**Submission ID #: 61482**
**Scriptwriter Name: Anastasia Gomez**
**Project Page Link:** https://www.jove.com/account/file-uploader?src=18752058

# Title: Oxygen-Induced Retinopathy Model for Ischemic Retinal Diseases in Rodents

## Authors and Affiliations:

Maria Vähätupa[1,2], Niina Jääskeläinen[2], Marc Cerrada-Gimenez[2], Rubina Thapa[2], Tero A.H. Järvinen[1], Giedrius Kalesnykas[2], Hannele Uusitalo-Järvinen[1,3]

[1]Faculty of Medicine and Health Technology, Tampere University & Tampere University Hospital, Tampere, Finland
[2]Experimentica Ltd, Kuopio, Finland
[3]Eye Centre, Tampere University Hospital, Tampere, Finland

**Corresponding Authors:**

Hannele Uusitalo-Järvinen (hannele.uusitalo-jarvinen@tuni.fi)

**Email Addresses for All Authors:**

maria.vahatupa@tuni.fi
niina.jaaskelainen@experimentica.com
marc@experimentica.com
rubina.thapa@experimentica.com
tero.jarvinen@tuni.fi
giedrius.kalesnykas@experimentica.com
hannele.uusitalo-jarvinen@tuni.fi

# Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **Yes**

   If **Yes**, can you record movies/images using your own microscope camera?

   **Yes**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**, all done

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. **Filming location:** Will the filming need to take place in multiple locations? **No**

**Current Protocol Length**

Number of Steps: 19
Number of Shots: 48

# Introduction

**1. Introductory Interview Statements**

**REQUIRED:**

1.1. **Maria Vähätupa:** After its development, the oxygen-induced retinopathy model has become widely used in preclinical studies of neovascular retinal diseasesIt has been used as a model to study pathological angiogenesis in response to hypoxia.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.2. **Niina Jääskeläinen:** The main advantage of this model is that it induces a robust neovascular response that is reproducible and easily quantifiable.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

**OPTIONAL:**

1.3. **Maria Vähätupa:** This model can be used as a preclinical model for studying the effects of possible therapeutic candidates. It is considered a relevant model for several human diseases, such as retinopathy of prematurity, diabetic retinopathy and wet age-related macular degeneration.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

**Ethics Title Card**

1.4. Procedures involving animal subjects have been approved by the National Animal Ethics Committee of Finland.

# Protocol

## 2. First Experimental Animals and Mouse OIR Model Induction

2.1. Record the weight of the animals before and after hyperoxia induction and at the time of sacrifice **[1]**. Make sure that there is enough food on the bottom of the cage **[2]** and add soda lime with color indicator **[3]** to the bottom of the chamber to absorb excess carbon dioxide when a filtration system is not used **[4]**. *Videographer: This step is important!*

2.1.1. LAB MEDIA: Maria Vähätupa - 1.1.mp4.

2.1.2. LAB MEDIA: Maria Vähätupa - 1.2.mp4.

2.1.3. LAB MEDIA: Maria Vähätupa - 1.3.mp4.

2.1.4. LAB MEDIA: Maria Vähätupa - 1.3 II (extra).mp4.

2.2. Monitor the humidity and temperature inside the chamber, keeping the humidity between 40 and 65% **[1]**. If needed, increase the humidity of the chamber by placing dishes with water on the bottom **[2]**.

2.2.1. LAB MEDIA: Maria Vähätupa - 1.4 I.mp4.

2.2.2. LAB MEDIA: Maria Vähätupa - 1.4 II.mp4.

2.3. Calibrate the oxygen sensor with normal room air and 100% oxygen **[1]**, then place the P7 mice into a chamber and set the oxygen level to 75% **[2]**. Keep the mice in the chamber for 5 days, until P12, monitoring the animals during the induction **[3]**. When finished, remove the mice from the chamber **[4]**.

2.3.1. Talent calibrating the oxygen sensor.

2.3.2. Talent placing the mice in the chamber.

2.3.3. Mice in the chamber.

2.3.4. Talent removing mice from the chamber.

## 3. Drug Administration (Optional)

3.1. Weigh the animals and make identification marks on the tail or ear **[1]**. After applying local analgesia, perform a toe pinch. A small reflex is acceptable **[2]**. Keep the animal on a heating pad during treatment **[3]**.

3.1.1. Talent marking the animal.

3.1.2. Talent performing a toe pinch.

3.1.3. Mouse on the heating pad.

3.2. Use a glass syringe with a 33 to 34-gauge needle for the ivt *(spell out 'I-V-T')* injection **[1]**. Apply a drop of iodine **[2]**, then press the eyelid down and grab the eyeball with forceps **[3]**. Make the injection posterior to the limbus, approximately at a 45-degree angle pointing towards the optic nerve **[4]**. *Videographer: This step is difficult!*

3.2.1. Syringe for injection.

3.2.2. Talent applying a drop of iodine.

3.2.3. Talent grabbing the eyeball.

3.2.4. Talent injecting the drug. NOTE: Use last take

3.3. Keep the needle in place for 30 seconds after injecting the drug to avoid reflux of the injected solution **[1]**. Examine the eye for any complications, such as hemorrhages or retinal damage, after removing the needle **[2]**. Apply antibiotic ointment on top of the eye after the injection **[3]**. *Videographer: This step is difficult!*

3.3.1. Talent holding the needle in place.

3.3.2. Talent examining the eye.

3.3.3. Talent applying antibiotic ointment.

3.4. If desired, conduct in vivo imaging on live animals during the follow-up period to record changes that develop in the retina during the angiogenic responses **[1]**. Use spectral domain optical coherence tomography to visualize retinal layers in vivo **[2]**.

3.4.1. LAB MEDIA: Maria Vähätupa - 4.1 I.mp4.

3.4.2. LAB MEDIA: Maria Vähätupa - 4.1 II.mp4.

## 4. Tissue Collection and Preparation of Retinal Flat Mounts

4.1. Collect the eyes of the animals by cutting the tissue around the eye and grabbing behind the eyeball with curved forceps, , and lifting the eye out from the orbit **[1]**.

4.1.1. Talent collecting the eye.

4.2. Incubate the eyeballs in freshly made, filtered 4% paraformaldehyde for 1 to 4 hours **[1]**. Remove the fixative **[2]** and wash the eyeballs 3 times with PBS for 10 minutes per wash. Dissect the retinas immediately or store them in PBS at 4 degrees Celsius **[3]**.

4.2.1. Talent placing the eyeballs in PFA.

4.2.2. Talent removing the PFA.

4.2.3. Talent washing the eyeballs in PBS.

4.3. Prepare retinal flat mounts to quantify the amount of neovascularization and the size of avascular areas, or AVAs. Dissect the retina under a stereo microscope using micro scissors and forceps **[1]**.

4.3.1. Talent at the dissection microscope.

4.4. Place the eyeball in PBS to keep it moist and puncture it at the limbus with a 23-gauge needle, then cut around the limbus with curved micro scissors to remove the iris and the cornea **[1]**.

4.4.1. SCOPE: Talent puncturing the eyeball and cutting around the limbus to remove iris and cornea. NOTE: All scope shots uploaded and files named according to shot number.

4.5. Carefully place the tip of the scissors between RPE and neural retina and cut the RPE/choroid/sclera complex towards the optic nerve **[1]**. Do the same to the other side of the eyeball, then gently cut or tear the tissueuntil the retinal cup is exposed **[2]**. Pull the lens out from the retinal cup and add PBS to the cup **[3]**.

4.5.1. SCOPE: Talent cutting the sclera.

4.5.2. SCOPE: Talent cutting or tearing the sclera.

4.5.3. SCOPE: Talent pulling out the lens and adding PBS to the retinal cup.

4.6. Remove all the hyaloid vessels, vitreous, and debris without damaging the retina **[1]**. Make four incision to the retina with straight micro scissors to create a flower-like structure **[3-TXT]**. Use a soft paintbrush or transfer pipette to transfer it to a well-plate for staining **[4]**. *Videographer: This step is important!*

4.6.1. SCOPE: Talent removing hyaloid vessels, vitreous, and debris.

~~4.6.2. SCOPE: Talent adding PBS to the retinal cup.~~

4.6.3. SCOPE: Talent making the incisions. **TEXT: 12, 3, 6 and 9 o'clock**

4.6.4. Talent transferring the retina to a well-plate.

4.7. Label the retinal vasculature with Isolectin $B_4$ *(pronounce 'B-four')*, which stains the surface of endothelial cells. Incubate the retinas in blocking buffer for 1 hour **[1]** and wash them with 1% NGS and 0.1% Triton in TBS for 10 minutes **[2]**.

4.7.1. Talent putting the retinas in the blocking buffer, with the blocking buffer container in the shot.

4.7.2. Talent washing the retinas, with the wash solution container in the shot.

4.8. Incubate the retinas with 5 to 10 micrograms per milliliter fluorescent dye conjugated Isolectin $B_4$ **[1]** overnight at 4 degrees Celsius while protected from the light **[2]**.

4.8.1. Talent placing the retinas in Isolectin $B_4$.

4.8.2. Talent putting the retinas in the refrigerator.

4.9. On the next day, wash the retinas 3 times with 1% NGS and 0.1% Triton in TBS for 10 minutes per wash **[1]**. Place the retinas on a microscope slide, inner retina facing

upwards **[2]**, then carefully spread out the retina using a soft paintbrush and remove any remaining hyaloid vessels or debris **[3]**.

4.9.1. Talent washing the retinas, with the wash solution container in the shot. NOTE: Use take 2.

4.9.2. Talent placing the retinas on a microscope slide. NOTE: Use take 2.

4.9.3. Talent spreading the retinas out and removing debris. NOTE: Use take 2.

4.10. Add mounting medium on top of the sample **[1]** and place cover slip on top of the slide. Store the retinas at 4 degrees Celsius protected from light **[2]**.

4.10.1. Talent adding mounting medium on top of the retina. NOTE: Use take 2.

4.10.2. Talent placing the coverslip on top of the retina.

4.11. Image the retinal flat mounts using a fluorescence microscope with a 10 X objective. Focus on the superficial vascular plexus and the preretinal neovascularization **[1]**. Make a tile scan image to capture the whole retina and merge the tile scans **[2]**.

4.11.1. SCREEN: Marc Cerrada-Gimenez - 6.1 I Screen capture.mp4.

4.11.2. SCREEN: Marc Cerrada-Gimenez - 6.1 II Screen capture.mp4.

4.12. Quantify the images by measuring the AVAs, area of neovascularization, and total retinal area using an image processing program **[1]**. Draw the AVAs and total retinal area using a free hand drawing tool **[2]** and select the neovascular areas using a selection tool **[3]**.

4.12.1. Talent at the computer using the image processing software.

4.12.2. SCREEN: AVA.mp4.

4.12.3. SCREEN: NV.mp4.

# Results

**5. Results: Rat and Mouse Models of OIR**

5.1. In the mouse oxygen-induced retinopathy model, the vaso-obliteration occurs in the central retina **[1]**, while in the rat model it develops in the periphery, similar to human ROP **[2]**. Preretinal neovascularization develops near the avascular areas, specifically the central retina in mouse and periphery in rats **[3]**.

5.1.1. LAB MEDIA: Figure 2 A and 3 A. *Video Editor: Emphasize 2 A.*

5.1.2. LAB MEDIA: Figure 2 A and 3 A. *Video Editor: Emphasize 3 A.*

5.1.3. LAB MEDIA: Figure 2 A and 3 A.

5.2. Histological analysis using either cross-sections or flat mounts can be done to evaluate morphological changes in OIR retinas or the presence of cell types of interest, for example inflammatory cells **[1]**.

5.2.1. LAB MEDIA: Figure 2 B.

5.3. Optionally, non-invasive in vivo imaging can be conducted during the OIR follow-up period. Retinal and hyaloid vasculature can be visualized with fluorescein angiography **[1]** and spectral domain optical coherence tomography can be used to evaluate structural changes in the retina **[2]**.

5.3.1. LAB MEDIA: Figure 4 A.

5.3.2. LAB MEDIA: Figure 4 B.

5.4. Functional changes can be measured by electroretinography **[1]**.

5.4.1. LAB MEDIA: Figure 5.

5.5. Aflibercept, a soluble VEGF-trap, inhibits both neovascular and physiological revascularization in OIR **[1]**. OIR eyes injected at P14 with a high dose of aflibercept had even bigger retinal AVAs than untreated eyes, suggesting that aflibercept also blocks physiological retinal revascularization driven by hypoxia **[2]**.

5.5.1. LAB MEDIA: Figure 6 A.

5.5.2. LAB MEDIA: Figure 6 B. *Video Editor: Emphasize the Aflibercept data.*

# Conclusion

## 6. Conclusion Interview Statements

6.1. **Niina Jääskeläinen:** When attempting this protocol, remember to consider the proper litter size for your experiment in advance and monitor the wellbeing of the dams and the pups in the oxygen chamber. Take into account that post-natal weight gain affects the outcome in the model.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.1.1, 2.1.2.*

6.2. **Maria Vähätupa:** Retina samples obtained from this model can be used for analytical methods such as immunohistochemistry, mRNA or protein analyses to study expression levels of genes and proteins and to measure the effects of potential therapeutic candidates on the retina at the molecular level.

6.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. NOTE: Text slightly edited during the shoot.