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RENEB Inter-Laboratory Comparison 2021: The Dicentric Chromosome Assay

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After large-scale radiation accidents where many individuals are suspected to be exposed to ionizing radiation, biological and physical retrospective dosimetry assays are important tools to aid clinical decision making by categorizing individuals into unexposed/minimally, moderately or highly exposed groups. Quality-controlled inter-laboratory comparisons of simulated accident scenarios are regularly performed in the frame of the European legal association RENEB (Running the European Network of Biological and Physical retrospective Dosimetry) to optimize international networking and emergency readiness in case of large-scale radiation events. In total 33 laboratories from 22 countries around the world participated in the current RENEB inter-laboratory comparison 2021 for the dicentric chromosome assay. Blood was irradiated in vitro with X rays (240 kVp, 13

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mA, ~75 keV, 1 Gy/min) to simulate an acute, homogeneous whole-body exposure. Three blood samples (no. 1: 0 Gy, no. 2: 1.2 Gy, no. 3: 3.5 Gy) were sent to each participant and the task was to culture samples, to prepare slides and to assess radiation doses based on the observed dicentric yields from 50 manually or 150 semi-automatically scored metaphases (triage mode scoring). Approximately two-thirds of the participants applied calibration curves from irradiations with γ rays and about 1/3 from irradiations with X rays with varying energies. The categorization of the samples in clinically relevant groups corresponding to individuals that were unexposed/minimally (0–1 Gy), moderately (1–2 Gy) or highly exposed (>2 Gy) was successfully performed by all participants for sample no. 1 and no. 3 and by ≥74% for sample no. 2. However, while most participants estimated a dose of exactly 0 Gy for the sham-irradiated sample, the precise dose estimates of the samples irradiated with doses >0 Gy were systematically higher than the corresponding reference doses and showed a median deviation of 0.5 Gy (sample no. 2) and 0.95 Gy (sample no. 3) for manual scoring. By converting doses estimated based on γ-ray calibration curves to X-ray doses of a comparable mean photon energy as used in this exercise, the median deviation decreased to 0.27 Gy (sample no. 2) and 0.6 Gy (sample no. 3). The main aim of biological dosimetry in the case of a large-scale event is the categorization of individuals into clinically relevant groups, to aid clinical decision making. This task was successfully performed by all participants for the 0 Gy and 3.5 Gy samples and by 74% (manual scoring) and 80% (semi-automatic scoring) for the 1.2 Gy sample. Due to the accuracy of the dicentric chromosome assay and the high number of participating laboratories, a systematic shift of the dose estimates could be revealed. Differences in radiation quality (X ray vs. γ ray) between the test samples and the applied dose effect curves can partly explain the systematic shift. There might be several additional reasons for the observed bias (e.g., donor effects, transport, experimental conditions or the irradiation setup) and the analysis of these reasons provides great opportunities for future research. The participation of laboratories from countries around the world gave the opportunity to compare the results on an international level.

INTRODUCTION

In the case of a large-scale radiological or nuclear (RN) incident it will be crucial to sort people according to their need for care. In the frame of radiological triage, early medical treatment decisions will be based on unspecific clinical and hematological parameters, e.g., vomiting and changes in blood cell count (*1*). Biological dosimetry can contribute significantly to the categorization of individuals into clinically relevant exposure groups based on estimates of the dose (*2*) and can provide evidence for or against an assumed exposure to ionizing radiation. The first group is comprised of the so-called ''worried-well'' persons (*3*), who believe that they were exposed, or even show early symptoms (e.g., vomiting or diarrhea) similar to that of a radiation exposure, but were actually not exposed or only exposed to low doses. Identifying those individuals will help to reduce the pressure on the healthcare system, to appropriately direct limited healthcare resources, and to alleviate fears in the population. The second group includes persons that were exposed to moderate doses and will not need immediate clinical care, but might have an increased long-term cancer risk and, therefore, require regular surveillance. For the third group of people, exposed to high doses, immediate medical care improves the prognosis and increases the survival probability. However, the diagnosis based on unspecific clinical symptoms alone will often not be sufficient for a successful categorization of individuals and requires support using more specific markers for an exposure to ionizing radiation. In most real-life radiation accidents, little will be known about the doses received by the individuals and retrospective dose assessment can be one factor to aid clinical decision making. Biological dosimetry provides a large toolbox of methods to assess whole- or even partial-body doses in the time frame of hours up to years after a potential exposure to ionizing radiation and has proven its usefulness in many cases of accidental exposures to radiation (*4–12*). The dicentric chromosome assay (DCA) is still considered as the ''gold standard'' in biological dosimetry and has been continuously improved and validated for several years (*13, 14*). However, for a large-scale radiation accident, the huge number of samples will quickly bring each single laboratory to its capacity limit. International networking among laboratories provides great opportunities to share the workload and to increase the number of samples that can be processed in a given time (*15*). In Europe, the legal association of the RENEB (Running the European Network of Biological and Physical retrospective Dosimetry) network has been established to ensure availability, quality, and efficiency of biological and retrospective physical dosimetry and to identify needs for training and harmonization for the member organizations (*16*). Regular quality-controlled inter-laboratory comparisons (ILCs) are important to allow the comparison of laboratories performance and to identify needs to optimize international networking and the workflows of the participants for preparedness to future RN events. ILCs simulating various real-life exposure situations are regularly performed in the frame of RENEB to validate and improve the procedures of the participants for various assays (*17–28*).

The current exercise was designed to simulate acute, homogeneous whole-body exposures with an X-ray source (240 kVp, 13 mA, ~75 keV, 1 Gy/min) and 33 RENEB member and non-member institutions from 22 countries from Europe, Asia and North America participated in the exercise for the DCA. The study design included the irradiation of three blood samples (0 Gy, 1.2 Gy and 3.5 Gy), blood shipment, sample processing, analysis of chromosome aberrations and dose assessment in triage scoring mode (50 cells for manual scoring and 150 cells for

semi-automatic scoring). The main aims included the categorization of samples into clinically relevant groups, to determine whether the estimated radiation doses of the participating laboratories were in good agreement with the reference doses, and to identify potential needs for further training and harmonization. Furthermore, as various assays for biological and retrospective physical dosimetry were included and performed in parallel, this exercise provided the opportunity to compare the performance of different assays in terms of response time and the accuracy of the provided dose estimates. The results from the DCA are shown in this paper and suggest that the categorization into clinically relevant groups was successfully performed by most participants. Interestingly, the results from the DCA unexpectedly revealed systematically higher dose estimates compared to the reference doses for test samples irradiated with 1.2 Gy and 3.5 Gy. The exact sources for the observed bias remain unknown, but serve as a very valuable basis for discussions on improving the design of future ILCs and for further research in the field of biological dosimetry.

This manuscript comprises DCA results only, generated in the context of the RENEB ILC 2021 exercise and all results from other assays used during the RENEB ILC 2021 are presented as a series of manuscripts in this special issue, including an inter-assay comparison article (*29*) where the results are compared between assays and details regarding radiation exposure, shipment and response times are discussed.

MATERIALS AND METHODS

Participating Laboratories, Irradiation and Shipment of Blood Samples

In total 33 laboratories from 22 countries (24 from Europe, 2 from North America, 7 from Asia) participated for the DCA in the frame of this RENEB exercise. Eighteen of the participants were RENEB member organizations and 15 were not members of RENEB. For this manuscript, the participating laboratories were anonymized and named L1-L33. These numbers do not correspond to the numbers from the affiliations of the co-authors. Blood samples from one healthy donor (male, 32 years) were taken in heparinized tubes with informed consent and the approval of a local ethics committee. The blood samples were taken in 2–3 mL vials and irradiated at room temperature at the Bundeswehr Institute of Radiobiology (BIR) in a Maxishot SPE X-ray cabinet (Yxlon, Hamburg, Germany) using 3 mm beryllium and 3 mm aluminum filters, an accelerating potential of 240 kVp and a 13-mA electron beam to simulate an acute, homogeneous whole-body X-ray exposure with a mean photon energy of approximately 75 keV. The kerma in air rate was approximately 1.0 Gy/min. Several blood tubes (8–9) were irradiated in parallel within a radiation field, the homogeneity of which was determined prior to irradiation by using Gafchromic®EBT3 films (Ashland Advanced materials, Bridgewater, NJ). Further details concerning radiation dosimetry and calibration procedure are included in the inter-assay paper of this exercise (*29*). Based on the MULTIBIODOSE project (*30*), in the current ILC, three reference doses were chosen (0, 1.2; and 3.5 Gy) to represent triage categories that enable the classification of individuals into clinically relevant groups: unexposed/minimally (0–1 Gy), moderately (1–2 Gy) and highly exposed individuals (>2 Gy). The reference doses were given as dose in water and transformations from kerma in air were performed as described in (*29*). Deviating from the requirements given by the IAEA (*31*), the 2-h repair time postirradiation was accidentally performed at room temperature and not at 37°C. For the DCA, three blood samples, one vial per dose point, were distributed to 26 teams from Europe (23), Canada (1), South Korea (1) and USA (1) by express service according to standard regulations under UN 3373 Biological Substance Category B (*31, 32*). The doses corresponding to the blood samples were blinded and coded and are referred to as test sample no. 1 (0 Gy), no. 2 (1.2 Gy) and no. 3 (3.5 Gy). The assignment of the blood tubes to the participants was done randomly. Six laboratories from Asia and one from the Ukraine received slides or images (Table 1) of metaphases generated by other laboratories because of shipment problems or logistical reasons. The delivery time of the blood samples to the partner laboratories by courier service and the report time for dose assessments considering the speed of method performance up to the submission of the dose estimate was documented (*29*). All participating laboratories had the possibility to provide information regarding the level of priority for the performance of the analysis.

Cell Culture and Dicentric Chromosome Assay

The laboratories that received blood samples were requested to set up lymphocyte cultures following their own standard protocols, considering the IAEA recommendations (*31*) and ISO standards (*32, 33*). Cell cycle-controlled scoring should be applied according to the standard procedure of each laboratory. Detailed information about culture and scoring variables and on the methods for generating the applied calibration curve were requested in a scoring sheet circulated to all participants in advance. The task of the participants was to prepare slides according to the standard staining method of the laboratory and manually and/or semi-automatically analyze dicentric chromosomes for dose estimation. For both scoring methods, only triage mode scoring was requested, comparable to large-scale emergency situations, where many samples must be analyzed. Therefore, for manual scoring, 50 cells or 30 dicentrics per dose point had to be analyzed by a human scorer. Depending on the quality of the slide and/or the radiation dose, a second (and/or third) slide could be scored. For semi-automated scoring 150 cell images had to be captured per dose point at high-resolution quality. Here, it was also possible to include a second (and/or third) slide if the number of cells was too low. The detection of the dicentric chromosomes was performed on a software-based procedure using the Metafer platform (MetaSystems, Germany). Some labs scored more cells than the requested in the guidelines of this ILC (Table 1). For both scoring modes the techniques applied had to be implemented and validated in the laboratories in advance.

Dose Assessment

For the DCA, no calibration samples were distributed in advance and the participants were asked to use their own calibration curves for dose assessment. All calibration curves were generated by fitting the yield of aberrations to linear-quadratic dose dependencies. Information on the details concerning the dose effect curves (source, radiation quality, dose rate, origin of curve, calibration of the source based on air kerma or dose in water, irradiation temperature, irradiation in water or air, coefficients, number of analyzed cells, number and distribution of dicentrics for the applied doses) was requested from the participants (see Tables 2 and 3 for summary). For dose estimation as well as for the corresponding uncertainties it was recommended to use the Biodose Tools software (*34*). All reported dose estimates were re-calculated to detect possible errors related to the calculation of dose estimates (Table 1). The dose estimates and the corresponding 95% confidence interval (CI) were to be provided in Gy and according to the number of dicentric chromosomes scored. Some participants provided several dose estimates for each test sample, e.g., based on different radiation qualities, scorers or software tools. In such cases only one result per lab was chosen based on the following criteria: 1. results based on X-ray curves were used if results were provided based on X-ray and γ-ray curves; 2. results from several scorers were combined into a single dose estimate based on the sums of

TABLE 1
Reported and Recalculated Dose Estimates with Cell Numbers, Scoring Mode and Material (Blood, Images, Slides) used for Dose Estimation by each Participant

Lab	Material	Scoring	Cell number scored	Reported dose (Gy)	Recalculated dose (Gy)	Comments
L1	blood	auto	132/150/160	0/1.34/3.92	0/1.34/3.92	-
L1	blood	manual	50/52/24	0/1.19/3.5	0/1.19/3.5	-
L2	blood	auto	123/117/124	0/1.21/2.59	0/1.21/2.59	-
L2	blood	manual	50/50/20	0/1.43/4.43	0/1.43/4.43	-
L3	blood	manual	**119/62/55**	0/1.75/4.26	0/1.75/4.26	> 50 cells
L4	blood	manual	50/50/50	0/2.06/4.53	0/2.06/4.53	-
L5	blood	manual	50/50/50	0/1.76/4.52	0/1.76/4.52	-
L6	blood	manual	50/50/18	0/1.47/4.03	0/1.47/4.03	-
L7	blood	manual	50/50/50	0/0.82/4.02	0/0.82/4.02	α and β mixed up X ray and Co-60 results
L8	blood	manual	50/51/45	0/1.51/**4.62**	0/1.51/**4.51**	Dic+r used on dic curve
L9	blood	auto	**2098/730/1676**	0.04/2.23/5.13	0.04/2.23/5.13	No triage scoring
L10	blood	manual	50/50/40	0/1.71/3.84	0/1.71/3.84	-
L11	blood	manual	50/50/17	0/1.11/5.05	0/1.11/5.05	-
L12	blood	manual	50/52/24	0/1.74/4.95	0/1.74/4.95	-
L13	blood	manual	50/50/24	0/1.55/5.03	0/1.55/5.03	-
L14	blood	manual	**150/150/59**	0/2.04/5.04	0/2.04/5.04	> 50 cells
L15	blood	manual	50/50/50	0/1.66/3.35	0/1.66/3.35	Several results submitted
L16	blood	auto	142/139/132	0/1.68/4.73	0/1.68/4.73	α and β mixed up
L16	blood	manual	50/50/55	0/2.1/4.4	0/2.1/4.4	α and β mixed up
L17	blood	manual	50/50/30	0/1.82/3.8	0/1.82/3.8	-
L18	images	manual	50/50/22	0/1.51/4.57	0/1.51/4.57	-
L19	blood	manual	50/50/50	0/1.78/4.34	0/1.78/4.34	-
L20	blood	manual	50/50/20	0/2.04/4.49	0/2.04/4.49	-
L21	blood	manual	**100/100/75**	0/1.59/4.9	0/1.59/4.9	2 scorers/dose
L22	images	manual	50/53/24	0/1.48/3.9	0/1.48/3.9	Several results submitted
L23	blood	manual	50/50/50	0/1.98/3.75	0/1.98/3.75	-
L24	blood	manual	50/50/17	0/2.02/5.47	0/2.02/5.47	-
L25	blood	auto	153/156/120	0/1.28/3.62	0/1.28/3.62	-
L26	images	manual	50/50/50	0/1.65/4.89	0/1.65/4.89	Several results submitted
L27	images	manual	51/50/27	0.72/1.65/4.45	0.72/1.65/4.45	-
L28	slides	manual	50/50/21	0.55/1.56/4.85	0.55/1.56/4.85	-
L29	images	manual	50/50/38	**0.26/2.63/5**	**0.32/1.9/3.46**	Unknown error
L30	blood	manual	50/50/50	0/1.7/3.93	0/1.7/3.93	-
L31	blood	manual	**143/140/121**	0/2.47/5.34	0/2.47/5.34	> 50 cells
L32	images	manual	50/50/23	0/0.95/4.02	0/0.95/4.02	X ray and Co-60 results
L33	blood	manual	**150/150/64**	0/1.73/4.83	0/1.73/4.83	> 50 cells

Notes. Doses were re-estimated based on the calibration curves coefficients provided by the participants. Deviating results between provided and recalculated dose estimates and scored cell numbers that were higher than requested for this ILC are shown in bold text. The numbers separated by ''/'' indicate the results for samples no. 1 (0 Gy), no. 2 (1.2 Gy) and no. 3 (3.5 Gy). The participants were labelled as L1-L33. Only one result was used for participants providing several results per dose point.

the dicentric counts; 3. only Biodose Tools results were used if results were provided based on several different software tools.

Statistical Analysis

In a first step, all provided dose estimates were quality checked by recalculating the dose estimates based on the provided calibration curve coefficients and the dicentric distribution of the test samples and participants were contacted if problems were observed. Next, dose estimates provided by the participants were categorized into clinically relevant groups of 0–1 Gy, 1–2 Gy and >2 Gy. The provided results were further evaluated by checking if the estimated 95% CIs included the reference dose or if dose estimates were within an uncertainty interval of ±0.5 Gy (reference doses ≤2.5 Gy) or ±1 Gy (reference doses >2.5 Gy) as described in the literature (*35*). The homogeneity between the results provided by the participants was assessed by the inter-quartile range (IQR) and by the coefficient of variation (CV). To assess whether the results were more heterogeneous than expected based on a Poisson distribution, dicentric counts were randomly drawn with a mean value corresponding to the dose-effect curves of the participants at a given dose (1.2 Gy and 3.5 Gy) using the number of cells scored by each participant. The median of the CV and IQR across 500 simulation runs was compared to the observed CV and IQR. To assess the effect of radiation type on the dose estimates, all dose estimates based on γ-ray curves were transformed to X-ray doses, using calibration curve coefficients from Schmid et al. (*36*). Due to the observed systematic shift of the provided dose estimates, no Z-scores or related statistics were calculated as usually in such ILCs.

RESULTS

Shipment, Reporting Time

Almost all blood samples for the DCA were delivered within 24 h inside the EU without any difficulties. For the EU member Croatia, the delivery time was 76 h. Two laboratories received the blood samples directly because

TABLE 2
Details about the Irradiation Conditions for the Calibration Curves of each Participant

Code	Own curve	Min (Gy)[a]	Max (Gy)[a]	Number doses[a]	Source	Dosimetry	Irradiated in	Temperature (°C)	Dose rate (Gy/min)
L1	yes	0 (0)	6 (5)	12 (10)	Cs-137	air kerma	?	37	0.49
L2	yes	0 (0)	5 (4)	10 (6)	X ray (240 kV)	air kerma	air	20	1
L3	yes	0 (-)	6 (-)	8 (-)	Cs-137	?	?	20	0.6
L4	yes	0 (-)	4 (-)	10 (-)	Co-60	air kerma	water	?	0.34
L5	yes	0 (-)	6 (-)	8 (-)	Cs-137	?	?	20	0.6
L6	yes	0 (-)	5 (-)	10 (-)	X ray (250 kV)	air kerma	air	20	0.37
L7	yes	0 (-)	4.5 (-)	7 (-)	Orthovoltage	water	water	37	1.27
L8	no	0 (-)	5 (-)	11 (-)	Co-60	?	water(?)	20	0.5
L9	yes	- (0)	- (5)	- (?)	Cs-137	water	water	37	0.5
L10	yes	0 (-)	3 (-)	7 (-)	Co-60	air kerma	air	20	0.180 - 0.126
L11	yes	0 (-)	5 (-)	10 (-)	Co-60	?	water	37	0.5
L12	yes	0 (-)	5 (-)	11 (-)	X ray (6 MV; 15MeV)	water(?)	water	?	?
L13	yes	0 (-)	4 (-)	8 (-)	Co-60	air kerma	air	20	0.745
L14	yes	0 (-)	6 (-)	9 (-)	Co-60	air kerma	air	20	0.3
L15	yes	0 (-)	4 (-)	5 (-)	X ray (200 kV)	?	water	37	0.485-0.99
L16	yes	0 (0)	5 (5)	8 (10)	Co-60	water	air	20	0.638
L17	yes	0.05 (-)	6 (-)	14 (-)	X ray (250 kV)	air kerma(?)	air(?)	37	1
L18	?	0 (-)	5 (-)	11 (-)	Co-60	air kerma	water	20	0.5
L19	no	0 (-)	5 (-)	11 (-)	Co-60	?	?	?	?
L20	yes	0 (-)	4 (-)	9 (-)	Co-60	water	?	20	0.24
L21	yes	0 (-)	4 (-)	9 (-)	Orthovoltage	air kerma	air	20	1
L22	no	0 (-)	5 (-)	11 (-)	?	?	?	?	?
L23	yes	0 (-)	4.9 (-)	10 (-)	Co-60	air kerma	air	37	0.46
L24	yes	0 (-)	5 (-)	11 (-)	Co-60	water	water	37	1.07-1.18
L25	yes	- (0)	- (4.5)	- (11)	Co-60	water	water	37	0.17
L26	yes	0 (-)	2 (-)	7 (-)	Co-60	?	water	37	0.5
L27	no	0 (-)	5 (-)	8 (-)	?	?	?	?	?
L28	no	0 (-)	5 (-)	8 (-)	?	?	?	?	?
L29	no	0 (-)	5 (-)	8 (-)	Co-60	air kerma	air	20	0.6
L30	no	0 (-)	5 (-)	11 (-)	Co-60	?	?	?	?
L31	yes	0 (-)	4 (-)	10 (-)	Co-60	water	?	21	0.86
L32	yes	0 (-)	5 (-)	11 (-)	X ray (200 kV)	air kerma	air	20	0.5
L33	yes	0 (-)	5 (-)	9 (-)	Co-60	air kerma	?	20	0.5

Notes. For columns min, max and number doses, ''-'' indicates that the scoring mode was not performed and ''?'' indicates that the information was not provided by the participants. The column ''own curve'' indicates whether a participant used a curve established in its own laboratory or a curve from another source. The column ''Dosimetry'' indicates whether the doses for the irradiations for the establishment of the calibration curve were given as dose in water or air kerma.

[a] Minimum (min), maximum (max) and number of dose points used for calibration curves for manual and semi-automatic (brackets) scoring.

they were next to the irradiation facility. For the shipments to USA and Canada about 50 h were necessary to deliver the blood samples to the laboratories. Similar to a recent RENEB ILC (*18*), the shipment to European countries outside the EU was rather time consuming because of logistical difficulties. For Serbia the package reached the destination after 68 h. For the shipment to Ukraine, an excessive delay of 30 days was too long to allow for the processing of blood samples. The thermologgers included in the packages showed temperature ranges between 5–33.5°C. The extreme high temperatures were monitored in most cases at the end of the journey and are probably related to an increase in temperature after unpacking the packages. The very low temperature was due to the special packaging equipment used by one laboratory. In most cases, the temperature profiles were quite smooth with an average temperature of about 24°C.

To get information about the required time frame until dose estimations can be delivered by the participating laboratories in an emergency, the reporting time was documented by the organizers of the exercise. Depending on the priority given to this task, the time range of the reporting between laboratories was quite variable. The earliest dose estimations arrived after 2.4 days the latest after 42 days. On average, the laboratories submitted their dose estimations 10 days after arrival of the blood sample in the laboratory. Further details on the reporting time can be found elsewhere (*29*).

Procedure

The details provided by the participants revealed that the scoring procedure was mostly performed using Giemsa-stained slides. Two participants used telomere/centromere staining with PNA FISH probes. The Quick Scan method

TABLE 3
Linear-Quadratic Calibration Curve Coefficients ($\lambda = C + \alpha D + \beta D^2$) and Corresponding Standard Errors for each Participant (L1-L33) and Scoring Mode

			Calibration curve coefficients					
Code	Scoring mode	Radiation source	$C \times 10^{-2}$	$\alpha \times 10^{-2}$	$\beta \times 10^{-2}$	$SE(C) \times 10^{-2}$	$SE(\alpha) \times 10^{-2}$	$SE(\beta) \times 10^{-2}$
L1	manual	Cs-137	0.18	1.43	10.82	0.01	0.52	0.52
L1	auto	Cs-137	0.11	1.37	2.61	0.01	0.26	0.14
L2	manual	X ray (240 kV)	0.08	6.12	6.5	0.04	0.97	0.62
L2	auto	X ray (240 kV)	0.03	3.21	2	0.03	0.53	0.22
L3	manual	Cs-137	0.13	8.96	8.02	0.19	2.69	0.99
L4	manual	Co-60	0.12	0.57	8.17	0.06	0.53	0.51
L5	manual	Cs-137	0.08	5.58	6.5	0.04	0.79	0.3
L6	manual	X ray (250 kV)	0.24	8.13	8.24	0.04	0.46	0.21
L7	manual	Orthovoltage	0.11	4.95	5.69	0.1	0.81	0.39
L8	manual	Co-60	0.13	2.1	6.31	0.05	0.52	0.4
L9	auto	Cs-137	0.12	1.47	1.65	0.12	0.44	0.15
L10	manual	Co-60	0.11	1.05	4.8	0.06	0.35	0.19
L11	manual	Co-60	0.11	3.55	6.44	0.01	0.43	0.29
L12	manual	X ray (6 MV; 15MeV)	0.07	4.13	4.44	0.06	0.58	0.33
L13	manual	Co-60	0.04	1.24	7.49	0.07	0.86	0.53
L14	manual	Co-60	0.04	1.79	5.66	0.04	0.39	0.24
L15	manual	X ray (200 kV)	0.14	6.08	10.07	0.14	9.23	9.25
L16	manual	Co-60	0.13	3.44	6.82	0.08	0.66	0.38
L16	auto	Co-60	0	6.81	2.86	0	1.62	0.58
L17	manual	X ray (250 kV)	0.05	4.6	6.5	0.05	0.5	0.3
L18	manual	Co-60	0.01	3.02	5.88	0.02	0.44	0.28
L19	manual	Co-60	0.13	2.1	6.31	0.05	0.52	0.4
L20	manual	Co-60	0.06	1.01	7.2	0.04	0.51	0.43
L21	manual	Orthovoltage	0.09	4.21	6.02	0.03	0.42	0.22
L22	manual	-	0.13	2.1	6.31	0.05	0.52	0.4
L23	manual	Co-60	0.05	2.09	7.11	0.02	0.57	0.25
L24	manual	Co-60	0.13	2.1	6.3	0.05	0.52	0.4
L25	auto	Co-60	0.09	2.56	2.66	0.08	0.45	0.16
L26	manual	Co-60	0.09	3.05	6.24	0.05	0.79	1.13
L27	manual	-	0.14	3.93	5.65	0.2	1.4	0.47
L28	manual	-	0.14	3.93	5.65	0.2	1.4	0.47
L29	manual	Co-60	0.14	3.93	5.65	0.2	1.4	0.47
L30	manual	Co-60	0.13	2.1	6.31	0.05	0.52	0.4
L31	manual	Co-60	1.16	0.26	2.52	0.49	1.13	0.35
L32	manual	X ray (200 kV)	0.03	5.91	7.13	0.04	0.83	0.59
L33	manual	Co-60	0.08	0.73	6.68	0	1.94	0.46

(*37*) for the detection of dicentric chromosomes was used by one lab while the others checked the number of chromosomes in the microscope or on the screen and included only cells with 46 or 45 centromeres. Automated metaphase finding systems are not available in all laboratories as are software tools like IKAROS (https://metasystems-international.com/en/products/ikaros/) to validate the dicentric chromosomes candidates.

Dose Effect Curves

The linear (α) and linear-quadratic (β) coefficients of the dose effect curves were relatively heterogeneous between the participants, with α ranging from 0.0026 to 0.086 and β from 0.025 to 0.11 for manual scoring (Table 3 and Fig. 1A) or α ranging from 0.014 to 0.068 and β from 0.017 to 0.029 for semi-automatic scoring (Table 3 and Fig. 1B). Most participants used curves based on γ-ray sources (55% ^{60}Co and 12% ^{137}Cs), some participants (24%) used curves based on X rays with varying energies and 9% of the participants did not provide information on the radiation type of the applied dose effect curve (Table 2 and Fig. 1C). The median of the α coefficients was at least two-fold lower for ^{60}Co curves than for other radiation types and the α coefficients were significantly lower (Wilcoxon-Mann-Whitney test $P = 0.001$) compared to X-ray curves from voltages of 200–250 kVp (Table 3 and Fig. 1D). Although the β coefficients seemed to be more similar between the different radiation types (Table 3 and Fig. 1E), significantly lower coefficients (Wilcoxon-Mann-Whitney test $P = 0.03$) were observed for participants using ^{60}Co curves compared to X-ray curves from voltages of 200–250 kVp. Most participants (76%) used their own dose effect curve, 12% used a curve provided in the IAEA manual (*31*), 8% used a curve from the Japanese Network for biological dosimetry and one lab did not provide information on the origin of the curve (Table 2). The reference dose of sample no. 3 (3.5 Gy) was

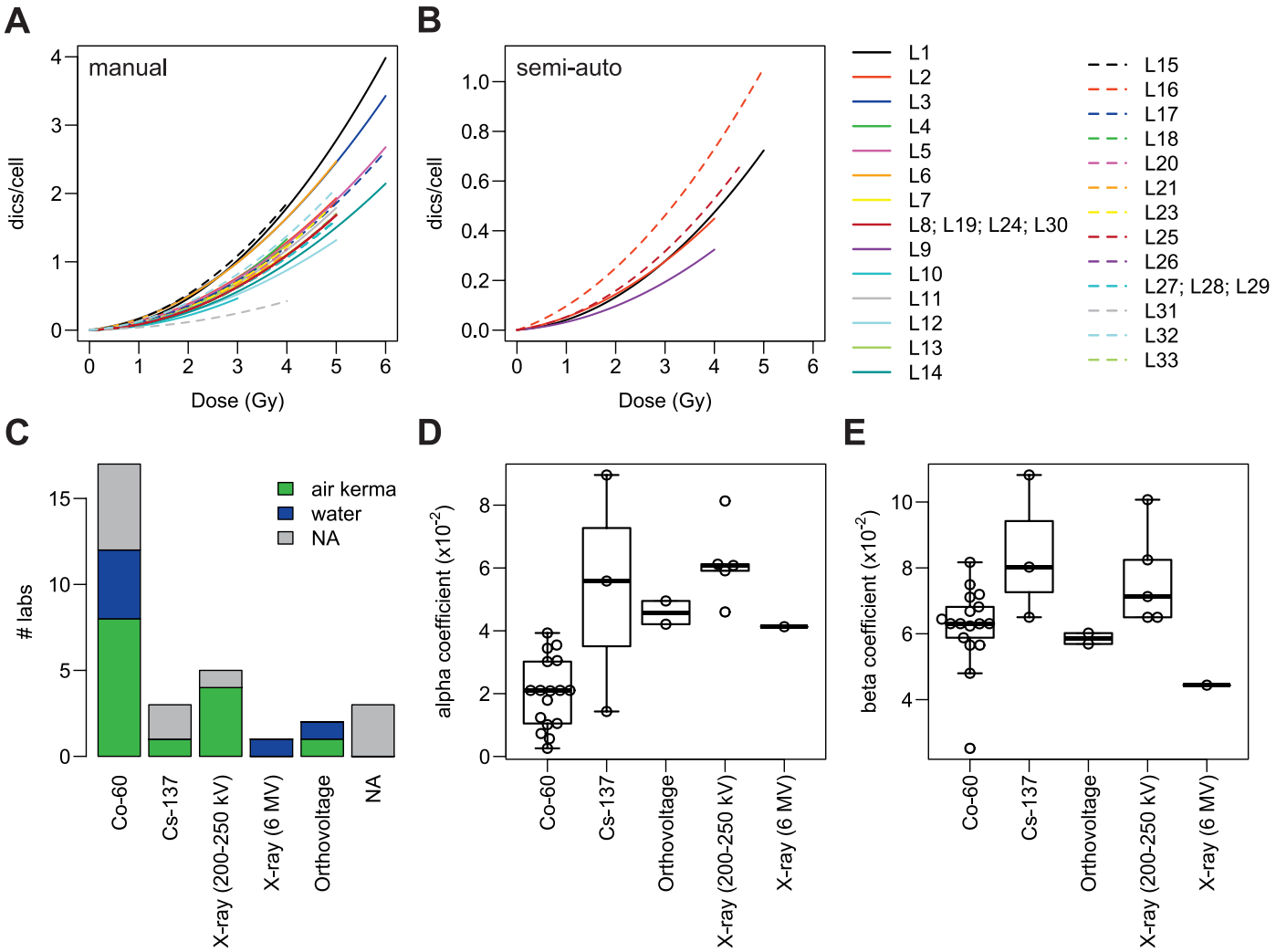

FIG. 1. Dose-effect curves from participating laboratories. Panels A and B: Manually and semi-automatically scored linear-quadratic dose-effect curves. The participants were labelled as L1-L33 and displayed by different colors and line types. L8, L19, L24 and L30 used the curve from Barquinero et al. (*31, 56*) Panel C: Number of labs (y-axis) for each radiation type used for the establishment of dose-effect curves. Colors indicate participants that performed the irradiations of their dose effect curve based on air kerma (green), dose in water (blue) or did not provide this information (gray). Panels D and E: Boxplots of the linear (α) and quadratic (β) coefficients by radiation type used for the establishment of dose-effect curves.

outside the range of the calibration curve for laboratories L10 (3 Gy) and L26 (2 Gy). The dosimetry for the calibration curve was based on air kerma by 42%, on dose in water by 24% and not provided by 33% of the labs, suggesting that this information is not available to many participants. The irradiation of blood samples for establishing the dose effect curves was performed in air by 33%, in water by 33% and for 33% of the participants this information was not provided. In total, 48% of the participants irradiated at room temperature, 30% at 37°C and for 21% this was not provided.

Classification of Test Samples into Clinically Relevant Groups

One of the main aims of this ILC was to categorize the test samples into clinically relevant groups of unexposed/minimally (category 1: 0–1 Gy), moderately exposed (category 2: 1–2 Gy) and highly exposed (category 3: >2 Gy). The reference doses of the test samples were chosen to represent these clinically relevant groups.

The unirradiated control was correctly classified in category 1 by all participants (Table 4) and 89% of the participants estimated a dose of exactly 0 Gy. Three (L27, L28, L29; Fig. 2) of the four labs that estimated a dose >0 Gy for sample no. 1 did not receive blood samples and performed their dose estimates based on the same set of images. Sample no. 2 (1.2 Gy) was classified in category 1 by 7% or 0%, in category 2 by 74% or 80% and in category 3 by 19% or 20% for manual or semi-automatic scoring, respectively (Table 4). Sample no. 3 (3.5 Gy) was correctly classified in category 3 by all participants.

TABLE 4
For each Test Sample, Numbers (and Percentages) of Dose Estimates Classified into each Clinically Relevant Group, with Reference Doses within the ±0.5 Gy or ±1 Gy Uncertainty Intervals or within the Estimated 95% Confidence Interval are Shown

	Clinically relevant groups			Reference dose within		
	0-1 Gy	1-2 Gy	>2 Gy	±0.5 Gy	±1 Gy	95% CI
Sample no. 1 (0 Gy)	31; 5 (100%; 100%)	0; 0 (0%; 0%)	0; 0 (0%; 0%)	29; 5 (94%; 100%)	31; 5 (100%; 100%)	30; 5 (97%; 100%)
Sample no. 2 (1.2 Gy)	2; 0 (7%; 0%)	23; 4 (74%; 80%)	6; 1 (19%; 20%)	15; 4 (52%; 80%)	30; 4 (97%; 80%)	16; 3 (52%; 60%)
Sample no. 3 (3.5 Gy)	0; 0 (0%; 0%)	0; 0 (0%; 0%)	31; 5 (100%; 100%)	8; 2 (26%; 60%)	17; 3 (55%; 60%)	11; 2 (35%; 40%)

Note. Numbers and percentages for manual or semi-automatic scoring mode are separated by semicolons.

Dose Estimation for Test Samples

All participants were asked to provide point estimates of the dose with corresponding uncertainties, given by the 95% CI. Based on the quality-check performed on the provided dose estimates, miscalculations in the estimated doses could be detected for two participants (L8 and L29, see Table 1). The provided dose estimates can be found in Table 1 and

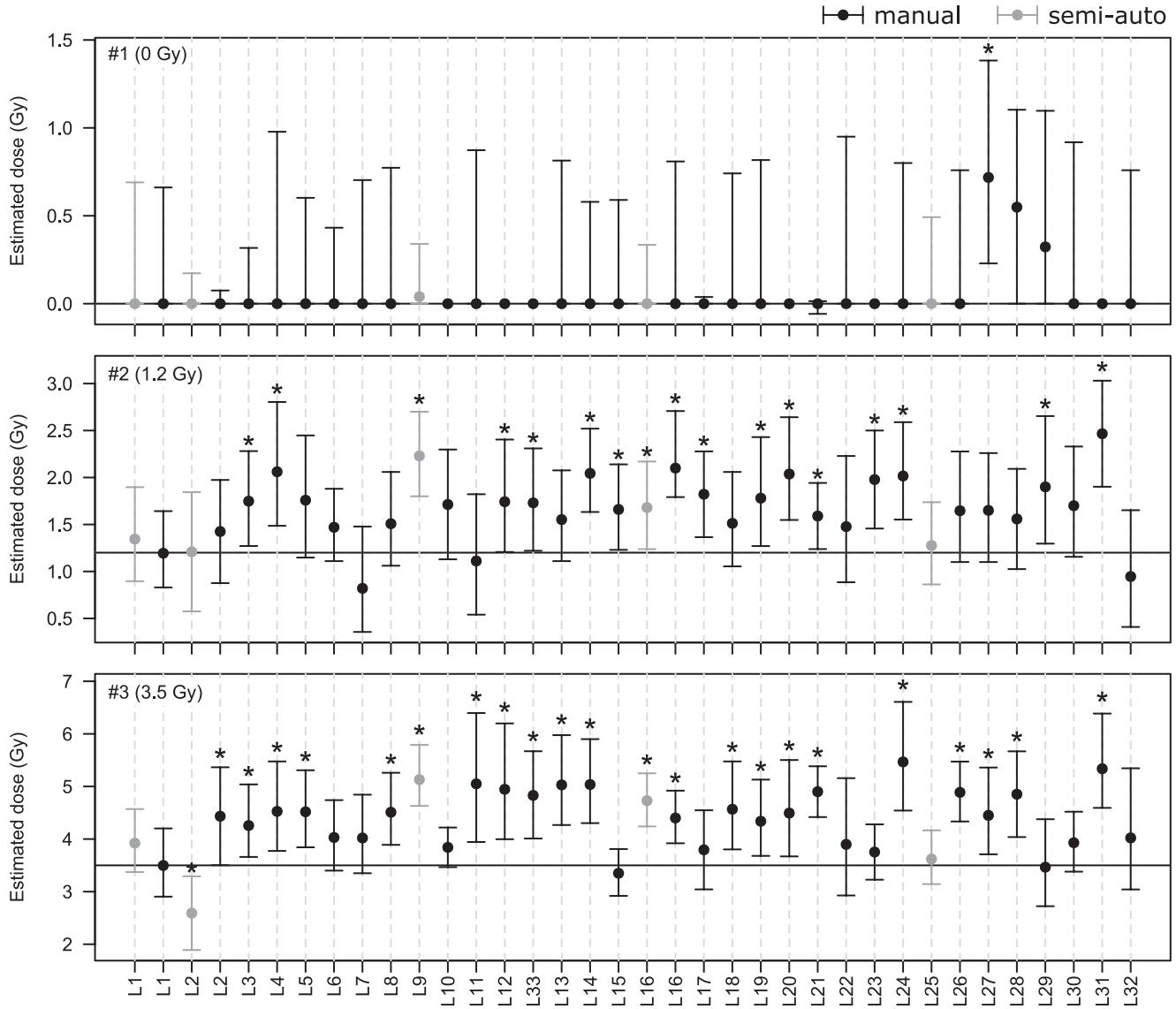

FIG. 2. Dose estimates and 95% confidence intervals. The figure shows the point estimates of the dose (circles) and the corresponding 95% confidence intervals (error bars) for each test sample (no. 1: 0 Gy; no. 2: 1.2 Gy; no. 3: 3.5 Gy) for each participating laboratory. Manually scored results are shown in black and semi-automatically scored results in gray. Results where the 95% confidence interval does not include the reference dose (black horizontal line) are indicated by asterisks.

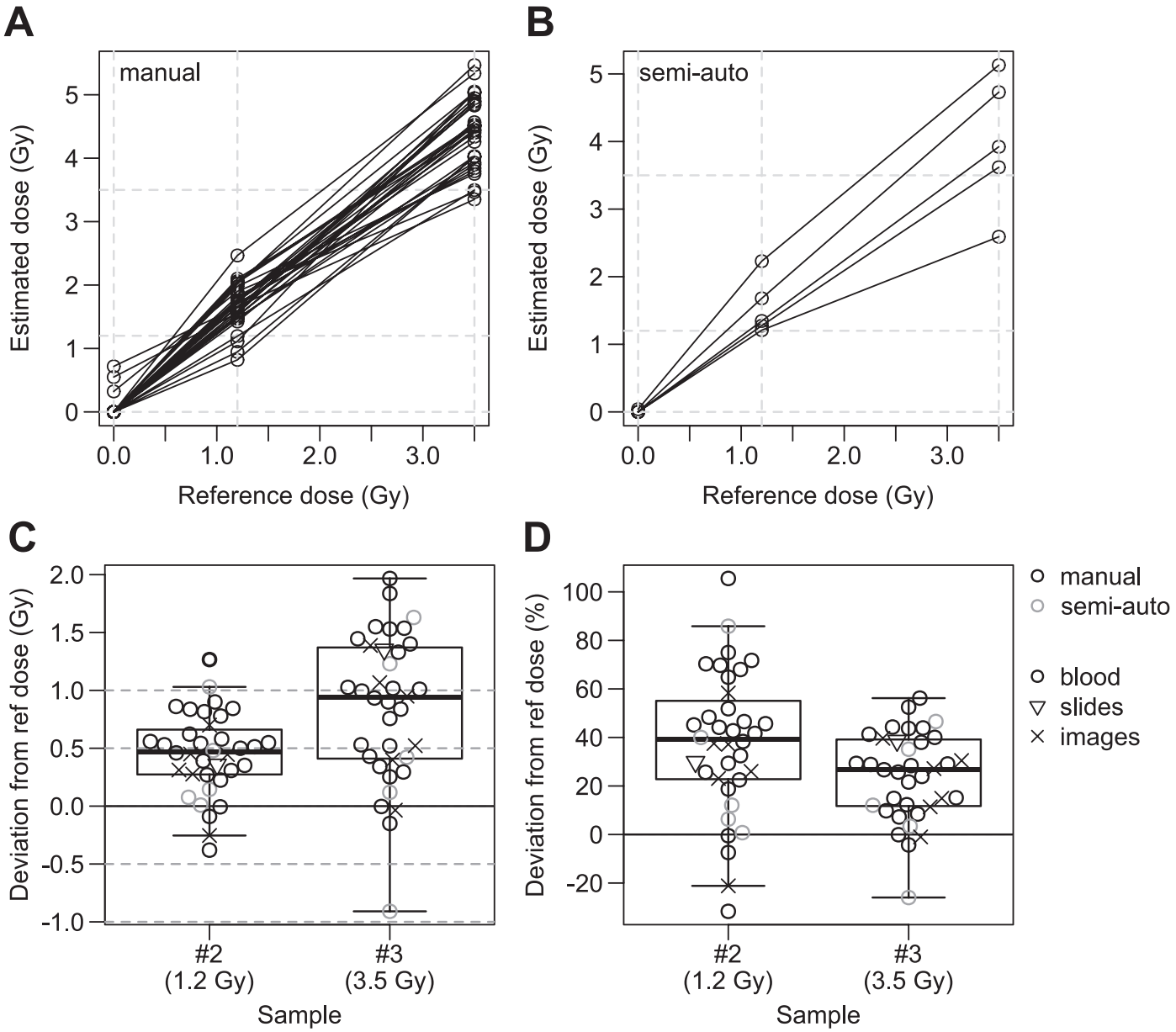

FIG. 3. Dose-effect relationships and deviations from reference doses. Panels A and B: The reference doses of the three test samples (x-axis) are shown versus the dose estimates from the DCA (y-axis) for manual (panel A) or semi-automatic scoring (panel B), respectively. Gray horizontal and vertical dashed lines refer to reference doses of 0 Gy, 1.2 Gy and 3.5 Gy. Panel C: Boxplot of the deviation (in Gy) of the DCA based dose estimates from the reference doses for test samples no. 2 and no. 3. Panel D: Boxplot of the deviation (in percent) of the DCA based dose estimates from the reference doses for test samples no. 2 and no. 3. In subpanels C and D manually scored results are shown in black and semi-automatically scored results in gray. Results from participants using blood samples, slides or images are labelled by circles, triangles or crosses, respectively.

the recalculated dose estimates were used for all following analyses. While the reasons for the problems remain unknown for L29, for L8 dicentrics including rings were used for the test samples with a calibration curve for dicentrics only. Three labs (Table 1) provided the α and β coefficients in the wrong order but the coefficients were used correctly for the estimation of the doses.

Most participants (N = 28) performed only manual scoring, three manual and semi-automatic scoring and two only semi-automatic scoring. All participants estimated the doses for the three test samples in the correct order (no. 1 < no. 2 < no. 3) for manual (Fig. 3A) and semi-automatic scoring (Fig. 3B). However, compared to the reference doses, systematically higher dose estimates were observed for samples no. 2 and no. 3 (Fig. 3). For sample no. 2 the median deviation from the reference dose was 0.5 Gy or 42% for manual scoring and 0.15 Gy or 12% for semi-automatic scoring (Fig. 3C and D). For manual and semi-automatic scoring the provided dose estimates were higher than the reference dose for 87% and 100% of the participants, respectively. The dose estimates exceeded the reference dose by more than 0.5 Gy for 48% (manual) or

20% (semi-automatic) or by more than 1 Gy for 3% (manual) or 20% (semi-automatic) of the participants (Fig. 3C and Table 4). Similarly, for sample no. 3 the median deviation was 0.95 Gy or 27% for manual scoring and 0.42 Gy or 12% for semi-automatic scoring (Fig. 3C and D). In total, 90% (manual) or 80% (semi-automatic) of the provided dose estimates were higher than the reference dose. The dose estimates exceeded the reference dose by more than 0.5 Gy for 74% (manual) or 40% (semi-automatic) or by more than 1 Gy for 45% (manual) or 40% (semi-automatic) of the participants (Fig. 3C and Table 4).

The participants were asked to provide estimates on the 95% CIs of the dose, to account for the uncertainty of the dicentric counts from the calibration curve and the test samples. Most participants used the recently developed Biodose Tools (version 3.5.0) software (*34*) to estimate doses and the 95% CIs, as recommended for this ILC. For the control sample (no. 1), for only one participant (L27) 0 Gy was not included in the 95% CI. In this case, the laboratory would have wrongly assumed that the observed frequency of dicentrics is significantly higher than the background frequency in the general population and that the individual was exposed to a dose > 0 Gy. In the remaining cases, the control sample was correctly identified, i.e., the dicentric frequency was not significantly different from the background frequency. Due to the systematic shift compared to the reference doses, for sample no. 2, 48% (manual) and 40% (semi-automatic) and for sample no. 3, 65% (manual) and 60% (semi-automatic) of the dose estimates did not include the reference dose in the 95% CI (Table 4 and Fig. 2).

Despite the observed shift, the dose estimates were relatively homogeneous between the participants for sample no. 2. This was indicated by an inter-quartile range (IQR) of 0.37 Gy (sample no. 2) and a CV of 21%. For sample no. 3, the variability between the participants was higher and an IQR of 0.95 Gy and a CV of 15% was observed. From simulations it can be expected that the IQR should be at approximately 0.36 Gy (median from 500 simulations) for sample no. 2 or 0.48 Gy for sample no. 3 and the expected CV should be approximately 18% for sample no. 2 or 10% for sample no. 3.

For sample no. 3, some labs (L4, L10, L20, L21, L24, L26, L31) estimated doses that were relatively far (0.47 to 2.9 Gy higher than the maximum dose used for the curve) or slightly (L9, 0.13 Gy) outside the range of their calibration curves. This extrapolation is generally not recommended (*32*).

Effect of Radiation Quality

One of the possible reasons for the observed systematic shift of the dose estimates is the fact that the test samples were irradiated with X rays (240 kVp, ~75 keV) but most participants used dose effect curves based on γ rays. For the manually scored dose estimates based on γ-ray curves, the median deviation from the reference dose was 0.55 Gy or 1.05 Gy for samples no. 2 or no. 3 (Fig. 4), respectively. In comparison, for the 5 participants using X-ray curves with voltages of 200–250 kVp, the median deviation was 0.27 or 0.52 for samples no. 2 or no. 3 (Fig. 4A and B), respectively. To further quantify the effect of the differences in radiation quality, the manually scored dose estimates based on γ-ray curves were transformed to X-ray doses of a comparable voltage and energy (220 kVp, 96 keV), as used for irradiation of the test samples, by using data from the literature (*36*). After converting the estimates based on γ-ray curves to X-ray doses, the median deviation reduced to 0.27 Gy or 0.6 Gy for samples no. 2 or no. 3 (Fig. 4), respectively. In addition, two participants provided estimates based on ^{60}Co and X-ray (L7: Orthovoltage; L32: 200 kVp) calibration curves. While the dose estimates based on the X-ray curve of L7 were only slightly lower for sample no. 2 (X ray: 0.82 Gy; ^{60}Co: 1.0 Gy) and no. 3 (X ray: 4.02; ^{60}Co: 4.16), the difference for L32 was larger for sample no. 2 (X ray: 0.95 Gy; ^{60}Co: 1.21 Gy) and sample no. 3 (X ray: 4.02 Gy; ^{60}Co: 4.77 Gy).

DISCUSSION

Networking between laboratories for biological and physical retrospective dosimetry provides great potential to share the workload in the case of a large-scale RN event and to validate the own workflow in preparation to such events. Regular exercises are required to train the logistics of sample shipment and sample processing, to test and improve the ability of network members to provide reliable dose estimates and to identify weaknesses. In the frame of RENEB, the European network for biological and physical retrospective dosimetry, a number of exercises have already been performed for the DCA (*17, 18, 23*) as well as for other assays (*20, 21, 24–28, 38*). The DCA assay is the biomarker of choice for investigations of recent exposure to ionizing radiation (*39*) and numerous applications have clearly shown the value of the method. Scoring dicentric chromosomes in triage mode based on a small number of cells was first introduced by Lloyd et al. (*35*) as a simplified and faster approach to provide early information about dose estimation to supplement medical management after an emergency situation. During the current exercise blood samples were irradiated with an X-ray source at the Bundeswehr Institute for Radiobiology, Germany, to simulate an acute, homogeneous whole-body exposure. Samples were distributed to RENEB members as well as to non-RENEB participants around the world. Similar to the situation during a real large-scale accident, the main focus of this exercise was the categorization of individuals into clinically relevant groups, as previously defined during the MULTIBIODOSE project (*30*). For the DCA, the participants were asked to provide dose estimates from triage scoring (*18, 40, 41*) of 50 manually or 150 semi-

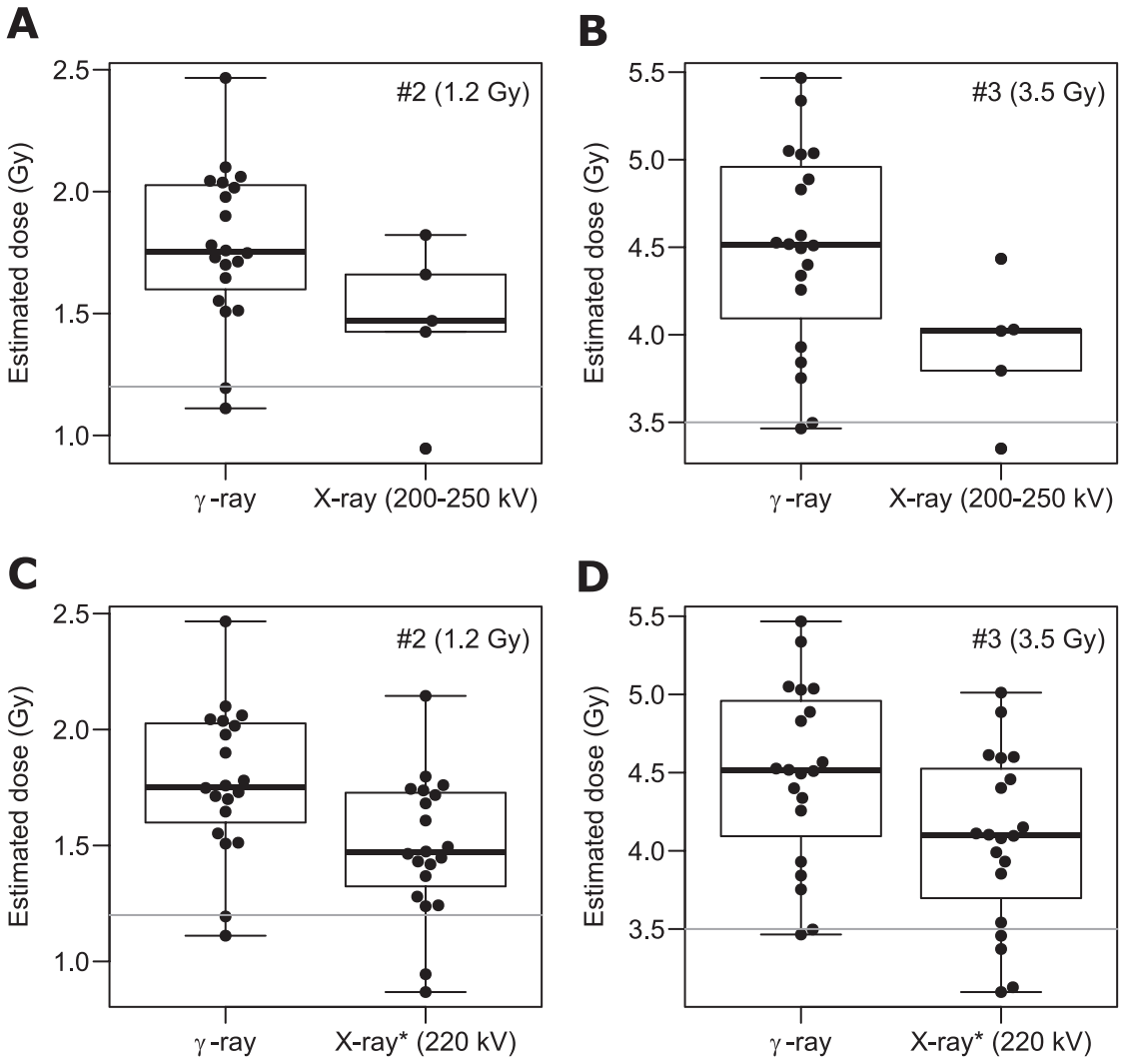

FIG. 4. Dose estimates γ ray vs. X ray based on manual scoring of dicentric chromosomes. Panels A and B: Boxplots of dose estimates from participants using dose-effect curves from γ rays (^{60}Co or ^{137}Cs) or X rays (200–250 kVp). Panels C and D: Dose estimates from participants using γ ray (^{60}Co or ^{137}Cs) dose-effect curves are compared to the same dose estimates transformed to X-ray doses based on the differences in the biological effectiveness published in Schmid et al. (*36*). The results from test sample no. 2 (1.2 Gy) or no. 3 (3.5 Gy) are shown on the left or right, respectively. The reference doses are indicated by gray horizontal lines.

automatically scored cells. The main aim of the categorization of the individuals into clinically relevant groups was successfully performed by almost all participants during this exercise. This result is in line with a previous RENEB exercise having a similar focus (*18*). Importantly, the sham irradiated sample was identified by all but one participant and all of the test samples irradiated with >0 Gy could significantly be distinguished from the unirradiated control. Similarly, all of the sham irradiated samples were successfully classified into the unexposed/minimally exposed group (0–1 Gy) and only two participants wrongly categorized the 1.2 Gy sample into this group. Hence, with regard to a real-life scenario, it can be assumed that the identification of the ''worried-well'' individuals can be successfully performed by the participants. About 20% of dose estimates for the 1.2 Gy sample were misclassified into the highly exposed group (>2 Gy) which can partly be attributed to the observed shift in dose estimates relative to the reference doses.

Unexpectedly, the point estimates of the doses and their corresponding 95% confidence intervals were systematically higher than the reference doses. The latter could only be revealed due to the high number of participating laboratories and was, interestingly, also observed for other cytogenetic methods (*29, 42, 43*). This systematic bias might be introduced due to differences regarding the irradiation or experimental setup between the calibration curves used by the participants and the test samples, by problems during the transport of the samples or by problems with the irradiation setup. However, in the case of a large-scale RN event, false-positive classifications (overestimation) of individuals can rather be tolerated than false-negative classifications (underestimation), as the latter could lead to delayed clinical interventions. Nevertheless, false-positive results might

contribute to increased anxiety and stress for the affected individuals.

This was the first RENEB exercise where X rays with relatively low-photon energies (~75 keV) were used and it is well-known from the literature that the biological effectiveness differs particularly between X rays of low-photon energies and γ rays (*36, 44, 45*). It is difficult to exactly quantify the effect of the differences between the radiation types for this ILC, as dose effect curves from the X-ray source used during the exercise and from a γ-ray source would be required, ideally scored by the same laboratory. Nevertheless, in Schmid et al. (*36*) an X-ray curve with an approximately comparable photon energy (96 keV) and voltage (220 kVp) as used during this ILC was published together with a ^{60}Co curve. Based on these data we would expect that the reference dose would be overestimated by approximately 22% and 11% for the 1.2 Gy (sample no. 2) and the 3.5 Gy (sample no. 3) samples, respectively. In comparison, the observed shift for the participants using γ-ray curves was in median 46% and 30% for samples no. 2 and no. 3, respectively. Hence, based on the published data (*36*) approximately one-third to one-half of the observed bias can be explained by differences in the biological effectiveness between X rays and γ rays, suggesting that other reasons also contributed to this observation. This exercise demonstrated that appropriate calibration curves for X rays seem to be missing for most of the participants. The RBE (relative biological effectiveness) of X rays strongly depends on the photon-energy spectrum (*36, 44, 45*). For most laboratories performing biological dosimetry, it might hardly be possible to establish calibration curves for X rays with a range of different energy spectra. The X-ray exposures during this exercise was mainly chosen because each ILC within RENEB should be organized by a different organization, often with limited access to sources from other radiation types, to train important logistical parameters, such as sample preparation and transport. In most cases, real radiation accidents with X rays will be on a relatively small scale and involve rather few individuals. Nevertheless, during an RN event, individuals will often, e.g., due to scattered radiation, be exposed to a mixture of lower and higher energy photons or neutrons. In the frame of the RENEB network, it is therefore important to study the effect of different radiation types with variable energies to be prepared for various exposure scenarios that might occur during large-scale RN events.

For this ILC, blood from a young healthy male donor (32 years) was irradiated for the test samples. Due to the high specificity of the DCA to ionizing radiation, it is generally assumed, that the individual variation between healthy adults can be neglected for the purpose of biological dosimetry (*46–48*). Based on this assumption, the dose-effect curves of most laboratories are only based on one or very few individuals. Due to the high workload of the DCA, literature comparing full calibration curves between donors can hardly be found. One recent publication from Saudi-Arabia compared dose effect curves based on a 320 kVp X-ray source between 10 adult donors and suggested that the differences between individuals can be neglected (*49*). Moreover, several exercises performed with variable donors have shown that doses can be successfully recovered based on the DCA (*17, 18, 41, 50, 51*). Nevertheless, it cannot be fully excluded that individual variations contributed to the observed bias.

Irradiations in the current study were performed at room temperature. However, as often recommended (*31*), some participants performed the irradiations for establishing their dose effect curves at 37°C. It is well known that very low temperatures applied during in vitro irradiation have an effect on the level of cytogenetic damage. Lisowska et al. (*52*) showed that the yields of dicentrics in lymphocytes irradiated at 0°C were significantly lower than after corresponding doses delivered at 37°C. Nevertheless, the difference in the aberration yield between irradiation temperatures of 20°C and 37°C should be negligible for an acute exposure (*53*). In support of these data, no significant differences could be found between manually scored dose estimates of labs with ^{60}Co curves irradiated at 37°C or 20°C for sample no. 2 and no. 3.

Deviating from the requirements given by the IAEA (*31*), during this ILC, the 2-h repair time postirradiation was accidentally performed at room temperature and not at 37°C. In 1986, Virsik-Peuckert et al. (*54*) showed in single exposure and split-dose experiments at different temperatures also applied after irradiation, that lesion repair is suppressed at temperatures below 21°C. The authors speculated, that postirradiation lesion interactions, such as the formation of dicentric chromosomes, are also suppressed at such temperatures, but can be restored, as soon as the necessary temperature is reached again. Although enzymatic processes like DNA repair are very complex and are not fully understood it seems not very likely that the influence of a repair at room temperature in the ILC can fully explain the shift in dose estimates. Additional experiments will be required to better understand if increasing the temperature 2 h after irradiation at room temperature might lead to enhanced dicentric yields. Differences in the temperature profiles during transport to the participants, after the 2-h repair time, might contribute to the variability between results of different participants. The latter also complicates the analysis regarding the association between dicentric yields and the 2-h repair time at room temperature and increased temperatures afterwards. However, for the DCA, this question is rather of theoretical interest as, in a real accident, blood will certainly be drawn more than 2 h after the exposure allowing enough time for repair at 37°C.

To mimic the irradiation scenario in the human body as closely as possible, the reference doses of this ILC were transformed to dose in water using appropriate correction factors [see Port et al.'s special issue inter-assay paper (*29*) for details]. In comparison, for many of the participants, the

calibration of the radiation source used for the dose effect curves was based on air kerma instead of dose in water (Table 2). It has previously been shown that this has rarely been accounted for by laboratories for biological dosimetry (*55*). However, instead of overestimation, underestimation would be expected if participants used a curve from a source calibrated in air kerma instead of dose in water. The observed shift of dose estimates for the test samples could therefore not be explained by these differences.

Due to the high number of participants and assays in this exercise (*29*) 8–9 blood samples were irradiated at the same time. Measurements of the homogeneity of the field prior to the exercise showed, that the applied doses can be ~5% lower for the blood samples farthest away from the center of the radiation field. Although, this can contribute to the observed heterogeneity between the dose estimates of the participants, it is very unlikely that inhomogeneities of the radiation field led to the observed systematic shift of the provided dose estimates. Similarly, the triage scoring of only 50 (manual) or 150 (semi-automatic) metaphases also contributes to increased variability between the participants but not to systematic deviations from the reference dose.

For the transport of the blood samples, thermoluminescent dosimeters (TLDs) were included as well as thermologgers. No additional dose that could have an influence on the observed shift of the dose estimates could be detected by the TLDs. The temperature was also in the acceptable range (mean 24°C) and transport did not have an observable effect on the dicentric yield in previous RENEB exercises (*18*).

The dicentric frequencies for samples no. 2 or no. 3 were in median 1.7 fold (range: 0.6–3.4 fold) or 1.6 fold (range: 0.6–2.4 fold) higher than expected based on dicentric yields obtained from the calibration curves (at doses of 1.2 Gy or 3.5 Gy) of each lab. Therefore, during discussion within RENEB, the concern was raised that there was a problem with the irradiation. However, the organizing institution put a lot of effort in design and dosimetry of the irradiation setup and performed many measurements prior to the exercise to guarantee the reliability of the reference doses [for details see Port et al. inter-assay paper (*29*)]. Nevertheless, it can never be fully excluded that problems during the irradiation contributed to the observed differences between DCA based dose estimates and the reference doses.

Despite the observed shift in the dose estimates, the variability of the provided results for sample no. 2 was in the range expected for triage scoring, suggesting a good homogeneity between laboratories. For sample no. 3, the heterogeneity between dose estimates was greater than expected for triage scoring. In addition to reasons discussed above, the extrapolation to doses outside the range of the applied calibration curve by some laboratories might have contributed to the observed heterogeneity. Generally, it cannot be recommended to estimate doses that are not covered by the dose effect curve. In contrast to the recommendations in papers (*32*) and (*31*), some laboratories used only relatively few dose points (e.g., L15; Table 2) for the construction of the dose effect curves or the highest applied doses were relatively low (e.g., L10 and L26; Table 2), which might further contribute to the observed heterogeneities. However, the deviation of the dose estimates from the reference doses in these laboratories were not higher than in other laboratories using more dose points or higher maximum doses (Fig. 2).

Similar to recent RENEB ILCs (*17, 18, 23*), heterogeneity was observed with regard to the calibration curves used by the participants. This strongly suggests that each laboratory should use its own calibration curve and regularly perform intra-laboratory quality checks to ensure if the scoring does still agree with the used calibration curve, especially when there are changes in the staff of the laboratory. Four laboratories used a curve published in (*31, 56*) and members from the Japanese network used a common curve established by the network members (Table 2). Using curves from other sources is generally not recommended and requires regular exercises whether the scoring and experimental procedures are in agreement with the applied curves.

In conclusion, the main aim of the clinical categorization of the test samples by triage scoring was successfully performed by most participants. Due to the accuracy of the dicentric chromosome assay and the high number of involved laboratories, it was possible to reveal a systematic shift of the dose estimates which could partly be attributed to differences in the biological effectiveness between X rays and γ rays. Currently, we can only speculate about additional sources contributing to the systematic shift, but, this observation offers many opportunities for future research which might help to further improve the workflow of biological dosimetry and the design of ILCs in the future. Although, the organization of such exercises requires a huge effort, regular ILCs are indispensable to validate the performance of laboratories and assays in preparation to future RN events and to identify potential sources for improvements.

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