TEST CODE:
CT-046

Vaginal Irritation Test

EPIVAGINAL™ ET-50 METHOD

OVERVIEW

The EpiVaginal™ ET-50 test provides a scientifically advanced approach for feminine product safety testing, using a human reconstructed vaginal epithelium. It enables the rapid generation of reliable, human-relevant data on the vaginal irritation potential of a product or ingredient, without the use of animals.

EpiVaginal™ utilises tissue engineering technology to create a human vaginal epithelium, which closely parallels both the structure and cellular physiology of the *in vivo* tissue. It can be used as a model for short or long-term exposure to diverse substances such as spermicides, hormones, anti-microbial agents, products of intimate hygiene, contraceptives and lubricants, as well as any personal care products applied to the skin in the intimate area.

The test item is applied topically to the surface of the model. 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 vaginal tissues.

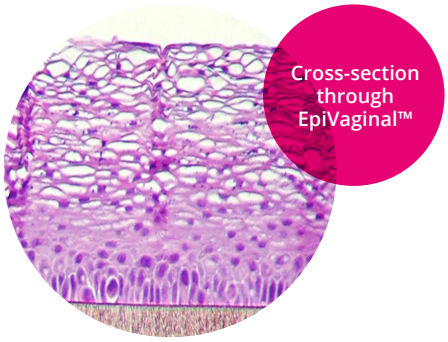
The exposure time causing a 50% reduction in tissue viability (ET-50) is determined and test items are assessed for vaginal irritation potential against well-defined, published benchmarks. The ET-50 value can also be used to place a series of items into a rank order of vaginal irritation potential.

A special extended protocol is also available for mild and ultra-mild test items. It is possible, upon special request, to save the culture medium from the basal side of the tissues for the analysis of inflammatory mediators such as LDH, PGE-2 or IL-1α.

TEST SYSTEM: RECONSTRUCTED HUMAN VAGINAL EPITHELIUM

EpiVaginal™ is a reconstructed human vaginal epithelium composed of normal human ectocervico-vaginal (ECV) epithelial cells. The *in vitro* model closely resembles the histological, ultrastructural, and protein expression properties of native tissue, including a multilayered three-dimensional morphology, inter-digitation of cells, glycogen production, and cytokeratin expression. The cells are metabolically active and can release inflammatory agents.

EpiVaginal™ is grown on a microporous membrane at the air-liquid interface, allowing for direct application of test items onto the tissue surface. It is therefore suitable for testing a wide variety of ingredients and formulations, irrespective of their solubility in aqueous cell culture medium.


Cross-section through EpiVaginal™


Preparation for dosing


MTT test

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XCellR8 Redefining testing

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SUMMARY OF THE TEST METHOD

- EpiVaginal™ models are pre-incubated for 60 minutes or overnight at 37°C/5% CO2.
- The test item is applied to the tissue surface (100µl or 100mg)
- Controls consist of ultrapure water (negative control) and 1.0% Triton X-100 (positive control).
- The dosed vaginal models are placed in a cell culture incubator for 1 hour, 4 hours and 18 hours, using triplicate models for each time point. For mild and ultra-mild test items, a 48 hour exposure time is included.
- After appropriate incubation period, tissues are gently rinsed to remove residual material.
- If required, culture medium is collected for subsequent analysis of LDH, PGE-2, IL-1α or other inflammatory markers.
- The viability of the vaginal models is assessed by enzymatic conversion of the vital dye MTT. A solution of MTT is applied to the basal side of the tissues, which are placed into a cell 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 or overnight.
- Duplicate samples of the extracted formazan solution are transferred to a microplate and the formazan product is quantified by absorbance spectrophotometry (wavelength 570nm)
- Absorbance readings quantifying the amount of formazan product are used to calculate the ET-50 value for test items and controls. (ET-50 is the time taken to reduce the viability of the EpiVaginal™ model by 50% compared to the negative control). In some cases, based on the initial range finding experiment, additional time points may be necessary (between 4-8 hours or 8-12 hours) to define an accurate ET-50 value.
- Acceptance criteria must be satisfied for the experimental run to be valid. The ET-50 value for the positive control should be between 0.75-1.75 hours.
- ET-50 values are compared to a range of published benchmark values, to assess the irritancy level of the test item.

TURNAROUND TIME	*6 – 8 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.*

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 you with test results that may be used at a regulatory level to demonstrate product safety, where the test is an approved regulatory method. The test method described here is non-regulatory but is conducted in our GLP-accredited laboratory.

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