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ABSTRACT

Inter-individual susceptibility to mutagens/carcinogens can be assessed by either genotyping DNA repair genes in different pathways or phenotyping DNA repair capacity (DRC) at the molecular or cellular level. Due to the large number of known DNA repair genes, and the interactions between repair pathways, phenotyping is becoming the preferred approach to measure DRC, and reliable assays are therefore increasingly needed. The use of a cellular phenotype comet assay for the nucleotide excision repair (NER) pathway using benzo[a]pyrene diol epoxide (BPDE) has been described in previous papers, but no thorough evaluation of its applicability in large genotype-phenotype studies has been presented.

Our aim was to evaluate the possibility of using cryopreserved instead of fresh peripheral blood mononuclear cells (PBMCs) to evaluate intra- and inter-assay variation, and inter-individual variation, for the aphidicolin (APC)-block NER comet assay. Moreover, we measured the variation for the designated internal standard (K562 erytroleukemia cell line) and we evaluated the feasibility to use lymphoblastoid cell lines (LCLs) as surrogate of PBMCs.

Our results showed a low intra- (coefficient of variation [CV] 19.9%) and inter-assay (CV 32.3%) variation, with a good inter-individual variation (122 subjects, mean ± standard deviation 7.38 ± 4.99; range 0.66-26.14; CV 67.63%). A significant correlation between results derived from cryopreserved and fresh PBMCs from the same individuals was found (10 subjects, r = 0.62; p = 0.05). Results from LCLs and cryopreserved PBMCs from the same subjects showed an inverse significant correlation (10 subjects, r = -0.712 p = 0.02). K562 cells as internal standard showed low intra-assay variation.

In the present study the APC-block NER comet assay on cryopreserved PBMCs seemed to be a reliable method to measure DRC variation in epidemiological studies; LCLs were not a good surrogate in this assay.

Keywords: DNA repair, nucleotide excision repair, comet assay, cryopreserved PBMCs

INTRODUCTION

Less efficient DNA repair capacity (DRC) is associated with increased cancer risk and other human diseases, such as neurodegenerative disorders, inflammatory disorders and ageing. DRC is an ubiquitous defense mechanism that plays an essential role in cell survival and genome stability (1). Moreover, it is an important factor of inter-individual variation in response to mutagen exposure and cancer susceptibility (2). Cells have developed a network of DNA repair mechanisms to ensure that the large variety of DNA lesions induced by exogenous and endogenous sources are effectively dealt with. At least five partly overlapping main repair pathways are active in the removal of DNA lesions: base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), double strand break repair (DSBR) and direct repair (DR) (1,3). The development and validation of functional assays that are able to assess individual DRC is notably important for identifying high-risk complex-disease susceptible subgroups within the general population (4).

The alkaline comet assay is a relatively inexpensive technique for the detection of DNA damage and repair. Since few cells are required for the assay, it is particularly advantageous for the analysis of human samples. The method has proven to be very sensitive, and can detect small phenotypic changes caused by genetic variation (5). An interesting possible epidemiological application of the assay is the determination of inter-individual variation in DRC (6). Most alkaline comet assay protocols used in biomonitoring employ peripheral blood mononuclear cells (PBMCs), in part because obtaining blood from human subjects is minimally invasive. However, the technical and logistical difficulties that arise when working with fresh PBMCs have rendered their use quite challenging in epidemiological studies of DRC, and no refined protocol exists for the use of viable cryopreserved PBMCs. In fact, previous papers have been published on the use of human PBMC extracts deriving from PBMC frozen at -80 ^{0}C (7), from cryopreserved PBMCs (8,9) or from dry PBMC pellets

(10,11). These *in vitro* assays consist of an incubation of lymphocyte extracts with substrate nucleoid DNA from cells pretreated with specific damaging agent: either photosensitizer plus light to induce 8-oxoguanine (8,9), or short wavelength ultraviolet light irradiation (7), or benzo[a]pyrene diol epoxide (BPDE) (10,11). The advantage of this kind of in vitro repair assays is that they use DNA substrates with few lesions that simulate the in vivo asset. However, the above assays have also some limitations due to imbalance between concentration in protein extracts, enzyme activity and substrate amount as well as in setting the right incubation time. Moreover, this kind of assays do not always allow discrimination between DNA breaks induced by the mutagen and those resulting from DNA repair incisions, or do not assess a cellular phenotype, but instead measure DRC in cell extracts. Standard cellular "challenge" assays using viable cells have the advantage that the endpoint is the removal of the lesions and restoration of normal DNA structure (4), but they require the sampling at several time-points. In our method we simplified the assay by adding the DNA polymerase inhibitor aphidicolin (APC) (12).

The APC-block NER comet assay is a cellular phenotype assay that measures DRC after BPDE treatment as model mutagen, and has already been developed and validated on fresh PBMCs by Vande Loock *et al.* (12). In the present study we validated the assay on cryopreserved PBMCs by comparing DRC in fresh and cryopreserved PBMCs, evaluating intra- and inter-assay variation and standardizing the use of an internal control to minimize the variation between different electrophoretic runs. Moreover, to evaluate inter-individual variation we performed the assay on a larger number of subjects.

MATERIALS AND METHODS

Lymphocyte isolation

PBMCs were separated from heparinized venous blood collected from healthy subjects by centrifugation with Ficoll Paque PLUS (GE Healthcare, Milan, Italy) at 400 x *g* for 30 minutes at room temperature. After two washes in a combination of RPMI 1640, 1% FBS and 25 mM EDTA, PBMCs were prepared for cryopreservation. Cells were resuspended at $10x10^6$ cells/ml in freezing medium (RPMI 1640, 50% FBS, 10% DMSO), aliquoted in cryovials and slowly frozen overnight at the rate of –1ºC/minute in isopropyl alcohol to –80ºC (Mr. Frosty containers, Nalgene, Roskilde, Denmark). Cryovials were then transferred into liquid nitrogen for long-term storage. All PBMCs used in the present study were cryopreserved before use, unless otherwise stated. Cells were stored in liquid nitrogen for a period ranging from one month to a maximum of one year.

Lymphoblastoid cell lines

Lymphoblastoid cell lines (LCLs) were derived from the PBMCs of 10 healthy subjects as previously described (13,14), and cryopreserved in the laboratory of the University of Turin. Briefly, an aliquot of PBMCs was incubated in RPMI 1640 medium supplemented with L-glutamine, 100 µg/ml streptomycin and 100 U/ml penicillin and 10% FBS (complete medium) for 15 h at 37°C and 5% CO_2, in the presence of EBV-containing supernatant (from B95-8 marmoset lymphoma cells, ATCC, Washington, DC, cat. no. CRL-1612) 1:1 (v/v). The cells were then washed and cultured ($1.5x10^6$/ml) in complete medium in the presence of irradiated (30 Gy) allogenic PBMCs ($0.5x10^6$/well) and 0.5 µg/ml cyclosporin A (Sigma Aldrich Italia, Milan, Italy). Cells were grown adding fresh medium every 3 days and fresh feeder cells after

one week. After 3-4 weeks, LCLs were established, and cells were cryopreserved as above described for fresh PBMC. Before performing the assay, cells were rapidly thawed and cultured in RPMI 1640, 10% FBS, 1% L-glutamine (Invitrogen, Carlsbad, CA), 1% Na-pyruvate (Invitrogen), 1% non-essential amino acids (Invitrogen), 1% kanamycin (Invitrogen). NER comet assay results from LCLs were compared with those from PBMCs from the same subjects. NER comet assay was performed as described below: cells from the same subject (LCLs and PBMCs) were treated on the same day and analyzed in the same electrophoresis run to avoid further variation.

Cell treatments

Cryopreserved PBMCs were thawed quickly in a 37°C water bath and suspended in 5 ml of medium at 4^0C containing 50% FBS, 49% RPMI 1640 and 1% dextrose. Treatment was performed as previously described (12), with minor modifications. Cells were spun down by centrifugation (10 minutes at 450 x g) at 4°C, and $5x10^6$ viable cells were suspended in 5 ml of F10 medium (Invitrogen, Paysley, UK) containing 10% FBS and 2.5 µg/ml phytohemoagglutinin (PHA) (Sigma-Aldrich Co, St Louis, MO, USA). Twenty-four hours later, samples were centrifuged (10 minutes at 450 x g) at 4°C, and cell pellets were resuspended in 5ml of serum-free F10 medium containing 2.5 µg/ml PHA. Cells were then aliquoted into 5 culture tubes for the following treatment conditions (tubes were named A, B, C, D, E for each sample): (A) F10 medium only; (B) 0.5% DMSO (solvent of APC and BPDE); (C) 2.5 µg/ml of APC (Sigma-Aldrich Co), added at time 0; (D) 0.5 µM BPDE (NCI Chemical Carcinogen Reference Standards Repository, Midwest Research Institute, Kansas City, MO, USA), added at time 30 min; (E) pretreatment with 2.5 µg/ml of APC (30 min) followed by 0.5 µM BPDE (2 hours). All the cell cultures were incubated for a total of 2.5 hours at 37°C, 5% CO_2. At the end of the treatment cells were centrifuged and pelleted cells

were processed for NER comet assay. When indicated, only the APC negative control was used (only treatments C, D and E were assayed for).

LCLs were seeded into T25 flasks at a density of $5x10^5$ cells per ml and grown at 37°C at 5% CO_2 for 24 hours, then treated with the same procedure performed for cryopreserved PBMCs.

Aphidicolin-block NER comet assay

The NER comet assay was performed according to the methods described by Singh *et al.* (15) and Vande Loock *et al.* (12), with slight modifications. After treatment, cells were mixed with low-melting-point agarose (0.75%; Sigma-Aldrich Co) and layered on 85x100 mm GelBond films (Lonza, Basel, Switzerland). Each GelBond film comprised eight 19x23 mm agarose gels. The GelBond films were immersed in lysis solution (2.5 M NaCl, 0.1 mM Na_2EDTA, 10 mM Tris, 1% Triton X-100, pH 10) for 1.5 hours at 4ºC, then placed in an electrophoresis tank for 40 minutes, submerged into electrophoresis buffer (0.3 M NaOH, 1 mM Na2EDTA, approximately pH 13). Finally, gels were electrophoresed at 30 V (0.8 V/cm) and 300 mA for 20 minutes. After neutralization with 0.4 M Tris-HCl (pH 7.5) gels were fixed in ethanol and dried at room temperature. The nuclei were visualized by a Leica fluorescence microscope at 40X magnification and two gels of 50 nuclei for each sample treatment were scored with Comet IV software (Perceptive Instrument, Suffolk, UK). The median tail intensity fluorescence (% Tail DNA) of 100 nuclei was used as a measure of DNA damage. For each electrophoresis run, a human K562 erytroleukemia cell line was included as an internal standard and used to normalize results. DRC was calculated for each subject as: % Tail $DNA_{APC+BPDE}$ - % Tail DNA_{BPDE} - % Tail DNA_{APC}.

Variation in K562 cells as the internal standard

A human K562 erytroleukemia cell line was included as an internal standard in each of the electrophoretic runs to account for variations across different runs. As the variation of background DNA damage in permanent cultures of K562 cells can be very wide, in order to eliminate variation due to the culture and to limit the overall variation in the electrophoresis run, we prepared a batch of cryopreserved K562 cells as follows. K562 cells were cultured in suspension in RPMI 1640 medium supplemented with 10% FCS, glutamine and penicillin/streptomycin. Cultures were incubated at 37^0C in 5% CO_2 and supplied with new medium twice a week. A uniform batch of K562 cells was obtained by growing up to $50x10^6$ cells and aliquoting them at $1x10^6$ cells/aliquot. All cells were cryopreserved the same day, and stored in liquid nitrogen until use.

To evaluate background levels of DNA damage in K562 cells, and to assess intra-assay variation, we prepared 24 NER comet assay gels from one of the K562 aliquots: cells were extracted from liquid nitrogen and rapidly thawed at room temperature. Cells were mixed with low-melting-point agarose (0.75%; Sigma-Aldrich Co) and layered on 85x100 mm GelBond films (Lonza, Basel, Switzerland). Each GelBond film comprised four 19x23 mm agarose gels. Comet assay was performed as described in "Aphidicolin-block NER comet assay" paragraph. The procedure was repeated on 4 different days, to test for inter-assay variation. A total of 96 gels (24 gels for each day, repeated in 4 different days) were scored, including 50 nuclei each. The position of each slide in the electrophoretic tank was recorded, to control for position effects. All the gels were prepared by a single operator, who also performed NER comet assays. Coded gels were scored blindly twice, by two different operators.

The batch of cryopreserved K562 cells was then used as the internal standard, with one K562 aliquot included in each electrophoresis run of assayed samples. NER comet assay scores were then normalized to those of the K562 cells used in the same electrophoresis run

by dividing TI values of samples with TI of K562 cells as suggested in (16). A reference value of the internal standard has been calculated to check whether outcomes from a given electrophoresis should be excluded from the analysis or not by using a similar approach as in (16). The mean±2 standard deviation (SD) of the TI of K562 cells was taken as reference. When internal standards values were lower or higher than this reference interval results of all samples included in the same electrophoresis run were excluded from the final analysis (16). In the present set of experiments no runs have been excluded from the analysis.

DRC in fresh and cryopreserved PBMCs

Comparison between fresh and cryopreserved PBMCs was performed on 10 subjects. PBMCs were isolated and cryopreserved as described above, while a part of them was immediately cultured with PHA for treatment of fresh PBMCs (see paragraph "Cell treatments").

Intra- and inter-assay variation

Cryopreserved PBMCs from three healthy subjects were used to evaluate intra- and inter-assay variation of the NER comet assay. Different aliquots of cryopreserved PBMCs were thawed on different days (inter-assay; donor 1: three experiments; donor 2: three experiments; donor 3: two experiments), and each aliquot was further divided into two different cell cultures for treatment (intra-assay).

Inter-individual variation in DRC

To evaluate inter-individual variation, NER comet assay was performed on PBMCs from 122 young healthy subjects (39 males, 83 females; mean age 24.5 years, range 19.1–48.4 years). Subjects provided written informed consent before inclusion in the present study. DRC

was calculated for each subject as explained below, and all values were normalized to the designated internal control (K562 cell aliquots prepared as cryopreserved batch with one aliquot thawed for each electrophoresis run, see above).

To validate the applicability of the NER comet assay to the analysis of a large number of subjects, we also tested the use of only one negative control for each subject instead of three, in order to reduce the overall number of gels to be scored in a large study on DRC. After experimental validation of a correlation between the different negative control treatments on 10 subjects, we performed the NER comet assay using APC as the only negative control, one single culture for each treatment and one aliquot from the same batch of cryopreserved K562 cells for each electrophoresis run.

Statistical analysis

Mean of median values of % Tail DNA, SD and percentage of coefficient of variation (CV %) were used for a statistical description of data, and the Pearson coefficient was used for correlation assessment.

Intra- and inter-assay analyses were performed with non-parametric tests (Kruskal Wallis and Wilcoxon signed rank test, as needed), with Bonferroni correction for multiple comparison.

We analysed for variation in K562 cells across different testing days, within different positions in the electrophoretic tank, and by scorer using the Wilcoxon Rank Sum test, Wilcoxon Signed Rank test and Kruskal-Wallis test as appropriate. All analyses were conducted using SAS V 9.2.

RESULTS

Comparison of DRC between cryopreserved and fresh PBMCs

We performed NER comet assay with both fresh and cryopreserved PBMCs from 10 healthy subjects and found a significant correlation between samples from the same subject ($r = 0.62$; $p = 0.05$, Figure 1). A very low DRC reflects in general a weak capacity to resolve the DNA damage, however, from a biological point of view (and from our formula), we should not expect negative values. On the other hand, a negative DRC could reflect a sensitivity limitation of the technique in measuring very low synthesis activity levels (Supplementary Table I).

Intra- and inter-assay variation

Optimal experimental set-up was chosen based on the validation previously described on fresh PBMCs (12). Intra- and inter-assay variation was measured and, no statistically significant differences were detected between the three repeated experiments (one day's results for Donor #3 are missing due to technical issues) for any of the three donors (nor between the two parallel cultures, data not shown), indicating low intra- and inter-assay variation (Table I, Supplementary Table II). CV % were between 5.12% and 44.10% for the intra-assay variation, and between 9.43% and 27.12% for the inter-assay variation. Intra-culture variation CV % ranged from 0.33% to 71.24%.

Inter-individual variation in DRC

DRC as evaluated in 122 healthy subjects by comet assay on PBMCs to measure inter-individual variation (median % Tail DNA) showed a CV% of 67.63%, with a broad range from 0.66 to 26.14 and a mean ± SD of 7.38 ± 4.99 (Supplementary Table III). Five to seven subjects were examined every day, for a total of 18 different experiments; K562 internal standard mean of median % Tail DNA was 8.41 ± 4.09.

Our experimental validation of the correlation between the different negative control treatments we performed in the NER comet assay showed a significant positive correlation between the three treatments used as negative controls (background, solvent and APC): solvent *versus* background (r = 0.80, p = 0.006); solvent *versus* APC (r = 0.84, p = 0.003); background *versus* APC (r = 0.71, p = 0.022). These results suggest it might be possible to use just one negative control when testing cryopreserved PBMCs, which has already been proposed for fresh PBMCs (12).This last approach was adopted in the present study when measuring inter-individual variation.

K562 cell variation as an internal standard

As the variation of background DNA damage in permanent cultures of K562 cells can be very wide (16), we prepared a batch of cryopreserved K562 cells in order to eliminate variation due to cell cycle in the culture, thus limiting the overall variation in the electrophoresis run. No statistically significant difference was evident across different operator scoring, testing days, or different electrophoresis positions (Supplementary Table IV).

Comparison between LCLs and PBMCs

We performed NER comet assay on both LCLs and PBMCs from 10 subjects to evaluate the suitability of LCLs as a surrogate tissue (Supplementary Table V). Figure 2 shows the comparison of DRC measured in the two cell types. LCLs show a lower DRC (mean ± SD: 4.30 ± 4.07) than PBMCs (9.73±3.51), with a statistically significant inverse correlation (r = -0.712; p = 0.02). Moreover LCLs showed a larger inter-individual variation (CV % 94.79) than PBMCs (CV % 36.11).

DISCUSSION

The alkaline comet assay is a sensitive and relatively inexpensive technique for the detection of DNA damage and DNA repair (6). Often, the assay is performed on PBMCs as blood sampling is minimally invasive. However, the use of PBMCs to examine DRC in epidemiological studies is still challenging. Indeed, there are many technical and logistical difficulties related to the use of fresh lymphocytes, and there is no refined protocol for the use of viable cryopreserved PBMCs.

Samples are usually collected over an extended period of time, and DRC can either be analyzed immediately after sampling, or retrospectively. The most efficient approach when using comet assay is generally a retrospective analysis using cryopreserved cells. This reduces any possible inter-batch variation by running more samples in the same experiment.

Recent studies have examined the inter-assay experimental variation of the comet assay (17,18), but only a few compared fresh and cryopreserved PBMCs. Trzeciak *et al*. showed that cryopreserved PBMCs can be used to measure DNA repair after treatment with γ radiation: DRC was assessed in a small cohort (10 subjects) measuring initial DNA damage, half-time of DNA repair and residual damage after 30 and 60 min (19). Duthie *et al*. evaluated in PBMCs the effect of cryopreservation on the measurement of endogenous and induced DNA strand breakage, altered bases, antioxidant capacity following incubation with the dietary flavonoid quercetin, and DRC. The authors demonstrated that frozen human lymphocytes can be suitable for most aspects of the detection of biomarkers of DNA damage, but not for oxidant-induced DNA strand break repair (20).

The objective of the present study was to validate the use of an APC-block NER comet assay on cryopreserved PBMCs and to show the assay's reliability and applicability to a large number of samples. This modified comet assay is based on the assessment of cellular DNA damage repair, and measures the capacity of donor cells to resolve DNA damage in case of *in vitro* challenging with BPDE in the presence or absence of APC, a potent and specific

inhibitor of DNA polymerases α and δ (21). The method has already been standardized on fresh PBMCs (12), and we then applied to cryopreserved PBMCs using K562 cells as an internal standard to avoid experimental variation during electrophoretic runs (16). With the present assay is possible to evaluate the number of damaged sites produced by NER incision that have been repaired after 2h BPDE exposure. This is a reflection also of the synthesis activity. Since it is not possible to distinguish the origin of DNA damage not repaired (if basal or induced by the treatment), some further improvements of the assay are needed in the future (i.e. BPDE treatment at different time).

Firstly, we compared DRC measured in fresh and cryopreserved PBMCs from 10 donors. As a significant correlation was found ($r = 0.62$, $p = 0.05$), we further validated the assay on cryopreserved PBMCs evaluating intra- and inter-assay variation. We obtained DRC CVs % (mean intra-assay CVs % for each donor) ranging from 14.2% to 35.1% for intra-assay variation, and from 9.4% to 27.1% for inter-assay variation. These ranges of variation are quite similar to those previously reported for fresh PBMCs (12).

To evaluate the suitability of NER comet assay to assess inter-individual variation in DRC, we performed the assay on cryopreserved PBMCs collected from 122 young healthy subjects. In this case, DRC ranged from 0.66 to 26.14, with a CV % of 67.6%. These results suggest that the methodology has the capacity to detect inter-individual variation in cryopreserved PBMCs as in fresh PBMCs, showing intra-individual, intra- and inter-assay variation that are lower than the inter-individual variability. Many studies during the last few years have analyzed the NER inter-individual variations in healthy individuals. Qiao *et al.* found a NER capacity variation of 4-7 times, measured by host cell reactivation assay (HCRA) in 102 healthy subjects (22). Tyson *et al.* showed an inter-individual variation of 11 times in the NER capacity, always measured by HCRA in 66 healthy young individuals (23). Moreover, the repair activity induced by UV ray damage, measured by unscheduled DNA

synthesis, varied with a range of approximately 8 times in healthy control subjects. The explanations of a substantial inter-individual variation in the ability to repair the DNA can include genetic factors, as polymorphisms of DNA repair genes and environmental factors, as diet and life style. Recently Slyskova *et al.* analyzed DRC in 100 healthy subjects by BPDE-challenge comet assay (24). They observed that the levels of single strand breaks that represents NER-mediated breaks originated from BPDE-adducts removal varied from 0 to 76 Tail DNA %. In our sample population, we observed a range of variability of about 40 times in inter-individual DRC. The wider range of variability observed by Slyskova *et al.* could be due to the different endpoint of their method and to the different BPDE treatment (i.e. different concentration) they applied.

To adapt the assay to the analysis of large sample sizes such as those regarding molecular epidemiological studies, we tried to reduce the burden of the work required by the assay itself, and evaluated the correlation between different negative controls usually included in each assay, with the final goal of selecting the most suitable for our purposes. We obtained a high correlation between solvent (DMSO) and background ($r = 0.79$, $p = 0.006$), background and APC ($r = 0.71$, $p = 0.02$) and solvent and APC ($r = 0.84$, $p = 0.003$). These results confirmed those obtained with fresh PBMCs (12), suggesting that it might be possible to choose only one treatment as a negative control for experiments on a large number of samples.

To account for inter-electrophoresis differences, a K562 erythroleukemia cell line was used as the internal standard (16). This allowed for the comparison of several different electrophoresis sessions, which are necessary when a large number of samples is processed. Nevertheless, the use of this particular cell line may not be completely straightforward, as the cell line genotype/genome may not be entirely stable. This may cause heterogeneity in the evaluation of DNA damage. In the present study we reduced the variation in the internal standard itself by preparing a large batch of cryopreserved cells from the same culture and

storing them immediately after. Moreover, we examined the variation of internal standard gels run in different positions of the electrophoretic tank, and variation in operator scoring. There were no statistically significant differences among the positions in the electrophoretic tank or between scores given by different operators, nor there was significant inter-day variation. Finally, when we used the K562 cell batch for inter-individual DRC analysis, performing 18 different experiments, K562 internal standard mean of median % Tail DNA ± SD was 8.41 ± 4.09, fully comparable with the results reported by De Boeck *et al.* (16).

The use of LCLs a surrogate of PBMCs for DRC evaluation has been previously tested (25-28). In the present study we performed the NER comet assay on LCLs obtained from 10 subjects and their corresponding cryopreserved PBMCs to evaluate whether LCLs were suitable as a surrogate tissue. In agreement with our previous findings on other DNA repair assays (13,14), LCLs are not a suitable surrogate of PBMCs for NER comet assay, since they showed a negative correlation with DRC obtained in corresponding PBMCs and a very high inter-individual variation. This result may be due to the genomic instability and DNA repair inhibition induced by Epstein-Barr virus (29).

The use of the NER comet assay on cryopreserved PBMCs to measure DRC in epidemiological studies is further supported by the possibility to use one, instead of three, negative controls, allowing for the analysis of a large number of samples simultaneously, and by the normalization to a K562 cell standard, that allows for comparison between different electrophoretic runs.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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FIGURE LEGENDS

Fig. 1

Correlation between fresh and cryopreserved peripheral blood mononuclear cells (PBMCs) in ten subjects (DRC: DNA repair capacity).

Fig. 2

Comparison of DNA repair capacity (DRC) evaluated in lymphoblastoid cell lines (LCLs) and peripheral blood mononuclear cells (PBMCs) obtained from ten subjects.