

Grading system for the evaluation of the structural damage to the corneal tissue

- Damage of the epithelium
 - Normal (NL)
 - Mild: vacuoles in the cytoplasm, sloughing $<1/3$ of the epithelium
 - Moderate: more changes in the staining characteristics of the epithelium, sloughing $1/3 \sim 2/3$ of the epithelium
 - Severe: not viable cells, sloughing $>2/3$ of the epithelium

- Damage of the collagen and the keratocytes
 - Normal (NL)
 - Mild: vacuoles in the cytoplasm, increased size in $<1/3$ of total keratocytes
 - Moderate: more noticeable changes than mild, changes in $1/3 \sim 1/2$ of total keratocytes
 - Severe: not viable keratocytes

- Quality of the endothelial cell layer
 - Normal (NL)
 - Mild: mild swelling, vacuoles in the cytoplasm
 - Moderate: more changes than mild
 - Severe: not viable endothelial cells

- Changes in the trabecular meshwork / the chamber angle
 - Normal (NL)
 - Mild: mild swelling in the endothelium, no change in the architecture
 - Moderate: slight disruption in the architecture
 - Severe: disruption of normal architecture, not viable endothelial cells

Evaluation of Cornea Histology

Layers	Cell Type/Area	Observation	Degree	Depth
Overall evaluation of the sections		<ul style="list-style-type: none"> • All layers present • Overall Staining • Tissue architecture • Thickness of Descemet's Membrane as an indicator of true cross section • Thickness of endothelium 		
Epithelium	Squamous	<ul style="list-style-type: none"> • Flattened • Limited cytoplasm and highly condensed nuclei • Detached 		
	Wing Cells	<ul style="list-style-type: none"> • Several layers • Cell nuclei with diffuse chromatin without clear nucleoli 		
	Basal Cells	<ul style="list-style-type: none"> • Well formed and columnar • Tightly attached to each other • Attached to Bowman's Layer 		
	Overall	<ul style="list-style-type: none"> • Cell loss • Coagulation • Lysed • Nuclear vacuolization (swelling) • Nuclear condensation (pyknosis or precipitation) • Cytoplasmic vacuolization • Cytoplasmic precipitation 	Number of cells showing lesion or magnitude	Number of cell layers impacted
Stroma	Extracellular collagen matrix fibers	<ul style="list-style-type: none"> • Well organized • Parallel • Swelling • Presence of vacuole like holes • Measurement (number) 	Relative number and size of vacuoles	% involved starting at Bowman's
	Keratocyte	<ul style="list-style-type: none"> • Morphology (pyknosis, karyorrhexis, vacuolization, chromatin condensation) • Staining (eosinophilic/basophilic) 		
	• Cytoplasm	<ul style="list-style-type: none"> • Staining (eosinophilic/basophilic, keratocyte eosinophilia) • Vacuole formation 		
	Descemet's Membrane	Prominent		
Endothelium	Cells	<ul style="list-style-type: none"> • Single layer • Flattened • Attached to Descemet's membrane • Nuclei elongated and flattened • Cytoplasmic degeneration 		
	Overall	<ul style="list-style-type: none"> • Cell loss • Stromal swelling in lower 1/3 		

Evaluation of Corneas by Research Pathology Labs (MB)

1. Three representative parallel cross-sections of each cornea, extending from one limbus to the contralateral limbus, are processed, embedded in paraffin, sectioned and stained with H&E.
2. Microscopic evaluation includes evaluation of the corneal epithelium (squamous cell layer, wing cell layer, and basal cell layer), basal lamina, Bowman's layer, stroma (substantia propria), Descemet's membrane and the endothelium.
3. Changes are graded using a four scale system ranging from minimal to marked with minimal being the least detectable change to marked being the most severe change that one could expect to occur within various areas of the cornea.

Test Article:	DI H ₂ O						
Cornea Number:	6	7	8	9	10	C1	C2
<u>Epithelium</u>	-	-	-	-	-	-	-
<u>Squamous-Cell Layer:</u>	-	-	-	-	-	-	-
<u>Wing-Cell Layer:</u>	-	-	-	-	-	-	-
<u>Basal-Cell Layer:</u>	-	-	-	-	-	-	-
<u>Basal Lamina:</u>	-	-	-	-	-	-	-
<u>Bowman's Layer</u>	-	-	-	-	-	-	-
<u>Stroma (Substantia Propria):</u>	-	-	-	-	-	-	-
<u>Descemet's Membrane:</u>	-	-	-	-	-	-	-
<u>Endothelium:</u>	-	-	-	-	-	-	-

Key: - = Within normal limits or indicated change or lesion not present
 1 = Minimal degree or amount of indicated change
 2 = Mild degree or amount of indicated change
 3 = Moderate degree or amount of indicated change
 4 = Marked degree or amount of indicated change

Scoring Codes for the BCOP Histology

Epithelium:

- 0) Normal epithelium
- 1) Damage or loss of squamous epithelium
- 2) Damage or loss of upper wing cell layer
- 3) Damage to lower wing cell layer
- 4) Loss of lower wing cell layer
- 5) Damage or loss of basal cells
- 6) Damage to basal lamina

Stromal Swelling:

- 0) Normal stroma
- 1) Mild to moderate swelling in apical 1/3 of stroma (very small vacuoles)
- 2) Moderate microscopic swelling through 1/2 of stroma
- 3) Severe microscopic swelling through 1/2 of stroma and observable overall thickening of corneal stroma (macroscopic)
- 4) Swelling extending to the posterior 1/3 of corneal stroma and overall thickening of the corneal stroma
- 5) Swelling extending to Descemet's Membrane and marked overall thickening of the corneal stroma

Keratocyte Damage or Loss

- 0) Normal keratocyte morphology and number
- 1) Observable decrease in cell number and/or increased number of necrotic/pyknotic cells in the apical 1/3 of the stroma
- 2) Moderate to marked (30-50%) decrease in cell number and/or increased number of necrotic/pyknotic cells in the apical 1/3 of the stroma
- 3) Moderate to marked (30-50%) decrease in cell number and/or increased number of necrotic/pyknotic cells in the apical 2/3 of the stroma. Note: Cells in the more apical regions may appear less damaged if rapid fixation by the test material has occurred.
- 4) Moderate to marked (30-50%) decrease in cell number and/or increased number of necrotic/pyknotic cells extending into the posterior 1/3 of the stroma.

Endothelial Cell Layer

- 0) Cells normal (similar to controls)
- 1) Cell vacuolization or other signs of toxicity in the dosing area (or over the whole area in controls)
- 2) General loss of endothelial layer in dosing area (or whole area in controls)
- 3) Complete/partial loss which precludes evaluation (either artifact or shipping changes)