



ULOGA KOMET I MIKRONUKLEUS TESTA U GENETIČKOJ TOKSIKOLOGIJI I BIOMONITORINGU POPULACIJA

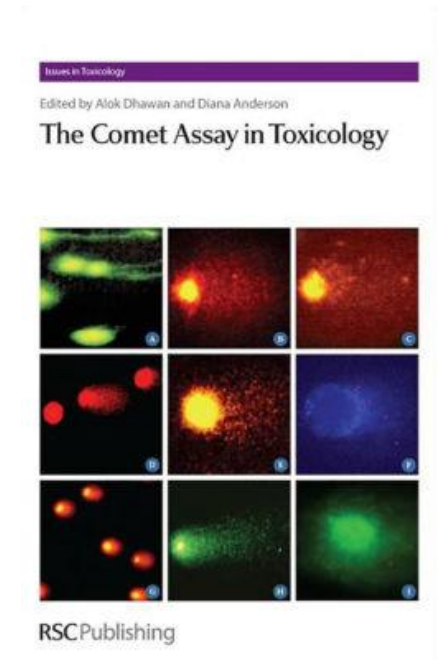
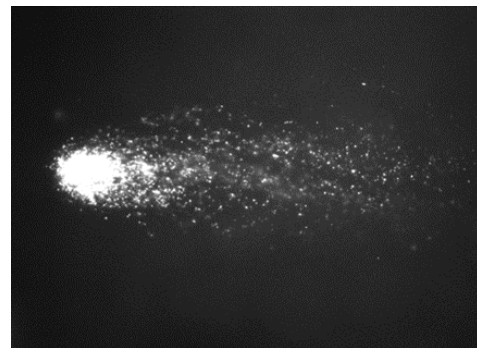
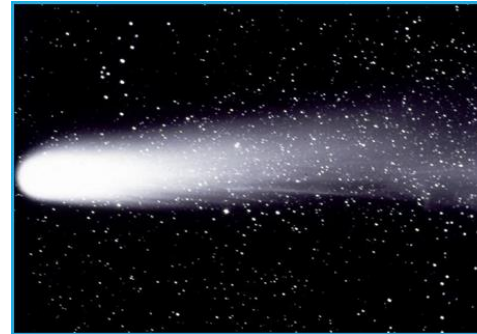
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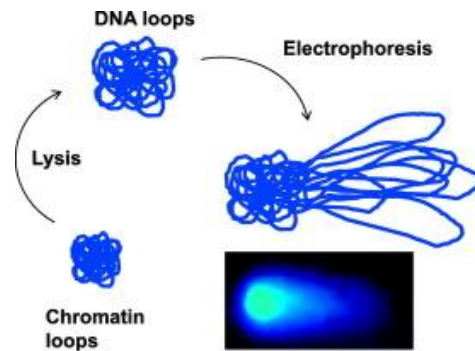
KOMET TEST - UVOD

- mikrogel elektroforeza pojedinačnih stanica
- tehnika za procjenu oštećenja i popravka DNA
- Östling i Johanson 1984. godine (neutralni komet)
- Singh 1988. godine (alkalni komet)



KOMET TEST - PROTOKOL

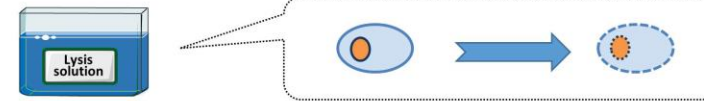
- uklapanje u troslojni agarozni gel
- liza stanica (membrana i citoplazma)
- denaturacija DNA
- elektroforeza
- neutralizacija
- bojanje
- analiza



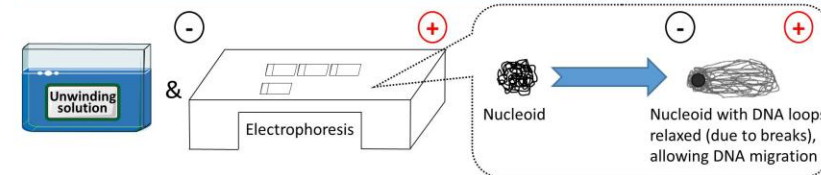
Step 1 : From blood or other isolated cells to cells embedded in gel and deposit on slides



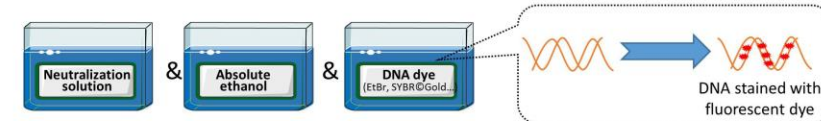
Step 2 : Alkaline lysis (membranes and soluble components removal)



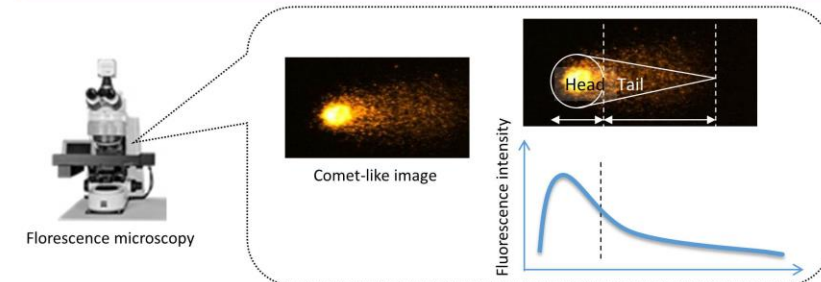
Step 3 : Electrophoresis (DNA denaturation, unwinding and migration)



Step 4 : Neutralization, Fixation, DNA staining



Step 5 : Image analysis



KOMET TEST - PROCJENA OŠTEĆENJA

- oštećenje se procjenjuje na osnovi udjela DNA u “repu” kometa i udjela DNA u “glavi” kometa
- **mjerenja se vrše:**
 - okometrijski
 - pomoću sustava za analizu slike u kojima je epifluorescencijski mikroskop povezan s računalom
 - pomoću računalnog programa za svaki pojedinačni komet istovremeno se mjeri više deskriptora » statistička obrada podataka
- **deskriptori komet testa:**
 - **dužina repa (μm)** = predstavlja udaljenost na koju su tijekom elektroforeze odputovali fragmenti DNA (proporcionalna je oštećenju DNA)
 - **% DNA u repu** = mjeri se pomoću sustava za analizu slike te ukazuje na količinu oštećene DNA
 - **repni moment** = izračunava se prema formuli: $\text{dužina repa} \times \% \text{ DNA u repu} / 100$

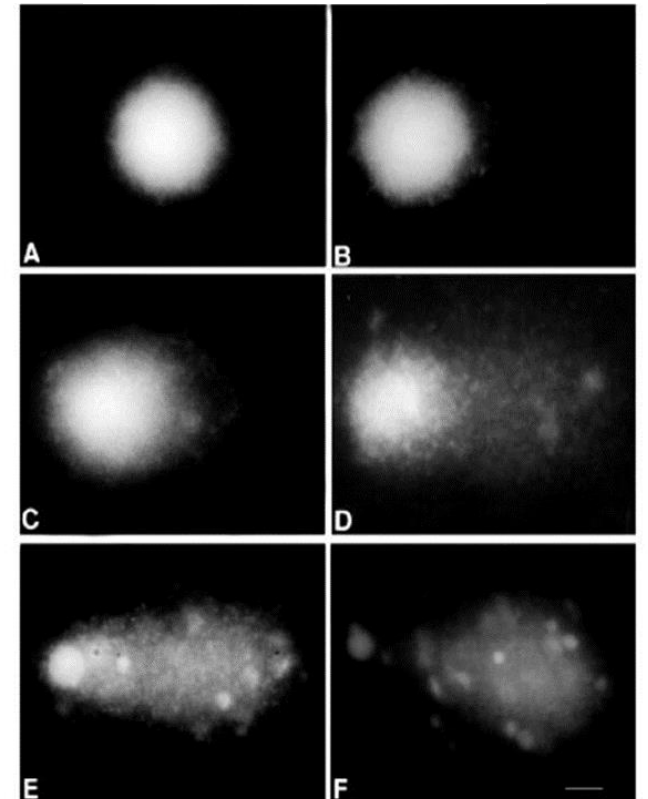
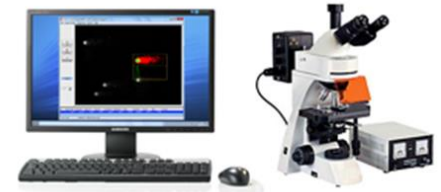
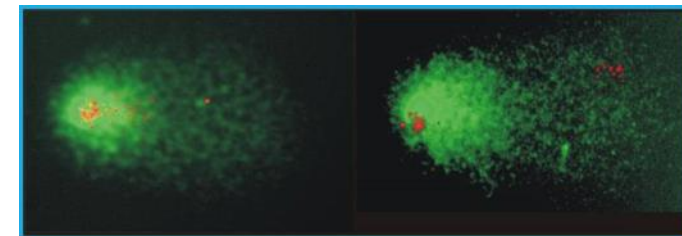
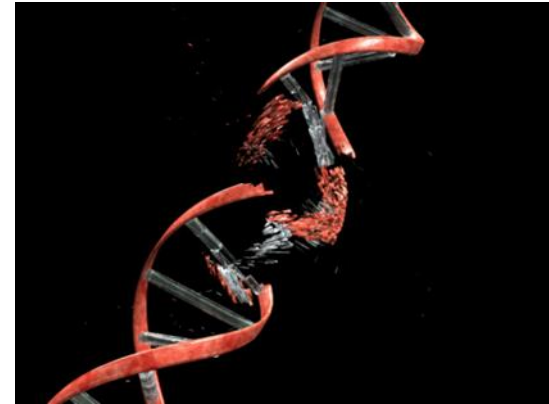


Figure 1 - Photomicrographs showing the classification of *Clonosty torquatus* blood cells in the comet assay. A, Class 0, undamaged; B, class 1; C, class 2; D, class 3; E, class 4, maximum damage; F, apoptotic cell. Bar = 10 μm .

KOMET TEST - TIPOVI OŠTEĆENJA

- jednolančani lomovi
- dvolančani lomovi
- mjesta osjetljiva na lužine (AP mjesta)
- ukriženo povezivanje DNA-DNA
- ukriženo povezivanje DNA-proteini
- nepotpuni ekscizijski popravak
- popravak DNA
- oksidacijska oštećenja (EndoIII, FPG, hOGG1)
- epigenetske promjene (Epi-Comet, Methy-sens Comet)



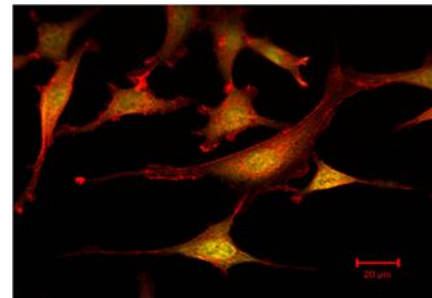
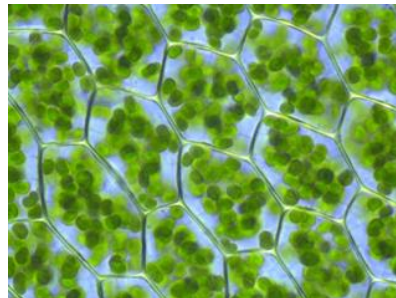
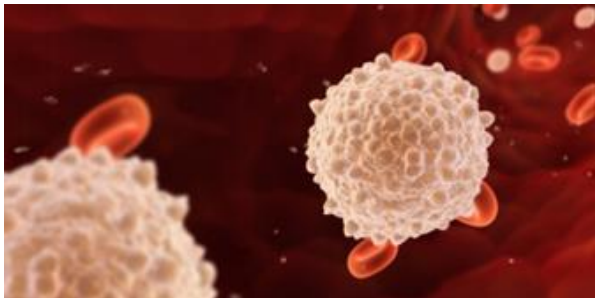
KOMET TEST

▪ PREDNOSTI

- osjetljivost
- ponovljivost rezultata
- jednostavan
- niski troškovi
- brzina izvođenja
- mali volumen uzorka (μL)

▪ TIPOVI STANICA

- bilnog podrijetla
- životinjskog podrijetla
- ljudskog podrijetla
- stanice (primarne, kultura, tkiva)



USPOSTAVA KOMET TESTA NA KVASCIMA



[DNA Repair Protocols](#) pp 101-109 | [Cite as](#)

Quantitative DNA Damage and Repair Measurement with the Yeast Comet Assay

Authors [Authors and affiliations](#)

Rui Oliveira, Björn Johansson

Protocol

First Online: 23 July 2012

6

Citations

3

Readers

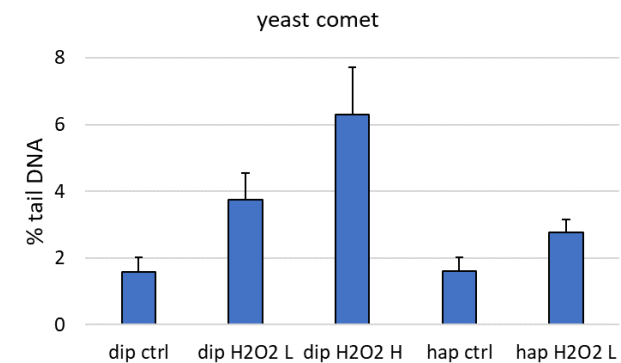
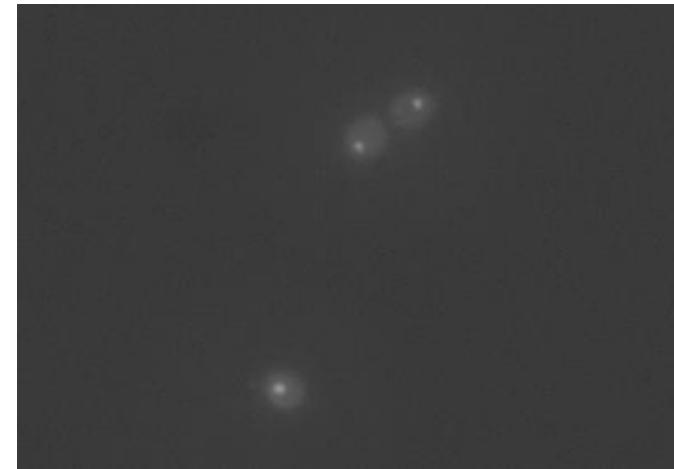
5.4k

Downloads

Part of the [Methods in Molecular Biology](#) book series (MIMB, volume 920)

Abstract

The yeast comet assay is a fast, sensitive, and inexpensive technique to measure oxidative DNA damage, DNA damage repair, and the genotoxic or protective effects of chemicals. The main advantage over the comet assay using cells of higher organisms is the genetic tractability and ease of cultivation of yeast. A drawback is the lower DNA content of the cells as well as the need for cell wall digestion prior to electrophoresis. Here, we describe in detail a recently developed protocol that permits sensitive and reproducible measurement of DNA damage and DNA repair using *Saccharomyces cerevisiae* as model system. The combination of this assay with yeast mutants affected in genome maintenance and the wide selection of available yeast molecular biology tools can contribute to illuminate fundamental mechanisms of DNA damage, repair, and activity of DNA protective compounds.

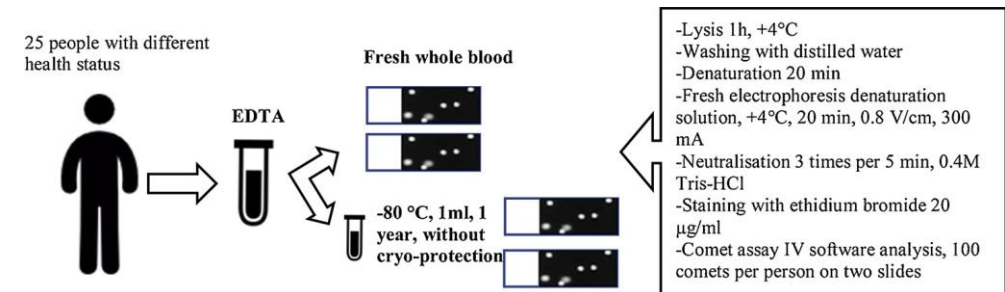


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Sveučilište
u Zagrebu

KOMET TEST - PRIMJENA



- genetička toksikologija
- ekotoksikologija
- kemijski i fizikalni agensi
- biomonitoring (ljudi, biljke, životinje)
- dijagnostika (her-2, p53)
- **smrznuti uzorci (biobanke, EPIC)**



International Agency for Research on Cancer
World Health Organization

EPIC study

ABOUT EPIC | CENTRES | RESEARCH | HIGHLIGHTS | PUBLICATIONS | ACCESS | CONTACT US | INTRANET

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The European Prospective Investigation into Cancer and Nutrition (EPIC) study is one of the largest cohort studies in the world, with more than half a million (521 000) participants recruited across 10 European countries and followed for almost 15 years.

EPIC was designed to investigate the relationships between diet, nutritional status, lifestyle and environmental factors, and the incidence of cancer and other chronic diseases. EPIC investigators are active in all fields of epidemiology, and important contributions have been made in nutritional epidemiology using biomarker analysis and questionnaire information, as well as genetic and lifestyle investigations. For details about research activities within EPIC, please refer to the [Research](#) and [Highlights](#) sections.

The EPIC study is jointly coordinated by Professor Elio Riboli, Director of the School of Public Health at [Imperial College London](#), United Kingdom, and Dr Marc Gunter and Dr Paul Brennan at the [International Agency for Research on Cancer](#) in Lyon, France.

To learn more about the resources available within EPIC, please see the [About EPIC](#) section.

NEWS



Important Announcements - ICAW 2019

For those who are planning to attend the International Comet Assav Workshon in



EEMGS 47th Annual Meeting

Dear all,

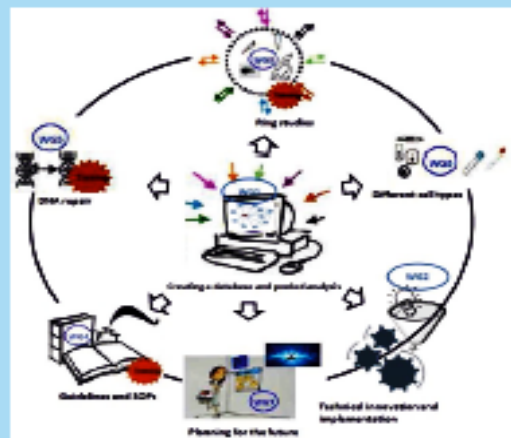
During the next Annual Meeting in May



Training School Announced

hCOMET-COST training course @ Riga, January 29th to February 1st - 2019
On the use of non-invasive sampled

hCOMET Working Groups



WG1 Creating a database and pooled analysis
(Mirta Milić and Stefano Bonassi)

WG2 Technical innovation and implementation
(Gunnar Brunborg and Kristine Bjerve Gutzow)

WG3 Ring Studies
(Peter Møller and Andrew Collins)

WG4 Guidelines and SOPs
(Amaya Azqueta and Elisa Boutet-Robinet)

WG5 DNA repair
(Roger Godschalk and Sabine Langie)

WG6 Different cell types
(Maria Dušinská and Solange Costa)

WG7 Planning for the future
(Sabine Langie and Blanca Laffon)

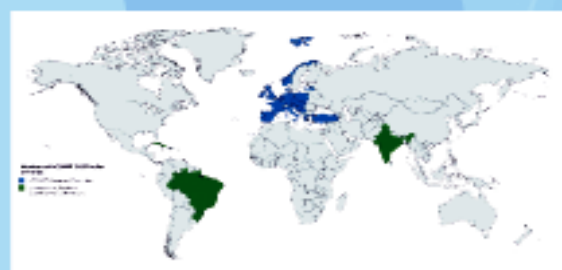
hCOMET Activities

- Training schools
 - DNA damage
 - DNA repair
 - Statistical and epidemiological issues in human biomonitoring studies: An hCOMET course
 - Use of different cell types in human biomonitoring
- Short term scientific missions
- Comet Assay Films-Tutorials
- Conference Grants
- Publications and Videos-JOVE

hCOMET Objectives

Research Coordination Objectives

- Network of researchers using the comet assay to assess DNA damage and repair in human population studies
- Collecting the results of individual human population studies. Creating a unified database of comet assay data relating to human health and disease.
- Establishing (by pooled analyses) the methodological, demographic, genetic and exposure variables that determine levels of DNA damage
- Determining the experimental factors affecting the performance of the assay, its reliability and reproducibility



Capacity-building Objectives

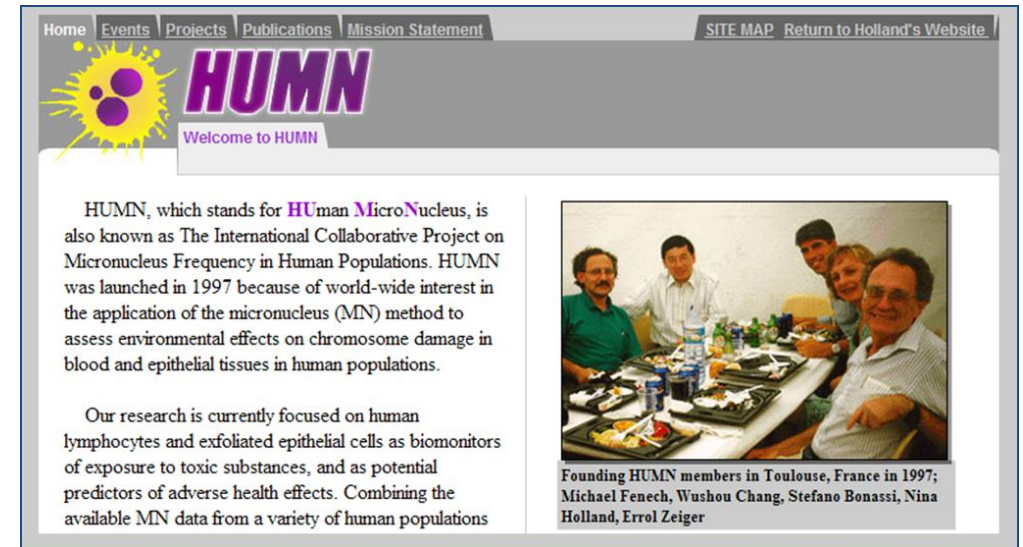
- Ring studies with standard comet assay protocols to identify best practice
- Guidelines/standard operating procedures (SOPs) for best practice in human population studies design and in performance of assay
- Comparing different biological source materials: lymphocytes, leukocytes from frozen blood, buccal epithelial cells, etc.
- Preparing for a subsequent prospective human trial to test DNA damage and DNA repair as predictive markers of disease, making use of technical and theoretical approaches developed in the COST Action
- Creating a cohort of skilled young molecular epidemiologists by providing training courses and supporting exchange visits between laboratories
- Supporting small and medium enterprises (SMEs) and other companies using the comet assay, by providing definitive guidelines and SOPs

Management Committee

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Denmark	Prof Peter MØLLER Dr Nicklas Raun JACOBSEN
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MIKRONUKLEUS TEST - UVOD

- Fenech i Morley 1985. godine
- HUman MicroNucleus project 1997. godine



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Mutation Research 534 (2003) 65–75



Genetic Toxicology and
Environmental Mutagenesis

www.elsevier.com/locate/gen tox

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HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures[☆]

M. Fenech^{a,*}, W.P. Chang^b, M. Kirsch-Volders^c, N. Holland^d, S. Bonassi^e, E. Zeiger^f

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^b Institute of Environmental Health Sciences, National Yang Ming University and National Miaoli Hospital, Taipei, Taiwan

^c Laboratory for Cell Genetics, Vrije Universiteit Brussel, Brussels, Belgium

^d School of Public Health, University of California, Berkeley, CA, USA

^e Department of Environmental Epidemiology, Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy

^f Errol Zeiger Consulting, Chapel Hill, NC, USA

PROTOCOL

Cytokinesis-block micronucleus cytome assay

Michael Fenech

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Published online 3 May 2007; doi:10.1038/nprot.2007.77

The cytokinesis-block micronucleus cytome assay is a comprehensive system for measuring DNA damage, cytostasis and cytotoxicity. DNA damage events are scored specifically in once-divided binucleated (BN) cells and include (a) micronuclei (MNi), a biomarker of chromosome breakage and/or whole chromosome loss, (b) nucleoplasmic bridges (NPBs), a biomarker of DNA misrepair and/or telomere end-fusions, and (c) nuclear buds (NBUDs), a biomarker of elimination of amplified DNA and/or DNA repair complexes. Cytostatic effects are measured via the proportion of mono-, bi- and multinucleated cells and cytotoxicity via necrotic and/or apoptotic cell ratios. Further information regarding mechanisms leading to MNi, NPBs and NBUDs formation is obtained using centromere and/or telomere probes. The assay is being applied successfully for biomonitoring of *in vivo* genotoxin exposure, *in vitro* genotoxicity testing and in diverse research fields such as nutrigenomics and pharmacogenomics as well as a predictor of normal tissue and tumor radiation sensitivity and cancer risk. The procedure can take up to 5 days to complete.

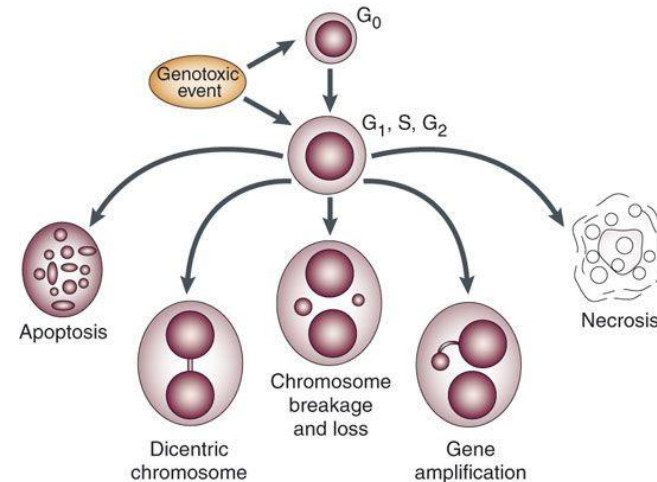
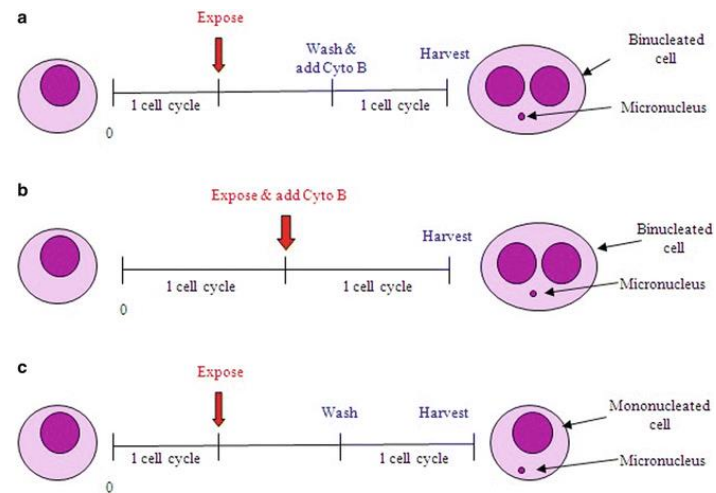


MIKRONUKLEUS TEST - PROTOKOL

- 72 h kultura limfocita
- phytohaemagglutinin
- cytochalasin-B
- bojanje 5% Giemson

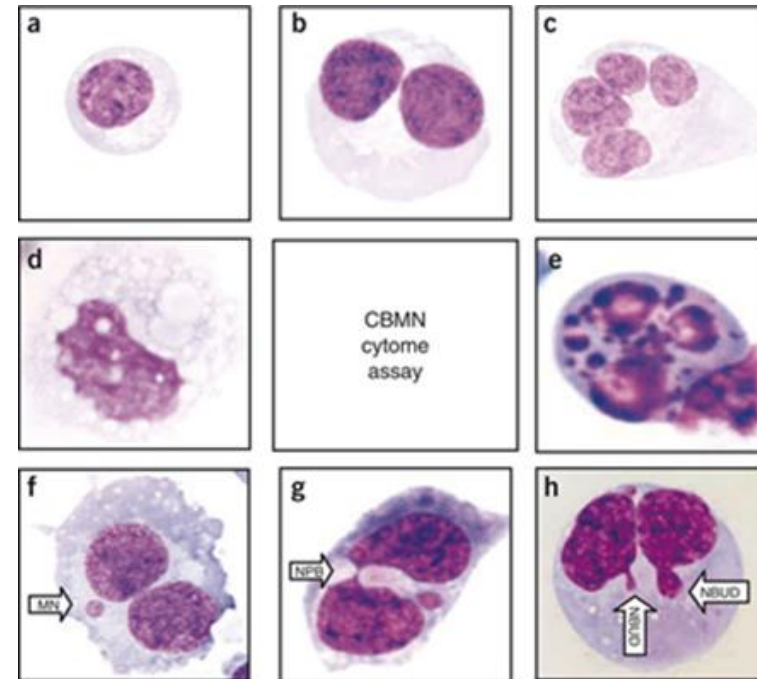
▪ parametri:

- mikronukleus
- nukleoplazmatski most
- jezgrin pup
- proliferacijski indeks



MIKRONUKLEUS TEST - TIPOVI OŠTEĆENJA

- **mikronukleusi** = potječu od acentričnih kromosomskih fragmenata ili čitavih kromosoma zaostalih u anafazi, a njihova prisutnost pokazatelj je postojanja aberacija nastalih u prethodnoj diobi stanice te se koriste kao mjera strukturnih i numeričkih aberacija kromosoma
- **nukleoplazmatski mostovi** = nastaju kao posljedica nastanka dicentričnih kromosoma, u kojima su centromere povučene na suprotne polove stanice
- **jezgrini pupovi** = nastaju kao posljedica amplifikacije gena, koja rezultira izbacivanjem amplificirane regije u jezgri pup
- **proliferacijski indeks** (CPBI, NDI)



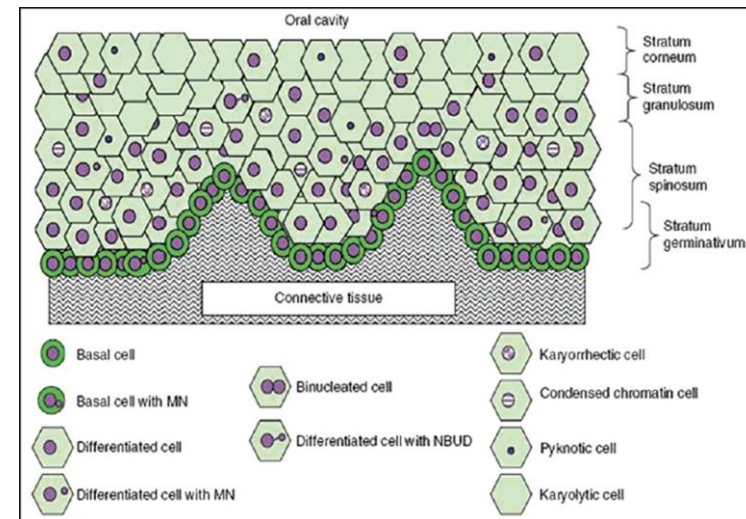
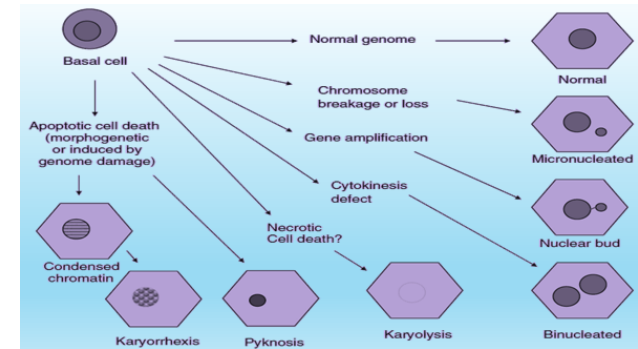
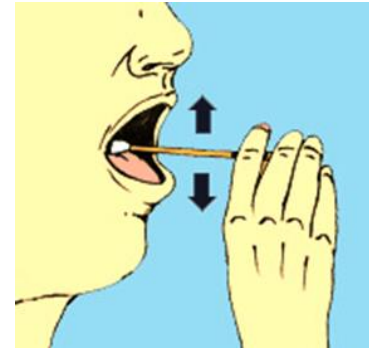
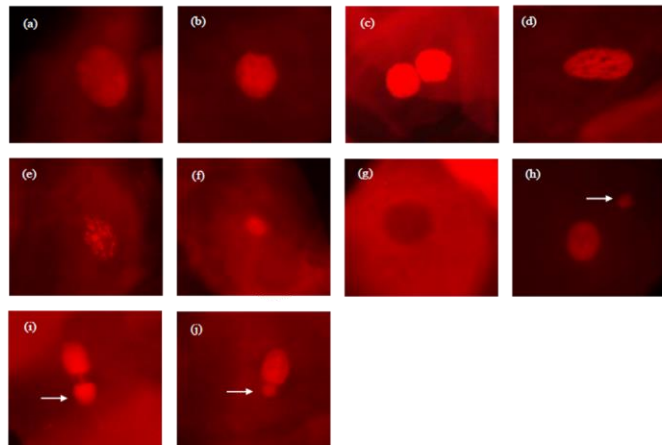
MIKRONUKLEUS TEST

▪ PREDNOSTI

- osjetljivost
- ponovljivost rezultata
- jednostavan
- niski troškovi

▪ TIPOVI STANICA

- životinjskog podrijetla
- ljudskog podrijetla
- stanice
 - primarne
 - kultura



PROTOCOL

Buccal micronucleus cytome assay

Philip Thomas¹, Nina Holland², Claudia Bolognesi³, Micheline Kirsch-Volders⁴, Stefano Bonassi⁵, Errol Zeiger⁶, Siegfried Knasmueller⁷ & Michael Fenech¹

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MIKRONUKLEUS TEST - PRIMJENA

- genetička toksikologija
- ekotoksikologija
- kemijski i fizikalni agensi
- biomonitoring (ljudi, životinje)
- dijagnostika

Research Article

Received: 16 August 2010, Revised: 6 September 2010, Accepted: 7 September 2010, Published online in Wiley Online Library: 19 November 2010

(wileyonlinelibrary.com) DOI 10.1002/jat.1603

Application of dosimetry systems and cytogenetic status of the child population exposed to diagnostic X-rays by use of the cytokinesis-block micronucleus cytome assay

Goran Gajski,^a Đurđica Milković,^b Mária Ranogajec-Komor,^c Saveta Miljanić^c and Vera Garaj-Vrhovac^{a*}

Journal of
Applied Toxicology



U okviru projekta u suradnji sa Laboratorijem za mutagenezu Instituta za medicinska istraživanja COMET ASSAY metodom istražuju se moguća oštećenja kromosoma nakon slikanja pacijenata u našoj dijagnostici.

oštećenja kromosoma nakon slikanja pacijenata u našoj dijagnostici. Rezultati iz tih mjerenja prikazuju se na brojnim simpozijima i kongresima, te se objavljuju radovi.

U okviru projekta u suradnji sa Laboratorijem za mutagenezu Instituta za medicinska istraživanja COMET ASSAY metodom istražuju se moguća oštećenja kromosoma nakon slikanja pacijenata u našoj dijagnostici.

Suvremenom opremom odjela za radiologiju i ultrazvuk idemo u korak sa drugim visoko specijaliziranih ustanovama za torakalnu obradu djece i mladeži. Educirano osoblje za rad u dječjoj ustanovi, tj. inženjeri medicinske radiologije, radiološki obrađuje djecu od najranije životne dobi upotrebom posebnih uređaja – baby-fix (za dojenčad i malu djecu); rotacioni stolic (za predškolsku djecu), a u svih pacijenata strogo se pridržavajući mjera zaštite od prekomjernog izlaganja ionizirajućem zračenju.

ODJELI

Dnevna bolnica

Odjel za pulmologiju i alergologiju dojenčadi i male djece

Pulmologija i alergologija predškolske i školske dobi

Odjel za tuberkulozu

Odjel intenzivnog liječenja

Odjel za poremećaje spavanja

HVALA NA POZORNOSTI!

