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International comparison exercise for biological dosimetry after exposures with neutrons performed at two irradiation facilities as part of the BALANCE project

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Author Contributions

Ulrike Kulka, Ursula Oestreicher, Ulrich Giesen, Guy Garty, Andrzej Wojcik organized and conducted the irradiations and distribution of blood samples. Ursula Oestreicher, Martin Bucher, Christina Beinke, Matthias Port, Gaëtan Gruel, Eric Gregoire, Georgia Terzoudi, Sotiria Triantopoulou, Elizabeth Ainsbury, Jayne Moquet, Mingzhu Sun, María Jesús Prieto, Mercedes Moreno Domene, Joan-Francesc Barquinero, Monica Pujol-Canadell, Anne Vral, Ans Baeyens provided dose estimates based on the DCA. David Endesfelder did the statistical evaluation of the ILC results and wrote the manuscript. All authors reviewed and revised the manuscript.

Statement of Ethics

In Germany, blood samples from healthy adult donors were obtained by physicians according to §15 of the code of medical ethics for physicians in Bavaria, Germany, following the principles of the Declaration of Helsinki. In the US blood was collected under Columbia University Institutional Review Board (IRB) protocol AAAS3035.

For blood samples taken in Germany, ethics approval was not required according to the Ethics Committee of the Bavarian State Medical Association (BLÄK). For blood samples taken in the US, the study protocol was reviewed and approved by the Institutional Review Board (IRB) under the protocol number AAAS3035.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Abstract

In the case of a radiological or nuclear event, biological dosimetry can be an important tool to support clinical decision-making. During a nuclear event, individuals might be exposed to a mixed field of neutrons and photons. The composition of the field and the neutron energy spectrum influence the degree of damage to the chromosomes. During the transatlantic BALANCE project, an exposure similar to a Hiroshima-like device at a distance of 1.5 km from the epicenter was simulated and biological dosimetry based on dicentric chromosomes was performed to evaluate the participants ability to discover unknown doses and to test the influence of differences in neutron spectra. In a first step, calibration curves were established by irradiating blood samples with 5 doses in the range of 0 Gy to 4 Gy at two different facilities in Germany (PTB) and USA (CINF). The samples were sent to eight participating laboratories from the RENEB network and dicentric chromosomes were scored by each participant. Next, blood samples were irradiated with 4 blind doses in each of the two facilities and sent to the participants to provide dose estimates based on the established calibration curves. Manual and semi-automatic scoring of dicentric chromosomes were evaluated for their applicability to neutron exposures. Moreover, the biological effectiveness of the neutrons from the two irradiation facilities was compared. The calibration curves from samples irradiated at CINF showed a 1.4 times higher biological effectiveness compared to samples irradiated at PTB. For manual scoring of dicentric chromosomes, the doses of the test samples were mostly successfully resolved based on the calibration curves established during the project. For semi-automatic scoring, the dose estimation for the test samples was less successful. Doses >2 Gy in the calibration curves revealed non-linear associations between dose and dispersion index of the dicentric counts, especially for manual scoring. The differences in the biological effectiveness between the irradiation facilities suggested that the neutron energy spectrum can have a strong impact on the dicentric counts.

Keywords

biological dosimetry; RENEB; neutrons; dicentric chromosomes; biological effectiveness

Introduction

In principle, various scenarios of large-scale radiological incidents are conceivable, ranging from a fire to an explosion in a nuclear power plant, a dirty bomb an improvised nuclear device (IND) or the detonation of a military grade nuclear weapon. In all these cases, a large number of individuals are potentially exposed to ionizing radiation [Buddemeier and Dillon

2009] and a quick and reliable dose assessment should be an essential part of radiation emergency management. In the case of a nuclear disaster, the high number of injured or worried-well will exceed the capacity of emergency preparedness of a single country. An effective strategy to enhance analysis capacity in the case of large-scale accidents is networking between experienced laboratories [Kulka et al. 2018]. In Europe, the RENEB association (RENEB e.V.), a network for biological dosimetry and physical retrospective dosimetry was founded in 2017 to act as a legal partner for organizations and platforms, active in emergency preparedness, radiation protection and research [Kulka et al. 2017]. The network provides rapid, comprehensive and standardized methodology for individualized dose estimation in the case of large-scale radiological events. Another strategy, RABiT (Rapid Automated Biodosimetry Tool), was developed at Columbia University and is a tool that was designed to allow fully automated analysis from the input of the blood samples into the machine to the output of a dose estimate [Garty et al. 2010]. This was combined with newer approaches using commercially available High Throughput/High Content Screening platforms [Repin et al. 2017; Royba et al. 2019]. The RABiT allows a high throughput processing of blood samples and dose estimates with minimal need for manpower. Both approaches have strengths and weaknesses, but could ideally complement each other, depending on the emergency scenario. This publication focuses on the project results based on the RENEB networking approach and the project results on the RABiT system will be published separately in later publications. Currently, the most qualified methods for biological dosimetry are based on cytogenetic biomarkers in human peripheral blood lymphocytes, such as dicentric chromosomes and micronuclei. These biomarkers have already been validated in various radiation incidents in which they proved to be reliable tools to detect an absorbed dose with sufficient precision [Beinke et al. 2015; Güçlü 2021; Salassidis et al. 1994; Tawn et al. 2018; Wernli et al. 2015]. In biological dosimetry, the dose received by an individual is estimated based on an *ex vivo* calibration curve which is prepared by each laboratory in advance. Calibration curves should be established for a range of radiation types with different biological effectiveness.

Following a nuclear detonation, people are exposed to a mixed field of neutrons and gamma-rays. In particular, in an IND scenario where the device is detonated at or near ground level, the higher shielding of photons, compared to neutrons, by construction material will result in an increased fraction of the dose delivered by neutrons [Kramer et al. 2016]. Additionally, the composition and energy distribution of the radiation field will depend on the distance from the epicenter and shielding. As an example, at a distance of 1.5 km from the epicenter of a Hiroshima-like device, the delivered dose is significant but survivable [Kramer et al. 2016]. In this case, the neutron energy spectrum is a relatively broad energy distribution peaking at around 1 MeV but spanning energies from thermal up to about 10 MeV [Egbert et al. 2007] and is markedly different from a standard reactor spectrum of fission neutrons [Garty et al. 2017; Xu et al. 2015b]. Currently, most research on the relative biological effectiveness (RBE) of neutrons with regard to the formation of dicentric chromosomes has been performed for different energies of monoenergetic neutrons, where the RBE showed a peak at approximately 0.4 MeV [Pandita and Geard 1996; Schmid et al. 2003; Tanaka et al. 1999], or for fission neutrons from different types of reactors [Fajgelj et al. 1992; Schmid et al. 2008; Schmid 1998]. There are also some publications exploring cytogenetic

data based on irradiations with neutrons with broad energy spectra comparable to a nuclear event from an A-bomb or an IND [Dobson et al. 1991; Heimers et al. 2005, 2006] or from Hiroshima and Nagasaki survivors [Bloom et al. 1966]. Nevertheless, it is currently not clear how far differences in the neutron energy spectrum and the composition of the mixed beam, as encountered after such an event, influence the level of cytogenetic damage in exposed lymphocytes. Data published by [Sasaki et al. 2006] suggested that the structure of the energy spectrum of fission neutrons has only little effect on the chromosomal effectiveness. Recently, a facility has been developed [Xu et al. 2015b] for simulating a neutron spectrum similar to the spectrum encountered at the Hiroshima bombing at 1.5 km from the epicenter. Calibration curves and the RBE based on the scoring of micronuclei [Xu et al. 2015b] and various transcriptomic [Broustas et al. 2018; Mukherjee et al. 2019] and metabolomic [Laiakis et al. 2019; Laiakis et al. 2017] endpoints have been evaluated. In the frame of biological dosimetry, where the dicentric chromosome assay (DCA) is the gold standard that is routinely used to evaluate dose in accidental and potential malicious exposures, it is essential that laboratories, such as those in the RENEB network, have appropriate calibration curves available.

The BALANCE project was a transatlantic cooperation between the European RENEB network and Columbia University in the United States. The main aim of the project was to simulate exposures in a nuclear event, with a relevant neutron spectrum and to improve, validate and compare different approaches to estimate doses based on biological markers. In the frame of the project, blood was irradiated at two different neutron/gamma sources: The Columbia IND Neutron Facility (CINF) at the Radiological Research Accelerator Facility, USA and the neutron facility at Physikalisch-Technische Bundesanstalt (PTB), Germany, both mimicking neutron spectra similar to the relevant spectrum. Irradiations at two different facilities with slightly different compositions of the energy spectra enabled the comparison of the biological effectiveness between the facilities. Irradiation at CINF yielded a neutron spectrum spanning 0.05–8 MeV [Xu et al. 2015a] with a photon component of approximately 18% and irradiation at the PTB yielded a neutron spectrum spanning 0.1–8 MeV with a photon component of 10%. During the first part of the project calibration curves were established by each participating laboratory based on each of the two different mixed-radiation fields to test the sensitivity to detect differences in the neutron energy spectra from CINF and PTB and to clarify if one calibration curve is sufficient to estimate the dose absorbed by people exposed to slightly different distributions of radiation energies and beam compositions. In the second part of the project, four blind-coded samples were irradiated in each of the two facilities and again distributed to the participating laboratories, to test the validity of the calibration curves established in part one of the project. Irradiated blood samples from both facilities were distributed among eight laboratories associated in the RENEB network. Each participating laboratory established calibration curves for samples irradiated at both facilities, enabling the evaluation of differences in the scoring of dicentric chromosomes between the participating laboratories as well as differences in the biological effectiveness of the neutron fields from the two facilities. Manual and/or semi-automatic scoring of dicentric chromosomes was performed by the participants and the performance of the two different scoring modes for exposures in the different mixed fields was assessed by estimating doses from blind-coded samples.

Material and Methods

Participating laboratories and tasks

In the frame of this project blood samples were exposed *ex vivo* at two different neutron irradiation facilities (PTB, Germany and CINF, USA). In the first part of the project calibration curves were established following the irradiation procedure at both facilities. In the second part, validation of these calibration curves was performed by dose estimation of blind-coded blood samples irradiated at both facilities. The blood samples were sent to the Federal Office for Radiation Protection (BfS), Germany and further processed. Eight partners of the European RENEB network were involved in the analysis of blood samples and provided results.

Irradiation conditions of blood samples

For irradiations at the PTB accelerator facility (PIAF) [Brede et al. 1980], the intense neutron field with a broad energy distribution was produced by a deuteron beam of 3.4 MeV with beam currents of up to 52 μA on a thick, water-cooled beryllium (Be) disc. The energy spectrum of the neutron beam starts from very low energies and ranges up to approximately 8 MeV [Meadows 1993]. The beam charge on the Be target served as neutron monitor. Before and after the irradiation of the samples, the total dose to tissue per target charge was determined according to ICRU recommendations [ICRU 1989] with a tissue-equivalent (A-150) gas ionization chamber (EXRADIN, T2-#381). The chamber had been calibrated in the ^{60}Co reference field of PTB. The photon component was determined with a Geiger-Müller counter (Type MX 163, Alrad Inst., Surrey England) to (10 ± 2) %. The dose rate was around 1.2 Gy/h. Three blood-filled tubes were irradiated simultaneously side-by-side (shown in Fig. 1a). A temperature of about 35°C was maintained during the irradiation by means of a heater plate and a Styrofoam box.

For irradiations at CINF, neutrons were generated by impinging a 28 μA mixed proton/deuteron beam with an energy of 5 MeV on a water-cooled 0.5 mm thick Be target on copper backing (Materion, Brewster, NY)[Xu et al. 2015b]. Prior to irradiation, dosimetry was performed using a custom-built tissue equivalent proportional counter [Rossi et al. 1960] that was calibrated to a NIST-traceable radium source. Because of the possible variation of the dose rate during the experiment, a second tissue-equivalent gas ionization chamber, placed downstream of the neutron target, was used as a monitor to halt the beam when the prescribed dose was reached. Twelve 5 mL vacutainers were mounted on a Ferris wheel rotating around the Be target with the samples at an angle of 60º to the primary beam position (shown in Fig. 1b). Dose rate at the vacutainers was 3 Gy/h of neutrons with a concomitant photon dose of 0.6 Gy/h. During irradiation, 3 tubes per dose were removed from the wheel (and replaced with water containing tubes) when each of the prescribed doses was achieved.

The neutron fields of PTB and CINF are qualitatively compared in Figure 1c with the Hiroshima field at 1.5 km from the epicenter. Shown are the neutron fluences multiplied by the tissue-kerma factors [Malmer 2001], which correspond to the dose distribution as a function of neutron energy. The curves are overlayed in arbitrary units for better

comparison of differences in shape and are, therefore, not to scale. The most important difference between the two irradiation platforms is that PTB uses a pure deuteron beam, filtered through a dipole magnet, whereas CINF uses the direct beam from the accelerator, containing protons, deuterons and molecular ions. This has the effect of significantly broadening the CINF energy spectrum due to the low energy neutrons generated by the Be(p,n) reaction, compared to Be(d,n), resulting in a large excess of <1 MeV neutrons as seen in Figure 1c, which have a higher RBE [Pandita and Geard 1996; Schmid et al. 2003]. The higher energy at CINF (5 MeV vs 3.4 MeV) also results in more high energy neutrons.

Each neutron facility provided (i) five blood samples that were irradiated with doses in the range of 0 Gy to 4 Gy (Table 1) for the establishment of calibration curves, (ii) three test samples irradiated with blinded doses and (iii) one unirradiated control sample. For this publication, the test samples were re-labeled in increasing order of the corresponding doses: Blind 1 (0 Gy), Blind 2 (CINF: 0.6 Gy; PTB: 0.654 Gy), Blind 3 (CINF: 1.2 Gy; PTB: 1.61 Gy) and Blind 4 (CINF: 2.4 Gy; PTB: 2.23 Gy). Blood from each test sample was provided to the laboratories without knowing the reference doses before the analysis.

Blood sampling and shipment of blood samples

Human blood samples were collected by venipuncture in 10 mL heparinized tubes from a total of 4 (one for each calibration curve and blind-coded test sample from each neutron facility) 3 male (31, 46 and 59 years) and one female (42 years) healthy adult human volunteers. As in a real emergency, blood samples used for the set-up of calibration curves were not from the same individuals as those used for blind-coded samples in the validation procedure. The blood samples were fully anonymized and as such not traceable to the individual participants. In Germany, blood samples from healthy adult donors were obtained, in heparinized tubes (Sarstedt AG & Co. KG., Germany) by venipuncture by physicians according to §15 of the code of medical ethics for physicians in Bavaria, Germany, following the principles of the Declaration of Helsinki. In the US blood was collected under Columbia University IRB protocol AAAS3035.

After irradiation, blood samples were kept for 2 h at 37 °C to allow DNA repair. Blood samples irradiated at PTB were transported to BfS in temperature-controlled boxes (15–25°C) within 24 h. Blood samples irradiated at CINF were placed in a 22 ºC passive temperature-controlled shipper (CREDO Cube; Pelican Biothermal, Maple Grove, MN) and sent to BfS by express service within 48 h.

Processing of blood samples at BfS and scoring procedure

At BfS, the cultivation and preparation of blood samples were performed according to standard procedures [IAEA 2011; ISO19238 2014; Oestreicher et al. 2018]. Whole blood (0.5 ml) was transferred to culture tubes containing RPMI-1640 culture medium (Biochrom, Berlin) supplemented with 10% FCS (Biochrom, Berlin), 2% PHA (Biochrom, Berlin) and antibiotics (Biochrom, Berlin). For cell-cycle controlled scoring, long-term Colcemid treatment (Roche, Mannheim) with a final concentration in culture of 0.08 µg/ml was added 24 h after culture set up. Blood samples were cultured in total for 48 h. For each dose point 20 parallel cultures were set up. The hypotonic treatment of cells was carried

out with 75 mM KCl. Cells were then fixed in methanol:acetic acid (3:1) three times. The suspension was stored in the freezer (–18°C) before aliquots of fixed cells were sent to 7 RENEB partners in the EU (BIR, Germany; UKHSA, UK; UAB, Spain; IRSN, France; SERMAS, Spain; UGent, Belgium and NCSRD, Greece). The task of each RENEB partner (8 laboratories in total) was to prepare Giemsa stained slides and manually analyze 1000 cells or 100 dicentric chromosomes per dose point to establish a calibration curve [ISO19238 2014]. In the case of semi-automated scoring [Romm et al. 2013], laboratories were asked to score as many cells as possible. Scoring was performed according to the standard and validated procedure of each particular laboratory.

For validation of the calibration curves based on blind-coded test samples, culturing, preparation and distribution of blood samples were performed according to the same procedure as for the calibration curves in the first part of the project. The task of each RENEB partner was to prepare Giemsa stained slides and manually and/or semi-automatically analyze dicentric chromosomes for dose estimation. For both scoring methods triage and full scoring mode were applied. For manual scoring requirements were to analyze 50 cells or 30 dicentrics per dose point (2 slides and 25 cells per slide) for triage mode and 500 cells or 100 dicentrics per dose point (2 slides and 250 cells per slide) for full mode. For semi-automated scoring the detection of dicentric chromosomes was performed on a software-based procedure, where 150 cells should be captured for triage mode and 1500 or more cells for full mode.

The semi-automatic scoring of dicentric chromosomes is a multi-step procedure performed in a similar way by all participating laboratories. The Giemsa stained slides were analyzed using the automatic scoring system Metafer 4 by MetaSystems Hard & Software GmbH (Altlussheim, Germany) including the software modules for metaphase finding (MSearch) to detect the metaphase spread in a first step. In a second step, additional software tools were applied for auto-capturing of high-resolution images at 63x magnification (with oil) (AutoCapt) and automatic detection of dicentric candidates (DCScore). In a third step, a human scorer evaluated the automatically detected dicentric candidates, in the metaphases selected by the software, on the screen of the PC to remove false positive dicentric candidates, thus resulting not in a full but in a semi-automated scoring approach [Romm et al. 2013]. Apart from L6, which scored tricentrics and tetracentrics as 1 dicentric, all other participants scored tricentrics as 2 dicentrics and tetracentrics as 3 dicentrics. A manual preselection of low-quality cells before running DCScore was performed by two laboratories (L4 and L6). Two laboratories (L1 and L4) used the BfS and two laboratories the IRSN (L2 and L6) classifiers as described in Romm et al. [Romm et al. 2013] and one lab used a laboratory specific classifier (L7). Apart from L7, all participants scored only dicentrics that belong to one metaphase and dicentrics that are on the image but likely belong to another metaphase were not considered.

Statistical analysis

The physical reference doses of samples irradiated at PTB were slightly updated after the participants provided the calibration curve estimates. All calibration curves and dose estimates for the test samples were therefore re-estimated after the participants

provided their estimates. As commonly accepted for high-LET exposures [IAEA 2011], the calibration curves were estimated assuming a linear dose-effect relationship. The calibration curves were estimated using generalized linear models (R function “glm”) with identity link. In a first step, overdispersion was accounted for by using a quasi-Poisson glm model. If the estimated dispersion of this model was ≤1, a Poisson glm model was used instead. The doses and corresponding 95% confidence intervals (CI) were estimated using the approach described in Savage et al. [Savage and Papworth 2000]. For the calculation of 95% CIs of the dose estimate, overdispersion of the dicentric yields of the test samples was accounted for by using the empirical standard deviation of the dicentric yield if the dispersion index $\delta > 1$ and the Poisson standard deviation if $\delta \leq 1$. To test whether the observed overdispersion is significantly different from 1, the U test was applied as described in [IAEA 2011] and results with U>1.96 were assumed to be significantly overdispersed (P<0.05).

In order to evaluate the performance of the participating laboratories the ζ-score was used and calculated as described in [ISO13528 2015]:

$$\zeta = \frac{D - D^*}{\sqrt{s_D^2 - s_{D^*}^2}}$$

where D is the dose estimated by the DCA, D^* is the physical reference dose, s_D is the estimated standard deviation corresponding to D and s_{D^*} is the standard deviation of the physical reference dose. The standard deviation s_D was calculated as described in Savage et al. [Savage and Papworth 2000], accounting for overdispersion of the test samples as described above. It was assumed that s_{D^*} is small relative to s_D and was set to $s_{D^*} = 0$. The critical values were defined as in [ISO13528 2015] and results with $|\zeta| < 2$ were considered as satisfactory, $2 \leq |\zeta| < 3$ as questionable and $|\zeta| \geq 3$ as unsatisfactory. ζ-scores were chosen to assess whether the deviation of the reference dose is higher than expected, given the standard error of the dose estimated by each laboratory. Generally, high ζ-scores can have two reasons, 1) the deviation of the reference dose is high or 2) the estimation of the standard errors by the participant did not include all sources of uncertainty. Here, the estimation of the standard error of the dose was performed in exactly the same way for all participants and it was assumed that all sources for uncertainties were accounted for.

The ratio between the slopes of the calibration curves from samples irradiated at CINF and PTB, where PTB was used as the reference, was calculated to provide a relative biological effectiveness [ICRP 2003] between the two neutron fields.

To analyze whether laboratory-specific effects have to be considered for the estimation of calibration curves or if the data from different laboratories can be pooled, quasi-Poisson regression models were applied using the data from all laboratories together, comparing two different models. For the first model, the data were pooled without considering a laboratory effect and for the second model, the laboratory effect was included into the model by modelling an interaction effect between lab and dose. The two models were compared by ANOVA F-Tests, where a significant result indicates that model 2 outperforms model 1

and laboratory effects should therefore be considered, i.e. there are systematic differences between dicentric counts provided by the laboratories.

Results

Comparison of calibration curves

In the first part of the project, seven laboratories provided manually, four laboratories manually and semi-automatically and one laboratory only semi-automatically scored calibration curve data. The full distribution of the detected dicentric chromosomes for the calibration curves of each lab can be found in Supplementary Tables 1 & 2, for manual and semi-automatic scoring, respectively. Besides L8 all laboratories manually scored at least the required 1000 cells for the 0 Gy data point (Table 1). As expected, the number of manually scored cells necessary to obtain 100 dicentrics decreased with increasing dose (Table 1). For all laboratories, the number of semi-automatically captured cells was higher for samples irradiated at PTB than at CINF for each of the analyzed doses (Table 1). Generally, the number of semi-automatically scored cells decreased strongly with increasing dose and some laboratories were not able to capture more than 500 cells for doses ≥ 2 Gy.

For manually scored calibration curves, the slopes (α coefficient in dicentrics per cell) ranged between 0.80 and 1.13 for samples irradiated at CINF and between 0.53 and 0.78 for samples irradiated at PTB (shown in Table 1 and Fig. 2a–c). The estimated slopes were significantly higher (paired t-test; $P<0.0001$, Fig. 2c) for samples from CINF than for samples from PTB and the relative biological effectiveness between the two neutron fields was very similar for all laboratories, ranging between 1.3 and 1.5 with a median of 1.4. Evaluation of differences between laboratories revealed a strong correlation (Spearman's $\rho=1$, $P=0.0004$) of slopes between calibration curves for samples irradiated at CINF and PTB, suggesting systematic differences in the analysis of dicentric chromosomes between the laboratories. To further test whether differences in the analysis of dicentric chromosomes between laboratories should be considered, calibration curves from the pooled data of all laboratories were estimated with and without including laboratory as a predictor variable in the regression models. The results suggested that there is a laboratory effect, resulting in differences in the slopes of the calibration curves between laboratories (ANOVA F-Test; $P<0.0001$).

The slopes for the semi-automatically scored calibration curves ranged between 0.23 and 0.33 for samples irradiated at CINF and 0.15 and 0.25 for samples irradiated at PTB (shown in Table 1 and Fig. 2d–f). Again, samples irradiated at CINF showed significantly higher slopes (paired t-test; $P=0.006$; Fig. 2f) compared to samples from PTB. The relative biological effectiveness between the two neutron fields was relatively consistent for the participating laboratories and ranged between 1.1 and 1.7 with a median of 1.3. Compared to manual scoring, the slopes from semi-automatically scored samples were on average consistently 70% lower. Consideration of differences between laboratories showed two clusters (shown in Fig. 2f and Table 1) of semi-automatically scored calibration curves for samples irradiated at CINF as well as for PTB with low slopes (L2 and L6) and higher slopes (L1, L4 and L7). In contrast to manual scoring, the correlation of slopes between calibration curves from samples irradiated at CINF and PTB was not significant

(Spearman's ρ=0.5, P=0.45). However, regression models including laboratory as a predictor variable again suggested that there is a laboratory effect (ANOVA F-Test; P<0.0001). The latter observation can very likely be attributed to the clustering of slopes between laboratories.

Most of the manually scored results from doses ≤1 Gy showed a tendency for overdispersion (δ > 1) with many reaching significance (Supplementary Figure 1a–d). In contrast, for doses >1 Gy, the dispersion levels decreased and at the highest dose, a tendency for underdispersion was often observed (Supplementary Figure 1a–d). Semi-automatically scored samples showed significant overdispersion for all doses >0 Gy and the dispersion levels increased with dose or were approximately constant (Supplementary Figure 1e–h).

Dose estimates for test samples

After the establishment of calibration curves, dose estimates for test samples with blinded doses were performed to validate the applicability of the calibration curves and to test the performance of the participating laboratories. Each neutron facility provided three irradiated test samples (Blind 2–4) and included one sham-irradiated sample (Blind 1). The dose estimates were obtained using the laboratory specific calibration curves established at the same irradiation facility as the test samples. The number of scored metaphases (in full mode) for the test samples can be found in Table 2 and the full dicentric distribution, estimated doses and confidence intervals for the test samples of each lab can be found in Supplementary Tables 3 & 4, for manual and semi-automatic scoring, respectively.

For manually scored dicentric chromosomes, a good agreement between biological dose estimates and the physical reference doses was observed for samples from both irradiation facilities for triage as well as for full scoring mode (shown in Fig. 3 and Table 3). For samples irradiated at CINF, all of the estimated doses included the reference dose in the 95% CI, all or almost all were within ±0.5 Gy or ±0.25 Gy of the reference dose (shown in Fig. 3 and Table 3) and all estimates showed $|\zeta| < 2$ (shown in Fig. 4 and Table 3). Details on the definition of ζ-scores can be found in Materials and Methods. For samples irradiated at PTB, dose estimates showed an increased deviation from reference doses in comparison to samples irradiated at CINF (shown in Fig. 3 and Table 3). Here, at least 50% (full) or 67% (triage) estimated doses included the reference dose in the 95% CI, were within ±0.25 Gy or within ±0.5 Gy of the reference dose (shown in Fig. 3 and Table 3) and showed $|\zeta| < 2$ (shown in Fig. 4 and Table 3). The control sample was detected by all participants for samples from CINF as well as from PTB.

For semi-automatically scored dicentric chromosomes, the agreement between the biological dose estimates and the physical reference doses was worse compared to manual scoring with regard to ζ-scores ($|\zeta| < 2$). Moreover, fewer dose estimates included the physical reference dose in the estimated 95% CI and fewer dose estimates were within ±0.25 Gy or ±0.5 Gy of the reference dose (shown in Fig. 3 & 4 and Table 3). Moreover, manually scored results of the irradiated test samples consistently showed lower variability in terms of the coefficient of variation (Table 3; CINF: CV between 0.07 and 0.12; PTB: CV between 0.14 and 0.17) than

semi-automatically scored results (CINF: CV between 0.21 and 0.28; PTB: CV between 0.26 and 0.42).

Test samples that were manually scored in full mode generally showed a tendency for overdispersion ($\delta>1$) for doses <1 Gy for both irradiation facilities (Table 3 and Supplementary Figure 2). The percentage of results with significant overdispersion decreased with increasing dose (Table 3). For semi-automatic scoring in full mode, all test samples with doses >0 Gy showed overdispersion, independent of dose and irradiation facility (Supplementary Figure 2 and Table 3). Correspondingly, significant overdispersion ($P<0.05$) was observed for 100% (CINF) and for 87% (PTB) of the irradiated (>0 Gy) test samples.

Discussion

In the case of a large-scale radiological or nuclear event, biological dosimetry can be an important tool to aid clinical decision-making and to identify non-exposed “worried-well” individuals. Networking between international laboratories is one approach to handle the large sample size to be analyzed during such an event. To enable reliable dose estimation or categorizations of individuals into clinically relevant groups, the laboratories for biological dosimetry must establish calibration curves from different radiation qualities. While most RENEB laboratories have well established calibration curves based on the DCA for low-LET γ-rays or X-rays which have been validated in several exercises [Endesfelder et al. 2021; Gregoire et al. 2021; Oestreicher et al. 2017], the situation is different for exposures with neutrons, where the number of laboratories with validated calibration curves is certainly lower. Moreover, the distribution of the energy spectrum of the neutrons has a significant influence on the biological effectiveness [ICRP 2003; Pandita and Geard 1996; Schmid et al. 2003; Tanaka et al. 1999], and it might therefore not be sufficient to have a single neutron calibration curve per laboratory. In the frame of the BALANCE project, an exposure similar to a Hiroshima-like device at a distance of 1.5 km from the epicenter was simulated. At this distance the neutrons have a broad energy spectrum, spanning energies from thermal up to about 10 MeV [Egbert et al. 2007], and the field is composed of a mixture of neutrons and photons. To enable the comparison of differences in the biological effectiveness between the two neutron sources resulting from differences in the shape of the applied energy spectra, blood samples were irradiated at two different facilities in Germany (PTB) and USA (CINF). The practicability of the shipment of blood samples between Germany and USA was tested by sending samples in both directions. In a first step calibration curves for the DCA were established by each participating RENEB laboratory and the differences in the biological effectiveness were evaluated between the two irradiation facilities. Next, to test the applicability of the calibration curves and to validate the performance of the participating RENEB laboratories four test samples with blinded doses were irradiated at each PTB and CINF and sent to the participants for dose estimation. To test the validity of the DCA for neutron exposures greater than 1 Gy, blood samples were exposed to doses ranging from 0 Gy to 4 Gy.

The neutron spectra from both irradiation facilities approximately cover the range of the energy spectrum from the Hiroshima bomb at a distance of 1.5 km from the epicenter.

Nevertheless, the calibration curves obtained by the participating laboratories from the RENEB network strongly suggested that the biological effectiveness of irradiations at CINF is in median 1.4 times higher compared to irradiations at PTB. This result was consistently observed by all participating laboratories, which strongly suggested a systematic difference that was not expected to this extent when the project started. A closer inspection of the neutron energy spectra revealed differences in the shape of the energy distributions. While the contribution of energies <0.7 MeV and >3 MeV is higher for CINF, the contribution of energies in the range of 1–3 MeV is higher for PTB. The tissue-kerma weighted mean energy of the PTB neutron field was about 2.5 MeV and the one at CINF was about 3.2 MeV. From the literature on monoenergetic neutrons, it can be expected, that the relative biological effectiveness compared to γ-rays should be increased if the contributions of energies in the range of approximately 0.2–0.5 MeV is higher compared to energies >1 MeV [Pandita and Geard 1996; Schmid et al. 2003; Tanaka et al. 1999]. Hence, it is likely that the observed differences in the biological effectiveness can be caused by differences in the distribution of the energy spectra of the two irradiation facilities. However, to exactly quantify the expected difference further research will be required in future. Although it can be assumed that donor effects and differences in transport times did not significantly influence the results, it should be noted that blood samples from different donors were used at PTB and CINF to simulate real accident scenarios and that the transport time from the irradiation facilities to BfS differed slightly, but within an acceptable range. Sending blood samples between EU and non-EU countries is always a challenge. In the BALANCE project, the transportation between USA and Germany was successfully completed within 48 h underlining the need to use specialized express services for diagnostic material to avoid any delays. For optimal shipment conditions the use of temperature-controlled thermoboxes can be recommended to prevent extreme temperatures which make the stimulation of lymphocytes more difficult.

The calibration curves of the participants showed a significant laboratory effect. The differences between the slopes of the manually scored calibration curves were highly correlated between samples irradiated at CINF and PTB, i.e. the laboratories that scored higher or lower numbers of dicentric chromosomes for samples irradiated at CINF scored also higher or lower numbers of dicentric chromosomes for samples irradiated at PTB. The latter strongly suggested a systematic laboratory effect, which is probably related to different scoring criteria. For semi-automatically scored calibration curves, two clusters of laboratories were observed, consistently having either lower or higher numbers of dicentric chromosomes, which again suggested a systematic difference between these laboratories. This might be related to the use of different classifiers for the automatic detection of dicentric chromosomes, to differences in the scoring of tri- and tetracentrics or to differences in the manual exclusion of low-quality metaphases. Due to the differences in the calibration curves between laboratories, which have also been reported in recent RENEB ILCs [Endesfelder et al. 2021; Gregoire et al. 2021; Oestreicher et al. 2017], it is strongly recommended that each laboratory uses its own calibration curves and performs regular inter- and intra-laboratory comparisons to ensure that the scoring is performed according to the laboratory specific curves.

The current recommendation for biological dosimetry for the exposure to high-LET radiation is to establish calibration curves for the DCA in the dose range 0 Gy to 2 Gy [IAEA 2011] and there is currently little research on the DCA for neutron doses greater than 2 Gy. One of the aims of this project was therefore, to establish calibration curves including higher doses of up to 4 Gy. Such high neutron doses will in most cases be lethal but might be relevant for biological dosimetry in the case of inhomogeneous or partial body exposures. For this purpose, each participating laboratory from the RENEB network used the calibration curves established in the first part of the BALANCE project to provide dose estimates for the test samples. While the manually scored results showed a good agreement with the physical reference doses in the whole dose range tested, especially for samples irradiated at CINF, the semi-automatically scored results revealed some problems. As a consequence, semi-automatic scoring for high-LET neutron exposures should be further validated and only be used if the validity of the approach was ensured. The good performance of most RENEB laboratories for dose estimates obtained based on manual scoring suggested that RENEB laboratories were able to successfully estimate neutron doses based on the pre-established calibration curves irradiated at the same source and conditions as the test samples. However, it should be noted that the exact neutron energy spectrum will in most cases not be known in a real-life scenario and could also vary based on the location. Calibration curves that exactly mimic the exposure situation might not be available to the laboratories performing biological dosimetry. Nevertheless, within the frame of the BALANCE project, all RENEB participants established new calibration curves for neutron exposures based on two different neutron spectra approximately simulating the spectrum of the Hiroshima bombing at a distance of 1.5 km from the epicenter, which can be further validated and potentially be used in real-life exposure scenarios in the future. The focus of the BALANCE project was rather on scientific questions regarding the difference between neutron spectra and the ability of RENEB laboratories to recover neutron doses, given that an adequate calibration curve was available. For the establishment of calibration curves for a real radiation accident, especially in the lower dose range, additional dose points <0.5 Gy should be included.

The distribution of dicentric chromosomes after high-LET neutron irradiation is generally known to show overdispersion [Heimers et al. 2005; Schmid et al. 2000; Schmid 1998], i.e. the variance is larger than the mean and the data is therefore not Poisson distributed as for acute whole-body low-LET exposures. However, most studies focusing on dicentric chromosomes for high-LET neutron exposures analyzed doses ≤1 Gy. In concordance with data shown elsewhere [Heimers et al. 2005; Schmid et al. 2000; Schmid 1998], most of the manually scored results from doses ≤1 Gy showed overdispersion ($\delta > 1$). In contrast, for doses >1 Gy, considerably fewer of the manually scored results showed overdispersion and dispersion levels seemed to decrease with increasing dose for doses >1 Gy. This observation might indicate that the exposure is more uniform for higher doses, i.e. each cell has the same probability to develop dicentric chromosomes. Another explanation is that the variance decreases relative to the mean due to saturation effects. The observation that the dispersion levels are not constant with doses complicates an adequate consideration of dispersion for the estimation of the uncertainties, as most models (e.g. quasi-Poisson regression models) assume constant overdispersion. In contrast, all semi-automatically

scored samples showed overdispersion over the whole dose range >0 Gy and 95% of the samples showed significant overdispersion. While a decreasing trend of dispersion levels with increasing dose was observed for manual scoring, the dispersion levels rather increased or were approximately constant for the semi-automatically scored data. This observation is in concordance with published data, where overdispersion was reported for semi-automatic scoring due to differences in the number of detected chromosomes related to variable quality of the metaphases [Endesfelder et al. 2020]. The differences in the dispersion patterns between manual and semi-automatic scoring strongly suggest that different methods for the assessment of uncertainties should be applied.

Conclusions

The research presented in this publication provides new insights into the applicability of cytogenetic biomarkers for dose estimations in the case of a neutron exposure with a spectrum similar to the Hiroshima bombing. Critical points, such as high doses and neutron energy spectra, practicability of the shipment of blood samples and the applicability of calibration curves for different emergency situations were tested and evaluated in a transatlantic cooperation of laboratories from Europe and the US. Interestingly, differences in the biological effectiveness of different neutron irradiation facilities could be revealed. While the manually scored results suggested that RENEB laboratories were able to successfully resolve the doses, the results based on semi-automatically scored data were more biased, suggesting that further research is needed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability statement

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

References

Beinke C, Ben-Shlomo A, Abend M, Port M: A Case Report: Cytogenetic Dosimetry after Accidental Radiation Exposure during192Ir Industrial Radiography Testing. Radiation Research 184:66–72 (2015). [PubMed: 26151173]

Bloom A, Neriishi S, Kamada N, Iseki T, Keehn R: Cytogenetic Investigation of Survivors of the Atomic Bombings of Hiroshima and Nagasaki. The Lancet 288:672–674 (1966).

Brede HJ, Cosack M, Dietze G, Gumpert H, Guldbakke S, Jahr R, Kutscha M, Schlegel-Bickmann D, Schölermann H: The Braunschweig accelerator facility for fast neutron research. Nuclear Instruments and Methods 169:349–358 (1980).

Broustas CG, Harken AD, Garty G, Amundson SA: Identification of differentially expressed genes and pathways in mice exposed to mixed field neutron/photon radiation. BMC Genomics 19:504 (2018). [PubMed: 29954325]

Buddemeier B, Dillon M: Key Response Planning Factors for the Aftermath of Nuclear Terrorism. Environmental Science (2009).

Dobson RL, Straume T, Carrano AV, Minkler JL, Deaven LL, Littlefield LG, Awa AA: Biological effectiveness of neutrons from Hiroshima bomb replica: results of a collaborative cytogenetic study. Radiat Res 128:143–149 (1991). [PubMed: 1947011]

Egbert SD, Kerr GD, Cullings HM: DS02 fluence spectra for neutrons and gamma rays at Hiroshima and Nagasaki with fluence-to-kerma coefficients and transmission factors for sample measurements. Radiat Environ Biophys 46:311–325 (2007). [PubMed: 17643260]

Endesfelder D, Kulka U, Einbeck J, Oestreicher U: Improving the accuracy of dose estimates from automatically scored dicentric chromosomes by accounting for chromosome number. Int J Radiat Biol 96:1571–1584 (2020). [PubMed: 33001765]

Endesfelder D, Oestreicher U, Kulka U, Ainsbury EA, Moquet J, Barnard S, Gregoire E, Martinez JS, Trompier F, Ristic Y, Woda C, Waldner L, Beinke C, Vral A, Barquinero JF, Hernandez A, Sommer S, Lumniczky K, Hargitai R, Montoro A, Milic M, Monteiro Gil O, Valente M, Bobyk L, Sevriukova O, Sabatier L, Prieto MJ, Moreno Domene M, Testa A, Patrono C, Terzoudi G, Triantopoulou S, Histova R, Wojcik A: RENEB/EURADOS field exercise 2019: robust dose estimation under outdoor conditions based on the dicentric chromosome assay. Int J Radiat Biol 97:1181–1198 (2021). [PubMed: 34138666]

Fajgelj A, Horvat D, Pucelj B: Chromosome aberrations induced in human lymphocytes by U-235 fission neutrons. Part II: Evaluation of the effect of the induced Na-24 activity on the chromosomal aberration yield. Strahlenther Onkol 168:406–411 (1992). [PubMed: 1496451]

Garty G, Chen Y, Salerno A, Turner H, Zhang J, Lyulko O, Bertucci A, Xu Y, Wang H, Simaan N, Randers-Pehrson G, Yao YL, Amundson SA, Brenner DJ: The Rabit: A Rapid Automated Biodosimetry Tool for Radiological Triage. Health physics 98:209–217 (2010). [PubMed: 20065685]

Garty G, Xu Y, Elliston C, Marino SA, Randers-Pehrson G, Brenner DJ: Mice and the A-Bomb: Irradiation Systems for Realistic Exposure Scenarios. Radiation Research 187:475–485 (2017).

Gregoire E, Barquinero JF, Gruel G, Benadjaoud M, Martinez JS, Beinke C, Balajee A, Beukes P, Blakely WF, Dominguez I, Duy PN, Gil OM, Güçlü I, Guogyte K, Hadjidekova SP, Hadjidekova V, Hande P, Jang S, Lumniczky K, Meschini R, Milic M, Montoro A, Moquet J, Moreno M, Norton FN, Oestreicher U, Pajic J, Sabatier L, Sommer S, Testa A, Terzoudi G, Valente M, Venkatachalam P, Vral A, Wilkins RC, Wojcik A, Zafiropoulos D, Kulka U: RENEB Inter-Laboratory comparison 2017: limits and pitfalls of ILCs. International Journal of Radiation Biology 97:888–905 (2021). [PubMed: 33970757]

Güçlü I: Cytogenetic follow-up of an individual after accidental exposure to industrial radiation using dicentric frequency in blood lymphocytes. Mutation research 861–862:503276 (2021).

Heimers A, Brede HJ, Giesen U, Hoffmann W: Influence of mitotic delay on the results of biological dosimetry for high doses of ionizing radiation. Radiat Environ Biophys 44:211–218 (2005). [PubMed: 16273382]

Heimers A, Brede HJ, Giesen U, Hoffmann W: Chromosome aberration analysis and the influence of mitotic delay after simulated partial-body exposure with high doses of sparsely and densely ionising radiation. Radiat Environ Biophys 45:45–54 (2006). [PubMed: 16565842]

IAEA: Cytogenetic Dosimetry: Applications in Prepardness for and Response to Radiation Emergencies. EPR-Biodosimetry, International Atomic Energy Agency, Vienna (2011).

ICRP: Relative Biological Effectiveness (RBE), Quality Factor (Q), and Radiation Weighting Factor (wR). ICRP Publication 92 Ann ICRP 33 (4) (2003).

ICRU: ICRU Report No.45, Clinical neutron dosimetry, part I: determination of absorbed dose in a patient treated by external beams of fast neutrons. ICRU, International Commission on Radiation Units and Measurements, Bethesda, Maryland (1989).

ISO13528: Statistical methods for use in proficiency testing by interlaboratory comparison Geneva (2015).

ISO19238: International Organization for Standardization (ISO) , Radiation protection performance criteria for service laboratories performing biological dosimetry by cytogenetics Geneva ISO 19238:2014 (2014).

Kramer K, Li A, Madrigal J, Sanchez B, Millage K: Monte Carlo Modeling of the Initial Radiation Emitted by an Improvised Nuclear Device in the National Capital Region (Revision 1), 2016).

Kulka U, Abend M, Ainsbury EA, Badie C, Barquinero JF, Barrios L, Beinke C, Bortolin E, Cucu A, De Amicis A, Domínguez I, Fattibene P, Frøvig AM, Gregoire E, Guogyte K, Hadjidekova V, Jaworska A, Kriehuber R, Lindholm C, Lloyd D, Lumniczky K, Lyng F, Meschini R, Mörtl S, Della Monaca S, Monteiro Gil O, Montoro A, Moquet J, Moreno M, Oestreicher U, Palitti F, Pantelias G, Patrono C, Piqueret-Stephan L, Port M, Prieto MJ, Quintens R, Ricoul M, Romm H, Roy L, Sáfrány G, Sabatier L, Sebastià N, Sommer S, Terzoudi G, Testa A, Thierens H, Turai I, Trompier F, Valente M, Vaz P, Voisin P, Vral A, Woda C, Zafiropoulos D, Wojcik A: RENEB – Running the European Network of biological dosimetry and physical retrospective dosimetry. International Journal of Radiation Biology 93:2–14 (2017). [PubMed: 27707245]

Kulka U, Wojcik A, Di Giorgio M, Wilkins R, Suto Y, Jang S, Quing-Jie L, Jiaxiang L, Ainsbury EA, Woda C, Roy L, Li C, Lloyd D, Carr Z: Biodosimetry and Biodosimetry Networks for Managing Radiation Emergency. Radiation Protection Dosimetry 182:128–138 (2018). [PubMed: 30423161]

Laiakis E, Canadell M, Grilj V, Harken A, Garty G, Astarita G, Brenner D, Smilenov L, Fornace A: Serum lipidomic analysis from mixed neutron/X-ray radiation fields reveals a hyperlipidemic and pro-inflammatory phenotype. Sci Rep 9:4539 (2019). [PubMed: 30872747]

Laiakis EC, Wang YW, Young EF, Harken AD, Xu Y, Smilenov L, Garty GY, Brenner DJ, Fornace AJ, Jr.: Metabolic Dysregulation after Neutron Exposures Expected from an Improvised Nuclear Device. Radiat Res 188:21–34 (2017). [PubMed: 28475424]

Malmer CJ: ICRU Report 63. Nuclear Data for Neutron and Proton Radiotherapy and for Radiation Protection. Medical Physics 28:861–861 (2001).

Meadows JW: The 9Be(d, n) thick-target neutron spectra for deuteron energies between 2.6 and 7.0 MeV. Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment 324:239–246 (1993).

Mukherjee S, Grilj V, Broustas CG, Ghandhi SA, Harken AD, Garty G, Amundson SA: Human Transcriptomic Response to Mixed Neutron-Photon Exposures Relevant to an Improvised Nuclear Device. Radiat Res 192:189–199 (2019). [PubMed: 31237816]

Oestreicher U, Endesfelder D, Gomolka M, Kesminiene A, Lang P, Lindholm C, Rossler U, Samaga D, Kulka U: Automated scoring of dicentric chromosomes differentiates increased radiation sensitivity of young children after low dose CT exposure in vitro. Int J Radiat Biol 94:1017–1026 (2018). [PubMed: 30028637]

Oestreicher U, Samaga D, Ainsbury EA, Antunes AC, Baeyens A, Barrios L, Beinke C, Beukes P, Blakely WF, Cucu A, De Amicis A, Depuydt J, De Sanctis S, Di Giorgio M, Dobos K, Dominguez I, Duy PN, Espinoza ME, Flegal FN, Figel M, Garcia O, Monteiro Gil O, Gregoire E, Guerrero-Carbajal C, Guclu I, Hadjidekova V, Hande P, Kulka U, Lemon J, Lindholm C, Lista F, Lumniczky K, Martinez-Lopez W, Maznyk N, Meschini R, M'Kacher R, Montoro A, Moquet J, Moreno M, Noditi M, Pajic J, Radl A, Ricoul M, Romm H, Roy L, Sabatier L, Sebastia N, Slabbert J, Sommer S, Stuck Oliveira M, Subramanian U, Suto Y, Que T, Testa A, Terzoudi G, Vral A, Wilkins R, Yanti L, Zafiropoulos D, Wojcik A: RENEB intercomparisons applying the conventional Dicentric Chromosome Assay (DCA). Int J Radiat Biol 93:20–29 (2017). [PubMed: 27766931]

Pandita TK, Geard CR: Chromosome aberrations in human fibroblasts induced by monoenergetic neutrons. I. Relative biological effectiveness. Radiat Res 145:730–739 (1996). [PubMed: 8643833]

Repin M, Pampou S, Karan C, Brenner DJ, Garty G: RABiT-II: Implementation of a High-Throughput Micronucleus Biodosimetry Assay on Commercial Biotech Robotic Systems. Radiat Res 187:502–508 (2017).

Romm H, Ainsbury EA, Barnard S, Barrios L, Barquinero JF, Beinke C, Deperas M, Gregoire E, Koivistoinen A, Lindholm C, Moquet J, Oestreicher U, Puig R, Rothkamm K, Sommer S, Thierens H, Vandersickel V, Vral A, Wojcik A: Automatic scoring of dicentric chromosomes as a tool in large scale radiation accidents. Mutation research 756:174–183 (2013). [PubMed: 23707243]

Rossi HH, Bateman JL, Bond VP, Goodman LJ, Stickley EE: The dependence of RBE on the energy of fast neutrons: 1. Physical design and measurement of absorbed dose. Radiat Res 13:503–520 (1960). [PubMed: 13743717]

Royba E, Repin M, Pampou S, Karan C, Brenner DJ, Garty G: RABiT-II-DCA: A Fully-automated Dicentric Chromosome Assay in Multiwell Plates. Radiat Res 192:311–323 (2019). [PubMed: 31295087]

Salassidis K, Schmid E, Peter RU, Braselmann H, Bauchinger M: Dicentric and translocation analysis for retrospective dose estimation in humans exposed to ionising radiation during the Chernobyl nuclear power plant accident. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 311:39–48 (1994). [PubMed: 7526173]

Sasaki MS, Endo S, Ejima Y, Saito I, Okamura K, Oka Y, Hoshi M: Effective dose of A-bomb radiation in Hiroshima and Nagasaki as assessed by chromosomal effectiveness of spectrum energy photons and neutrons. Radiat Environ Biophys 45:79–91 (2006). [PubMed: 16807767]

Savage JRK, Papworth DG: Constructing a 2B Calibration Curve for Retrospective Dose Reconstruction. Radiation Protection Dosimetry 88:69–76 (2000).

Schmid E, Regulla D, Guldbakke S, Schlegel D, Bauchinger M: The effectiveness of monoenergetic neutrons at 565 keV in producing dicentric chromosomes in human lymphocytes at low doses. Radiat Res 154:307–312 (2000). [PubMed: 10956437]

Schmid E, Schlegel D, Guldbakke S, Kapsch RP, Regulla D: RBE of nearly monoenergetic neutrons at energies of 36 keV-14.6 MeV for induction of dicentrics in human lymphocytes. Radiation and Environmental Biophysics 42:87–94 (2003). [PubMed: 12844222]

Schmid E, Wagner FM, Romm H, Walsh L, Roos H: Dose–response relationship of dicentric chromosomes in human lymphocytes obtained for the fission neutron therapy facility MEDAPP at the research reactor FRM II. Radiation and Environmental Biophysics 48:67–75 (2008). [PubMed: 18979115]

Schmid HS M. Bauchinger, E.: Chromosome aberration frequencies in human lymphocytes irradiated in a phantom by a mixed beam of fission neutrons and gamma -rays. International Journal of Radiation Biology 73:263–267 (1998). [PubMed: 9525254]

Tanaka K, Gajendiran N, Endo S, Komatsu K, Hoshi M, Kamada N: Neutron Energy-Dependent Initial DNA Damage and Chromosomal Exchange. Journal of Radiation Research 40:36–44 (1999).

Tawn EJ, Curwen GB, Riddell AE: Chromosome aberrations in workers occupationally exposed to tritium. J Radiol Prot 38:N9–N16 (2018). [PubMed: 29589589]

Wernli C, Eikenberg J, Marzocchi O, Breustedt B, Oestreicher U, Romm H, Gregoratto D, Marsh J: 30-y follow-up of a Pu/Am inhalation case. Radiat Prot Dosimetry 164:57–64 (2015). [PubMed: 25527180]

Xu Y, Randers-Pehrson G, Marino SA, Garty G, Harken A, Brenner DJ: Broad Energy Range Neutron Spectroscopy using a Liquid Scintillator and a Proportional Counter: Application to a Neutron Spectrum Similar to that from an Improvised Nuclear Device. Nucl Instrum Methods Phys Res A 794:234–239 (2015a). [PubMed: 26273118]

Xu Y, Randers-Pehrson G, Turner HC, Marino SA, Geard CR, Brenner DJ, Garty G: Accelerator-Based Biological Irradiation Facility Simulating Neutron Exposure from an Improvised Nuclear Device. Radiation Research 184:404–410 (2015b). [PubMed: 26414507]

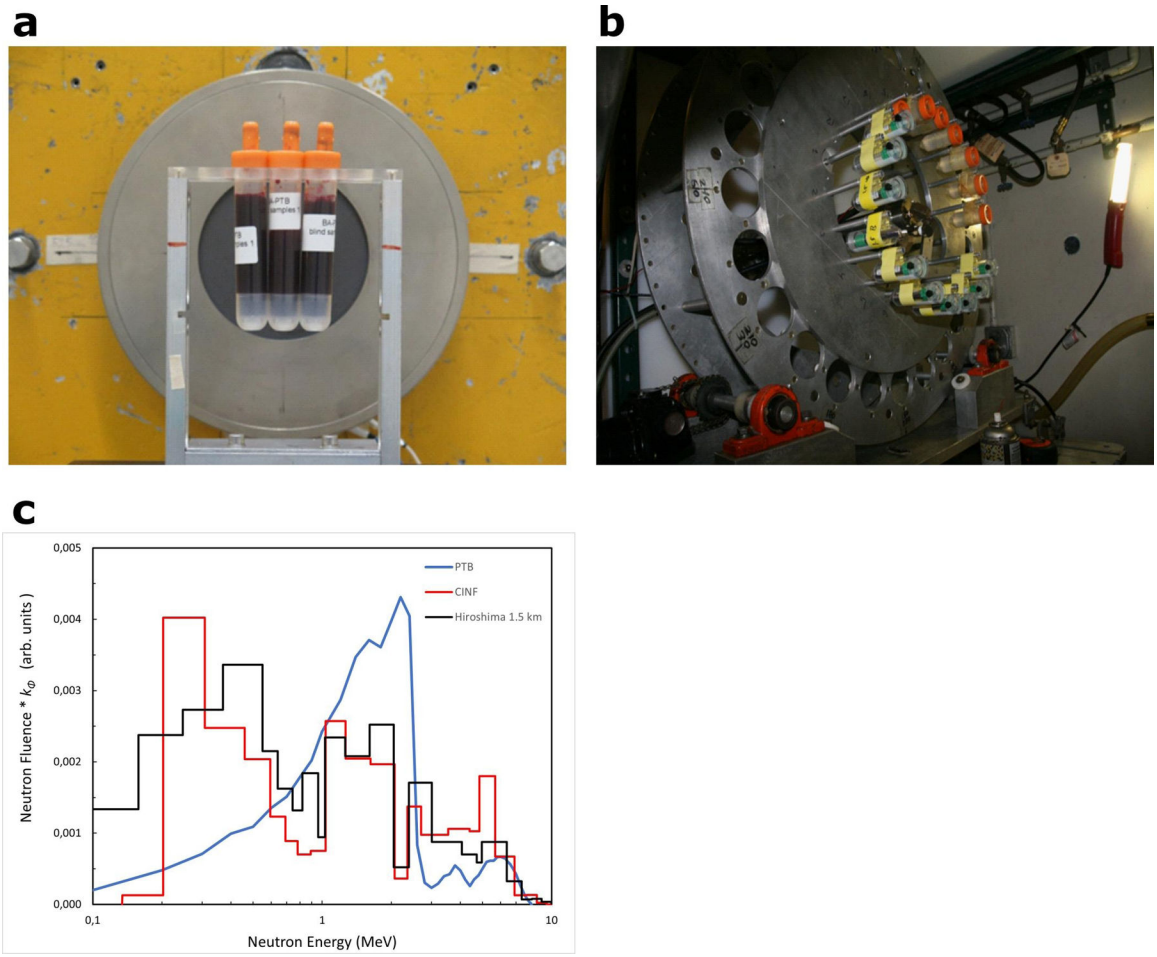

Figure 1: Irradiation of samples and comparison of neutron fields for the two irradiation facilities.

a&b: Position and mounting of the samples at PTB (**a**) and CINF (**b**). **c:** Calculated tissue-kerma-weighted relative energy distributions of the neutron fields (neutron fluence * kerma factors k_Φ), PTB (blue), CINF (red) and for the Hiroshima bombing at approximately 1.5 km from the epicenter (black).

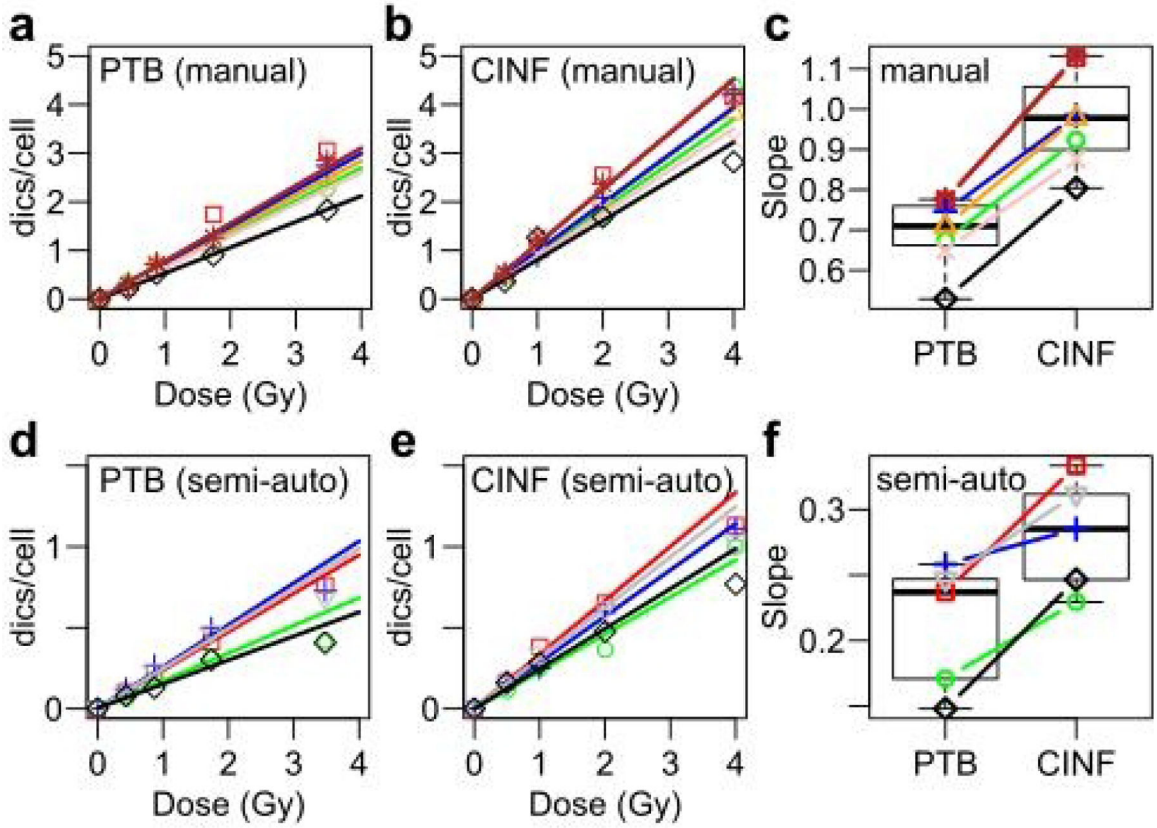

Figure 2: Calibration curves from RENEB participants for irradiations at PTB and CINF and comparison of slopes.

a&b: Linear calibration curves from manual scoring for irradiations performed at PTB (**a**) and CINF (**b**). **c:** Boxplots comparing the slopes of manually scored calibration curves from irradiations performed at PTB and CINF. **d&e:** Linear calibration curves from semi-automatic scoring for irradiations performed at PTB (**d**) and CINF (**e**). Different line colors and symbols indicate the participating laboratories from the RENEB network. **f:** Boxplots comparing the slopes of semi-automatically scored calibration curves from irradiations performed at PTB and CINF.

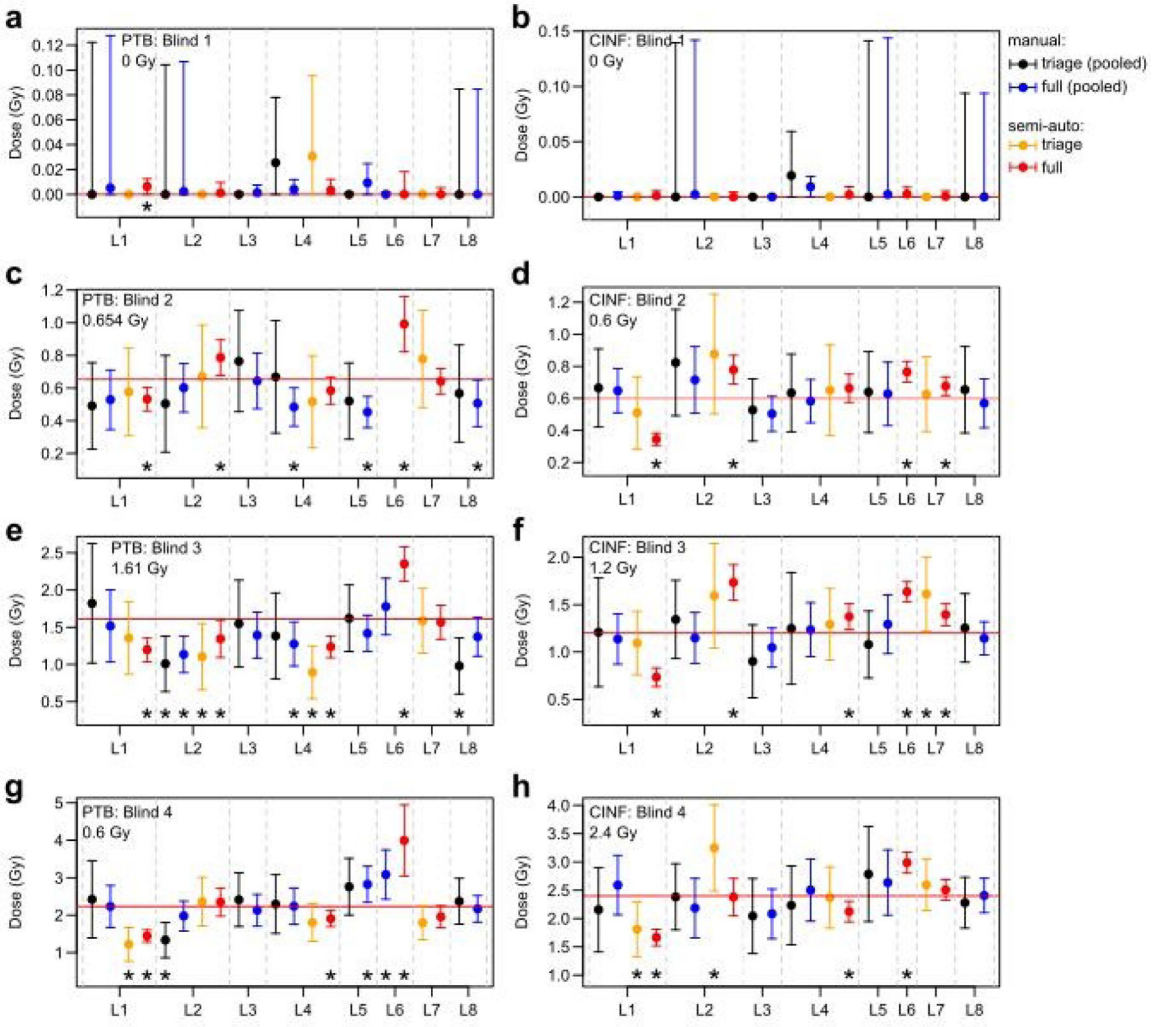

Figure 3: DCA-based dose estimates for test samples provided by the participating RENEB laboratories.

Each plot shows the dose estimates and the corresponding 95% confidence intervals (error bars) provided by the eight participating RENEB laboratories (x-axis). The results for manual scoring are shown in black (triage mode scoring) and blue (full mode scoring) and the results for semi-automatic scoring in orange (triage mode scoring) and red (full mode scoring). The asterisks indicate dose estimates where the corresponding 95% confidence intervals did not include the physical reference dose. Data from replicate slides was pooled for each test sample.

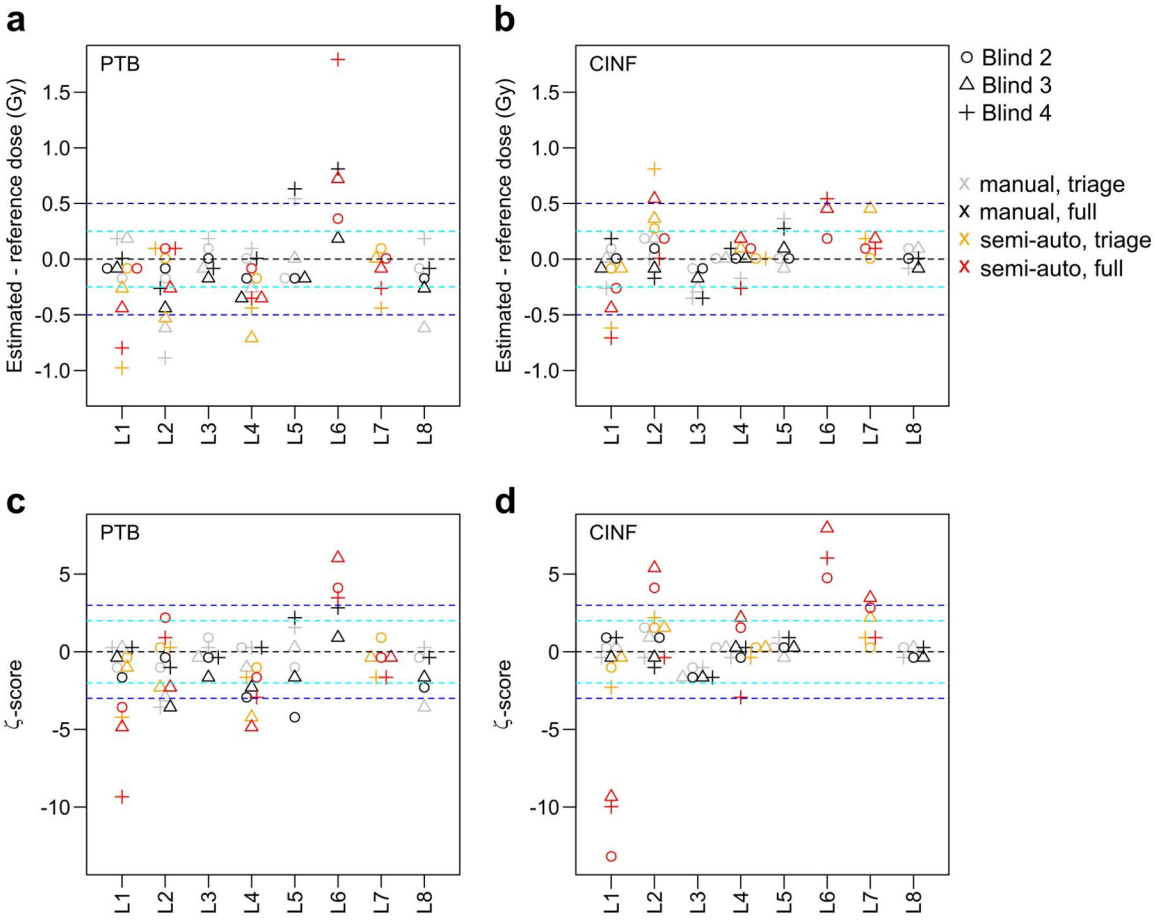

Figure 4: Comparison of DCA-based dose estimates with physical reference doses.

a&b: Difference between DCA-based dose estimates and physical reference doses (y-axis) for the eight participating RENEB laboratories (x-axis) from test samples irradiated with doses >0 Gy at PTB (**a**) and CINF (**b**). The horizontal lines show the intervals of ±0.25 Gy (cyan) or ±0.5 Gy (blue) around the physical reference dose. **c&d:** ζ -score (y-axis) for the eight participating RENEB laboratories (x-axis) from test samples irradiated with doses >0 Gy at PTB (**c**) and CINF (**d**). The horizontal lines indicate ζ -scores of ±2 (cyan) or ±3 (blue), respectively. Results with $|\zeta| < 2$ are considered as satisfactory, $2 \leq |\zeta| < 3$ as questionable and $|\zeta| \geq 3$ as unsatisfactory. The results for manual scoring are shown in gray (triage mode scoring) and black (full mode scoring) and the results for semi-automatic scoring in orange (triage mode scoring) and red (full mode scoring). Some laboratories performed only manual (L3, L5, L8) and some only semi-automatic scoring (L6 for samples irradiated at CINF and L7) and one lab (L6) performed the manual scoring only in full mode for samples irradiated at PTB.

Table 1:

Number of manually and semi-automatically scored metaphases per dose and calibration curve coefficients for calibration samples irradiated at CINF and PTB.

The number of scored metaphases and the number of dicentric chromosomes (in brackets) are shown for each dose point and each participating laboratory. The intercept (C) and slope (α) of the linear calibration curves and their corresponding standard errors (SE) are shown for each participating laboratory.

Facility: CINF (manual scoring)									
Lab	**0 Gy**	**0.5 Gy**	**1 Gy**	**2 Gy**	**4 Gy**	**C**	**α**	**SE(C)**	**SE(α)**
L1	1009 (1)	245 (118)	96 (101)	125 (318)	25 (104)	0.0010	1.1268	0.0010	0.0463
L2	1000 (0)	220 (100)	110 (102)	104 (175)	26 (114)	$9.8x10^{-8}$	0.9229	0.0657	0.0698
L3	1025 (1)	263 (118)	100 (107)	49 (100)	30 (115)	0.0010	0.9778	0.0010	0.0467
L4	1000 (1)	204 (100)	113 (100)	49 (102)	24 (101)	0.0010	0.9845	0.0010	0.0501
L5	1000 (0)	245 (102)	116 (108)	55 (104)	37 (121)	$6.4x10^{-6}$	0.8762	0.0632	0.0710
L6	1002 (4)	283 (101)	77 (97)	80 (135)	67 (189)	0.0040	0.8042	0.0028	0.0499
L8	150 (0)	346 (185)	195 (247)	199 (473)	100 (415)	$1.1x10^{-5}$	1.1321	0.0542	0.0501
Facility: CINF (semi-automatic scoring)									
L1	11803 (10)	6002 (994)	4548 (1740)	2868 (1865)	759 (858)	0.0009	0.3335	0.0003	0.0047
L2	2221 (1)	2632 (299)	1770 (442)	413 (151)	90 (90)	0.0005	0.2294	0.0005	0.0078
L4	3051 (2)	1708 (279)	1437 (361)	1057 (628)	310 (345)	0.0007	0.2852	0.0005	0.0075
L6	4617 (4)	2864 (460)	1663 (469)	2161 (1030)	563 (433)	0.0011	0.2465	0.0005	0.0053
L7	4445 (3)	4741 (756)	5453 (1836)	1164 (719)	624 (641)	0.0007	0.3117	0.0004	0.0053
Facility: PTB (manual scoring)									
Lab	**0 Gy**	**0.435 Gy**	**0.869 Gy**	**1.74 Gy**	**3.48 Gy**	**C**	**α**	**SE(C)**	**SE(α)**
L1	1000 (0)	414 (105)	164 (107)	61 (106)	38 (116)	$8.5x10^{-8}$	0.7737	0.0483	0.0632
L2	1012 (0)	350 (119)	303 (191)	218 (234)	98 (222)	$3.0x10^{-6}$	0.6743	0.0359	0.0405
L3	1000 (1)	430 (130)	159 (113)	121 (134)	45 (115)	0.0010	0.7096	0.0010	0.0332
L4	1000 (1)	334 (100)	141 (101)	83 (102)	38 (104)	0.0010	0.7464	0.0010	0.0380
L5	1000 (2)	429 (100)	145 (103)	82 (102)	50 (105)	0.0019	0.6494	0.0014	0.0330
L6	1002 (1)	450 (96)	188 (98)	131 (118)	52 (95)	0.0010	0.5290	0.0010	0.0268
L8	250 (0)	500 (145)	450 (323)	300 (379)	200 (570)	$1.5x10^{-7}$	0.7759	0.0336	0.0330
Facility: PTB (semi-automatic scoring)									
L1	22439 (19)	18706 (1978)	13333 (2928)	9004 (3726)	3297 (2512)	0.0009	0.2369	0.0002	0.0025
L2	6954 (5)	5940 (394)	3906 (697)	1179 (373)	485 (204)	0.0007	0.1708	0.0003	0.0044
L4	3527 (2)	2720 (331)	1997 (531)	1316 (652)	973 (710)	0.0007	0.2582	0.0004	0.0057
L6	3076 (7)	6891 (562)	4344 (563)	5039 (1522)	2432 (989)	0.0043	0.1479	0.0012	0.0027
L7	5232 (5)	5740 (607)	5820 (1361)	3451 (1629)	1331 (913)	0.0010	0.2470	0.0005	0.0039

Table 2:

Number of manually and semi-automatically scored metaphases and the number of dicentric chromosomes (in brackets) per dose for test samples with blinded doses irradiated at CINF and PTB.

Facility: CINF (manual scoring; full mode)				
Lab	**Blind 1 (0 Gy)**	**Blind 2 (0.6 Gy)**	**Blind 3 (1.2 Gy)**	**Blind 4 (2.4 Gy)**
L1	514 (1)	141 (103)	85 (109)	38 (111)
L2	500 (1)	250 (165)	200 (212)	60 (121)
L3	513 (0)	215 (106)	123 (126)	53 (108)
L4	500 (5)	174 (100)	83 (101)	41 (101)
L5	500 (1)	358 (197)	165 (187)	84 (194)
L8	200 (0)	197 (127)	399 (517)	200 (545)
Facility: CINF (semi-automatic scoring; full mode)				
L1	2481 (3)	3386 (392)	1081 (265)	1217 (676)
L2	2872 (1)	2715 (486)	1514 (603)	560 (306)
L4	1588 (2)	1470 (279)	1587 (623)	1558 (944)
L6	4747 (8)	4197 (796)	4144 (1675)	3008 (2218)
L7	2255 (2)	3241 (685)	1586 (691)	1462 (1144)
Facility: PTB (manual scoring; full mode)				
Lab	**Blind 1 (0 Gy)**	**Blind 2 (0.654 Gy)**	**Blind 3 (1.61 Gy)**	**Blind 4 (2.23 Gy)**
L1	501 (2)	247 (101)	86 (101)	66 (114)
L2	618 (1)	557 (226)	217 (166)	183 (244)
L3	509 (1)	155 (71)	104 (103)	88 (133)
L4	500 (2)	276 (100)	106 (101)	61 (102)
L5	500 (4)	500 (148)	221 (204)	110 (202)
L6	501 (0)	-	123 (113)	78 (127)
L8	150 (0)	267 (105)	199 (212)	148 (249)
Facility: PTB (semi-automatic scoring; full mode)				
L1	4608 (11)	1978 (251)	992 (282)	1144 (392)
L2	2163 (2)	1894 (256)	621 (143)	555 (223)
L4	1312 (2)	1503 (228)	1142 (365)	818 (403)
L6	2668 (10)	1172 (177)	1515 (533)	158 (94)
L7	3139 (3)	2026 (323)	523 (203)	424 (205)

Table 3.

Summary of dose estimates from manual and semi-automatic scoring in full mode for blind samples irradiated at CINF and PTB.

The column “Dose” shows the physical reference doses, the column “CV” shows the coefficient of variation, “δ” the percentage of results with overdispersion (dispersion index δ>1), “U” the percentage of results with significant (Papworth’s U test P<0.05) overdispersion and |Δ| the absolute average difference to the reference dose in mGy. The subsequent columns show the percentage of participants that included the physical reference dose within the estimated 95% confidence interval (CI), or within an interval of ±0.25 Gy or ±0.5 Gy, respectively, based on full mode scoring.

Code	Dose (Gy)	CV	$\delta > 1$ (%)	$U > 1.96$ (%)	$\lvert\zeta\rvert < 2$ (%)	$\lvert\Delta\rvert$ (mGy)	95% CI (%)	±0.25 Gy (%)	±0.5 Gy (%)
Facility: CINF (manual scoring)									
Blind 1	0	-	-	-	-	2	100	100	100
Blind 2	0.6	0.12	100	33	100	56	100	100	100
Blind 3	1.2	0.07	50	33	100	75	100	100	100
Blind 4	2.4	0.09	0	0	100	178	100	83	100
Facility: CINF (semi-automatic scoring)									
Blind 1	0	-	-	-	-	1	100	100	100
Blind 2	0.6	0.27	100	100	20	148	20	80	100
Blind 3	1.2	0.28	100	100	0	361	0	40	80
Blind 4	2.4	0.21	100	100	40	346	40	40	60
Facility: PTB (manual scoring)									
Blind 1	0	-	-	-	-	3	100	100	100
Blind 2	0.654	0.14	100	50	50	118	50	100	100
Blind 3	1.61	0.14	100	29	71	245	71	71	100
Blind 4	2.23	0.17	71	14	71	268	71	57	71
Facility: PTB (semi-automatic scoring)									
Blind 1	0	-	-	-	-	2	80	100	100
Blind 2	0.654	0.26	100	100	40	135	40	80	100
Blind 3	1.61	0.31	100	80	20	367	20	20	80
Blind 4	2.23	0.42	100	80	40	654	40	20	60