Micronucleus Assay

1. Introduction

The micronucleus (MN) test is part of a battery of tests that many new products must go through prior to bringing them to market.
The *in vitro* micronucleus assay is a mutagenic test system for the detection of chemicals which induce the formation of small membrane bound DNA fragments i.e. micronuclei in the cytoplasm of interphase cells. These micronuclei may originate from acentric fragments (chromosome fragments lacking a centromere) or whole chromosomes which are unable to migrate with the rest of the chromosomes during the anaphase of cell division.
The purpose of the micronucleus assay is to detect those agents which modify chromosome structure and segregation is such a way as to lead to induction of micronuclei in interphase cells.

2. Principle of test method

Cell cultures (V79^{TM}) are exposed to the test substances both with and without metabolic activation. After exposure to a test substance, and addition of cytochalasin B for blocking cytokinesis cell cultures are grown for a period sufficient to allow chromosomal damage to lead to the formation of micronuclei in bi- or multinucleated interphase cells. Harvested and stained interphase cells are then analysed microscopically for the presence of micronuclei. Micronuclei are scored in those cells that complete nuclear division following exposure to the test item.

For more information please contact us!

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3. Exposure concentrations

In consideration of solubility and cytotoxicity the highest test item concentrations are 10 mM, 5 mg/ml or 5 µl /ml. At least three analysable concentrations are tested.

4. Controls

Concurrent negative (solvent or vehicle) and positive controls both with and without metabolic activation are included in each experiment.

positive controls	**w/o metabolic activation**	**w metabolic acitvation**
clastogen	mitomycin C	cyclophosphamide
aneugen	colchicine	-

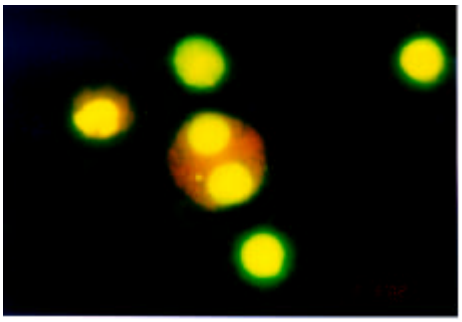
5. Evaluation/Analysis

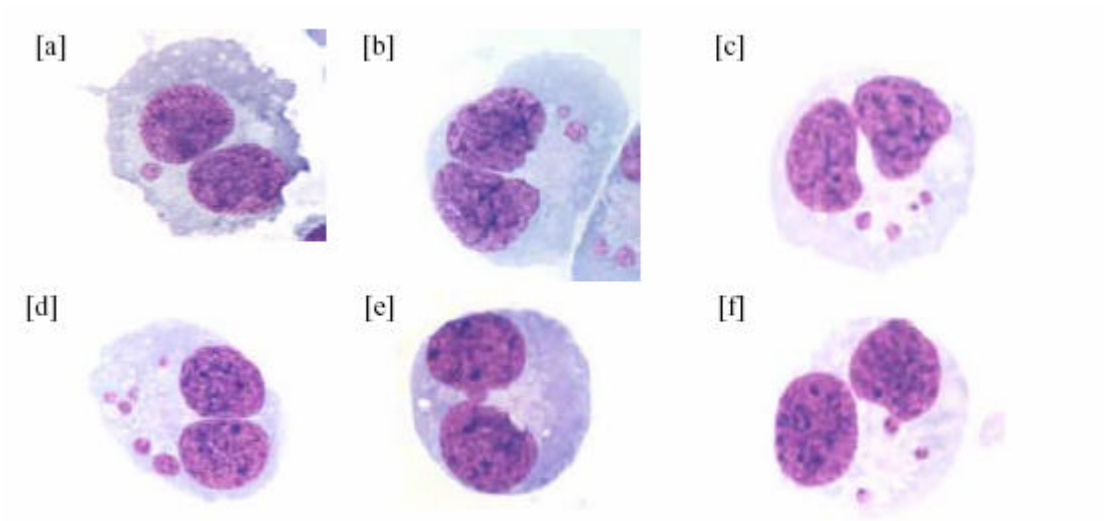
At least 1000 binucleated cells per duplicate cell culture are scored to assess the frequency of cells with one, two ,or more than two micronuclei. Additionally, the cells are classified as mononucleates, binucleates or multinucleates to estimate the proliferation index as a measure of toxicity.

6. Interpretation of results

If a substance induces a concentration-related increase or a reproducible increase in the number of cells containing micronuclei, it is classified as a positive result.

A positive result from the *in vitro* micronucleus test indicates that the test substance induces chromosome damage or damage to the cell division apparatus.
If there is a clear cut positive result there is no requirement for verification. Equivocal results are clarified by further testing using modified experimental conditions.
Negative results have to be confirmed.





BN Cells with MN

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7. References

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- Pary J: A proposal for a new OECD Guideline for the in vitro micronucleaus test, UKEMS (1997).