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Seeing green

Clinical applications of fluorescein in optical practice

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Sodium fluorescein, usually simply referred to as fluorescein, is an orange-red dye and is the most commonly used ocular surface stain in optical practice. It is a water-soluble pH indicator dye with a small molecular weight (376.27g/mol). It is typically supplied as sterile, fluorescein-impregnated paper strips – although it is also available in minims in one and two per cent weight per volume (w/v) unit dose options.

Fluorescein minims and fluorescein-impregnated paper strips are classed as a pharmacy (P) medicine but can be used by contact lens opticians on the General Optical Council (GOC) specialty register[1]. The tip of the strip is moistened with sterile saline or artificial tears, and can be applied to either the lower or upper conjunctival sac, where it mixes with the tear film and spreads across the ocular surface.

As a hydrophilic molecule, fluorescein cannot penetrate healthy, intact epithelial cell membranes. Its primary route of entry is through areas where the tight junctions of the epithelial barrier are compromised or disrupted, typically through damage, dryness or disease[2-5]. Fluorescein also mixes with the tears and fluoresces, making it visible and allowing diagnostic assessment of tear film stability.

For best results, it should be viewed under specific lighting conditions. It fluoresces under cobalt blue light on the slit lamp biomicroscope, with a peak excitation at 490nm and emits a bright green fluorescence at approximately 530nm[4].

By using a yellow barrier filter, either hand-held or integral to the slit lamp, visibility of this fluorescence is enhanced. These filters block out excess reflected blue light, increasing contrast and allowing clearer visualisation of subtle ocular surface defects and tear film disruptions that may not be apparent in the absence of the filter.

This article discusses the diagnostic applications of fluorescein in routine clinical practice – from assessing the tear film and anterior ocular surface integrity to evaluating the fitting of rigid corneal and mini-scleral contact lenses.

TEAR FILM ASSESSMENTS

Fluorescein highlights tear film disturbances, allowing for the assessment of tear stability, dry eye evaluation and ocular surface damage. It is an essential component for ocular surface examination, and an effective means of evaluating the integrity and function of the tear film.

FLUORESCEIN TEAR BREAK-UP TIME TEST (FTBUT)

The installation of a small amount of fluorescein onto the ocular surface allows for a dynamic evaluation of the tear film. Using a minimal amount of sterile saline, or non-preserved artificial tears, is essential to minimise disruption of the tear film.

The patient is asked to blink fully before holding their eye open. The time

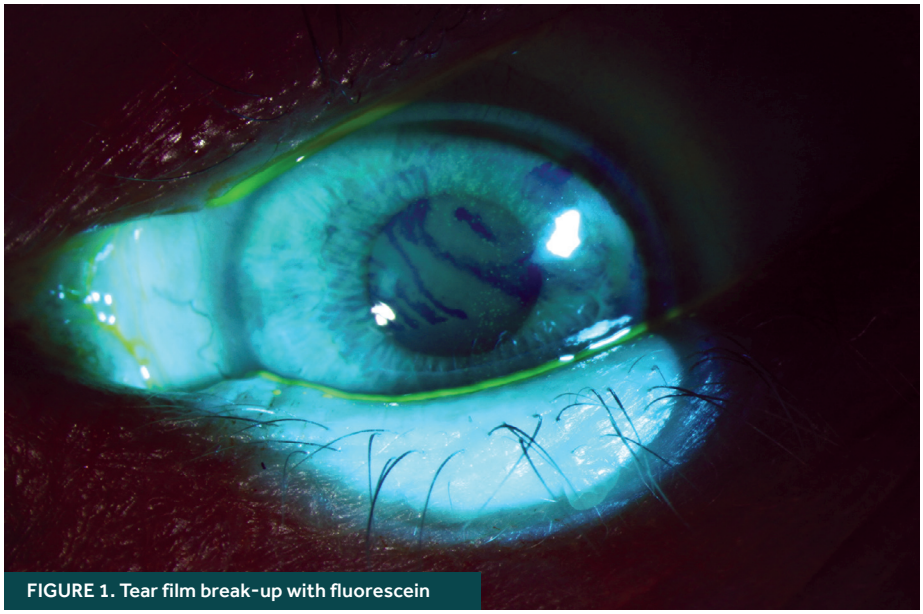

FIGURE 1. Tear film break-up with fluorescein

interval between the last blink and the first appearance of a dark area (the first break in the tear film) on the cornea is recorded in seconds[2,5] (**Figure 1**). A reduced tear break-up time (TBUT) (less than 10 seconds or less than five seconds when smaller volumes of fluorescein are used)[6] could indicate one or more layers of the tear film is deficient and that further dry eye assessment is needed.

The Tear Film and Ocular Surface Society Dry Eye Workshop III (TFOS DEWS III) recently published an updated definition for dry eye disease (DED), emphasising that dry eye and ocular surface disease are multifactorial conditions requiring comprehensive examination[6,7].

The TFOS DEWS III definition[6] of dry eye is as follows: *'Dry eye is a multifactorial, symptomatic disease characterised by a loss of homeostasis of the tear film and/or ocular surface, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities are etiological factors'*.

When using FTBUT, careful consideration is needed interpreting the results, as it is an invasive test. Instillation of fluorescein can disrupt tear film stability, dilute tear film components, and stimulate reflex tearing – potentially leading to a shorter break-up time than the true physiological TBUT. The FTBUT for this reason is 10th in the sequence of tests, following non-invasive assessments proposed by TFOS DEWS III[6].

Full assessment of the tear film would include the use of other techniques discussed later in the article, tear osmolarity and examining the patency of the meibomian glands. An impaired lipid layer due to dysfunctional or blocked meibomian glands causes reduced lipid secretion and, ultimately, increased tear evaporation and overall instability[6].

TEAR MENISCUS HEIGHT

Tear meniscus height (TMH) is used to assess the tear volume and is easier to measure when the uppermost edge of the meniscus is more visible. Ideally, this is measured using infrared or white light – and directly below the pupil centre as the height may vary towards the periphery due to evaporation and punctal drainage[6].

Anterior segment optical coherence tomography (AS-OCT) can also be used as this provides a cross-sectional view along the lower lid, which will highlight if the tear meniscus is regular or contains any gaps.

While non-invasive techniques are preferred, fluorescein still plays a valuable role in tear assessment – particularly for interpreting corneal staining and enhancing visibility of the tear reservoir where AS-OCT is not available or the TMH is difficult to measure using white light.

A normal TMH is generally considered to be 0.2-0.3mm, with values ≤0.2mm potentially indicating reduced tear volume and suggesting an aqueous-deficient dry eye[6].

LID WIPER EPITHELIOPATHY

Lid wiper epitheliopathy (LWE) is staining of the tissue of the palpebral marginal conjunctiva at the lid wiper region. It can be seen with either fluorescein or lissamine green, and typically indicates increased friction between the lid and the ocular surface. It is often associated with dry eye. Higher LWE grades correlate with tear film instability, whereas minimal or no staining is considered normal[6].

ANTERIOR EYE ASSESSMENT

The unique properties of fluorescein make it ideal for assessing the integrity of the anterior ocular surfaces. Interpreting the intensity and pattern of staining provides clinical insight into not only the severity of any damage, but also if there are indicative signs of underlying pathology. Corneal staining also provides clinicians with essential information as to whether a patient can be managed in practice or requires referral for a potentially sight-threating keratitis. Herpes simplex virus keratitis staining is shown in **Figure 2**.

While various conditions exhibit distinct staining patterns, accurate diagnosis relies on the correct interpretation of these patterns. Staining should be evaluated with consideration to the appearance, location and depth – and in conjunction with the patient's history and symptoms. It is important to remember that while ocular surface staining is a valuable clinical indicator, it does not provide a diagnosis in isolation.

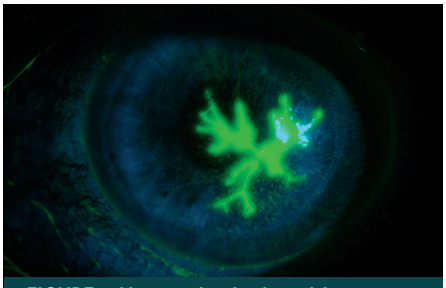

FIGURE 2. Herpes simplex keratitis

FOREIGN BODY TRACKS

The staining caused by a foreign body can appear as straight vertical lines or zigzag staining on the corneal surface. These staining patterns are typically produced as the foreign body moves across the corneal surface during blinking, particularly if trapped under the upper lid. This highlights the importance of a thorough investigation of any discomfort including eversion of the upper lid.

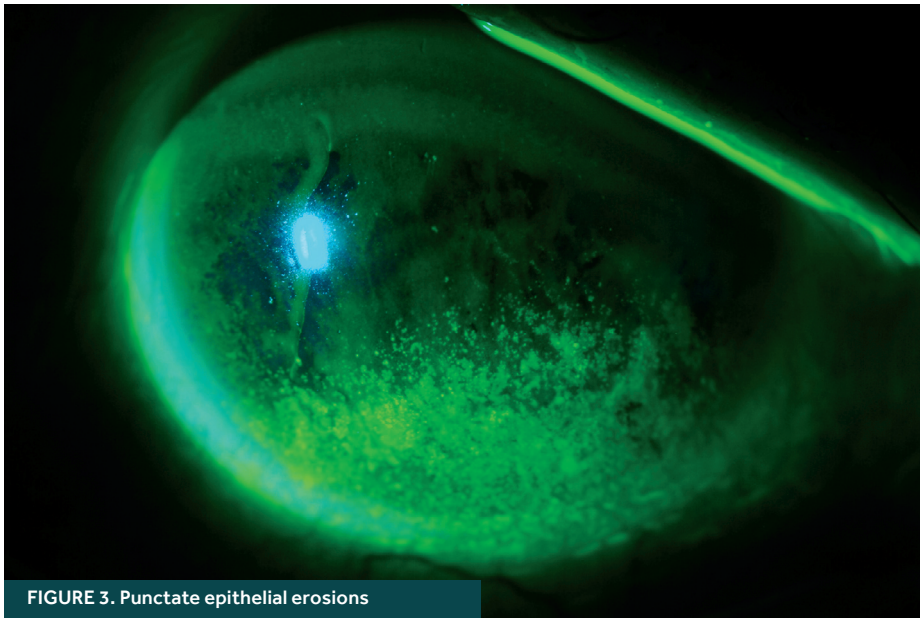

FIGURE 3. Punctate epithelial erosions

DIFFUSE PUNCTATE STAINING

This pattern is characterised by numerous small, dot-like areas of staining scattered across the cornea and/or conjunctiva. Often associated with DED, the compromised tear film can lead to widespread superficial epithelial damage. Also, solution, preservative or chemical toxicity can cause diffuse staining but usually appears denser or more coalesced in areas of greater exposure.

Chronic exposure to preservatives in topical medications, including some artificial tears or glaucoma drops, can give dry eye symptoms, discomfort and ocular surface staining[8].

Viral conjunctivitis can cause a fine, diffuse punctate keratitis particularly in the more serious epidemic keratoconjunctivitis caused by the adenovirus, which presents with punctate staining along with corneal inflammation and discharge[9].

Inferior staining occurring predominantly on the lower part of the cornea and conjunctiva not covered by the eyelid can be indicative of exposure keratopathy, often due to incomplete eyelid closure (lagophthalmos) during sleep or conditions affecting lid position (**Figure 3**).

Blepharitis, chronic inflammation of the eyelids, can release inflammatory mediators into the tear film — also leading to inferior corneal and conjunctival irritation and staining. Posterior blepharitis or meibomian gland dysfunction can lead to a deficient lipid layer in the tear film, resulting in increased evaporation and desiccation of the interpalpebral surface[6].

Superior staining, occurring primarily in the upper part of the cornea and conjunctiva, is less common but can suggest superior limbic keratoconjunctivitis, mechanical or contact lens-induced trauma such as superior epithelial arcuate lesions. A poorly fitting contact lens, for example, transition curves on older silicone hydrogel soft lenses, or lens deposits can cause mechanical irritation and arcuate staining on the superior cornea, especially under the upper eyelid.

Floppy eyelid syndrome, a relatively rare condition caused by abnormal lid laxity often associated with sleep apnoea, can lead to chronic rubbing of the superior conjunctiva and cornea against the pillow during sleep, resulting in staining[10]. This condition is also often associated with keratoconus.

CONTACT LENS-ASSOCIATED STAINING

- Dimple veiling due to air bubbles under rigid corneal lenses (RCLs) lenses or mucin balls under soft silicone hydrogels, causing indentations in the corneal epithelium
- Mechanical from defined transition curves on older generation silicone hydrogels, deposits, or damaged lens edges, particularly under the top lid
- Three and nine o'clock staining due to disruption of the epithelium at the limbus from consistent dryness, poor lens fitting or incomplete blinking, commonly seen with RCLs

OTHER STAINING PATTERNS

Interpreting fluorescein staining patterns can be complex, particularly in conditions such as recurrent corneal erosions (**Figure 4**). This is a painful condition, often occurring spontaneously on waking or during sleep. It is caused by an underlying defect in the adhesion between the corneal epithelium and its basement membrane[11].

Confluent staining refers to broad, continuous areas of fluorescein uptake, indicating moderate to severe epithelial disruption. This pattern may arise from a variety of underlying causes, and typically reflects more extensive damage to the ocular surface.

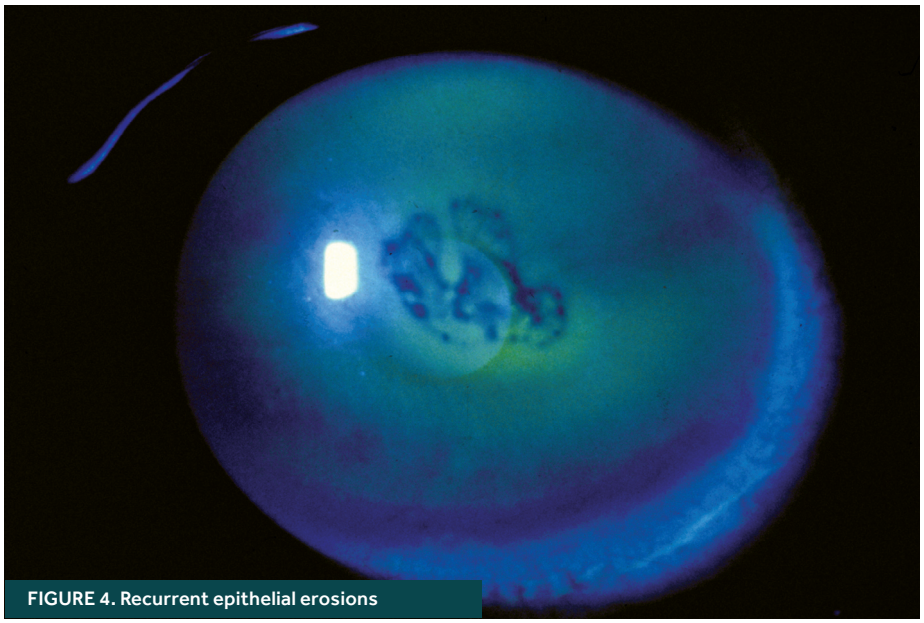

FIGURE 4. Recurrent epithelial erosions

GRADING SCALES

The severity of staining can be graded using standardised grading scales, such as the Efron or Brien Holden Vision Institute (formerly CCLRU) grading scales. These provide a consistent method of objectively recording the condition severity, allowing practitioners to monitor any changes over time[5]. It is recommended to grade to the nearest 0.1 and to note which grading scale has been used.

The mnemonic PEDAL can be used in conjunction with the staining appearance to aid the differentiation and management of sterile or infectious corneal conditions, where P = pain, E = epithelial defect, D = discharge, A = anterior chamber involvement and L = location (**Table 1**).

P	**Pain** – should be graded from 0-10 where 0 = no pain and 10 = the most severe pain	*Generally, with infected corneal lesions pain is graded 6-10. Photophobia and excess lacrimation are also likely*
E	**Epithelial defect** – size, depth, and appearance of edges are all important features	*Epithelial staining may be present with both non-infected infiltrates and infected lesions, but 'generally' infected lesions have a crater-like appearance with rolled edges, and are usually >2mm*
D	**Discharge** – colour, texture and quantity should be recorded	*Discharge is only present with infectious keratitis*
A	**Anterior chamber reaction** – the presence of white cells in the anterior chamber and hypopyon	*Flare and hypopyon may be present with infectious keratitis*
L	**Location of lesion** – is it in the periphery or central?	*Infectious lesions are usually but not always located in the mid periphery or centrally*

TABLE 1. PEDAL mnemonic[12]

CONTACT LENS ASSESSMENT

When assessing the fitting of mini-scleral lenses, fluorescein is essential for visualising the tear layer shape and aiding measurement of how much the lens vaults the central cornea as well as checking there is full limbal clearance. **Figure 5** shows a large air bubble under a mini-scleral lens after insertion. When fitting RCLs, the brighter the green, the greater the gap between the lens and the cornea; and where there is touch with the corneal tissue underneath this would appear black as well as where the tear layer is less than approximately 15-20µ (the threshold detectable to the human eye)[13].

Standard fluorescein easily penetrates the porous structure of soft

FIGURE 5. Air bubble under a mini-scleral lens after initial insertion

hydrogel contact lenses, leading to permanent staining. Alternatively, high molecular weight fluorescein can be used, which has a significantly larger molecular structure (Fluorexon, 622.53g/mol)[14]. Due to its larger molecular size, it does not readily penetrate the matrix of soft contact lens materials, meaning contact lens wearers can reapply their lenses following an aftercare appointment. However, patients should always attend such appointments with their spectacles

GOLDMANN APPLANATION TONOMETRY

Accurate measurement of intraocular pressure (IOP) is a fundamental aspect of glaucoma management, and Goldmann applanation tonometry is regarded as the gold standard for IOP measurement. Due to the invasive nature of the measurement technique, a topical anaesthetic eyedrop is instilled (either in combination with fluorescein or applied separately) before the probe touches the cornea. As the fluorescein mixes with the tear film, it makes the mires of the instrument more visible and allows examination of the cornea following the procedure[15].

SEIDEL'S TEST

In cases of ocular trauma where a full-thickness penetrating injury is suspected, there may be leakage of aqueous humour from the anterior chamber. Seidel's test is used to detect such leaks by applying a high concentration of fluorescein to the ocular surface. A positive result is indicated by the dilution of the fluorescein by the clear aqueous humour, creating a distinct streaming or waterfall pattern[4,16].

LACRIMAL DRAINAGE AND TEAR TURNOVER

The fluorescein dye disappearance test is a simple but crude method for screening lacrimal drainage function and tear turnover. The procedure involves instilling fluorescein into the conjunctival sac, assessing the appearance and thickness of the tear meniscus of each eye, then reassessing after five minutes. A thinner, or absent, fluorescein appearance typically suggests a normal tear drainage. However, delayed clearance may indicate nasolacrimal duct obstruction, impaired tear turnover or reduced basal tear secretion[3,4]. Alternatively, asking the patient to blow their nose, and noting any presence of fluorescein on the tissue also provides evidence of drainage function.

SAFETY CONSIDERATIONS

While fluorescein is considered a safe and well-tolerated diagnostic agent, it is crucial for practitioners to be aware of potential safety considerations and rare contraindications to ensure patient well-being and minimise the risk of adverse events.

Always ensure that the fluorescein strips are within the expiry date to ensure best results and patient safety, and never touch the tip of the saline bottle with the fluorescein strip.

HYPERSENSITIVITY AND ALLERGIC REACTIONS

Hypersensitivity reactions to topically applied fluorescein are very rare and usually mild. Signs and symptoms of a hypersensitivity reaction may include conjunctival hyperaemia and a transient burning or stinging sensation upon instillation, which usually resolves quickly. With more significant reactions, periorbital rash or oedema may develop – but again this is incredibly rare[17].

In the event of a suspected allergic reaction, the examination should be discontinued, the eye thoroughly irrigated and management of the reaction fully documented[18].

INFECTION CONTROL

Maintaining strict hygiene and infection control practices is paramount. The primary risks of infection transmission relate to contamination of the fluorescein preparation or contact with non-sterile surfaces.

To minimise these risks, the following protocol should be adhered to:

- Exercise standard hygiene practices, such as hand washing before and after seeing a patient
- Use single-use fluorescein impregnated strips due to the susceptibility of fluorescein solution to pseudomonas aeruginosa infection[19]
- Once a fluorescein impregnated strip has been moistened and touched the eye, it should be discarded immediately and never re-used on another patient
- Fluorescein impregnated strips and minims are considered clinical waste and need to be disposed of in clinical waste bins after use[20]

INFORMED PATIENT CONSENT

Instilling fluorescein is considered an invasive procedure; therefore, explicit informed consent should be obtained prior to its administration. This process also requires clinicians to enter the patient's personal space, emphasising the need for clear communication.

The GOC provides clear guidance on obtaining informed consent, including distinctions between the different types of consent required[21]. It is the responsibility of each registrant to exercise professional judgement in determining which type of consent is required for the situation. Beyond the legal and ethical implications, obtaining informed consent fosters patient trust and contributes to a more positive patient experience.

The GOC Principles of Consent guidance states: *'Explicit consent is when a patient gives you specific permission to do something, either oral or written. You should obtain explicit consent where the procedure, treatment or care being proposed is more invasive and/or has greater risks involved'*[21]. And*: 'Implied consent is when consent can be assumed from a patient's actions, for example, by placing their chin on an instrument such as an autorefractor following an explanation of the test involved'*[21].

Association of British Dispensing Opticians Advice and Guidelines also advise: *'Explicit and informed consent must be obtained and noted before administration of any topical preparations'*[22].

To ensure informed consent:

- Instruct patients wearing soft contact lenses to remove them before the dye is instilled, and advise on when they can be reapplied
- Explain why fluorescein is being used, for example, to check the anterior surface of the eye or to help measure IOP
- Describe how the dye will be administered; a drop of liquid or a moistened impregnated paper strip
- Inform the patient of any temporary side-effects they might experience, such as slight yellow staining of the tears, which will dissipate quickly

PREGNANCY AND BREAST-FEEDING

Pregnant and breast-feeding women represent a special case in the use of topical fluorescein, as careful consideration of potential risks and benefits is required. Although systemic absorption of topical fluorescein is considered minimal, the lack of robust clinical studies involving these populations means that its safety remains inconclusive.

However, it should also be noted that there have not been any case reports published on the adverse effects of topical fluorescein. As such, clinicians should discuss the benefits and potential uncertainties with the patient, ensuring they are informed of the limited safety data available for use during pregnancy and breast-feeding. Using the smallest effective amount of fluorescein and applying nasolacrimal occlusion would also reduce systemic absorption through the nasal mucosa[23-25].

FIGURE 6. Lissamine green conjunctival staining

LISSAMINE GREEN

While fluorescein remains a cornerstone in ocular surface assessment, it does have certain limitations. Although it can stain conjunctival cells, the reduced contrast against the white sclera can make conjunctival staining more difficult to detect.

Historically, rose bengal was used to assess conjunctival staining, particularly because it stains devitalised epithelial cells and, in cases of mucus-deficient tear film, even viable cells. However, its use has declined due to the significant discomfort and irritation it causes upon instillation. In clinical practice, it has largely been replaced by lissamine green[26].

This dye has a peak absorption at approximately 630nm and, like rose bengal, stains devitalised epithelial cells and mucus. However, it is generally better tolerated by patients, making it a more suitable option (**Figure 6**). Lissamine green is particularly valuable in patients who report dry eye symptoms in the absence of fluorescein detectable signs, and is especially effective for evaluating conjunctival staining and the lid wiper region[27,28].

More lissamine green is needed to effectively view the ocular surface compared to fluorescein, and further instillation may be needed[29]. Instilling the dye onto the superior bulbar conjunctiva helps it spread down across the conjunctiva, particularly if the patient has reduced tear volume. The effect of lissamine green can fade quickly for some patients, although in severe dry eye may encourage lasting staining[29]. Using white illumination in conjunction with a red filter improves the visibility[30,31].

CONCLUSION

Fluorescein is an essential diagnostic tool in the assessment of the anterior ocular surface, playing a critical role in evaluating tear film integrity, fitting contact lenses, and performing Goldmann applanation tonometry. Its capacity to reveal subtle epithelial disruptions and tear film irregularities highlights its clinical value, particularly in the diagnosis and monitoring of DED and other anterior surface pathologies.

Selecting the appropriate ocular surface staining agent should be guided by the specific clinical context. For instance, lissamine green offers enhanced diagnostic utility in evaluating conjunctival health and detecting lid wiper epitheliopathy through its affinity for devitalised epithelial cells.

Although both fluorescein and lissamine green are generally well-tolerated, obtaining informed consent is essential particularly in patients who are pregnant or breast-feeding.

Fluorescein remains an indispensable diagnostic tool in routine clinical practice due to its versatility, ease of use, and strong safety profile.

LEARNING OUTCOMES FOR THIS CPD ARTICLE

DOMAIN: Communication

3.1: *Obtain valid and informed consent before using ocular surface staining agents such as sodium fluorescein ensuring patients are aware of the risks and benefits when used for anterior eye assessment.*

DOMAIN: Clinical practice

5.3: *Recognise the clinical applications of sodium fluorescein in anterior eye assessments and evaluation of rigid corneal and mini-scleral contact lens fitting, applying current good practice to support the care you provide.*

DOMAIN: CL speciality

Critically evaluate anterior surface staining when using sodium fluorescein for tear film assessment, contact lens fitting and to aid differentiation and management of sterile versus infectious corneal lesions.

COMMUNICATION

CLINICAL PRACTICE

SPECIALITY: CONTACT LENS OPTICIANS

REFERENCES

References can be found when completing this CPD module. For a PDF of this article with references email, abdocpd@abdo.org.uk

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