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

Commonly used acronym: *COM, MN*

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SCOPE OF THE METHOD

The Method relates to	Human health
The Method is situated in	Basic Research
Type of method	In vitro - Ex vivo
Specify the type of cells/tissues/organs	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 access to what extend 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% CO2 (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 (AxioImager.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

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Links

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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 µT 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 µT. 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 up-right position efficiently shield the background MF inside the incubator.

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