## GENOTOXICITY : THE MICRONUCLEUS TEST

A micronucleus (MN) is formed during the metaphase/anaphase transition of mitosis (cell division). It may arise from a whole lagging chromosome (aneugenic event leading to chromosome loss) or an acentric chromosome fragment detaching from a chromosome after breakage (clastogenic event) which do not integrate in the daughter nuclei.

Scoring of micronuclei can be performed relatively easily and on different cell types relevant for human biomonitoring: lymphocytes, fibroblasts and exfoliated epithelial cells, without extra in vitro cultivation step. MN observed in exfoliated cells are not induced when the cells are at the epithelial surface, but when they are in the basal layer.

An ex vivo/ in vitro analysis of lymphocytes in the presence of cytochalasin-B (added 44 hours after the start of cultivation), an inhibitor of actins, allows to distinguish easily between mononucleated cells which did not divide and binucleated cells which completed nuclear division during in vitro culture. Indeed, in these conditions the frequencies of mononucleated cells provide an indication of the background level of chromosome/genome mutations accumulated *in vivo* and the frequencies of binucleated cells with MN a measure of the damage accumulated before cultivation plus mutations expressed during the first *in vitro* mitosi

The criteria for selecting binucleated cells to score are the following:
Score binucleated cells with

- main nuclei that are separate and of approximately equal size,
- main nuclei that touch and even overlap as long a nuclear boundaries are able to be distinguished, and
- main nuclei that are linked by nucleoplasmic bridges

Do not score:

- trinucleated, quadranucleated, or multinucleated cells or
- cells where main nuclei are undergoing apoptosis (because MN may be gone already or may be caused by apoptotic process)

In the absence of cytochalasin B, mononucleated cells are analyzed for the presence of micronuclei
In the presence of cytochalasin B, mononucleated cells are recommended to be harvested at 24 hours post-PHA stimulation as there can be no doubt at this time-point that MN within such a cell are a result of in vivo rather than ex vivo division. Binucleated cells are recommended to be harvested at 72 hours post-PHA. Moreover, 24 hour post-PHA time-point may be the right time to count apoptotic/necrotic cells (Kirsch-Volders et al, 2001)

For the scoring of micronuclei the following criteria were adopted from Fenech et al, 2003:

- the diameter of the MN should be less than one-third of the main nucleus
- MN should be separated from or marginally overlap with main nucleus as long as there is clear identification of the nuclear boundary
- MN should have similar staining as the main nucleus

**Characteristics of the MN-test**

- Biomarker of effect: relevant for risk assessment of cancer
- Endpoint: identification of chromosome + genome mutations
- Expression of MN requires cell division
- MN contain either a whole chromosome or an acentric fragment
- Discrimination between mononucleated cells and binucleated cells in the cyto-B assay

Mononucleated cells → damage accumulated before cultivation of the cells

Binucleated cells → damage accumulated before cultivation of the cells + damage expressed during cultivation

**Advantages/ Disadvantages**

| MN-assay | Advantages | Disadvantages |
| --- | --- | --- |
| With/without cyto-B | • Cell/cell approach<br>• Simultaneous detection of chromosome + genome mutations<br>• Discrimination between clastogen/ aneugen<br>• Possible co-detection of apoptosis/necrosis<br>• Applicable on many cell types<br>• Rapidity<br>• Cheap<br>• Simplicity<br>• Potential for automation<br>• Statistical power | • Does not detect all structural chromosome aberrations (only acentric fragments)<br>• Requires cell division for expression of MN |
| With cyto-B | • Discrimination between cells which underwent nuclear division and cells which did not<br>• Enables detection of dicentric bridges as | • Possible interference of cyto-B with test chemical; like spindle poisons<br>• Possible interference with other inhibitors of |

| | nucleoplasmic bridges<br>• Assessment of cell proliferation (% binucleated cells) | cytokinesis<br>• Cytoxicity of cytochalasin B itself varies between cell types and sometimes even between subtypes of the same cell type |
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**Confounding factors:**

age
gender
smoking?

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***References:***


- Fenech M, Chang WP, Kirsch-Volders M, Holland N, Bonassi S, Zeiger E; HUman MicronNucleus project. "HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures."; *Mutat Res.,* 2003, **534**(1-2):65-75
- Kirsch-Volders M, Fenech M. Inclusion of micronuclei in non-divided mononuclear lymphocytes and necrosis/apoptosis may provide a more comprehensive cytokinesis block micronucleus assay for biomonitoring purposes;*Mutagenesis*, 2001, **16(1)**:51-58