gentronix

MNT

In vivo micronucleus testing of rodent peripheral blood samples using flow cytometry

- **Conforms to ICH Guidelines (20,000 RETs scored per sample)**
- **Staining procedure restricts analysis to the newly formed RETs**

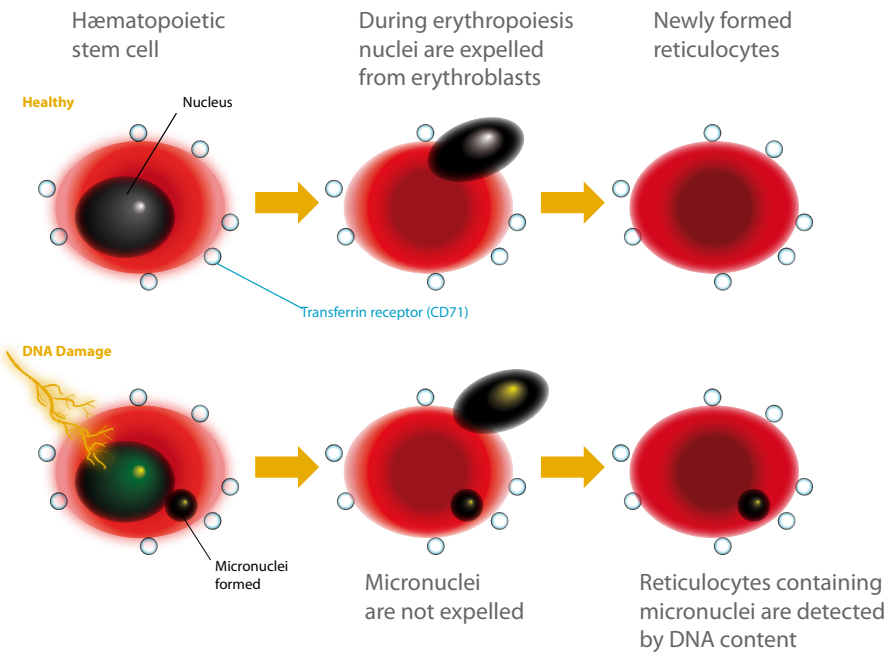
The rodent peripheral blood micronucleus test provides an assessment of the ability of a test compound to induce either chromosomal damage or mis-segregation in haematopoietic cells in rodent bone marrow. During the generation of red blood cells, these progenitor stem cells differentiate to form erythroblasts, which in turn expel their nuclei to form immature red blood cells (reticulocytes, RETs). If these cells have previously suffered chromosomal damage, nuclear membranes form around the resulting fragments, generating micronuclei. These micronuclei are not expelled with the nucleus of the erythroblast during differentiation, resulting in RETs that contain micronuclei and therefore DNA (MN-RETS).

Whilst MN-RETs from peripheral blood can be easily scored by microscopy, given the relative rarity of these events this is a time-consuming process that requires the manual scoring of thousands of reticulocytes per sample. Using a flow cytometric approach allows the robust & rapid enumeration of tens of thousands of RETs from a small aliquot of rodent blood.

The flow cytometric rodent peripheral blood micronucleus test is included in the recent ICH revision to the guidelines on genotoxicity , with a requirement for restriction of analysis to the RETs population required for non-mouse in vivo studies – the offering from Gentronix meets this criteria.

Regulatory Acceptance

The US FDA accepts pre-clinical MicroFlow data, and this method adheres to the necessary guidelines as stated by the International Workshop on Genotoxicity Test Procedures (IWGTP).



Additionally, the most current Organization for Economic Co-operation and Development (OECD) guidelines regarding micronucleus testing, guideline 474, indicate that flow cytometry is an acceptable alternative to manual evaluation.

With regard to rat blood analysis, Section 4 of the OECD Guideline 474 states "…any appropriate mammalian species may be used provided it is a species in which the spleen does not remove micronucleated erythrocytes or a species which has shown an adequate sensitivity to detect agents that cause structural or numerical chromosome aberrations." Accumulating data suggests that rat blood is an adequately sensitive compartment for analysing micronucleus formation if restricted to the newly, despite the ability of the rat spleen to remove micronucleated erythrocytes from circulation.

Method

Gentronix Ltd. utilises MicroFlow® technology, developed by Litron Laboratories, to perform *in vivo* micronucleus testing of rodent peripheral blood. This method uses a staining procedure that restricts analysis to the newly formed RETs population (using anti-CD71) and thus avoids the problems caused by splenic clearance of MN-RETs in many animal species. These immature RETs are then assessed for DNA content using a nucleic acid stain, allowing MN-RETs to be differentiated from the healthy background population.

Whilst Gentronix does not provide animal testing services, we are happy to work with the client's chosen provider. Peripheral blood samples from rat or mouse studies (~100µl) will be stabilised by them using a kit provided, and samples are then shipped to Gentronix for analysis.

Samples are then labelled for flow cytometric data collection, with 20,000 RETs scored for micronuclei.

Results

A study report is provided and data reported on the percentage of RETs and MN-RETs observed in each treatment group, with further statistical analysis also available. Flow cytometer standards and quality controls ensure robustness in the collection of in vivo micronucleus test data.

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innovation and service

Gentronix is an established biotechnology innovation and service company specialising in early screening for genotoxicity 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 assays, Gentronix offers GreenScreen®HC and BlueScreen™HC which are novel, patented systems that, unlike earlier assays, detect all known classes of genotoxin.

In addition to early screening, Gentronix provides 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.

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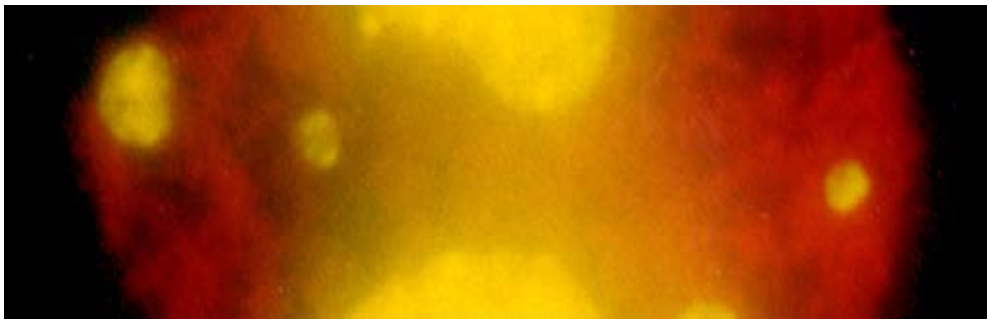
Study turnaround times are sample size dependent, although a typical 60 sample study completes within 2-weeks. In addition to providing this service, Gentronix is also a distributor of the Litron Laboratory kits throughout Europe and Japan.



References

.For details of the latest publications, visit our website at: **www.gentronix.co.uk**