1.0 OBJECTIVE

1.1 The objective of this procedure is to describe the procedures for examination of eye using a Micron IV multi-modal (Phoenix research Lab) with technology of slit lamp, funduscope, Optical Coherence Tomography (OCT), or Electroretinography (ERG) in rats and mice

2.0 SCOPE

2.1 This procedure applies to animals used for experimentation.

3.0 POLICY

3.1 It is the policy of MMRI to establish written and approved procedures to ensure that the health and well being of employees is protected, and that potentially hazardous procedures are performed in a safe manner.

4.0 RESPONSIBILITIES

4.1 It is the responsibility of Manager of Animal Research or designated alternate to implement this procedure and revise it when necessary.

5.0 REFERENCES

5.1 Video

5.1.1. Macton Multi-modal: https://www.youtube.com/watch?v=SWfYGnyZy04
5.1.2 Retinal Imaging Microscope:
https://www.youtube.com/watch?v=6CBfDaLyrrI
5.1. 3 Micron IV multi-modal:
https://www.youtube.com/watch?v=SWfYGnyZy04
5.1.4 OCT: https://www.youtube.com/watch?v=tx6N0-TeV6Q

5.2 ANP016 Animal Anesthesia

6.0 SAFETY PRECAUTIONS

6.1 It is the responsibility of all personnel to use good judgment and safe practices in the laboratories. Protective clothing (e.g., laboratory coats, coveralls, boots, face masks, aprons, rubber gloves and safety glasses) are provided by the company.

6.2 Sterile rubber gloves, facemasks, bouffants and safety glasses are worn when performing surgery.

6.3 Used disposable scalpel blades, hypodermic needles and syringes are placed in a disposable sharps container located in the procedure room. When full, the container is disposed of safely.

6.4 All injury accidents are promptly reported to the appropriate Supervisor.

7.0 EQUIPMENT AND MATERIALS

- Corneal lubricant Gel (Novartis,drug stores)
- Phenylephrine (Akorn, cat. no. NDC: 17478-200-12, *http://www.akorn.com*)
- Tropicamide (Akorn, cat. no. NDC: 17478-101-12, *http://www.akorn.com*)
- AK-FLUOR® (fluorescein injection, USP) NCD: 17478-253-10, *http://www.akorn.com*
- Ketamine
- Xylazine
- Sodium Fluorescent solution (AK-FLUOR, Akorn, cat. No.FI00N R07-08) *http://www.akorn.com*
- Phoenix Micron IV multi- modal – http://www.phoenixreslabs.com
  - Retinal Imaging Microscope
  - Slit-lamp
  - Optical Coherence Tomography (OCT)
  - Electroretinography (ERG)
- Insulin syringe
- Heating plate/mat

8.0 PROCEDURE

8.1 Preparation

8.1.1 Dilate pupils using one drop of phenylephrine (quick and short effect) and one drop of Tropicamide (slow and long effect).

8.1.2. A corneal lubricants (2.5% hypromellose ophthalmic gel will be used on the surface of cornea to keep cornea moist during the period of examination.

8.1.3 Once the pupils are dilated (5 to 10 min later), anesthetize animal by an intraperitoneal or intramuscular injection of a ketamine/xylazine (up to 100 mg/kg and 30 mg/kg) mixture (Donovan and Brown, 2006a). The depth of anesthesia will be observed by pinching animal's foot to see the reaction to pain. If animal is not under deep anesthesia, another dose at 1/3 to ½ of the original dose will be given.

8.1.4 Topical cornea anesthetic eye drop (0.5% Proparacaine HCl Ophthalmic Solution Sterile) may be used if the eye blinking during the period of eye examination.

8.2 **Slit Lamp Examination:**

Phoenix Micron IV –Slit lamp will be used for visualization and photo of the anterior segment of the eye including eyelids, conjunctiva, cornea, anterior chamber, iris, and lens

8.2.1 Place animal on Slit lamp platform. A corneal lubricants (2.5% hypromellose ophthalmic gel will be used on the surface of cornea to keep cornea moist during the period of anesthesia.

8.2.2 Place Slit lamp directly toward one eye by moving the platform into the appropriate position

8.2.3 Select appropriate light source and focus image.

8.2.4 Move the platform around to allow you to see different areas of the eye.

8.2.5 Acquire individual images

8.2.6 Place animal back into its cage and place the cage onto a warm heating plate (37◦C) until the animal starts to have blink response and then move in cage.

8.3 **Funduscope Examination:**

Phoenix Micron IV –fundus scope will be used for visualization and photo of posterior segment of the eye including vitreous, retina, retinal cells, and subretinal space. Retinal vascular will also be observed by fundus scope after an intraperitoneal injecting with 5% Sodium Fluorescent solution.

8.3.1 Place animal on funduscope platform. A corneal lubricants (2.5% hypromellose ophthalmic gel will be used on the surface of cornea to keep cornea moist during the period of examination.

8.3.2 Place camera directly on one eye by moving the platform into the appropriate position

8.3.3 Select appropriate filter and focus image.

8.3.4 Move the platform around to allow you to see different areas of the retina

8.3.5 IP injection of 0.1- 0.2 mL of AK-FLUOR® as needed.

8.3.6 Acquire movie or individual images

8.3.7 Place animal back into its cage and place the cage onto a warm heating plate (37◦C) until the animal starts to have blink response and then move in cage.

8.4 **Optical Coherence Tomography** (**OCT**)

Phoenix Micron IV – Optical Coherence Tomography for visualization and photo of fundus structural analysis

8.4.1 Place animal on Slit lamp platform. A corneal lubricants (2.5% hypromellose ophthalmic gel will be used on the surface of cornea to keep cornea moist during the period of examination.

8.4.2 Place camera directly on one eye by moving the platform into the appropriate position

8.4.3 Select appropriate wavelength and focus image.

8.4.4 Move the platform around to allow you to see different areas of the retina

8.4.5 Acquire movie or individual images

8.4.6 Place animal back into its cage and place the cage onto a warm heating plate (37◦C) until the animal starts to have blink response and then move in cage

8.5 **Electrophysiology (ERG) Examination:**

Phoenix Micron IV – **Electrophysiology** with both the global (Ganzfeld ERG) and regional (image guided focal ERG) will be performed for vision functional analysis.

8.5.1 Place animal on funduscope platform. A corneal lubricants (2.5% hypromellose ophthalmic gel will be used on the surface of cornea to keep cornea moist during the period of examination

8.5.2 Place camera directly on one eye by moving the platform into the appropriate position

8.5.3 Select appropriate filter and focus image.

8.5.4 Move the platform around to allow you to see different areas of the retina

8.5.5 Acquire movie or individual images

8.5.6 Place animal back into its cage and place the cage onto a warm heating plate (37◦C) until the animal starts to have blink response and then move in cage