

# Combined in vitro cytogenetic tests to study long-term exposure to ELF-MF

*Commonly used acronym: COM, MN*

*Created on: 10-09-2020 - Last modified on: 18-02-2022*

## SCOPE OF THE METHOD

<b>The Method relates to</b>	Human health
<b>The Method is situated in</b>	Basic Research
<b>Type of method</b>	In vitro - Ex vivo
<b>This method makes use of</b>	Human derived cells / tissues / organs
<b>Specify the type of cells/tissues/organs</b>	human lymphoblastoid cells (TK6)

## DESCRIPTION

### Method keywords

alkaline comet assay

micronucleus test

human lymphoblastoid cell line

micronuclei

percentage of DNA damage

### **Scientific area keywords**

long-term exposure

non-ionizing radiation

50Hz magnetic field

background frequency

mu-metal shielding

### **Method description**

Alkaline comet assay (COM) and Micronucleus test (MN) are well-established cytogenetic tests that are used to detect both immediate damages i.e. DNA fragmentation, and permanent damage which results in micronuclei formation. These techniques are often applied separately or, sometimes, in combination to thoroughly assess to what extent a damaging agent could affect genetic materials. So far, *in vitro* cytogenetic studies towards the long-term effects of extremely low-frequency (electro)magnetic fields (ELF-(E)MFs) are less common and a consistent methodology is lacking. To our knowledge, this is the first optimized protocol for a combined *in vitro* cytogenetic study (i.e. COM and MN) in the human lymphoblastoid cell line (TK6) to investigate the effects associated with long-term effects of ELF-MF exposure has been published. Moreover, by including some additional experimental conditions, the protocol can also be applied to examine the impact of long-term pre-exposure to ELF-MFs on the sensitivity of cells to damage induced by known chemical mutagens.

### **Lab equipment**

- Exposure systems (Coil configuration was used to generate 50 Hz MF at different flux densities) ;
- Mu-metal cylinder (This cylinder can shield the control cells against background ELF-MFs) (Meca Magnetic) ;

- Incubator at 37°C, 5% CO<sub>2</sub> (Binder incubator, VWR) ;
- Biological safety cabinet (class II; BioVanguard Green Line, Telstar) ;
- Chemical hood ;
- Water bath at 37°C (Sub Aqua Pro, Grant) ;
- Benchtop centrifuge (Heraeus, Multifuge 1S, ThermoFisher Scientific) ;
- Heating block at 36°C (QBD2, Grant) ;
- Electrophoresis chamber with power supply (COMET-40 system, SCIE-PLAS, LTD) ;
- pH meter (pH7110, Inolab) ;
- Fluorescent microscope (Axiolmager.Z2) supplied with a camera and connected to the Automated Scanning System Metafer4 (Metasystems).

## **Method status**

History of use

Internally validated

## **PROS, CONS & FUTURE POTENTIAL**

### **Advantages**

- Well-validated tests ;
- Applicable in different cell types ;
- Fast and easy ;
- Reproducible results can be obtained if testers strictly follow the protocol ;
- Highly sensitive for detecting low-level DNA damage.

### **Challenges**

- Result variation due to possible confounding factors that link to sample handling and gel electrophoresis ;
- Need to ensure that cells underwent cell division in the micronucleus test ;
- In the micronucleus test, cytochalasin B is spindle poison, which might interfere with

the test results.

## **Modifications**

With regards to alkaline comet assay, it is possible to detect different endpoints (i.e. global DNA methylation status or oxidative damage) by treating the cells with different restriction enzymes (i.e. HpaII, MspI, or Fpg). Currently, methylation-sensitive comet assay on TK6 is developing to investigate the global methylation status of unexposed cells vs cells exposed ELF-MFs. Micronucleus test can be coupled with fluorescence *in situ* hybridization (FISH) to reveal the capability of an agent in inducing structural chromosome aberrations (clastogenic activity) and/or numerical chromosome changes (aneugenic activity)

## **Future & Other applications**

These methods can be applied to investigating the genotoxicity of different agents i.e human biomonitoring studies on effects of exposure to nanomaterials,...

## **REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION**

### **References**

- Collins, A. R., Oscoz, A. A., Brunborg, G., Gaivao, I., Giovannelli, L., Kruszewski, M., Smith, C.C & t tina, R. (2008). The comet assay: topical issues. *Mutagenesis*, 23(3), 143-151.
- Tice, R. R., Agurell, E., Anderson, D., Burlinson, B., Hartmann, A., Kobayashi, H., Miyamae, Y., Rojas, E., Ryu, J.C & Sasaki, Y. F. (2000). Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. *Environmental and molecular mutagenesis*, 35(3), 206-221.
- Fenech, M. (2007). Cytokinesis-block micronucleus cytome assay. *Nature protocols*, 2(5), 1084.

OECD. 2014. OECD TG487: In vitro Mammalian Cell Micronucleus Test.

## Associated documents

## Links

[In vitro 50 Hz magnetic field long-term exposure: Cytogenetic tests on human ly...](#)

## Other remarks

The main remark relates to the validation of the exposure system and the exposure environment. In a biological experiment designated to investigate the effect of MF exposure, it is important to include a good negative control which has the exposure level close to 0  $\mu\text{T}$  to observe the effect of MF exposure. However, the measurements in incubators revealed non-negligible background levels of ELF-MFs ranging from 2.2 to 17  $\mu\text{T}$ . The control cells might thus be exposed to higher MF than those reported. Consequently, efficient shielding of control cells against unintentional MF is a key factor in this type of study. We confirm that the mu-metal cylinder putting in an upright position efficiently shield the background MF inside the incubator.

## PARTNERS AND COLLABORATIONS

### Organisation

**Name of the organisation** Université Libre de Bruxelles (ULB)

**Department** Medicine

**Country** Belgium

**Name of the organisation** Sciensano

**Department** Chemical and physical health risks

**Country** Belgium

*Coordinated by*



*Financed by*

