Optimized protocol for human eye preparation to obtain high-quality retina specimens

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Abstract

Histological evaluation of retina is an important part of cross reactivity and various toxicology studies required for novel drug development. Dissection, processing and embedding of the eye often produces a total retinal detachment resulting in significant morphology artifacts and limited quality of specimens. We modified an existing protocol for sandwich eyeball preparation and achieved minimal retina artifacts and better histological evaluation of the stained specimens.

Historical method review

Sandwich Embedding of Eyeball Wall for Optimal Paraffin Sections of Retina
E. Sutter; W. Meier-Ruge
Pathological Institute, University of Basle, Basle, Switzerland. January 1965

After fixing for 2-4 hr, the cornea is cut off and the lens extruded to accelerate fixation of the retina. Extraocular muscle, peribulbar fat and associated connective tissue are then removed, and the anterior half of the eyeball cut away to allow inspection of the retina.

The sandwich consists of two slices of **fixed,** partially dehydrated liver with a piece of eyeball wall between them. The preparation is wrapped with thread, which is removed just before the paraffin block

Materials

- Whole fresh human eye;
- Histology preparation workstation;
- Eye forceps;
- Eye scissors;
- Paper filter; paper clip;
- 10% buffered formaldehyde solution;
- Tissue Tek VIP tissue processor;
- Tissue embedding medium Paraplast plus;
- Tissue Tek embedding center.

Method

Whole human eye specimens were collected from consented donors in collaboration with a certified eye bank in the USA and delivered to Cureline histology laboratory within 12 hours of procurement (Fig.1).


Figure 1

Sectioning of an eyeball started from lateral upper surface using eye scissors and forceps (Fig.2; Fig.3; Fig.4).


Figure 2

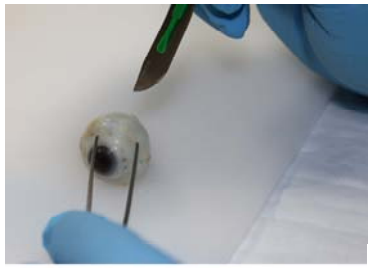
Figure 3

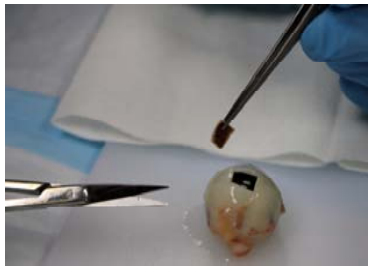
Figure 4

Method

A few small eye wall fragments were dissected and gently placed on the preparation board (internal surface up) (Fig.5). Retinal detachment did not occur due to presence of vitreal fluid.

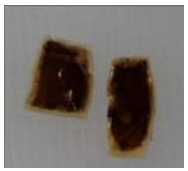
Figure 5

Fold the paper filter into a cone shape to make a pocket (Fig.6; Fig.7).

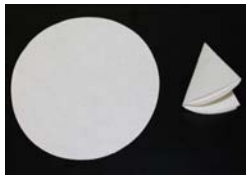
Figure 6

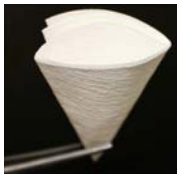
Figure 7

Two or more eye wall fragments were put together as a sandwich and placed into the pocket of the paper filter (Fig.8; Fig.9).


Figure 8

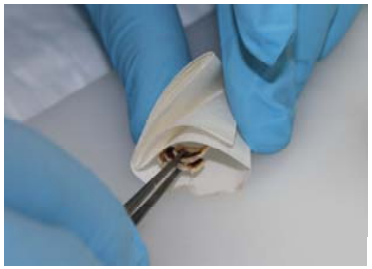
Figure 9

Method

The pocket was closed using a paperclip, tissue was fixed in 10% buffered formaldehyde for 24h, and processed in an automated tissue processer (Tissue-Tek VIP). The paper filter was removed from the specimen before embedding in IHC grade paraffin (Fig.10; Fig.11).


Figure 10


Figure 11

Conclusion

This procedure helped us to develop a complete eye wall fragment specimen with perfect retina histology (Fig.12).

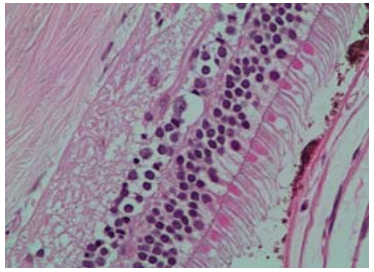
Figure 12

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