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GLP MNT

GLP OECD Guideline 487 Micronucleus Test using Primary Human Lymphocytes

The *in vitro* micronucleus assay is used to detect chemicals which induce the formation of small membrane-bound DNA fragments (micronuclei) in the cytoplasm of interphase cells.

Chromosomal damage is detected by the presence of micronuclei in the cytoplasm of interphase cells that originate from acentric fragments, or whole chromosomes that failed to segregate correctly during anaphase. This means the micronucleus test is sensitive to both clastogenic and aneugenic mechanisms of genotoxicity[1].

This cytogenetic test is designed to meet the requirements of the current international guidelines issued by the Organization for Economic Cooperation and Development (OECD; Test Guideline 487[2]).

Process

When requesting a GLP study, the sponsor will typically provide test item characterisation and formulation information, and analytical methods for formulation analysis.

A draft study plan is prepared for review by the sponsor, Gentronix, and an independent QA group. Once this is approved and the test items supplied, the study can begin.

If no toxicity or solubility information has been provided, a range-finder test is carried out before the main assay is performed. If formulation analysis is required, this is carried out immediately after dosing.

The sponsor is notified of the results of the study.

A draft report is prepared and reviewed by the sponsor, Gentronix and the QA group. Once approved, a QA statement is added before the final report is issued.

Number of dose levels

At least 3 analysable dose levels will be carried out.

Number of replicates

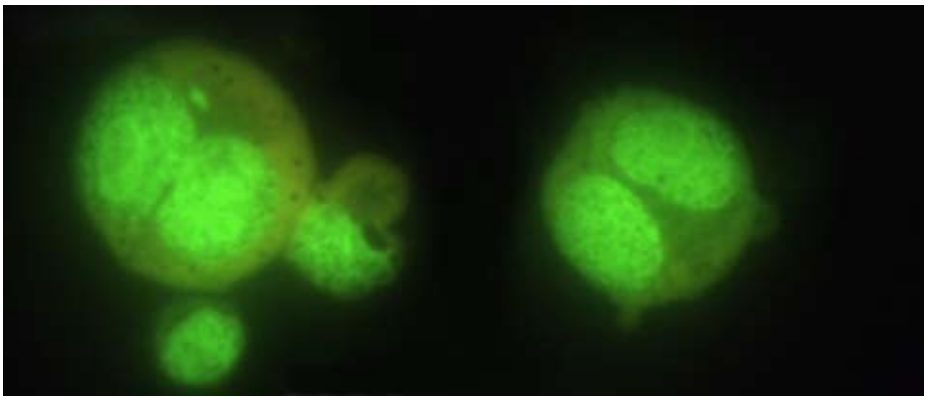
Duplicate cultures will be tested.

Test compound requirement

1.5g of test substance will be required (includes range-finder testing and formulation analysis).

Turnaround time

Turnaround time from receipt of test article and characterisation/ formulation information, to submission of the final report for sponsor approval is typically 12 weeks.



Assay Principles

Cell cultures of freshly isolated human lymphocytes are exposed to the test item both with and without an exogenous source of metabolic activation (S9 fraction). Solvent/vehicle and positive controls are included in all tests. During exposure to the test item, the cells are grown for a period sufficient to allow chromosome or spindle damage to lead to the formation of micronuclei in interphase cells. Harvested and stained cells are analysed for the presence of micronuclei.

Micronuclei are only scored in cells that have completed mitosis during exposure to the test item. In this version of the assay cytokinesis is blocked using cytochalasin B.

Cell preparation

Whole blood is drawn from young, healthy, non-smoking volunteers with no known recent exposures to genotoxic chemicals or radiation. Typically at least 2 donor samples are pooled for each assay. Lymphocyte cells are isolated using standard methods and are cultured in the presence of a mitogen for between 44 and 48 hours prior to exposure to the test item.

Dosing

Cells are exposed to the test item along with solvent/vehicle and positive controls. A preliminary dose range finding assay can be set up to assess the toxicity of the test item. From the information provided by the initial test, at lease 3 analysable concentrations are evaluated, in duplicate, with the highest concentration aiming to produce 55 ±5% cytotoxicity.

Incubation

The assay is carried out both in the presence and absence of a rat-liver S9-mediated metabolic activation system. Typical treatment periods are outlined in Table 1.

Cell Harvesting

Following the treatment period, cells are washed, enumerated and adjusted to the same cell density. A thin monolayer of cells is produced on a glass slide which is then fixed and stained using a DNA specific stain to allow the straightforward detection of nuclear material.

Analysis

All slides are independently coded prior to analysis to ensure there is no operator

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innovation and service

Gentronix is an established biotechnology innovation and service company specialising in early screening, mechanistic follow-up and regulatory genotoxicity assays for a range of industries including; pharmaceuticals, chemicals, agrochemicals, personal care, consumer products, flavours, fragrances and taste enhancers, and medical devices.

In addition to classical genotoxicity screening assays, Gentronix offers GreenScreen®HC and BlueScreen™HC which are novel, patented systems that, unlike earlier assays, detect all known classes of genotoxin.

Gentronix can provide assays and advice on follow-up strategies for positive results, and mechanism elucidation to help chemists modify compounds to eliminate genotoxicity early in product discovery and development thereby preventing late stage failure.

Gentronix is GLP compliant and offers regulatory assays to OECD and other test guidelines.

Culture Description	S9
Treat for 3 h in the presence of S9; replace medium; add cytoB; harvest 1.5 – 2.0 normal cell cycles later.	✓
Treat for 3 h; replace medium; add cytoB; harvest 1.5 – 2.0 normal cell cycles later.	✗
Treat for 1.5 – 2.0 normal cell cycles in the presence of cytoB; harvest at the end of the exposure period.	✗

Table 1: Cell treatment periods used in the micronucleus assay in human lymphocytes

bias. The cytokinesis-block proliferation index (CBPI) is determined to demonstrate cell proliferation using at least 500 cells per test item concentration, vehicle and positive control. Micronucleus frequencies are analysed in at least 2000 binuclear cells per test item dose level.

Data and Reporting

The final test report generated includes all of the information required by OECD Guideline 487. Individual culture data are provided and all data are summarized in tabular form. There are several criteria for the determination of a positive result such as a concentration-related increase of a statistically significant increase in the number of cells containing micronuclei. In addition, the biological relevance of the results is evaluated by comparing the test data to the appropriate historical control ranges.

References

[1]*Parry, J., Parry, E., Bourner, R., Doherty, A., Ellard, S., O'Donovan, J., Hoebee, B., et al. (1996) The detection and evaluation of aneugenic chemicals. Mutation Research, 353: 11-46.*

[2]*www.oecd-ilibrary.org/environment/test-no-487-in-vitro-mammalian-cell-micronucleus-test_9789264091016-en*

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