Eye Irritation Test:

Reconstructed Human Cornea-like Epithelium Method (*draft* OECD Test Guideline)

Test code: CT-033

Quality Statement

XCELLR8 is accredited by the UK Medicines and Healthcare Products Regulatory Authority (MHRA) for the conduct of *in vitro* safety testing in compliance with Good Laboratory Practice (GLP). This means that we are able to provide our clients with test results that may be used at a regulatory level to demonstrate product safety, where the test is an approved regulatory method.

Overview

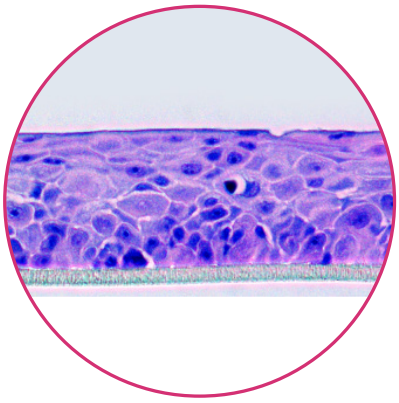
Eye irritation is defined as changes in the eye following the application of a test chemical to the surface, which are fully reversible within 21 days of application.[1]

The test described here has been internationally validated and currently forms a draft OECD Test Guideline (published 25 July 2014). Full acceptance of the test as a regulatory method is expected in 2015. The test provides a method for the hazard identification of irritant chemicals in accordance with the UN Globally Harmonized System (GHS) of Classification and Labelling Category 2. It can be used for single substances and for mixtures including finished products. It is appropriate for compliance with a range of legislation including REACH (Registration, Evaluation, Authorisation and restriction of CHemicals) and the EU Cosmetics Regulation 1223/2009. This test method is not intended to differentiate between UN GHS Category 1 (serious eye damage) and UN GHS Category 2 (eye irritation).

The test is based on the depth of injury model or Maurer and Jester, using the rationale that the degree of irritation caused by a test substance correlates with the degree of penetration into cell layers and subsequent impact on cell viability.

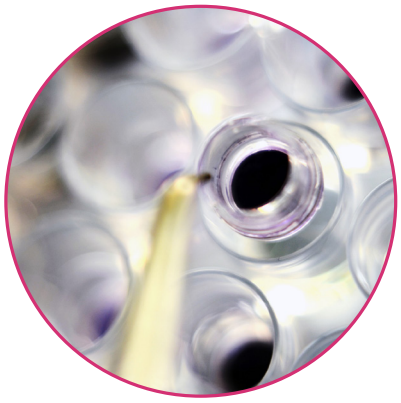
The method utilises reconstructed human cornea-like epithelium (RhCE), which in its overall design mimics the biochemical and physiological properties of the corneal epithelium of the human eye. The test item is applied directly to the cornea surface, providing a good model of "real life" exposure. Cell viability is measured by enzymatic conversion of the vital dye MTT into a blue formazan salt that is quantitatively measured after extraction from the skin tissues. Irritant test items are identified by their ability to decrease cell viability below defined threshold levels (below or equal to 60% for UN GHS Category 2). If the viability is greater than 60%, the test item is classified as Non-Irritant ("no-label", or UN GHS No Category).

Test System: Reconstructed Human Cornea-like Epithelium



Cross section through reconstructed human cornea-like epithelium

Application of a test item to the model surface



MTT test

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Eye Irritation Test (cont):

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Test System: Reconstructed Human Cornea-like Epithelium

Reconstructed human cornea-like epithelium (EpiOcular™) is a corneal model composed of living human cells which have been cultured to form a multi-layered, differentiated corneal epithelium. The levels of differentiation obtained are at the cutting edge of *in vitro* tissue technology. The model consists of highly organized basal cells which progressively flatten out as the apical surface of the tissue is approached, analogous to the normal human *in vivo* corneal epithelium. The profiles of key differentiation markers also mirror those seen *in vivo*. The cells are both metabolically and mitotically active, and release many of the pro-inflammatory agents (cytokines) known to be important in eye irritation and inflammation. EpiOcular™ is grown on special platforms at the air-liquid interface, allowing for direct application of test items in a way that accurately models "real life" eye exposure.

Turnaround Time

4 – 6 weeks

Amount of Sample Required

Minimum 10ml (liquids) / 10g (solids)

Price

Our test prices are dependent on the quantity of test items. Please enquire for a quote using the contact information shown below, or the contact form on our website.

Summary of the Test Method

- EpiOcular™ models are pre-warmed in a cell culture incubator (37°C / 5% CO_2) for 60 minutes or overnight. The culture medium is replaced prior to applying treatment.
- The test item is applied to the surface of the EpiOcular™ models: triplicate models are dosed at the apical surface with 50µl (liquid) or 50mg (solid).
- Controls consist of ultrapure water (negative control) and methyl acetate (positive control).
- The dosed cornea models are placed into a cell culture incubator for 30 minutes (liquids) or 6 hours (solids).
- Test items and control substances are removed from the cornea models' surface by washing.
- Following a post-exposure soak in culture medium, the viability of the EpiOcular™ models is assessed by MTT conversion. MTT solution is applied to the surface of the models and placed into a cell culture incubator for 3 hours. The blue formazan metabolite produced by viable cells is then extracted into isopropanol by incubation at room temperature for 2 hours.
- Triplicate samples of the extracted formazan solution are transferred to a microplate and the formazan product is quantified by absorbance spectrophotometry (wavelength 570nm).
- Absorbance readings of the formazan product from EpiOcular™ models incubated with test items are compared with those of negative controls to calculate percentage viability.
- A range of acceptance criteria must be satisfied in accordance with the draft OECD Test Guideline.
- If the viability is less than or equal to 60%, the test item is classified as an Eye Irritant (UN GHS Category 2). If the viability is greater than 60%, the test item is classified as Non-Irritant ("no-label" or UN GHS No Category).

Further Downloads

XCELLR8 Good Laboratory Practice (GLP) Compliance Certificate.

References

UN (2009), United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Third revised edition, UN New York and Geneva.

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