Cosmetic Safety Testing

Soaps, Dyes, Perfumes, Raw Materials

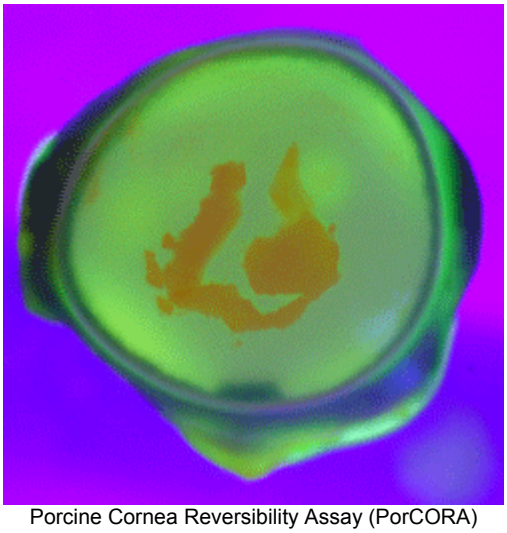
Since 1988, MB Research Labs has pioneered the use and development of non-animal testing models for eye irritancy, dermal corrosivity, irritation and sensitization.

MB Research Labs
Experience and Innovation

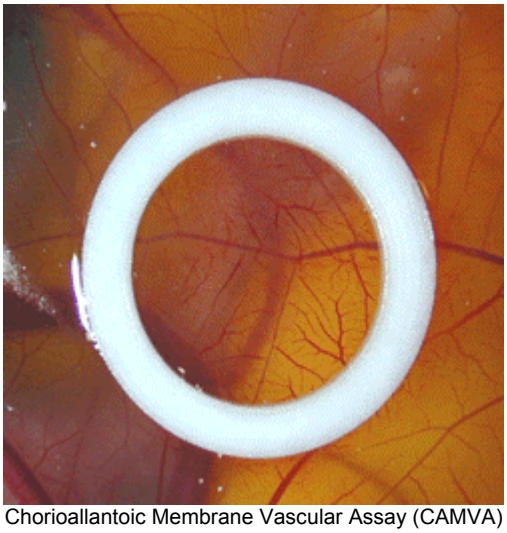
The Experience and Innovation that you've been looking for.

MB Research Labs
Experience and Innovation

Alternative Toxicology Test Models



Porcine Cornea Reversibility Assay (PorCORA)



Chorioallantoic Membrane Vascular Assay (CAMVA)

Available alternative eye irritation testing models:
BCOP – Bovine Cornea Opacity/Permeability Test – Assesses bovine corneal tissue for opacity and permeability

CAMVA – Chorioallantoic Membrane Vascular Assay – Uses vascularized membrane of fertile chicken egg to screen for ocular irritation

HET-CAM – Hen's Egg Test – Chorioallantoic Membrane test – Uses vascularized membrane of fertile chicken egg to screen for ocular irritation

PorCORA – Porcine Cornea Reversibility Assay – Screen for determination of reversibility after exposure to severe ocular irritant or ocular corrosive

PorFOCAL – Porcine Confocal Microscopy Assay – Screen for low-level irritation.

Experience.

Our experience is what differentiates MB Research from other labs. MB has been conducting alternative toxicology studies for over 20 years and has performed over 10,000 ocular irritation studies using alternative models for the cosmetic, chemical and consumer product industries. Our study directors and technicians have been trained in specific areas of data interpretation and assay performance.

Innovation.

The Draize Rabbit Eye Test has been used since 1944 in ocular toxicity screening, but little progress has been made to replace the Draize test. The Draize is used to determine effects on key ocular tissues after exposure to irritants.

Over the years, MB Research has worked closely with industry leaders as well as other laboratories to develop and optimize testing protocols that reduce the number of animals needed in ocular irritation studies.

One of our main goals is to use our experience and look at things in a new way. Over 36 years of experience gives us a unique perspective on viable alternatives to *in vivo* toxicology tests. Because these new assays don't use live animals, they are well suited for cosmetics and consumer product safety evaluations.

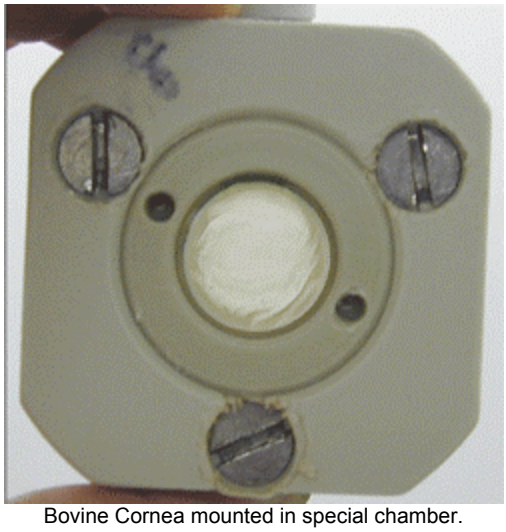
MB Research recently completed a 2 year research project funded by the Society of Toxicology Colgate-Palmolive Grant for Alternative Research to develop and validate a unique assay - PorCORA for ocular irritation that can determine the recovery potential of corneal epithelia after exposure to possible irritants, thus allowing one to classify test articles as severely irritating or as an ocular corrosive.

Pushing the Envelope

We love what we do and take pride in our work in the advancement of the 3Rs – Reduce, Refine and Replace. We are constantly working towards this goal by practicing good science.

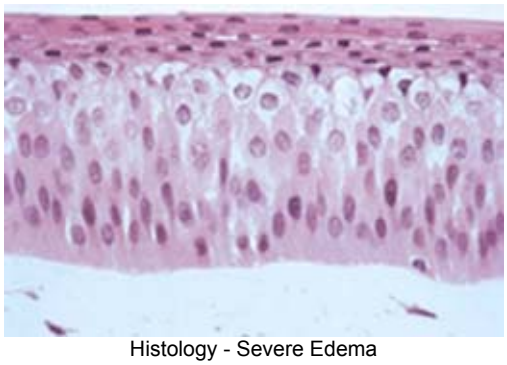
MB Research Labs, PO Box 178, 1765 Wentz Road, Spinnerstown, PA 18968
Tel: 1-215-536-4110 Fax: 1-215-536-1816 Email: mbinfo@mbresearch.com
www.mbresearch.com

BCOP – Bovine Cornea Opacity/Permeability Test



Bovine Cornea mounted in special chamber.

BCOP – Opacity



Histology - Severe Edema

Background

Since the cornea is of primary interest in the assessment of ocular irritancy, the BCOP provides a useful parallel to possible human exposure.

Summary of Experimental Procedures

This assay is conducted on intact bovine corneas that would normally be discarded by abattoirs. Results from the opacity and permeability scores are used to calculate an In Vitro Score, which is correlated to ocular injury.

Irritation is assessed through corneal opacity (light transmittance) and permeability (loss of cell to cell membrane junctions and barrier properties of the corneal epithelium). Corneas are prepared and mounted in special chambers, which creates two separate chambers. Test article is introduced to the corneal epithelium and then rinsed. Opacity or light transmittance is measured by using an opacitometer. The clearer the cornea, the more light passes through.

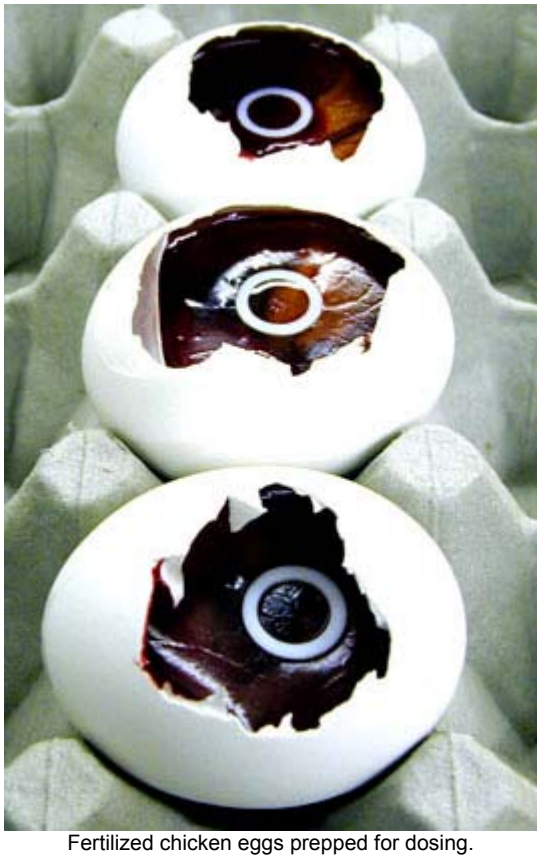
Permeability is determined by introducing fluorescein to the epithelium side of the chamber and measuring the amount of fluorescein that passes through the corneal tissue. The less damage, the less fluorescein permeability. The amount of fluorescein is measured by a spectrophotometer.

Assay Specifics and Advantages:

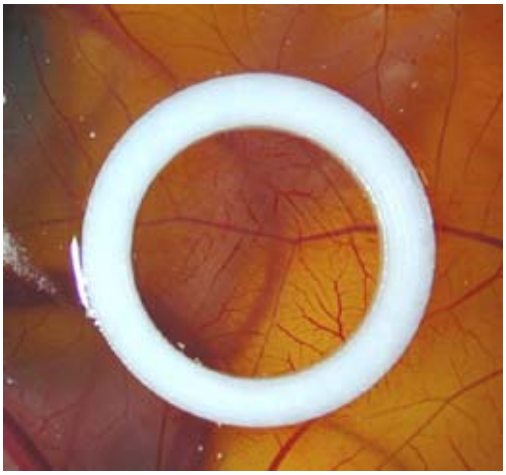
- Two Main Endpoints
  - Opacity - Changes in Light Transmission
  - Trans-corneal Fluorescein permeability
- Histological evaluation is also possible by a board certified veterinarian pathologist
- Capable of testing Liquids and Solids
- Rapid assay (usually one day)
- Accepted by regulatory agencies for use as alternative screen
- Cost-efficient
- MB has performed over 5000 BCOP assays

MB Research Labs, PO Box 178, 1765 Wentz Road, Spinnerstown, PA 18968
Tel: 1-215-536-4110 Fax: 1-215-536-1816 Email: mbinfo@mbresearch.com
www.mbresearch.com

CAMVA – Chorioallantoic Membrane Vascular Assay



Fertilized chicken eggs prepped for dosing.



Chorioallantoic Membrane of fertilized chicken egg – Capillary Injection.

Background

The chorioallantoic membrane (CAM) is a vascularized membrane in the inside of a fertilized chicken egg, which has been widely used in research for many years.

The objective of the CAMVA is to determine the potential for ocular irritation using an alternative to the Draize methodology. The methodology is based on that described **in An improved CAM Method for Predicting Ocular Irritation**, Bagley, D.M., Rizvi, P.Y., Kong, B.M., and De Salva, S.J. (1988), Alternative Methods in Toxicology, Vol. 6, Progress in In vitro Toxicology, pp. 131-138.

Summary of Experimental Procedures

- Fertile chicken eggs are received, cleaned and incubated for 10 – 14 days*
- On day 4, a dosing windows is cut into the shell exposing the CAM
- On Day 10 (or 14)*, a Teflon ring is inserted onto the CAM, isolating a test area from the remaining portion of the CAM which is used for control purposes
- Test article concentrations are topically dosed inside the ring
- Positive responses or changes in vascular blood flow are recorded after 10 – 30 mins of exposure*

* Protocol dependant

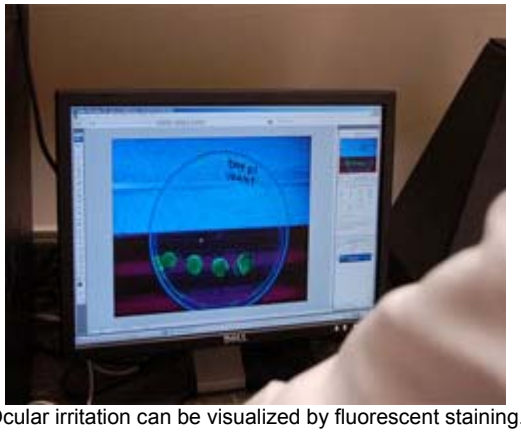
Assay Specifics and Advantages:

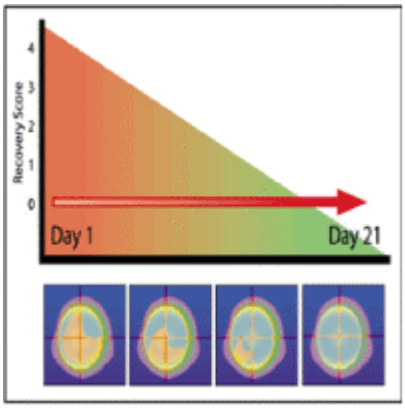
- Widely used animal alternative
- MB has performed over 7000 CAMVAs
- Topical dosing of opaque, viscous and powder materials is possible
- Endpoint is changes in vascular blood flow/hemorrhaging
- Dose Response Curve of % positive used to calculate RC50
- Correlation: 84–92% rabbit Draize
- Rapid and Inexpensive

MB Research Labs, PO Box 178, 1765 Wentz Road, Spinnerstown, PA 18968
Tel: 1-215-536-4110 Fax: 1-215-536-1816 Email: mbinfo@mbresearch.com
www.mbresearch.com

PorCORA – Porcine Cornea Reversibility Assay

Porcine corneas.


Ocular irritation can be visualized by fluorescent staining.



Stained corneas allow for easy identification of tissue damage.

Background

There are several alternative methods to characterize aspects of eye irritation and damage, but no established method can model recovery after injury as in a Draize test. PorCORA[1] was developed to fill this void by measuring corneal damage and recovery for extended periods in excised porcine corneas.

Summary of Experimental Procedures

- Excised porcine corneas normally discarded are prepared and placed in culture dishes
- Corneas are topically dosed and stained with a fluorescent dye revealing loss of tight cellular junctions or opacities
- Corneas are cultured using a unique air interface system
- Corneas are evaluated over time up to 21 days to assess reversal of damage

Assay Specifics and Advantages:

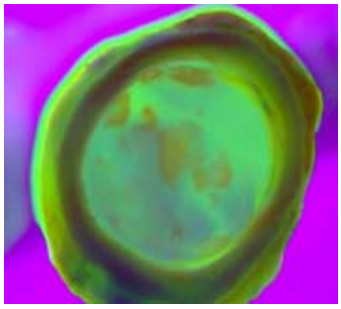
- Uses excised corneal tissue
- Uses unique air interface tissue culture method
- Topical dosing possible
- Very effective in discriminating between severe ocular irritant and ocular corrosives

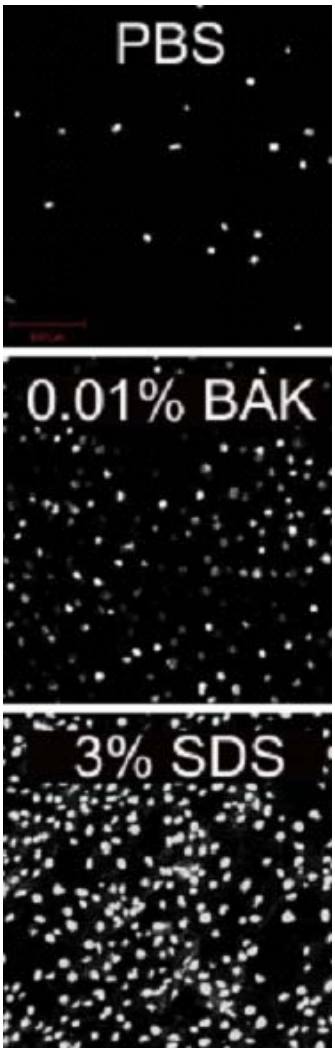
Fluorescent stain retention by corneal epithelium has proven to be an effective tool in the assessment of ocular damage after exposure to irritants. This method is non-destructive and allows for re-staining of the same tissue over the course of the study. The area of damage is clearly defined and easily observed almost immediately after staining.

1. Patent pending.

MB Research Labs, PO Box 178, 1765 Wentz Road, Spinnerstown, PA 18968
Tel: 1-215-536-4110 Fax: 1-215-536-1816 Email: mbinfo@mbresearch.com
www.mbresearch.com

PorFocal – Porcine Confocal Assay





Background

MB is now in the early stages of developing a variation of the PorCORA: a high-sensitivity Porcine Confocal assay (PorFocal) that uses individual cell death visualized and quantified with fluorescence confocal microscopy as an endpoint.

Summary of Experimental Procedures

- Excised porcine corneas normally discarded are prepared and placed in culture dishes
- Corneas are cultured using unique Air Interface Method
- Corneas can be topically dosed multiple times a day (1 – 3x per day)
- Corneas can be kept viable for up to 4 weeks
- Ethidium Homodimer, a fluorescent dye that attaches to cellular DNA, is used to stain cells with compromised cell membranes.
- Tissue is “optically sectioned” and viability of each tissue is assessed by confocal microscopy

Assay Specifics and Advantages:

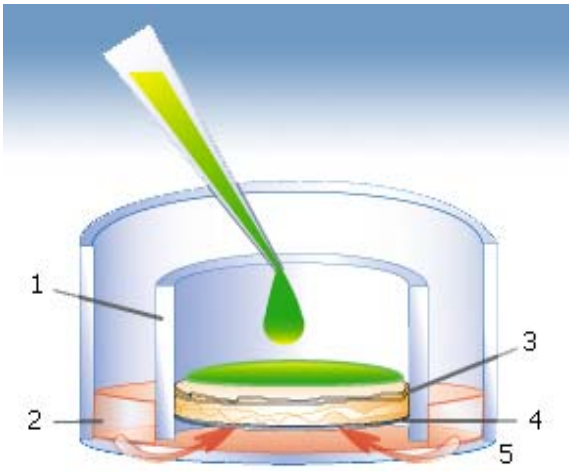
- Uses excised corneal tissue that would normally be discarded
- Uses unique Air Interface tissue culture method
- Topical dosing possible multiple times a day
- Incubation possible up to 4 weeks
- Very effective in discriminating between slightly/mildly irritating and non-irritating test articles.

MB Research Labs, PO Box 178, 1765 Wentz Road, Spinnerstown, PA 18968
Tel: 1-215-536-4110 Fax: 1-215-536-1816 Email: mbinfo@mbresearch.com
www.mbresearch.com

MatTek EpiDerm™

3D Human Tissue Constructs

MatTek EpiDerm™



1. Cell Culture insert
2. Media
3. Human keratinocytes, Air Liquid Interface
4. Micro-porous membrane
5. Tissue is fed thru micro-porous membrane

Background

MB Research has vast experience with 3-D tissue constructs. We have evaluated over 2500 test articles using ocular, dermal, vaginal, full-thickness and phototoxicity models.

The MatTek EpiDerm™ tissue model is a three-dimensional tissue constructed of stratified, differentiated human keratinocytes on a micro-porous membrane. EpiDerm™ has barrier function properties similar to normal skin and an Air Liquid Interface (ALI) that exposes the surface of the tissue to the air and allows for topical dosing.

EpiDerm™ is an excellent solution for screening cosmetics and other industries for irritancy and toxicity. Recently, EpiDerm™ has been recommended as an alternative for dermal corrosivity by US and EU regulatory agencies.

Assay Specifics and Advantages

- Cytotoxicity via MTT
- Skin Irritation Testing (recently validated as alternative by ECVAM)
- Inflammatory mediator and cytokine release (PGE_2, TNFa, IL1a, and other interleukins)
- Histological examination (H & E) of the epidermal layers, including the stratum corneum
- EpiDerm results can be correlated to *in vivo* irritation classifications
- Validated for corrosivity testing by US and EU

Other MatTek Tissue Models Available

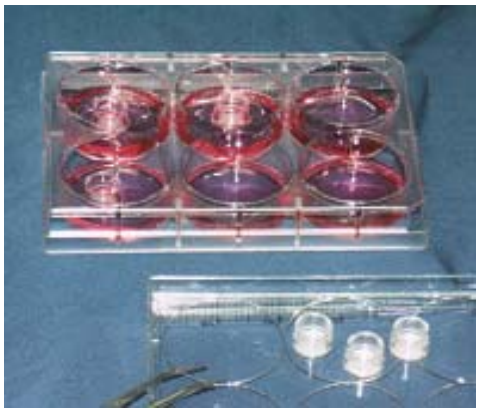
- EpiOcular™
- EpiVaginal™
- EpiOral™
- EpiAirway™
- Full Thickness
- MelanoDerm™

MB Research Labs, PO Box 178, 1765 Wentz Road, Spinnerstown, PA 18968
Tel: 1-215-536-4110 Fax: 1-215-536-1816 Email: mbinfo@mbresearch.com
www.mbresearch.com

MatTek EpiOcular™

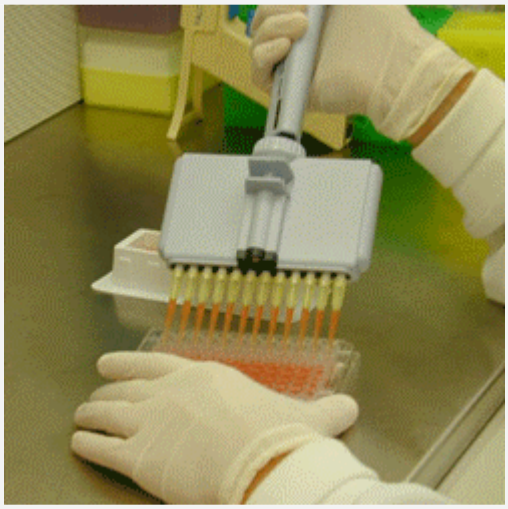
3D Human Tissue Constructs

EpiOcular™ is cultured to form a stratified, squamous epithelium. They consist of basal, spinous, granular, and cornified layers analogous to those found *in vivo*.



Assay Specifics and Advantages

- Cytotoxicity via MTT
- Skin Irritation Testing (recently validated as alternative by ECVAM)
- Inflammatory mediator and cytokine release (PGE_2, TNFa, IL1a, and other interleukins)
- Histological examination (H & E) of the epidermal layers, including the stratum corneum
- EpiOcular™ results can be correlated to *in vivo* irritation classifications
- Excellent for assessing ocular irritancy for cosmetic formulations



Other In Vitro Assays Offered:

- Agar Overlay Cytotoxicity (ISO 10993-5 and USP 23)
- Cytotoxicity – based on ICCVAM protocol
- Corrositex – alternative to Draize – DOT Packing Groups
- Mechanistic
  - Nitric Oxide Sythase (Irritation/Inflammatory Mediator)
  - Peroxide Production Assay (Lipid Peroxidation)
  - Tyrosinase Inhibition Assay
  - Angiogenesis/Anti-Angiogenesis
- Photobiology/Phototoxicity
  - 3T3 Neutral Red Uptake Phototoxicity Test – OECD 432
  - EpiDerm™ Phototoxicity

MB Research Labs, PO Box 178, 1765 Wentz Road, Spinnerstown, PA 18968
Tel: 1-215-536-4110 Fax: 1-215-536-1816 Email: mbinfo@mbresearch.com
www.mbresearch.com