

In vitro mammalian cell micronucleus test

Commonly used acronym: *In vitro MN test*

Created on: 22-02-2019 - Last modified on: 23-11-2022

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Organisation

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SCOPE OF THE METHOD

The Method relates to	Human health
The Method is situated in	Basic Research, Regulatory use - Routine production
Type of method	In vitro - Ex vivo
Species from which cells/tissues/organs are derived	Hamster
Type of cells/tissues/organs	TyCHO-K1 cells

DESCRIPTION

Method keywords

DNA damage

micronuclei

chromosomal damage

OECD TG 487

structural and numerical aberration chromosomal aberration

automated scoring

Scientific area keywords

in vitro toxicology

genotoxicity

regulatory toxicology

hazard assessment

Method description

The *in vitro* micronucleus test is a genotoxicity test for the detection of micronuclei in the cytoplasm of interphase cells and has been described in detail in OECD TG 487. Micronuclei may originate from acentric chromosome fragments (i.e. lacking a centromere), or whole chromosomes that are unable to migrate to the poles during the anaphase stage of cell division. Consequently, this *in vitro* method can detect both structural and numerical chromosome aberrations in cells that have undergone cell division during or after exposure to the test chemical. Within our lab, the *in vitro* micronucleus test with the actin polymerisation inhibitor cytochalasin B is applied. Cytochalasin B allows for the identification and selective analysis of micronucleus frequency in cells that have completed one mitosis, because such cells are binucleate. Scoring of the micronuclei in the binucleated cell is done in an automated way using the Metafer system. Other imaging software or flow cytometry can also be used as well as manual scoring.

Lab equipment

- Standard equipment for working with cell cultures;
- Fluorescence microscope;
- Analysis software for automated scoring (e.g. metafer 4).

Method status

Published in peer reviewed journal

Validated by an external party (e.g. OECD, EURL ECVAM,...)

PROS, CONS & FUTURE POTENTIAL

Advantages

- Simple and easy to identify endpoint.

Challenges

- Distinction between structural and numerical chromosome aberrations requires combination with other techniques (e.g. Fluorescence In Situ Hybridization - FISH);
- Detection of artefacts during automated scoring, manual verification needed;
- Automated flow cytometric detection for faster scoring of more test concentrations with more cells (benchmark dose approach) Flow Cytometry for automated cytotoxicity detection along with micronuclei [scoring](#).

Modifications

- Use of other cell types, preferably p53-competent human-derived cell lines;
- Combination with Fluorescence In Situ Hybridization (FISH) to distinguish between structural and numerical chromosome aberrations;
- Use of flow cytometry for benchmark dose approach and simultaneous detection of cytotoxicity and MN scoring.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

Fenech, M. (1997), The advantages and disadvantages of cytokinesis-blood micronucleus method, Mutation Research, Vol. 392, pp. 11-18.

Kirsch-Volders, M. et al. (2011), The *in vitro* MN assay in 2011: origin and fate, biological significance, protocols, high throughput methodologies and toxicological relevance, Archives of Toxicology, Vol. 85/8, pp. 873-99.

Associated documents

[OECD in vitro mammalian cell micronucleus test.pdf](#)

Links

[OECD Test guideline](#)

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