Cytogenetic damage analysed by the dicentric assay

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Summary. Biological dosimetry, based on the analysis of dicentric chromosomes, is an internationally established, independent method applied in the area of radiation protection. The biodosimetry is mainly performed, in addition to physical dosimetry, with the aim of individual dose assessment, especially after unclear or suspected radiation dose exposures. However, a new biodosimetrical challenge has emerged in recent years in the form of a possible large scale radiation accident potentially involving large numbers of exposed persons. In order to be prepared to act in an efficient manner in such an accident, the established cytogenetic laboratories have increased their cooperation at the national and international level. General experience and results of intercomparisons are reported and future options to increase sample throughput are outlined.

Key words: chromosome aberrations, biological assay, cytogenetic analysis, mass casualty incidents, radiation dose-response relationship.

Riassunto *(Il danno citogenetico analizzato con il test dei dicentrici).* La dosimetria biologica basata sull'analisi dei cromosomi dicentrici è un metodo internazionalmente riconosciuto nell'ambito della radioprotezione. Insieme alla dosimetria fisica, questo metodo è applicato alla stima della dose individuale, specialmente in caso di esposizioni non chiare o sospette a radiazioni ionizzanti. Negli anni recenti una nuova sfida per la biodosimetria è venuta dalla possibilità di un incidente radiologico su ampia scala che coinvolga potenzialmente un gran numero di persone. Per predisporre una risposta efficace a situazioni di questo tipo, i laboratori di citogenetica stanno aumentando la collaborazione a livello sia nazionale che internazionale. In questo lavoro si descrive l'esperienza acquisita e i risultati ottenuti negli interconfronti effettuati e si delineano possibili opzioni atte ad aumentare la capacità di misura del metodo.

Parole chiave: aberrazioni del cromosoma, saggio biologico, analisi citogenetica, eventi con vittime in massa, radiazioni relazione dose-risposta.

INTRODUCTION

The conventional analysis of dicentric chromosomes in the indicator system "peripheral blood" has achieved significance for radiological protection purposes as a useful technique to supplement physical dose measurements in the event of suspected or confirmed accidental radiation exposures.

In 1962 it was suggested by Bender and Gooch [1] that dicentric chromosomes in peripheral lymphocytes could well be used for the detection and dose assessment of human radiation exposures and these authors have used this method for the first time in the sense of biological dosimetry at the occasion of the so-called Recuplex criticality accident at Hanford, USA. A further significant development of the method was the introduction of the fluorescence plus Giemsa (FPG) staining [2]. With this technique it became possible to distinguish between the first and subsequent mitotic divisions after culture initiation. This discrimination of the first and the following cell divisions is important as dicentric chromosomes are lost at cell division. Following the development of the fluorescence in situ hybridisation (FISH) technique [3] symmetrical translocations can easily be detected. This more persistent aberration type has the advantage that it is not lost during cell divisions. In consequence, dicentric chromosomes are the indicator of choice for recent and acute radiation exposures and symmetrical translocations for past and chronic radiation conditions.

There are several essential requirements for biological parameters as meaningful dosimeters:

- low background level;
- clear dose effect relationship for different radiation qualities and dose rates;
- specific to ionising radiation;
- non-invasive;
- fast availability of dose estimate;
- good reproducibility;
- comparability of *in vitro* and *in vivo* results.

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The dicentric assay conforms to all of those points which makes this assay a robust and "gold standard" biodosimetry method [4]. Up to date the dicentric assay is the most specific and sensitive biological indicator of dose in case of an acute radiation overexposure [5].

To be prepared for radiation threats a scoring procedure was developed to allow high throughput analysis in a triage mode [6]. Using this method in case of a radiation mass casualty a biodosimetry screening of a high number of persons could be performed in a fast and reliable manner by a network of cytogenetics laboratories.

This paper outlines the current status of dicentric chromosome assay for the purpose of biological dosimetry in case of a small radiation accident and the requirements to the assay, when it is used in triage mode for mass radiation casualty.

BLOOD SAMPLING AND CULTURE CONDITIONS

For the analysis of dicentric chromosomes, blood should be sampled within four weeks post exposure since after this time the frequency of dicentrics begins to decrease [7]. If the radiation exposure occurred with lower doses the sampling time can be extended to about six months. In the case of partial-body or non-uniform exposure the sample should not be drawn before about 24 h after the exposure in order to assure a homogeneous mixture of irradiated and unirradiated lymphocytes.

The best anticoagulant for lymphocyte cultures is preservative free lithium heparin. T-lymphocytes are generally stimulated *in vitro* to proliferate by the mitogen phytohaemagglutinin (PHA) during a culture period of about 48 hrs at 37 °C. In order to discriminate between first and following cell divisions bromodeoxyuridine (BrdU) is added to the culture medium. After the induction of a mitotic block by colcemid for the last 3 hrs of culture time and subsequent fixation, the suspension can be dropped onto slides. The metaphase preparations can be further processed for conventional Giemsa staining (FPG) or for fluorescence in situ hybridisation (FISH). A detailed protocol for culture set up is given by Stephan [8].

In recent publications [9, 10] modifications of the culture conditions were described. These include adding colcemid either for the whole duration of culture or for the last 24 h. The modification allows to identify first division mitoses, but also results in a higher frequency of more condensed chromosomes. The main advantage of the method is a faster staining procedure of the cells in case of the need for high throughput. All cells can be analysed by using automatic procedures.

BACKGROUND FREQUENCY OF DICENTRIC CHROMOSOMES

An important prerequisite for dose reconstruction of exposed persons is the knowledge of the background levels of the corresponding chromosome aberration types, determined in blood samples of healthy unexposed persons. A careful analysis of the baseline frequency of chromosome aberrations is important because this information has a direct bearing on the precision of dose estimate, especially when individuals are suspected to have been exposed to a low dose of radiation.

Numerous studies have been performed to evaluate the spontaneous background levels of dicentric chromosomes. The frequencies vary extensively among different studies [11, 12]. Values for dicentric frequencies between 0.09 [13] and 2.99 per 1000 cells [14] are reported. It was expected that a cell cycle controlled scoring, together with standardization of culture conditions would eliminate most interlaboratory variations. However, the factors responsible for the interlaboratory differences remain unexplained and can be attributed, at least partly, to the scoring criteria of the observer. In consequence, for purposes of biological dosimetry, each laboratory should have its own control data and its own dose-response curves. If so, in case of a chromosome analysis of an exposed person all conditions (culture, preparation, scoring) are identical for the three samples (control data, dose response curve, exposed person). Since the quantification of a radiation exposure in the low dose range is strongly influenced by the background frequency of chromosome aberrations, a wrong dose assessment for subjects who received low-level exposure may occur when the background dicentric levels are chosen from the literature.

The control group of the BfS laboratory includes 53 healthy individuals (54 689 scored cells) and the mean frequency of dicentrics is 1.15 ± 0.15 per 1000 cells [15] *(Figure 1)*. In this group 7 smokers with a daily consumption of > 30 cigarettes are included.

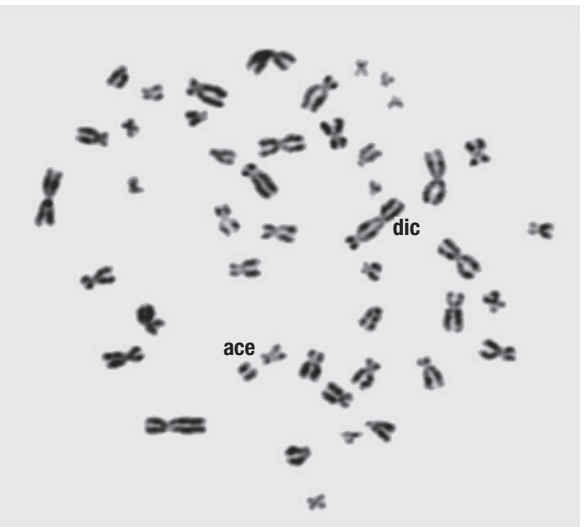

Fig. 1 | *Dicentric chromosome (dic) and acentric fragment (ace) in first division mitosis of human peripheral lymphocyte.*

Without the heavy smokers the mean frequency of dicentrics is 0.96 ± 0.14 (47 593 scored cells). In the control group with an age range from 20 years to 73 years no relation to age was observed, furthermore, there was no difference between males and females in the frequency of dicentric chromosomes. In children, however, the background frequency of dicentrics was found to be lower (0.4 ± 0.2 dic/1000 cells) than in adults [16]. Similar low control values for children could be observed by Padovani *et al*. [17].

Sometimes the question is posed whether an increased level of chromosome aberrations may have some health consequences. Cells with dicentrics chromosomes and associated fragments are regarded as unstable cells, because they are eliminated during cell proliferation. Thus, they pose no risk of giving rise to cancer. However, dicentric chromosomes are induced with the same frequency as symmetrical translocations. And since symmetrical translocations can be transmitted to descendent cells they may bear a potential risk of inducing cancer. It is well documented that cancer can be initiated by chromosomal changes. It is, however, not possible to predict a given risk associated with an increased level of radiation-induced chromosome aberrations on the individual level. On the other hand it was shown that in populations, individuals with an elevated background frequency of chromosomal aberrations show a higher cancer risk than individuals with a low frequency [18, 19].

DOSE RESPONSE RELATIONSHIPS

Dose response curves for the yield of exchange aberrations are generated by irradiation of unstimulated lymphocytes *in vitro*. The relation between acute doses of low LET radiations and yield of exchange aberrations can be described by the following equation:

$$y = c + \alpha D + \beta D^2$$

where y is the yield of aberrations per cell, c the background frequency, α is the coefficient for exchange aberrations produced by single electron tracks, and β, the corresponding coefficient for aberrations produced as consequence of two electron tracks. D is the absorbed dose in the cell. The quotient α/β is equal to the dose at which the linear and quadratic components contribute equally to the formation of exchange aberrations. Below this dose the majority of aberrations will be produced by single tracks. As the interacting lesions must be produced almost simultaneously when the ionising particle traverses the nucleus, aberrations associated with the linear term are independent of the effects of dose rate or fractionation. Fractionating and dose rate of low LET radiation affect the yield of aberrations mainly at higher doses where the damage is associated with the dose squared term (βD^2) of the above mentioned equation. The reason for this is that the two breaks in two different chromosomes which are the supposition for forming exchange aberrations must be induced temporally and locally close enough for a random pairwise interaction. With increasing LET there is an increased likelihood that two breaks are produced by a single ionising track and the relative contribution of αD becomes more prominent. For high LET radiations, the dose-response relation for exchange aberrations tends therefore to be linear and the yield is independent of exposure time and fractionation

$$y = c + \alpha D.$$

It is of practical importance to know the different relative biological effectiveness (RBE) of the various radiation qualities that are in use, or are likely to be in use, in medicine, in research laboratories, in nuclear installations, or are in everyday use in industry. It is known that the X-rays possess a higher effectiveness for the induction of dicentric chromosomes than Cs-137 γ-rays. The biological efficiency of X-rays is influenced by filtration since different filter/filter combinations produce various photon energy spectra and the biological effectiveness of photon radiation depends on the photon energy [20]. It is well established that neutrons have a higher RBE than photons but also the RBE of neutrons is influenced by their energy [21]. In consequence, for biological dosimetry purposes an appropriate calibration curve must be used for dose estimation.

A considerable part of the available knowledge on the relative biological effectiveness of different radiation qualities has been derived from experimental studies on the dose-response relationship for the production of dicentric chromosomes in human lymphocytes. In recent publications, the relative biological effectiveness of different radiation qualities were investigated in more detail [22]. The variations in RBE at different energies and dose rates are of interest in understanding the basic mechanisms of radiation biology [23]. Another aspect of interest is the depth-dependence of the biological effectiveness of various radiation qualities [10, 24].

IN VITRO AND *IN VIVO* RADIATION INDUCED ABERRATION FREQUENCIES

The advantages of measuring radiation-induced chromosome aberrations produced in circulating human lymphocytes are obvious. The cells are all in the G0 phase of the cell cycle at the time the blood sample is taken. Therefore, only chromosome-type aberrations are produced. This fact makes quantification of the data reasonably simple. The circulating lymphocytes offer the opportunity to do direct quantitative studies of the effect of ionising radiation on chromosomes. For the conversion of an observed frequency of aberrations into a dose, calibration curves are used which are established by *in vitro* exposures of whole blood. In order to extrapolate

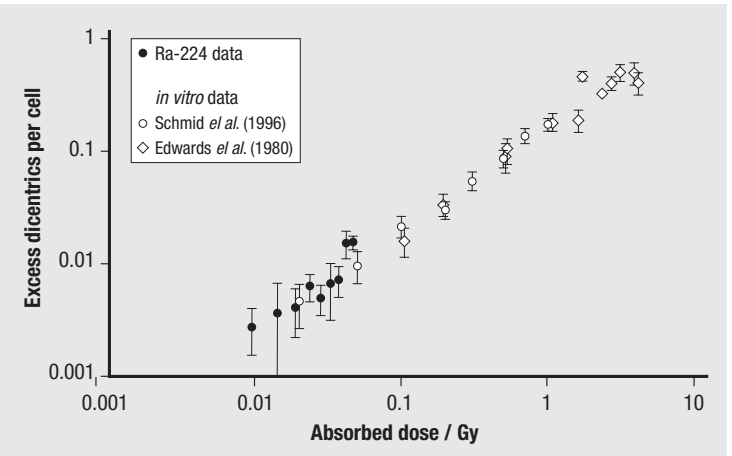

Fig. 2 | *Comparison of the* in vivo *dose response curve of patients with ankylosing spondylitis (treated with Ra-224) with data obtained by exposure of human lymphocytes irradiated with* α*-particles* in vitro.

to man from this system with confidence, results from controlled comparisons of *in vivo* and *in vitro* exposures must be known. There are some data which show that there is no significant difference in the aberration frequencies after whole body irradiation of patients and their blood *in vitro* with X-rays [25, 26]. *In vivo* and *in vitro* investigations after Co-60 γ-irradiation, again, yielded comparable results for the two different exposure conditions [27]. In a more recent paper [28] analysis performed with ankylosing spondylitis patients who have been treated with Ra-224 are in good agreement with published dose response curves *(Figure 2)* established by *in vitro* exposure with α-particles [29, 30]. These data demonstrate that calibration curves established by *in vitro* exposure can be used for the conversion of an observed frequency of exchange aberrations in an exposed individual into a dose.

DOSE ESTIMATION

Biological dosimetry using the measurement of the yield of dicentric chromosomes in human lymphocytes is an established technique. It works well for acute radiation exposures when the blood sample is taken within a few weeks after exposure. This technique is capable of estimating whole body doses of low LET radiation down to about 100 mGy based on the analysis of 1000 metaphases. It is less sensitive, however, for chronic exposures or if the exposure occurred years prior to the blood sampling. This is because the yield of dicentric chromosomes decreases with time after exposure depending on the cell turnover characteristics of the exposed person. A half-time of about 3 years [11] (or about 1.5 years after high dose exposure [31]) can be assumed when estimating the initial dicentric yield for dose estimation. But those estimates may be affected by uncertainties resulting from individual factors. In this cases the investigation of symmetrical translocations detected with the FISH technique can give important additional information [32].

DOSE ASSESSMENT IN PARTIAL BODY EXPOSURE

Experience has shown that most accidents occur in an inhomogeneous manner resulting in partial- body or non-uniform exposures. Irradiated and non-irradiated populations of lymphocytes are mixed and undamaged lymphocytes outside the irradiation field will dilute the aberration yield and make the interpretation more difficult. Due to this the size of the irradiated volume has a significant influence on the yield of scored aberrations. For partial-body exposure with a larger volume mathematical-statistical methods were developed which allow the calculation of a partial body dose. The "contaminated Poisson" method first proposed by Dolphin [33] allows an estimation of the unexposed part of the body by analysing the overdispersed distribution of dicentrics in the scored cells in relation to the expected Poisson distribution of the irradiated cells.

The Qdr method is another approach proposed by Sasaki and Miyata [34]. Here the yield of dicentrics and rings are considered only from those cells that contain unstable aberrations and assumes that these cells were present at the time of the accident. A more detailed description of these two methods is given in the IAEA Technical Report 405 [7].

The Dolphin method was applied for the dose estimation of three cancer patients after partial body exposure [35]. The blood samples were taken 24 hours after the first fraction. Each patient was irradiated with 1.8 Gy of 15 MeV photons and the partial-body volume lay within 12 to 16% of the whole body. For the calculation of the whole body doses the mean frequencies of dicentric chromosomes were used. For the calculation of the partial body dose the distribution of dicentric chromosomes in about 500 cells was fitted for a truncated Poisson distribution with missing zero class. The dose estimations were performed using the fitted rates. The estimated partial body dose agreed with the actually applied dose to a level of ± 30% (1.43 to 2.36 Gy). Similar results were obtained with the Qdr method. The calculated whole body dose was by a factor of

about two lower in comparison to the received partial body dose. The estimated partial body volumes are 1.6 to 2.0 times higher than was expected from the clinical estimations. The calculations work well but are only useful when a significant part of the body has received a relatively high dose. The results with radiotherapy patients confirm the practical applicability of these mathematical methods, but the impact of the irradiated volume and the dose of exposure is obvious and turns out to be a limiting factor because a sufficiently large number of aberrations is required.

EXAMPLES FOR THE PRACTICAL USE OF CHROMOSOME ABERRATIONS FOR DOSE ESTIMATION AFTER REAL OR SUSPECTED RADIATION OVEREXPOSURES

Since 1982 the cytogenetic laboratory of the Federal Office for Radiation Protection is the official laboratory authorised to perform biological dosimetry in Germany. Up to now, more than 300 blood samples in connection with acute or assumed exposures were analysed. The analysed subjects came from industry, power plant facilities, research units and public health sections. In many cases a personal dosimeter was worn by the exposed person. In these cases the chromosome analysis provides an additional independent source of information to confirm or reject the physical dose. Sometimes it was not clear whether the dosimeter reflects the real dose received by the exposed person because the exposure conditions could not be reconstructed or it seemed unlikely that they represent the real situation. There were other cases where the personal dosimeter was definitively not worn or it was not possible to evaluate it for some reasons. In these cases the biological dosimetry was the only possibility to get information about the assumed radiation exposure. When an acute exposure occurred and a blood sample for chromosome analysis should be obtained as soon as possible, the dicentric assay represents the method of choice. Some examples for the application of biological dosimetry are given in the following:

- a worker in a nuclear power plant, performing routine inspection of the technical installations during his shift work, recorded an unexplained dose of 0.63 Gy gamma rays on his film badge. There was no hint of any radiation exposure by the result of chromosome analysis (1dic in 1000 cells). The cause of the badge recording remains unresolved;
- during the revision in a nuclear power plant a technician did not wear his film badge. An exposure was suspected. Based on the chromosome analysis a whole body dose of 0.13 Gy with a confidence interval of 0.06 – 0.26 Gy was estimated and confirmed a radiation exposure.

The analysis of chromosomal aberrations in human peripheral blood lymphocytes plays an important role in radiological protection. After acute exposure to ionising radiation the dicentric assay can help to get important additional and independent information which helps to evaluate and clarify an unclear radiation exposure.

INFLUENCE OF SCORED CELLS

The statistical uncertainty of whole body dose estimation includes the uncertainties of the dose response relationship as well as of the dicentric measurement. For establishing a dose response curve more than 7 dose points should be included which results in a large number of cells (about 10 000) to be scored. In this case the uncertainty to the dose estimation contributed by the dose effect curve is negligible. The number of analysed cells from the exposed person influences directly the statistical accuracy of the whole body dose estimation. The 95% confidence intervals will express the quality of the calculation. An example of the influence of the sample size on the dose estimation is given in *Table 1*. Here the confidence limits are shown for estimated whole body doses on the basis of Cs-137 gamma radiation as a function of the number of scored cells. The confidence limits are markedly influenced up to a number of 500 to 1000 analysed cells. Scoring more than 1000 cells has a minor impact on the confidence limits. In the case of an assumed or actually occurred radiation overexposure 1000 scored cells will permit to estimate a whole body dose after acute radiation exposure down to about 0.1 Gy. Because chromosome analysis is a time consuming

Table 1 | *Influence of sample size on the confidence intervals of the dose estimate*

Scored cells	Estimated dose (Gy)	95% Confidence interval		Dicentric	Dicentrics / 100 cells
		LCI	UCI		
250	0.25	0.05	0.58	2	0.8
500	0.25	0.09	0.47	4	0.8
750	0.25	0.12	0.43	6	0.8
1000	0.25	0.14	0.40	8	0.8
1500	0.25	0.16	0.37	12	0.8
2000	0.25	0.17	0.35	16	0.8

work, for practical purposes 500 cells are generally considered as sufficient to obtain a reliable result. In case of very low dose exposure it might be helpful to increase the number of cells.

Microscope analysis is a time consuming procedure. Skilled cytogeneticists or technicians need about 1 day to analyse about 200 cells. Since the 1980-ties computer-aided systems of metaphase search and dicentric chromosome detection are available which reduce the scoring time. Since 1989 the automatic detection and location of suitable metaphase plates for scoring is routinely applied in the BfS laboratory. In comparison with the conventional analysis without computer assistance this new method is faster by a factor of about 2 [36].The development and validation of the semi automated dicentric scoring system gives promising results. A comparison between results achieved by automatically and manually scoring revealed a good correlation. The frequency of dicentrics detected by the automated system was always lower than manual scoring. An explanation for this is the challenge to separate all 46 chromosomes in a metaphase plate. Overlapping chromosomes and clusters of chromosomes are to be distinguished from other objects. In consequence, the automatic scoring system can not analyse the whole genome (detect all chromosomes) of one cell and is not programmed to do so. Therefore, this system is not in use for small radiation accidents until now. In case of a large scale radiation accident the uncertainties revealed with this kind of analysis could be compensated by scoring a higher number of cells. Preliminary results in the BfS laboratory gave promising results for a reliable and high throughput.

LARGE SCALE RADIATION ACCIDENT

The dicentric assay has been shown to provide good individual dose estimation in a number of accidents, i.e. during the Bialystock accident an assessment of doses by radio therapy patients was performed [37]. Partial body and protracted exposures can be detected and evaluated in more detail using mathematical models [7], here a special software was developed to facilitate this special kind of statistics [38]. Furthermore, it was demonstrated that the dicentric assay is able to assess health risks and guide medical treatment decisions in large scale radiation accidents like Chernobyl [39] or Goiânia [40]. In a large scale radiation accident, a multi parameter approach will be needed [41] and several biological assays will be applied [42]. In this context, the dicentric assay was described as the gold standard of biological dosimetry [4] for large scale accidents.

In the case of an emergency situation with a large-scale public radiation disaster for the first step it will be important to separate a small number of radiation exposed individuals who require medical intervention from the large population of "concerned public" [43]. One important strategy for increasing the throughput in hospitals is biological dosimetry triage [44].

In order to cope with a high number of exposed people, different strategies were developed in recent years. The concept of analysing 20 to 50 metaphases or 30 dicentrics is one of them. [6]. This procedure was experimentally examined and permits an individual dose estimation with an uncertainty of ± 0.5 Gy. This confidence interval is sufficient for the triage mode and acceptable in an emergency situation [7, 45]. However, a single cytogenetic laboratory does not have the capacity to analyse sufficient number of cells in adequate time. Thus, in a large scale accident, there is a need for collaboration within an established network [46].

During the last years, several cytogenetic reference laboratories have established networks on a national (*i.e.* Canada and Japan) [47-49] or international level (*i.e.* the European tripartite Network from the United Kingdom, Germany and France, or the south American network) to enhance their capabilities.

In 2006 the International Atomic Energy Agency (IAEA) has established a Response Assistance Network (RANET), which was developed out of the global Emergency Response Network (ERNET), to cope with radiation emergencies. RANET includes biodosimetry and promotes emergency preparedness among its Member States. IAEA is organizing training activities for biodosimetry laboratories [50].

In 2007 the World Health Organization (WHO) conducted a survey of biological dosimetry laboratories and their emergency-response capabilities in selected regions and established a global Network in 2008. WHO *BioDoseNet* is a global network of biodosimetry laboratories whose role is to support management and decision-making in cases of large radiation emergency events where the capability of individual laboratory is likely to be overwhelmed [51, 52]. The *BioDoseNet* has been established to fulfil WHO's mandate under the International Health Regulations [53] and its implementation plan.

In general, the cooperation within a network includes standardisation of techniques with detailed description of the laboratory protocols, logistic organisation planning and emergency response, internal and external quality assurance programs and stockpiling of consumables. Here, the progress in standardisation and harmonisation of the dicentric assay within the ISO working groups is very helpful and gives good orientation for small [54] and large accidents [45].

EXPERIENCE WITH INTERCOMPARISONS

Exercises and training are mandatory to establish and guarantee harmonized quality assurance and quality control procedures. Based on the new ISO standard for biological dosimetry an international collaborative study on the analysis of dicentric chromosomes was carried out in 2007 [9]. The 5 participating biodosimetry laboratories were located in countries on different continents (North America,

Asia and Europe). The aim of the intercomparison was to establish own dose effect curves for gamma rays in each laboratory. Based on this dose assessments for four different irradiated blind samples were performed. Whole blood was irradiated in one lab and dispatched to the participating laboratories by air express mail service.

The results revealed a good consistency, only differences in the high dose range between two laboratories appeared. The applied dose always matched the dose estimate based on the established dose effect curve of each laboratory. It was demonstrated that whole blood samples irradiated with doses from 0.75 to 4.5 Gy can quickly and reliably be detected by each participant in the frame of this large-scale accident training [15]. Thus, these labs can distribute blood samples between each other in an emergency case.

FUTURE

Parallel to creating networks, other efforts to establish rapid sample analysis focus on automation as a tool to develop a flexible, scalable and high-sample-throughput laboratory. The components of an automated cytogenetic biodosimetry laboratory include robotic instruments, metaphase harvester and spreaders [55]. With this attempt up to 500 samples per week can be processed in triage mode with little staff interactions.

Furthermore, new strategies of dicentric scoring need to be investigated in order to receive a considerably higher throughput of the analysis. The results obtained so far with dicentric automation look promising and must now be validated as a technique for population triage in a large scale accident.

Therefore the aim will be to test whether the automated system is able to distinguish between different doses and exposure conditions. Initially, the scoring criteria for dicentrics must be adjusted. The dicentric scoring system will work with very low human interaction and will include automation of metaphase finding, high resolution image capturing, automatic chromosome separation and dicentric candidate detection. Finally, the dicentric candidates will be evaluated by experienced scorers, which can be performed very rapidly. Preliminary results look promising.

Another possibility to increase the speed of analysis is scoring of high resolution metaphase images via the internet (telescoring). This is a new approach which has not yet been tested, but which could be used in a large scale accident. The possibilities and limitations of this method have to be explored. However, it opens a new perspective regarding cooperation between trained cytogenetic laboratories working in the field of biodosimetry and in clinical diagnostic, which can be accomplished without sending blood or slides.

The new approaches (automation and telescoring) need to be validated for different radiation exposure conditions using the conventional scoring method which remains the gold standard of biological dosimetry.

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