

Bovine Corneal Opacity and Permeability Assay

Theory: The purpose of this assay is to evaluate the potential ocular irritancy/toxicity of a test article as measured by the test article's ability to induce either 1) corneal opacity, which may be caused by protein denaturation and cell precipitation, or the induction of stromal swelling, and 2) corneal permeability to fluorescein, reflecting a loss in the cell to cell membrane junctions and barrier properties of the corneal epithelium.

Applications and Use

- The BCOP model is a biologically complex ex vivo model with endpoints similar to many human corneal responses, yet is relatively inexpensive.
- Histological evaluation of the tissue may be performed.
- BCOP is particularly suited for moderate to severely aggressive materials, where other models may not be suitable.
- Multiple endpoints allow the investigation of mechanisms of action.
- The BCOP currently provides the best depth of injury and recovery predictions.

Experimental Procedure

Bovine Cornea Preparation

- Bovine eyes are received shortly after the slaughter of the animal so the cells are still viable.
- The corneas are excised, mounted in the BCOP chambers and allowed to equilibrate in MEM supplemented with 1% FBS and without phenol red (Complete MEM) for one hour.

Sample and Positive Control Preparation

- The samples can be tested undiluted (neat) or at any concentration in a variety of solvents.
- Positive and negative controls are tested and are the basis for assay acceptance criteria, and reproducibility.
- Individual positive controls are used for the testing of either liquid or solid test materials. Ethanol at ~100% is used for the liquid protocol and Imidazole at 20% in Complete MEM for the solid protocol.

Assay Procedure

- The bovine eyes are harvested shortly after the animal is terminated, and shipped in cold HBSS supplemented with 1% Pen/Strep.
- The bovine corneas are carefully checked for any damage (opacity, cuts, vascularization, etc.) before being excised and mounted in the special BCOP chambers with Complete MEM and incubated at 32°C for one hour.
- After the one hour incubation an initial opacity for each cornea is read in an opacitometer and recorded.
- The corneas are divided into groups between 3-6 corneas per test article per exposure time and exposed to the test article at the concentration requested.
- A 750 µl aliquot of test material is exposed to the cornea. The material can be tested at any concentration and over a range of exposure times.
- The test article is rinsed from the cornea and opacities read and recorded before an additional 32°C incubation.
- Post treatment incubation times may be varied to enhance post exposure expression of irritancy.
- A post incubation opacity reading is taken on each cornea and recorded.
- The initial opacity reading is subtracted from the post incubation opacity to calculate the final opacity reading.
- A permeability test is performed to measure the passage of fluorescein stain through the cornea. A medium sample is removed from the posterior end of the chamber and measured spectrophotometrically (490nm) to determine the amount of fluorescein leakage.
- Both opacity and permeability scores are used to calculate the final BCOP score.
- Corneas may also be fixed, sectioned, and examined in histopathology.

Data Evaluation

- 0-25 is considered a mild irritant, 25.1-55 is considered moderate, and 55.1 and above is considered severe.
- It is important to address the individual contributions of opacity and permeability relative to the chemical class tested.