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To cite this article: Andrzej Wojcik, Ursula Oestreicher, Leonard Barrios, Anne Vral, Georgia Terzoudi, Elizabeth Ainsbury, Kai Rothkamm, Francois Trompier & Ulrike Kulka (2017) The RENEB operational basis: complement of established biodosimetric assays, International Journal of Radiation Biology, 93:1, 15-19, DOI: [10.1080/09553002.2016.1235296](https://doi.org/10.1080/09553002.2016.1235296)

To link to this article: <http://dx.doi.org/10.1080/09553002.2016.1235296>



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Published online: 25 Oct 2016.



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RESEARCH ARTICLE

 OPEN ACCESS

The RENEB operational basis: complement of established biodosimetric assays

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ABSTRACT

Purpose: To set up an operational basis of the Realizing the European Network of Biodosimetry (RENEB) network within which the application of seven established biodosimetric tools (the dicentric assay, the FISH assay, the micronucleus assay, the PCC assay, the gamma-H2AX assay, electron paramagnetic resonance and optically stimulated luminescence) will be compared and standardized among the participating laboratories.

Methodology: Two intercomparisons were organized where blood samples and smartphone components were irradiated, coded and sent out to participating laboratories for dosimetric analysis. Moreover, an accident exercise was organized during which each RENEB partner had the chance to practice the procedure of activating the network and to handle large amounts of dosimetric results.

Results: All activities were carried out as planned. Overall, the precision of dose estimates improved between intercomparisons 1 and 2, clearly showing the value of running such regular activities.

Conclusions: The RENEB network is fully operational and ready to act in case of a major radiation emergency. Moreover, the high capacity for analyzing radiation-induced damage in cells and personal electronic devices makes the network suitable for large-scale analyses of low doses effects, where high numbers of samples must be scored in order to detect weak effects.

ARTICLE HISTORY

Received 31 March 2016
Revised 4 August 2016
Accepted 7 September 2016

KEYWORDS

Biological dosimetry;
dosimetry network;
intercomparison; radiation
emergency

Introduction

The aim of the Realizing the European Network of Biodosimetry (RENEB) project was to set up and run a European network of laboratories dealing with biological dosimetry (Kulka et al. 2012, 2016). An essential element of such a network is its operational basis, i.e. the biodosimetric assays. Seven biodosimetric assays were tested and harmonized within RENEB: five using human peripheral blood lymphocytes (PBL) and two using smartphones. The former five included the dicentric test (DIC), chromosome painting (FISH), the micronucleus test (MN), premature chromosome condensation by cell fusion (PCC) and the gamma H2AX focus test (gH2AX). The two latter methods electron paramagnetic resonance spectroscopy (EPR) in display glass of smartphones and optically stimulated luminescence (OSL) in resistors. EPR and OSL are physicochemical methods, so strictly speaking, cannot be regarded as biodosimetric assays. However, since they are designed for retrospective assessment of the individual dose, we will continue using this term for the sake of simplicity.

The applicability of some of the assays (DIC, MN, gH2AX, OSL and EPR) as quick, triage tools for large scale

emergencies was tested and optimized in an earlier European Union-funded project MULTIBIODOSE (Wojcik et al. 2014). In RENEB, we included FISH and PCC and focused on harmonizing the use of all assays so that, in case of a large emergency, laboratories can effectively collaborate to estimate doses and to triage a large number of people. To this end we carried out two intercomparisons during which irradiated blood samples and smartphones were sent to the RENEB members for analysis. We also organized an accident simulation exercise, where the communication and collection of results were tested. The detailed results of these activities are described in separate publications included in this special issue. The aim of this publication is to give an overview of these activities.

The biodosimetric tools used in RENEB

DIC is regarded as the gold standard for biological dosimetry because it was invented more than 50 years ago (Bender and Gooch 1962) and its excellent ability to detect an absorbed dose was demonstrated on numerous occasions (Romm et al. 2009). The signal stability is not precisely known, but the half-

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Table 1. General characteristics of the biodosimetric assays used in RENEb. Sensitivity is given for low LET radiation. See text for explanation of assay acronyms.

Assay	Time span after exposure during which the assay can yield usable results				Exposure scenario that can be detected by each method alone			Specific for ionising radiation	Sensitivity of the assay (dose range in Gy)
	Days	Weeks	Months	Years	Acute	Pro-tracted	Partial body		
DIC	✓	✓	✓		✓	✓	✓	✓	0.1–5
FISH	✓	✓	✓	✓	✓	✓			0.3–5
MN	✓	✓	✓		✓	✓	✓		0.3–5
PCC	✓				✓		✓	✓	0.3–>10
gH2AX	✓				✓		✓		0.2–5
EPR	✓	✓	✓	✓	✓	✓		✓	1–>10
OSL	✓	✓			✓	✓		✓	0.01–>10

life of dicentric chromosomes is estimated to be *ca.* 1.5 years (International Atomic Energy Agency [IAEA] 2011). FISH is used to detect aberrations in selected, painted chromosomes and its major advantage is the ability to visualise stable-type aberrations called translocations which are not visible with conventional staining (Whitehouse et al. 2005). The signal stability of translocations is superior to that of DIC and is in the range of years. MN can be regarded as an outcome of DIC, because micronuclei arise as consequence of chromosomal aberrations. Its advantage is speed of analysis and very good possibility of automation (Willemis et al. 2010). PCC is analogous to DIC; however, thanks to fusion of target interphase cells with mitotic cells, chromosomes can be visualized without the necessity to wait until the target cell reaches mitosis (Terzoudi and Pantelias 1997). Similar to PCC, gH2AX allow visualizing DNA damage shortly after radiation exposure (Horn et al. 2011). Inherent to both methods is a significant decline of the signal within 24 h post exposure. Hence, a good knowledge of the time of exposure is necessary for a proper doses assessment. EPR spectroscopy allows radiation-induced signals to be detected in inert materials such as liquid crystal display and touch screens of smartphones (Trompier et al. 2011; Fattibene et al. 2014). The main advantages of EPR are its high radiation specificity of radio-induced signals and long-term signal stability (up to several years). OSL is used to assess the dose of ionizing radiation by measuring luminescence emitted from irradiated objects under optical stimulation such as smartphone resistors (Woda et al. 2009). Its advantage is high specificity and sensitivity to radiation. The signal half-life is *ca.* 10 days. A summary of the main characteristics of the assays is given in Table 1.

The distribution of biodosimetric assays among the RENEb partner laboratories is shown in Table 2. It is clear that most partners rely on the DIC assay, followed by MN, FISH, gH2AX, PCC, EPR and OSL. This distribution of preferences probably reflects various factors such as experience of the laboratory staff and the belief in versatility of the assay for retrospective dose assessment. Moreover, it must be borne in mind that a laboratory dealing with biological dosimetry cannot exclusively deal with radiation emergency preparedness. The reason for this is that there are too few emergencies to justify financing such a laboratory. Hence, the established tools must be applicable to the type of research other than retrospective dosimetry that is carried out in a laboratory. The involvement of laboratories in research outside the field of biological dosimetry can only be encouraged because the

Table 2. Biodosimetric assays established in the RENEb partner laboratories (as of 2015) and used in the intercomparisons. See text for explanation of assay acronyms and Kulka et al. (2016) in this issue for the explanation of laboratory acronyms.

Partner and country	Biodosimetric assay						
	DIC	FISH	MN	PCC	gH2AX	EPR	OSL
BFS, Germany	X	X	X		X		
CEA, France	X						
ENEA, Italy	X		X				
HMGU, Germany						X	X
PHE, UK	X	X	X		X		
ICHTJ, Poland	X	X	X	X	X		
INSP, Romania	X		X				
IRSN, France	X	X				X	
ISS, Italy							X
ITN, Portugal	X		X		X		
LAFE, Spain	X						
NCCRP, Bulgaria	X	X	X	X	X		
NCSR D, Greece	X			X			
NRIRR, Hungary	X		X				
NRPA, Norway							
SERMAS, Spain	X	X			X		
SU, Sweden	X		X				
UAB, Spain	X	X		X			
UGent, Belgium			X		X		
UNITUS, Italy	X						
UULM, Germany	X	X	X				
Total	17	8	11	4	7	2	2

laboratory staff is trained in research approaches and methods that may then be applied in biological dosimetry, leading to its further development and perfection.

How the biodosimetric tools complement each other

The scenario of a large-scale radiation emergency is difficult to predict. It may involve hiding a high activity sealed source in a public space until many hundreds or thousands of people are irradiated or spreading of radioactive material leading to mass contamination (Rojas-Palma et al. 2009). In any case, it can easily be imagined that people will be exposed at different time-points and to different degrees without the information about the exposure scenarios being available. With this in mind, already members of the MULTIBIODOSE project recommended the parallel application of as many biodosimetric assays as possible after a radiation emergency (Ainsbury et al. 2014; Wojcik et al. 2014). The RENEb team supported this approach and expanded the number of assays as compared with the MULTIBIODOSE project. Each assay has its specific characteristics (listed in Table 2) so the total

Table 3. Summary of work carried out during the first intercomparison. See text for explanation of assay acronyms.

Biodosimetric tool	Content of the intercomparison
DIC	Part A: Telescoring: manual scoring of DIC from images provided by BFS. Two galleries of images were prepared from cells irradiated with 1.3 and 3.51 Gy. 50 images per gallery were scored by each laboratory. Reports included DIC frequencies and doses estimated based on own calibration curve. See Romm et al. (2016) for details. Part B: Blood from one donor was irradiated by BFS and sent to laboratories. Doses were 0, 0.94, 3.27 and 4.75 Gy mixed 1:1 with control blood (partial body exposure simulation). Reports included DIC frequencies and doses estimated based on own calibration curve. Only manual scoring. 50 cells scored per dose by each laboratory. See Oestreicher et al. (2016) for details.
FISH	Blood irradiated by UAB and sent to laboratories. The dose was 2 Gy. Laboratories used their own FISH cocktails and calculated genomic translocation frequencies. Reports included genomic frequencies of total translocations and doses estimated based on own calibration curves (in some cases calibration curves for DIC were used). See Barquinero et al. (2016) for details.
MN	Blood was irradiated by BFS and sent to laboratories. Doses were 0, 0.94, 3.27 and 4.75 Gy mixed 1:1 with control blood (partial body exposure simulation). Reports included MN frequencies and doses estimated based on own calibration curve. Some laboratories used manual scoring, other fully automatic or semi-automatic. 500 cells scored per dose. See Depuydt et al. (2016) for details.
PCC	Part A: Scoring of PCC from images provided by LUMC. 10 images were scored per dose of 0, 1, 2, 4 and 6 Gy to set up calibration curves. Part B: Blood irradiated at NCSR with 0, 0.5, 1, 2, 3, 4, 5 and 6 Gy, PCC slides prepared and sent to laboratories for analysis. 100 cells per point were analyzed. Part C: Blood irradiated at NCSR with 2 and 4 Gy, mixed 1:1 with control blood. PCC slides prepared and sent to laboratories for PCC for analysis. 100 cells per point were analyzed.
gH2AX	Part A: Telescoring. Blood irradiated by PHE with 0, 0.5, 1, 2 and 4 Gy and fixed for gH2AX focus scoring after 4 or 24 h. Images sent to laboratories who scored them manually or automatically. Part B: Cell scoring. Isolated lymphocyte samples irradiated by PHE with 0, 1, 2, 3 and 4 Gy incubated for 4 or 24 h and sent to laboratories who scored foci manually or automatically. Laboratories with existing calibration curves scored only 2 and 4 Gy samples. See Barnard et al. (2015) for details.
EPR	Part A: Uniform samples. Samples of bulk glass from 3 smartphones of the same model were irradiated by IRSN with 0.8, 2, 4 and 10 Gy for the calibration curve and with 0.9, 1.3 and 3.3 Gy for the blind test. Samples sent to laboratories, including selected EURADOS members. Part B: non-uniform samples. Glass from 9 smartphones of the same model were irradiated separately with by IRSN with 0.8, 2, 4 and 10 Gy for the calibration curve and with 0.9, 1.3 and 3.3 Gy for the blind test. Samples sent to laboratories, including selected EURADOS members. See Trompier et al. (2016) for details.
OSL	Smartphones of the same model were irradiated by IRSN with 0.3, 1.7 and 3.3 Gy and sent to laboratories, including selected EURADOS members. Analysis of OSL signals in resistors in 'triage mode' and 'full mode'. See Trompier et al. (2016) for details.

results can give valuable information about the exposure scenario and its time-point. The time of exposure can be deduced based on the short signal stability of the gamma-H2AX and PCC assays and the decay of the OSL signal. Partial body exposure can be detected if a personal electronic device (ped) was outside the radiation field while the majority of lymphocytes were exposed, leading to a deviation of doses assessed by EPR/OSL and the other assays. Alternatively, a ped could be inside the radiation field while the majority of lymphocytes receive a lower dose leading again to a deviation of doses assessed by EPR/OSL and the other assays.

The possibility of deducing information about the exposure scenario and time-point from a comparative analysis of doses assessed by the various assays was trained during the final year of the RENEB project in a radiation accident exercise. The details of this exercise and its results are described elsewhere in this special issue (Brzowska et al. 2016).

Intercomparisons

Maintaining a network of laboratories that will collaborate in case of a large radiation emergency will only make sense if all laboratories are similarly proficient in retrospective dose assessment. This proficiency must be regularly tested and trained. With this in mind, two intercomparisons were organized during the RENEB project during which blood samples and elements of smartphones were irradiated, coded and sent out to partners for dose assessment. The intercomparisons were carried out separately for each assay, whereby one partner could, and in fact most did, participate in several comparisons. As shown in Table 1, most partners have several biodosimetric assays established in their laboratories, some of which were in fact established thanks to the RENEB

network which offered the possibility of learning new methods.

The first intercomparison was carried out shortly after the project kicked off. An overview of how the performance of laboratories for each assay was tested is shown in Table 3. Details are described separately for each assay in reports included in this issue. The intercomparison was followed by a round of training activities during which partners could learn new assays and revise the ones requiring improvement. After that a second intercomparison was organized (Table 4).

The proficiency of each laboratory to correctly assess a dose was tested either by checking whether a reported dose fitted within a defined confidence interval of the true dose or the standard score of the dose. Relative numbers of correct dose assessments per laboratory are shown in Figure 1 for both intercomparisons. The values must be regarded with caution because they represent the total results from all assays and doses analyzed by each laboratory. Various laboratories were engaged in various numbers of assays; hence, the results are not appropriate for comparing the performance of the laboratories. Rather, the aim of this crude assessment was to verify if there was an improvement in dose assessment between intercomparisons 1 and 2, which were separated by a round of training activities. The results confirm that this was the case, clearly reinforcing the rationale behind running regular intercomparisons.

Conclusions

The RENEB consortium tested the collaborative effort of 23 laboratories to assess absorbed doses to blood samples by five biodosimetric assays and to smartphone components by two physical assays. Moreover, an accident simulation

Table 4. Summary of work carried out during the second intercomparison. See text for explanation of assay acronyms.

Biodosimetric tool	Content of the intercomparison
DIC	Blood irradiated by BFS and sent to laboratories. Doses were 0.85 and 2.7 Gy. Reports included DIC frequencies and doses estimated based on own calibration curve. Manual scoring and automated scoring. 200 cells scored per dose, whereby 50 cells were scored per one of four parallel slides for comparison of interslide variability. See Oestreicher et al. (2016) for details.
FISH	Blood irradiated by BFS and sent to laboratories. Doses were 0.85 and 2.7 Gy. Laboratories used their own FISH cocktails and calculated genomic translocation frequencies. Reports included genomic frequencies of total translocations and doses estimated based on own calibration curves (in some cases calibration curves for DIC were used). See Barquinero et al. (2016) for details.
MN	Blood irradiated by BFS and sent to laboratories. Doses were 0.85 and 2.7 Gy. Reports included MN frequencies and doses estimated based on own calibration curve. Manual scoring and automated scoring was carried out. Manual scoring: 2000 cells scored per dose (500 slides per parallel slide from two blood culture). Automated scoring: 4000 cells scored per dose (1000 slides per parallel slide from two blood culture). See Depuydt et al. (2016) for details.
PCC	Part A: Blood irradiated by BFS and sent to laboratories. Doses: 0.85 and 2.7 Gy. 40 cells were scored per dose. Part B: Two galleries of PCC images were prepared by NCSR and distributed among five additional RENEB participants who did not participate in part A. Also, a calibration curve was distributed and doses estimates based on this calibration curve were reported. See Terzoudi et al. (2016) for details.
gH2AX	Whole blood was irradiated by PHE with 0.5 and 2.5 Gy, incubated for 4 or 24 h and sent to laboratories who scored them manually or automatically. In parallel to whole blood samples lymphocytes were isolated and shipped together with whole blood. See Moquet et al. (2016) for details.
EPR	A new analysis method of EPR spectra was tested. The method overcomes the problem of confounding influence of sun light on the irradiated glass samples. Spectra generated during the first intercomparison were re-analyzed by the two RENEB partners IRSN and ISS without participation of EURADOS.
OSL	OSL was tested in a realistic accident exercise that was carried out within the FP7 CATO project. Participants received smartphone components exposed to various doses of radiation. Dose estimates were carried out similarly as during intercomparison 1.

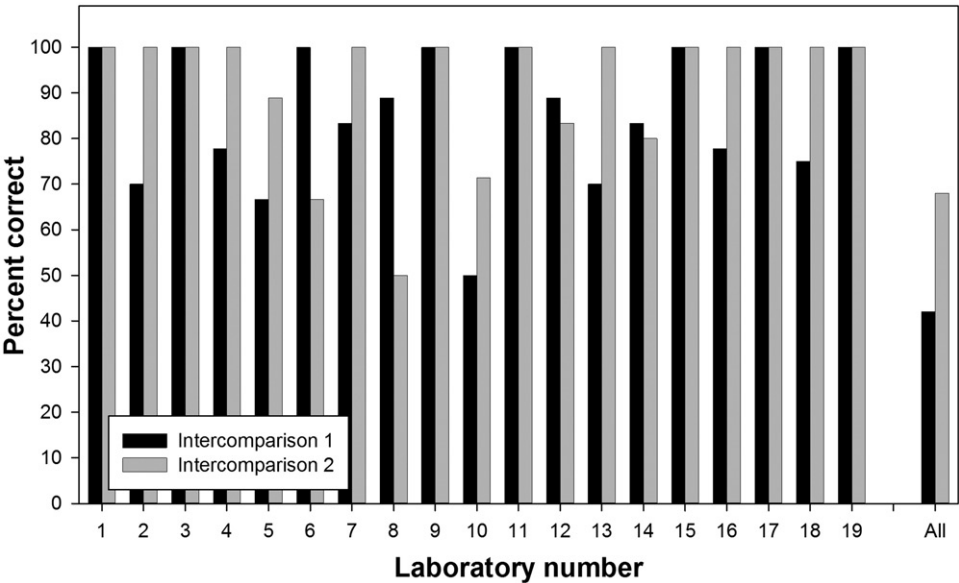


Figure 1. Performance of RENEB laboratories during the two intercomparisons. Values refer to the relative number of doses correctly estimated by a laboratory. Results from all assays and dose-points were pooled. EPR and OSL analyses are excluded. ‘All’ refers to percentage of all 19 laboratories that reached the value of 100.

exercise was carried out to train how the network is activated in case of an emergency and how large amounts of dosimetric data are interpreted and collated. The RENEB network is thus fully operational and ready to act in case of a major radiation emergency. The improvement of precision of dose estimates from intercomparison 1 to intercomparison 2 clearly demonstrated the necessity of carrying out regular intercomparison exercises. Such exercises are a part of the long-term training programme and are included in the RENEB quality manual for the future activity of the network. It should not remain unmentioned that the high capacity of the network can be applied not only for retrospective dose assessment following radiation emergencies, but also for large-scale analyses of low doses effects, where high numbers of samples must be scored in order to detect weak effects.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Funding

This work was supported by the EU within the 7th Framework Programme [EURATOM grant agreement No. 295513]

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