Laboratory Animals

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Lab Animal Ophthalmology

Dog

Rabbit

Primate

Rat

Guinea Pig

Pig

Mouse
Acknowledgements

- Hiroshi Kuno
  - Merck/Banyu Pharmaceutical
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  - Alcon Pharmaceutical
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  - NAMSA
- Dr. Tom Kern
  - Cornell University
Lab Animal Ophthalmology

- Ethics
  - This is beyond the scope of this course, but is a concern for many and must be considered based on personal beliefs and opinions.
ACVO Position Statement:

“It is the position of the ACVO that to ensure public safety ACVO Diplomate status is the minimum qualification for those performing ocular evaluations and assessment of findings in laboratory animal studies that are intended to support applications to the FDA (or other similar regulating agencies) for entry into human clinical trials.”
Industry

- Laboratory animal ophthalmology as it pertains to industry, not to pocket pets
Industry

- Service Provided to:
  - Contract Research Organizations (CRO’s)
Industry

- Service Provided to:
  - Pharmaceutical Industry
Industry

- Service Provided to:
  - Researchers in an academic environment that may require the expertise of a Board-Certified Veterinary Ophthalmologist.
Contract laboratories (CROs) are governed by the FDA and Good Laboratory Practices (GLP).

“Good Laboratory Practice (GLP) embodies a set of principles that provides a framework within which laboratory studies are planned, performed, monitored, recorded, reported and archived. These studies are undertaken to generate data by which the hazards and risks to users, consumers and third parties, including the environment, can be assessed for pharmaceuticals (only preclinical studies), agrochemicals, cosmetics, food additives, feed additives and contaminants, novel foods, biocides, detergents etc. GLP helps assure regulatory authorities that the data submitted are a true reflection of the results obtained during the study and can therefore be relied upon when making risk/safety assessments.”
GLP

Basic Research | Disease Development | Drug Development | Preclinical Development | Clinical Trials I, II, III | Manufacturing incl. APIs QC Laboratories

Not Regulated → GLP → GCP → GMP → 21 CFR Part 11 → Study Based → Process Based

Part 11 applies for computers that are used in FDA regulated areas.
Industry

- Contract laboratories (CROs) are governed by the FDA and Good Laboratory Practices (GLP)
  - governs non-clinical laboratory studies for products regulated by the FDA including:
    - food and color additives
Industry

- Contract laboratories (CROs) are governed by the FDA and Good Laboratory Practices (GLP)
  - governs non-clinical laboratory studies for products regulated by the FDA including:
    - food and color additives
    - animal food additives
Industry

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    - animal food additives
    - human and animal drugs
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    - food and color additives
    - animal food additives
    - human and animal drugs
    - medical devices for human use
Industry

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    - food and color additives
    - animal food additives
    - human and animal drugs
    - medical devices for human use
    - biologic products and electronic products
Contract Research Laboratories evaluate products for pharmaceutical and agricultural use and are governed by FDA and USDA guidelines.
Industry

- Contract Research Laboratories evaluate products for pharmaceutical and agricultural use and are governed by FDA and USDA guidelines.
- In addition, they may test cosmetics, contact lenses and associated materials, intraocular devices and a host of other products that might have an ocular use, contact the eye or be applied topically, inhaled, ingested or injected.
Industry

- Contract Research Laboratories evaluate products for pharmaceutical and agricultural use and are governed by FDA and USDA guidelines.
- In addition, they may test cosmetics, contact lenses and associated materials, intraocular devices, and a host of other products that might have an ocular use, contact the eye or be applied topically, inhaled, ingested, or injected.
- Contract laboratory study is overseen by a Study Director, all associated personnel must be adequately trained, and a Quality Assurance Unit is responsible for monitoring each study to ensure compliance.
  - Must follow SOP
Industry

- Toxicology Study Types
  - LD-50
  - Maximum tolerated dose (MTD)
  - Acute Toxicity
  - SubAcute Toxicity
  - Subchronic Toxicity
  - Chronic Toxicity
  - Single dose range-finding and acute toxicity studies
  - Repeated Dose
  - Sensitization
  - Irritation
  - Carcinogenicity studies
  - Teratogenic
Industry

- Dosing Regimes
  - Oral
  - Oral Gavage
Industry

- Dosing Regimes
  - Oral
    - Oral Gavage
  - Intranasal
Industry

- Dosing Regimes
  - Oral
    - Oral Gavage
  - Intranasal
  - IV, IM, IP, SQ
Industry

- Dosing Regimes
  - Oral
    - Oral Gavage
  - Intranasal
  - IV, IM, IP, SQ
  - Dermal
Industry

- Dosing Regimes
  - Oral
    - Oral Gavage
  - Intranasal
  - IV, IM, IP, SQ
  - Dermal
  - Inhalation
Industry

- Dosing Regimes
  - Oral
    - Oral Gavage
  - Intranasal
  - IV, IM, IP, SQ
  - Dermal
  - Inhalation
  - Dietary
Industry

- Dosing Regimes
  - Oral
    - Oral Gavage
  - Intranasal
  - IV, IM, IP, SQ
  - Dermal
  - Inhalation
  - Dietary
  - Device implantation
Industry

- Dosing Regimes
  - Oral
    - Oral Gavage
  - Intranasal
  - IV, IM, IP, SQ
  - Dermal
  - Inhalation
  - Dietary
  - Device implantation
  - Topical - ocular
Industry

- Dosing Regimes
  - Oral
    - Oral Gavage
  - Intranasal
  - IV, IM, IP, SQ
  - Dermal
  - Inhalation
  - Dietary
  - Device implantation
  - Topical
  - Intravitreal - for AMD
Species - Normative Data

- Ophthalmologist must be familiar with:
  - What is normal for the species in question
  - What are the common spontaneous abnormalities for that species, age and breed/strain
Species - Normative Data

- Ophthalmologist must be familiar with what is normal for the species in question and what are the common abnormalities for that species, age and breed/strain
- Albino vs. pigment

Wistar

Long-Evans
Species - Normative Data

- Ophthalmologist must be familiar with what is normal for the species in question and what are the common abnormalities for that species, age and breed/strain
- Albino vs. pigment
- Type of retinal vasculature, ranging from anangiotic to merangiotic to holangiotic
Species - Normative Data

- Ophthalmologist must be familiar with what is normal for the species in question and what are the common abnormalities for that species, age and breed/strain
- Albino vs. pigment
- Type of retinal vasculature, ranging from anangiogenic to merangangiogenic to holangangiogenic
- Presence or absence of a tapetum
Species - Normative Data

- Ophthalmologist must be familiar with what is normal for the species in question and what are the common abnormalities for that species, age and breed/strain
- Albino vs. pigment
- Type of retinal vasculature, ranging from anangiotic to merangiotic to holangiotic
- Presence or absence of a tapetum
- Foveated vs Non-foveated
Examination techniques

- With respect to toxicology - the eye must be considered in 3 ways:
  - undesirable ocular effects when the eye is the target organ of interest with the drug of interest applied to the eye
  - undesirable systemic effects associated with an ocularly applied agent
  - undesirable ocular effects from an agent applied in a systemic manner (oral, dermal, injection, inhalation) with resulting ocular effects
Examination techniques

- Basic
- Advanced
Examination technique - Basic

- Brand of biomicroscope
  - Zeiss HSO-10
  - Kowa SL-14/15
- Indirect ophthalmoscope
  - Keeler All-Pupil
  - Size of indirect lens
    - 30, 40, 60D
- Direct ophthalmoscope - Primates
Examination technique

- Some may advocate for indirect examination only:
- Report of 100 rats with lens abnormalities by biomicroscopy
  - 65 seen with direct ophthalmoscope
  - 35 seen with indirect ophthalmoscope
- **Standard of care:**
  - Biomicroscopy
  - Indirect Ophthalmoscopy
Examination technique - Basic

- Number of animals that can be examined in an hour must be understood – to be discussed....
Examination technique - Advanced

- Intraocular Pressure – Tonopen/Tonovet, pneumotonometer, Goldmann
GLP compliant
Non-GLP compliant
Rabbit
Advanced Examination

- **Applanation tonometry**
  - **Tonopen**
    - demonstrated to be applicable to determination of rat IOP (19.9 +/- 4.7 mmHg)
    - demonstrated to be applicable to determination of mouse IOP (19.9 +/- 4.7 mmHg).
    - Please remember that there can be a significant variation in IOP according to the circadian rhythm and the season of the year. This true even for animals housed indoors all their lives on a 12:12 hour light:dark cycle.

- **Tonovet**
  - Better for rabbits?
  - Non-GLP compliant
Advanced Examination

- Pachymetry
Examination technique - Advanced

- Photography
You need an ID photo and a photo log that indicates which eye and how many photos were taken.
Examination technique - Advanced

- Fluorescein angiography
Examination technique - Advanced

- Electroretinogram
Examination technique - Advanced

- Specular Microscopy
Optical Coherence Tomography

- Utilizes light wave interferometry to produce high resolution cross sectional images, similar to a computed tomography (CT) scan, of the retina.
- Measure the thickness of the retina.
- Useful for macular disease.
- Study glaucoma.
Biomicroscopy
Biomicroscope

- Zeiss HSO-10
- Kowa SL-14
Biomicroscopy

- Indications
  - Examination of the anterior segment of the eye:
    - Adnexa
    - Conjunctiva
    - Cornea
    - Aqueous
    - Iris
    - Lens
    - Anterior Vitreous
Digital Documentation
Biomicroscopy grading criteria:

- **Grade**
  - 0: No observable lesion
  - 1: Some loss of transparency. The underlying structures are clearly visible with diffuse illumination.
  - 2: Moderate loss of transparency. With diffuse illumination the underlying structures are barely visible, but can still be examined and graded.
  - 3: Severe loss of transparency. With diffuse illumination the underlying structures are not visible when viewed through the lesion and evaluation of them is impaired.

- This grading system is based on the modified McDonald-Shadduck scoring system.
Modified Hackett-McDonald scoring

Conjunctival congestion

0¼ Normal. May appear blanched to reddish pink without perilimbal injection (except at 12:00 and 6:00 o'clock positions) with vessels of the palpebral and bulbar conjunctiva easily observed.

1¼ A flushed, reddish color predominantly confined to the palpebral conjunctiva with some perilimbal injection but primarily confined to the lower and upper parts of the eye from the 4:00, 7:00, 11:00, and 1:00 o'clock positions.

2¼ Bright red color of the palpebral conjunctiva with accompanying perilimbal injection covering at least 75% of the circumference of the perilimbal region.

3¼ Dark, beefy red color with congestion of both the bulbar and the palpebral conjunctiva along with pronounced perilimbal injection and the presence of petechiae on the conjunctiva. The petechiae generally predominate along the nictitating membrane and the upper palpebral conjunctiva.

Conjunctival swelling (there are five divisions from 0 to 4)

0¼ Normal or no swelling of the conjunctival tissue.

1¼ Swelling above normal without eversion of the lids (can be easily ascertained by noting that the upper and lower eyelids are positioned as in the normal eye); swelling generally starts in the lower cul-de-sac near the inner canthus, which needs slit lamp examination.

2¼ Swelling with misalignment of the normal approximation of the lower and upper eyelids; primarily confined to the upper eyelid so that in the initial stages the misapproximation of the eyelids begins by partial eversion of the upper eyelid. In this stage, swelling is confined generally to the upper eyelid, although it exists in the lower cul-de-sac (observed best with the slit lamp).

3¼ Swelling definite with partial eversion of the upper and lower eyelids essentially equivalent. This can be easily ascertained by looking at the animal head-on and noticing the positioning of the eyelids; if the eye margins do not meet, eversion has occurred.

4¼ Eversion of the upper eyelid is pronounced with less pronounced eversion of the lower eyelid. It is difficult to retract the lids and observe the perilimbal region.

Conjunctival discharge—Discharge is defined as a whitish-gray precipitate, which should not be confused with the small amount of clear, inspissated, mucoid material that can be formed in the median canthus of a substantial number of rabbit eyes. This material can be removed with a cotton swab before the animals are used.

0¼ Normal. No discharge.

1¼ Discharge above normal and present on the inner portion of the eye but not on the lids or hairs of the eyelids. One can ignore the small amount that is in the inner and outer canthus if it has not been removed prior to starting the study.

2¼ Discharge is abundant, easily observed, and has collected on the lids and around the hairs of the eyelids.

3¼ Discharge has been flowing over the eyelids so as to wet the hairs substantially on the skin around the eye.

Aqueous flare—The intensity of the Tyndall phenomenon is scored by comparing the normal Tyndall effect observed when the slit lamp beam passes through the lens with what is seen in the anterior chamber. The presence of aqueous flare is presumptive evidence of break down of the blood-aqueous barrier.

0¼ The absence of visible light beam in the anterior chamber (no Tyndall effect).

1¼ The Tyndall effect is barely discernible. The intensity of the light beam in the anterior chamber is less than the intensity of the slit beam as it passes through the lens.

2¼ The Tyndall beam in the anterior chamber is easily discernible and is equal in intensity to the slit beam as it passes through the lens.

3¼ The Tyndall beam in the anterior chamber is easily discernible; its intensity is greater than the intensity of the slit beam as it passes through the lens.

Pupillary light reflex—The pupillary diameter of the iris is controlled by the radial and sphincter muscles. Contraction of the radial muscle due to adrenergic stimulation results in mydriasis while contraction of the sphincter muscle due to cholinergic stimulation results in miosis. As an ophthalmic drug can exert potential effects on these neural pathways, it is important to assess the light reflex of an animal as part of the ophthalmic examination. Using full illumination with the slit lamp, the following scale is used.

0¼ Normal pupillary response.

1¼ Sluggish pupillary response.

2¼ Maximally impaired (i.e., fixed) pupillary response.

Iris involvement—In the following definitions, the primary, secondary, and tertiary vessels are utilized as an aid to determining a subjective ocular score for iris involvement. The assumption is made that the greater the hyperemia of the vessels and the more the secondary and tertiary vessels are involved, the greater the intensity of iris involvement. The scores range from 0 to 4.

0¼ Normal iris without any hyperemia of the iris vessels. Occasionally around the 12:00–1:00 o'clock position near the pupil border and the 6:00 and 7:00 o'clock position near the pupil border, there is a small area around 1–3 mm in diameter in which both the secondary and tertiary vessels are slightly hyperemic.

1¼ Minimal injection of secondary vessels but not tertiary. Generally, it is uniform, but may be of greater intensity at the 1:00 or 6:00 o'clock position. If it is confined to the 1:00 or 6:00 o'clock position, the tertiary vessels must be substantially hyperemic.

2¼ Minimal injection of tertiary vessels and minimal to moderate injection of the secondary vessels.

3¼ Moderate injection of the secondary and tertiary vessels with slight swelling of the iris stroma (this gives the iris surface a slightly rugose appearance, which is usually most prominent near the 3:00 and 9:00 o'clock positions).

4¼ Marked injection of the secondary and tertiary vessels with marked swelling of the iris stroma. The iris appears rugose; may be accompanied by hemorrhage (hyphema) in the anterior chamber.

Cornea cloudiness—The scoring scheme measures the severity of corneal cloudiness and the area of the cornea involved. Severity of corneal cloudiness is graded as follows:

0¼ Normal cornea. Appears with the slit lamp adjusting to a narrow slit image as having a bright gray line on the epithelial surface and a bright gray line on the endothelial surface with a marble-like appearance of the stroma.

1¼ Some loss of transparency. Only the anterior half of the stroma is involved as observed with an optical section of the slit lamp. The underlying structures are clearly visible with diffuse illumination, although some cloudiness can be readily apparent with diffuse illumination.
Modified Hackett-McDonald scoring

2¼ Moderate loss of transparency. In addition to involving the anterior stroma, the cloudiness extends all the way to the endothelium. The stroma has lost its marble-like appearance and is homogeneously white. With diffuse illumination, underlying structures are clearly visible.

3¼ Involvement of the entire thickness of the stroma. With the optical section, the endothelial surface is still visible. However, with diffuse illumination, the underlying structures are just barely visible (to the extent that the observer is still able to grade flare and iris, observe for pupillary response, and note lenticular changes).

4¼ Involvement of the entire thickness of the stroma. With the optical section, cannot clearly visualize the endothelium. With diffuse illumination, the underlying structures cannot be seen. Cloudiness removes the capability for judging and grading flare, iris, lenticular changes, and pupillary response.

Corneal area—The surface area of the cornea relative to the area of cloudiness is divided into five grades from 0 to 4.
0¼ Normal cornea with no area of cloudiness.
1¼ 1–25 % area of stromal cloudiness.
2¼ 26–50 % area of stromal cloudiness.
3¼ 51–75 % area of stromal cloudiness.
4¼ 76–100 % area of stromal cloudiness.

Pannus—Pannus is vascularization or the penetration of new blood vessels into the corneal stroma. The vessels are derived from the limbal vascular loops. Pannus is divided into three grades.
0¼ No pannus.
1¼ Vascularization is present but vessels have not invaded the entire corneal circumference. Where localized vessel invasion has occurred, they have not penetrated beyond 2 mm.
2¼ Vessels have invaded 2 mm or more around the entire corneal circumference.

Fluorescein—For fluorescein staining, the area can be judged on a 0 to +4 scale using the same terminology as for corneal cloudiness. The intensity of fluorescein staining can be divided into 0–4 scale.
0¼ Absence of fluorescein staining.
1¼ Slight fluorescein staining confined to a small focus. With diffuse illumination, the underlying structures are easily visible. (The outline of the pupillary margin is where there were no fluorescein staining.)
2¼ Moderate fluorescein staining confined to a small focus. With diffuse illumination the underlying structures are clearly visible, although there is some loss of detail.
3¼ Marked fluorescein staining. Staining may involve a larger portion of the cornea. With diffuse illumination the underlying structures are barely visible but are not completely obliterated.
4¼ Extreme fluorescein staining. With diffuse illumination the underlying structures cannot be observed.

Lens—The crystalline lens is readily observed with the aid of the slit lamp biomicroscope, and the location of lenticular opacity can readily be discerned by direct and retro illumination.

The lens is normal (N) or abnormal (A).

If abnormal, the location and severity of lenticular opacities are noted in the comments.
## Modified Hackett-McDonald scoring

### MICROSCOPIC GRADING DATA
(Hackett and McDonald)

<table>
<thead>
<tr>
<th>Study Number:</th>
<th>Study Day:</th>
<th>Group:</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Conjunctival</th>
<th>Aqueous</th>
<th>Iris</th>
<th>Corneal</th>
<th>Cloudiness</th>
<th>Time</th>
<th>Fluor. Exam</th>
<th>Time</th>
<th>Lens</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conges.</td>
<td>Swell</td>
<td>Dis.</td>
<td>Light</td>
<td>Reflex</td>
<td>Involvement</td>
<td>Sev.</td>
<td>Area</td>
<td>Pannus</td>
<td>Time</td>
</tr>
<tr>
<td>(Test) Left Eye</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Control) Right Eye</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Comments:**

Tech Date/Initials: ____________________________

Ophthalmologist Date/Initials: ____________________________

Conges. = congestion  Sev. = Severity
Swell = swelling  Fluor. Exam = Fluorescein staining  Dis. = Discharge
### SUN grading system for aqueous

<table>
<thead>
<tr>
<th>Grade</th>
<th># cells in the field</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&lt;1</td>
</tr>
<tr>
<td>0.5+</td>
<td>1–5</td>
</tr>
<tr>
<td>1+</td>
<td>6–15</td>
</tr>
<tr>
<td>2+</td>
<td>16–25</td>
</tr>
<tr>
<td>3+</td>
<td>26–50</td>
</tr>
<tr>
<td>4+</td>
<td>&gt;50</td>
</tr>
</tbody>
</table>

Cells should be counted at the same location, usually the central anterior chamber [1, 12]

### Aqueous Cells

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td>1+</td>
<td>Faint</td>
</tr>
<tr>
<td>2+</td>
<td>Moderate (iris and lens details clear)</td>
</tr>
<tr>
<td>3+</td>
<td>Marked (iris and lens details hazy)</td>
</tr>
<tr>
<td>4+</td>
<td>Intense (fibrin or plasmoid aqueous)</td>
</tr>
</tbody>
</table>

### Using a biomicroscope

A biomicroscope is used to observe and grade the number and appearance of cells in the aqueous humor.

### Aqueous Flare

- **Grade 0**: None
- **Grade 1+**: Faint
- **Grade 2+**: Moderate (iris and lens details clear)
- **Grade 3+**: Marked (iris and lens details hazy)
- **Grade 4+**: Intense (fibrin or plasmoid aqueous)

A biomicroscope is used to observe and grade the appearance of aqueous flare.
NEI grading system for vitreous inflammation

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td>1+</td>
<td>Posterior pole clearly visible</td>
</tr>
<tr>
<td>2+</td>
<td>Posterior pole details slightly hazy</td>
</tr>
<tr>
<td>3+</td>
<td>Posterior pole details very hazy</td>
</tr>
<tr>
<td>4+</td>
<td>Posterior pole details barely visible</td>
</tr>
<tr>
<td>5+</td>
<td>Fundus details not visible</td>
</tr>
</tbody>
</table>

Using an indirect ophthalmoscope
Indirect Ophthalmoscope

- Keeler All-Pupil
- Size of indirect lens
  - 30, 40, 60D
Indirect Ophthalmoscope

- Keeler All-Pupil
- Size of indirect lens
  - 30, 40, 60D
1% - dog, pig, monkey, rabbit
0.5% - rat, mouse
Role of the ophthalmologist

- Perform a pretest examination to eliminate those animals not suited to the study
Role of the ophthalmologist

- Perform a pretest examination to eliminate those animals not suited to the study
- Establish a **baseline database**
Role of the ophthalmologist

- Perform a pretest examination to eliminate those animals not suited to the study
- Establish a baseline database
- Animals are then subsequently examined during, at the conclusion and recovery phase of the study
  - Masked to compound and dose group
Role of the ophthalmologist

- The ophthalmologist must then interpret findings in light of the:
  - species
  - age
  - expected spontaneous abnormalities
  - study design – i.e. orbital bleeding?
  - pretest data
  - compound evaluated
  - dose group outcome
Species

- Canine - Beagle
Species

- Canine - Beagle
- Rat
  - Sprague-Dawley
  - Fisher 344
  - Wistar
  - Long Evans
Species

- Canine - Beagle
- Rat
  - Sprague-Dawley
  - Fisher 344
  - Wistar
  - Long Evans
- Mouse
Species

- Canine - Beagle
- Rat
  - Sprague-Dawley
  - Fisher 344
  - Wistar
  - Long Evans
- Mouse
- Rabbit - NZW
Species

- Canine - Beagle
- Rat
  - Sprague-Dawley
  - Fisher 344
  - Wistar
  - Long Evans
- Mouse
- Rabbit
- Swine - mini vs farm pigs
Species

- Canine - Beagle
- Rat
  - Sprague-Dawley
  - Fisher 344
  - Wistar
  - Long Evans
- Mouse
- Rabbit
- Swine
- Primate - Cyno, Rhesus
Species

- Canine - Beagle
- Rat
  - Sprague-Dawley
  - Fisher 344
  - Wistar
  - Long Evans
- Mouse
- Rabbit
- Swine
- Primate - Cyno, Rhesus
- Other - Cat, Guinea Pig
The beagle is a canine like any other and most veterinary ophthalmologists are both familiar and comfortable with the examination and abnormalities of this breed.
Canine - Beagle

- Breed-related abnormalities will vary according to the supplier and are often seen with a prevalence that waxes and wanes according to the current sires and dams.
  - Marshall farms
  - Ridglan farms
  - Covance
In my experience, the most common abnormalities are:
- prolapse of the gland of the nictitans
- retinal dysplasia (folds)
- optic nerve micropapilla/hypoplasia – Marshall farms
- cataract
Canine - Beagle

- In my experience, the most common abnormalities are:
  - prolapse of the gland of the nictitans
  - retinal dysplasia (folds)
  - optic nerve micropapilla/hypoplasia
  - cataract
- In addition:
  - Persistent hyaloid
  - Distichia
  - Corneal Dystrophy
  - PPM
  - KCS (dry eye)
In my experience, the most common abnormalities are:
- prolapse of the gland of the nictitans
In my experience, the most common abnormalities are:
- retinal dysplasia (folds)
In my experience, the most common abnormalities are:
- optic nerve micropapilla/hypoplasia
- Micropapilla is #1
- Occasionally hypoplasia
- Marshall Farms especially
Canine - Beagle

- In my experience, the most common abnormalities are:
  - Cataract
Canine - Beagle

- In my experience, the most common abnormalities are:
  - Cataract
    - Post. Cortical
    - Anterior Suture
    - Nuclear
    - Other
Canine - Beagle

- PPM

- Persistent hyaloid
### Normative Data - Beagle

<table>
<thead>
<tr>
<th>Finding</th>
<th># dogs (479 examined)</th>
<th>% total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prolapsed gland</td>
<td>4</td>
<td>0.83</td>
</tr>
<tr>
<td>Corneal opacity</td>
<td>7</td>
<td>1.46</td>
</tr>
<tr>
<td>PPM</td>
<td>1</td>
<td>0.21</td>
</tr>
<tr>
<td>Lens cortical vacuoles/opacity</td>
<td>23</td>
<td>4.8</td>
</tr>
<tr>
<td>Fetal nucleus opacity</td>
<td>7</td>
<td>1.46</td>
</tr>
<tr>
<td>Prominent nucleus</td>
<td>2</td>
<td>0.42</td>
</tr>
<tr>
<td>Peripheral capsular opacity</td>
<td>1</td>
<td>0.21</td>
</tr>
<tr>
<td>Prominent post. sutures</td>
<td>25</td>
<td>5.23</td>
</tr>
<tr>
<td>Post. capsular opacity</td>
<td>27</td>
<td>5.64</td>
</tr>
<tr>
<td>Persistent hyaloid</td>
<td>15</td>
<td>3.13</td>
</tr>
<tr>
<td>Tapetal scar/pigment</td>
<td>13</td>
<td>2.71</td>
</tr>
<tr>
<td>Micropapilla</td>
<td>22</td>
<td>4.59</td>
</tr>
</tbody>
</table>

From: Bellhorn RW: Survey of ocular findings in 8-10 month old beagles. JAVMA 164:1114-1116, 1974.
Canine - Beagle

- Study-Related Findings
  - Cataract
    - Incipient to Mature
  - Corneal Dystrophy
  - Retinal Degeneration
  - Keratoconjunctivitis Sicca
Amiodarone-Keratopathy
NSAID
New Pharmaceutical
Rat

- WHAT IS THE MOST IMPORTANT THING TO KNOW ABOUT THE RAT??
Really Big Teeth!
Rat: Albino vs Pigmented
Rat/Mouse

- Orbital venous plexus
  - Used for blood collection
- Three lacrimal glands/eye
  - Intraorbital, extraorbital, harderian
Rat/Mouse

- Hyaloid artery
- Large spherical lens
- Holangiotic (rod) retina
- Atapetal
- Eyelid separation 12-16 days (13-14 d mouse)
- Normal IOP-Tonopen
  - 13.9 (+/-4.2) mmHg - Lewis rat
  - 17.3 (+/- 5.3) mmHg
Rat

Mouse  Rat
In my experience, the most common abnormalities are:

- Corneal dystrophy
- Persistent hyaloid (with or without hemorrhage)
- Persistent pupillary membranes
- Synechiae
- Cataract (often nuclear)
- Retinal hemorrhage
- Saccular aneurysm of retinal vessels
- Choroidal and optic nerve coloboma
Rat

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Corneal dystrophy - Rat

- Sprague-Dawley
- Fisher 344
- Wistar
- Lewis
- Long-Evans
Rat

- Corneal dystrophy
  - Common
  - Varies by:
    - Strain
    - Supplier
    - Age
    - Male vs female
Rat: Corneal Dystrophy
Rat

- Keratitis, most often nasal cornea can occur secondary to corneal dystrophy (especially Fischer 344), anesthesia, SDA, environment (dust, irritants), conjunctivitis, etc.
Rat

- If a study requires general anesthesia the ophthalmologist can expect exacerbation of corneal dystrophy and in some instances cataract formation.
Rat

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- Young animals are more likely to have PH or PPM
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Rat

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- In one report the prevalence of hyaloid remnants may be as high as 60% at 6 weeks of age.
- On a pretest examination, I will eliminate animals with intravitreal hemorrhage. In addition, anterior synechia, microophthalmos and anterior cleavage anomalies are also occasionally seen.
Rat

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- Xylazine has been incriminated as being cataractogenic in one study.
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Choroidal Coloboma
Coloboma
Long-Evans rat
Optic nerve/Choroidal coloboma
Non-pigmented rat
Optic nerve/Choroidal coloboma
Iris Coloboma
Long Evans Rat
Aberrant Myelination
Microophthalmos, cryptophthalmos
Spontaneous posterior lens rupture
Rat

- I have also seen buphthalmos/megaloglobus in rats, but have not determined IOP in affected animals.
Rat

- Retinal degeneration:
  - spontaneous
  - associated with aging
  - result of orbital bleeding techniques
  - toxicologic effect
Retinal degeneration:
- spontaneous
- associated with aging
- result of orbital bleeding techniques
- toxicologic effect.

Care should be taken to evaluate retinal "blanching" in combination with the temperament and restraint required to examine a particular animal. Excessive restraint will result in apparent retinal degeneration.
Rat: Retinal Degeneration
Rat

- Orbital bleeding may be used to collect venous blood during a study.
- Most often one side is preferred, usually the right.
Orbital Bleeding

- Technique
  - Capillary Tube
  - Superolateral conjunctival sac puncture
  - Occluded jugular
The effects of orbital bleeding can be severe and include:
- exophthalmos
- corneal rupture
- exposure keratitis
- retinal degeneration
- hyphema
- cataract
- phthisis bulbi
Rat

- Chromodacryorhea appears as a reddish-brown periorbital discharge.
Rat

- Chromodacryorhea appears as a red-brown periorbital discharge.
- Ocular irritation, respiratory infection, stress and sialodacryoadenitis (coronavirus) can all result in chromodacryorhea.
Rat

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- Ocular irritation, respiratory infection, stress and sialodacryoadenitis (coronavirus) can all result in chromodacryorhea.
- Irritation can occur from something as simple as a diet formulation change in an effort to get a compound into the feed.
Chromodacryorrhea

- **Infectious**
  - *Mycoplasma*
  - SDAV
  - Other
- **Non-infectious**
  - Nutritional?
  - “Stress”
  - Malocclusion
  - Excess light exposure
Rat – specific breeds

- Sprague-Dawley
- Fisher 344
- Wistar
- Lewis
- Long-Evans
Corneal dystrophy is common affecting 20-70% of animals in my experience. This is higher than what is reported in the literature.
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It appears more prevalent in males than females.
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Rats as young as a few weeks of age can be affected.
Fisher 344

I have never examined a Fisher 344 that did not have corneal dystrophy!!!
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As they age, keratitis, corneal vascularization and ulceration may occur.
Fisher 344

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- Please note that papers citing prevalence below this fail to examine using biomicroscopy.
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Please note that papers citing prevalence below this fail to examine using biomicroscopy.

In addition to corneal dystrophy, affected rats were also found to have basement membrane changes in other organs.
Corneal changes are characterized histologically by thickened, fragmented and mineralized corneal basement membranes.
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Limbal inflammatory reaction precursor to the keratitis and vascularization.
Fisher 344

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Dystrophy may result in the loss of the overlying corneal epithelium exposing the underlying basement membrane and possibly the corneal stroma.
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Epithelium overlying a severe area of dystrophy will be thin and possibly keratinizing.

Dystrophy may result in the loss of the overlying corneal epithelium exposing the underlying basement membrane and possibly the corneal stroma.

Severity may be more severe in males.
Corneal dystrophy has been reported to affect 65% of Wistar rats
Rat

- Study Related Findings
  - Corneal Dystrophy
    - Exacerbation of pre-existing abnormalities
  - Primary
    - Cataract
    - Retinal Degeneration
    - KCS
    - Optic Nerve Degeneration
Rabbit

- Most rabbits I examine are NZW and are normal.
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Most rabbits I examine are NZW and are normal. Optic nerve coloboma, cataract, glaucoma, epiphora, pseudopterygium and dacryocytitis are all seen sporadically. Corneal dystrophy has been reported in Dutch Belted rabbits and as a result of diet. Inherited glaucoma in the NZW rabbit is the result of goniodysgenesis and the bu/bu gene (autosomal recessive). IOP increases beginning at 1-3 months of age with resulting buphthalmia.
Normal NZW rabbit
Normal NZW rabbit
Normal Dutch Belted rabbit
Normal rabbit
Coloboma
Corneal dystrophy rabbit
Cataract rabbit
Watanabe Rabbit - hyperlipidemia
Endophthalmitis NZW rabbit
Conjunctival overgrowth rabbit
Conjunctival overgrowth rabbit
Conjunctival overgrowth rabbit
Rabbit

- Rabbits are often the model used for:
  - Topical ophthalmic drugs
    - Draize - technicians
    - Modified Hackett McDonald - ophthalmologist
  - Contact lens studies
  - IOL implantation
  - Drug delivery
  - Vitreal replacement devices
  - Intravitreal drug testing
24 hr post IOL rabbit
3 month post IOL rabbit
3 month post IOL rabbit
6 month post IOL rabbit
Silicone Oil testing - rabbit
Silicone Oil testing - rabbit
Macular Degeneration Inhibitor
Study-associated cataract rabbit
Spontaneous lens capsule rupture rabbit
The mouse is difficult to examine owing to its size and restraint.

Many examiners use only an indirect ophthalmoscope and a condensing lens to examine mice.

This is not the best approach.
Mouse

- Corneal dystrophy and cataracts are the most common finding on pretest examination.
Mouse

- Pigmented easier to examine than albino
- Condensing lense?
  - 40D
    - Dr. Bromberg
    - My personal choice
  - 60D
    - Dr. Kern
Mouse

- Corneal Dystrophy
- Cataract
  - Nuclear
  - Diffuse
- Microophthalmia
- Retinal Degeneration
  - Spontaneous vs Restraint
Mouse Restraint

Secure tail with finger

University of Iowa Animal Care Unit
3/28/96
Normal pigmented mouse
Normal albino mouse
Normal albino mouse
Retinal degeneration pigmented mouse
Corneal dystrophy - Mouse

- DBA
- C3H
- BALB/C
- Others
Ocular Abnormalities - Mice

- Microphthalmos/anophthalmos
  - C57BL, Sey
- Eyelid defects
- Cataracts
  - Nakano, Philly, Fraser, Nop, WBN/KOB diabetic, Lop, ICR, Cts, SAM-R3
- KCS
  - NZB/NZW F
  - NOD
  - HTLV-1 tax TG
Cataract - Mice

- Inherited
- Secondary
  - Uveitis
  - Retinal degeneration
  - Trauma
- Lens capsule rupture
  - Neonate
  - Autosomal recessive
Retinal Degeneration - Mice

- Phototoxic
- Inherited
Mice

- Harderian gland
Mice

- Age associated lesions
  - Harderian gland adenoma/adenocarcinoma
    - >24 months
Mouse fundus photography

Mouse fundus photography and angiography: A catalogue of normal and mutant phenotypes

Norman L. Hawes,1 Richard S. Smith,1,2 Bo Chang,1 Muriel Davisson,1 John R. Heckenlively,3 Simon W. M. John1,2,4

http://www.molvis.org/molvis/v5/p22/
http://www.molvis.org/molvis/v5/p22/
Mouse Gonioscopy

- A goniolens for clinical monitoring of the mouse iridocorneal angle and optic nerve
  - Richard S. Smith,1,2 Donald Korb,3 Simon W. M. John1,2,4
http://www.molvis.org/molvis/v8/a4/
Normal pigmented mouse
Guinea pig

- Eyelids open at birth
- Rudimentary NM
- Orbital glands
  - Large lacrimal - lateral, anterior, ventral
- Zygomatic salivary - posterior, medial, superior
- Paurangiotic retina
- Atapetal
Normal Pigmented Guinea Pig

Normal Albino Guinea Pig
Normal Albino Guinea Pig
Blepharitis

CN VII palsy

“Pea” Eye

Osseous choristoma
Hamster and Gerbil

- Holangiocytic retina
- Atapetal
- Eyelid separation
  - Hamster (Syrian) - 15 days
  - Hamster (Chinese) - 10-14 days
  - Gerbils - 16-22 days
Gerbil
Hamster
Chinchilla

- Small third eyelid
- Pupil - vertical slit
- Retina - anangiotic
- Atapetal
Chinchilla
Ferret

- Nictitating membrane
- Pupil - horizontal ellipse
- Retina - holangiotic
- Tapetum
- Optic disc - small, slightly myelinated
- IOP - 22.8 (+/- 5.5) mmHg Tonopen
- Eyelid separation - 34 days
Ferret
Primates

- Prosimians
- Anthropoids
Prosimians

- Large cornea
- Tapetum
- Open orbit
- Afoveate
  - Exception - Tarsier

Bush baby
Bush baby
Tree Shrew
Tree Shrew
Slow Loris
Slow Loris

Is there a fast loris??
Tarsier
-higher Prosimian
-foveal spot
Marmoset
Ring-tailed Lemur
Owl Monkey

- Afoveate - anthropoid
- Rod Retina
Anthropoids

- Cornea - small
- Schlemm’s canal, scleral spur
- Ciliary body - large musculature
- Closed orbit
- Retina - holangiotic
  - Atapetal
  - Macula lutea/fovea
  - Central retinal artery
Human

Rhesus monkey
Human

Rhesus monkey
Rhesus monkey
Rhesus monkey

Stump tail macaque
Rhesus monkey
Baboon
Gibbon
Non-human primates are generally examined under a short acting general anesthetic.

- Ketamine is most common
Non-human primates are generally examined under a short acting general anesthetic.

Traumatic lesions are most common
- eyelid lacerations and corneal scar.

In addition:
- Cataract
- Retinal scars
- Iris nevi
- Optic nerve coloboma
Diff Dx?

- 4 yr cynomolgus
If this was a Human:
- Choroidal osteoma
- Amelanotic melanoma
- Metastasis
- Other
In one study of 2100 wild-caught Cynomolgus monkeys 167 animals (7.95%) had 185 findings the majority of which involved the posterior segment.
Primate

- Wear double gloves
- Mask or respirator
- Face shield
- Tyvek suit
- Boots
- TB test prior to study
  - Animal
  - Examiner
Squirrel monkey
TB test
There is now a TB blood test:
- QuantiFERON TB test
- You will need to be current on your TB test
Swine
- Most are normal
- Cataract
- Coloboma
Guideline/Regulations

- Federal
- Quality Assurance - In-house
- GLP
- SOP
Routine Examination

- Since most laboratory studies involve a large number of animals, organization and efficiency become key to success.
Routine Examination

- Since most laboratory studies involve a large number of animals, organization and efficiency become key to success.
- In general, most beagle, primate, swine and rabbit studies I examine have 40-80 animals, rats 250-1200 animals and mice 500-1500 animals.
Routine Examination

- A single ophthalmologist requires 2-3 animal handlers, a data entry individual and in studies over 250 animals 1-2 individuals to go ahead of the handlers to dilate the pupils.
Routine Examination

- A single ophthalmologist requires 2-3 animal handlers, a data entry individual and in studies over 250 animals 1-2 individuals to go ahead of the handlers to dilate the pupils.

- An animal should be in front of the ophthalmologist at all times and findings are reported verbally and then either entered in a computer program or onto a paper record for later entry into a computer database.
Routine Examination

- Animals are dilated with 1.0% tropicamide for dogs and primates and 0.5% tropicamide for rabbits, rats and mice.
Routine Examination

- Animals are dilated with 1.0% tropicamide for dogs and primates and 0.5% tropicamide for rabbits, rats and mice.
- Some advocate use of 10% phenylephrine in pigmented rodents, but I have not found dilation to be a concern.
Routine Examination

- With larger studies, the ophthalmologist should have the handlers dilate approximately the number of animals that the examiner can evaluate in 1 hour.
  - In non-pigmented rats and mice this is approximately how long the mydriasis will last.
Routine Examination

- When examining with both a biomicroscope and indirect ophthalmoscope:
  - I can examine 300-350 rats or mice per hour. When you are just beginning you should estimate less than half this amount.
Routine Examination

- When examining with both a biomicroscope and indirect ophthalmoscope:
  - I can examine 300-350 rats or mice per hour. When you are just beginning you should estimate less than half this amount.
  - With beagles, primates, swine and rabbits, 60-150 animals per hour is reasonable.
Routine Examination

- Dogs and rabbits are typically examined on a table.
Routine Examination

- Dogs and rabbits are examined on a table.
- I prefer to stand for dogs and sit for rabbits.
Routine Examination

- Dogs and rabbits are examined on a table.
- I prefer to stand for dogs and sit for rabbits.
- Rabbits are more manageable when placed on a table with a soft cloth or towel. A high table with the ophthalmologist seated in a low chair works best.
Routine Examination

- Rats and mice are held in a dose hold and presented to the ophthalmologist. The head and legs are restrained and the eyes will partially proptose in this position. The ophthalmologist can be seated or stand.
Routine Examination

- Rats and mice are held in a dose hold and presented to the ophthalmologist. The head and legs are restrained and the eyes will partially proptose in this position. The ophthalmologist can be seated or stand.
- Sit with your legs apart...They will pee on you
Routine Examination

- Room lights should be off, but the handlers will require some light to collect and handle to animal and identify it by tattoo or ear tag.
  - Use an anteroom if possible
Routine Examination

- Room lights should be off, but the handlers will require some light to collect and handle to animal and identify it by tattoo or ear tag.
- Many are now microchipped
Routine Examination

- The ophthalmologist should expect to wear a lab coat, gloves and shoe covers as a minimum and potentially a Tyvec® suit, mask and occasionally a full respirator.
Biomicroscopy

- The slitlamp of choice will depend on the ophthalmologist
- I prefer the Zeiss HSO-10
  - 12X magnification, 125 mm working distance, portability and light weight make it an excellent choice for examination of large numbers of animals.
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  - 12X magnification, 125 mm working distance, portability and light weight make it an excellent choice for examination of large numbers of animals.
- It can be laid down on a table between animals which is easier than hanging a Kowa SL model
Indirect Ophthalmoscopy

- The indirect headset of choice should be lightweight, comfortable, easy to manipulate out of the way with one hand and have a small pupil setting.
Indirect Ophthalmoscopy

- The indirect headset of choice should be lightweight, comfortable, easy to manipulate out of the way with one hand and have a small pupil setting.
- Again, the indirect ophthalmoscope of choice will depend on the ophthalmologist.
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Indirect Ophthalmoscopy

- The indirect headset of choice should be lightweight, comfortable, easy to manipulate out of the way with one hand and have a small pupil setting.
- Again, the indirect ophthalmoscope of choice will depend on the ophthalmologist.
- I prefer the Keeler All-Pupil.
  - This differs from my clinical indirect of choice in clinical practice, which is a Heine. The reason for the difference is the easy of use and lightweight of the Keeler.
Indirect Ophthalmoscopy

- The indirect condensing lens of choice varies by species examined. In general, I feel comfortable with a 30D or 2.2 panretinal lens for routine examination of dogs, a 20D for NHP and a 40D for rats & mice.
Advanced Examination

- Flourescein for evaluation of the cornea
- Flourescein angiography
- Tonometry
- Pachymetry
- Electroretinography
- Photography
- Gonioscopy
- OCT
Marshall beagles have no bones!