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Investigation of the influence of calibration practices on cytogenetic laboratory performance for dose estimation

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ABSTRACT

Purpose: In the frame of the QA program of RENEB, an inter-laboratory comparison (ILC) of calibration sources used in biological dosimetry was achieved to investigate the influence of calibration practices and protocols on the results of the dose estimation performance as a first step to harmonization and standardization of dosimetry and irradiation practices in the European biological dosimetry network.

Materials and methods: Delivered doses by irradiation facilities used by RENEB partners were determined with EPR/alanine dosimetry system. Dosimeters were irradiated in the same conditions as blood samples. A short survey was also performed to collect the information needed for the data analysis and evaluate the diversity of practices.

Results: For most of partners the deviation of delivered dose from the targeted dose remains below 10%. Deviations larger than 10% were observed for five facilities out of 21. Origins of the largest discrepancies were identified. Correction actions were evaluated as satisfactory. The re-evaluation of some ILC results for the fluorescence in situ hybridization (FISH) and premature chromosome condensation (PCC) assays has been performed leading to an improvement of the overall performances.

Conclusions: This work has shown the importance of dosimetry in radiobiology studies and the needs of harmonization, standardization in irradiation and dosimetry practices and educational training for biologists using ionizing radiation.

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Alanine dosimetry; biological dosimetry; calibration; inter-laboratory comparison; quality assurance

Introduction

Since the 1980s, a few programs have aimed to harmonize irradiation practises and dosimetry protocols in radiobiology (Zoetelief et al. 1985, 1997, 2001; Coleman et al. 2003; Desrosiers et al. 2013) and related reference documents have been published as an attempt to harmonize practises (Bond et al. 1979; International Atomic Energy Agency [IAEA] 2011). As radiobiology aims to establish relationship between delivered doses and biological effects, the problem of dosimetry and irradiation protocols has to be considered as an essential part of experimental designs, especially in quantitative radiobiology, as for example for biological dosimetry. In biological dosimetry assays, to assess a dose from the cytogenetic analysis of an irradiated blood sample, each laboratory has to establish its own calibration curve (IAEA 2011). Calibration curves are based on the analysis of in vitro blood samples irradiated in known conditions. Biological dosimetry techniques began several years ago to establish harmonization and standardization processes of practice (Voisin et al. 2002). Several International Organization for Standardization (ISO) standards have been published, but none has addressed the specific problem of the choice of the beam,

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the irradiation configuration, the dose quantity, the dose rate, the beam quality and the appropriate method for measuring and reporting dosimetric parameters (International Organization for Standardization [ISO] 2008, 2014a, 2014b). If the importance of the dosimetry standardization in radiobiology studies reaches generally consensus, it should be noted that, in the current literature, very few papers actually describe properly the dosimetric parameters with sufficient details (Desrosiers et al. 2013, Pedersen et al. 2016). The evaluation of the quality of dosimetry description in radiobiology studies made by Pedersen et al. (2016) provides an objective alert for the needs for more detailed reporting. Yoshizumi et al. (2011) considered that improper description of dosimetry makes most of the published work in radiobiology useless. The lack of details in the description of dosimetry does not mean that the dosimetry is incorrectly performed, but it makes difficult or impossible to reproduce the described experiments, to re-analyse published data and above all it prevents the possibility to compare results from different publications or works. This last statement is particularly important for quantitative methods, especially when important resources are spent to organize inter-laboratory comparison (ILC) programs. It is highly desirable to compare what can be compared, especially for highly standardized and accurate methods of biological dosimetry. As a matter of fact, discrepancies in dose estimation among participants in ILC programs can certainly be at least partly explained by the use of different dose quantities, beam qualities, dosimetric parameters, dosimetry protocols and irradiation set-ups. The aim of this paper is to underline the influence of calibration practises on dose estimation performances with cytogenetic assays. To demonstrate the importance of all these parameters to the radiobiologists' community, an ILC was elaborated in the frame of the work package of the RENEB (Realizing the European Network of Biodosimetry) project dedicated to Quality Assurance and Quality Management (QA&QM) (Gregoire et al. 2016). A survey among participants was conducted to collect the minimum information on the irradiation facilities and on the reference dosimetry to be able to analyze the evaluation of the doses delivered by the different facilities investigated. The results of this ILC are presented and discussed. Based on these data, some of the results of latest biological dosimetry ILC presented in this issue were discussed and re-evaluated (Barrios et al. 2016; Terzoudi et al. 2016). The usefulness of recommendations of the QA&QM RENEB manual regarding dosimetry and irradiation practises is evaluated. The utility of such comparison programs at a European level but also within educational, training, practise harmonization and assistance programs, not only for biological dosimetry but for the whole field of radiobiology, is also promoted.

Materials and methods

ILC set-up and organization

Alanine pellets, as described below, were distributed by express mail to participants during the first week of September 2015. In order to irradiate pellets in the same conditions as blood, it was requested to place pellets in the tube used for blood irradiation. Only one pellet was irradiated per tube. The tubes containing the pellets were filled with water to mimic the presence of blood in the tube in order to be as close as possible to actual conditions of blood irradiation. The tubes containing the alanine pellet were irradiated in the set-ups currently used by the participants. It was demanded of the participants that were in charge of the irradiations to keep the temperature of water in the tube and in the water bath (if used) between 20 and 25 °C and to report the temperature, in order to be able to correct the measured dose for any temperature effect during irradiation. After irradiation at a dose of 10 Gy, immediate return of the dosimeter was required. A procedure describing the conditions of irradiation and storage was sent with each pellet. Taking into account the amount of dose demanded and the low sensitivity of alanine, no additional dosimeters were supplied to control possible doses delivered from X-rays control during transportation. This type of controls generates dose of a few μSv to a few mSv (Zhumadilov et al. 2008). A questionnaire was also distributed to participants to collect the information necessary for the analysis of the alanine data: dose quantity used, beam characteristics, type of set-up for irradiation, including pictures. At this stage, we did not envisage to survey the dosimetry techniques and protocols used to calibrate the beams. However this will very probably be necessary as the next step in the harmonization process. The ILC was originally considered more as an educational action to focus attention on the importance of physical dosimetry in the calibration of cytogenetic assays. Three participants requested additional dosimeters, because they are using currently several irradiation facilities. For one participant, the dose evaluation was performed twice, because of unexpected results and to evaluate corrective actions undertaken in dose delivery.

EPR alanine dosimetry

To be able to compare the dose delivered by the different irradiation facilities used by the RENEB partners to irradiate blood samples, an alanine dosimetry system was selected. It presents the advantages of having low energy dependence when calibrated in terms of dose in water over a large range of photon energy (Olsen et al. 1990; Bergstrand et al. 2003). For gamma-rays from radionuclide sources (^{60}Co and ^{137}Cs) and X-rays produced by electrons accelerated in the MV range, a unique calibration factor can be defined. For photon energy below 100 keV, correction factors or specific calibration are necessary. Moreover, the fading is limited and the signal intensity can be corrected if needed (Anton 2008). As the reading is non-destructive, the dosimeter can be read several times allowing re-evaluation of EPR signals if it is necessary.

Pellets of alanine used in this work were purchased to Gamma Services Company (Germany). The participants were requested to irradiate the pellets in the same conditions as for blood samples, but 'with a dose' of 10 Gy. The dose of 10 Gy was selected to be able to provide data with reasonable uncertainties and to define a dose not too far from

doses used for blood irradiation for calibration purposes that usually never exceeds 5 Gy. Alanine pellets were irradiated in tubes used for blood irradiation filled with water. For this reason, each pellet was wrapped and sealed in a thin plastic film to avoid humidification of the pellet, which causes an increase of the fading rate (Sleptchonok et al. 2000).

Measurements of the pellets were performed at room temperature with an X-band EPR spectrometer (Bruker EMX) equipped with a high Q cavity. Recording of EPR spectra was performed with a microwave power of 2 mW, a modulation depth of 0.3 mT, a modulation frequency of 100 kHz and a magnetic field sweep of 12 mT. Ten EPR spectra were recorded for each pellet at minimum. Peak-to-peak amplitude of the central peak was reported for each spectrum and then related to dose. When repeating measurements of a pellet, between each measurement, the pellet was removed and replaced in the measurement tube in order to attempt to account for the contribution of the pellet positioning and spectrometry tuning in the uncertainty budget. To establish the calibration curves based on alanine spectra, pellets were irradiated at known doses (5, 10 and 20 Gy) with ^{60}Co gamma-rays to the French primary standard facility (Laboratoire National Henry Becquerel, CEA, Saclay). The beam was calibrated in terms of absorbed dose in water. As the temperature during irradiation was not always reported; it was decided to not correct the results from the temperature irradiation effect. Therefore, the uncertainties budget was increased because of the non-correction of this effect. This effect was evaluated at about 0.2% per °C (Schaeken et al. 2011). A contribution of 1% was added in the total uncertainty budget. In order to be able to correct the response of the dosimeters for the irradiations with orthovoltage X-rays facilities, a set of pellets were irradiated in terms of dose in water at the reference German facility (Physikalisch-Technische Bundesanstalt, Braunschweig) with the following beams: TH-250, TH-200, TH-150, TH-120 and TH-70. The characteristics of these X-rays beams are given in ISO standard 4037-1 (1996). Alanine pellets for calibration purposes were analysed during the same measurement session as the dosimeters sent back by the participants. The total uncertainty on dose is estimated at 5.5% (k = 2). Additional irradiations with MV X-rays were performed to illustrate the low energy dependence in this range compared to ^{60}Co gamma rays. Irradiations were performed with a linear accelerator at 4, 6, 10 and 18 MV (Clinac 2100) in the reference conditions described in IAEA (2000) with dose rate in terms of dose in water of 2 Gy per min.

Re-evaluation of data of the second ILC

Only the data of the second ILC were re-evaluated. For the second ILC, all samples were irradiated in air at the same facility with dosimetric references expressed in terms of air kerma, whereas some participants reported results in terms of absorbed dose in water. This fact is not taken into account in the analysis provided in papers submitted in this issue (Barrios et al. 2016; Oestreicher et al. 2016; Terzoudi et al. 2016). The re-evaluation of data from the cytogenetic ILC was limited to fluorescence in situ hybridization (FISH) and premature chromosome condensation (PCC) assays. The re-evaluation was done in two steps. Firstly, the doses delivered to blood samples distributed to participants were corrected and the Z-scores were recalculated. Secondly, for a few laboratories, the calibration curves were re-evaluated based on the dose measurements performed with alanine and the reported doses recalculated. The calibration source ILC was proposed only to RENEB members. Therefore, it was not possible to perform this exercise with the data from all participants since a large number of laboratories were not RENEB members.

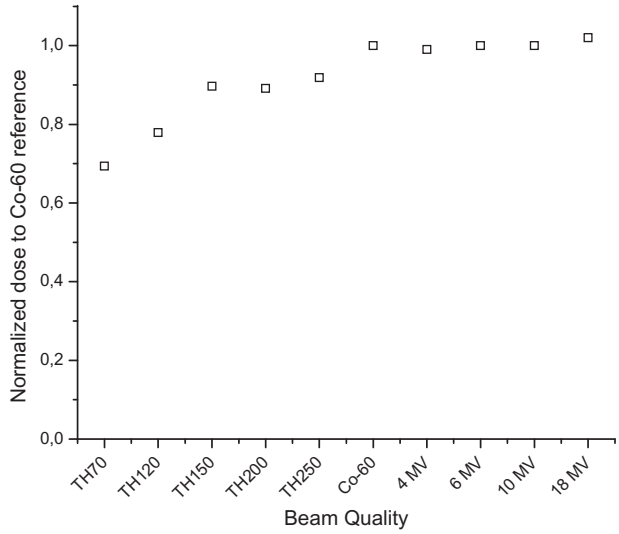

Figure 1. Variation of the relative dose response of alanine pellets normalized to ^{60}Co gamma-rays for different beam qualities.

Results

Before presenting the results of comparison of the calibration beam dosimetry, correction factors for the alanine dosimetry have to be determined for the orthovoltage facilities. The next section presented the results of the energy dependence of the alanine response.

Alanine energy dependence

Figure 1 presents the energy dependence of the dose response of the alanine pellets for various beam qualities. The dose response is normalized to the irradiation performed with ^{60}Co gamma-rays. The associated uncertainties are estimated at 5.5% (k = 2), for doses determined without energy correction factors. As expected for the most energetic photons, the observed variation remains largely within the expected uncertainties. For the orthovoltage X-rays irradiations between with TH150, TH200 and TH250, the alanine response decreases by about 10% relatively to ^{60}Co gamma-rays. With these data, it was therefore possible to evaluate the results obtained with alanine irradiated by participants with voltage ranging between 160 kV and 250 kV.

Overview of the questionnaire results

Table 1 summarizes the main information obtained from the questionnaire on irradiation facilities and dosimetry.

Table 1. Summary of the main information collected from the questionnaire.

	Beam characteristic		Dose quantity				Set-up			
Facility types	Reported	Not reported	D_w	k_{air}	k_{tissue}	Not reported	Water tank	air	10 cm Perspex	Not reported
Orthovoltage	2	2		2	0	2	0	4	0	0
LINAC – MW X-rays	4	0	4	0	0	0	4	0	0	0
Radionuclide sources	9	4	6	2	0	5	3	6	1	3

About half of the RENEB partners have access to an irradiation facility with radionuclide sources, ^{137}Cs and ^{60}Co gamma-rays sources. Another half has access to X-rays irradiation facilities. Half of the X-rays facilities are orthovoltage units while the other are linear accelerators (LINAC) operating with MV accelerating tension.

For all orthovoltage facilities investigated, irradiations were performed by participants in terms of air kerma and free in air. For LINAC facilities, all irradiations were performed in a water tank in terms of absorbed dose in water. For radionuclide sources facilities, various set-ups were used: free in air, in a water tank or behind a 10 cm thick Perspex plate to simulate water. For orthovoltage facilities, Half Layer Value (HLV) was also requested to verify that the alanine correction could be applied. This information was not always available.

The choice of the beam used for cytogenetic calibration seems chiefly to be driven by ease of access rather than the suitability to the expert needs. Therefore, facilities located close to the laboratory seemed to be preferred. In future questionnaires, it would be interesting to survey the motivation that has led to the selection the facility.

Overview of results of the exercise

Figure 2 presents the overview of the obtained results for the all investigated facilities. The uncertainty on reported dose is estimated at 5.5%. Taken into account the energy dependence correction for the orthovoltage irradiations, total uncertainties reach 6%.

Over the 21 facilities investigated in this survey, seven facilities presented measured doses that differed by more than 10% from the targeted dose of 10 Gy. For two of these facilities, that are radionuclide source facilities, differences exceeded 20%. For one of these facilities, a re-evaluation of the delivered dose led to correction of 30% (facility no. 6). For the facility no. 16, the origin of the discrepancy was not understood. For facility 14 and 15, an error was detected by the local physicist and corrected. The second dose evaluation showed a much better agreement for these two facilities. Doses delivered by irradiation facilities in radiobiology may be sometimes significantly different from the targeted doses. Surprisingly, systematic large deviations for orthovoltage facilities as reported in Pedersen et al. (2016) were here not observed. As a matter of fact, as dosimetry for orthovoltage facilities are considered to be complicated, a similar tendency was expected. It would be very interesting to investigate the reason(s) of these better results within RENEB partners to possibly derive some recommendations.

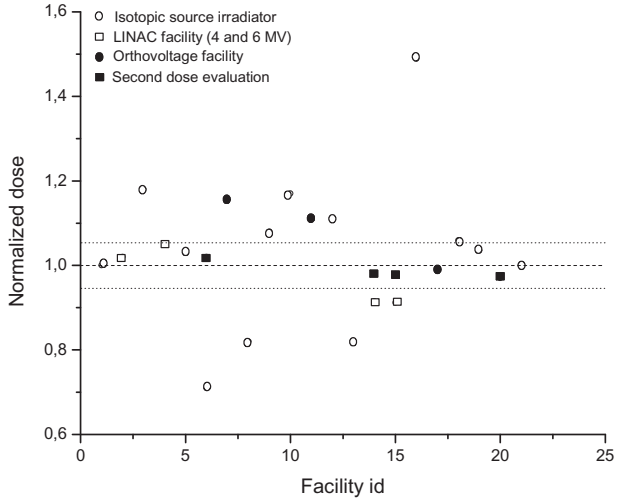

Figure 2. Doses measured on the 21 facilities with alanine with a calibration in terms of dose in water and normalized to the dose delivered at the French primary laboratory.

However, is should be pointed out that part of the laboratories have performed irradiation according to references expressed in terms of air kerma due to difference between mass energy absorption coefficients between air and water for the considered energies (cf. Table 1). For these laboratories, a systematic error of about 10% is expected. Taking into account this difference, four facilities presented a deviation from the targeted dose (also named error or accuracy in older publication) larger than 10%, to be compared to a number of seven without this correction. As a matter of fact, it was intentionally asked of the participants to irradiate the alanine dosimeters in accordance with their current practises. This observation points out the fact that by reporting the same dose quantity, laboratories can easily improve the accuracy of dose assessment. Therefore, in the RENEB QA&QM manual, a recommendation regarding dose quantity to be used has been included. It is recommended to perform the calibration and to report dose estimates in terms of dose in water.

Re-evaluation of results of the second RENEB ILC

Based on previous results, the influence of the dosimetry on the laboratories performances was evaluated. The doses delivered to the blood samples for the second ILC were re-evaluated accordingly the results of the alanine dosimetry. References doses for irradiation of samples labelled Re5 and Re6 were corrected by a multiplicative factor of 1.17. A summary of uncorrected and corrected doses is given in Table 2. This correction factor is based on the ratio between the targeted dose and measured dose by alanine. Without accurate information, it is assumed, here, that the conditions of all irradiations were similar.

As an example of the influence of the reference dosimetry on ILC statistical performance parameters, Figure 3 provides

Table 2. Summary of reference doses used for the second inter-laboratory comparison (ILC) as in Barrios et al. (2016) and after correction based on alanine dosimetry.

Sample code	Delivered dose expressed in air kerma based on dosimetry of the facility (Gy)	Delivered dose expressed in dose in water based on alanine dosimetry (Gy)
Re5	0.85 ± 0.03	0.99 ± 0.05
Re6	2.7 ± 0.08	3.2 ± 0.18

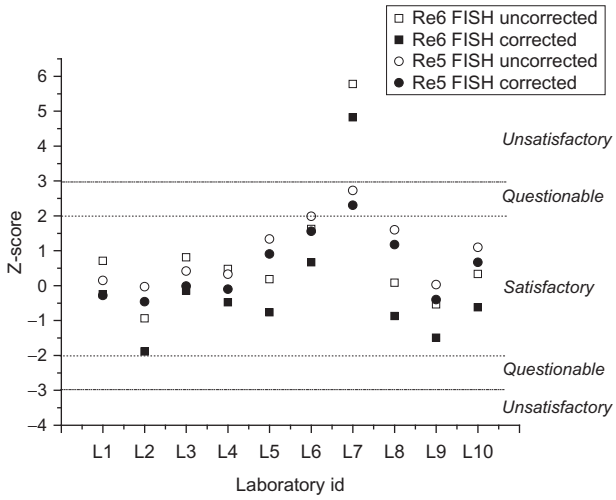

Figure 3. Frequency analysis of the Z-scores for the dose estimation reported for samples Re5 and Re6. Dashed lines indicate boundaries of classification according to Z-scores.

the Z-score calculated for the different participants of the second FISH ILC for the uncorrected and corrected value of the reference doses and for samples Re5 and Re6. In order to put highlight the influence of the reference dose on the performance, Z-score was calculated as proposed in ISO 13528 (2005) using the reference dose instead of the average dose.

Overall, the correction on the reference dose induced a decrease on the Z-score for all participants. The number of laboratories with unsatisfactory results based on this criterion remained the same and the questionable results were reduced from two to one. Overall, the dose correction did not induce significant improvement of the performance evaluated with Z-score; only the Z-score distribution was modified as shown in Figure 4. The Figure gives the frequency analysis of the Z-score for the two samples (Re5 and Re6) of the second ILC before and after dose reference correction.

In addition to Z-score, the Root Mean Square Error (RMSE) was also evaluated for the different set of data as presented in Table 3. The RMSE was reduced for the data for the two samples (*cf.* Table 3) when reference doses were corrected. This data show that the agreement was found to be better in average with the reference dose, when these doses were corrected.

This example shows that the FISH technique was sufficiently accurate and that the overall performance could be significantly affected by inaccurate reference doses. In such ILC, attention should be paid to irradiation and dosimetry of the irradiation in order to reduce uncertainties and improve the accuracy of references doses.

A similar study was performed with the results of the second PCC ILC based on the results presented in Terzoudi et al. (2016). For this ILC, two samples per dose were distributed to the participants (samples respectively labelled Re5a, Re5b, Re6a and Re6b). Figures 5 and 6 compare the Z-scores calculated for samples Re5 and Re6 (a and b), with the uncorrected and corrected reference doses.

For the PCC assay results, the dose correction decreased the number of questionable data points for Re5, whereas it had the inverse effect for Re6. Only based on Z-score, the influence of the reference dose correction on the laboratory performance was not obvious. As for the FISH assay, the RMSE was also evaluated as shown in Table 4. RMSE evaluations showed that the agreement was generally similar for the Re5 samples, but much worse for Re6 (the largest dose). It is important to note that for PCC, samples irradiated at known dose were also distributed to establish a calibration curve. The irradiation was performed on another facility than the one used to irradiated Re5 and Re6. This latter facility according to Terzoudi et al. (2016) was also a radionuclide facility (gamma cell 220 supplied with ^{60}Co sources) with dose rate about 10 times higher than the dose rates used for blind dose. Regarding the RMSE, the reference dosimetry on the gamma cell is perhaps also questionable.

It was intended to collect additional information on irradiation facilities and calibration curves to provide a more detailed re-analysis of the whole data from first and second RENEB ILC. With the data available, it would have been possible only for a few partners to recalculate the calibration curves and to re-evaluate the reported doses. This future work is out of the objectives of this paper that was only aiming to raise the attention of the importance of having accurate and traceable dosimetry in biological assays.

Discussion

The ILC of calibration beams presented in this paper has shown that large differences can be sometimes observed between the targeted doses and the actual delivered dose. By means of the current work, the largest differences (>20%) observed were identified and corrective actions have been applied or are in progress. For two other facilities, shortcomings were also detected and corrected based on the current findings, which again has led to the improvement of the accuracy on delivered doses. This is the first benefit of this work. For most of facilities the differences between targeted and delivered dose ranges between 2 and 10%.

It has been suggested by Zoetelief et al. (1997) 'that an accuracy of better than ±5% is required for radiobiological studies'. This value of 5% may be somewhat questionable. Dosimetry techniques that could be used to evaluate the accuracy within the frame of ILC program have associated uncertainties (k = 2) usually larger than 4%, as for routine EPR alanine or thermoluminescence dosimetry (TLD) (Zoetelief et al. 1997, ISO/American Society for Testing and Materials [ASTM] 2004, 2013). Efforts could be made to reduce uncertainties, but one might wonder if this is really necessary, regarding the contribution of the reference

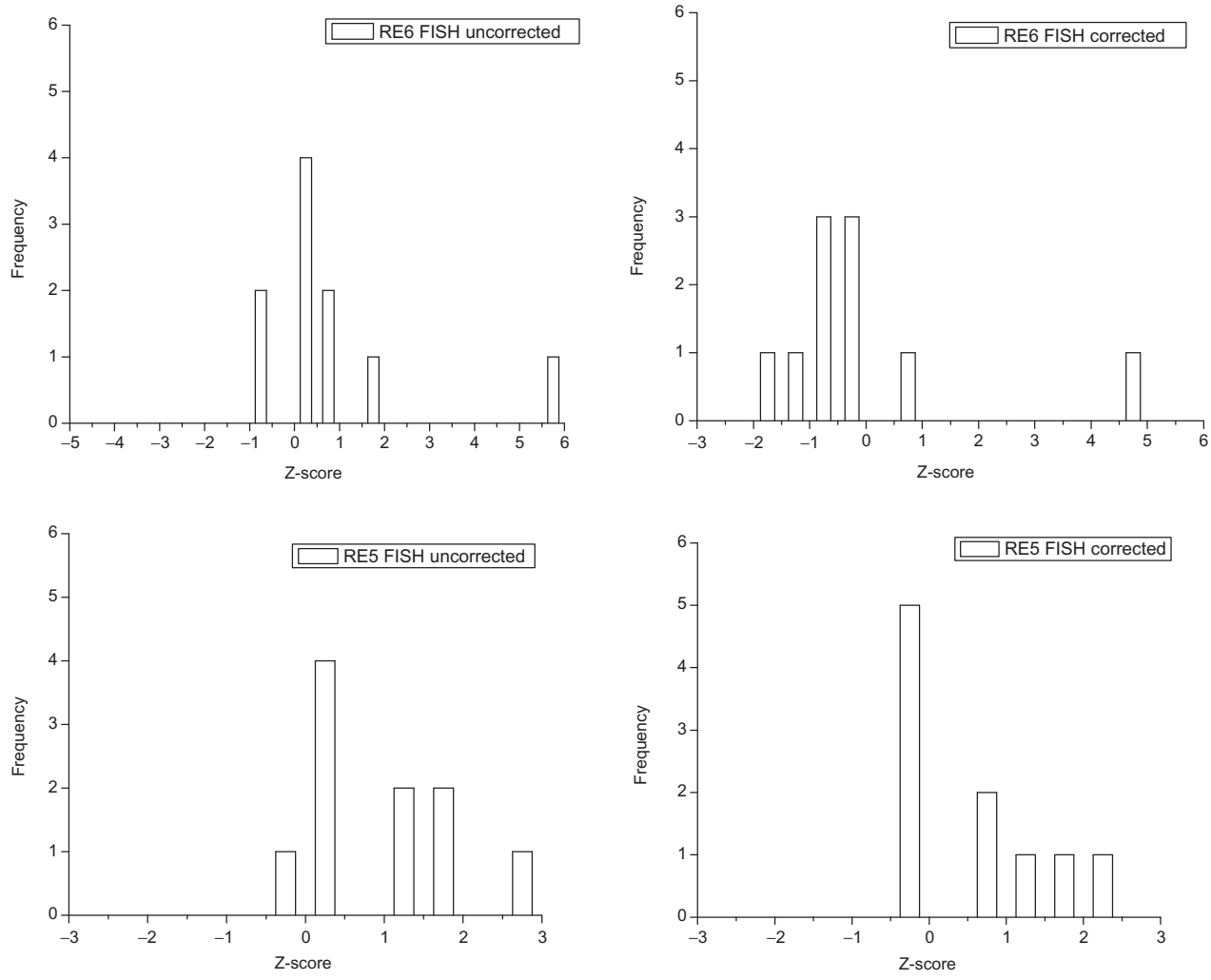

Figure 4. Frequency distribution of the Z-score for the samples Re5 and Re6 analyzed by fluorescence in situ hybridization (FISH).

Table 3. Root Mean Square Error (RMSE) for the reported results of samples Re5 and Re6 of the second inter-laboratory comparison (ILC) for fluorescence in situ hybridization (FISH) with reference dose as in Barrios et al. (2016) and with correction of reference dose.

Samples	RE5 uncorrected (0.85 Gy)	RE5 corrected (0.99 Gy)	RE6 uncorrected (2.7 Gy)	RE6 corrected (3.2 Gy)
RMSE	0.44	0.35	0.95	0.85

dosimetry to the total uncertainties budget in biological dosimetry. The target in terms of accuracy for such ILC programs remains to be discussed for biological applications and a reasonable target for accuracy has to be defined. Specifically for the QA&QM RENEB program, the pertinence to perform periodically such ILC also still needs to be discussed. If it is foreseen to repeat such ILC for QA purposes, then criteria must be determined to indicate whether dosimetry for a given facility should be questioned or not.

One of the other positive outputs of this ILC was to raise awareness within the biological dosimetry community of the importance of accurate dosimetry, and to report sufficient information on dosimetry and irradiation to ensure traceability of calibration curves and to be able to properly compare data from different laboratories. As a matter of fact, the short

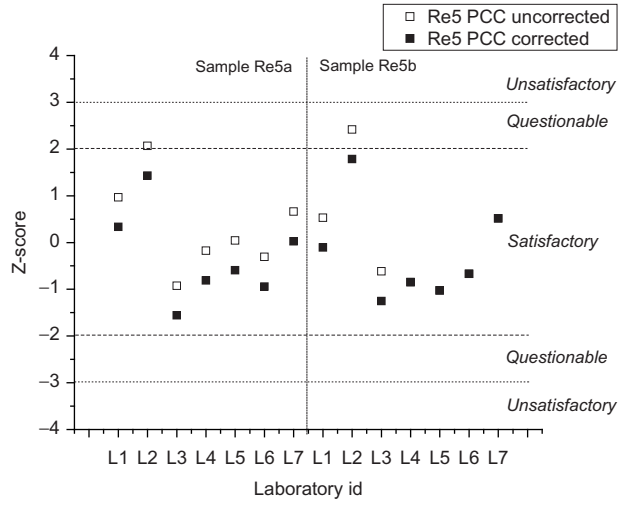

Figure 5. Z-scores for estimated doses based on 30–40 cells calculated for samples Re5a and Re5b. Dashed lines indicate boundaries of classification according to Z-scores.

survey conducted within this ILC has shown some differences in calibration practises. The calibration curves are established versus 'doses' expressed in air kerma or in absorbed dose in water when this parameter is known. From a methodological

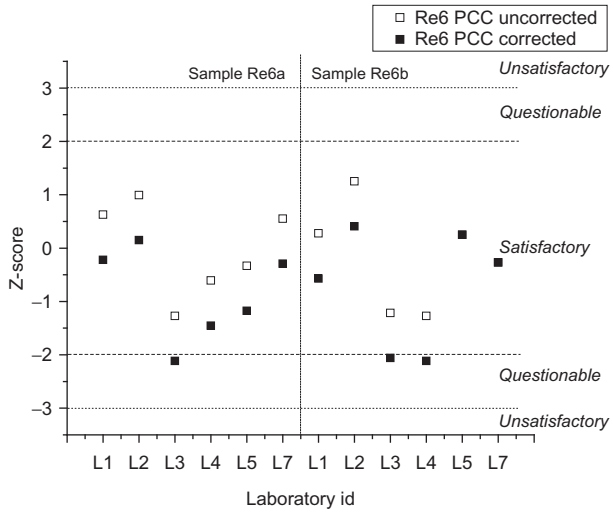

Figure 6. Z-scores for estimated doses based on 30–40 cells calculated for samples Re6a and Re6b. Dashed lines indicate boundaries of classification according to Z-scores.

Table 4. Root Mean Square Error (RMSE) for the reported results of samples Re5 and Re6 of the second ILC for premature chromosome condensation (PCC) with reference dose as in Terzoudi et al. (2016) and with correction of reference dose.

Samples	RE5 uncorrected (0.85 Gy)	RE5 corrected (0.99 Gy)	RE6 uncorrected (2.7 Gy)	RE6 corrected (3.2 Gy)
RMSE	0.28	0.29	2.56	5.69

point of view, corrections on reporting dose should be applied before any attempt to analyze data from ILC. If such corrections can be performed, significant improvement of ILC performances is expected, with a minimum of effort. In the QA&QM manual, to harmonize dose reporting and calibration practise, it is now recommended to calibrate and report doses for cytogenetic assay in terms of dose in water (Gregoire et al. 2016). Depending on the facility, this recommendation would necessitate additional dosimetry work. Desrosiers et al. (2013) insist on the necessary collaboration with physicists to define the most suitable irradiation set-up and dosimetry protocol. For example, for orthovoltage dosimetry, at least six different protocols can be used for the reference dosimetry (Deutsches Institut für Normung [DIN] 1988, 1996; Institution of Physics and Engineering in Medicine and Biology [IPEMB] 1996; Nederlandse Commissie voor Stralingsdosimetrie [NCS] 1997; IAEA 2000; Ma et al. 2001). For MV X-rays dosimetry, different protocols are also proposed (Almond et al. 1999, IAEA 2000). The difference in terms of dose rate values between these protocols can reach 7% (Peixoto and Andreo 2000). Therefore the choice of a protocol is also of importance for traceability purpose. It would be useful to provide a few recommendations in the RENEB QA&QM manual regarding the choice of dosimetry protocols and also a list of parameters and dosimetric data that have to be recorded when a dosimetry protocol is applied. An example of minimum of parameters to be recorded can be found in Desrosiers et al. (2013).

With some facilities, the application of reference dosimetric protocols may be difficult and some compromises have to be made, which usually increase uncertainties on dose rate. It should be clear that the dosimetry results provided by the manufacturer cannot usually be used directly. Masterson and Febo (1992), after having studied the dosimetry of irradiators for blood, recommend not using them, because significant variations in delivered doses were found in this study. Moreover, the dosimetric quantity may not be adapted. It does not take into account the effect of the set-up used for irradiation and the radiation field heterogeneity. Some irradiation facilities are designed to deliver large amounts of dose in short times. Even if the dose rates are correctly measured, irradiating at lower doses may introduce problems. Irradiations of a few seconds, whatever the facility considered, should be avoided. For example, with some radionuclide source irradiators, the source may travel few seconds up to about tens of seconds, before it reaches the irradiation position. During transit, it starts to irradiate the samples in an inhomogeneous way, delivering a quasi-constant dose whatever the duration of the irradiation. In the softwares used to determine the irradiation duration, this additional dose is usually not determined and therefore not taken into account. As an end result, for short irradiation duration, the difference between the targeted doses and delivered doses can be up to tens of %. It is recommended therefore to irradiate with a dose rate that makes it possible for the desired delivered dose to be achieved in irradiation times of at least tens of seconds. Even with orthovoltage X-ray irradiators, irradiations of shorter than 10 sec should be avoided to minimize the effect due to the tube stabilization.

Therefore, a certain number of criteria should be evaluated when choosing an irradiation facility. As a matter of fact, it seems that the choice of facility to calibrate the assays is mainly driven by the ease of access. As establishing a calibration curve necessitates a very large amount of work and as curves for this same reason are not regularly re-evaluated, it makes sense to select the irradiation facility(ies) used for assay calibration on according to much more rigorous criteria. In general, in dosimetry, especially if the linear energy transfer (LET) has an influence on the dose response of the assay, the beam quality selected for the assay calibration should be as close as possible to the beam quality to which persons were exposed. It is reported that the variation in LET between orthovoltage and MV X-rays or ^{60}Co gamma-rays is sufficient to induce significant changes in the parameters of the calibration curves. The effect is obviously more important between gamma-rays and fission neutrons, for examples, calibration curves for neutron become almost linear for dicentric assay. The same type of remarks could be made regarding the dose rate effect. In physical dosimetry, it is usually necessary to correct the response of the dosimeter depending on LET of beam quality as has been done here with alanine dosimeters. It is possible to define energy or LET correction factors, avoiding establishing a full calibration curve for each beam quality. In physical dosimetry, the type of fitting curve does not usually depend on the beam quality; most of detectors respond linearly with dose whatever the beam type and within a large range of dose rates. For most of cytogenetic assays, this is not the case and as a consequence, there is no alternative than to establish several calibration curves.

Regarding the work needed to establish one calibration curve, it can be easily understood that the choice of the beam quality becomes crucial regarding the biological dosimetry expertise that are usually demanded. Another obvious criterion would be the quality of the dosimetry, its traceability, the beam homogeneity, the use of an adapted set-up that respects the conditions in which reference dose quantities have been measured.

Future, perspectives and needs

Desrosiers et al. (2013) underlined the necessary collaboration between physicists and radiobiologists, especially for the design and the realization of irradiation and dosimetry and also to ensure that the minimum of necessary information is made available and reported. In its early years, EURADOS, which was originally an association of physicists, has developed harmonization and survey programs for dosimetry in radiobiology together with the European Late Effects Project Group (EULEP). This action was not maintained and a long period has followed without further action at European level. The working group 10 on retrospective dosimetry of EURADOS was initially aimed at development of the cooperation and exchange between biologists and physicists. Later, MULTIBIODOSE and RENEB EC projects have followed the same philosophy (Kulka et al. 2012; Wojcik et al. 2014). It is desirable to create conditions for such cooperation in order to improve the quality of realization of radiobiology experiments and to make all the published results useful.

Regarding the results obtained within RENEB, with a minimum of investment, it could be highly interesting to propose such calibration beam ILC to all of the participants of the second RENEB ILC which has involved 42 laboratories from 35 countries. The international biological dosimetry community should decide whether a formal beam dosimetry ILC program needs to be periodically implemented and, if so, how it could be established and sustained.

Taking this opportunity, a more detailed survey should be conducted to investigate the variety of dosimetric protocols and irradiation set-ups and as well the need for educational programs in dosimetry for biologists. Educational and training actions would be a natural follow-up of the work already conducted within RENEB.

Conclusions

Within the RENEB QA&QM work-package, an ILC program was conducted to evaluate possible sources of systematic bias in dose evaluation due to difference in calibration procedures among cytogenetic laboratories members of RENEB. This work has shown the importance of dosimetry in quantitative radiobiology and the necessity to conduct such ILC program in radiobiology.

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