scored manually in a total of 50 to 100 cells per blind coded sample and at least 100 cells for each of the calibration samples. However, one laboratory scored automatically using Metacyte software (Metasystems, Altlussheim, Germany) with at least 200 and 500 cells being scored for the blind coded and calibration samples, respectively. The participants reported foci numbers and dose estimates in a standardized scoring sheet, which was returned to the coordinating laboratory at BIR, together with an indication of the priority assigned to scoring the blind coded samples e.g., high (scored immediately) or low priority (scored when other work permitted). In addition, the participants also recorded a qualitative assessment of the temperature of the samples on arrival e.g., cold/not cold and provided details of laboratory reagents in the scoring sheet. All dose estimates were returned before the exercise was closed, six weeks from the dispatch of the blind coded samples.

Data Analysis

The software package Dose Estimate_v5.1 (*37*) was used to fit the calibration data using iteratively reweighted least squares, according to standard practice (*35*), although one laboratory (lab 2) used R program (www.r-project.org). Poisson statistics, which are assumed to dominate the random error (*35, 38*), were used to calculate standard errors. The participants used their own calibration curves to convert the foci counts from the blind coded samples into whole-body dose estimates.

Minitab® 18 was used to carry out general linear model analysis of variance (GLM ANOVA) and post hoc testing (Tukey's pairwise comparisons) for the calibration samples foci yields tested against dose and laboratory. The dose estimates were tested against sample, laboratory and transport temperature.

RESULTS

Participants, Sample Transport and Time to Provide Dose Estimates

The laboratory number used in this paper corresponds to those allocated to the 46 institutions that took part in the entire RENEB 2021 ILC (*24*). In total, 6 laboratories took part in the gamma-H2AX inter-comparison exercise. Four laboratories were experienced in using the gamma-H2AX assay for biodosimetry and had taken part in previous RENEB ILCs. One laboratory was relatively new to using the assay for biodosimetry and the one had not used the assay to produce dose estimates for some years. Three laboratories made the ILC a priority and reported dose

TABLE 1
Laboratory Reagents and Antibodies Used in Key Processing Steps of the Gamma-H2AX Assay by the Participants

Lab	Fixation at room temperature		Permeabilization at room temperature		Antibody supplier	Antibody incubation times	Incubation temperature
2	2% formaldehyde in PBS	5 min	0.15% Triton X in PBS	3 × 5 min	Cell signaling	Primary - 2 h; Secondary - 45 min	Room temperature
5	2% formaldehyde in PBS	5 min	0.25% Triton X in PBS	5 min	Primary - AbCam; Secondary - Fisher Scientific	Primary - 45 min; Secondary - 30 min	Room temperature
6	1% formaldehyde in PBS	5 min	0.5% Triton X in PBS	20 min	Primary - Sigma-Aldrich; Secondary - Dianova	Primary - 90 min; Secondary - 60 min	37°C
15	2% formaldehyde in PBS	5 min	0.25% Triton X + 0.1% glycin in PBS	5 min	Primary - Biolegend; Secondary - Sony Biotechnology Inc.	Primary - 45 min; Secondary - 30 min	Room temperature
17	2% formaldehyde in PBS	5 min	0.25% Triton -X in PBS	5 min	Primary - Upstate; Secondary- Invitrogen	Primary - 45 min; Secondary - 30 min	Room temperature
18	2% formaldehyde in PBS	5 min	0.25% Triton X in PBS	5 min	Primary - Millipore; Secondary - Invitrogen	Primary - 50 min; Secondary -30 min	Room temperature

estimates in 4.6–7.3 h and the reporting time for the other laboratories ranged from 193 to 723 h, as shown in Table 2.

Four laboratories received samples via a courier and two were in close enough proximity to the lead institution where blood sampling and irradiation occurred that staff were able to collect the samples in person, with transport times of 30 mins or less. Transport times using a courier ranged from 20.7 to 44.7 h for the blind coded samples. All participants received the calibration samples within 24 h except one laboratory (lab 18) where the package was delayed for two days. Maximum temperatures during transport recorded by the thermo-logger included in the shipment ranged from 9 to 12°C for calibration (excluding lab 18) and 14 to 23°C for blind coded samples. Physical dosimeters in each package showed no evidence of irradiation during transport.

Calibration and Dose Estimates

On receipt of all the gamma-H2AX results from the reference laboratory, the assay lead performed a quality check on all the calibration and blind coded sample data prior to analysis. Where necessary (labs 6 and 15) the dose-response curves, and hence the dose estimates were re-evaluated and are different from those reported to the reference laboratory.

The yield of foci in the calibration samples increased with dose and each laboratory used their own approach and choice of software to produce their calibration curve. Unfortunately, laboratory 18 was unable to produce a calibration curve as the samples were delayed in transit for several days and were unusable. Instead, the laboratory had to use a ^{137}Cs gamma-ray curve prepared during a previous RENEB exercise (*35*). Table 2 shows the calibration coefficients obtained by each laboratory and the software employed, that were subsequently used to convert foci counts to dose estimates.

GLM ANOVA carried out on the X-ray calibration data (excluding lab 18) revealed that both dose and laboratory had a significant effect on the foci yield ($P < 0.001$). Post hoc testing established that the foci yield for the 3 and 4 Gy dose points were not significant ($P > 0.999$) and the scoring of laboratory 17 was significantly different from all the other participants ($P \leq 0.001$). However, the foci yields obtained by laboratory 6 was not significantly different from 2 and 5 ($P = 0.842$ and 0.797, respectively); while laboratory 15 was not significantly different from 5 ($P = 0.149$).

Prior to reporting any dose estimates two laboratories (2 and 15) ranked the samples based on average foci per cell into lowest, medium, and highest radiation exposure. Even without a calibration curve the laboratories were able to distinguish low to high exposures and this initial assessment was reported 20% (high priority) to 60% (low priority) quicker than the laboratories triage dose estimates. The dose estimates for the three blind coded samples from each laboratory are shown in Table 3. GLM ANOVA analysis showed the dose estimates were significantly different for sample ($P = 0.002$), but not for laboratory ($P = 0.101$). Temperature during transit was shown to have borderline significance on the dose estimates ($P = 0.049$). The participants correctly identified sample no. 1 as not irradiated. All laboratories except one produced dose estimates lower than the true dose for samples no. 2 and no. 3. The laboratories dose estimates were compared to the error accepted for triage dosimetry (*4, 39*) of ±0.5 Gy or ±1.0 Gy for reference doses <2.5 and >3 Gy, respectively. Dose estimates were also assigned to the three MULTIBIODOSE triage categories of low (<1 Gy), medium (1–2 Gy) and high (>2 Gy) exposure, which do not consider the confidence interval on the dose estimates (*1*). Table 3 shows the number of dose estimates with 95% confidence intervals that do not include the true dose, the number of dose estimates outside of ±0.5 or ±1.0 Gy (depending on the reference dose) and the number assigned to the wrong triage category.

TABLE 2
Temperature of the Blind Coded Samples Reported by Each Laboratory, Transit Time, Report Time for Dose Estimates and Scoring Method

Lab	Temp reported by lab	Transit time (h)	Report time (h) and priority	Scoring method	Sample no.1 Foci per cell ± SE	Sample no.2 Foci per cell ± SE	Sample no.3 Foci per cell ± SE	Calibration curve	Software
2	cold	0.3	7.3 (H)	M	0.00 ± 0.00	7.72 ± 0.21	13.04 ± 0.26	$Y = 15.29(\pm0.88) \cdot \exp(-2.52(\pm0.25) \cdot 0.28(\pm0.05))D$	R script
5	cool	43.8	4.6 (H)	M	0.08 ± 0.06	2.82 ± 0.43	1.02 ± 0.29	$Y = 0.3614(\pm0.1700) + 2.7710(\pm0.2386)D$	DE
6	cold	0.2	7.3 (H)	M	0.23 ± 0.30	6.57 ± 0.30	12.26 ± 0.30	$Y = 0.1640(\pm0.2313) + 6.5340(\pm0.6032)D$	DE
15	cold	20.7	216 (L)	M	0.64 ± 0.11	4.96 ± 0.32	7.04 ± 0.38	$Y = 0.1630(\pm0.1947) + 1.7040(\pm0.2118)D$	DE
17	cold	20.7	723 (L)	A	4.44 ± 0.07	9.66 ± 0.07	14.58 ± 0.07	$Y = 7.0980(\pm0.3332) + 3.6920(\pm0.4084)D$	DE
18*	not cold	44.7	193 (L)	M	0.28 ± 0.07	0.94 ± 0.14	0.98 ± 0.14	$Y = 0.4191(\pm0.1156) + 1.3320(\pm0.1169)D$	DE

Notes. In addition, calibration curves and associated standard errors produced by the laboratories and used to convert foci counts to dose estimates, together with the software used and scoring method. H = high priority; L = low priority; M = manual scoring; A = automated scoring. Report time = time from sample receipt to receiving dose estimates. DE = DoseEstimatev5.1 software.

* ^{137}Cs gamma-ray curve, 24 h postirradiation incubation.

DISCUSSION

As defined in the RENEB QA/QM manual, in a real radiological incident or accident and on activation of the RENEB network one institution would be appointed as the ''reference laboratory'', with responsibility for administration and deciding the appropriate assays to be used. The reference lab would be responsible for organizing sample collection, sending blind coded samples to the ''service laboratories'' within the network and collating all the triage dose estimates (https://cordis.europa.eu/docs/results/295/295513/final1-reneb-qa-and-qm-manual.pdf). In this ILC, BIR acted as the ''reference laboratory'' by organizing the exercise, irradiating/sending samples and collating the dose estimates from all the participants. On closure of the ILC, the results from individual assays were sent to the lead laboratory for that assay for further analysis. The RENEB ILC 2021 has tested the ability of six laboratories to provide triage dose estimates using the gamma-H2AX assay and the ability to report these findings within the time frame of the inter-comparison.

After an incident involving ionizing radiation a quick, approximate dose estimate for the secondary triage of casualties would be needed to inform medical decision making and reassure unexposed persons (the 'worried well'). All 18 dose estimates were reported on time to the institute organizing the ILC. Gamma-H2AX dose estimate reporting times for this ILC were 4.6 to 7.3 h for laboratories making analysis a priority, which is similar to previous timed inter-comparisons (*34, 40*) and this demonstrates the assay can provide rapid screening for the detection of exposed individuals. Even without reference to a calibration curve, two laboratories ranked the samples in order of low to highly exposed reducing the reporting time further e.g., from 7.3 to 6.0 h for high priority analysis.

Large variations in foci yields were seen in the calibration and blind coded samples between the laboratories (see

TABLE 3
Triage averaged Whole-Body Dose Estimates and 95% Confidence Intervals, Rounded to One Decimal Place, Reported by the Laboratories

Laboratory	Sample no. 1 = 0.0 Gy Dose estimate [95% CI] (Gy)	Sample no. 2 = 1.2 Gy Dose estimate [95% CI] (Gy)	Sample no. 3 = 3.5 Gy Dose estimate [95% CI] (Gy)	Number of dose estimates: 95% CI includes true dose	Outside ± 0.5 or ± 1.0 Gy^	Outside triage category
2	0.0 [0.0–0.0]	1.0 [0.9–1.3]	2.2 [1.7–3.0]	2	1	0
5	0.0 [0.0–0.0]	0.9 [0.7–1.1]	0.2 [0.1–0.7]	1	1	2
6	0.0 [0.0–0.0]	1.0 [0.9–1.1]	1.9 [1.7–2.1]	1	1	1
15	0.0 [0.0–0.2]	2.2 [1.6–2.8]	3.5 [2.8–4.1]	2	1	1
17	0.0 [0.0–0.0]	0.7 [0.6–0.8]	2.0 [1.9–2.2]	1	1	1
18	0.0 [0.0–0.0]	0.4 [0.2–0.6]	0.4 [0.2–0.7]	1	2	2
No. of estimates 95% CI includes true dose	6	1	1			
No. of estimates outside ± 0.5 or ± 1.0 Gy^	0	2	5			
No. estimates outside triage category	0	4	3			

Note. The number of dose estimates outside ±0.5 or ±1.0 Gy of the reference dose and the MULTIBIODOSE triage category are also shown.

[a] Dose estimates considered outside the error accepted for triage (4). Samples no.1 and no.2 the accepted error is ±0.5 Gy (reference doses <2.5 Gy). Sample no.3 the accepted error is ± 1.0 Gy (reference dose >3.0 Gy).

[b] MULTIBIODOSE triage categories of low (<1 Gy), medium (1–2 Gy) and high (>2 Gy) exposure (1).

Table 2) and suggests there are substantial differences in foci detection and identification. Differences in the number of observed foci between laboratories have been noted in previous inter-comparisons (*21, 32, 35, 40*). This is most likely due to factors such as foci loss during transport or the day-to-day variability in the staining quality and the scoring method (*36*) or the criteria used by the individual performing manual enumeration, although the experienced RENEB partners have undergone training in foci scoring (*35*). In addition, as show in Table 1, the gamma-H2AX immunofluorescent staining process varies between laboratories as antibodies and reagents are obtained from different suppliers depending on availability, resulting in modifications to the standard protocol to suit the specific manufacturers requirements. This variability in experimental factors supports the requirement for the assay to be regularly re-calibrated (*40*) and for laboratories not to use a common calibration curve (*21, 35*). Furthermore, gamma-H2AX dose estimates currently cannot be considered as reliable as the ''gold standard'' dicentric assay, but can provide a means of fast screening for radiation emergency response to identify exposed casualties from the ''worried well'' and aid the prioritization of cytogenetic biodosimetry.

The results of the gamma-H2AX assay presented here (Table 3) show that every participant correctly distinguished the unirradiated sample from the two irradiated samples. However, the dose estimations for samples no. 2 and no. 3 were noticeably varied, with a tendency for lower dose estimates, especially for the highest dose with all but one laboratory underestimating the true dose. In relation to the triage categorization of dose estimates considered here, the results demonstrate that all the participants successfully placed sample no. 1 into the low exposure group. Not surprisingly, 95% confidence limits performed poorly when compared to the broader measures used to categorize dose, with only one dose estimate for both sample no.2 and no.3 correctly within the interval. The number of dose estimates per sample was small (6 per category), however, for sample no.3 the number outside the MULTIBIODOSE triage categorization was fewer compared to the accepted triage measure (3 vs.5), but the reverse was true for sample no.2 (4 vs. 2).

Underestimation of the dose and hence triage categorization was probably caused by several factors. During the transport of samples for gamma-H2AX analysis it is critical to keep the temperature below ambient by sending samples with ice packs, to prevent or slow down DNA repair. Circumstances beyond the control of the organizing laboratory and the participants resulted in some blind coded samples being delayed in transit by about 24 h to laboratories 5 and 18, which also may occur during a real-life incident. The temperature during transit for laboratories 5 and 18 peaked at 17 and 23°C, respectively, for several hours. In addition, the temperature during transport to the other laboratories was on average several degrees higher for the blind coded samples compared to those for calibration and ANOVA analysis indicated that temperature during transit had a significant effect on the dose estimate ($P = 0.049$). The rate at which radiation induced foci are lost due to DNA repair follows a bi-exponential decay that has both a fast and a slow element (*31*). The 2 h postirradiation time point used in this ILC lies within the fast component of the decay curve, which has a half time of ~1.6 h (*31*), so small changes in post-exposure incubation or holding temperature can have a relatively large effect on foci numbers. The dose estimates from laboratories 5 and 18, using the delayed samples, miscategorized the highest exposed sample into the lowest triage category, which could have unintended consequences on the clinical management of an exposed casualty. In a real-life event, precise temperature measurements may not be available, and a qualitative assessment of the sample temperature can provide information quickly. In the ILC the reported condition of the samples on arrival (e.g., cold/not cold) matched the data from the temperature loggers. If gamma-H2AX samples are delayed in transit and they do not arrive cold any dose estimate must be viewed with caution and will probably be an underestimate of the true dose; although it should be noted that the laboratories were able to identify that an exposure had occurred. Further investigation of the results from laboratory 5 also revealed that for sample no.3 the recovery of lymphocytes and subsequent staining was poor, which should also be reported as a reason to suspect underestimation of the dose. Another factor is the saturation seen in foci numbers at higher doses (e.g., 3 and 4 Gy), especially at short post exposure incubation times (*40*). At these doses and a short time after exposure when yields are high, discrimination between foci in different focal planes becomes more difficult (*41*). When such high numbers of foci are seen a precise estimation of dose cannot be made and it would be advisable, when reporting a dose estimate to the reference laboratory, to highlight this as a possible indication of a higher dose and the patient may need further clinical evaluation.

The aim of this ILC was not only to test the RENEB networks ability to respond and produce triage dose estimates to simulated over exposures, but to provide a training opportunity for participants; especially laboratories 6 and 15 taking part in a RENEB gamma-H2AX exercise for the first time. Reassuringly, the ANOVA analysis of the gamma-H2AX assay blind coded samples results showed no significant effect of laboratory on the dose estimate ($P = 0.101$). However, the quality check of the calibration data revealed that the dose response curves from laboratories 6 and 15 needed to be recalculated and hence the dose estimates. The recalculated dose estimates for laboratory 6 only made a marginal different to that reported for sample no.3 (1.9 vs. 2.0 Gy). Larger differences in the recalculated and reported dose estimates from laboratory 15 were seen for samples no.2 (2.2 vs. 1.7 Gy) and no.3 (3.5 vs. 3.7 Gy).

To determine calibration curve coefficients for biodosimetry it is recommended that the maximum likelihood iteratively reweighted least squares method is used (*38*), whereas laboratory 6 had originally used a linear regression model in Excel® to calculate their dose response curve. The data from laboratory 15 was calculated using the maximum likelihood method but had been fitted to a linear-quadratic curve with a negative beta coefficient. A linear quadratic with negative quadratic term will never be appropriate for biodosimetry purposes, because after the point at which the curve starts to turn over, there will be two solutions to the quadratic equation for dose, i.e., two doses estimated. The drop or levelling off in foci yield, which is seen at doses around 3 to 4 Gy, is most likely due to saturation, as discussed above, which presents difficulties on how best to approach curve fitting. In such a case as this, there are three possible approaches:

1. Fit a linear curve. This is only appropriate, however, if the linear term is statistically significant.
2. Remove the highest dose point and refit the curve to a linear, repeating this until the linear coefficient (or indeed a linear quadratic fit with positive quadratic term) becomes significant.
3. Do more scoring/add more dose points in the hope that a linear curve can be reached.

The recalculated linear curve using the data from laboratory 15 provided a satisfactory fit with the P value for the F-test on the linear term of <0.05. This ILC provided a useful learning experience for laboratory 15, where the gamma-H2AX assay has only been recently introduced. This demonstrates the importance of ensuring any laboratory within a network can carry out the laboratory ''wet work'' and scoring proficiently, but also understands the statistical requirements of producing calibration curves and associated dose estimates.

CONCLUSION

The RENEB network of laboratories employing the gamma-H2AX assay has successfully distinguished between irradiated and unirradiated samples in this intercomparison. The laboratories can quickly give a triage dose categorization for a recent acute whole-body exposure, although the dose estimates themselves may not be as accurate as conventional biodosimetry assays. It is important for laboratories to provide dose estimates to the reference laboratory with caveats regarding potential factors that may have resulted in an under estimation i.e., sample temperature during transit or high numbers of coalescing foci. It is evident that the gamma-H2AX assay can be used by the RENEB laboratories to prioritize patients with high foci counts for further clinical and/or cytogenetic dosimetry and that inter-comparisons provide a useful training tool.

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