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Impact of Logistical Variation in Sample Handling On Comet Results in Human Leukocytes. Toraason, M[1], Krieg, E[1], Singh, N[2]. [1]National Institute for Occupational Safety and Health, Cincinnati, OH, United States. [2]University of Washington, Seattle WA, United States.

Epidemiological studies employing biomarkers such as DNA damage often encounter logistical restrictions in the field that unavoidably lead to variation in the handling of biological samples. This may lead to variation in sample analysis outcome. The present study assessed potential sources of variation in comet assay results (% tail DNA and DNA tail moment) in human leukocytes arising from variations in day and time of blood draw, freezing protocols, pre-freezing duration, freezing duration, and analysis lot. Two experimental designs were used to assess variability. Under the first design, blood was drawn at the same time on three sequential days from a single individual in the laboratory where comet analyses were performed. Following blood draw, a subset of samples were exposed to 0 or 200 rads of X rays and analyzed in the comet assay as fresh blood or after being frozen for 1 month at – 80 °C using three freezing protocols. The protocols evaluated provide both advantages and disadvantages depending on whether blood was irradiated or not. Adding 100 µl of whole blood directly to 900 µl of RPMI 1640 containing 20% DMSO at 4 °C was optimum in terms of accurately replicating the overall outcome obtained with fresh blood. Comet results from three sequential blood samples drawn over as many days and frozen for 1 month did not vary significantly regardless of whether samples were analyzed on a single day in a single analysis lot or over three sequential days in three separate analyses lots. Results also indicate that maintaining blood on ice prior to freezing increases DNA damage in time-dependent fashion with small but significant effects becoming evident at 8 hrs. Under the second design, morning and evening blood samples were acquired on the same day from 10 individuals and duplicate samples were analyzed 1 or 3 months after freezing and shipping across the US. Comet results were analyzed using a mixed model to calculate variance components and to test for effects of time of blood draw and month of analysis. Time of blood draw was without significant effect. Month of analysis had a small but significant effect on % tail DNA (1 month, 50.4 vs 3 months, 48.6; P = 0.044), but not on DNA tail moment (1 month, 2034 vs 3 months, 1965; P = 0.313).

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Polymorphisms Of XRCC1, XRCC3 and XPD and the Frequency of Micronuclei in Human Lymphocytes of Industrial Radiographers. Cho, YH, Choi, JY, Ahn, YS, Woo, HD, Kang, SJ, Chung, HW, School of Public Health, Seoul National University, Seoul, Korea, South.

Aim: In order to evaluate the influence of polymorphisms in DNA repair genes ($XRCC1^{399}$, $XRCC3^{241}$ and XPD^{751}) on the frequency of micronuclei in industrial radiographers and to validate micronucleus-centromere assay as a sensitive method for biomonitoring of industrial radiographers. Methods: The cytokinesis-block micronucleus (CBMN) assay in combination with FISH technique using pan-centromeric probes and genotyping of DNA repair genes by PCR-RFLP were performed in 47 radiographers and 47 controls. Results: The frequencies of centromere negative micronuclei (MNC-) were significantly higher in radiographers than in the controls (14.6 vs 4.2 per 1000 binucleated cells; p<0.0001), whereas similar frequencies of centromere positive micronuclei (MNC+) were observed in both groups (6.9 vs 6.2; p=0.395). The frequencies of MNC- was significantly associated with radiation doses after adjusting confounding variables such as age, smoking, alcohol intake, duration of work in the poisson regression analysis. A significantly higher frequency of MNC- was observed in radiographers with variant $XRCC3^{241}$ genotype. Similar result was also observed in radiographers with variant alleles for $XRCC1^{399}$ whereas the radiographer with variant alleles for XPD^{751} showed higher frequency of MNC+. Conclusion: Scoring of MNC- is recommended to improve sensitivity of CBMN assay as a useful cytogenetic biomonitoring method for radiation workers and polymorphisms in the DNA repair genes influence on the frequency of micronuclei in radiographers.

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Chronic Low Dose Radiation Effects on Radiation Sensitivity (Adaptive Response, Low Dose Hypersensitivity) and Genomic Instability in TK6 Cells. Schwartz, JL[1], Jordan, R[1], Slovic, J[1], Moruzzi, A[1], Liber, HL[2]. [1]University of Washington, Seattle, WA, United States. [2]Colorado State University, Fort Collins, CO, United States.

There are a number of cell responses that can be detected after low dose radiation exposures. These include the adaptive response, low dose hypersensitivity, and induced chromosome instability. It is unclear whether there is any relationship between the three phenomena. In this study, we examined the relationship between the adaptive response, low dose hypersensitivity, and induced chromosome instability in a human B-lymphoblastoid cell model, TK6. One of the difficulties in studying these phenomena is that results are highly variable. One possible explanation for the variability is that the phenomena are cell cycle phase specific. Small changes in growth characteristics might change the proportion of cells that are susceptible to the induction of one or more of these processes. To overcome this potential problem, we studied the effects of daily low dose exposures given over a three week period. Our initial studies focused on cells exposed to daily doses of 10 cGy gamma rays. This chronic low dose exposure induced a significant increase in resistance in the TK6 cells (adaptive response), but had no significant effect on sensitivity in a TP53 knockout of TK6 or in TK6 cells over-expressing Bcl-2. Low dose hypersensitivity was prominent in TK6 cells but absent in the TP53-knockout and Bcl-2 over-expressing variants. Chronic exposure to 10 cGy eliminated the low dose hypersensitivity response in TK6 cells. Background levels of chromosome instability were low in TK6. They were significantly higher in the TP53-knockout and Bcl-2 over-expressing variants. Induced levels of instability were similar in all three cell lines. These results suggest a link between the adaptive response, low dose hypersensitivity, and baseline chromosome instability levels. Radiation-induced chromosome instability appears to involve a different process. The research described herein was supported by the Low Dose Radiation Research Program, Biological and Environmental Research (BER), U.S. Department of Energy, grants DE-FG03-003462908 and DE-FG03-02ER63365. The authors thank Dr. A. Kronenberg for sharing with us the TK6 cell line that over-expresses Bcl-2.

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An International, 4 Laboratory Assessment of the Robustness and Reproducibility of the GADD45a Genotoxicity Assay, GreenScreenHC. Billinton, N[1], Beerens, D[4], Birrell, L[1], van Gompel, J[4], Hastwell, PW[2], Rees, R[2], Scott, A[3], Walmsley, RM[5,1], Webster, TW[2], Windebank, S[3], Woestenborghs, F[4]. [1]Gentronix Ltd, Manchester, United Kingdom. [2]GSK, Ware, Herts, United Kingdom. [3]SEAC, Unilever Colworth, Sharnbrook, Beds, United Kingdom. [4]Johnson & Johnson Pharmaceutical Research & Development, Beerse, Belgium. [5]University of Manchester, Manchester, United Kingdom.

The recent paper from Kirkland and co-workers (Kirkland *et al*, 2005) highlighted the lack of specificity in *in vitro* mammalian cell assays. This leads to an unacceptably high incidence of false positive indications: compounds uniquely positive in the *in vitro* tests, and negative in the *in vivo* micronucleus test or long term carcinogenicity studies. A new *in vitro* mammalian cell test that exploits the expression of the GADD45a gene has been developed (Hastwell *et al*, 2006). The combination of a p53 competent host cell and the full array of upstream and intra-gene regulatory elements for the GADD45a gene has produced a convenient microplate assay that provides both high specificity and sensitivity. In order to assess the routine utility of the assay, an inter-laboratory trial was carried out. Four participating laboratories were chosen: 1. Gentronix Ltd, Manchester, UK; 2. GSK, Ware, UK; 3. Unilever Colworth, Shambrook, UK; 4. J&J, Beerse, Belgium. The Manchester and Ware laboratories had prior experience of the assay, so the trial experiments were carried out by a recently appointed technician an undergraduate 'sandwich' student respectively. 16 compounds were chosen on the basis of mechanism of genotoxic damage (without S9), toxicity profile and stability in DMSO. Non-genotoxins were included in the trial. The compounds were coded, prepared as single batches, then aliquoted out for distribution. Passage and assay medium was similarly standardised. Frozen cell cultures were sent out at least 2 weeks in advance of trial commencement in order to establish stable passage. The study director (from Gentronix Ltd) provided protocols, and visited each laboratory to demonstrate the assay in order to establish that the instrumentation and other local technologies were able to reproduce results for reference compounds. The participating labs were required to test each of the 16 coded compounds 4 times on different days. The results and conclusions of this trial will be presented and discussed. D. Kirkland *et al.*, Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 584 (2005) 1-256. P.W. Hastwell *et al.*, Mut. Res. (in press)