Arh. farm. 2024; 74: 556 – 568 *Review article/Pregledni rad*

The basic principles of DNA damage detection by the alkaline comet assay

Lada Živković[1], Dijana Topalović[1, *], Ninoslav Đelić[2], Petar Popović[1], Milica Marković[1], Ilija Gunjić[1], Biljana Spremo-Potparević[1]

[1]University of Belgrade – Faculty of Pharmacy, Department of Pathobiology, Vojvode Stepe 450, 11221 Belgrade, Serbia

[2]University of Belgrade – Faculty of Veterinary Medicine, Department of Biology, Bulevar Oslobođenja 18, 11000 Belgrade, Serbia

*Corresponding author: Dijana Topalović, e-mail: dijana.topalovic@pharmacy.bg.ac.rs

Received: 17 April 2024; Revised in revised forme: 31 May 2024; Accepted: 5 June 2024

Abstract

The Comet assay is a highly sensitive and rapid method for detecting DNA damage at the level of individual cells. It measures single-stranded and double-stranded DNA breaks, alkali-labile, incomplete excision repair and cross-linking sites. A major advantage of the Comet assay is its applicability to both proliferating and non-proliferating cells, and it can be performed on all types of eukaryotic cells. The basic principle of the alkaline Comet assay is to lyse the biological membranes to release proteins bound to the DNA, followed by a short-term electrophoresis at a pH above 13. Through this process, the DNA fragments migrate and form a comet-like shape, which is visualized by staining with a DNA-binding fluorescent dye. The extent of electrophoretic migration is proportional to the amount of DNA damage. Key advantages of the Comet assay include sensitivity - the assay can detect low levels of DNA damage, versatility – it can be used with a small amount of test substance, speed – results are usually available within 24 to 48 hours, and broad applicability – the method is suitable for numerous purposes, including molecular epidemiology, occupational exposure studies, environmental biomonitoring, antigenotoxicity assessments and clinical research. Overall, the Comet assay provides valuable data for a range of scientific and clinical fields, making it a versatile and powerful tool for assessing DNA damage.

Key words: DNA damage, comet assay, eukaryotic cells

---

https://doi.org/10.5937/arhfarm74-50506

Introduction

Since DNA is a storehouse of genetic information, the integrity and stability of DNA molecules in all cells of an organism are fundamental to life. Every day, numerous lesions in the genome occur in every cell of an organism, caused by natural or artificial exogenous genotoxic agents, endogenously generated reactive oxygen species, or defective replication. Most of the DNA damage gets repaired, but even if it is not repaired, it does not necessarily lead to mutations. Mutations in genetic material can lead to various diseases, including cancer. Consequently, DNA damage could be considered a useful biomarker for its role in these processes. However, in the absence of compelling evidence, the individual risk of cancer depending on the extent of DNA damage is controversial (1, 2, 3).

"DNA damage" refers to a change in the chemical structure of DNA in the form of single and double strand breaks, removal of bases from the DNA backbone or chemically altered bases (4, 5). Various methods can be used to assess DNA damage or the effects on its repair, such as the Ames test (6), alkaline elution (7), chromosomal aberrations (8), sister chromatid exchanges (SCE) (9), the cytokinesis block micronucleus assay (CBMN) (10, 11, 12), and the γ-H2AX test (13, 14).

In addition, the Comet assay has become the most popular method for measuring DNA damage and repair in cells and tissues of eukaryotic organisms. It was introduced by Ostling and Johanson (15) and modified to an alkaline version by Singh et al. (16). The name "comet assay" is derived from the specific shape of the damaged cellular DNA after electrophoresis, which allows visualization of the fragmented DNA. It measures the relaxed supercoiling of damaged DNA loops, which expand under alkaline conditions in electrophoresis and form comet-like figures (17). The relative intensity of fluorescent migrated DNA indicates the degree of DNA breaks, including single- and double-strand breaks and alkali-labile sites (18). By modifying the comet assay method, other DNA lesions such as oxidation of DNA bases or DNA cross-links can also be measured (19, 20, 21). The oxidized pyrimidines and purines could be evaluated by incorporating enzyme digestion of DNA with the following enzymes: endonuclease III (EndoIII), formamidopyrimidine DNA glycosylase (Fpg) and human 8-oxoguanine DNA glycosylase 1 (hOGG1) (19, 22). In addition, a modified high-throughput comet assay was introduced to assess DNA methylation status in a human cell suspension to detect aberrant hypo- and hypermethylation. The use of specific restriction endonucleases (HpaII and MspI) enables the assessment of aberrant methylation, which is highly associated with carcinogenesis (23, 24, 25, 26). In addition, the comet FISH method is an extension of the comet assay by fluorescence *in situ* hybridization (FISH), which improves the detection of specifically labeled DNA sequences of interest. The position of a particular DNA sequence in fragmented or non-fragmented DNA can be important for the development of various human diseases (27, 28, 29).

The present review addresses strategies that utilize the comet assay *in vitro* and *in vivo,* as well as *in situ,* to provide reliable results for various studies.

In situ, *in vivo*, and *in vitro* comet test

The fundamental challenge of *in situ* biomonitoring is to establish a clear relationship between the analytical data, the exposure dose and the biomarker response. Biomarkers can be considered to be any substances, structures or processes that can be objectively quantified and evaluated as a possible indication of a normal or abnormal pathophysiological process or response to therapy (30, 31).

In environmental risk assessment, the evaluation of the genotoxic potential of different xenobiotics plays an important role in determining the impact of pollutants in ecosystems (32). In a well-designed *in situ* biomonitoring study, the level of DNA damage as a valuable biomarker of environmental contamination could allow the assessment of genotoxic effects on the species being studied. This biomarker can establish quantitative correlations between the causes and effects of genotoxicity at different levels of biological organization, from tissues and organs of a single organism to a population or an ecosystem (33, 34, 35, 36). The use of wild species in *in situ* environmental biomonitoring is challenging because exposure duration and concentration of genotoxic substances are uncontrolled, making it difficult to establish a clear relationship between analytical data, exposure dose and biomarker response (37). The Comet assay in human biomonitoring studies requires a small sample size to estimate the effects of hazard and risk assessment (17). Therefore, the Comet assay performed *in vivo* and *in vitro* could be used as part of the regulatory biomonitoring test strategy.

Furthermore, the *in vitro* comet assay has been used in different human and animal cell lines to determine the genotoxic effects of different xenobiotics. A small amount of the test substance is required for the *in vitro* Comet assay. If the result of the *in vitro* study is positive, the *in vivo* comet assay is recommended as an accompanying test strategy (38, 39, 40).

The *in vivo* comet assay is performed to determine DNA damage in cells isolated from different tissues that have been exposed to a genotoxic substance or several substances at a certain concentration and duration of exposure (41). The comet assay is a useful tool for evaluating the genotoxic properties of test substances in a given organ, as the assay can be performed on cells from any tissue, both proliferating and non-proliferating (42, 43). For the assessment of genotoxic hazard, the *in vivo* comet assay is the most important tool to evaluate the cellular responses that depend on *in vivo* ADME (absorption, distribution, metabolism and excretion). In addition, the *in vivo* ADME may differ depending on the species, tissue and form of DNA damage (44).

Overall, various guidelines for biomonitoring studies and *in vitro* and *in vivo* Comet assays in genotoxicology are the result of international working groups (45, 46, 47, 48) and OECD guidelines.

In general, the Comet assay has been used for the assessment of genome damage in humans and in various animal models worldwide, including invertebrate species, which are recognized as valuable models due to their number and importance in

ecosystems. Most invertebrate studies have been performed on planarians, cnidarians, molluscs, annelids, arthropods and/or echinoderms, using a variety of cell types such as hemolymph, gills, digestive glands and embryo cells. Moreover, vertebrates, including cyclostomata, fish, amphibians, reptiles, birds and mammals are models where the comet assay is also commonly used. The comet assay is performed on different cell types such as blood, liver, kidney, brain, gill, bone marrow and sperm cells. The most commonly used vertebrate models are laboratory animals such as mice, rats and zebrafish, which are suitable for *in vivo* studies (49, 50).

Cell lines from Chinese hamster ovary/lung cells (CHO/CHL, V79), human breast cancer cells (MCF7), human lymphoblasts (TK6), mouse lymphoma cells (L5178Y) or cultured human peripheral lymphocytes are frequently used for *in vitro* comet assay studies. Since the S9 fraction renders the cells metabolically competent, the *in vitro* tests should also include the comparison of the genotoxic effects of the tested substances with and without the S9 fraction. It should be noted that human lymphocytes, TK6 and HepG2, are metabolically competent and can be used for testing without external metabolic activation (43). In addition, the earliest life stage of the zebrafish (embryo) is considered as an *in vitro* test model for the evaluation of xenobiotics, supporting the idea of 3R (replace, reduce and refine) in animal testing (51).

The methodology of the alkaline Comet assay

When using the comet assay to evaluate a wide range of genotoxic physical, chemical and/or biological agents, both *in vitro* and *in vivo*, including *in situ* studies, a cell suspension (or nuclei) must be used. The interaction of genotoxic substances with DNA can cause DNA damage, which can lead to DNA breaks.

A small number of viable cells per sample from a desired source are suspended in a low melting point agarose (LMPA), applied to a slide previously coated with normal melting point agarose (NMPA), and allowed to solidify in the cold. The slides are then immersed in a cooled lysis solution. The lysis step (pH 10) in the comet assay protocol enables the removal of cell membranes, histone proteins and nucleosome disruption, so that the DNA loops pulled out during electrophoresis are no longer damaged. Otherwise, the introduction of a higher pH value (>13) in the lysis step leads to denaturation of the DNA (with disruption of the hydrogen bonds between the double-stranded DNA) and the appearance of alkali-labile sites as DNA breaks. The negatively charged DNA migrates to the anode and enables the differentiation of the DNA fragments in the electrophoresis field, which form a special "comet" with a head (intact DNA) and a tail (damaged DNA). The extent of electrophoretic migration is proportional to the extent of DNA damage – the more DNA in the comet tails, the more DNA damage. For each experimental condition, the procedure is performed in duplicate to obtain meaningful results.

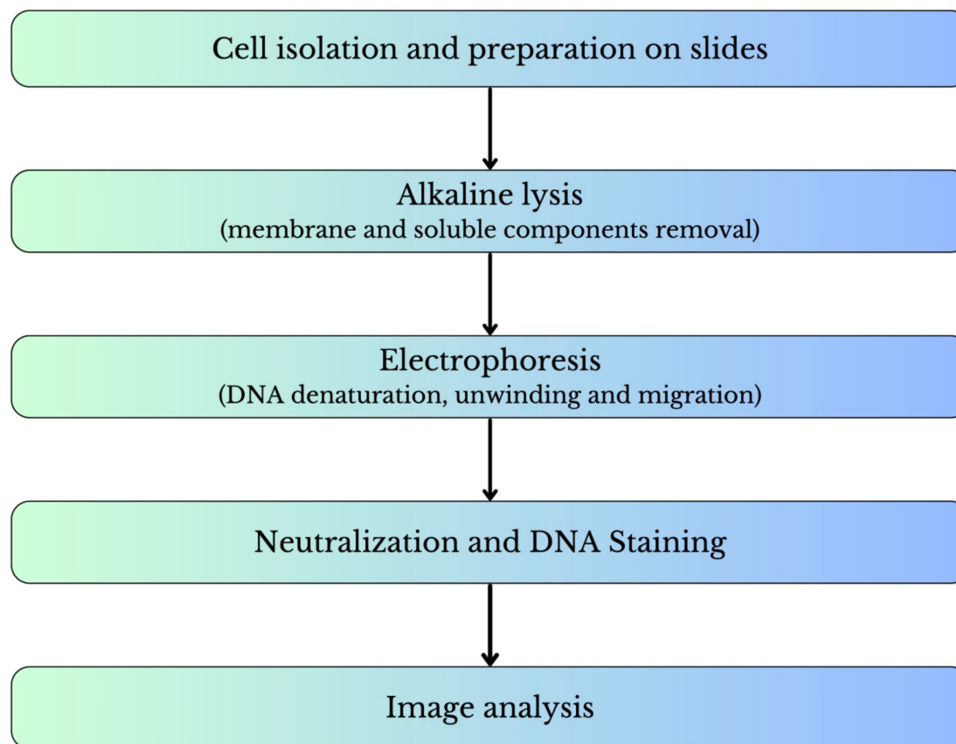

Figure 1. Scheme of the alkaline Comet assay procedure
Slika 1. Šema procedure alkalnog komet testa

Alkaline Comet assay step by step

1. Preparations for solutions and components for the Comet assay:
   a. Normal melting point agarose: 1% NMPA.
   b. Low melting point agarose: 0.5% - 1% LMPA.
   c. Stock Lysing solution: 2.5 M NaCl, 100 mM disodium ethylenediaminetetraacetic acid (EDTA disodium salt), 10 mM Trizma base. The pH value is adjusted to 10 with NaOH and the solution is stored at room temperature.
   d. Final lysing solution: stock Lysing solution, 1% Triton X-100, 10% dimethyl sulfoxide (DMSO). Refrigerated at 4°C for one hour before use.
   e. Electrophoresis buffer: Stock solutions: 10 M NaOH, stored at 4°C; 200 mM EDTA, pH to 10 with NaOH pellets, also stored at 4°C.
   f. Working electrophoresis buffer: 300 mM NaOH/1 mM EDTA from the stock solutions, mixed well and the final volume adjusted with chilled dH2O.
   g. Neutralization buffer: 0.4 M Tris base, pH 7.5 adjusted with concentrated HCl, stored at 4°C.

h. Staining solution: 2 μg/mL ethidium bromide (EtBr), stored at room temperature.

2. Slide stock preparation: Slides should be precoated with 1% NMPA and air dried a few days before performing the comet assay. If the slides need to be prepared one day before the experiments, the slides precoated with 1% NMPA should be dried faster at 37°C overnight.
3. Slide preparation: LMPA must be melted at 37°C. The desired cell suspension is mixed with LMPA at a final concentration of 0.5%-1% and placed on a previously coated slide. A coverslip is placed on top to spread the mixture of agarose and cells and form a thin layer. The slide is laid flat at 4°C until the agarose layer has solidified.
4. Lysis: The coverslip is removed from the slides and the slides are placed in fresh, cold final lysis solution for at least one hour to overnight, or in the refrigerator. Protection from light is required during this step.
5. DNA unwinding: The slides are transferred to a horizontal electrophoresis apparatus and left in freshly prepared and cold 1× electrophoresis buffer (pH > 13) for 20-30 minutes to unwind the DNA and allow the detection of alkali-labile damage.
6. Electrophoresis: The voltage (~0.74 V/cm) and the current of 300 mA are set by increasing or decreasing the buffer level. Electrophoresis duration is 20-40 minutes, depending on the extent of migration in control samples.
7. Neutralization: After electrophoresis is complete, the neutralization buffer (pH 7.5) is added and left for 5 minutes, two or more times.
8. Schedule for comets evaluation: After neutralization, the slides can be stained and the comets evaluated. In addition, the gel can be dehydrated in methanol and the slides can be stored so that the comets can be evaluated when needed.
9. Comets staining: The most commonly used DNA-specific fluorescent dyes are ethidium bromide (EtBr), propidium iodide, 4,6-diamide- ino-2-phenylindole (DAPI), SYBR Green and YOYO-1. For the most commonly used dye (EtBr), slides are stained with 2 μg/mL for 15 minutes. The excess staining must be removed by washing with distilled water, after which the coverslip must be mounted.
10. Visualization of DNA damage: A fluorescent microscope with a final magnification of 400× is used to assess DNA damage. At least 50 random comets per slide are evaluated. DNA migration can be determined visually by categorizing the comets into different migration classes or by using image processing software for quantitative and qualitative analysis of DNA damage (olive tail moment (OTM), tail DNA (%) and tail length (TL)).

11. Statistical analyses: Parametric tests, such as Student's t-tests and ANOVA, as well as non-parametric alternatives, such as Mann-Whitney, Wilcoxon and Kruskal-Wallis tests, are suitable for data analysis (52). For the evaluation of dose-dependent effects, linear trends can be used to obtain higher statistical power (53).

Application of the Comet assay

A single cell suspension obtained from different tissues/organs is required for the application of the Comet assay. It is mainly used with human cells, but can also be used with yeasts (54, 55), plants (56, 57, 58) and animals (42, 59, 60, 61). Moreover, proliferating cells are not required for the application of the Comet assay, and non-proliferating cells are even preferred (62). Accordingly, the Comet assay has been used extensively for studies in different fields. This technique plays an important role in the field of genotoxicology and ecotoxicology, in the evaluation of environmental pollution and occupational exposure. The Comet assay is also used in human epidemiology and biomonitoring (63, 64). In addition, this method is used to investigate the antigenotoxic, antimutagenic and/or anticarcinogenic effects of different natural and artificial substances. Furthermore, this method is essential for the assessment of genome damage in human and animal models under different physiological and pathological conditions (49, 50, 65). The comet assay could be considered as a useful tool in the field of clinical medicine. For the clinical application of the comet assay, it is necessary to assess the extent of DNA damage and repair mechanisms in various diseases and conditions. Elevated levels of DNA damage have been found in a number of diseases such as cardiovascular diseases, neurodegenerative diseases (Alzheimer's and Parkinson's), diabetes, kidney diseases, chronic obstructive pulmonary diseases, multiple sclerosis and cancers (65-71). In terms of comet assay applications in drug testing, it has previously been used to test the genotoxicity of therapeutics such as Melphalan, Mechlorethamines (alkylating agents), doxorubicin (in breast cancer), and cisplatin (in colon cancer cell lines) (72), confirming its use as a valuable tool in pharmacology.

Conclusion

Considering the number of publications using the Comet assay method *in situ*, *in vivo* and *in vitro* in both animals and humans, as well as the worldwide interest in this method, it is clear that the Comet assay will be of great value in the future. Although the Comet assay is a long-established technique, there is a need to reduce inter-laboratory variability and mitigate the variation in the individual steps through the development of reproducible protocols. Therefore, the international network must continue to collaborate in the future to provide more reliable results and eliminate the differences in the common steps and interpretation of the Comet assay results.

Acknowledgements

This research was funded by the Ministry of Science, Technological Development and Innovation, Republic of Serbia through Grant Agreement with the University of Belgrade – Faculty of Pharmacy No. 451-03-65/2024-03/ 200161 and Grant Agreement with the University of Belgrade – Faculty of Veterinary Medicine No. 451-03-66/2024-03/200143.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

Conceptualization, Živković L, Topalović D; Data curation; Gunjić I, Marković M; Resources; Marković M; Supervision, Spremo-Potparević B; Validation, Đelić N; Visualization; Gunjić I; Roles/Writing - original draft, Živković L, Topalović D, Popović P; and Writing - review & editing, Đelić N, Spremo-Potparević B.

References

1. Chatterjee N, Walker GC. Mechanisms of DNA damage, repair, and mutagenesis. Environ Mol Mutagen. 2017;58(5):235-263.
2. Cheong A, Nagel ZD. Human Variation in DNA Repair, Immune Function, and Cancer Risk. Front Immunol. 2022;13:899574.
3. Talseth-Palmer BA, Scott RJ. Genetic variation and its role in malignancy. Int J Biomed Sci. 2011;7(3):158-171.
4. Helleday T, Eshtad S, Nik-Zainal S. Mechanisms underlying mutational signatures in human cancers. Nat Rev Genet. 2014;15:585-598.
5. Chatterjee N, Walker GC. Mechanisms of DNA damage, repair, and mutagenesis. Environ Mol Mutagen. 2017;58(5):235–263.
6. Vijay U, Gupta S, Mathur P, Suravajhala P, Bhatnagar P. Microbial Mutagenicity Assay: Ames Test. Bio Protoc. 2018;8(6):e2763.
7. Koch CJ, Giandomenico AR. The alkaline elution technique for measuring DNA single strand breaks: increased reliability and sensitivity. Anal Biochem. 1994;220(1):58-65.
8. Registre M, Proudlock R. Chapter 7 - The In Vitro Chromosome Aberration Test. In: Proudlock R, editor. Genetic Toxicology Testing. Academic Press; 2016; p. 207-267.
9. Tumini E, Aguilera A. The Sister-Chromatid Exchange Assay in Human Cells. Methods Mol Biol. 2021;2153:383-393.

10. Sioen S, Cloet K, Vral A, Baeyens A. The Cytokinesis-Block Micronucleus Assay on Human Isolated Fresh and Cryopreserved Peripheral Blood Mononuclear Cells. J Pers Med. 2020;10(3):125.
11. Fenech M, Kirsch-Volders M, Natarajan AT, Surralles J, Crott JW, Parry J, et al. Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. Mutagenesis. 2011;26:125-132.
12. Kopjar N, Kašuba V, Milić M, Rozgaj R, Želježić D, Gajski G, et al. Normal and cut-off values of the cytokinesis-block micronucleus assay on peripheral blood lymphocytes in the Croatian general population. Arh Hig Rada Toksikol. 2010;61:219-234.
13. Valdiglesias V, Giunta S, Fenech M, Neri M, Bonassi S. γ-H2AX as a marker of DNA double strand breaks and genomic instability in human population studies. Mutat Res. 2013;753:24-40.
14. Gerić M, Gajski G, Garaj-Vrhovac V. γ-H2AX as a biomarker for DNA double-strand breaks in ecotoxicology. Ecotoxicol Environ Saf. 2014;105:13-21.
15. Ostling O, Johanson KJ. Microelectrophoretic study of radiation - induced DNA damage in individual mammalian cells. Biochem Biophys Res Commun. 1984;123:291-298.
16. Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res. 1988;175:184-191.
17. Azqueta A, Ladeira C, Giovannelli L, Boutet-Robinet E, Bonassi S, Neri M, et al. Application of the comet assay in human biomonitoring: An hCOMET perspective. Mutat Res. 2020;783:108288.
18. Costa S, Teixeira JP. Comet Assay. In: Wexler P, editor. Encyclopedia of Toxicology (Third Edition). Academic Press; 2014; p. 1020-1023.
19. Collins AR. Measuring oxidative damage to DNA and its repair with the comet assay. Biochim Biophys Acta. 2014;1840:794-800.
20. Collins AR. Investigating oxidative DNA damage and its repair using the comet assay. Mutat Res. 2009;681:24-32.
21. Wu JH, Jones NJ. Assessment of DNA interstrand crosslinks using the modified alkaline comet assay. Methods Mol Biol. 2012;817:165-181.
22. Dhawan A, Bajpayee M, Parmar. Comet assay: a reliable tool for the assessment of DNA damage in different models. Cell Biol Toxicol. 2009;25:5-32.
23. Townsend TA, Parrish MC, Engelward BP, Manjanatha MG. The development and validation of EpiComet-Chip, a modified high-throughput comet assay for the assessment of DNA methylation status. Environ Mol Mutagen. 2017;58:508-521.
24. Lewies A, Van Dyk E, Wentzel JF, Pretorius PJ. Using a medium-throughput comet assay to evaluate the global DNA methylation status of single cells. Front Genet. 2014;5:215.
25. Ramos AA, Pedro DFN, Lima CF, Collins AR, Pereira-Wilson C. Development of a new application of the comet assay to assess levels of O6-methylguanine in genomic DNA (CoMeth). Free Radic Biol Med. 2013;60:41-48.
26. Wentzel JF, Gouws C, Huysamen C, van Dyk E, Koekemoer G, Pretorius PJ. Assessing the DNA methylation status of single cells with the comet assay. Anal Biochem. 2010;400:190-194.
27. Spivak G, Cox RA, Hanawalt PC. New applications of the Comet assay: Comet- FISH and transcription-coupled DNA repair. Mutat Res. 2009;681:44-50.
28. Glei M, Schloermann W. Analysis of DNA damage and repair by comet fluorescence in situ hybridization (Comet-FISH). Methods Mol Biol. 2014;1094:39-48.

29. McKenna DJ, Doherty BA, Downes CS, McKeown SR, McKelvey-Martin VJ. Use of the Comet-FISH Assay to Compare DNA Damage and Repair in p53 and hTERT Genes following ionizing radiation. PLOS ONE. 2012;7(11):e49364.
30. Ahmad A, Imran M, Ahsan H. Biomarkers as Biomedical Bioindicators: Approaches and Techniques for the Detection, Analysis, and Validation of Novel Biomarkers of Diseases. Pharmaceutics. 2023;15(6):1630.
31. Aryutova K, Stoyanov DS, Kandilarova S, Todeva-Radneva A, Kostianev SS. Clinical Use of Neurophysiological Biomarkers and Self-Assessment Scales to Predict and Monitor Treatment Response for Psychotic and Affective Disorders. Curr Pharm Des. 2021;27:4039–4048.
32. Plaza G, Nalecz-Jawecki G, Ulfig K, Brigmon RL. The application of bioassays as indicators of petroleum-contaminated soil remediation. Chemosphere. 2005;59(2):289-296.
33. Mouneyrac C, Amiard-Triquet C. Biomarkers of Ecological Relevance in Ecotoxicology. In: Ferard JF, Blaise C, editors. Encyclopedia of Aquatic Ecotoxicology. Amsterdam, Netherlands: Springer; 2013; p. 221–236.
34. Cant A, Bonnard M, Porcher JM, Prygiel J, Catteau A, Delahaut L, et al. Integration of Genotoxic Biomarkers in Environmental Biomonitoring Analysis Using a Multi-Biomarker Approach in Three-Spined Stickleback (Gasterosteus aculeatus Linnaeus, 1758). Toxics. 2022;10(3):101.
35. Bickham JW, Smolen MJ. Somatic and heritable effects of environmental genotoxins and the emergence of evolutionary toxicology. Environ Health Perspect. 1994;102(S12):25-28.
36. Lam P. Use of biomarkers in environmental monitoring. Ocean Coast Manag. 2009;52(7):348-354.
37. Barbee GC, Barich J, Duncan B, Bickham JW, Matson CW, Hintze CJ, et al. In situ biomonitoring of PAH-contaminated sediments using juvenile coho salmon (Oncorhynchus kisutch). Ecotoxicol Environ Saf. 2008;71(2):454-464.
38. COM (United Kingdom Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment). Guidance on a strategy for testing of chemicals for mutagenicity, 2000.
39. US Food and Drug Administration [Internet]. Guidance for industry; 2012 [cited 2024 Aug 3]. Available from: www.fda.gov/down- loads/Drugs/Guidances/ucm074931.pdf.
40. International Committee on Harmonization (ICH) guideline S2(R1) [Internet]. Guidance on genotoxicity testing and data interpretation for pharmaceuticals intended for human use; 2011 [cited 2024 Aug 3]. Available from: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S2_ R1/Step4/S2R1_Step4.pdf.
41. Rothfuss A, Honma M, Czich A, Aardema MJ, Burlinson B, Galloway S, et al. Improvement of in vivo genotoxicity assessment: combination of acute tests and integration into standard toxicity testing. Mutat Res. 2011;723:108-120.
42. Dhawan A, Bajpayee M, Parmar D. Comet assay: a reliable tool for the assessment of DNA damage in different models. Cell Biol Toxicol. 2009;25:5-32.
43. Bajpayee M, Kumar A, Dhawan A. The Comet Assay: Assessment of In Vitro and In Vivo DNA Damage. Methods Mol Biol. 2019;2031:237-257.
44. Ding W, Bishop ME, Lyn-Cook LE, Davis KJ, Mugimane G. Manjanatha MG. In Vivo Alkaline Comet Assay and Enzyme-modified Alkaline Comet Assay for Measuring DNA Strand Breaks and Oxidative DNA Damage in Rat Liver. J Vis Exp. 2016;111:53833.

45. Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, et al. Single cell gel/Comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environ Mol Mutagen. 2000;35:206-221.
46. OCDE, Test No. 489: In Vivo Mammalian Alkaline Comet Assay, OECD Guidelines for the Testing of Chemicals, Section 4. Paris: Editions OCDE, 2016.
47. Burlinson B, Tice RR, Speit G, Agurell E, Brendler-Schwaab SY, Collins AR, et al. In vivo comet assay workgroup, part of the fourth international workgroup on genotoxicity testing. Fourth International Workgroup on Genotoxicity testing: results of the in vivo Comet assay workgroup. Mutat Res. 2007;627:31-35.
48. Vasquez MZ. Recommendations for safety testing with the in vivo comet assay. Mutat Res. 2012;747:142-156.
49. Gajski G, Žegura B, Ladeira C, Pourrut B, Del Bo' C, Novak M, et al. The comet assay in animal models: From bugs to whales - (Part 1 Invertebrates). Mutat Res. 2019;779:82-113.
50. Gajski G, Žegura B, Ladeira C, Novak M, Sramkova M, Pourrut B, et al. The comet assay in animal models: From bugs to whales - (Part 2 Vertebrates). Mutat Res. 2019;781:130-164.
51. EU Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes; 2010 [Internet] [cited 2024 Aug 3]. Available from: http://data.europa.eu/eli/dir/2010/63/oj.
52. Armitage P, Berry G, Matthews JNS. Statistical Methods in Medical Research, 4th edition. UK: Blackwell Publishing Ltd; 2002.
53. Patel S, Pandey AK, Bajpayee M, Parmar D, Dhawan A. Cypermethrin-induced DNA damage in organs and tissues of the mouse: evidence from the comet assay. Mutat Res. 2006;607(2):176-183.
54. Oliveira R, Johansson B. Quantitative DNA damage and repair measurement with the yeast comet assay. Methods Mol Biol. 2012;920:101-109.
55. Azevedo F, Marques F, Fokt H, Oliveira R, Johansson B. Measuring oxidative DNA damage and DNA repair using the yeast comet assay. Yeast. 2011;28:55-61.
56. Santos CLV, Pourrut B, Ferreira de Oliveira JMP. The use of comet assay in plant toxicology: recent advances. Front Genet. 2015;6:216.
57. Lanier C, Manier N, Cuny D, Deram A. The comet assay in higher terrestrial plant model: review and evolutionary trends. Environ Pollut. 2015;207:6-20.
58. Ventura L, Giovannini A, Savio M, Donà M, Macovei A, Buttafava A, et al. Single Cell Gel Electrophoresis (Comet) assay with plants: research on DNA repair and ecogenotoxicity testing. Chemosphere. 2013;92:1-9.
59. de Lapuente J, Lourenço J, Mendo SA, Borràs M, Martins MG, Costa PM, Pacheco M. The Comet Assay and its applications in the field of ecotoxicology: a mature tool that continues to expand its perspectives. Front Genet. 2015;6:180.
60. Guidi P, Lyons BP, Frenzilli G. The Comet Assay in Marine Animals. Methods Mol Biol. 2019;2031:275-286.
61. Augustyniak M, Gladysz M, Dziewięcka M. The Comet assay in insects-status, prospects and benefits for science. Mutat Res Rev. 2016;767:67-76.
62. Albertini RJ, Anderson D, Douglas GR, Hagmar L, Hemminki K, Merlo F, et al. IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. Mutat Res. 2000;463:111-172.

63. Ladeira C, Smajdova L. The use of genotoxicity biomarkers in molecular epidemiology: applications in environmental, occupational and dietary studies. AIMS Genet. 2017;4(3):166-191.
64. Gunasekarana V, Gladwin VR, Parkash C. Comprehensive Review on Clinical Applications of Comet Assay. J Clin Diagn Res. 2015;9(3):GE01–GE05.
65. Kuchařová M, Hronek M, Rybáková K, Zadák Z, Štětina R, Josková V, Patková A. Comet assay and its use for evaluating oxidative DNA damage in some pathological states. Physiol Res. 2019;68(1):1-15.
66. Živković L, Spremo-Potparević B, Siedlak SL, Perry G, Plećaš-Solarović B, Milićević Z, Bajić VP. DNA Damage in Alzheimer Disease Lymphocytes and Its Relation to Premature Centromere Division. Neurodegener Dis. 2013;12(3):156-63.
67. Milić M, Frustaci A, Del Bufalo A, Sánchez-Alarcón J, Valencia-Quintana R, Russo P, Bonassi S. DNA damage in non-communicable diseases: A clinical and epidemiological perspective. Mutat Res. 2015;776:118-27.
68. Møller P, Stopper H, Collins AR. Measurement of DNA damage with the comet assay in high-prevalence diseases: current status and future directions. Mutagenesis. 2020;35(1):5-18.
69. Djelić N, Radaković M, Borozan S, Dimirijević-Srećković V, Pajović N, Vejnović B, et al. Oxidative Stress and DNA Damage in Peripheral Blood Mononuclear Cells From Normal, Obese, Prediabetic and Diabetic Persons Exposed to Adrenaline in Vitro. Mutat Res. 2019;843:81-89.
70. Borisovs V, Ļeonova E, Baumane L, Kalniņa J, Mjagkova N, Sjakste N. Blood Levels of Nitric Oxide and DNA Breaks Assayed in Whole Blood and Isolated Peripheral Blood Mononucleated Cells in Patients With Multiple Sclerosis. Mutat Res. 2019;843:90-94.
71. Russo P, Lamonaca P, Milić M, Rojas E, Prinzi G, Cardaci V, et al. Biomarkers of DNA damage in COPD patients undergoing pulmonary rehabilitation: Integrating clinical parameters with genomic profiling. Mutat Res. 2019; 843: 111-117.
72. Yadam S. The Comet Assay: A Straight Way to Estimate Geno-Toxicity. 21st Century Pathology. 2023;3(2):145.

Osnovni principi otkrivanja oštećenja DNK pomoću alkalnog komet testa

Lada Živković[1], Dijana Topalović[1,*], Ninoslav Đelić[2], Petar Popović[1], Milica Marković[1], Ilija Gunjić[1], Biljana Spremo-Potparević[1]

[1]Univerzitet u Beogradu – Farmaceutski fakultet, Katedra za patobiologiju, Vojvode Stepe 450, 11221 Beograd, Srbija

[2]Univerzitet u Beogradu – Fakultet veterinarske medicine, Katedra za biologiju, Bulevar Oslobođenja 18, 11000 Beograd, Srbija

*Autor za korespondenciju: Dijana Topalović, e-mail: dijana.topalovic@pharmacy.bg.ac.rs

Kratak sadržaj

Komet test je veoma osetljiva i brza metoda za otkrivanje oštećenja DNK na nivou pojedinačnih ćelija. Meri jednolančane i dvolančane DNK prekide, alkalno labilna mesta, mesta nepotpune ekscizione reparacije i mesta unakrsnog povezivanja. Značajna prednost komet testa je njegova primenjivost na proliferišuće i neproliferišuće ćelije, a može se primeniti na gotovo bilo kom tipu eukariotskih ćelija. Osnovni princip alkalnog komet testa uključuje ugradnju ćelija u agarozni gel praćen lizom ćelijskih membrana i uklanjanjem proteina kako bi se oslobodila ukupna DNK. Zatim se denaturiše DNK i podvrgava elektroforezi, nakon čega se vrši neutralizacija. Ćelije se boje različitim fluorescentnim bojama kako bi se obezbedila vizuelizacija i procena intenziteta nivoa DNK oštećenja koriščenjem fluorescentnog mikroskopa. Stepen elektroforetske migracije DNK vizuelizovane u vidu repa komete je srazmeran količini oštećenja DNK. Ključne prednosti komet testa su osetljivost – test može da otkrije niske nivoe DNK oštećenja, raznovrsnost – može se koristiti sa malom količinom test supstance i na različitim tipovima eukariotskih ćelija, brzina – rezultati su obično dostupni u roku od 24 do 48 sati i široka primenjivost – metoda je pogodna za više namena, uključujući molekularnu epidemiologiju, studije profesionalne izloženosti, biomonitoring životne sredine, procene antigenotoksičnosti i klinička istraživanja. Sve u svemu, komet test pruža vredne podatke u širokom spektru naučnih i kliničkih oblasti, što ga čini moćnim alatom za procenu DNK oštećenja.

Ključne reči: DNK oštećenja, komet test, eukariotske ćelije