What We Have Learned from RENEB Inter-Laboratory Comparisons Since 2012 With Focus on ILC 2021

Authors: Endesfelder, D., Oestreicher, U., Barquinero, J.F., Vral, A., Terzoudi, G., et al.

Source: Radiation Research, 199(6) : 616-627

Published By: Radiation Research Society

URL: https://doi.org/10.1667/RADE-22-00204.1

RADIATION RESEARCH **199**, 616–627 (2023)
0033-7587/23 $15.00

DOI: 10.1667/RADE-22-00204.1

What We Have Learned from RENEB Inter-Laboratory Comparisons Since 2012 With Focus on ILC 2021

D. Endesfelder,[a,1] U. Oestreicher,[a] J.F. Barquinero,[b] A. Vral,[c] G. Terzoudi,[d] J. Moquet,[e] F. Trompier,[f] A. Wojcik,[g,h] M. Abend,[i] M. Port[i]

[a]Bundesamt für Strahlenschutz, BfS, Oberschleissheim, Germany; [b]Department of Animal Biology, Plant Biology and Ecology, Universitat Autònoma de Barcelona, Bellaterra, Spain; [c]Faculty of Medicine and Health Sciences, Radiobiology Research Unit, Ghent University, Ghent, Belgium; [d]National Centre for Scientific Research "Demokritos", Health Physics, Radiobiology & Cytogenetics Laboratory, Athens, Greece; [e]UK Health Security Agency, Radiation, Chemicals and Environmental Hazards Directorate, Chilton, Oxfordshire, United Kingdom; [f]Institut de Radioprotection et de Sûreté Nucléaire, Fontenay-aux-Roses, France; [g]Stockholm University, Department of Molecular Biosciences, The Wenner-Gren Institute, Sweden; [h]Institute of Biology, Jan Kochanowski University, Kielce, Poland; [i]Bundeswehr Institute of Radiobiology, Munich, Germany

Endesfelder D, Oestreicher U, Barquinero JF, Vral A, Terzoudi G, Moquet J, Trompier F, Wojcik A, Abend M, Port M. What We Have Learned from RENEB Inter-Laboratory Comparisons Since 2012 With Focus on ILC 2021. Radiat Res. 199, 616–627 (2023).

Inter-laboratory exercises are important tools within the European network for biological dosimetry and physical retrospective dosimetry (RENEB) to validate and improve the performance of member laboratories and to ensure an operational network with high quality standards for dose estimations in case of a large-scale radiological or nuclear event. In addition to the RENEB inter-laboratory comparison 2021, several inter-laboratory comparisons have been performed in the frame of RENEB for a number of assays in recent years. This publication gives an overview of RENEB inter-laboratory comparisons for biological dosimetry assays in the past and a final summary of the challenges and lessons learnt from the RENEB inter-laboratory comparison 2021. In addition, the dose estimates of all RENEB inter-laboratory comparisons since 2013 that have been conducted for the dicentric chromosome assay, the most established and applied assay, are compared and discussed.

INTRODUCTION

Biological and physical retrospective dosimetry can be important tools to supplement clinical decision-making in the case of a large-scale radiological or nuclear (RN) event. International networking between several organizations provides the opportunity to increase the capacity of single national laboratories by sharing the immense workload caused by a large number of potentially exposed individuals. In the frame of 7th EU framework EURATOM Fission Programme, the EU project RENEB (Realizing the European Network of biological dosimetry and physical retrospective dosimetry, GA 295513) was started in 2012. Based on this project a Memorandum of Understanding for mutual assistance in individual dose estimation in large scale RN emergencies was signed in 2016 by 26 organizations from 16 European countries and RENEB (Running the European Network of biological dosimetry and physical retrospective dosimetry) became an operational network. In 2017, the RENEB legal association was founded and currently has 16 voting members (organizations) and 48 associate members (individuals) from 17 countries in total.

It is crucial to ensure high and consistent quality, capacity and efficiency of biological dosimetry and physical retrospective dosimetry for all partners in a network. Moreover, established assays for biological and physical retrospective dosimetry must be constantly validated and improved for different scenarios. Depending on the stage of development of an emerging technique, the focus of inter-laboratory comparisons (ILCs) should foremost be on the evaluation of the possible capacity as well as the identification and investigation of the main pitfalls and problems of the method. The thorough evaluation of emerging techniques enables decisions if those methods meet RENEB requirements and can be integrated in the operational basis of the network. ILCs with a different focus must be performed such that the network is prepared if a RN event occurs and to prove the proficiency of each laboratory in the network. In the frame of RENEB a number of ILCs have already been performed since 2012 for various assays and different research questions, including the activation of the network, field exercises, web-based scoring, establishment of calibration curves, triage dose assessment or harmonization of SOPs between network members (*1–17*). Each RENEB ILC is

[1] Corresponding author: Dr. David Endesfelder, Federal Office for Radiation Protection, Ingolstaedter Landstraße 1, Oberschleissheim, Germany; email: dendesfelder@bfs.de.

organized by a different RENEB member, to allow the partners to train also logistical aspects such as the handling and shipment of a large number of samples (blood or other materials). Furthermore, differences in the experimental setup or radiation source used by the organizing institutions can help to identify potential issues and to improve and optimize the performance of the network.

The RENEB ILC 2021 was the first ILC organized within RENEB where the irradiation of biological samples and samples used for physical retrospective dosimetry was carried out simultaneously by one institution. The general study design included blood sampling, irradiation and shipment of the samples for a significant number of biological [dicentric chromosome assay (DCA), cytokinesis-block micronucleus (CBMN), fluorescence in situ hybridization (FISH), gene expression (GE), phosphorylated histone variant H2A.X foci (yH2AX), premature chromosome condensation (PCC))] and physical retrospective [(electron paramagnetic resonance (EPR), optically stimulated luminescence (OSL), thermoluminescence (TL)] dosimetry assays. In contrast to other RENEB ILCs, where γ-ray sources or high-energy X-ray sources were used, samples were irradiated with an X-ray source with a comparably low energy. The results led to discussions within the network which will help to further improve the interpretation and reporting of dose estimates for biological dosimetry in the future. The RENEB ILC 2021 was designed to simulate a large-scale scenario and to compare the performance within and between assays with regard to response time, triage dose assessment and the categorization into clinically relevant dose groups. This paper starts with an overview of the development of RENEB ILCs in the past and provides a summary of the main results and the lessons learnt from this ILC.

RENEB ILCs from 2012–2021

Since the RENEB project started, a number of ILCs with focus on different research questions have been performed for various assays. A summary of RENEB exercises with assays used for biological dosimetry that were published in recent years can be found in Table 1.

ILCs 2012–2015

The first round of ILCs for the cytogenetic assays comprised telescoring exercises (ILC 2012), a first exercise (ILC 2013) for dose assessment and after harmonization and training of the participating laboratories, a second exercise (ILC 2014) for dose assessments was conducted (*2, 4–6, 17*). The participating laboratories were RENEB members only for ILC 2013 or the exercise was opened to networks and institutions outside of RENEB for ILC 2014 (Table 1). For DCA and CBMN, the doses for the irradiation of blood samples ranged between 0 Gy and 3.27 Gy for ILC 2013 and ILC 2014, and simulated a homogeneous whole-body exposure (*2, 4*). The exercise also included one sample to simulate a partial body exposure by mixing equal amounts of blood exposed to 4.75 Gy with unexposed blood.

For the DCA (*4*), scoring was performed in manual triage mode (20–50 cells) and the performance of laboratories in dose estimation was compared by Z-scores (*18, 19*). In addition, the performance was assessed by grouping dose estimates into intervals of 0–1 Gy, 1–2 Gy and >2 Gy, corresponding to the clinically relevant groups defined during the MULTIBIODOSE project (*20*). Generally, the classification into clinically relevant groups was successfully performed by most participants. The accuracy of dose estimations for the dose points >2.5 Gy improved from ILC 2013 to ILC 2014. The Z-scores of most participants were satisfactory, although a tendency for overestimation was observed. In 2015 a validation exercise for candidate methods, such as DCA-TC (DCA with telomere and centromere probes), GE, Raman spectroscopy and TL on mobile phone glass, was organized in cooperation with EURADOS WG10 (*16*). Samples were irradiated with doses of 0, 0.44, 1.08 and 1.89 Gy and 5 participants conducted the DCA as a reference technique. Based on the Z-test only 2 of 32 submitted results showed questionable results. Simultaneously, a new method based on dicentric chromosomes detected with telomere and centromere probes (DCA-TC) was performed (*16*). Images from samples irradiated during ILC 2013 for the DCA were sent to 17 participants and doses were estimated based on the calibration curve published in (*21*). As the same set of images was used by the participants and most participants did not have own calibration curves for this method, the results have only limited value. Nevertheless, the provided dose estimates were highly homogeneous and each partner correctly identified the sham-irradiated sample and the simulated partial-body irradiation.

For CBMN (*2*) in ILC 2013 and ILC 2014 scoring was performed in full mode (manual scoring of 500 binucleated (BN) cells per slide, 2 slides per duplicate culture) or triage mode (automatic/semi-automatic scoring of 1,000 BN cells per slide, 2 slides per duplicate culture). Lab performances in dose estimation were compared using Z-scores and triage uncertainty intervals of ±0.5 Gy for doses <2.5 Gy and of ±20% deviation for doses >2.5 Gy. Moreover, the results from scoring 1, 2 and 4 slides were compared. The categorization in triage uncertainty intervals was very successful for the dose points <2.5 Gy, where between 84% and 88% of the results were within the defined triage uncertainty interval. Higher variation was observed for dose points >2.5 Gy where 47% (ILC 2013) or 74% (ILC 2014) of the results were within the triage uncertainty interval. Similar to the DCA, the accuracy of the dose estimates for samples irradiated with doses >2.5 Gy increased from the ILC 2013 to the ILC 2014 exercise. Compared to manual or fully automatic scoring, higher accuracy was observed for the semi-automatic scoring of micronuclei. The Z-scores of most RENEB labs were

TABLE 1
Overview of Published RENEB ILCs from 2012–2019

Assay	Year	Labs	Source	Dose (Gy)	Purpose	Material	Articles
DCA	2012	18	^{60}Co	1.3,[a] 3.5[a]	Telescoring	images	(5)
DCA	2013	18	^{137}Cs	0, 0.94,[a] 3.27,[a] 4.75[b]	Dose assessment	blood	(4)
DCA	2014	42	^{137}Cs	0.85,[a] 2.7[a]	Dose assessment	blood	(4)
DCA	2015[e]	5	^{60}Co	0, 0.44,[a] 1.08,[a] 1.89[a]	Dose assessment	blood	(16)
DCA	2017	38	X ray (4 MV)	0, 0.4,[a] 1.8[a]	Dose assessment	blood	(7)
DCA	2019[e]	17	1.36 TBq ^{192}Ir	0.05–2.17 (n = 16),[a] 0.18/1.91,[c] 0.1/0.38,[c] 0.18/0.26,[c] 0.05/1.73[c]	Field exercise; Dose assessment	Blood, suspension	(8)
DCA-TC	2015[e]	17	^{137}Cs	0, 0.94,[a] 3.27,[a] 4.75[b]	Dose assessment	images	(16)
CBMN	2013	12	^{137}Cs	0, 0.94,[a] 3.27,[a] 4.75[b]	Dose assessment	blood	(2)
CBMN	2014	16	^{137}Cs	0.85,[a] 2.7[a]	Dose assessment	blood	(2)
FISH	2012	11	-	-	Telescoring	images	(6)
FISH	2013	10	^{137}Cs	2[a]	Dose assessment; Harmonization	blood	(6)
FISH	2014	10	^{137}Cs	0.85,[a] 2.7[a]	Dose assessment	blood	(6)
PCC	2013	4	^{60}Co	0–6 (n = 5)[a,d]	Telescoring; DEC establishment; Harmonization	images	(17)
PCC	2013	4	^{60}Co	0–6 (n = 8),[a] 2,[b] 4[b]	Dose assessment	slides	(17)
PCC	2014	10	^{137}Cs	0.85,[a] 2.7[a]	Telescoring; Dose assessment	images	(17)
yH2AX	2013	8	^{137}Cs	0.5,[a] 1,[a] 2,[a] 4[a]	Telescoring	images	(1)
yH2AX	2014	8	^{137}Cs	0–4 (n = 5),[d] 0, 1,[a] 4,[a] 3,[b] 2/3[c]	DEC establishment; Dose assessment	lymphocytes	(1)
yH2AX	2015	7	^{60}Co	0,[a,d] 2,[a,d] 0.5,[a] 2.5[a]	Dose assessment	lymphocytes vs. blood	(3)
GE	2015	4	X ray (240 kV), LINAC	0–4 (n = 7,[a,d] n = 10[a]), 0, 2 (in vivo, pelvis)	DEC establishment; Dose assessment	whole blood cell culture, peripheral blood	(13)
GE	2015	5	^{60}Co	0–2.92 (n = 6),[a,d] 0, 0.44,[a] 1.08,[a] 1.89[a]	DEC establishment; Dose assessment	blood	(12)
GE	2019[e]	6	X ray (240 kV), 1.36 TBq ^{192}Ir	0–4 (n = 6),[d] 0–2, (n = 6),[d] 0.18,[a] 0.25,[a] 1.6,[a] 2.4[a]	DEC establishment; Field exercise; Dose assessment	blood	(14)

[a] Homogeneous exposure.
[b] partial body (50:50 mixture).
[c] heterogeneous exposure with two doses (50:50 mixture).
[d] used for establishment of dose effect curves (DEC) or reference samples with known doses.
[e] Exercise jointly organized with EURADOS WG10.

satisfactory and out of 106 submitted results only 7 showed questionable and 1 unsatisfactory Z-scores. In contrast, for the non-RENEB partners, out of 10 submitted results 2 showed questionable and 1 unsatisfactory Z-scores.

For FISH (*6*), a first telescoring exercise (ILC 2012) was performed to harmonize the scoring criteria and chromosome aberration description based on the modified PAINT nomenclature (*22*). This was followed by a first exercise for dose assessment (ILC 2013) where blood samples were only irradiated with 2 Gy and training was provided afterwards. The second exercise for dose assessment (ILC 2014) was carried out in parallel and with the same doses as for DCA and CBMN (0.85 Gy and 2.7 Gy). The dose estimation was performed based on the scoring of approximately 500 cells and results of the dose assessments were mainly compared based on Z-scores. The level of experience of the participants was very different before these exercises started. Thus, it was important to harmonize the description of aberrations in a first step. Some participants showed questionable (20% for ILC 2013 and 10% for ILC 2014) or unsatisfactory (5% for ILC 2014) results in terms of Z-scores. FISH results of the ILC 2014 were later reevaluated considering both, that the blood samples were irradiated in air and dosimetric references expressed in terms of air kerma. In addition, some dose-effect curves were recalculated based on their results during the investigation on the influence of calibration practices on biodosimetry. The reevaluation of the ILC 2014 resulted in a reduction of questionable or unsatisfactory results from 15% to 5% (*23*).

When the first rounds of RENEB ILCs started, the level of experience for PCC was low for many labs and the cell

fusion PCC assay had not been widely used for biological dosimetry. Hence, the aim of the first round of exercises (2012–2014) was to harmonize and standardize the SOPs for the PCC assay and to construct calibration curves (*17*). In a first step, captured images based on the PCC assay applied shortly (a few minutes) after irradiation were prepared by one laboratory and were sent to the participants. Calibration curves were estimated to compare the scoring between participants and were in relatively good agreement between the participants. In the second step, slides for several dose points simulating homogeneous whole-body or partial-body exposures were sent to the participants and doses were estimated based on an established calibration curve from one experienced lab. Differences in dose estimates were observed among participating laboratories, as well as between the different scorers, particularly for doses above 2 Gy. Using the same slides and based on the analysis of a larger number of lymphocyte PCC spreads per dose point, more precise dose-response curves were established by some of the participating labs. In a third step, the aim was to estimate doses after a repair period of 24 h postirradiation. For this purpose, images of two test samples irradiated with doses of 0.85 Gy and 2.7 Gy were sent to the participants and dose estimates were performed based on a calibration curve shared by one of the participants, since only one lab had a calibration curve after a 24 h repair period. Results of the dose assessments were compared using Z-scores. Although two participants had questionable Z-scores for the lower dose points, the results were mostly in good agreement between the participants. Similar to the FISH assay, the results of the ILC were reevaluated afterwards. While the Z-scores improved for the lower dose point, three Z-scores became questionable for the higher dose point (*23*).

The first two RENEB exercises for the GE assay were performed in the year 2015 and published in two separate papers (*12, 13*). The first exercise (*13*) included in vivo-irradiated blood samples from prostate cancer patients as well as ex vivo-irradiated blood samples from healthy donors. For the study performed on healthy donors, samples from whole blood cell cultures irradiated with known doses (0–4 Gy) were sent to the participants for the establishment of calibration curves as well as 10 test samples irradiated with unknown doses (0–4 Gy) for dose estimations. Generally, most of the dose estimates for reference doses $\leq$2 Gy were inside the $\pm$0.5 Gy triage uncertainty interval and unexposed samples could be distinguished from exposed samples. For reference doses $>$2 Gy the number of results outside the $\pm$0.5 Gy triage uncertainty interval increased. For some participants the results varied considerably for different gene sets. For the study carried out in prostate cancer patients, peripheral blood was collected from the patients before and 24 h after the first in vivo fraction (2 Gy) of localized radiotherapy to the pelvis. Different GE platforms used by the participants were analyzed and radiation-induced gene expression changes were compared between ex vivo and in vivo irradiations. The in vivo-irradiated samples from prostate cancer patients could be successfully distinguished from the unexposed samples. However, the in vivo results were out of range of the ex vivo calibration curves and an adjustment was therefore necessary to enable dose estimations. For the second RENEB GE exercise (*12*) blood samples were irradiated for the construction of calibration curves (0–2.92 Gy) and four test samples (0–1.89 Gy) with unknown doses were sent to the participants for dose estimations. One of the main aims of this exercise was to compare the effect of two different culture conditions on the dose estimates. While the shape of the calibration curves was similar between the two different culture conditions, the baseline expression levels were different. Independent of the platforms used, the provided dose estimates were in good agreement with each other. However, while the unexposed samples could, in most cases, be successfully distinguished from exposed samples, many of the dose estimates for reference doses $>$1 Gy were not within the $\pm$0.5 Gy triage uncertainty interval. The authors suggested that this observation might be due to a plateau of the gene expression signal at higher doses.

The first RENEB exercise for the yH2AX assay consisted of a telescoring exercise (2013) which was followed by a comparison of dose estimates (2014) from isolated blood lymphocyte samples that were shipped overnight to the participants after incubation of 4 or 24 h at 37°C after exposure (*1*). For the telescoring exercise, lymphocytes were exposed to doses of 0.5, 1, 2, and 4 Gy and images were sent electronically to the participants for foci scoring. The results between the participants showed relatively large variability in the number of scored foci, however, the samples could be successfully ranked from lowest to highest dose. For the ILC 2014, lymphocyte samples irradiated with doses from 0–4 Gy and incubated at 37°C for 4 and 24 h were sent to the participants for the establishment of calibration curves. Next, coded lymphocyte samples that were uniformly irradiated with doses of 0, 1 and 4 Gy or samples irradiated with doses of 0 + 3 Gy or 2 + 3 Gy simulating heterogeneous exposures were sent to the participants for dose estimation. Again, calibration curve coefficients showed considerable variation in foci yields between the participants. Dose estimates were classified in triage groups of 0–1 Gy, 1–2 Gy and $>$2 Gy. Manual scoring of samples incubated for 4 h postirradiation achieved the highest accuracy. The results of samples incubated for 24 h and scored automatically were less reliable and the usefulness for the assessment of triage categories was doubted by the authors. The identification of heterogeneously exposed samples based on the analysis of dispersion levels deviating from a Poisson distribution was difficult, as homogeneously exposed samples also frequently showed overdispersion. For the second RENEB exercise for the yH2AX assay (2015) whole blood and separated

lymphocyte samples were irradiated with 0.5 and 2.5 Gy doses (blind samples), incubated for 4 and 24 h, and sent to the participants for dose estimation. While a tendency for overestimation was observed for the 0.5 Gy sample, a tendency for underestimation was observed for the 2.5 Gy sample. Based on the analysis of Z-scores 26% or 17% of the dose estimates for the 0.5 Gy sample were classified as questionable or unsatisfactory, respectively. For the 2.5 Gy sample, 4% or 35% of the dose estimates were classified as questionable or unsatisfactory, respectively. The most accurate classification of dose estimates into triage groups of 0–1 Gy, 1–2 Gy and >2 Gy was achieved by manual scoring of the 4 h whole blood or lymphocyte samples. The data available for 24 h incubation time and for automatically scored foci indicated a higher variability of the categorization in dose groups.

ILC 2017

The next RENEB ILC was done in 2017 after the legal RENEB association was established and samples were irradiated (0, 0.4, 1.8 Gy) with a LINAC X-ray source and dose assessments were performed using several assays (DCA, CBMN, PCC, yH2AX and GE). While the results for the DCA were published (*7*), the results for the remaining assays (CBMN, PCC, yH2AX, GE) have so far only been evaluated internally within RENEB. For the DCA, the scoring was performed in "full mode" (500 cells per slide) and dicentric frequencies as well as dose estimates were compared between laboratories using Z and U-scores and the limits and pitfalls of ILCs were discussed. Relatively high heterogeneity was observed with regard to the statistical methods used by the participants for the assessment of the uncertainties based on 95% confidence intervals (CIs). Therefore, the authors re-calculated the dose estimates and corresponding 95% CIs using the same statistical method for all participants. After this step, between 90% and 100% of the provided dose estimates included the reference dose for the three test samples in the 95% CI and most participants showed satisfactory Z and U-scores. The observed heterogeneity of the applied methods for uncertainty assessment lead to the development of a new software (Biodose Tool) tool to harmonize the application of statistical methods for dose and uncertainty assessment (*24*).

RENEB/EURADOS Field Exercise 2019

In 2019 RENEB and EURADOS WG10 organized a joint field exercise where samples from a number of different materials (including blood) were located at different positions on anthropomorphic phantoms placed at different angles and distances from a 1.36 TBq ^{192}Ir source (*25*). In the frame of RENEB, GE and DCA analyses were performed, and the resulting dose estimates were compared to reference doses from RPL glass dosimeters (GDs) placed on the tubes of the blood samples (*8, 14*). In contrast to other RENEB ILCs, the irradiation and the first handling of the samples was performed outdoors and therefore deviated from typical indoor laboratory conditions. In addition, the exposure was not acute but delivered over 1 and 2.5 h. For the irradiation, blood tubes were placed in thermos flasks filled with heated water to keep the temperature at approximately 37°C. The reference dosimeters were placed directly on different sides of each blood tube. Thus, blood samples for the same exposure could have different reference doses according to the different RPL GDs attached. For the DCA, blood tubes were distributed across 8 thermos flasks and positioned at the hips and shoulders of the phantoms. The delivered doses ranged between 0.05 and 2.17 Gy and the resulting RPL GD reference dose estimates suggested a significant dose gradient within and among tubes. Blood samples irradiated at different positions of the phantom were also mixed to simulate heterogeneous exposures with two different doses. Due to logistic reasons, blood samples were distributed to three partner laboratories which provided cell suspension of the samples to the rest of the participating laboratories. Protraction was initially not considered for the dose estimates which caused a systematic underestimation compared to the RPL GD reference doses. A relatively high heterogeneity between tubes within the same thermos flasks was detected for samples close to the source for the RPL GD reference dosimeters as well as for the DCA dose estimates. Results were compared based on the percentage of estimates within ±0.25 Gy and ±0.5 Gy of the reference doses and by the percentage of 95% confidence intervals including the RPL GD reference dose. While the dose estimates for reference doses <1 Gy were within ±0.25 Gy (90–95%) and ±0.5 Gy (95–100%) for most participants, systematic underestimation was observed for samples with reference doses >1 Gy, located closer to the source. After considering protraction time in the dose estimation, the results improved considerably for samples with reference doses >1 Gy. The authors summarized that the participants were able to provide valid dose estimates under conditions closely resembling a real-life exposure scenario.

For the GE assay, the irradiation conditions were similar to the DCA and samples were irradiated simulating 4 different exposure scenarios with protracted doses between 0.2 and 2.4 Gy. Calibration samples were sent to the participants prior to the exercise. The dose estimates provided by the participants strongly underestimated the reference doses. Due to the weather conditions, the temperatures of the blood samples could not be held constant inside the thermos flasks and varied between 39°C and 27.7°C. Moreover, the incubation time was <4 h for the samples irradiated during this exercise. Based on additional experiments performed afterwards under controlled laboratory conditions, these two factors were identified as the most likely reasons for the observed underestimation of the reference doses by the GE assay.

TABLE 2
Overview of RENEB ILC 2021

Assay	Labs	Source	Doses (Gy)	Purpose	Material	Article
DCA	33	X ray (240 kV)	0, 1.2,[a] 3.5[a]	Dose assessment	Blood	(27)
CBMN	14	X ray (240 kV)	0, 1.2,[a] 3.5[a]	Dose assessment	Blood	(29)
FISH	7	X ray (240 kV)	0, 1.2,[a] 3.5[a]	Dose assessment	Blood	(26)
GE	8	X ray (240 kV)	0–4 (n = 7),[c] 0, 1.2,[a] 3.5[a]	DEC establishment; Dose assessment	Blood	(31)
yH2AX	6	X ray (240 kV)	0–4 (n = 7),[c] 0, 1.2,[a] 3.5[a]	DEC establishment; Dose assessment	Blood	(30)
EPR	5	X ray (240 kV)	0, 1.2,[b] 3.5[b]	Dose assessment	Watch/gorilla/phone glass	(28)
EPR	5	X ray (240 kV)	0, 1.2,[b] 3.5[b]	Dose assessment	enamel	(28)
OSL	4	X ray (240 kV)	0, 1.2,[b] 3.5[b]	Dose assessment	Watch/phone glass	(28)
OSL	5	X ray (240 kV)	0, 1.2,[b] 3.5[b]	Dose assessment	resistor	(28)
TL	1	X ray (240 kV)	0, 1.2,[b] 3.5[b]	Dose assessment	Phone glass	(28)

[a] Dose in water.
[b] Kerma in air.
[c] Used for establishment of dose-effect curves (DEC) or reference samples with known doses.
[d] The publication provides and overview over all assays performed during the RENEB ILC 2021.

ILC 2021

In total 46 organizations from 27 countries participated in the RENEB ILC 2021. Several assays for biological dosimetry (DCA, CBMN, FISH, GE, yH2AX, PCC) and physical retrospective dosimetry (EPR, OSL, TL) were applied (Table 2) and the results for most of the assays are published in a number of papers in this special issue (*26–31*). Blood was irradiated in terms of dose in water and various different materials (electronic components and glass from mobile phones, watch glass and mini-biopsies of tooth enamel) were irradiated in terms of air kerma with 0, 1.2 and 3.5 Gy X-ray doses (240 kVp, 1 Gy/min, ~75 keV, 13 mA, HVL = 0.63) at the Bundeswehr Institute of Radiobiology, Munich, Germany and sent to the participating laboratories. The reporting time was relatively variable between assays and participants, ranging from hours (EPR, OSL, TL, GE, yH2AX) to days (DCA, CBMN, PCC) or even weeks for FISH (*28*). Besides general differences in the time needed for conducting the assays, not all laboratories assigned the highest priority to the task. The main goal of the exercise, the categorization of samples into clinically relevant groups, was generally very successful for all applied assays. Although the categorization into triage uncertainty intervals and the estimation of doses were mostly successfully performed, relatively strong outliers were observed for some assays (GE, FISH). For yH2AX, the highest dose point was underestimated, due to saturation of the foci signal (yH2AX). In the assay specific papers (*26, 27, 29, 30, 31*) of this special issue and the inter-assay comparison paper (*28*) these observations are discussed in detail. In most cases it was possible to identify methodological problems which can help the participants with problematic results to improve their workflow in future. In the case of EPR on mini-biopsies of tooth enamel, the results of the participants were reported in terms of enamel kerma as recommended in ISO standard 13304-2 (*32*). Therefore, a direct comparison to the reference doses given in terms of air kerma and to results from other assays reported either in air kerma or absorbed dose in water was not possible without applying additional conversions. Using a conversion factor between enamel/air kerma of 6.5 (*28*), the results agreed well with the air kerma references. Moreover, for cytogenetic assays (DCA, CBMN, FISH and PCC) the dose estimates were systematically higher than the reference doses (*26–29*). This systematic shift can partly be attributed to differences in the biological effectiveness between the irradiation sources used for the establishment of calibration curves (mostly ^{60}Co or ^{137}Cs gamma rays) and for the exposure of the samples of this ILC (X rays, 240 kVp). The other factors contributing to this shift remain unknown and possible reasons are discussed in detail elsewhere (*27*) and will be the basis for future research in the field of biological dosimetry to improve the design of future ILCs.

ILCs Over Time for the DCA

Up to now, the DCA, which is considered as the "gold standard" for biological dosimetry, is the assay that has been evaluated in most RENEB ILCs and performed by most laboratories (Table 1). This provides the opportunity to compare the dose estimation results over time on a relatively large dataset. However, as described above, blood samples for these exercises were irradiated with different sources and the exercises addressed different research questions, including telescoring, triage scoring, full mode scoring, acute and protracted exposures, heterogeneous and homogeneous whole-body exposures and a field exercise. Therefore, the results of these ILCs are only partly comparable. Nevertheless, the comparison of the ILC results from the DCA assay over time is important to further improve the performance of the method and the

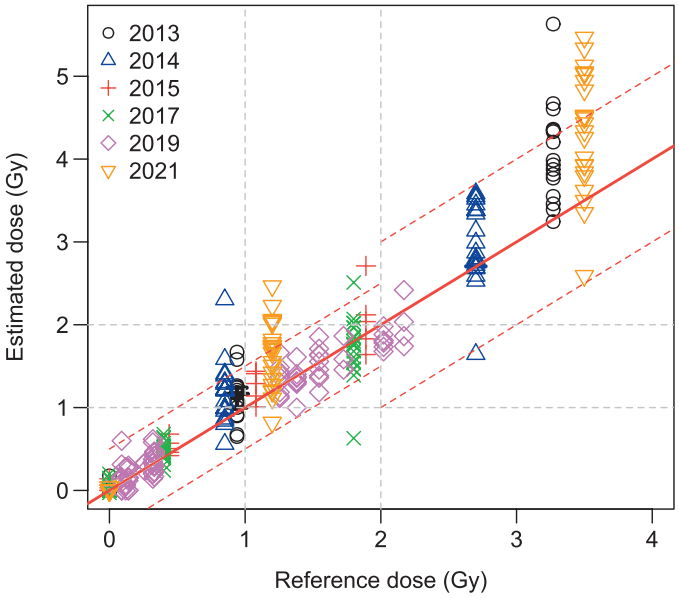

FIG. 1. Comparison of DCA dose estimates for RENEB ILCs over time. The reference doses (x-axis) and the corresponding dose estimates (y-axis) from RENEB member laboratories are shown for all ILCs conducted from 2013–2021. Only samples simulating homogeneous whole-body exposures are shown for each exercise. For the field exercise (2019), protraction times were considered for the estimation of doses for samples with reference doses >1 Gy. The red solid line shows the bisecting line and the red dashed lines show the triage uncertainty intervals of ±0.5 Gy for doses ≤2.5 Gy and ±1 Gy for doses >2.5 Gy as suggested elsewhere (*37*). The dashed gray lines indicate the clinically relevant groups of <1 Gy, 1–2 Gy and >2 Gy. For participants with dose estimates based on manual and semi-automatic scoring, only the manual result is shown.

design of future ILCs. For this comparison, only samples simulating a homogeneous whole-body exposure were used and only the results from RENEB members were considered. A control (0 Gy) sample was included in four of the six exercises conducted so far and the dose estimates were always classified in the correct triage uncertainty interval (0–0.5 Gy) and the correct clinically relevant group (0–1 Gy), suggesting that the identification of unexposed individuals can be successfully performed by the RENEB partners (Fig. 1 and Table 3). Similarly, samples exposed to doses <1 Gy (and >0 Gy) were also successfully categorized in the correct triage uncertainty interval (±0.5 Gy) in most of the cases (79% to 100%; Table 3). Samples irradiated with doses <0.5 Gy were always classified in the correct clinically relevant group. The samples irradiated with 0.85 Gy (ILC 2014) and 0.94 Gy (ILC 2013) showed a tendency for overestimation and were therefore often classified in the 1–2 Gy group. For samples irradiated with doses between 1 Gy and 2.5 Gy, the estimates were also within the triage uncertainty interval (±0.5 Gy) in most cases. Here, the exception is the current RENEB ILC 2021 where only 43% of the estimates for the sample irradiated with 1.2 Gy were within ±0.5 Gy of the reference dose and 28% of the estimates were wrongly classified in the >2 Gy group (Fig. 1 and Table 3). While samples irradiated with doses >2.5 Gy were mostly categorized in the correct clinically relevant group (95–100%), the results were sometimes less reliable in terms of the triage uncertainty intervals (±1 Gy). While the dose estimates of the sample irradiated with 2.7 Gy during the ILC 2014 were within the triage uncertainty interval in 95% of the cases, the dose estimates for samples irradiated with 3.27 Gy (ILC 2013) or 3.5 Gy (ILC 2021) were only in 63% or 52% of the cases within the triage uncertainty interval, respectively (Fig. 1 and Table 3). Moreover, for both samples irradiated with doses >3 Gy a considerable degree of overestimation could be observed (Fig. 1). In contrast to ILCs in the past, the observed shift was much higher during the RENEB ILC 2021 and a similar result was also observed for the sample irradiated with 1.2 Gy, suggesting that this observation is related to sample handling or the irradiation conditions (e.g. X ray vs. γ ray).

Different Phases of ILCs and Challenges during RENEB ILC 2021

The organization of an ILC is very labor intensive and involves several phases (planning, reference dosimetry, irradiation, control dosimetry, shipment, dose estimation, data collection and analysis, interpretation and publishing). All phases of the RENEB ILC 2021 required several rounds of discussion between the organizing institution, all contributing partners and the RENEB task leaders of each particular assay and the task leader of the statistical evaluation of ILCs. A good cooperation between physicists in charge of the dosimetry of the irradiation facility and the organizers is highly needed.

The first phase of an ILC involves the whole planning. Firstly, it must be decided which assays should be included in the exercise and what are the common aims of the ILC for all assays and the specific research questions for each single assay. As the stage of development is very different between established and emerging assays, it is difficult to define common strategies for evaluating and comparing the results between different assays. For instance, during the planning of the RENEB ILC 2021, it became clear, that for some assays (GE with one exception, yH2AX, EPR on glass), calibration samples will be sent to the participants prior to the exercise while for other assays (DCA, CBMN, TRANS, PCC, EPR enamel mini-biopsy) pre-established calibration curves or sample re-irradiation methods (OSL and TL) were used. If calibration samples are exposed to the same source as the test samples, the situation will significantly deviate from a real accident, where the source used for the calibration samples will mostly be different from the source a person was exposed to. In the case of EPR on glass, calibration samples were sent prior to the exercise because some of the participants did not have any experience with this material. The purpose here was not to evaluate individual lab performance (proficiency test), but to evaluate the method and the ability of the EPR dosimetry

TABLE 3
DCA Results from RENEB ILCs since 2013

		Clinically relevant groups				
	Ref dose (Gy)	0–1 Gy	1–2 Gy	>2 Gy	Accuracy	Triage category
2013	0	100%	0%	0%	100%	100%
	0.94	29%	71%	0%	29%	88%
	3.27	0%	0%	100%	100%	63%
2014	0.85	37%	58%	5%	37%	79%
	2.7	0%	5%	95%	95%	95%
2015	0	100%	0%	0%	100%	100%
	0.44	100%	0%	0%	100%	100%
	1.08	0%	100%	0%	100%	100%
	1.89	0%	40%	60%	40%	80%
2017	0	100%	0%	0%	100%	100%
	0.4	100%	0%	0%	100%	100%
	1.8	5%	75%	20%	75%	90%
2019	0.09–0.14	100%	0%	0%	100%	94%
	0.26–0.33	100%	0%	0%	100%	100%
	1.5–2.2	0%	87.5%	12.5%	56%	100%
	1.3–1.7	6%	94%	0%	94%	100%
2021	0	100%	0%	0%	100%	100%
	1.2	5%	67%	28%	67%	43%
	3.5	0%	0%	100%	100%	52%

Notes. The percentage of dose estimates categorized in one of the three clinically relevant groups (<1 Gy, 1–2 Gy, >2 Gy) or in the correct triage category considering defined uncertainty intervals (±0.5 Gy for doses <=2 Gy or ±1 Gy for doses >2 Gy) is shown for all samples simulating a homogeneous whole-body exposure (last column). The table shows only the results from RENEB member laboratories.

community to provide consistent results. Before the exercise it was discussed if the FISH assay should be included as it will usually not be applied in large-scale emergency scenarios but rather for exposures that occurred several months or years in the past. Eventually, although the design of this ILC did not exactly fit the purpose of the FISH assay, it was decided to include it, to validate and improve the performance of the participants. To enable an efficient evaluation of the results of this ILC, harmonized excel templates to collect the results of the participants had to be prepared for all assays. It must also be decided who will participate in the ILC, RENEB members only or, also other laboratories. Obviously, increasing the number of participants and the number of assays will significantly increase the amount of workload during almost all phases of the ILC (irradiation, shipment and evaluation of the results). Moreover, including less experienced teams in the ILC might lead to higher variability in the results, but can also help to identify training needs to improve the workflow for less experienced teams.

The next phase of an ILC involves the planning and execution of the irradiation of the test samples. Depending on the aim of the exercise, dose ranges for the irradiation of the test samples need to be decided prior to the exercise. For the ILC 2021, it was decided to include one control sample, one sample in the 1–2 Gy dose range and one sample >2 Gy, corresponding to the clinically relevant groups defined during the MULTIBIODOSE project (*20*). The exact doses of the blind samples were decided by the organizing institution and only communicated to the participants after the deadline for submitting the results. It is crucial to ensure that the irradiation source is appropriately calibrated, and that the irradiation setup cannot lead to a bias between the physical reference doses and the doses absorbed by the samples. If several samples are irradiated at once, it needs to be ensured that the samples are homogeneously exposed. Necessarily, a traceable reference dosimetry has to be performed for the irradiation facility used with appropriate means of measurements and reference protocols for the different set-up used to define the reference doses in the different irradiated materials. A control dosimetry during the irradiation of samples is highly recommended (e.g., TLDs, alanine, ionization chambers), in the eventuality of a questionable irradiation. It is also desirable to perform the irradiation with set-up and reference dosimetry in strict accordance with the RENEB QA manual (absorbed dose in water for biological samples) or ISO standard (enamel kerma for tooth enamel). During this ILC, the radiation source was calibrated in air kerma and associated absorbed doses in water were calculated. Clearly, experienced physicists must be involved in the planning phase of the irradiation to ensure appropriate reference dosimetry. The SOPs for sample handling prior, during and after irradiation of the samples are variable between different assays. Often the organizing institution does not have the same level of experience for all assays involved in an ILC and it is therefore crucial that the correct sample handling (e.g., temperature, incubation time) is communicated before the ILC to ensure that the results are not biased. However, even when doing so, unwanted failures happen. For instance, during the RENEB ILC 2021, samples for the cytogenetic

assays were accidentally not incubated for 2 h at 37°C but at room temperature, although outlined in corresponding protocols. It was difficult to assess the effect of this deviation from the recommended SOP on the results submitted by the participants, but this failure stimulated further research to examine the impact of repair at different temperatures.

After the samples are irradiated, they need to be shipped to the participants. This is a crucial interface and although defined by many regulations, it is highly recommended to involve experienced shipment companies before and during the procedure to minimize delays and problems during the transport and delivery of samples. The shipment of blood samples must be performed by express service labeled as UN3373 category B using packaging instructions P650. In emergencies the infection status of the blood donor is usually unknown. Therefore, for safe transport blood samples are sent according to the regulations for dangerous goods (Division 6.2 - infectious substances). Each package should include a temperature logger and a dosimeter to monitor the temperature and any dose received by the samples during transport. For the gene expression assay, frozen samples should be shipped on wet ice under defined conditions and packing instruction P650. For lithium-ion batteries (e.g., mobile phones) also dangerous goods regulations have to be considered.

After the samples are shipped, the participants apply their SOPs and should provide dose estimates for each sample. All partners should use the "sample code" given by the organizing lab and not use an internal lab code. Otherwise, reported dose estimates cannot be allocated to the corresponding test samples. It is important to fix a deadline for the reporting of the results and results submitted after the deadline should not be accepted, as the reference doses might already be known to the participants. It should be decided prior to the exercise which information should be collected from the participants to enable quality checks of the provided results and to identify potential reasons for deviations from the reference dose. Ideally, the participants provide the dose estimates with a corresponding assessment of the uncertainty (e.g., 95% confidence interval). The participants should clearly communicate if they encountered any problems, to simplify the final interpretation of the results.

The final phase of the ILC includes the evaluation, interpretation and publishing of the results. For the ILC 2021 it was decided that the organizing institution collects the results and that the data analysis was to be coordinated by the task leaders of each particular assay supported by the RENEB task leader for statistical evaluation. Moreover, each task leader of an assay had the responsibility to write an assay specific paper for this special issue and the organizers of the ILC were responsible to write an additional "inter-assay" paper to summarize and compare the results over all assays. While the aim of the inter-assay paper was to show all results as initially submitted, the aim of the assay specific papers was to provide a detailed analysis, to detect problems and to identify and discuss the reasons for outliers or unexpected results. Several rounds of discussion were required to provide an interpretation of the results that was accepted by the participants for the different assays. To enable an effective data analysis, it is crucial that the results are provided in a standardized manner by all participants. For this ILC, excel templates were provided prior to the exercise which could then be imported by using a data analysis software (e.g., R). Some participants submitted several dose estimates for one test sample, e.g., evaluated by different scorers or software tools or by using different gene sets. In the future, it must be clearly communicated prior to the exercise, if only one result per team is accepted or if several results are accepted to allow comparisons between different methods. After the initial evaluation of the results, unexpected results need to be discussed within the group of experts for each assay. It is crucial to identify the reasons for outliers and biased dose estimates to improve the accuracy of dose estimates and the design of ILCs in the future.

CONCLUSIONS AND LESSONS LEARNED

In the frame of RENEB, several ILCs have been conducted in the past for various assays. The focus of these ILCs was very variable to address a number of different research questions. Moreover, the ILCs were organized by different institutions and the samples were irradiated with different sources and radiation qualities. This low level of standardization between the different ILCs has advantages and disadvantages. The main advantage is clearly, that variations in the design of the ILCs enable the identification of problems related to different exposure conditions, which might help to improve the workflow for various situations encountered in real life scenarios. The main disadvantage of a low degree of standardization between ILCs is that it is difficult to compare the development of results over time and that certification of participants with regard to providing satisfactory dose estimates is only partly possible. In contrast to the RENEB approach, the Canadian Network for biological dosimetry performs annual inter-laboratory comparisons for DCA and CBMN assays with a higher degree of standardization which enables a more meaningful analysis of the development of the results over time (*33*). Nevertheless, the comparison of the RENEB ILCs over time for the DCA showed that the dose estimates were very reliable for doses <2 Gy and especially for the identification of the unexposed samples. Although only few homogeneously irradiated samples with doses >2.5 Gy were so far included in RENEB ILCs, a tendency for overestimation was observed for higher doses. Future ILCs should include a number of doses >2.5 Gy to evaluate whether this is a systematic trend or whether this observation was rather related to the exposure conditions

than to the biased dose estimates. For the more emerging assays in biological dosimetry (e.g., GE, yH2AX, PCC), ILCs in the past were rather designed to further develop and harmonize methodological aspects and the development is still in progress. For the more established assays (e.g., DCA and CBMN), the level of experience is much higher for most RENEB members and most exercises in the past were therefore designed to simulate accident situations, to validate and optimize the performance of the participants for different scenarios.

One of the aims of the ILC 2021 was to compare the results between different assays. However, it became obvious, that this comparison is only partly possible as the assays are used for different exposure situations and the level of development is very different between the assays. Due to the following reasons, the comparison of results between different assays should be interpreted with care:

1. For some assays (yH2AX, GE, EPR on glass) calibration curves were not available for most of the participants and calibration samples irradiated with the same X-ray source as used for the test samples were sent to most participants prior to the exercise. In contrast, for cytogenetic assays and EPR on enamel, calibration curves were available for most participants prior to the exercise. However, for cytogenetic assays the curves are mostly not based on the same radiation quality or energy spectrum as the source used during an ILC, which can potentially lead to biased results. Moreover, some laboratories use dose-effect curves with reference doses given in terms of air kerma and others in terms of dose in water and dose-effect curves are established with different beam qualities. These parameters will influence the resulting dose estimates and this should be kept in mind when results are compared between laboratories.
2. For some assays (yH2AX, PCC, GE, TL, OSL and EPR on glass) the signal decreases faster over time than for other assays and as an extreme, dose estimates for approximately more than 24 h postirradiation will either not be possible or require special correction factors. These assay dependent differences have to be considered in a real large-scale accident as it will, in most cases, not be possible to obtain samples so shortly after the accident and also to know when individuals could have been irradiated. Nevertheless, molecular biological driven assays have the advantage that dose estimates can be provided within hours after irradiation and many samples can be processed simultaneously (*34*).
3. While dose estimates based on blood samples give an average whole-body dose (or even a partial-body dose with the fraction irradiated), EPR and OSL based on mobile phone components, watch glass or tooth enamel provide a local body dose. Hence, ideally, as proposed in the output of the MULTIBIODOSE-Project (*20*), the combination of several methods can provide crucial and pertinent indication on the level of heterogeneity of the dose distribution in the organism.
4. For some assays (e.g., GE, yH2AX, PCC, EPR on glass, OSL and TL) there are currently no standardized and harmonized statistical methods for the estimation of calibration curves, doses and confidence intervals. Hence, comparisons can be biased by differences in statistical methods and point estimates without an assessment of the corresponding uncertainty do have only limited value. It should be the aim to harmonize the methods in the future and to develop approaches for the assessment of uncertainties, as already practiced by some but not all labs.
5. The number of participants was very different between the assays. For assays with very few participants (e.g., PCC) the possible conclusions are much more limited compared to assays with many participants (e.g., DCA).
6. The level of development and the degree of standardization and harmonization is much higher for the established cytogenetic methods (e.g., DCA and CBMN) and EPR on enamel (even if here only mini-biopsy of 5 mg were used, compared to the classic 100 mg mass used in support of epidemiology studies) and among cytogenetic methods DCA and CBMN should, therefore, still be the first choice for a reliable assessment of doses, in particular, if the time since exposure was $>$24 h. However, when early dose estimates for triage purposes (1–2 days postirradiation) are required, GE and yH2AX could be valuable tools for a first screening and categorization of individuals immediately after an accident if timely blood sampling is manageable.
7. For EPR on glass, many participants did not have experience with this material and the aim of the exercise was therefore not to evaluate the lab performance but to generally evaluate the ability of the EPR community to provide consistent results.

About the physical dosimetry methods (EPR/TL/OSL) it has not yet been decided which materials should be implemented in the operational basis of RENEB in future. Here, EPR on enamel is the most established method with $>$40 years of experience and an associated ISO standard and an IAEA manual (*32, 35, 36*). Especially, if minimally invasive methods with small sample volumes or mass can be applied by using higher frequency EPR spectrometers. This technique is currently only available to a limited number of laboratories and the performance of the method using conventional spectrometers with small sample mass or volume must be further evaluated. Moreover, the method is very sensitive to low energy X rays ($<$100 keV) as used during the RENEB ILC 2021 and the performance might be less satisfactory if the irradiation was performed with photons of higher energy. Although physical dosimetry on components from mobile phones measured by TL/OSL or EPR can provide interesting possibilities, there are many difficulties, such as signal fading, sample handling and the

availability of the materials after an accident which limit the applicability within the RENEB network, especially for large-scale radiation accidents. The future selection of a physical retrospective dosimetry method for the operational basis of RENEB is complex and is, in addition to the performance of the laboratories, a function of the number of laboratories performing the method, the availability of samples, the invasiveness of sample collection, the possibility to timely collect and transport samples, the sample preparation time, the stability of the signal, the considered scenario as well as the possibility of harmonization and standardization.

The RENEB ILC 2021 was open for RENEB and non-RENEB laboratories and various assays were included. The exercise showed that the level of experience was very different between participants for some of the assays, which can lead to increased variability in the results. The identification of methodological problems helps the teams to improve the results in the future. Due to the high number of participants and assays, the evaluation and interpretation of the results was not always trivial and was very labor intensive. It is important to verify prior to the exercise that enough personnel resources for data analysis are available. Otherwise, the data cannot be appropriately analyzed and reporting or publication will not be possible. Moreover, the communication was often difficult, as many partners with different scientific backgrounds were involved and it was often challenging to find common solutions for data analysis and reporting. However, the big advantage of the design of this ILC was, that from the discussions between experts for the different assays a lot can be learnt about the applicability of the assays for a large-scale RN event.

This exercise did grow over time, because more and more institutions worldwide became aware of this activity and asked for permission to participate. This generated a very unique exercise where a great number of expert labs worldwide participated and it provided an overview of the current status of biological and physical retrospective dosimetry. This also reflects the will of the society for training and challenging their expertise to be better prepared for future RN events.

In conclusion, the current and past exercises showed that the panel of methods for biological dosimetry and physical retrospective dosimetry included in the operational basis in RENEB can successfully be used to categorize individuals into clinically relevant groups under controlled laboratory conditions. However, for a real large-scale event, the limitations of each of the assays have to be considered and the best combination of assays should be chosen depending on the exposure scenario.

Received: November 28, 2022; accepted: February 3, 2023; published online: Month 0, 2023

REFERENCES

1. Barnard S, Ainsbury EA, Al-hafidh J, Hadjidekova V, Hristova R, Lindholm C, et al. The first gamma-H2AX biodosimetry intercomparison exercise of the developing European biodosimetry network RENEB. Radiation Protection Dosimetry. 2015; 164(3): 265-70. doi: 10.1093/rpd/ncu259
2. Depuydt J, Baeyens A, Barnard S, Beinke C, Benedek A, Beukes P, et al. RENEB intercomparison exercises analyzing micronuclei (Cytokinesis-block Micronucleus Assay). International Journal of Radiation Biology. 2017; 93(1):36-47. doi: 10.1080/09553002.2016.1206231
3. Moquet J, Barnard S, Staynova A, Lindholm C, Monteiro Gil O, Martins V, et al. The second gamma-H2AX assay inter-comparison exercise carried out in the framework of the European biodosimetry network (RENEB). International Journal of Radiation Biology. 2017; 93(1):58-64. doi: 10.1080/09553002.2016.1207822
4. Oestreicher U, Samaga D, Ainsbury EA, Antunes AC, Baeyens A, Barrios L, et al. RENEB intercomparisons applying the conventional Dicentric Chromosome Assay (DCA). Int J Radiat Biol. 2017; 93(1):20-29. doi: 10.1080/09553002.2016.1233370
5. Romm H, Ainsbury EA, Barquinero JF, Barrios L, Beinke C, Cucu A, et al. Web based scoring is useful for validation and harmonisation of scoring criteria within RENEB. International Journal of Radiation Biology. 2017; 93(1):110-17. doi: 10.1080/09553002.2016.1206228
6. Barquinero JF, Beinke C, Borràs M, Buraczewska I, Darroudi F, Gregoire E, et al. RENEB biodosimetry intercomparison analyzing translocations by FISH. International Journal of Radiation Biology. 2017; 93(1):30-35. doi: 10.1080/09553002.2016.1222092
7. Gregoire E, Barquinero JF, Gruel G, Benadjaoud M, Martinez JS, Beinke C, et al. RENEB InterLaboratory Comparison 2017; limits and pitfalls of ILCs. International Journal of Radiation Biology. 2021; 97:888-905.
8. Endesfelder D, Oestreicher U, Kulka U, Ainsbury EA, Moquet J, Barnard S, et al. RENEB/EURADOS field exercise 2019: robust dose estimation under outdoor conditions based on the dicentric chromosome assay. Int J Radiat Biol. 2021; 97(9):1181-98. doi: 10.1080/09553002.2021.1941380
9. Trompier F, Burbidge C, Bassinet C, Baumann M, Bortolin E, De Angelis C, et al. Overview of physical dosimetry methods for triage application integrated in the new European network RENEB. International Journal of Radiation Biology. 2017; 93(1):65-74. doi: 10.1080/09553002.2016.1221545
10. Bassinet C, Woda C, Bortolin E, Della Monaca S, Fattibene P, Quattrini MC, et al. Retrospective radiation dosimetry using OSL of electronic components: Results of an inter-laboratory comparison. Radiation Measurements. 2014; 71:475-79. doi: 10.1016/j.radmeas.2014.03.016
11. Fattibene P, Trompier F, Wieser A, Brai M, Ciesielski B, De Angelis C, et al. EPR dosimetry intercomparison using smart phone touch screen glass. Radiat Environ Biophys. 2014; 53(2): 311-20. doi: 10.1007/s00411-014-0533-x
12. Manning G, Macaeva E, Majewski M, Kriehuber R, Brzóska K, Abend M, et al. Comparable dose estimates of blinded whole blood samples are obtained independently of culture conditions and analytical approaches. Second RENEB gene expression study. International Journal of Radiation Biology. 2017; 93(1):87-98. doi: 10.1080/09553002.2016.1227105
13. Abend M, Badie C, Quintens R, Kriehuber R, Manning G, Macaeva E, et al. Examining Radiation-Induced In Vivo and In Vitro Gene Expression Changes of the Peripheral Blood in Different Laboratories for Biodosimetry Purposes: First RENEB Gene Expression Study. Radiation Research. 2016; 185(2):109. doi: 10.1667/rr14221.1
14. Abend M, Amundson SA, Badie C, Brzoska K, Hargitai R, Kriehuber R, et al. Inter-laboratory comparison of gene expression biodosimetry for protracted radiation exposures as part of the RENEB and EURADOS WG10 2019 exercise. Sci Rep. 2021; 11(1):9756. doi: 10.1038/s41598-021-88403-4
15. Brzozowska B, Ainsbury EA, Baert A, Beaton-Green L, Barrios

L, Barquinero JF, et al. RENEB accident simulation exercise. International Journal of Radiation Biology. 2017; 93(1):75-80. doi: 10.1080/09553002.2016.1206230

16. Ainsbury EA, Badie C, Barnard S, Manning G, Moquet J, Abend M, et al. Integration of new biological and physical retrospective dosimetry methods into EU emergency response plans - joint RENEB and EURADOS inter-laboratory comparisons. Int J Radiat Biol. 2017; 93(1):99-109. doi: 10.1080/09553002.2016.1206233

17. Terzoudi GI, Pantelias G, Darroudi F, Barszczewska K, Buraczewska I, Depuydt J, et al. Dose assessment intercomparisons within the RENEB network using G0-lymphocyte prematurely condensed chromosomes (PCC assay). International Journal of Radiation Biology. 2017; 93(1):48-57. doi: 10.1080/09553002.2016.1234725

18. ISO13528. Statistical methods for use in proficiency testing by interlaboratory comparisons. Geneva. 2005.

19. Di Giorgio M, Barquinero JF, Vallerga MB, Radl A, Taja MR, Seoane A, et al. Biological dosimetry intercomparison exercise: an evaluation of triage and routine mode results by robust methods. Radiat Res. 2011; 175(5):638-49. doi: 10.1667/RR2425.1

20. Jaworska A, Wojcik A, Ainsbury EA, Fattibene P, Lindholm C, Oestreicher U, et al. Guidance for using MULTIBIODOSE tools in Emergencies for adiation Emergency Response Organisations in Europe. RENEB: RENEB, 2017; 52 p. (https://www.reneb.net/wp-content/uploads/2021/02/multibiodose-guidance-small.pdf)

21. M'Kacher R, El Maalouf E, Terzoudi G, Ricoul M, Heidingsfelder L, Karachristou I, et al. Detection and Automated Scoring of Dicentric Chromosomes in Nonstimulated Lymphocyte Prematurely Condensed Chromosomes After Telomere and Centromere Staining. International Journal of Radiation Oncology Biology Physics. 2015; 91(3):640-49. doi: 10.1016/j.ijrobp.2014.10.048

22. Knehr HZ, M. Bauc S. FISH-based analysis of radiation-induced chromosomal aberrations using different nomenclature systems. International Journal of Radiation Biology. 2009; 73(2):135-41. doi: 10.1080/095530098142509

23. Trompier F, Baumann M, Barrios L, Gregoire E, Abend M, Ainsbury EA, et al. Investigation of the influence of calibration practices on cytogenetic laboratory performance for dose estimation. International Journal of Radiation Biology. 2016; 93(1):118-26. doi: 10.1080/09553002.2016.1213455

24. Hernandez A, Endesfelder D, Einbeck J, Puig P, Benadjaoud MA, et al. Biodose Tools: an R shiny application for biological dosimetry. Int J Radiat Biol. 2023 Feb 7;1-13. doi: 10.1080/09553002.2023.2176564. Online ahead of print.

25. Waldner L, Bernhardsson C, Woda C, Trompier F, Van Hoey O, Kulka U, et al. The 2019-2020 EURADOS WG10 and RENEB Field Test of Retrospective Dosimetry Methods in a Small-Scale Incident Involving Ionizing Radiation. Radiat Res. 2020; 195:253-64. doi: 10.1667/RADE-20-00243.1

26. Barquinero JF, Abe Y, Aneva N, Endesfelder D, Georgieva D, Goh VST, et al. RENEB Inter-Laboratory Comparison 2021: The FISH-based translocation assay. Radiat Res. 2023; 199:583–590.

27. Endesfelder D, Oestreicher U, Bucher M, Beinke C, Siebenwirth C, Ainsbury E, et al. RENEB Inter-Laboratory Comparison 2021: The Dicentric Chromosome Assay. Radiat Res. 2023; 199:556-570.

28. Port M, Barquinero JF, Endesfelder D, J. M, Oestreicher U, Terzoudi G, et al. RENEB Inter-Laboratory Comparison 2021: Interassay comparison of eight dosimetry assays. Radiat Res. 2023; 199:535–555.

29. Vral A, Endesfelder D, Balázs K, Beinke C, Cuceu Petrenci C, Finot F, et al. RENEB Inter-Laboratory Comparison 2021: The Cytokinesis-Block Micronucleus Assay. Radiat Res. 2023; 199:571–582.

30. Moquet J, Ainsbury E, Balázs K, Barnard S, Hristova R, Lumniczky K, et al. RENEB Inter-Laboratory Comparison 2021: The gamma-H2AX foci assay. Radiat Res. 2023; 199:591–597.

31. Abend M, A. AS, Badie C, Brzoska K, Kriehuber R, Lacombe J, et al. RENEB Inter-Laboratory Comparison 2021: The gene expression assay. Radiat Res. 2023; 199:598–615.

32. ISO13304-2. Radiological protection — Minimum criteria for electron paramagnetic resonance (EPR) spectroscopy for retrospective dosimetry of ionizing radiation — Part 2: Ex vivo human tooth enamel dosimetry. Geneva. 2020.

33. Wilkins RC, Beaton-Green LA, Lachapelle S, Kutzner BC, Ferrarotto C, Chauhan V, et al. Evaluation of the annual Canadian biodosimetry network intercomparisons. International Journal of Radiation Biology. 2015; 91(5):443-51. doi: 10.3109/09553002.2015.1012305

34. Port M, Ostheim P, Majewski M, Voss T, Haupt J, Lamkowski A, et al. Rapid High-Throughput Diagnostic Triage after a Mass Radiation Exposure Event Using Early Gene Expression Changes. Radiat Res. 2019; 192(2):208-18. doi: 10.1667/RR15360.1

35. ISO13304-1. Radiological protection — Minimum criteria for electron paramagnetic resonance (EPR) spectroscopy for retrospective dosimetry of ionizing radiation — Part 1: General principles. Geneva. 2020.

36. IAEA. Use of electron paramagnetic resonance dosimetry with tooth enamel for retrospective dose assessment. Dosimetry and Medical Radiation Physics Section International Atomic Energy Agency, Vienna. 2002.

37. Lloyd DC, Edwards AA, Moquet JE, Guerrero-Carbajal YC. The role of cytogenetics in early triage of radiation casualties. Appl Radiat Isot. 2000; 52(5):1107-12. doi: 10.1016/s0969-8043(00)00054-3