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Development of an automatable micro-PCC biodosimetry assay for rapid individualized risk assessment in large-scale radiological emergencies

Antonio Pantelias and Georgia I. Terzoudi

Laboratory of Health Physics, Radiobiology & Cytogenetics, Institute of Nuclear & Radiological Sciences & Technology, Energy & Safety, National Centre for Scientific Research “Demokritos”, Athens, Greece

Abstract

In radiation accidents and large-scale radiological emergencies, a fast and reliable triage of individuals according to their degree of exposure is important for accident management and identification of those who need medical assistance. In this work, the applicability of cell-fusion-mediated premature chromosome condensation (PCC) in G_0 -lymphocytes is examined for the development of a rapid, minimally invasive and automatable micro-PCC assay, which requires blood volumes of only 100 μ l and can be performed in 96-well plates, towards risk assessments and categorization of individuals based on dose estimates. Chromosomal aberrations are visualized for dose-estimation analysis within two hours, without the need of blood culturing for two days, as required by conventional cytogenetics. The various steps of the standard-PCC procedure were adapted and, for the first time, lymphocytes in blood volumes of 100 μ l were successfully fused with CHO-mitotics in 96-well plates of 2ml/well. The plates are advantageous for high-throughput analysis since the various steps required are applied to all 96-wells simultaneously. Interestingly, the use of only 1.5ml hypotonic and Carnoy's fixative per well offers high quality PCC-images, and the morphology of lymphocyte PCCs is identical to that obtained using the conventional PCC-assay, which requires much larger blood volumes and 15ml tubes. For dose assessments, appropriate calibration curves were constructed and for PCC analysis specialized software (MetaSystems) was used. The micro-PCC assay can be combined with fluorescence in situ hybridization (FISH), using simultaneously centromeric/telomeric (C/T) peptide nucleic acid (PNA) probes. This allows dose assessments on the basis of accurate scoring of dicentric and centric ring chromosomes in G_0 -lymphocyte PCCs, which is particularly helpful when further evaluation into treatment-level categories of exposed individuals is needed. The micro-PCC assay has significant advantages for early triage biodosimetry when compared to other cytogenetic biodosimetry assays. It is rapid, cost-effective, and could pave the way to its subsequent automation.

Corresponding author's : gterzoudi@rrp.demokritos.gr, Tel: +30 210 6503865, +30 210 6503848 / Fax: +30 210 6534710.

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1. INTRODUCTION

Following a large-scale radiological event, hundreds or thousands of people could have been potentially exposed to unknown and variable doses of radiation. It is a high priority, therefore, to use biomedical tools, sensitive biomarkers and automatable methods in order to reflect promptly the biological importance of the radiation exposure [1–3]. At present, it is estimated that the throughput of a cytogenetic laboratory is a few tens of samples per day and even large cytogenetic laboratory networks can only analyse a few hundred of samples per day [4–6]. For large-scale incidents, rapidity and ease of screening are essential in order to obtain quick radiological dose and risk assessments [7]. This will enable categorization of individuals according to the degree of their exposure and, subsequently, identification of those who need medical assistance, which is essential for optimal post-exposure management [8–10]. Towards this goal, two main approaches of biodosimetry, biologically-based and physically-based, have been developed and essentially three criteria are minimally necessary for an effective biodosimetric technique: the dose can be assessed promptly after-the-fact; the technique can assay at the level of an individual; and the technique can provide information sufficient to determine what actions should be taken for that individual [11].

Biologically-based biodosimetry approaches are based on biological processes or biomarkers that can be affected by ionizing radiation allowing thus a dose of radiation to be estimated. The status and suitability of current biomarkers for radiation exposure have been reviewed recently [12–15]. Particularly, cytogenetic biomarkers are the most widely used and, at present, several well-established biological dosimeters exist. They can offer accurate dose estimates but, are both time-and labour-consuming and therefore not ideal for use in radiological mass-casualty scenarios where short turnaround times and high throughput are of prime importance [9]. When an ideal biomarker is used for early triage biodosimetry in radiological events, the collection of the required biological samples from the potentially exposed individuals should be easy and non-invasive, while the procedures involved for dose and risk assessments should be rapid and automatable. The latter will pave the way to the subsequent automation of the assay's workflow so that it could be used in the event of a large-scale radiological emergency. The state-of-the-art advances in radiation biodosimetry for mass casualty events involving radiation exposure have been also reviewed recently [16, 17].

Among the different cytogenetic assays used for triage biodosimetry and the estimation of absorbed doses in exposed individuals, the dicentric chromosome (DC) assay remains the most widely used. The DC assay is essentially based on the analysis of dicentrics and centric ring chromosomes present in the peripheral blood metaphase lymphocytes of the exposed individuals. Nevertheless, this assay has a significant drawback with respect to the time needed to obtain dose estimates for rapid decision on the right line of medical treatment. It requires culturing of peripheral blood T-lymphocytes for two days before allowing the analysis of chromosomes at metaphase. Hence, it fails the requirement of rapid dose estimation, which is a high priority in radiation emergency medicine.

On the other hand, it is noteworthy that following radiation-induced DNA damage and the enzymatic repair processing of the lesions, which depend on the quality of radiation and the

complexity of DNA damage [18], chromosomal rearrangements in the nuclei of blood lymphocytes are well known to be formed within 8h post-irradiation, without requiring blood culture, T-lymphocyte stimulation and DNA replication for their formation. Following accidental exposure, the biomarkers of exposure i.e. dicentric and centric ring chromosomes as well as residual chromosomal fragments, will be present, therefore, as single chromatid chromosomes in the blood lymphocytes by the time the blood samples reach a reference Biodosimetry Laboratory for dose estimation. Consequently, rapid dose and risk estimates can be achieved by taking advantage of the unique features of cell fusion mediated premature chromosome condensation (PCC) in blood lymphocytes for biological dosimetry [19–21]. This phenomenon enables visualization, analysis and quantification of chromosomal aberrations directly in unstimulated G₀-peripheral blood T- as well as B-lymphocyte prematurely condensed chromosomes (PCCs), without requiring a two-day blood culture [22–24].

In the present study, the main objective is to explore the applicability of the PCC phenomenon in order to devise an automatable micro-PCC assay using very small blood sample volumes of 100µl and 96-well plates to obtain rapid dose estimates and risk assessments for the categorization of a large number of individuals according to the degree of their exposure. For high-throughput triage biodosimetry there are previous interesting attempts using other cytogenetic assays [25–29] including chemically-induced PCCs in G₂-phase cultured lymphocytes [30]. Here cell fusion-mediated induction of PCCs in non-stimulated G₀-phase lymphocytes is used, and for the standardization of the proposed micro-PCC assay the morphology of the lymphocyte PCCs obtained for non-irradiated and irradiated blood samples was first compared to those obtained using the conventional PCC assay. Subsequently, appropriate calibration curves for dose assessments were constructed, while the applicability and reliability of the micro-PCC assay was compared to the conventional DC assay, through the evaluation of speed of analysis and minimum number of cells required to be analyzed for each method. The development and standardization of such a micro-PCC assay for high-throughput analysis could pave the way to its subsequent automation, which is critically needed for timely triage biodosimetry in mass-casualty radiological emergencies.

2. Materials and methods

2.1. Blood samples, irradiation conditions and the conventional DC assay

Peripheral blood samples in heparinized tubes were obtained from healthy donors and used after their informed consent, according to our institutional ethics procedures. Irradiation of whole blood samples was carried out in vitro using a Co-60 Gamma Cell 220 irradiator (Atomic Energy of Canada Ltd., Ottawa, Canada) at room temperature and at a dose rate of 20 cGy/min. Different irradiation times were applied in order to administer to the whole blood samples doses ranging from 0.5 to 6 Gy. Subsequently, irradiated blood samples were processed according to the experimental design and, for comparison purposes, radiation-induced chromosomal aberrations were visualized and quantified by applying the conventional DC assay, the conventional PCC assay, as well as the micro-PCC assay.

For the conventional DC assay, whole blood cultures were set up by adding 0.5 ml of whole blood to 5ml of RPMI-1640 medium (Gibco) supplemented with 10% fetal bovine serum (FBS), 1% phytohemagglutinin (PHA), 1% glutamine and 1% antibiotics (penicillin, streptomycin). Cultures were then incubated at 37°C in a humidified atmosphere with 5% CO₂. After 48–50h at 37°C, cell cultures were harvested, treated with hypotonic solution KCl (0.075 M) and fixed with methanol:glacial acetic acid (3:1, v/v), following standard cytogenetic procedures with centrifugation at 250g. Chromosome spreads were prepared, slides were air-dried and stained with 3% Giemsa solution. Only metaphases with 46 centromeres were analyzed and for the irradiated samples, the yield of dicentric plus centric ring chromosomes was obtained for each experimental point using light microscopy and appropriate image analysis system (Ikaros MetaSystems).

2.2. Preparation of the PCC-inducer mitotic CHO cells

Chinese hamster Ovary (CHO) cells were grown in McCoy's 5A (Biochrom), culture medium supplemented with 10% FBS, 1% l-glutamine and 1% antibiotics (Penicillin, Streptomycin), incubated at 37 °C in a humidified atmosphere with 5% CO₂. CHO cultures were maintained as exponentially growing monolayer cultures in 75 cm² plastic flasks at an initial density of 4×10^5 cells/flask. For optimizing harvest of mitotic cells via cell synchronization, routinely the cells in a flask were allowed to grow until confluence and subcultured equally into three new 75 cm² plastic flasks. Following a 24–30h incubation at 37 °C, Colcemid (Gibco) at a final concentration of 0.1 µg/ml was added to CHO cultures for 4 hours and the accumulated mitotic cells were harvested by selective detachment. Once a sufficient number of mitotic cells had been obtained, they were used as supplier of mitosis promoting factors (MPF) to induce PCC in human lymphocytes by both the conventional and the micro-PCC assay.

2.3. Conventional cell fusion-mediated induction of premature chromosome condensation

Human lymphocytes were separated from heparinized irradiated whole blood samples using Biocoll separating solution (Biochrom). The blood sample was diluted 1:2 in RPMI-1640 without FBS, and was carefully layered on top of an equal amount of Biocoll in a test tube before centrifugation at 400g for 20 min. Collected lymphocytes were washed with 10 ml culture medium, centrifuged at 300g for 10 min and kept in culture medium (RPMI-1640 supplemented with 10% FBS, 1% glutamine and antibiotics) to be mixed and fused with mitotic CHO cells. When the conventional PCC assay is applied, the mitotic CHO cells harvested from a 75 cm² flask were used for 2–3 fusions using lymphocytes isolated from 1–2ml of blood for each experimental point. The conventional procedure for cell fusion mediated PCC-induction in non-stimulated G₀ lymphocytes was carried out as described earlier [22, 24, 31].

2.4. The micro-PCC assay using very small blood volumes and 96-well plates

The various steps of the conventional PEG-mediated cell fusion and PCC induction mentioned above were adapted for the development of a micro-PCC assay that could be applied to the very small blood volumes of 100µl using 96-well plates of 2ml per well. Briefly, whole blood samples of 100µl for each experimental point were transferred to each

well in 96-well plates and 1.5ml of cold red blood cell (RBC) lysing solution of ammonium chloride was added for 10 min. For the preparation of lysing solution, 8.02g ammonium chloride, 0.84g sodium bicarbonate and 0.37g disodium EDTA were dissolved in 1lt of distilled water. The RBC lysing solution is used as an alternative to the Biocoll gradient required by the conventional PCC assay for the separation of lymphocytes from whole blood before their fusion to the mitotic CHO cells.

Following centrifugation of the 96-well plates at 200g for 6min, the supernatants were discarded all at once and 1.5ml of mitotic CHO cells (2×10^5 cells) in serum-free RPMI-1640 medium with Hepes (25mM) and colcemid (0.1 μ gr/ml) was added to the lymphocyte pellet in each well. Subsequently, the plates were centrifuged for 6min at 200g and supernatants were discarded without disturbing the cell pellets, keeping the plate inverted on a paper towel to drain well the pellets from excess liquid. Immediately after the plates were turned in up-right position and 100 μ l of 45% (w/v) PEG (mol wt 1,450, Sigma-Aldrich/serum-free RPMI 1640 with Hepes) was injected into each well and held for about 1 min. Subsequently, 1ml of phosphate buffered saline (PBS) was slowly added to each well of the plate, shaken gently and centrifuged at 200g for 6 min. The supernatants were discarded and 0.3 ml RPMI-1640 complete growth medium containing 2% phytohemagglutinin (PHA) and 10% FBS, was added to each well.

After culturing for 75 min at 37°C, 1.5 ml of hypotonic KCL (0.075 M) was added to each well, and cells were fixed twice (1.5 ml/well) with methanol:glacial acetic acid (3:1 v/v), following standard procedures for chromosome preparation. Fixed cells were resuspended in 50 μ l of fixative and three microscope slides with chromosome spreads were prepared by dropping 15 μ l aliquots at the centre of each pre-cleaned slide. Finally, air-dried slides were stained with 3% Giemsa solution in Sorensen's buffer (pH 6.8) and covered with cover slips using Entellan mounting medium. The analysis of PCC spreads on the microscope slides and the quantification of radiation-induced excess chromosomal fragments in lymphocyte PCCs was greatly facilitated by combining light microscopy with image analysis systems (Ikaros, MetaSystems). The various steps required by the micro-PCC assay are summarized in the scheme below presented as Figure 1.

2.5. Analysis and scoring criteria

Lymphocyte PCC spreads were located manually and their analysis was facilitated by the use of a semiautomated image analysis system (Ikaros, MetaSystems). Specifically, the analysis of excess PCC fragments in lymphocyte PCC spreads stained with Giemsa was greatly facilitated by the appearance of the PCCs, which are single chromatid chromosomes and lighter stained than the double chromatid chromosomes of metaphase CHO cells. The lymphocyte PCCs are, therefore, easily distinguished from the darkly stained chromosomes of the PCC-inducer mitotic CHO cells. In unirradiated lymphocytes, 46 single chromatid elements can be scored in the PCC spreads and, in order to calculate the frequency of excess PCC fragments following exposure to ionizing radiation, the number 46 was subtracted from the mean value of lymphocyte PCCs per cell obtained in the irradiated blood samples. Generally, a number of 20–30 PCC-spreads was considered adequate for dose estimation

following a single exposure, as we have already shown previously via inter-laboratory accident simulation exercises [31].

2.6. Construction of dose-response curves for dose assessment using the micro-PCC assay

For dose assessment purposes by means of the micro-PCC assay, three different dose-response calibrations curves were constructed to be used depending on whether the blood samples from potentially exposed individuals are received within 6, 12 or 24 hours post-exposure. Specifically, whole blood samples were exposed *in vitro* to doses of 0, 1, 2, 3, 4, 5, and 6Gy as described earlier, and allowed to repair radiation-induced chromosomal damage for 6, 12 or 24 hours at 37 °C. Subsequently, the blood samples were processed for cell fusion, PCC induction and preparation of slides with lymphocyte PCC spreads as described earlier in this section and summarized in Figure 1. The frequencies of excess PCCs per cell for the different doses used were obtained by the analysis of 30 PCC spreads for each experimental point, and mean values $\pm$ SD were calculated from two to three independent experiments.

3. Results

3.1. Development of an automatable micro-PCC assay for early triage biodosimetry

The original protocol for the conventional PCC assay uses 1–2ml blood sample volumes per experimental point, lymphocyte isolation by means of Ficoll-Paque or Biocoll gradients, as well as 15ml round-bottom culture tubes for cell fusion, PCC induction, and chromosome preparation. The various steps of the procedure were adapted successfully towards the development of an automatable micro-PCC assay using blood volumes of only 100 μ l, and multi-tube racks or 96-well plates of 2ml. In order to comply with the requirements of a high-throughput blood sample collection based on small volumes of around 100 μ l, we experimented with two methods for lymphocyte isolation, namely the Biocoll separating solution and the ammonium chloride red blood cells lysing solution. Even though the isolation of lymphocytes by means of Biocoll was feasible, it was time-consuming and less efficient than the ammonium chloride solution, which we adopted.

In this way, the entire procedure including blood sample collection, red blood cell lysis, lymphocyte fusion to mitotic CHO cells, culturing of fused cells for PCC induction, and cell fixation were carried out in a single 2ml well. This improvement reduces the number of sample transfers, minimizes cell loss, and simplifies tracking operations. Our results demonstrate for the first time that lymphocytes isolated from 100 μ l blood sample via ammonium chloride lysing solution in 96-well plates can be fused with CHO mitotic cells.

3.2. Morphology of the lymphocyte PCCs obtained using the micro-PCC assay

The morphology of the lymphocyte PCCs obtained with the above micro-PCC procedure is practically identical to that obtained using the conventional PCC assay. It allows, therefore, the analysis of radiation induced excess PCC fragments stained with Giemsa, which is simple, rapid and cost-effective. Interestingly, the use of only 1.5ml of hypotonic solution and the fixation of cells twice with 1.5ml of Carnoy's fixative in 96-well plates of 2ml/well

offers high quality PCC images as shown in Figure 2. Figure 2A presents a typical image of a non-irradiated lymphocyte (46 PCCs), whereas Figures 2B and 2C enable visualization and quantification of radiation-induced PCC fragments following 0.5Gy (3 excess PCCs) and 2Gy (10 excess PCCs) of Co-60 γ -irradiation, respectively.

3.3. Construction of appropriate dose response calibration curves

Following the protocol of the micro-PCC assay described in the previous section, three dose-response calibration curves were constructed using doses of 0 to 6Gy of γ -irradiation, for dose assessment according to the time elapsed from irradiation to sample processing for biodosimetry purposes. Figures 3A, 3B, 3C present these calibration curves for 6, 12 or 24 hours post-exposure repair times, respectively. A comparison of the dose-response curves obtained under the different post-exposure repair times is depicted in Figure 3D.

3.4. Dose estimates by means of the micro-PCC and DC assay

To evaluate the applicability and reliability of the micro-PCC as compared to the conventional DC assay, we examined the speed of analysis and the minimum number of cells required to be analyzed for each method. For this purpose, a simulation of an accident was performed by irradiating blood samples from healthy individuals with doses of 0.5Gy, 1Gy and 2Gy of γ -rays, coded blindly and, subsequently, used for dose estimation by means of the two assays.

Table 1 presents the yields of excess fragments in G₀-Lymphocyte PCCs obtained by scoring 10, 20 or 30 cells, while the yields of dicentrics plus centric rings analysed at metaphase were obtained (with a delay of two days due to lymphocyte culturing) by scoring 50, 100, 200 or 300 cells, and are presented in Table 2. The results obtained suggest that the analysis of only 20–30 cells by the micro-PCC assay offers dose estimates with an accuracy that would require the analysis of 200–300 cells by the DC assay.

4. Discussion

The main goal of biodosimetry is to utilize biological changes caused by ionizing radiation in an individual and use them as biomarkers of exposure in order to estimate the dose received and to predict its clinically relevant consequences. The development of rapid, accurate, and reliable biodosimetry tools has been primarily motivated by the potential need to confront large-scale radiological events. In such cases, it is crucial to be able to identify the exposed individuals who would benefit from receiving urgent medical care.

To this end, it is imperative to set a reasonable cut-off dose of absorbed radiation as a threshold that will allow the categorization of the exposed population. Specifically, for doses below such a cut-off value, countermeasures are not immediately needed and medical treatment would not be expected to impact mortality. On the other hand, for individuals with absorbed doses above the cut-off value, medical treatment would be necessary to improve survival rates. This cut-off is generally set at 2 Gy, yet this threshold could be set higher if the number of affected individuals is beyond the capacity of the available medical facilities [4, 7, 32–34]. Such biodosimetry screening enables the categorization of the exposed individuals into three categories: those who have suffered radiation injury, for whom

immediate medical intervention is vital; those with intermediate exposure close to the threshold cut-off dose, for whom medical intervention is necessary to mitigate the short, medium and long term effects of exposure, and the “worried well” with probable low doses, for whom no deterministic effects are expected but long term monitoring may be required [35].

For timely biodosimetry dose assessments, the 48hour peripheral blood lymphocyte culture required for the standard DC analysis at metaphase remains the major obstacle for rapid dose estimation and the use of the dicentric assay for triage of a population after a mass exposure event. Alternatively, the conventional PCC fusion technique, which is based on the induction of premature chromosome condensation in unstimulated G₀-peripheral blood lymphocytes, allows a rapid visualization of radiation-induced chromosomal aberrations enabling their analysis. However, the use of this technique for triage biodosimetry has been restricted so far, since it requires 1–2ml blood samples per exposed individual, so that fingerstick blood sampling, which is crucial for large population screening, cannot be utilized.

To overcome this obstacle, we developed in the present work, an automatable micro-PCC assay that is appropriately designed to be suitable for triage biodosimetry to obtain rapid individualized dose estimates in cases of large-scale radiological emergencies or accidental overexposures. Indeed, this new method has the potential to screen fingerstick derived blood samples, in order either to estimate past radiation exposure, or to sort a large number of individuals exposed above or below a pre-set cut-off dose. Compared to the dicentric chromosome assay, which at present is the standard technique for biological dosimetry, our results demonstrate that the micro-PCC assay is quicker as well as reliable and cost effective for early triage biodosimetry. Indeed, Giemsa-stained excess PCC fragments can be visualized for scoring within 2 hours from the moment blood samples are available and dose estimations can be obtained subsequently using the appropriate calibration curves that we have constructed for standardization purposes of this assay.

Specifically, we have introduced for the first time the use of blood samples of 100µl for PCC induction in 96-well plates. Interestingly, the morphology of the lymphocyte PCCs so obtained is practically identical to that obtained using the conventional PCC assay. This innovation in the PCC protocol has two main advantages for its use in early triage of radiation emergencies involving large populations, as such very small amounts of blood need to be sampled together with the use of the 96-well plates, allowing thus a fast and reliable triage of many individuals simultaneously. Indeed, the analysis of only 20–30 lymphocyte PCC spreads are sufficient to obtain reliable dose estimates, as presented in Table 1. This is because the analysis is based on radiation-induced chromosomal fragments in excess of 46 PCCs. This number of 46 PCCs constitute the human genome, which is remarkably stable in healthy individuals. This allows to link every single excess fragment above 46 to radiation exposure. In contrast, using the conventional DC analysis at metaphase, radiation-induced dicentric chromosomes are rare, especially at low doses, and therefore many more cells must be analysed for reliable dose assessment. Therefore, the micro-PCC technique has the potential to deliver data for dose assessment in a significantly shorter period of time than any other biological assay being used currently, as only few lymphocyte PCC spreads need to be

analysed in order to detect exposed individuals, in agreement with previously reported results [31]. Indeed, the results presented in Table 1 and Table 2 suggest that the analysis of only 20–30 cells by the micro-PCC assay offers dose estimates with an accuracy that would require the analysis of 200–300 cells by the DC assay.

While overexposed individuals are identified and categorised using the micro-PCC assay through the analysis of Giemsa stained excess lymphocyte PCCs, centromeric and telomeric staining could be applied to lymphocyte PCCs using PNA probes and the FISH technique (C/T-PCC-FISH) for the accurate scoring of dicentric and centric ring chromosomes. This additional analysis would permit the reliable detection of all unstable chromosomal aberrations in lymphocyte PCCs with high level of precision and sensitivity, as we have previously reported [21, 22]. Therefore, it can be carried out in order to confirm doses in the overexposed individuals only, given that it is quite an expensive procedure. Additionally, in the event of an accident, the micro-PCC assay combined with C/T FISH staining for the accurate scoring of dicentric and centric ring chromosomes in Go-lymphocyte PCCs, would enable further evaluation of exposed individuals into treatment-level categories.

To conclude, the micro-PCC assay developed in this work has the potential to deliver data for dose assessment in a significantly shorter period of time than any other cytogenetic assay currently being used, as it does not require lymphocyte culture and only a few cells need to be analysed in order to detect exposed individuals. In addition, this automatable assay has the potential to discriminate between whole- and partial-body exposure, based on the frequency between damaged and not damaged lymphocytes encountered in the PCC spreads, as we have previously demonstrated for the conventional PCC assay [31]. Indeed, when all the analysed lymphocyte PCCs exhibit chromosomal damage, whole body exposure is confirmed, which is valuable for designing the treatment of individuals exposed to life threatening doses of radiation. Finally, the analysis of Giemsa-stained excess PCC-fragments using the micro-PCC assay is a simple and cost-effective biodosimetry tool that enables the rapid estimation of absorbed doses within 2–3 hours. The automation of the micro-PCC assay remains a challenge for the near future as it would increase its throughput and scoring objectivity.

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Highlights

- Lymphocytes were fused with CHO-mitotics for PCC induction using only 100µl blood
- An automatable micro-PCC assay was developed for early triage using 96-well plates
- The assay has the potential to deliver timely dose assessments for many individuals
- Dose response calibrations curves were constructed for 6, 12 and 24h repair time
- Scoring only 20 PCC-spreads gives accuracy obtained by 200 cells in the DC-assay

Figure 1:
Scheme of the automatable micro-PCC assay for simultaneous analysis of 96 individuals.
Added volumes refer to each well of the 96-well plate.

Figure 2:

Giemsa stained PCCs, obtained by means of the micro-PCC assay, demonstrating 46 single chromatid chromosomes in a non-irradiated lymphocyte (A). Three excess (over 46) PCC fragments can be visualized in an irradiated lymphocyte with 0.5Gy of Co-60 γ -irradiation (B). Ten excess PCC fragments can be scored following irradiation with 2Gy (C).

Figure 3:

Dose-response curves are presented for Giemsa-stained excess lymphocyte PCC fragments per cell for post-exposure repair time of 6h (Linear, $a_1 = 1.70 \pm 0.08$, $y_{01} = 0.075$) (A), 12h (Linear, $a_2 = 1.54 \pm 0.08$, $y_{02} = -0.20$) (B) and 24h (Linear, $a_3 = 1.41 \pm 0.06$, $y_{03} = 0.06$) (C). Figure 3D depicts a comparison of the dose-response curves obtained under the different post-exposure repair times. Mean values $\pm$ SD are calculated from three independent experiments.

Table 1:

Simulated whole body exposure to 0.5, 1 and 2Gy and dose estimation using the micro-PCC assay for the analysis of Giemsa-stained excess PCC fragments by scoring 10, 20 or 30 PCC spreads in non-stimulated lymphocytes. Mean doses are shown with low- (LCL) and upper-(UCL) confidence limits.

True Physic alDose(Gy)	Analysis of 10 Cells					Analysis of 20 Cells					Analysis of 30 Cells				
	Excess frags	Excess frags/cell	Estimated Dose (Gy)			Excess frags	Excess frags/cell	Estimated Dose (Gy)			Excess frags	Excess frags/cell	Estimated Dose (Gy)		
			Dose	LCL	UCL			Dose	LCL	UCL			Dose	LCL	UCL
0.5	5	0.5	0.31	0.15	0.49	13	0.65	0.42	0.25	0.60	19	0.63	0.41	0.24	0.58
1.0	11	1.1	0.74	0.56	0.93	23	1.15	0.77	0.59	0.97	42	1.40	0.95	076	1.16
2.0	24	2.4	1.66	1.44	1.90	57	2.85	1.98	1.75	2.23	91	3.03	2.11	1.87	2.36

Table 2:

Simulated whole body exposure to 0.5, 1 and 2Gy and dose estimation using the Dicentric plus centric ring (Dic +CR) analysis at metaphase lymphocytes. Mean doses are represented with low- (LCL) and upper- (UCL) confidence limits.

True Physical Dose(Gy)	Analysis of 50 Cells				Analysis of 100 Cells				Analysis of 200 Cells				Analysis of 300 Cells			
	Estimated Dose (Gy)				Estimated Dose (Gy)				Estimated Dose (Gy)				Estimated Dose (Gy)			
	Dic+CR	Dose	LCL	UCL	Dic+CR	Dose	LCL	UCL	Dic+CR	Dose	LCL	UCL	Dic+CR	Dose	LCL	UCL
0.5	0	0	0	0.95	1	0.26	0	0.81	3	0.36	0.10	0.70	7	0.47	0.26	0.74
1.0	2	0.66	0.15	1.38	7	0.92	0.54	1.38	18	1.06	0.79	1.37	24	0.99	0.76	1.24
2.0	10	1.65	1.10	2.30	16	1.46	1.07	1.91	44	1.74	1.46	2.04	82	1.96	1.73	2.20