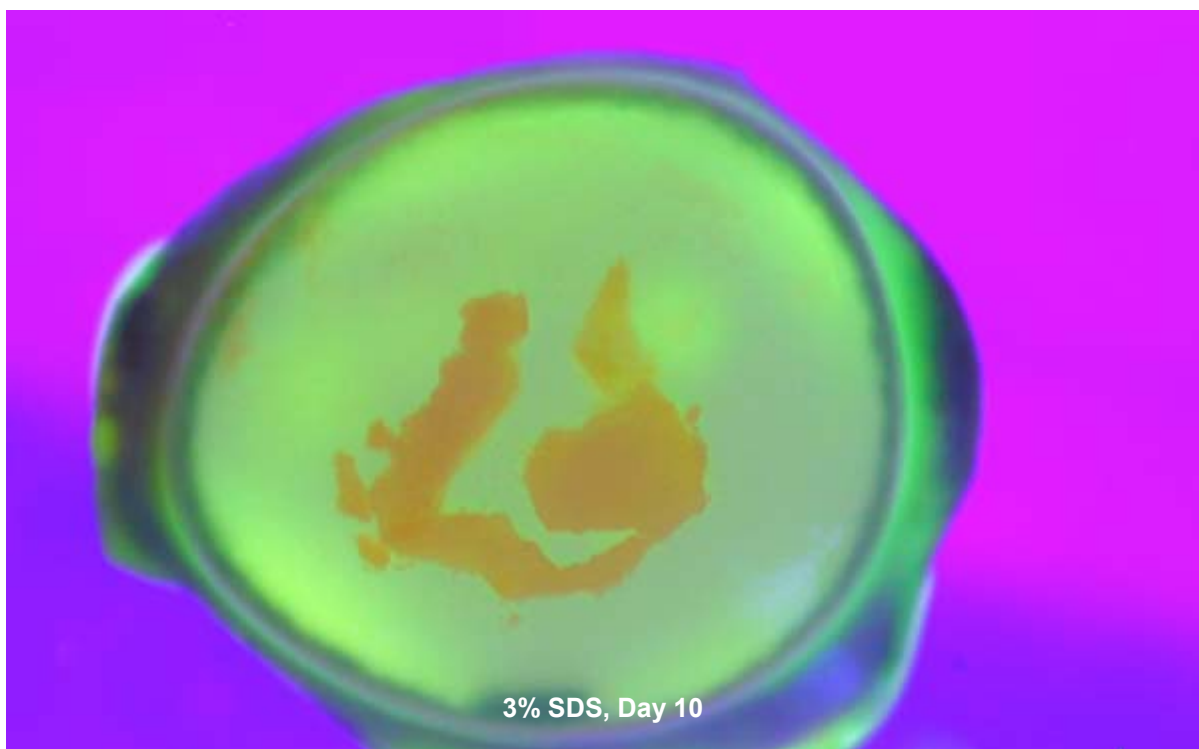


TOXNOTE

PorCORA

Porcine Cornea Opacity/Reversibility Assay
An Alternative to the Draize Ocular Irritation Assay



Protocol Now Available.

The new PorCORA protocol has been designed to allow the measurement of reversibility of corneal damage, using fluorescein retention.

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Introduction

MB Research has developed the Porcine Cornea Opacity/Reversibility Assay (PorCORA) - an alternative testing protocol for eye irritant reversibility in excised porcine corneas. *Ex vivo* corneal epithelium is dosed topically, similar to the BCOP, but by culturing the excised corneas for up to 3 weeks, the potential for the damage reversal can be measured.

The Missing Puzzle Piece

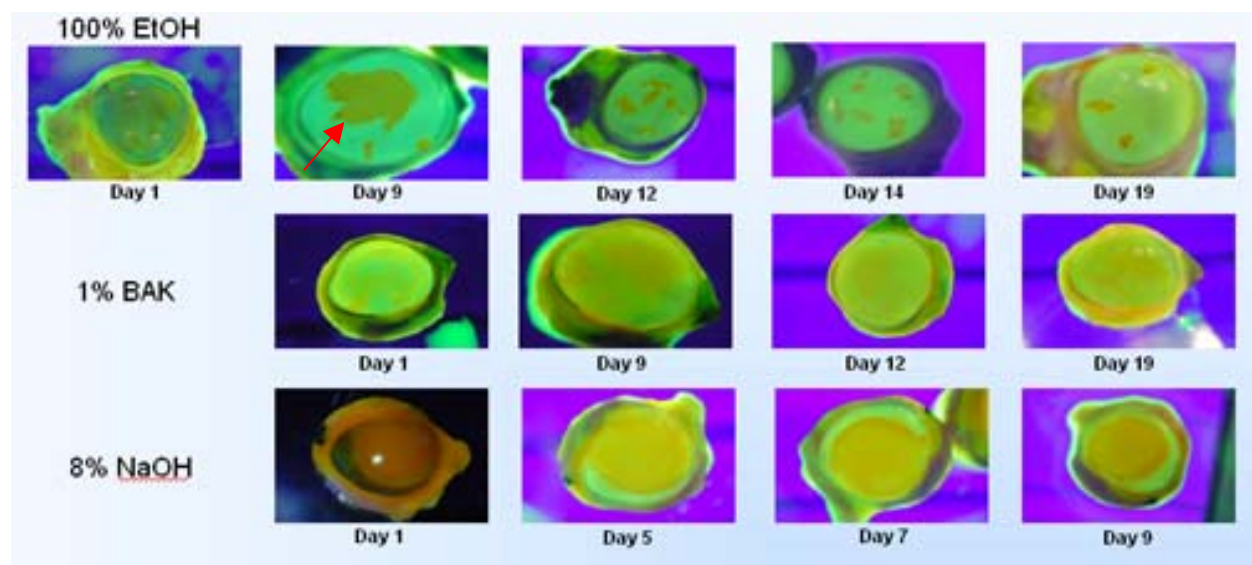
There are several alternative methods to characterize aspects of eye irritation and damage, but no established method can model recovery after injury as in a Draize test. PorCORA was developed to fill this void by measuring corneal damage and recovery for extended periods in excised porcine corneas. Combined with other alternative assays, such as the **HET-CAM** or **CAMVA** for assessment of conjunctival injury and vascular damage and the **BCOP** for assessment of acute ocular irritation, PorCORA is the missing piece that allows evaluation of corneal healing.

PorCORA evaluates similar mechanisms to those occurring in an *in vivo* Draize assay, in which test substances are placed directly onto corneal tissue. Any damage to the tissue is visualized by the retention of fluorescein. Tissues can be repeatedly stained with fluorescein to assess changes in the area of damage over time. Therefore, the potential for recovery in ocular tissue is monitored using the percent of area of the cornea that retains fluorescein in an *ex vivo* alternative model. Because corneal damage is generally the most persistent of ocular injuries, the time course measurements in the PorCORA are justifiable in modeling both reversible and non-reversible eye damage.

Current Validation Developments

Tracking Reversibility

For almost two years, MB Research Scientists have been developing assay methods in order to create a cost-effective protocol to offer you a viable alternative to the Draize Rabbit Eye Test. In validation experiments, we have been able to maintain excised corneas for over three weeks with minimal morphological changes to the corneal epithelium. The photos below show that the area of fluorescein staining decreased over time for the same cornea treated with 100% EtOH indicating reversibility; the stained areas become smaller with time in culture. In 8% NaOH-treated corneas, the area that retained the stain did not decrease over time, indicating irreversible damage. Although the corneas treated with 1% BAK showed some recovery, significant damage was still observed at Day 19. No significant fluorescein retention was visualized in corneas treated with DPBS (data not shown). These early results show PorCORA's potential to discriminate eye irritants that cause reversible damage (100% EtOH) from those that irreversibly damage corneal epithelia (8% NaOH).



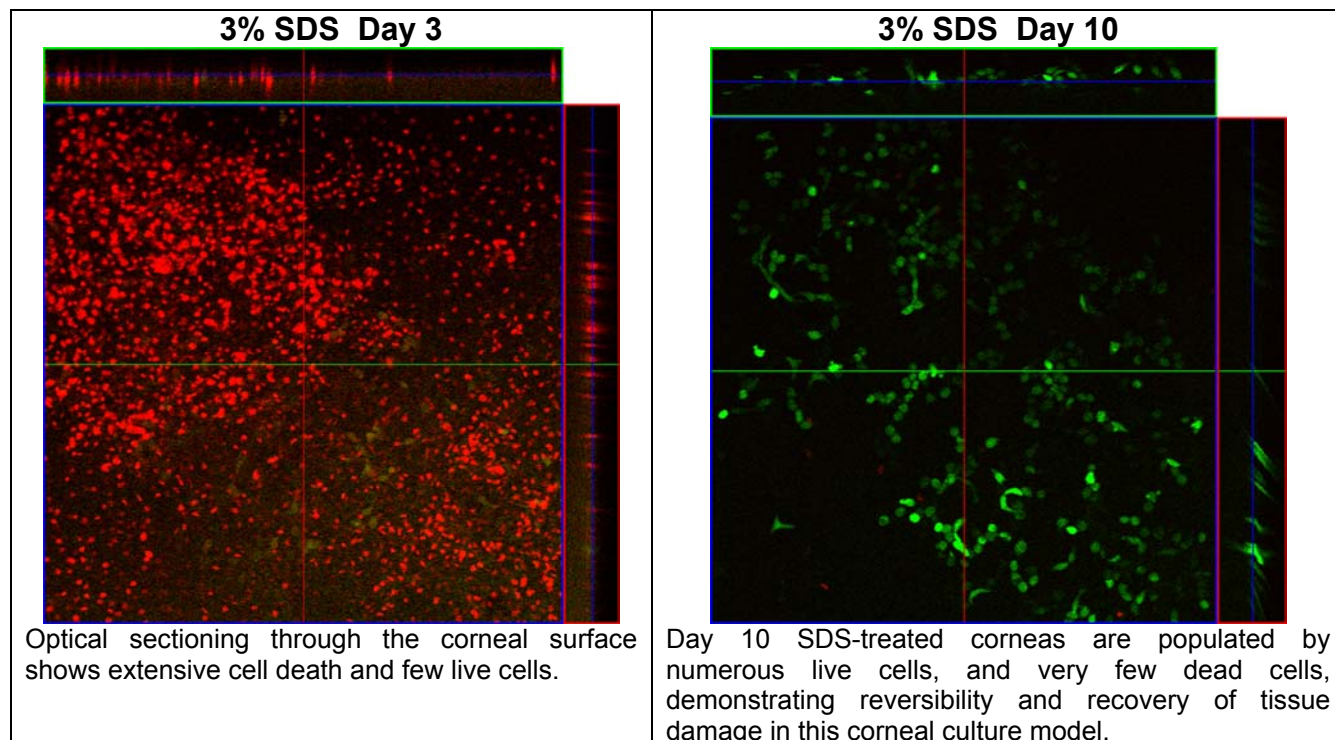
Current Validation Developments (cont'd)

Tracking Reversibility (cont'd)

Confocal Microscopy and Live/Dead Staining.

To confirm recovery of corneal epithelial cells post-dose in PorCORA, cell viability was visualized by using confocal microscopy in conjunction with a live/dead fluorescent staining kit.

Corneal cell viability was consistent with typical rates of recovery from SDS treatment seen by fluorescein retention. We also observed a dense area of live cells localized adjacent to a dense area of dead cells on a recovering corneal surface.



Confocal microscopy results support the current model of corneal recovery by epithelial sheet migration. At Day 10, the SDS-treated corneas were populated with many live epithelial cells and shows that re-epithelialization occurs during the time course of this culture. Similar validations were performed in DPBS.

Conclusions

- **PorCORA is an alternative test for eye irritancy and reversibility that does not use live animals.**
- **Re-epithelialization of the corneal surface can be measured with fluorescein stain.**
- **Fluorescein stain measurements are representative of actual tissue damage determined by a live/dead staining kit and observation by confocal microscopy.**
- **PorCORA may fill the gap that is left by current alternative eye irritation assays since it has the potential to determine recovery after initial eye irritation.**

If you would like to learn more about **PorCORA** or would like a copy of **Protocol 441C**, please feel free to contact MB Research at: **215-536-4110** or by sending an email to: **mbinfo@mbresearch.com**.