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The Micronucleus Test for Occupational Safety†

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Summary

Although less commonly used than internal dose indicators, biological effect indicators can be valuable for Risk Assessment. Among the numerous biomarkers used to date, those that indicate DNA damage could be especially useful for occupational safety, as they predict the risk of carcinogenesis. The most common among these assays is the micronucleus test. Unfortunately, this test cannot be performed on human erythrocytes; to apply it to lymphocytes, it requires invasive blood sampling, limiting its use to scientific research. We have developed a new method that enables the micronucleus test to be conducted in a rapid, non-invasive, and cost-effective manner. The test is performed on immature erythrocytes (reticulocytes) in human blood smears collected via a finger prick and stained with Acridine Orange. This new protocol allows the micronucleus test to be applied to human blood samples in a manner compatible with occupational safety procedures.

1. Introduction

Biological monitoring enhances exposure assessment by incorporating fundamental measures of absorbed dose and biological effects. In Italian legislation, Legislative Decree 81/08 mandates, within the framework of risk assessment activities, not only the evaluation of exposure to chemical and physical agents but also the conduct of biological monitoring. This is compulsory for workers exposed to agents for which biological limit values have been established. In addition to "exposure monitoring", Legislative Decree 81/08 also specifies procedures and reference values for "monitoring of the absorbed dose" (Annex XXXIX). As previously noted, however, biomonitoring can encompass both the use of "internal dose indicators" and "biological effect indicators." The latter are referenced in Title IX of Legislative Decree 81/08 but lack detailed procedural or quantitative guidance. Integrating biological effect indicators into occupational health surveillance could enable the detection of early damage, allowing for preventive or protective interventions prior to the onset of occupational pathologies.

Although, as already mentioned, "biological effect indicators are not yet implemented in routine health surveillance activities, they have been extensively developed within scientific research. Among the numerous biomarkers employed, those that indicate DNA damage may be particularly relevant to workplace safety, given their predictive value for carcinogenesis [1]. Most existing literature on human biomonitoring evaluates biomarkers of genotoxic damage—i.e., DNA damage—using peripheral blood samples [2]. This approach is favored due to the lower invasive nature of blood collection

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compared to tissue biopsies. The tests employed include detection of chromosomal aberrations, sister chromatid exchanges, DNA repair proteins, and the comet assay and micronucleus test [3].

However, these analyses often require specialized skills and resources that are not readily available outside research settings. They require non-negligible blood volumes for lymphocyte culture, preparation of metaphases for chromosomal aberration and sister chromatid exchange assessments, immunofluorescence techniques for DNA repair proteins, and gel electrophoresis for the comet assay. Consequently, although these tests are widely used in scientific studies—including investigations of cohorts with occupational exposure and significant associations between exposure and DNA damage—there is no application outside the field of scientific research. Despite the extensive scientific literature on the subject, these test are not currently employed by health surveillance or occupational safety practitioners. Nonetheless, developing accessible, non-invasive, and cost-effective biomonitoring methods could substantially advance occupational health practices.

Among these, the micronucleus test represents the most straightforward and cheapest assay for genotoxicity assessment. When performed on erythrocytes (red blood cells), it obviates the need for blood culture—necessary for lymphocyte-based tests—and can be conducted on blood smears. The micronucleus test consists in assessing the frequency of cells containing micronuclei, known in hematology as Howell-Jolly bodies. Micronuclei form when a chromosome fragment or an entire chromosome (Figure 1A) is not incorporated into one of the daughter nuclei at the end of cell division, and is therefore the product of genotoxic damage.

Therefore, it is feasible (at least in theory) in every proliferating tissue. In practice, both in human and animal studies, it is often performed on red blood cells (erythrocytes). This is due not only to the ease with which blood can be obtained, but also because, being erythrocytes enucleated (and thus containing no DNA), any nuclear material inside of them (i.e., micronuclei) can be easily recognized. In fact, during the transition from erythroblast (precursor) to erythrocyte, the nucleus is expelled, but not the possible micronucleus (Figure 1B). For these reasons, as well as for its proven sensitivity and specificity, the micronucleus test in animals (in vivo) is internationally standardized (OECD n°474) and recognized for the evaluation of induced DNA damage [4].

In certain species, however, micronuclei are extruded from erythrocytes during their passage through the spleen [5]. Consequently, the micronucleus test is not applicable in these species. This limitation prompted the Organization for Economic Cooperation and Development (OECD) to specify that "any appropriate mammalian species may be used provided it is a species in which the spleen does not remove micronucleated erythrocytes" [4]. Unfortunately, human spleens do remove micronuclei. Nonetheless, the test is feasible if only immature erythrocytes (reticulocytes) are scored, because reticulocytes have not yet passed through the spleen (where they develop into mature erythrocytes) and thus can be scored.

As noted above, many articles in the scientific literature describe occupational biomonitoring studies in which genotoxicity assays, including the micronucleus test (on lymphocytes), have been performed [3]. Nonetheless, they are not used by safety and prevention personnel and are not part of the risk assessment or health surveillance protocols. This could be mainly ascribed to three reasons: 1) invasiveness of the sampling; 2) technical skills required; 3) cost and time required for the assays. In this article, we describe a new protocol we have developed to address and resolve these issues. As a first validation, we used blood samples from X-irradiated mice to assess the method's sensitivity.

2. Methods

First, we introduce a simple and minimally invasive sampling technique. Most assays typically require several milliliters of blood, usually 5 ml, thus obtained via venipuncture. In contrast, our novel approach involves collecting a blood drop through digital puncture, specifically with a finger prick (Figure 2A-2B). While this volume is insufficient for other assays, such as those necessitating lymphocyte culture preparation, it is more than adequate for conducting the micronucleus test on reticulocytes. Indeed, human blood contains approximately

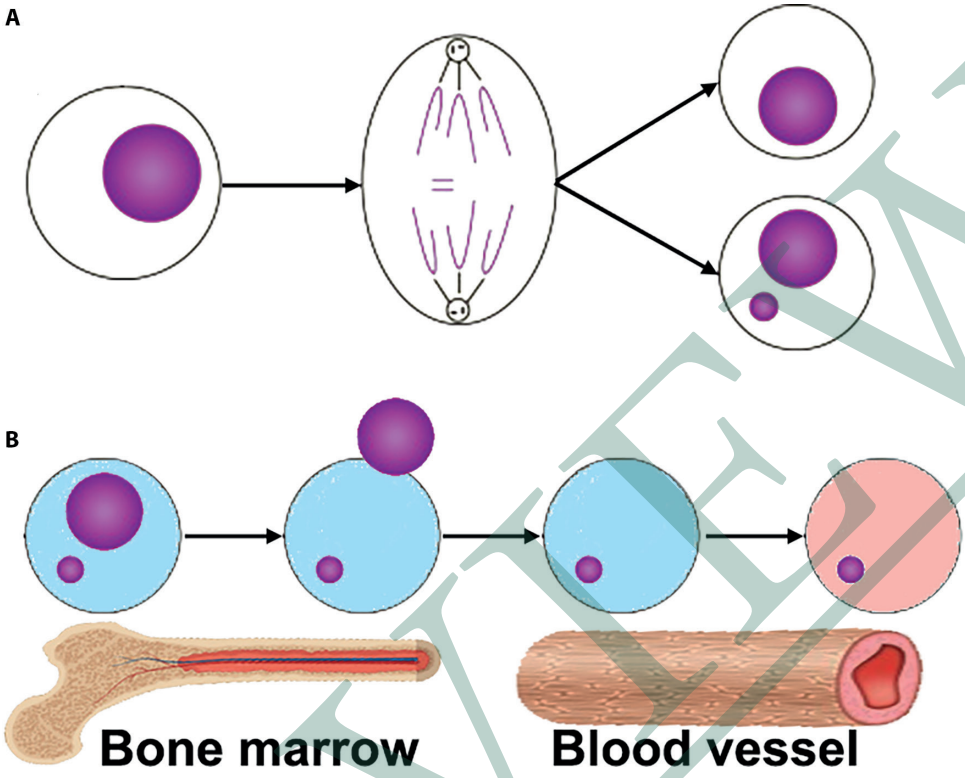

Figure 1. (**a**) During mitosis a chromosome fragment is not segregated in the two nuclei of the daughter cells, thus forming a micronucleus; (**b**) During the maturation of a micronucleated erythroblast in the bone marrow, the nucleus is expelled, resulting in a micronucleated reticulocyte released in the circulation; this will further lose all RNA and organelles, becoming a mature (and in this case micronucleated) erythrocyte.

5 million erythrocytes per microliter [6], with reticulocytes constituting nearly 1% of these cells. This corresponds to tens of thousands of reticulocytes per microliter, whereas the test requires only 1000-2000 reticulocytes for scoring. A blood drop obtained by finger prick typically ranges from 30 to 50 microliters. We recommend using no more than 10 microliters of blood (see below).

For each subject, three to four blood smears can be prepared (Figures 2C-2F) to ensure technical replicates for the staining procedure. To prevent the formation of overly dense smears with piled erythrocytes that are non-scorable, it is recommended to use no more than 10 microliters of blood. The smears are subsequently allowed to dry at room temperature and then fixed in absolute methanol for 10 minutes. Once dried, they may be stored at room temperature.

A stock solution of Acridine Orange (1 mg/mL) in deionized water should be prepared and stored at +4 °C in the dark. For staining, fresh solutions are prepared by diluting aliquots of the stock solution in Sørensen's buffer at pH 6.8 to a final concentration of 20 µg/mL. Blood smears are stained with 50 microliters of this freshly prepared Acridine Orange solution at room temperature, then covered with a coverslip.

Acridine Orange binds to both DNA and RNA, emitting yellow-green fluorescence (520 nm) and red-orange fluorescence (680 nm), respectively. Thus, reticulocytes, which contain RNA, are stained in red/orange, whereas mature erythrocytes are not stained. The test is performed using a fluorescent microscope by scoring only reticulocytes (orange-stained cells). Nucleated blood cells, identifiable by their size and the yellow-stained nucleus occupying

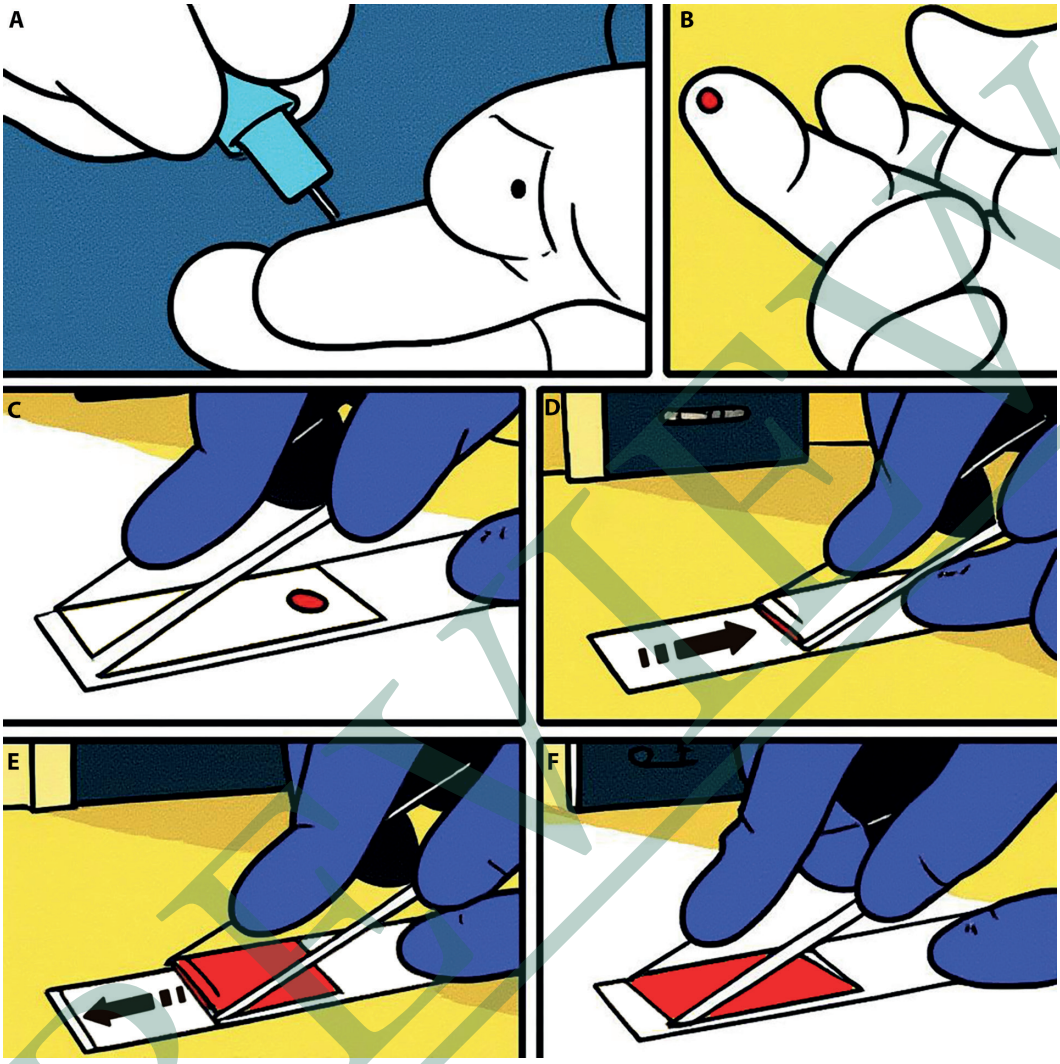

Figure 2. Non-invasive blood sampling. A finger prick (**a**) is used to collect a drop of blood (**b**), which is put on a smear glass (**c**). Another glass is put at 45° and pulled backward until it touches the drop (**d**), then it is quickly moved forward (**e**), creating the blood smear (**f**).

most of the cell, must not be scored. Micronuclei are classified as such if they are stained yellow, are clearly situated within reticulocytes, and are circular (Figure 3). Micronuclei that appear oblong or irregular must be discarded as debris or aggregates.

The assessment of micronuclei frequency then consists of scoring 1500 reticulocytes and noting how many are micronucleated. Summing up, the proposed protocol is the following:

1. Sample a drop of blood with a finger prick.
2. Smear blood on glass.
3. Air-dry the smears at room temperature.
4. Fix the smears in absolute methanol at room temperature for 10 minutes, then air-dry them (and, if needed, store the smears at room temperature).
5. Stain the smears with 50 μL of Acridine Orange in Sørensen's buffer at pH 6.8 (final concentration of 20 μg/mL) (remember Acridine Orange must be at room temperature!);
6. Close the smears with coverslips.
7. Analyze the sample with a fluorescent microscope, scoring 1500 reticulocytes.

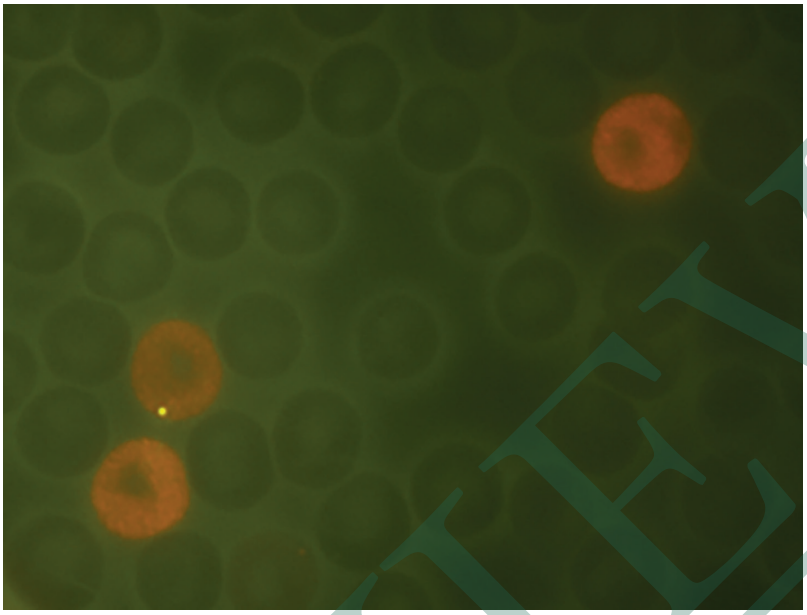

Figure 3. The erythrocyte micronucleus test with Acridine Orange staining. Mature erythrocytes (barely visible) are not stained and must not be scored. Reticulocytes are stained in orange and one of them bears a yellow-stained micronucleus.

As an initial validation of the method's feasibility and sensitivity, the erythrocyte micronucleus test coupled with Acridine Orange staining was performed on murine blood samples. These samples were derived from a prior experiment [7], approved by the Animal Research Ethical Committee of the Italian Ministry of Health (approval ID: 10.10.15). In brief, two groups of five female CD-1 Swiss mice each were subjected to X-irradiation at doses of 0.1 and 1 Gy, using a Gilardoni apparatus (Gilardoni, Italy) with 250 kV, 6 mA, 3 mm Al filter, and at a dose rate of 0.5 Gy/min. An additional group of five female mice served as unirradiated controls. Blood samples were collected via tail puncture 24 hours post-irradiation. The blood smears were fixed, stained, and analyzed as previously described. Micronucleus frequencies between the irradiated and unirradiated groups were compared using Welch's t test. Results are expressed as mean ± standard deviation.

3. Results

The mean frequency of micronucleated reticulocytes in blood samples from unirradiated mice was 1.5 ± 0.9‰ (Figure 4). In contrast, irradiated mice exhibited a mean micronucleated reticulocyte frequency of 5.6 ± 1.6‰ in the 0.1 Gy group and 23.6 ± 6.4‰ in the 1 Gy group.

These findings are consistent with results from previous studies that used Giemsa staining to evaluate micronuclei frequency in the blood of X-irradiated mice [8, 9]. Statistically significant differences were observed between the unirradiated group and the 0.1 Gy group ($p=0.0021$), as well as between the unirradiated group and the 1 Gy group ($p=0.0013$).

4. Discussion

The protocol presented herein is part of an ongoing research project funded by INAIL, the Italian National Institute for Insurance against Accidents at Work (grant number BRIC 2022 ID 54), aimed at facilitating the application of the micronucleus test within the field of occupational safety and preventive measures. During the initial validation phase, blood samples from X-irradiated mice served as positive controls to establish the sensitivity of the method. Both our findings and those of previous investigations [9] confirm that the micronucleus test

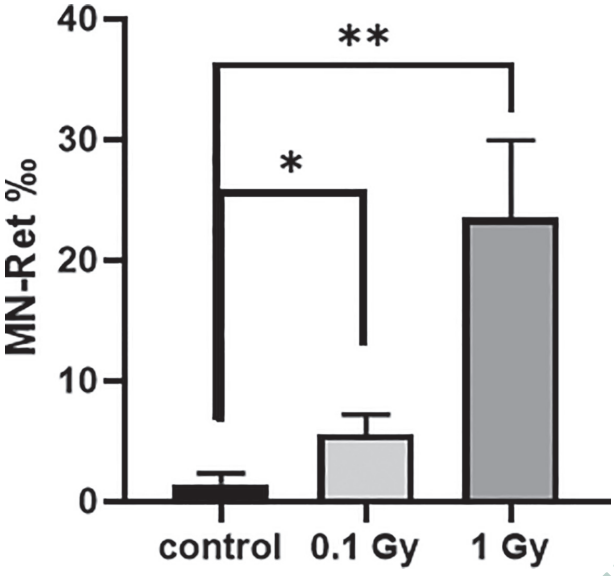

Figure 4. Frequencies of micronucleated reticulocytes (MN-Ret) in peripheral blood from unexposed (control), 0.1 Gy and 1 Gy X-irradiated mice. *: p=0.0021; **: p=0.0013.

on reticulocytes can detect genotoxic damage at low X-ray doses (0.1 Gy). Consequently, this assay is applicable in occupational safety contexts to identify exposure to various genotoxic agents beyond X-ray radiation, especially when damage levels are anticipated to be low or moderate. Although capable of detecting higher levels of genotoxicity typically associated with acute exposures, such as post-accident scenarios requiring immediate intervention rather than prevention, the test's primary utility lies in low-level exposure assessment.

The subsequent phase involves applying the test to blood samples from patients treated with iodine-131. Similar to the current study, a positive control group exposed to a known genotoxic agent will be included; however, this group will comprise human subjects. Based on our preliminary results and the extensive, decades-long application of the micronucleus assay in various contexts, we are confident that this protocol will prove to be both feasible and effective.

This methodology has the potential to be integrated into routine occupational health surveillance protocols. Although various other genotoxicity assays exist—possessing comparable sensitivity—their invasiveness, resource requirements, and need for specialized expertise render them less suitable for workplace monitoring.

Micronuclei frequency data from the worker cohort should be compared with those from a control group matched for gender, age, and habits. Alternatively, a database of unexposed individuals could be established by study personnel for use as a reference in future investigations, or comparable data could be sourced from the existing literature. A provisional threshold of >2‰ micronuclei (equivalent to three micronuclei per 1500 reticulocytes) is suggested as an indicator of concern—based on prior studies employing flow cytometry, which reported frequencies of 1.2±0.5‰ [10], 0.9±0.6‰ [11], 0.4±0.1‰ [12], and 1.3±0.5‰ [13] in unexposed populations.

This protocol is characterized by its simplicity and cost-effectiveness, requiring minimal reagents and no cell culture, antibodies, or DNA probes. The approximate cost per sample is only a few cents. Additionally, the sampling and staining procedures are straightforward and do not necessitate highly trained personnel, nor are they time-intensive. The primary time investment is in the scoring process, which typically takes 2 to 3 hours per sample. Overall, this timing is shorter than that required for other genotoxicity assays.

The ongoing development of deep neural network-based software aims to streamline the analysis of reticulocytes and micronucleated reticulocytes. Although the necessary automated microscopy equipment may be cost-prohibitive for individual facilities, this challenge can be mitigated by establishing national or regional centers. Such a centralized approach would allow users to leverage automated analysis via smear transmission, ensuring that high-standard diagnostics remain accessible and economically sustainable.

5. Conclusion

Some techniques and ideas used in this protocol are not new: Acridine Orange has been used to perform the micronucleus test on rodent samples [14], and antibody-stained human reticulocytes have been used to perform the flow cytometry micronucleus assay [10-13]. What is new is the entire procedure, combining an easy staining method with non-invasive sampling of human blood. Its minimal

invasiveness (requiring only one drop of blood per individual), ease of execution, very low cost, and potential for integration with dedicated automated software will also enable the test to be conducted by personnel responsible for the health surveillance of professionally exposed workers.

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Declaration of Interest: The authors declare no conflict of interest.

Author Contribution Statement: I.U. contributed to the design and implementation of the research, A.S., C.G. and I.U. contributed to the writing of the manuscript.

References

1. Choy WN. Genetic Toxicology and Cancer Risk Assessment. CRC Press: Boca Raton, USA, 2001.
2. Albertini RJ, Kaden DA. Mutagenicity monitoring in humans: global versus specific origin of mutations. *Mut Res Rev*. 2020;786:108341. Doi: 10.1016/j.mrrev.2020.108341
3. Ladeira C, Smajdova L. The use of genotoxicity biomarkers in molecular epidemiology: applications in environmental, occupational, and dietary studies. *AIMS Genetics*. 2017;4:166-191. Doi: 10.3934/genet.2017.3.166
4. OECD Guideline for the Testing of Chemicals: Mammalian Erythrocyte Micronucleus Test, Guideline 474. OECD. Paris, France, 1997.
5. Udroiu I. Feasibility of conducting the micronucleus test in circulating erythrocytes from different mammalian species: an anatomical perspective. *Environ Mol Mutagen*. 2006;47(9):643-646. Doi:10.1002/em.20258
6. Keohane E, Smith L, Walenga J. Rodak's Hematology: Clinical Principles and Applications, 5th edition. Elsivier: Amsterdam, Netherlands, 2016.
7. Udroiu I, Antoccia A, Sgura A. Long-term genotoxic effects in the hematopoietic system of prenatally X-irradiated mice. *Int J Radiat Biol.* 2017;93:261-269. Doi: 10.1080/09553002.2017.1239137
8. Chaubey RC, Bhilwade HN, Joshi BN, Chauhan PS. Studies on the migration of micronucleated erythrocytes from bone marrow to the peripheral blood in irradiated Swiss mice. *Int J Radiat Biol.* 1993;63:239-245. Doi: 10.1080/09553009314550311
9. Lenarczyk M, Slowikowska MG. The micronucleus assay using peripheral blood reticulocytes from X-ray-exposed mice. *Mutat Res Genet Toxicol Environ Mutagen*. 1995;335:229-234. Doi: 10.1016/0165-1161(95)00025-9
10. Abramsson-Zetterberg L, Zetterberg G, Bergqvist M, Grawé J. Human cytogenetic biomonitoring using flow-cytometric analysis of micronuclei in transferrin-positive immature peripheral blood reticulocytes. *Environ Mol Mutagen*. 2000;36:22-31. Doi: 10.1002/1098-2280(2000)36:1<22::AID-EM4>3.0.CO;2-U
11. Dertinger SD, Torous DK, Hall NE, et al. Enumeration of micronucleated CD71-positive human reticulocytes with a single-laser flow cytometer. *Mutat Res.* 2002;515:3-14. Doi: 10.1016/s1383-5718(02)00009-8
12. Offer T, Ho E, Traber MG, Bruno RS, Kuypers FA, Ames BN. A simple assay for frequency of chromosome breaks and loss (micronuclei) by flow cytometry of human reticulocytes. *FASEB J.* 2005;19:485-487. Doi: 10.1096/fj.04-2729fje
13. Stopper H, Hempel K, Reiners C, et al. Pilot study for comparison of reticulocyte-micronulei with lymphocyte-micronuclei in human biomonitoring. *Toxicol Lett.* 2005; 156: 351-60. Doi: 10.1016/j.toxlet.2004.12.007
14. Tinwell H, Ashby J. Comparison of acridine orange and Giemsa stains in several mouse bone marrow micronucleus assays--including a triple dose study. *Mutagenesis*. 1989;4:476-481. Doi: 10.1093/mutage/4.6.476