GUIDELINES FOR HISTOPATHOLOGICAL EVALUATION OF
BOVINE CORNEAS AS AN ENDPOINT OF THE BOVINE CORNEAL
OPACITY AND PERMEABILITY (BCOP) ASSAY

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1. **Brief Introduction of the Bovine Corneal Opacity and Permeability Assay**

The Bovine Corneal Opacity and Permeability (BCOP) assay was developed by Drs. Pierre Gautheron (Gautheron, Dukic et al. 1992) and Joe Sina (Sina, Galer et al. 1995) to address the ocular irritation potential of pharmaceutical intermediates for industrial hygiene purposes. The assay measures changes in corneal opacity (determined by changes in light transmission) and permeability to fluorescein (measured by increases in permeability to fluorescein) as a result of chemical insults. The opacity and permeability values are used to calculate an *in vitro* score, in order to evaluate the ocular irritation potential. The method is now widely applied across industries and chemical/formulation classes. For many chemical/formulation classes, the mode of action(s) of the test material is generally known. Membrane lysis, protein coagulation, and saponification are common modes of action that lead to ocular irritation. The BCOP assay closely models not only most of the initial stages of interaction of an eye irritant with the cornea, but also some of the more latter occurrences in eye irritation such as gross tissue changes in the corneal stroma. In our experience at the Institute for In Vitro Sciences, Inc. (IIVS), the opacity and permeability endpoints have generally reflected the epithelial and stromal changes associated with these types of damage. However, the prediction of ocular irritation from chemicals that react with nucleic acids, mitochondrial proteins, or other cellular targets, that do not lead to immediate loss of cellular integrity (particularly in the epithelium) has proven more difficult using only the opacity and permeability endpoints, and the short time period that the cornea can be kept in organ culture limits the amount of recovery, if any, which may occur, prompting the investigation of histopathology as an additional predictive endpoint.

The BCOP assay may be ideally suited for histopathology since it uses *ex vivo* corneal tissue that can be maintained fully viable using the standard organ culture procedures of the BCOP protocol, and in fact can be maintained for at least 24 hours with repeated medium changes. The bovine corneas contain the three layers of interest in the cornea; the epithelium, stroma, and endothelium, requisite for differentiating among minimal, mild, moderate and severe ocular irritants. Using standard histology preparation techniques, cellular and architectural changes to the three layers of the cornea can be observed. Lastly, whereas initial injury after chemical exposure can be determined using the standard BCOP assay and complementary histopathology for most chemistries, the progression of nuclear and cellular changes from chemistries inducing delayed effects can be monitored for up to 20 hours after exposure using a modified extended post-exposure protocol.

2. **Depth of Injury as a Predictor of Degree and Duration of Ocular Injury**

Jester *et al.* (1998), Maurer *et al.* (1996, 2002) and others have shown for a range of chemical classes that depth of injury in the early hours after exposure can be predictive of the eventual degree and duration of the ocular lesions. Epithelial damage alone, in the rabbit cornea, is associated with expected recovery provided the basal lamina is intact. Deeper injury into the stroma has more serious consequences, while a full thickness injury including damage or loss of the endothelium would be predictive of a severe injury. With the addition of the histopathological assessment of depth of injury, the BCOP would seem to address most of the requirements proposed by Maurer and Jester. If the hypothesis of Jester, Maurer, and others that initial area and depth of injury is predictive of time to, and extent of recovery, then the measurements made by the BCOP assay may have a relationship to recovery as
well.

Since test materials are applied topically on the outer corneal epithelium of the bovine cornea in the BCOP assay, a top-down evaluation follows the potential for lesions to occur dependent upon the penetration of the test chemical into the cornea, and the potential for toxic effects to be induced. Therefore, the evaluation of changes in the treated corneas would be performed starting superficially with the squamous epithelium at the site of test material exposure, and progressing deep into the cornea to the endothelium.

Redden et al. (2009) presented on an evaluation of the BCOP assay with corneal histopathology for predicting the eye irritation potential of a series of anti-microbial products with certain cleaning claims which had been previously classified in vivo, where the depth of injury model was fundamental to the in vitro classifications. The BCOP histopathology findings showed that anti-microbial cleaning products predicted in vivo to have an EPA category IV classification (approximately consistent with a Globally Harmonized System (GHS) No Label classification) generally induced cellular damage or corneal changes in the in vitro assay no deeper than midway through the epithelium. Furthermore, those products predicted in vivo to have an EPA category III classification (approximately consistent with a GHS 2B classification) generally induced cellular damage or corneal changes in vitro in the corneal epithelium and extending no deeper than the upper third of the stroma. Those products predicted in vivo to have an EPA category II classification (approximately consistent with a GHS 2A classification) generally induced corneal changes in vitro extending no deeper than two thirds of the stroma. Finally, those products predicted in vivo to have an EPA category I classification (approximately consistent with a GHS 1 classification) generally induced corneal changes in vitro extending into the lower third of the stroma.

3. Application of Histopathology to the Determination of Ocular Irritation Potential

Not all BCOP studies require histopathological evaluation. Where the mode of action of the active chemical(s) on the cornea is well understood, the opacity and permeability endpoints typically provide sufficient predictive capacity to determine ocular irritation potential. However, histopathology may be included in the BCOP study protocol for several reasons. For hazard assessments, histopathology is performed to determine whether any test material-induced changes in the treated corneas are evident, relative to the negative control-treated corneas, and in the presence of such changes, the depth and degree of these changes are reported. Histopathology can either complement the BCOP assay by elucidating the cellular and architectural changes associated with the increases in opacity and fluorescein permeability, or it could provide evidence of corneal injury not revealed by the BCOP endpoints alone. In product development and research settings, histopathology may be helpful to understand the types of lesions that a material might induce, or to elucidate modes of action.

The inclusion of chemically-relevant concurrent benchmark formulations and chemistries may help facilitate interpretation of the study data. When formulations contain reactive chemicals (e.g., peroxides, bleaches, etc.) where immediate changes in corneal transparency or barrier function may not be evident, histopathology is highly recommended. Maurer, Molai et al. (2001) have shown that oxidizing agents induce a delayed toxicity in vivo and act more profoundly on the stromal keratocytes. Our experience has shown that peroxide-containing formulations require histopathological assessment to elucidate the full depth of
injury (Swanson, White et al. 2003) since it is the cellular/nuclear changes observed histologically that are predictive of downstream corneal degeneration. Accordingly, as a rule, new chemistries where the mode of action cannot be predicted, or when “reactive chemistries” may be expected, may require including a modified extended post-exposure protocol and histopathology (Curren, Evans et al. 2000) to the standard BCOP protocol. Finally, positive and negative control corneas are always used (Cuellar, Merrill et al. 2002). The positive controls provide a measure of reproducibility of the test system and assay execution, while the negative controls provide the baseline against which histological changes are compared.

4. Overview of the Histology Procedures Used at IIVS

4.1 Corneal Accession Numbers

During the normal course of a BCOP assay, each cornea is identified by the individual corneal holder number. For those corneas requiring histology, each cornea is further assigned a unique histology accession number, which relates the corneal holder number to the accession number, test treatment or control designation, exposure time, harvest date, and any relevant comments. The documentation is maintained in the study notebook and in the histology logbook.

4.2 Fixation of the Corneas

The corneas are fixed after the completion of the fluorescein penetration step of the BCOP assay to allow the histological evaluation to be performed on the same corneas from which the opacity and permeability values were taken. In-house evaluation has shown that the fluorescein exposure does not impact tissue morphology. Once the posterior chamber of the corneal holder has been sampled for fluorescein penetration, the remaining fluids are removed and the corneal holder is dismantled. Each cornea is placed into a tissue cassette that has been pre-labeled with its accession number, and which ash been fitted with a “histology sponge” to help protect the endothelial surface. The cornea will be placed onto the sponge with the epithelium facing up. The tissue handling should be conducted carefully but expediently to prevent the cornea from drying during the transfer process. The cassette is closed and immersed in 10% neutral buffered formalin. It is important to assure that the tissue is fully submerged in the formalin. Approximately 20 cassettes are fixed in a volume of 300 mL. Corneas should be fixed at room temperature for at least 24 hours before processing.

Whereas the use of a Davidson's fixative is frequently recommended for the rapid fixation of whole globe eye tissue, in-house comparisons of excised bovine corneas fixed in Davidson's fixative and 10% neutral buffered formalin have shown that the latter has provided a better fixation of the tissues.

4.3 Preparation of the Slides

Corneas are trimmed, embedded, sectioned and stained by a qualified histology laboratory. The following general guidance is provided to the histology laboratory:

The actual size of the cornea and the treatment area are shown below. The center shows the area treated and the area of interest for histological examination.
The fixed corneas are transferred to the histology laboratory. Placement paperwork will be sent with the corneas. Upon receipt by the histology lab, the samples will be received into their tissue accession system.

The corneas may be trimmed to remove some of the excess scleral tissue outside of the outer crush zone if necessary; however, the cornea will be infiltrated whole. Before the corneas are placed on the tissue infiltration machine, a second sponge will be placed on the anterior (epithelial) surface of the cornea. This second sponge is intended to reduce the chances of tissue warping during infiltration. Corneas are always mounted in the cassette at IIVS with the anterior surface facing the upper lid. Elevated temperatures should not be used with the infiltrating solvents.

Once infiltrated with paraffin, the cornea will be bisected so that the two halves of the cornea can be embedded in the same block. The cornea will have some wrinkles and so it is often helpful to cut across the wrinkles (if they fall in a particular orientation) so that a good cross section can be obtained when the tissue is sectioned.

The microtome cuts must produce as close to true cross sections (anterior to posterior) of the cornea as possible. The true cross section allows us to better measure increases in the thickness of the corneas (expansion as a result of test material exposure) relative to the thickness of the negative control-treated corneas. Large, deep molds must be used and great care in orienting the tissue is required. The two halves of the cornea are placed with the cut side down in the mold and aligned vertically and with their long axis in parallel with the long axis of the mold.

If the tissue is too long to fit into the mold, the outer edges (shown as the dark outer area above) may be trimmed. Ideally, one would like to see some of the dark “crushed” tissue in the section so as to be assured that one is evaluating the whole cross section of the cornea. Having the two tissue strips oriented in parallel along the long axis of the slide (once they are cut) makes scoring much easier.

The tissues are sectioned to approximately 5 microns. Two tissue sections (one section from each half of a cornea) are mounted on a slide. Sectioning the tissue requires that the area damaged by the bisecting cut be trimmed away (with the microtome) so that the artifacts introduced by the cutting are not mistaken for changes associated with the test material exposure. The sections must include the full cross section of the cornea (epithelium,
stroma, and endothelium). The corneal stroma is quite delicate and prone to artifacts from over-stretching of the sections on the water bath. In addition, over-stretching will induce breaks between the epithelium and stroma that might be mistaken for test material-induced damage. Finally, the tissue sections are stained with hematoxylin and eosin (H&E).

5. Evaluating the Corneal Histology

5.1 Evaluation of the Corneal Sections (Overview)

The goal of the histopathology is to determine whether any changes from the control corneas are evident, and to determine the nature, depth and degree of the observed histologic changes within the treated corneas. The three tissue layers of the cornea are evaluated for histologic changes. Typically, the evaluations are conducted top-down, starting with the upper epithelium and progressing through the epithelial layers, through the stroma, and down to the corneal endothelium. Since test materials are applied topically on the outer corneal epithelium, the top-down evaluation follows the potential for histologic changes to occur dependant upon the penetration of the test chemical into the cornea, and the potential for toxic effects to be induced.

In some cases, quite evident or notable changes are observed in the treated corneas. For example, gross changes or erosion of the corneal architecture, whether limited in depth of injury or as a full corneal thickness injury would be readily apparent. Other lesions may be a bit more subtle, and may simply be limited to changes in nuclear staining, perhaps as an apoptotic event. In such cases, these changes may be expected to result in downstream loss of corneal function, which may not be evident by the opacity and permeability endpoints in the standard short-term BCOP assay. While in general, the depth of observed changes in the bovine cornea can be reported, the degree and impact of many of the observed changes may not be fully assessed or known. For example, the observation of abnormal nuclear staining in stromal keratocytes is not readily described in terms of a scale or degree of abnormality. Rather, an attempt is made to present the relative frequency of such observed changes relative to the negative control corneas. As another example, stromal edema may be presented in terms of the degree of stromal expansion, but should be characterized by the relative degree of stromal expansion in the upper, mid and lower stroma. Furthermore, stromal edema, resulting from loss of epithelial barrier function, in and of itself may not have the same consequences to corneal recovery as stromal edema with loss of keratocytes, or stromal edema occurring in concert with stromal protein precipitation. Therefore, the interpretation of the histopathology should allow for an integration of all of the observed changes, prior to the definitive assessment.

5.2 Evaluation of the Quality and Acceptability of the Corneal Sections

Negative control corneas were processed with the test article-treated corneas as a common histology batch process. The histology of the negative control corneas may thus be used to evaluate the quality/acceptability of the slides within the processing batch. Prior to conducting the evaluation of test article-induced histopathology, the quality of the H&E stained corneal sections must be evaluated, so that the nature and degree of the artifacts of both the BCOP assay and the histology processing can be assessed. To this end, the negative control slides are used to detect artifacts at the batch level. They are also used to assess “normal” staining (degree of hematoxylin or eosin in each layer/cell type), tissue
architecture and general thickness. Slides are normally stained with hematoxylin and eosin (H&E) although other stains may be requested. The following photographs are of negative control (tissue culture-grade deionized water) treated corneas.

Slides are thoroughly examined by microscopic evaluation. Each cross section of each cornea within a treatment group is observed first under low magnification for an overall assessment of the quality of the tissue sections for conducting the histopathology. Corneal sections are evaluated across the entire section from one crush zone to the other (the crush zone is the outer corneal perimeter where the cornea was mounted against an O-ring within the corneal chamber, and is readily apparent in the histology sections). It is not uncommon for areas within a tissue section to have aberrations or artifacts of processing which preclude those areas from being used in the histopathology. Furthermore, occasional processing artifacts may render an entire tissue section, or both sections from a cornea, unacceptable for use in the histopathology.

In the ideal, the corneal sections are prepared as true cross sections rather than tangential sections. However, some fraction of the sections (or portions of a section) will not be true cross sections and so the overall thickness and tissue architecture will be distorted from the ideal. There may also be some fields in a section that are tangential even when most of the section is a good cross section. In control or minimally damaged corneas, stromal thickness provides a good indication of how true the cross section is. In addition, the thickness of Descemet’s Membrane may provide a good measure of a true cross section. The Descemet’s Membrane in a good section of a control cornea can be used to compare with treated corneas. This measure can be especially helpful in sections of treated corneas where collagen matrix expansion and stromal swelling are evident.

![Bovine cornea treated with tissue culture grade deionized water cut on a tangent such that Descemet’s Membrane has two distinct sections.](image)

It is essential that all layers of the cornea be included in the section. Poorly trimmed blocks may produce sections where the full depth of the cornea is not present (e.g., the lower stroma and endothelium missing). It may be necessary to request recuts of such slides.
Bovine cornea treated with tissue culture grade deionized water with lower stroma and endothelium missing (left figure), and epithelium missing (right figure).

Slides are usually stained with an automated slide stainer. The intensity of staining depends on several factors and may vary slightly across studies. Decreased hematoxylin or eosin staining can markedly compromise interpretation and photography. Such slides should be returned for restaining or recutting. Note that highly swollen stromal collagen will appear to be poorly stained but, in fact, there is a great deal of empty space between the fibers that is not stained.

Bovine cornea treated with tissue culture grade deionized water with hypoeosinophilic staining of the epithelium (left figure) and areas of hypereosinophilic staining of the stroma (right figure).

There are many other types of histological artifacts that are not presented here. Below is a selection of figures from tissue culture grade deionized water-treated bovine corneas which show histological artifacts which result in difficulty interpreting the test material-induced damage. These artifacts are, but not limited to, folding of the tissue, vibration of the blade, nick in the knife blade, and inadequate dehydration.
Folding of the tissue obscuring the epithelium and stroma

Microtomy artifacts (cuts in the endothelium, separation of the epithelium from the Anterior Limiting Lamina)

Vibration of the tissue specimen causing separation of the collagen fibers in the stroma.

Nick in the knife black causing separation throughout the entire tissue.

Microtomy artifact causing separation of the epithelium from Anterior Limiting Lamina and halo around the epithelium nuclei.

Inadequate dehydration of tissue causing artifacts appearing as bubbles.
5.3 Recording Observations

Observations of treated corneas are recorded. The data include the treatment group, slide (cornea accession) numbers, exposure time, post-exposure expression time, date of test material application, observations on each corneal tissue layer, and the related figure numbers (where appropriate). Once the observations are completed they are formalized in the Histology Report. The observations are typically peer reviewed, and upon consensus the Histology Report is signed and dated by both the Principle Investigator/Lead Histopathologist and the Peer Reviewer.

5.3.1 Evaluating the Negative Control-Treated Corneas

Negative control corneas are treated with sterile, deionized water or saline in parallel with the positive control and test material-treated corneas. The overall thickness of the bovine cornea is generally between 850-1000 µm.

Epithelium: The negative control-treated epithelium is composed of three layers. The basal cell layer is a well-formed, columnar-cell region directly attached to the basement membrane above the Anterior Limiting Lamina. The basal cells were always tightly attached to each other. Several layers of wing cells covered the columnar basal layer. In both of these layers, the cell nuclei showed diffuse chromatin without clear nucleoli. Rare mitotic figures were seen in the basal layer. The squamous layer was flattened with limited cytoplasm and highly condensed nuclei.

Stroma: The stromal elements begin with the Anterior Limiting Lamina and are composed of well-organized collagen matrix fibers with dispersed keratocytes. Keratocyte nuclei show a range of morphologies from moderate sized (smaller than the epithelial nuclei) with diffuse basophilic staining to narrow, elongated and condensed with dark basophilic staining. Cytoplasmic staining, when visible, is moderately basophilic. Rarely, cells with eosinophilic cytoplasmic staining may be observed. Collagen bundles are generally parallel and well ordered. The Descemet’s Membrane is prominent and forms the bottom of the stroma. The overall thickness of the stroma is approximately one 20x field when a good cross section is obtained.

Endothelium: The endothelium is a single layer of flattened cells attached to the basal surface of the Descemet’s Membrane. Nuclei are elongated and flattened. In a cross section, little cytoplasm is visible. Generally, the cells are firmly attached to the Descemet’s Membrane but in some areas (or fields), they may be detached or lost through mechanical damage.

5.3.2 Evaluating the Corneal Histologic Changes

The goal in scoring the corneal damage and changes is to record the nature, degree and depth of the changes in each tissue layer. In most cases, the individual corneas in a treatment group will not be reported separately but rather they will be “averaged” to highlight the predominant lesions. The opacity and permeability values should be reviewed before scoring.
the slides. If there are wide variations among the corneas in either the indirect measures or histological changes, it may be necessary to report on some individual corneas within the treatment group.

**Epithelium:** Characteristic histologic changes observed in the epithelium are **cell loss**, **cell coagulation** (especially in the squamous epithelial layer), **nuclear vacuolization** (swelling), **nuclear condensation** (pyknosis or precipitation of nuclear proteins and/or DNA), **cytoplasmic vacuolization**, **cytoplasmic precipitation** (leaving only the cytoskeleton), and separation of the cells from the Anterior Limiting Lamina. The degree of the changes may be somewhat subjective. It may be the fraction of the cells showing a histologic change or the magnitude of the histologic change within the cells or cell layer (e.g., degree of cytoplasmic vacuolization). The **depth of the histologic change** relates to the number of cell layers impacted. Damage to or loss of the Anterior Limiting Lamina will be reported with the epithelium.

**Stroma:** The histologic changes of the stroma are reported in two parts; those of the **extracellular collagen matrix** and those of the **keratocytes**. The predominant histologic changes seen in the **extracellular collagen matrix** are expansion (loss of the normal ordered array of the fibers), and **stromal coagulation** or denaturation. Stromal swelling may be detected by the presence of expansion “holes” in the organized collagen matrix. Their appearance suggests that liquid has entered the matrix, expanding space between the fibers. The **depth** (see below) and **degree of expansion** are reported. The **degree refers to the relative frequency and size of the expanded “holes” in the matrix.** As the degree and/or depth of expansion increases, the overall thickness of the stroma would be expected to increase. **Stromal coagulation** appears frequently as areas with tightly bound collagen fibers with **hypereosinophilic** staining. The tightly bound collagen fibers may be interspersed between areas of stromal swelling or expansion.

Since depth of injury (both extracellular and cellular) to the stroma is important in the analysis, a means to describe the depth of injury is necessary as part of this analysis. Determining the depth is not always a straightforward process. Because of the topical application of the test material to the epithelium, one would expect that exposure to the stroma would progress from the area just under Anterior Limiting Lamina down through the stroma to Descemet’s Membrane. There is no external inflammatory process in the isolated corneas, so one might also expect the progression of damage to follow the progression of exposure. That means that damage to the stroma should first appear close to Anterior Limiting Lamina. As the damage to the stroma increases, deeper layers might be involved. This implies that one would want to **express damage as progressing from the anterior (Anterior Limiting Lamina) to the posterior (Descemet’s Membrane) and express it as a fraction of the total stromal depth involved.** However, collagen matrix expansion can increase stromal thickness. Thus, measurements of the depth of a stromal lesion can be complicated by the change in overall stromal thickness. Depth of stromal damage is reported as the percentage of the normal corneal depth (cross section) involved, starting from the anterior.
border (Anterior Limiting Lamina). However, to account for stromal swelling or expansion, this depth is actually estimated from the percentage of the stromal cross section that remained undamaged (starting at the posterior border). For example, a cornea reported to show collagen matrix expansion to 30% depth would mean that 70% of the cross section of that cornea (starting at Descemet’s Layer) did not show expansion.

An exception to the anterior to posterior progression of stromal expansion is caused by the loss of the endothelial cell layer. Since the endothelium is responsible for maintaining balanced hydration in the lower stroma, its loss (either through mechanical damage or test material toxicity) can lead to appreciable deep stromal swelling. It is important to differentiate between endothelial damage and expansion caused by the test material exposure and damage from other sources (e.g., mechanical). In the case of mechanical damage, the deep swelling can occur in the absence of expansion in the anterior stroma. Test material-induced damage should progress through the cornea and be manifested in both the anterior and posterior stroma. Sections or portions of sections where the endothelium is lost and posterior stromal swelling (collagen matrix expansion) is observed without similar anterior stromal swelling are likely the result of mechanical damage to the endothelium that occurred early in the assay (incubation). An effort should be made to score corneal sections that do not show such damage.

Histologic changes in the keratocytes are manifested in both the cytoplasm and nucleus. Rapid necrotic cell degeneration, as might follow exposure to a strong alkaline, organic solvent or surfactant, is quite apparent because the cellular components rapidly breakdown. Oxidative damage or DNA alkylation might produce more subtle damage (initially) but could also lead to cell death (delayed) and release of inflammatory mediators. Nuclear changes (pyknosis or karyorrhexis) are signs of this process. Progressive nuclear pyknosis or complete destruction are also signs of this process. Cytoplasmic changes include vacuole formation or loss of basic elements (mRNA for example) that are also indicative of the beginning of the degenerative process. The cell cytoplasm normally stains with both basophilic (hematoxylin) and acidophilic (eosin) stains. When the basic elements are lost, eosinophilic staining predominates. This change is termed keratocyte eosinophilia.

**Endothelium:** Histologic changes in this layer include cell loss and cytoplasmic degeneration (vacuolization). Since this layer is only one cell thick, mechanical damage has the potential to confound the evaluation. Where there is endothelial cell loss, it is important to evaluate surrounding fields for the presence of normal endothelium. Since the whole corneal surface is treated, a lack of a uniform changes to most of the endothelium would suggest mechanical damage to isolated patches rather than test material-induced damage. When mechanical damage occurs late in the assay or after fixation (e.g., during processing), little or no deep stromal swelling or expansion is expected.
5.4 Preparation of the Photomicrographs

Photomicrographs of the histologic changes are made to be “representative” of the observations and the degree of damage at the indicated depth observed in the treatment group. They are not intended to document damage, or be considered raw data. Images are prepared using a Spot Insight Digital Camera and Spot 4.0.8 software (Diagnostic Instruments, Inc., Sterling Heights, MI). The color balance of the images is sometimes corrected to better represent the colors that would be seen through the microscope. Each photomicrograph is documented in a study-associated digital image log. Once finalized, the image log for the study is printed, signed and dated by the scientist responsible. The finalized copy is placed into the study notebook. The photomicrographs are “pasted” electronically into the Histology Report.

6. A Short Compendium of Micrographs to Illustrate Negative Control-Treated (Normal) and Select Histologic Changes in Bovine Corneal Tissue

The following series of photomicrographs are intended to illustrate normal bovine corneal morphology and provide examples of the types of histologic changes that might be observed in the epithelium, stroma, and endothelium. It is by no means a complete listing of all histologic changes, but is intended to illustrate the types of changes mentioned in the discussion of corneal lesions. In some figures, the chemical and exposure are provided. When photomicrographs have been taken from unpublished client studies, test material information is omitted.

6.1 Negative Control Corneas

Figure 1 Negative control cornea (sterile, deionized water, 10-minute exposure, 2 hour post exposure) – Full thickness (4X)
Figure 2 Negative control cornea (sterile, deionized water, 10-minute exposure, 2 hour post-exposure) – Epithelium (20X)

Figure 3 Negative control cornea (sterile, deionized water, 10-minute exposure, 2 hour post-exposure) – Upper stroma showing normal collagen matrix organization and keratocyte morphology (40X)
Figure 4 Negative control cornea (sterile, deionized water, 10-minute exposure, 2-hour post-exposure) - Deep stroma and endothelium (40X)
6.2 Epithelial Damage

Figure 5 Sodium Lauryl Sulfate (1% SLS, 30-minute exposure) - Epithelial cell loss induced by surfactant exposure (40X)

<table>
<thead>
<tr>
<th>Opacity</th>
<th>Permeability</th>
<th>In Vitro Score</th>
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<tr>
<td>3.2</td>
<td>0.772</td>
<td>14.7</td>
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Figure 6 Sodium Lauryl Sulfate (5% SLS, 30-minute exposure) – Epithelial cell loss induced by surfactant exposure (40X)

Opacity 37.7 Permeability 2.538 In Vitro Score 45.7

Figure 7 Ethanol (neat ETOH), 10-minute exposure, 2-hr post-exposure – Squamous layer coagulation and cytoplasmic and nuclear vacuolization in the wing and basal layers (20X)

Opacity 24.8 Permeability 1.476 In Vitro Score 47.0
Figure 8 Acid formulation “A” (neat), 3-minute exposure, 2-hr post-exposure. Moderate coagulation, abnormal chromatin condensation and cytoplasmic vacuolization in the squamous and upper wing cell layers (40X).

Opacity 53.3  Permeability 0.533  In Vitro Score 61.3

Figure 9 Acid formulation “B” (neat), 3-minute exposure, 2-hr post-exposure. Marked squamous cell coagulation, abnormal chromatin condensation and breakdown of basal cell adhesion to the basal lamina (40X).

Opacity 34.0  Permeability 0.911  In Vitro Score 47.7
Figure 10 Acid formulation “B” (neat), 10-minute exposure, 2-hr post-exposure. Severe squamous cell coagulation, abnormal chromatin condensation (precipitation) and marked cytoplasmic eosinophilia (40X)

Opacity 46.7 Permeability 1.682 In Vitro Score 71.9

Figure 11 (neat) 10-minute exposure, 2-hr post-exposure. Hypo-chromic staining in the upper epithelium and marked nuclear pyknosis and upper hyper-eosinophilia in the deep epithelium (20X)

Opacity 15.2 Permeability 0.512 In Vitro Score 22.9
Figure 12 Organic solvent-induced loss of cellular structure and cytoplasmic contents in the epithelium

Opacity 42.2  Permeability 0.556  In Vitro Score 50.5

Figure 13 Overall destruction of the epithelium and separation from the basal lamina. Note the eosinophilic precipitate between the Anterior Limiting Lamina and basal cells (20X)

Opacity 18.3  Permeability 0.453  In Vitro Score 25.1
Figure 14 Damage to the epithelium and upper stroma resulting in a complete loss of viability and reduced staining.

Opacity 14.7 Permeability 0.082 In Vitro Score 15.9

Figure 15 Bleach Mixture (neat), 10-minute exposure, 2-hour post exposure. Epithelium showing complete loss of nuclear and cytoplasmic staining in the squamous cell layer and upper wing layer.
Figure 16 Bleaching Agent (neat), 3-minute exposure, 2-hr post-exposure. Epithelium showing microvacuolation of cellular structure and cytoplasm

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<thead>
<tr>
<th>Opacity</th>
<th>Permeability</th>
<th>In Vitro Score</th>
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<td>89.2</td>
<td>2.145</td>
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6.3 Stromal Histologic changes

Figure 17 Sodium Lauryl Sulfate (1.5% SLS), 10-minute exposure, 2-hr post-exposure. Example of slight collagen matrix expansion directly below Anterior Limiting Lamina.

![Image 1](image1.png)

Opacity 2.2  Permeability 0.379  *In Vitro* Score 7.8

Figure 18 Sodium Lauryl Sulfate (1% SLS), 30-minute exposure. Example of moderate collagen matrix expansion in the upper stroma.

![Image 2](image2.png)

Opacity 3.2  Permeability 0.772  *In Vitro* Score 14.7
Figure 19 Sodium Lauryl Sulfate (5% SLS), 30-minute exposure. Example of moderate to marked collagen matrix disorganization and expansion in the upper stroma. Note also the keratocyte nuclear changes.

Figure 20 Sodium Lauryl Sulfate (10% SLS), 30-minute exposure. Severe collagen matrix expansion following destruction of the epithelium. Note also the destruction of the upper keratocytes.
6.4 Endothelial Cell Histologic changes

Figure 21 Quinacrine (20% Quinacrine in MEM, 4-hour exposure) Endothelial cell vacuolation (severe)

![Endothelial cell vacuolation](image)

Opacity 10.5  Permeability -0.040  *In Vitro* Score 9.9

Figure 22 Bleach Mixture, 10-minute exposure, 2-hour post exposure. Damage to the endothelial cell layer, cytoplasmic, and deep stromal collagen matrix expansion (severe).

![Damage to endothelial cell layer](image)

Opacity 14.7  Permeability 0.082  *In Vitro* Score 15.9
Figure 23 Ethanol (neat ETOH), 10-minute exposure, 2-hr post-exposure) Severe damage to the epithelium and expansion of the upper stroma. The endothelium was intact and little expansion was observed in the deep stroma.

Figure 24 Example of massive stromal swelling resulting from the loss of both the epithelium and endothelium (severe).
7. References


