STM JOURNALS

Emerging Trends in
Personalized Medicines

ISSN: 3049-1347
Volume 2, Issue 2, 2025
July–December
DOI (Journal): 10.37591/ETPM

https://journals.stmjournals.com/etpm

Review ETPM

Exploring the Dynamics of Eye Fluid: Lymphatics, Aqueous Humor, and Intraocular Pressure

Maya Sharma[1,*], Satyavijay Singh[3], Ranu Sharma[1], P. K. Choudhury[4], Ankita Joshi[1], Amol Ghodke[2]

Abstract

Lymphatic vessels are essential for regulating interstitial fluid balance, facilitating immune cell transport, supporting lipid absorption, and playing significant roles in tumor development and metastasis. Recent findings have expanded their recognized functions, particularly in pathological contexts. One notable discovery is the involvement of lymphatics in draining aqueous humor from the eye, a process closely linked to the regulation of intraocular pressure (IOP) and its implications for glaucoma progression. This review provides an overview of the dynamics of ocular fluids, examines the distribution and structure of ocular lymphatics, and highlights studies that have advanced the three-dimensional visualization of the ocular vasculature. Additionally, it explores the molecular mechanisms governing the development of ocular lymphatic vessels and investigates how lymphatic pathways may influence aqueous humor outflow. The interaction between lymphatic and glymphatic systems is also discussed, particularly in relation to retinal clearance and diseases affecting the posterior eye. The review concludes by addressing unresolved questions and suggesting research directions to further elucidate the interplay between these systems in maintaining ocular fluid homeostasis.

Keywords: lymphatic vessels, lymphatic system, aqueous humor drainage, intraocular pressure, eye disease, glaucoma

INTRODUCTION

The human eye is filled with a viscoelastic substance known as vitreous humor and maintains a specific intraocular pressure (IOP) that gives the eye its characteristic spherical shape. This pressure is vital for normal eye function, as deviations from the optimal range can lead to various ocular disorders, such as glaucoma, ocular hypertension, and retinal detachment. Glaucoma, often resulting from impaired fluid drainage within the eye, stands as the second leading cause of blindness globally. It occurs when increased IOP damages the optic nerve, disrupting its connection to the brain and affecting the retinal nerve fibers responsible for transmitting visual information [1].

***Author for Correspondence**
Maya Sharma
E-mail: maya.sharma1703@gmail.com

[1]Associate Professor, Faculty of Pharmacy, Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan, India
[2]Lecturer, Maharashtra Polytechnic D Pharmacy Institute, Nilanga, Maharashtra, India
[3]Faculty of Pharmacy, Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan, India
[4]Professor, Faculty of Pharmacy, Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan, India

Received Date: November 04, 2024
Accepted Date: April 19, 2025
Published Date: April 23, 2025

Citation: Maya Sharma, Satyavijay Singh, Ranu Sharma, P. K. Choudhury, Ankita Joshi, Amol Ghodke. Exploring the Dynamics of Eye Fluid: Lymphatics, Aqueous Humor, and Intraocular Pressure. Emerging Trends in Personalized Medicines. 2025; 2(2): 31–44p.

While glaucoma and related diseases can be managed using eye drops, laser therapy, or surgical intervention, early diagnosis remains critical because any optic nerve damage is irreversible. Various diagnostic tests, such as tonometry, are available to measure IOP accurately. Non-contact tonometry, particularly the air puff test, is widely used for its non-invasive nature. Beyond its ophthalmic applications, air puff techniques have been employed in other biomedical fields, such as assessing the mechanical properties of soft tissues, embryonic development, and even food texture. However, the accuracy of IOP assessments using non-contact methods can be influenced by external pressure and variations in corneal biomechanics

among individuals [2].

Chronic dehydration, particularly extracellular dehydration, has been linked to several systemic diseases, including hypertension and type 2 diabetes, which may also impact ocular physiology. The renin-angiotensin-aldosterone system (RAAS) is activated in dehydration states, potentially contributing to disease progression. Water, a major component of the eye, plays an integral role in maintaining visual function. Despite this, the effects of hydration on ocular health have not been thoroughly examined. This review aims to investigate how hydration status influences ocular physiology and its association with common eye diseases [3].

FLUID COMPARTMENTS OF THE EYE AND DYNAMICS OF OCULAR FLUIDS

The maintenance of fluid balance is critical for the anatomy and functionality of the eye. In mammals, the anterior segment, situated between the cornea and lens, is filled with aqueous humor, a transparent fluid produced by the ciliary processes. This fluid flows from the posterior chamber through the pupil into the anterior chamber, with its production relying on a combination of passive and active processes, including diffusion, filtration, and secretion. Plasma diffusion and ultrafiltration through the fenestrated capillaries of the ciliary body facilitate the movement of water, solutes, and large molecules. Active ion transport across the ciliary epithelial cells generates osmotic pressure gradients, driving aqueous humor secretion [4].

In contrast, the vitreous humor occupies the posterior segment of the eye, situated between the lens and retina. This gel-like substance is composed of highly hydrated extracellular matrix proteins and polysaccharides, which contribute to its viscoelastic properties. Unlike aqueous humor, vitreous humor does not undergo continuous outflow but experiences gradual remodeling over time, with partial liquefaction occurring during aging [5].

Both aqueous and vitreous humor are essential for eye health, as they provide nutrients to avascular structures, clear metabolic waste, regulate intraocular oxygen levels, and maintain appropriate IOP to preserve the eye's shape. Aqueous humor supports corneal convexity, while the gelatinous consistency of the vitreous humor stabilizes the retinal layers and lens [6].

Dysfunction in ocular fluid regulation can lead to severe pathologies, especially in older individuals. Aqueous humor dynamics are central to IOP regulation, with outflow primarily occurring through two pathways: the trabecular meshwork and Schlemm's canal in the iridocorneal angle, and the uveoscleral route via the ciliary body. Impaired drainage due to increased resistance in the trabecular meshwork or dysfunction of the uveoscleral pathway can result in elevated IOP and subsequent glaucoma (Figure 1).

While the vitreous humor has historically been considered static, recent studies suggest the possibility of fluid flow through it. Evidence of drug diffusion and removal from the posterior to the anterior segment supports the hypothesis of limited but functional fluid movement within the vitreous. These findings have implications for the delivery and efficacy of treatments targeting diseases of the posterior segment [7].

Water Balance in the Eye

Aqueous humor is primarily produced by the ciliary processes in the posterior chamber and flows into the anterior chamber. Most (90%) of its outflow occurs through the trabecular meshwork, Schlemm's canal, and associated collector channels, while the remaining 10% exits via the uveoscleral route. The sclera, which is approximately 10% less hydrated than the cornea, plays a role in fluid dynamics influenced by proteoglycan concentrations.

Compared to plasma, aqueous humor contains significantly lower protein levels and higher concentrations of ascorbate and lactate. This composition reflects its role in protecting the eye from

oxidative stress and supporting anterior segment metabolism. The blood-aqueous barrier (BAB), consisting of tight junctions in the ciliary epithelium, regulates ionic gradients and restricts passive permeability to small, non-ionic molecules [8].

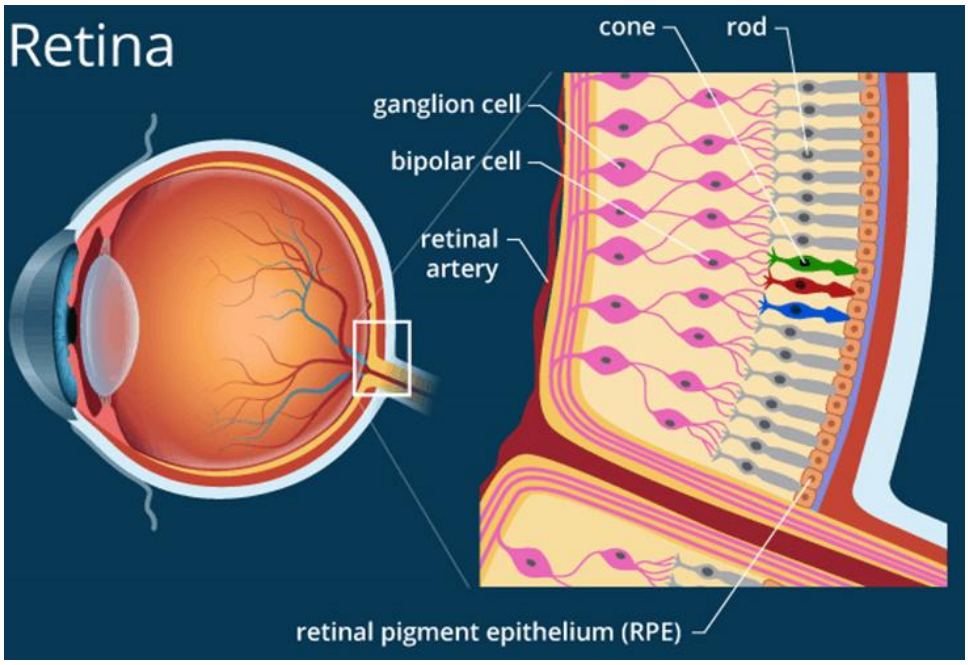

Figure 1. Retina of Eye.

Vitreous humor, on the other hand, restricts the movement of substances between the anterior and posterior eye due to its gel-like consistency. Aging and pathological conditions can lead to liquefaction, facilitating faster exchange and affecting ocular physiology. Collagen fibrils and glycosaminoglycans within the vitreous form a matrix that maintains its structural integrity and regulates diffusion gradients.

Physiological mechanisms, including active transport across blood-ocular barriers and metabolic processes in the ciliary body and retina, maintain water balance in the eye. Disruptions to these processes, such as those caused by hyperosmotic agents or disease states, can alter ocular fluid dynamics and impact visual function. Notably, the RAAS has been implicated in regulating fluid homeostasis in the eye, with components, like pro-renin, found in higher concentrations in the vitreous compared to plasma. This system's involvement in diabetic retinopathy and other ocular diseases highlights its significance in maintaining ocular health [9].

ANATOMY OF THE EYE LYMPHATIC VASCULAR SYSTEM

Lymphatic vessels in the eye were first identified over two centuries ago, with historical perspectives documented extensively by researchers, such as Grüntzig and Hollmann. Recent decades have seen remarkable advancements in the understanding of ocular lymphatic structures, enabled by innovations in imaging techniques and the discovery of specific molecular markers. These markers, such as lymphatic vessel endothelial hyaluronic acid receptor 1 (LYVE-1), podoplanin, vascular endothelial growth factor receptor 3 (VEGFR3), and prospero-related homeobox protein 1 (PROX-1), have facilitated the distinction between lymphatic and blood endothelial cells [10].

The presence of a surface network of lymphatic vessels in mammals has been confirmed through immunostaining studies and dye injection experiments. These vessels are located at the corneolimbus surrounding the avascular cornea and in both the bulbar and palpebral conjunctiva. Immunohistological analysis of dissected eye sections has provided detailed insights into this distribution. Furthermore, advanced imaging techniques, such as light-sheet fluorescence microscopy (LSFM) and fluorescent reporter transgenic animal models, have enhanced the visualization of these lymphatic networks.

Studies on corneal lymph angiogenesis have significantly contributed to understanding the role of Corne limbal lymphatics. These LYVE-1-positive vessels exhibit characteristic features, such as oak leaf-shaped endothelial cells and button-like interen dothelial junctions. Additionally, they may develop valves, suggesting their function as precollectors. Connections between limbal lymphatics and the conjunctival lymphatic network have been established, with denser lymphatic vessel distributions observed on the nasal side of the eyeball [11].

Recent research has further clarified the organization of ocular surface lymphatics, revealing dorsal-ventral and temporal-nasal polarization. For instance, dense, looped networks of lymphatic vessels drain the ventral part of the eyeball, while less dense networks converge into larger lymphatic trunks in the dorsal region. These structures exhibit symmetry between the left and right eyes and converge at the nasal canthus. The nictitating membrane, present in some species, also contains lymphatic structures; however, in humans, this membrane is vestigial [12].

LYMPHATIC VESSEL DEVELOPMENT AND FUNCTION

The development of ocular lymphatic vessels, or lymphangiogenesis, typically begins in early postnatal stages. Initial lymphatic vessels emerge from parent structures located near the nasal canthus and expand around the eyeball through sprouting lymphangiogenesis. Intussusception, a process where new vessels form via transcapillary pillar creation, may also play a role in lymphatic expansion. Evidence of this mechanism has been observed in lymphatic malformations and the formation of lymph node sinuses.

While traditional lymphatic vessels are largely absent in the internal eye tissues, certain structures, such as the ciliary body and iris have shown the presence of LYVE-1-positive cells. However, these cells are often identified as macrophages rather than true lymphatic endothelial cells. This distinction is crucial, as it highlights potential interspecies differences in ocular lymphatic systems. For example, studies on rodents suggest the absence of internal lymphatics, whereas similar investigations in sheep and humans provide conflicting results.

Despite these findings, the existence of unconventional or organ-specific lymphatic subtypes in the eye cannot be entirely ruled out. Further research is necessary to determine whether these unique structures exist and how they may contribute to ocular fluid regulation and immune responses. Advances in molecular and imaging technologies will undoubtedly aid in resolving these questions, providing a clearer understanding of lymphatic functions in the eye (Figure 2).

MOLECULAR MECHANISMS REGULATING EYE LYMPHATIC VESSEL DEVELOPMENT

The development of the ocular lymphatic vasculature likely utilizes some of the common signaling pathways that govern lymphatic development in other organs. In mammals, the formation of lymphatic vessels begins during mid-gestation (around E9.5 in mice), originating from the cardinal vein wall. Lymphatic endothelial differentiation occurs from a specific subset of venous endothelial cells, with the process regulated by various transcription factors, including SOX18 (sex determining region Y-box 18), COUP-TFII (chicken ovalbumin upstream promoter transcription factor 2), HHEX (hematopoietically expressed homeobox), and GATA-2 (GATA binding factor 2). These factors work together to activate PROX-1, a key transcription factor responsible for specifying lymphatic endothelial identity and maintaining lymphatic function. The subsequent budding of these endothelial cells to form lymph sacs, followed by the expansion of the lymphatic vasculature, is driven by the VEGFC/VEGFR3 signaling pathway, a major regulator of lymphangiogenesis in vertebrates. Another important pathway involved in lymphatic vascular remodeling and maturation is the angiopoietins/TIE receptor system. Angiopoietin-1 and angiopoietin-2 interact with the TIE-2 receptor to regulate both blood and lymphatic vascular development. In certain organs, such as the heart, lymphatic vessels have been shown to arise from both venous and non-venous origins and form postnatally, illustrating organ-specific differences in the cellular origins and regulatory mechanisms during lymphatic development. Notably, the

Schlemm's canal, a specialized vessel responsible for aqueous humor outflow, has a hybrid identity, exhibiting both blood venous and lymphatic characteristics. It expresses lymphatic markers, such as PROX-1 and VEGFR3 and shares regulatory pathways common to lymphatic vessel development. Both the VEGFC/VEGFR3 and angiopoietin/TIE pathways are essential for the formation and maintenance of Schlemm's canal.

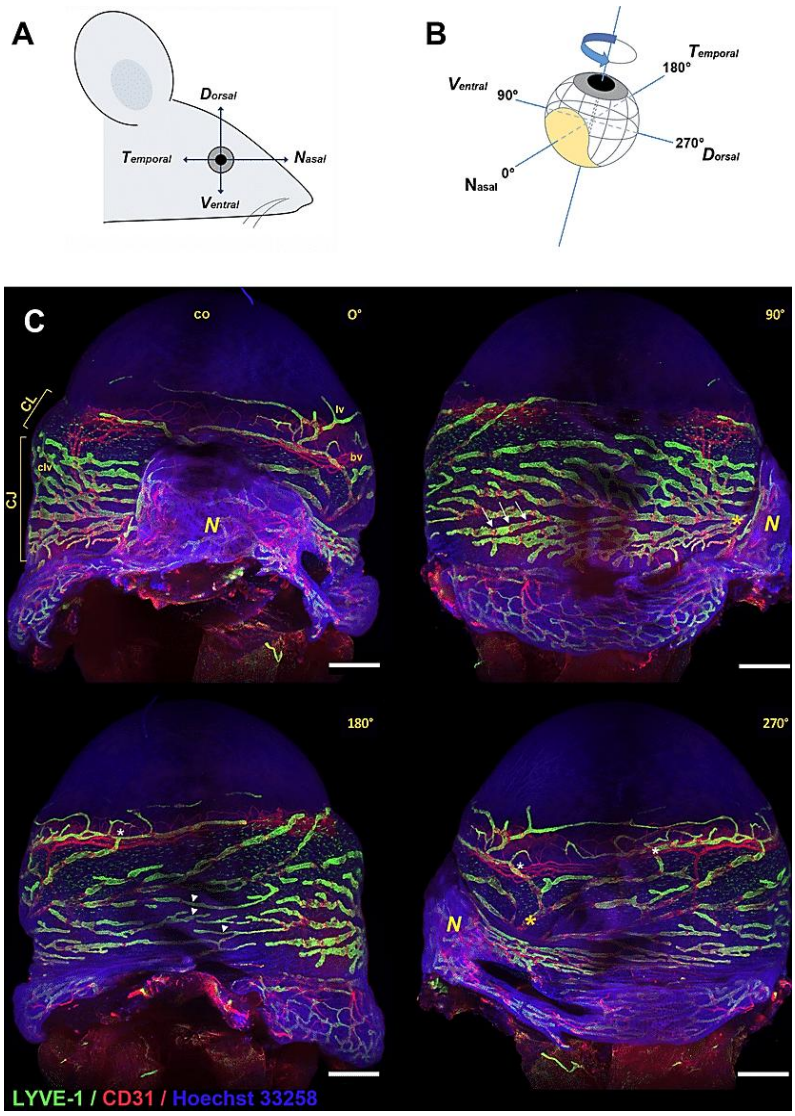

Figure 2. Visualization of Ocular Surface Lymphatics Through Whole-Mount Immunostaining. (A) Light sheet fluorescence imaging of the temporal side of the left mouse eye, using LYVE-1 (green) and CD31 (red) antibodies. Hoechst 33258 (blue) was used for counterstaining the nuclei. The conjunctiva (CJ), corneolimbus (CL), and cornea (co) are indicated. The inset, adapted from, provides a schematic representation of the cardinal axes of the left eye and the location of the nictitating membrane (shown in yellow). (B–D) High-magnification images of the corneolimbal (B) and conjunctival (C, D) vasculature after whole-mount fluorescence immunostaining and flat-mounting of the anterior segment of the dissected eye. LYVE-1-positive staining highlights lymphatic vessels (yellow asterisk), while blood vessels in the corneolimbus are identified solely by CD 31 staining (blue star). In (C), the gap in LYVE-1 staining corresponds to a valve, which is confirmed by VE-cadherin staining in a similar field shown in (D). The white arrow points to the valve in both images. The pattern of VE-cadherin staining, which appears punctuated or continuous depending on whether it's pre- or post-valve, reflects the characteristic structure of lymphatic button-type or intermediate button/zipper-type endothelial cell junctions (Figure 3).

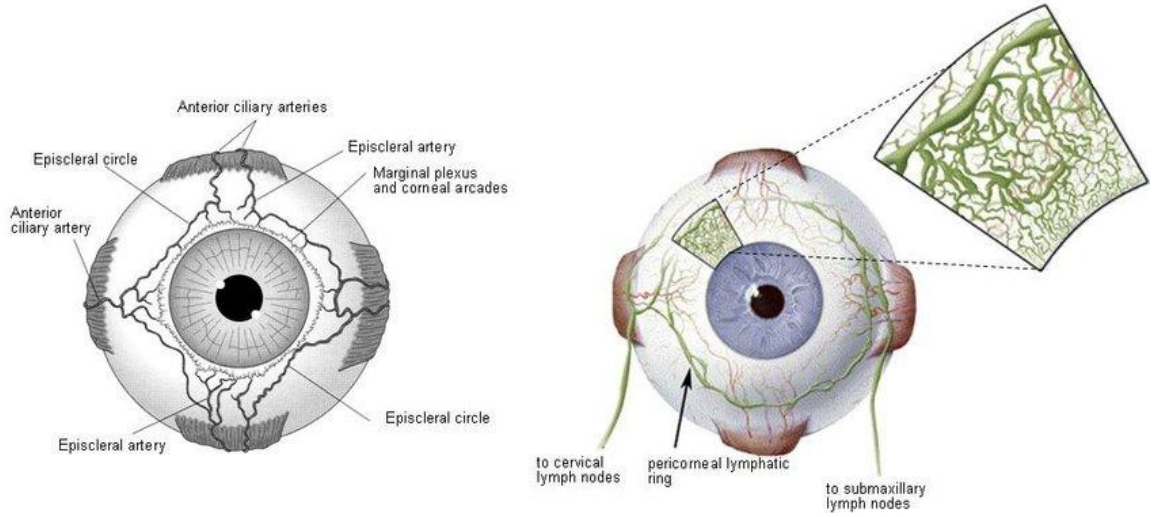

Figure 3. Schematic representation of the lymphatic ocular surface network.

This review focuses on the classical mechanisms underlying ocular lymphatic vessel formation. As anticipated, the VEGFC/VEGFR3 signaling pathway is crucial for lymphangiogenesis in the eye. VEGFC is essential for limbal lymphatic vessel formation during development. In VEGFC-deleted mutant mice, limbal lymphatics were nearly absent at postnatal stage P7, and their formation was significantly inhibited in wild-type mice treated with soluble VEGFR3 (sVEGFR3) between P1 and P6. The angiopoietins/TIE-2 pathway also plays a key role in ocular lymphatic development. Research from Susan Quaggin's group identified this pathway as critical for both ocular lymphatic vessel formation and Schlemm's canal development. In transgenic mice, conditional deletion of both angiopoietin-1 and angiopoietin-2 at late embryonic stages (Post E16.5) led to the failure of ocular lymphatic development. Interestingly, no lymphatic defects were observed when only one of the angiopoietin ligands was conditionally deleted, indicating a compensatory effect between the two ligands. Future studies may explore the involvement of angiopoietin-4, another ligand for the TIE-2 receptor that plays a role in venous development.

In addition to their roles in development, these pathways are involved in regulating the ocular lymphatic vasculature under normal and pathological conditions. VEGFC is a major regulator of corneal lymphangiogenesis, and its inhibition has been shown to prevent inflammation-induced corneal invasion by lymphatic vessels. Macrophages in the limbus, which contribute to corneal inflammation, are a source of VEGFC, promoting corneal lymphangiogenesis. Conversely, sVEGFR3 secreted by the corneal epithelium helps maintain corneal transparency by inhibiting lymphangiogenesis. Angiopoietin-2 also promotes corneal lymphangiogenesis from the existing corneolimbal lymphatic vessels, and inhibiting angiopoietin-2 has been linked to improved corneal graft survival.

A recently identified regulatory pathway in ocular lymphatic maturation involves Bone Morphogenetic Protein 9 (BMP9), a factor secreted by the liver and a member of the TGFβ superfamily. BMP9 activates the ALK1 receptor, primarily found on endothelial cells, and is known for its role in blood vascular quiescence. It also regulates blood retinal vascularization during postnatal development. BMP9 deficiency has been shown to affect lymphatic maturation and valve formation, leading to reduced lymphatic drainage efficiency without impacting blood vessels. In the conjunctiva, a significant reduction in lymphatic valve numbers was observed, accompanied by an enlargement of the lymphatic vessels draining the dorsal part of the eyeball, which may affect drainage efficiency. Unlike the VEGFC and angiopoietin pathways, BMP9 signaling specifically affects lymphatic vessels without influencing Schlemm's canal morphogenesis [13]. This suggests that BMP9 could be targeted for therapies focusing specifically on ocular lymphatics, while sparing the Schlemm's canal. Ephrin B2/EPHB4 signaling has also been implicated in eye lymphatic valve formation, like its role in mesenteric lymphatics [14].

CRITICAL EYE SPECIMEN COLLECTION

The collection of eye specimens is typically carried out when there is suspicion of an ocular infection or disease, and prompt, accurate diagnostic testing is necessary. Common specimens collected include conjunctival swabs, corneal scrapings, or aqueous/vitreous fluid. It is essential to follow established protocols to ensure accurate diagnosis and safeguard patient well-being. The following is a general outline of the procedure:

Preparation

Gather Necessary Materials

- Sterile gloves.
- Sterile cotton-tipped swabs, spatulas, or loop (for corneal scraping).
- Sterile specimen containers or transport media (e.g., viral transport media, bacterial culture tubes).
- Glass slides (If Microscopy is Required).
- Anesthetic drops (e.g, Proparacaine).
- Appropriate labeling materials.
- Disinfectants for the external eye (If Applicable) [15].

Patient Preparation

1. Explain the procedure to the patient.
2. Ensure the patient is seated comfortably with good head support.
3. If necessary, administer topical anesthetic drops to minimize discomfort [16].

Conjunctival Swab Collection

1. *Positioning:* Gently pull down the lower eyelid to expose the conjunctival sac.
2. *Swabbing:* Use a sterile swab to collect the specimen by gently rolling it over the conjunctiva.
3. *Storage/Transport:* Immediately place the swab into the appropriate transport medium for bacterial, fungal, or viral cultures [17].

Corneal Scraping (If Required)

Note: This procedure is more invasive and often performed by an ophthalmologist.

1. *Anesthetize the Eye:* Apply 1–2 drops of topical anesthetic.
2. *Sterilization:* Clean the eyelid margins and surrounding skin with an antiseptic solution if necessary.
3. *Collection:*
   - Use a sterile spatula or blade to scrape the affected area of the cornea.
   - Collect material for culture, slide preparation, or other diagnostic tests.

4 *Storage/Transport:* Transfer material directly onto slides or into appropriate media for microbiology or pathology evaluation [18].

Aqueous or Vitreous Fluid Collection

Note: Typically performed in sterile settings by ophthalmologists using aseptic techniques.

1. *Topical Preparation:* Clean the eyelids and administer anesthetic drops.
2. *Collection via Paracentesis:*
   - Insert a sterile needle (Fine-Gauge) into the anterior chamber or vitreous cavity.
   - Collect a small volume of fluid (e.g., 0.1–0.2 mL).
3. *Storage/Transport:* Place fluid in sterile tubes for analysis (e.g., culture, PCR, cytology) [19].

Labeling and Documentation

1. Label specimens with patient details (name, date of birth, collection site, and time).
2. Document the procedure in the patient's medical record, noting:
   - Type of specimen.

- Collection site.
- Method of collection.
- Any observations (e.g., purulent discharge, corneal opacity) [20].

Transport

1. Transport specimens to the laboratory promptly, ideally within 2 hours of collection.
2. Ensure specimens are maintained under appropriate conditions (e.g., refrigerated for viral specimens, room temperature for bacterial cultures) [21].

Post-Procedure Care

1. Advise the patient on any post-procedure care or symptoms to watch for (e.g., increased pain, redness, or discharge).
2. Follow up with the patient as required, depending on diagnostic results [22].

Important Considerations

- Use aseptic techniques throughout to prevent contamination.
- For suspected ocular fungal infections, use specialized fungal media or stains.
- If Acanthamoeba infection is suspected, collect specimens for direct microscopy and culture on non-nutrient agar seeded with *E. coli* [23].

Critical Eye Specimen Collection Containers

The choice of critical eye specimen collection containers depends on the type of specimen collected and the suspected pathogen (bacterial, viral, fungal, or parasitic). Proper selection ensures the viability of the specimen during transport and accurate diagnostic results. Below is a list of commonly used containers for various eye specimens:

Swabs for Conjunctival Specimens

- *Container Type:* Sterile swabs with transport media.
- *Bacterial cultures:* Amies or Stuart transport media.
- *Viral cultures:* Viral transport media (VTM) with antibiotics.
- *Chlamydia or Mycoplasma:* Specialized transport media (e.g., Universal Transport Medium or Mycoplasma transport media).
- *Fungal cultures:* Sterile swabs; no transport medium required (or fungal-specific medium, if available) [24].

Corneal Scrapings

- *Container Type:* Sterile tubes, slides, or direct inoculation
- *Transport/Preparation:*
- Directly inoculate the sample onto appropriate culture media (e.g., blood agar, chocolate agar, Sabouraud agar).
- Place additional scraps onto glass slides for Gram stain, Giemsa stain, or calcofluor white stain.
- Use sterile dry containers if direct inoculation is not possible [25].

Aqueous or Vitreous Fluid

- *Container Type:* Sterile tubes or syringes
- *Transport/Storage:*
  - Use sterile, screw-cap tubes or syringes (with the needle capped securely) for bacterial and fungal cultures.
  - Transport in sterile tubes for PCR, cytology, or biochemical analysis.
  - Avoid preservatives unless instructed by the laboratory [26].

Special Cases

- *For Acanthamoeba Detection:*
  - *Container Type:* Sterile dry containers or transport medium.
- *Transport Instructions:*
  - Place corneal scrapings or contact lens cases in non-nutrient agar plates seeded with *E. coli* (if immediate culture is needed).
  - Alternatively, use sterile saline for storage and transport [27].
- *For Histopathology:*
  - *Container Type:* Formalin-filled specimen container.
- *Instructions:*
  - Collect tissue biopsies (e.g., scleral or conjunctival biopsy) and immediately immerse them in 10% neutral buffered formalin [28].

Additional Considerations

- *Universal Transport Medium (UTM):*
  - Suitable for multiplex testing (e.g., viral PCR, Chlamydia, or atypical bacteria).
- *Dry Containers:*
  - Ideal for direct microscopy (e.g., fungal elements or parasites) (Table 1).
- *Preservatives:*
  - Only use preservatives (e.g., formalin or saline) if required by the laboratory for specific diagnostic tests [29].

Labeling and Transport

Clearly label containers with:

- Patient name, date of birth, and unique identifier.
- Specimen type (e.g., conjunctival swab, corneal scraping).
- Date and time of collection.
- Suspected diagnosis (to guide laboratory testing).
- Transport specimens under recommended conditions (e.g., refrigerated, room temperature) as specified for the suspected pathogen [30].

Routine Eye Specimen Collection Procedure

Routine collection of eye specimens is carried out to diagnose non-critical ocular conditions, such as conjunctivitis, blepharitis, or mild corneal infections. Employing the correct technique is crucial to prevent contamination and ensure the accuracy of diagnostic results [31].

PREPARATION

Materials Needed

- Sterile cotton-tipped swabs or brushes.
- *Transport media:*
  - *Bacterial culture:* Amies or Stuart transport medium.
  - *Viral detection:* Viral Transport Medium (VTM).
  - *Fungal identification:* Dry sterile containers or fungal-specific media.
- Glass slides (if required).
- Personal protective equipment (PPE): Gloves, face mask.
- Disinfectants (e.g., alcohol swabs for equipment).
- Anesthetic drops (if needed) [32].

Patient Preparation

1. Explain the procedure to the patient to obtain informed consent.
2. Ensure the patient is seated comfortably with good head support.
3. For young children, ensure proper assistance to stabilize their head.

4. Administer topical anesthetic drops if discomfort is expected [33].

Table 1. Critical eye specimen collection containers.

Critical Eye Specimen Collection Containers (Fluids, Scrapings, Tissues, Contact Lenses)			
Specimen	***Test Required***	***Collection Container***	***Notes***
a. Vitreous/aqueous fluid. b. Corneal scraping. c. Tissued. Other fluid.	Bacterial culture	*PRAS (Pre-Reduced Anaerobic Sterilized) Transport Media:* Use to collect Fluids <5 mL. Inject collected fluid through vial stopper. 	*Media to Inoculate at Bedside* • Blood agar plate • Chocolate agar • Thioglycollate broth • Slide for gram stain
		Sterile Container Use to collect Fluids >5 mL, scrapings, tissues	
			
	Fungal Culture	**Sterile Container** (all specimens)	*Media to Inoculate at Bedside* • Inhibitory Mold Agar (IMA). • Slide for Calcofluor white stain. • Submit a second slide if both gram and fungal stains required.
			
	Mycobacteria culture (Acid-fast bacilli, AFB)	**Sterile Container** (all specimens) 	Dedicated specimen and Prov Lab requisition required. Testing performed at Prov Lab.
• Vitreous/ • aqueous fluid	Acanthamoeba culture	Submit specimen in sterile container with10 mL Pages Amoeba Saline	
• Corneal scraping • Tissue • Other fluid • Contact lens • Contact lens • solution			
Corneal scraping	Viral conjunctivitis panel	Universal Transport Medium (UTM) 	Dedicated specimen and Prov Lab requisition required. Testing performed at Prov Lab.
• Vitreous/aqueous fluid	chorioretinitis Panel	**Sterile Container**	Dedicated specimen and Prov Lab requisition required. Testing performed at Prov Lab.

			

SPECIMEN COLLECTION

Conjunctival Swabs

1. *Purpose:* Diagnosis of conjunctivitis (bacterial, viral, fungal, or chlamydial).
2. *Procedure:*
   - Pull down the lower eyelid gently to expose the conjunctival sac.
   - Use a sterile swab to gently roll over the palpebral conjunctiva or the fornix.
   - Avoid touching eyelashes or eyelid margins to prevent contamination.
   - *Storage/Transport:* Place the swab in the appropriate transport medium [34].

Eyelid Margin Swabs

1. *Purpose:* Diagnosis of blepharitis or eyelid infections.
2. *Procedure:*
   - Moisten a sterile swab with sterile saline if necessary.
   - Gently roll the swab along the eyelid margin where the infection is localized.
3. *Storage/Transport:* Use appropriate medium for bacterial or fungal cultures [35].

Corneal Scraping (for Non-Critical Cases)

1. *Purpose:* To identify superficial corneal infections.
2. *Procedure:*
   - Apply topical anesthetic drops (e.g., Proparacaine).
   - Use a sterile spatula or blade to gently scrape the superficial layer of the affected corneal region.
   - Avoid excessive pressure to minimize patient discomfort.

3 *Storage/Transport:*
   - Transfer the specimen directly onto slides for staining (e.g., Gram or Giemsa stain).
   - Alternatively, inoculate directly onto culture media (e.g., blood agar, chocolate agar) [36].

Labeling and Transport

1. Clearly label all specimens with:
   - Patient's name.
   - Date of birth.
   - Collection site and type.
   - Date and time of collection [37].

2 Transport specimens to the laboratory as quickly as possible:
   - Maintain room temperature for bacterial and fungal cultures.
   - Refrigerate viral specimens if a delay is unavoidable [38].

Documentation

1. Record the procedure in the patient's chart, including:
   - Site and method of collection.
   - Any observed abnormalities (e.g., discharge, inflammation, lesions) [39].

Post-Procedure Care

1. Advise the patient on any expected sensations (e.g., mild irritation from scraping).
2. Instruct the patient to avoid touching or rubbing their eyes.
3. Schedule a follow-up appointment if necessary, depending on the results.

Common Diagnostic Applications

- *Bacterial infections:* Identified via culture or Gram staining.
- *Viral infections:* Detected through PCR or immunofluorescence assays.
- *Fungal infections:* Diagnosed via microscopy and fungal culture (Table 2).
- *Chlamydial infections:* Confirmed using PCR or special stains [40].

Table 2. Routine Eye Specimen Collection Containers.

Routine Eye Specimen Collection Containers(Swabs)			
Specimen	***Test Required***	***Collection Container***	***Notes***
• Conjunctival swab • Corneal swab	Bacterial Culture	**Liquid Amies Swab (Red lid)** 	Direct Bed Side in Oculation of Media/Slides is not Required.
	Gonorrhea Culture	**Liquid Amies Swab (Red lid)** 	Performed only if <3 months old, treatment failure, sexual exposure/assault, environmental exposure
	Chlamydia/Gonorrhea Naat	**Aptima Unisex Blue Swab** (Discard white Swab in Package, Collect Specimen with the Blue Swab) 	
	Viral conjunctiv it is panel	**Universal Transport Medium (utm)** 	Dedicated Specimen Required. Testing Performed at Prov Lab. Prov Lab Requisition Required.

CONCLUSIONS

The collection and analysis of eye fluids (aqueous or vitreous humor) are vital in diagnosing infectious, inflammatory, and malignant conditions affecting the eye. These specimens provide direct insight into intraocular pathologies, aiding in the identification of pathogens, such as bacteria, viruses, fungi, or parasites, as well as autoimmune or neoplastic disorders. Proper collection techniques, performed under strict aseptic conditions by trained ophthalmologists, are essential to avoid contamination and complications. Despite challenges, like limited sample volume and the need for rapid processing, eye fluid analysis is invaluable. Techniques, such as microbiological cultures, PCR, cytology, and biochemical assays enable precise and timely diagnosis, facilitating targeted therapies that reduce morbidity and preserve vision. Ultimately, the careful handling and study of eye fluids are integral to advancing ophthalmic care and improving patient outcomes.

REFERENCES

1. Goldmann Applanation Tonometry. Multiparameter Correction Equation. 2011;88(1):102–12.
2. Boyer G, Pailler-Mattei C, Molimard J, Pericoi M, Laquieze S, Zahouani H. Non contact method for in vivo assessment of skin mechanical properties for assessing effect of ageing. Med Eng Phys. 2012;34(2):172–8.
3. Fleury V, Al-Kilani A, Boryskina OP, Cornelissen AJM, Nguyen TH, Unbekandt M, et al. Introducing the scanning air puff tonometer for biological studies. Phys Rev E. 2010;81(2):021920.
4. Kling S, Marcos S. Finite-Element Modeling of Intrastromal Ring Segment Implantation into a Hyperelastic Cornea. 2013;54(1):881–9.
5. Wang S, Li J, Manapuram RK, Menodiado FM, Davis R, Twa MD, et al. HHS Public Access. 2015;37(24):5184–6.
6. Lee YS, Owens CM, Meullenet JF. A Novel Laser Air Puff and Shape Profile Method for Predicting Tenderness of Broiler Breast Meat. 2005.

7. Swan L. Origin(s) of Design in Nature.
8. Valero C, Homer I. Non-destructive fruit firmness sensors: A review. 2005;3:61–73.
9. Jequier E, Constant F. Water as an essential nutrient: the physiological basis of hydration. Eur J Clin Nutr. 2010; 64:115–23.
10. Manz F. Hydration and disease. J Am Coll Nutr. 2007;26:535S–41S.
11. Stookey JD, Brass B, Holliday A, Arieff A. What is the cell hydration status of healthy children in the USA? Preliminary data on urine osmolality and water intake. Public Health Nutr. 2012;15:2148–56.
12. Bonnet F, Lepicard EM, Cathrin L, et al. French children start their school day with a hydration deficit. Ann Nutr Metab. 2012;60:257–63.
13. Fadda R, Rapinett G, Grathwohl D, et al. Effects of drinking supplementary water at school on cognitive performance in children. Appetite. 2012;59:730–7.
14. McCauley LR, Dyer AJ, Stern K, Hicks T, Nguyen MM. Factors influencing fluid intake behavior among kidney stone formers. J Urol. 2012;187:1282–6.
15. Fischbarg J. Water channels and their roles in some ocular tissues. Mol Aspects Med. 2012;33:638–41.
16. To C-H, Kong C-W, Chan C-Y, Shahidullah M, Do C-W. The mechanism of aqueous humour formation. Clin Exp Optom. 2002;85:335–49.
17. Kiel J, Hollingsworth M, Rao R, Chen M, Reitsamer HC. Ciliary blood flow and aqueous humor production. Prog Retin Eye Res. 2011;30:1–17.
18. Sacco R, Guidoboni G, Jerome JW, Bonifazi G, Marazzi NM, Vercellin ACV, et al. A theoretical approach for the electrochemical characterization of ciliary epithelium. Life. 2020;10:8.
19. Monteiro JP, Santos FM, Rocha AS, Castro-De-Sousa JP, Queiroz JA, Passarinha LA, et al. Vitreous humor in the pathologic scope: Insights from proteomic approaches. Proteomics Clin Appl. 2014;9:187–202.
20. Nawaz M, Rezzola S, Cancarini A, Russo A, Costagliola C, Semeraro F, et al. Human vitreous in proliferative diabetic retinopathy: Characterization and translational implications. Prog Retin Eye Res. 2019;72:100756.
21. Wang Q, Thau A, Levin AV, Lee D. Ocular hypotony: A comprehensive review. Surv Ophthalmol. 2019;64:619–38.
22. Wang Q, Thau A, Levin AV, Lee D. Ocular hypotony: A comprehensive review. Surv Ophthalmol. 2019;64:619–38.
23. Johnson M, McLaren JW, Overby D. Unconventional aqueous humor outflow: A review. Exp Eye Res. 2017;158:94–111.
24. Smith DW, Lee C-J, Gardiner BS. No flow through the vitreous humor: How strong is the evidence? Prog Retin Eye Res. 2020;78:100845.
25. Riva CE, Alm A, Pournaras CJ. Ocular circulation. In: Kaufman PL, Alm A, editors. Ocular Circulation. 11th ed. New York: Saunders Elsevier; 2011. p. 243–73.
26. Foster C, de la Maza M. The Sclera. New York: Springer-Verlag; 1994.
27. Krause U, Raunio V. Proteins of the normal human aqueous humour. Ophthalmologica. 1969; 159:178–85.
28. DiMattio J. Active transport of ascorbic acid into lens epithelium of the rat. Exp Eye Res. 1989;49:873–85.
29. Lightman SL, Palestine AG, Rapoport SI, Rechthand E. Quantitative assessment of the permeability of the rat blood-retinal barrier to small water-soluble nonelectrolytes. J Physiol. 1987;389:483.
30. Stamer WD, Snyder RW, Smith BL, Agre P, Regan JW. Localization of aquaporin CHIP in the human eye: implications in the pathogenesis of glaucoma and other disorders of ocular fluid balance. Invest Ophthalmol Vis Sci. 1994;35:3867–72.
31. Sebag J. Age-related changes in human vitreous structure. Graefes Arch Clin Exp Ophthalmol. 1987; 225:89–93.
32. Lund-Andersen H, Sander B. The Vitreous. In: Kaufman PL, Alm A, editors. The Vitreous. 11th ed. New York: Saunders Elsevier; 2011. p. 164–81.

33. McNeil AR, Gardner A, Stables S. Simple method for improving the precision of electrolyte measurements in vitreous humor. Clin Chem. 1999;45:135–6.
34. Reddy DV, Kinsey VE. Composition of the vitreous humor in relation to that of plasma and aqueous humors. Arch Ophthalmol. 1960;63:715–20.
35. Fatt I. Flow and diffusion in the vitreous body of the eye. Bull Math Biol. 1975;37:85–90.
36. Duncan LS Jr, Ellis PP, Paterson CA. Effect of hyperosmotic agents on vitreous osmolality. Exp Eye Res. 1970;10:129–32.
37. Oster JR, Singer I. Hyponatremia, hyposmolality, and hypotonicity: tables and fables. Arch Intern Med. 1999;159:333–6.
38. Galin MA, Aizawa F, Mc LJ. Urea as an osmotic ocular hypotensive agent in glaucoma. Arch Ophthalmol. 1959;62:347–52.
39. Nakao S, Hafezi-Moghadam A, Ishibashi T. Lymphatics and Lymphangiogenesis in the Eye. J Ophthalmol. 2012;2012:783163.
40. Danser AH, Derkx FH, Admiraal PJ, Deinum J, de Jong PT, Schalekamp MA. Angiotensin levels in the eye. Invest Ophthalmol Vis Sci. 1994;35:1008–18.

