Best Practices for Using Human Eye Tissue in Research
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Obtaining human eye tissues for research

Working with eye and tissue banks

- Most eye and tissue banks are willing to help you obtain the tissue and/or fluids you require. They can provide whole globes, corneal rims, anterior segments, and posterior poles. With special training, they can collect aqueous humor, isolate specific ocular tissue, and may be willing to isolate tissue and store for DNA, RNA or protein isolation as long as you are willing to train and supply the reagents.

- There are over 600 eye banks around the world; most are members of one of six multinational eye bank associations.
  - Eye Bank Association of America
  - European Eye Bank Association
  - Eye Bank Association of Australia and New Zealand
  - Association of Eye Banks of Asia
  - Similar organizations operate India and Latin America.

  Contact your local city, state or regional eye bank to discuss your needs. You can use EyeFind to locate an eye bank that currently provides research tissue.

- When evaluating eye and tissue banks, consider costs of obtaining any tissue. While tissue may be donated, there are costs for processing, treating, testing, etc. that is intrinsic to the process.

- Schedule a time to meet with the eye bank’s CEO or its Technical Director to discuss tissue needs (normal tissues, diseased tissues—see below), recovery costs, and specialized training. Have a short presentation ready that can be used to show the eye bank how the use of the tissue/fluids will enhance your research. In most situations, they are eager to learn about your research and it can also help them when discussing donation with patients’ families as it provides examples of how the tissue will be used. It may be necessary to meet and train the technicians performing enucleations and/or phone center personnel obtaining consent.

- Establish protocols defining types of eye tissues (normal vs disease; whole eyes vs. corneal rims vs. posterior poles), acceptable age range, time from death to enucleation, preservation options (fresh, on ice, fixed, stored in specific media or reagent), and numbers of eyes/tissues over a period of time (provide example protocol). Also mention donor exclusion criteria.

- Protocols should include well-documented discard procedures. Consult with your eye and tissue bank for any local guidelines for disposal of tissue after project is complete.
If diseased tissue is required, determine the protocol for establishing the presence and severity of the disease (medical records, donor history from family, examination by eye bank personnel, etc.).

Discuss pathogen testing options and procedures (regardless of test results, treat all tissues as infectious - use universal precautions). It is also helpful to request a blood sample with eye donations that can be used for genetic testing or stored in case of an emergency (needle stick, etc.)

Obtain letter of support if needed for grant application/budget. Provide a draft letter with an overview of the project, including the inclusion and exclusion criteria, number of eyes/tissues required over a specific period of time (typically less than the length of the grant) and other relevant requirements.

Establish a process that includes communication between eye bank personnel and laboratory personnel (PI and technical staff), transport carriers, and delivery instructions. Make the process simple and reproducible so that it can be routinely followed.

For consenting of eye donor’s family, make sure eye bank does not have age limitations (e.g.: <4 or >70 years old) for eye donor (their triage protocol can be changed). Also, ask about research only consent. This is generally much simpler than consenting for transplant tissue (e.g.: An eye bank’s Donor Risk Assessment Interview, the tool used to screen prospective donors, can be over 100 questions for donors for transplant, but fewer than 20 questions for donors specifically for research (plus any specific questions needed for the project).

Make good use of the tissue and acknowledge the source of the tissue in any subsequent publications. It is the donor’s wish that their ocular tissues be used for research, and some patient groups follow-up on such information. Make this wish come true for the donor and their family.

Consult with local regulations and requirements regarding the approval status, or ethical clearance, of the use of human tissue in a research project. For example, consider whether an institutional review board (IRB) approval or exemption is necessary.

Consider geographical logistics and regulations regarding the transportation of tissue, especially across country borders. This consideration should also be a factor in the protocols when determining the time interval between processing at the eye bank and delivery to the researcher.

**Working with pathologists**

Find eye pathologists via national or international ophthalmic pathology organizations, such as:

- [The American Association of Ocular Oncologists and Ophthalmic Pathologists](https://www.aoop.org/)
- [The Verhoeff-Zimmerman Society](https://www.vzs.org/)

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Meet with your pathologist and discuss your tissue needs. Ascertain which pathologist(s) may have an ocular tissue biobank, and/or harvests specific tissue types from consented samples.

Establish contact with respective pathologists and obtain information/availability to receive tissues between technical staff members, and transport arrangements, etc.

Fresh tissue may be sampled in the operating room. However, it is important that a pathologist approves the methods by which any surgical pathology specimens are utilized in this manner, as they may need specific tissues for diagnosis. Importantly, even the collection of surgical material that would otherwise be discarded requires approval of the local ethical authority.

Organize simultaneously a Material Transfer Agreement between Institutions, if required.

Talk to pathologist to determine if formalin fixed paraffin embedded archival tissue may be available for research pending appropriate IRB approval

Organize any documentation concerning ethical approval required (such as an institutional review board, research ethics board, human tissue authority).

Obtain a letter of support from the pathologist for a grant application. Provide a draft letter with an overview of the project, including the inclusion and exclusion criteria, number of eyes/tissues required over a specific period of time (typically less than the length of the grant).

Why is this resource needed in our field?

In many studies, there is inconsistent and insufficient reporting of information about human donor/condition of eyes, making it challenging to interpret experimental results using tissues or primary cells isolated from these eyes. This is particularly true with donor eyes having a putative eye disease, often without sufficient documentation or characterization.

— W. Daniel Stamer, PhD, FARVO

Working with surgeons (surgical discard tissues)

Communication between the researchers and surgeons is one of most important factors in a successful partnership in this method of obtaining human tissue. Robust procedures for the execution of obtaining, handling, and transportation of surgical discard tissues are vital to the success of such partnerships.
**Corneal Rims (post corneal button removal)**

- Meet with corneal transplant surgeon(s) to discuss research needs, frequency of surgeries
- After surgeon agrees to collaborate, meet with operating room staff manager to obtain weekly surgery schedule and establish logistics (put corneal rim back into original eye bank container, place container in biohazard Ziploc bag, store bag in refrigerator, etc.)
- Establish time for daily pickups by a laboratory team member
- Contact eye bank to (i) verify that eye donor consented to both transplant and research before using tissue and (ii) obtain available information about eye donor
- Distribute tissues on a rotating basis if there is more than one PI user at an institution

**Surgical samples**

- Contact surgeons to discuss research study involving collection of disease tissue.
- After surgeon(s) agrees to collaborate, write up protocol and obtain IRB approval.
- Discuss protocol with study coordinators and inform them of the inclusion/exclusion criteria and any specific information that will enable you to obtain the appropriate tissue.
- Incorporate a process where surgeon notifies PI/technical staff that a surgical procedure will be taking place.
- PI/technical staff should provide on day of surgery the necessary containers that the tissue should be stored in (1.5 ml tubes, etc.) and storage conditions (ice, dry ice, liquid nitrogen).
- Establish the process of surgical staff notifying the laboratory personnel that the tissue is ready to be picked up.

**Aqueous humor/vitreous humor/lens capsules**

- IRB approved protocol is required.
- Discuss reasonable practices in collection techniques with the surgeon (such not removing all the aqueous humor)
- The PI (usually the patient’s ophthalmologist but can also be nurse, technician or study coordinator) informs the patient about the study and obtains written consent prior to surgery.
- Protocol is established for collecting and storing samples.
- If material is deemed waste, as in aqueous humor that needs to be removed from the anterior segment prior to cataract surgery, protocols can be set up for surgeon to collect and provide to research lab.
- Determine local guidelines for consent needed for use of any surgical discard.
Best Practices needed to validate human tissue for research by scientific area

Age-related macular degeneration (AMD)
(Phenotype donor for the stage of AMD)
For assessing disease status there are a number of excellent classification systems currently in use and that continue to be developed

- Eyes can be phenotyped using clinical records when available. In some cases (and with some consent processes), decades of clinical records may be available, which can be used to assign AREDS grades.
- If ocular imaging of the donor eye is available, the investigator can use a postmortem phenotyping scale such as the Minnesota, Alabama, Utah, or other grading systems using photomicrography and/or OCT.
- Phenotyping of histological samples can employ a system such as the Alabama Grading System using the defined features of AMD (confluent basal laminar deposits across the macula)

Additional information
- Treatments for neovascular AMD (anti-VEGF injections, photodynamic therapy, even thermal laser); length of treatment time, time since last treatments
- Donor consumption of supplements (AREDS or other vitamins); length of treatment time
- Documentation of ocular history (years since AMD diagnosis, other ocular diseases)
- Time of death to enucleation; and
- Time of death to fixation/culture

Cornea altered by Refractive Surgery
- Screen by history (history of LASIK, PRK)

Other Corneal Dystrophies
- Screen by history (history of dystrophies such as basement membrane dystrophy, stromal dystrophies, etc)
- Any characteristic features (characteristic stromal deposits/opacifications) seen on microscopy or OCT

Who will benefit from the resource?
The eye research community as a whole, especially reviewers and readers of papers and grants.

— W. Daniel Stamer, PhD, FARVO
**Diabetic Retinopathy**

**Evaluation if medical history is available (documented evidence of the following in EMR)**

- Evidence of DR treatment (e.g., evidence of anti-VEGF injections, laser treatment, membrane peel, etc.)
- Evidence of DR pathology (e.g., OCT, fundus imaging, angiograms, etc.)
- Evidence of retinal hemorrhage
- Metabolic disease status and time course (e.g., duration of diabetes [time since initial diagnosis], BMI, blood pressure, HDL cholesterol, triglycerides, etc.)
- Diabetes metrics and time course (e.g., fasting plasma glucose, HbA1c, glucose tolerance, etc.)
- Evidence of other diabetes complications (e.g., nephropathy, neuropathy, etc.)

**Evaluation methodology in the laboratory if no medical history is available (or to complement medical history)**

- Post-mortem retinal biopsy
- Confirm retinal vasculopathy in eye cup under dissecting or operating microscope with transillumination or using other imaging methods (i.e., postmortem phenotyping)
  - Bleeding into the vitreous
  - Dilated retinal blood vessels
  - Retinal lipid deposits
  - Retinal vascular abnormalities
  - Retinal neovascularization
  - Retinal vascular fibrosis/scarring
  - SD-OCT imaging

**Other considerations**

- Type 1 or 2 diabetes
- Time from initial diabetes diagnosis
- Time from death to enucleation
- Time from death to fixation/culture

**Dry Eye**

- Dry eye medications (artificial tears, Restasis, Cequa, Xiidra, Eyesuvis, Loteprednol, doxycycline, fish oil)
- History of dry eye, eye irritation, eye redness documented in medical record
- History of refractive surgery or systemic autoimmunity
- Punctate epithelial erosions on slit lamp exam

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Fuchs Endothelial Dystrophy

- History of or clinically detected guttata or corneal edema (biomicroscopy or central corneal thickness > 600μm by pachymetry or OCT), use of hypertonic saline
- Family history of Fuchs dystrophy
- History of corneal transplant
- Specular microscopy: Guttata, low endothelial cell count (< 1500/mm²)
- OCT or microscopy showing penetrating or endothelial keratoplasty (note: the recovered PK or DSEK will not have Fuchs)
- OCT or microscopy showing thickened Descemets membrane and/or guttata

Glaucoma

- Glaucoma medication list, type(s) and duration; and/or
- Documented or visualized glaucoma surgery (e.g., trabeculectomy, stent, shunt, etc); and/or
- Documented visual field defects, retinal nerve fiber thinning and/or optic nerve cupping.
- Evaluation methodology in the laboratory if no medical history is available
- Confirm optic nerve head cupping by histology, optic nerve axon counting and/or retinal ganglion cell body counting in laboratory

Additional information

- History of IOP elevation (and/or ex vivo measurement of outflow facility)
- Documentation of type of glaucoma (POAG, Angle-closure, trauma-induced, etc.)
- Family history of glaucoma
- Time of death to enucleation; or
- Time of death to fixation/culture
- If enucleation only, time of enucleation to fixation/culture
- COD

Inherited Retinal Disease

- Obtain genotype, clinical history, and images if available.
- Collect blood or other suitable tissue for genotyping if not already known.
- Identify appropriate subjects and approach patient and family about potential donation. Consider having research eye donation brochures available in waiting rooms.
- Eye Bank should work with family to promptly collect tissue if IRD patient has a known terminal illness.
- Documentation with gross photographs can be very helpful.
Develop standard protocols for processing of fixed and unfixed tissues.

Coordinating sharing of tissue, especially for particularly rare diseases.

**Keratoconus**

- History of keratoconus, topographic signs of keratoconus, high astigmatism (> 3 diopters), scleral contact lens wear, ocular allergy
- Family history of keratoconus
- History of cornea transplant (as long as not sole criteria)
- History of cross-linking or intrastromal rings
- OCT or Microscopy showing apical corneal scarring, Descemet’s membrane break, central or midperipheral stromal thinning

**Lens**

**Preservation Time (DPT)**

The time between death and recovery and stabilization of the tissue is critical for most uses of lens material. Use of the tissue will dictate how much latitude can be tolerated in DPT.

- For baseline analysis of lens tissue e.g. gene or protein expression or for culturing of whole lenses, lenses should be less than 24 hours post-mortem.
- For use in imaging of cataracts, whole globes can be used at up to 48 hrs DPT. Posterior poles (globs with cornea removed) often cannot be used for imaging due to opacification of the lens during storage and transport.
- For stretching experiments using dissected whole globe where the lens along with the accommodation apparatus (zonules, ciliary body and sclera) is stretched to simulate disaccommodation, tissues less than 96 hr DPT should be used.

**Shipping Conditions**

- For eyes destined for imaging studies, globes should be wrapped in gauze soaked with PBS and kept submerged in PBS in a sealed container; Materials should not be refrigerated or frozen. If shipping is necessary, the container may be kept cool by including a cold pack in the packaging, but the globe should not be allowed to undergo “cold cataract”.
- DMEM or BSS may also be used as an immersion medium.
- The key is to minimize movement within the transport box and the storage container. If whole lenses (with or without the cornea) are transported, it is good to tether them to a support structure if available e.g clamping/securing the optic stalk, in order to prevent movement. If support structures are not available, both eyes from a donor pair are placed in the same container, which is filled to the brim with fluid. The containers are then taped down within the transport box. Together these reduce impact of the tissue against the container during transport and markedly improve the
quality received. The transport box is chilled using ice blocks and packed with filler to further ensure the tissue containers remain in place.

- When establishing tissue preparations for culture it is always best to bring the tissue up to room temperature and transfer the preparation to medium also at room temperature to prevent a temperature shock prior to placing in the incubator.

**Preservation and fixation for microscopy**  
The simple initial fixation protocol of 10% neutral buffered formalin for 24 hours followed by fresh 4% paraformaldehyde works well for most human specimens, as described in Mohamed et al., Mol Vis 19:2352-9, 2013.

**Processing for RNA extraction**  
Collect <24h DPT human lenses in RNA Protect solution (Qiagen, Cat No./ID: 76104). Trizol (Sigma-Aldrich, 93289) can also be used for collection of human lenses. Lenses may also be flash frozen with dry ice and stored at -80C (preferred) and kept under dry ice during shipping.

**Processing for immunofluorescence.**  
For immunofluorescence, wash lenses in 1XPBS and fix for 30-45 minutes in 4% para-formaldehyde (PFA) and then immerse in tissue freezing media, OCT (Tissue Tek, Torrance California). Alternatively, the lenses can be directly immersed in OCT without prior fixation (and after sectioning, they can be subject to different fixation conditions) — preferred for different antibodies.

**Myopia**
- Age of onset of myopia, family history of myopia, documented refraction and/or corrective wear (glasses/contacts).
- Documentation of refraction so can further classify the degree of myopia (low/moderate/high)
- Any history of any non surgical refractive interventions such as atropine use, OrthoK, etc
- History of retinal detachment/tears, history of glaucoma, history of cataract, history of retinal degenerations
- Any documented ocular surgery (e.g., trabeculectomy, stent, shunt, retinal detachment surgery, cataract extraction, etc.) or refractive surgery (cataract surgery, LASIK/PRK), documented refraction prior to surgery.
- Evaluation methodology in the laboratory if no medical history is available
  - Obtain axial length of the eye (surrogate for refractive error)
  - Confirm optic nerve head cupping by histology, optic nerve axon counting and/or retinal ganglion cell body counting in laboratory (would help to distinguish from axial elongation due to early-onset glaucoma)
Additional Information
- History of IOP elevation
- Time of death to enucleation; or
- Time of death to fixation/culture
- If enucleation only, time of enucleation to fixation/culture
- Obtain genotype, clinical history, and images if available.
- Collect blood for genotyping if not already known.

Ocular Hypertension
- Glaucoma medication list, type(s), and duration
- History of IOP elevation (at least 3 IOP measurements >22 mm Hg)
  - If not available, perform outflow facility measurements (bucket and stop watch method) in laboratory (<0.1 µl/min/mmHg is pathologically low outflow facility)

Additional Information
- Documentation of type of glaucoma (POAG, Angle-closure, trauma-induced, etc.)
- Time of death to enucleation; or
- Time of death to fixation/culture
- If enucleation only, time of enucleation to fixation/culture

Ocular Tumors

Intraocular tumors:
- Uveal Melanoma — tumor tissue may be harvested by the eye pathologist on receipt of a fresh enucleation taken from a consented patient. This tumor material may be flash frozen for later use, with implementation of a wide range of technologies. In small (posteriorly located) uveal melanomas, material may be harvested from the non-tumor parts of the globe — e.g. anterior segment structures, lens, peripheral retina, and peripheral choroid. Following completion of the diagnostic report, access to the corresponding formalin-fixed paraffin embedded (FFPE) tissue may be possible. This FFPE tumor material can be used for morphological-, digital imaging-, immunohistochemical-, genomic- and other downstream studies.
- Retinoblastoma — in contrast to uveal melanoma eyes, retinoblastoma globes are less commonly dissected fresh. This is because of the ease with which the retinoblastoma cells scatter, and thereby compromise the sample for diagnostics and reporting of certain features (e.g. choroidal invasion). Hence, tumor harvesting may be performed in consented samples after globe transillumination (to establish location of the main tumor) via a small transcleral flap and using a fine needle aspiration procedure. Following completion of the diagnostic report, access to the
corresponding formalin-fixed paraffin embedded (FFPE) tumor tissue may be possible. This FFPE tumor material can be used for morphological-, digital imaging-, immunohistochemical-, genomic- and other downstream studies.

**Extraocular tumors**

- **Conjunctival melanoma** – harvesting of fresh conjunctival melanoma tissue for research is difficult and must be undertaken by the reporting pathologist only, in order to ensure that there is no compromise of the surgical margins. Fresh tumor harvesting is likely to be undertaken in large tumors only, e.g. an orbital exenteration sample or an excision specimen of a large nodular tumor, where apical sampling is possible. Otherwise, following completion of the diagnostic report, access to the corresponding formalin-fixed paraffin embedded (FFPE) tissue may be possible in consented patients. This FFPE tumor material can be used for morphological-, digital imaging-, immunohistochemical-, genomic- and other downstream studies.

- **Orbital tumors** – e.g. orbital carcinomas associated with the lacrimal gland or orbital lymphomas - harvesting of fresh orbital tumor tissue for research is difficult and must be undertaken by the reporting pathologist only, in order to ensure that there is no compromise of the surgical margins or that there remains sufficient tumor material to obtain a diagnosis. Orbital surgeons may provide an extra fine needle biopsy, in addition to the diagnostic biopsy, for research purposes in consented patients. Otherwise, following completion of the diagnostic report, access to the corresponding formalin-fixed paraffin embedded (FFPE) tissue may be possible in consented patients. This FFPE tumor material can be used for morphological-, digital imaging-, immunohistochemical-, genomic- and other downstream studies.
Publishing data derived from human eye tissues or cells

Scientific rigor is critically important to the advancement of research and discovery. The areas addressed in this section and not solely for record keeping but are for the assurance of reliability and reproducibility of a study. Overall, any data that would be useful for research to be repeatable should be included in a publication. Data inclusion in a publication can also impact the confidence a reviewer, and ultimately a reader, has in the results of the research.

Any publication of the data included should state where the information is gathered, such as from family interviews, eye bank records, or patient medical records. The publication should include an explanation of why any of the minimum requirements were not available.

**Minimum requirements***

- Age, sex, and other readily available or relevant demographic information (i.e. ethnicity of donor)
- Source of the tissue or cells
  - Examples: Eye bank or other repository, surgical discard, medical examiner, etc.
- Time interval from death to preservation
- Time interval from death to receipt (or initiation of experiment)
- Method of preservation
  - Examples: ice, fixation, etc.
- Cause of death
- Statement on the ethical approval or exemption of use of human tissue
- Consideration of individuals or groups that provided an intellectual or other significant contribution to the research project at all stages, whether through an authorship or acknowledgment. Acknowledgement of any known donors should be included.

*Fetal tissue will not require the same minimum requirements, due to availability

**Additional information, where available**

- Donor history of ocular disease, or systemic disease
- Ocular disease noted from evaluation of tissue
- Medication list relevant to ocular health
  - Examples: eye drops, diabetes medications, chemotherapy, intravitreal injections with anti-VEGF medication, supplement use, etc.

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What impact do you hope to see as a result of the best practices?

Our goal is to improve the awareness of eye researchers and consequently the quality of data that will appear in presentations and in the literature as a result of these guidelines.

— W. Daniel Stamer, PhD, FARVO
- Evidence of prior ocular surgery
  - Examples: scleral buckle, trabeculectomy, corneal refractive surgery, etc.
- Co-morbidities
- Reason for enucleation, if obtained via surgeon/pathologist
- Time on ventilator (if relevant)