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# Crystalline lens capsule staining with trypan blue

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A 1-step method for staining the anterior lens capsule with trypan blue is described. The dye is instilled via a paracentesis port at the start of cataract extraction. As aqueous humor is allowed to exit the anterior chamber (AC), which consequently shallows, the resulting pupil block confines the dye to the AC. An ophthalmic viscosurgical device (OVD) is used to flush dye-stained aqueous from the AC, circumventing the need for AC washout. Although the OVD may be tinged with dye, this does not impede performing capsulorhexis. This method does not add to the surgical time, requires no additional instruments or materials, and is safe.

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The long-term safety of trypan blue in the anterior chamber (AC) in eyes having cataract surgery<sup>1</sup> has recently been exploited by using the dye to stain the anterior crystalline lens capsule to facilitate capsulorhexis.<sup>2–4</sup> The ideal method of dye application must still be determined.

Two techniques are common. In the first, the AC is filled with air and dye is injected onto the capsule.<sup>4</sup> In the second, dye is injected after the AC is filled with an ophthalmic viscosurgical device (OVD).<sup>2–6</sup> More recently, other techniques have been described in which the dye is mixed into an OVD or is painted directly onto the anterior capsule with a special cannula<sup>7</sup> or swept across the capsule from a reservoir under the iris<sup>6</sup> under cover of an OVD. With dilute solutions, it is possible to stain the capsule under OVD cover without

replacing the OVD; there is sufficient staining of the capsule but negligible staining of the OVD, enabling good visualization of the capsule.<sup>5</sup>

In our early work with capsule dyes, a central issue was confining the dye to the AC,<sup>2,3</sup> since it was possible to inject too much dye, resulting in posterior capsule staining (and, consequently, a reduced red reflex). Our early observation has since been reported.<sup>8</sup> We developed a technique of injecting a viscous OVD into the AC followed by a less viscous OVD over the pupil, thus pushing the viscous OVD peripherally over the pupil margin and iris (reverse soft shell). Trypan blue was then injected directly onto the capsule. This induced some blocking by a viscous barrier at the pupil margin. This method is time consuming and expensive as it involves the use of 2 OVDs, but it is preferable to a relatively uncontrolled method that subjects the corneal endothelium to potential injuries from air.<sup>9,10</sup>

We present an alternative method in which trypan blue is introduced into the eye through a paracentesis port without first injecting air or an OVD.

## Surgical Technique

Aqueous is drained via the paracentesis port before the dye is injected. The dye remains mainly in the pupil area as the AC shallows (Figure 1) and is allowed to remain for 30 seconds. An OVD is injected by fully inserting the cannula into the AC while placing pressure on the posterior lip of the paracentesis port. This dis-

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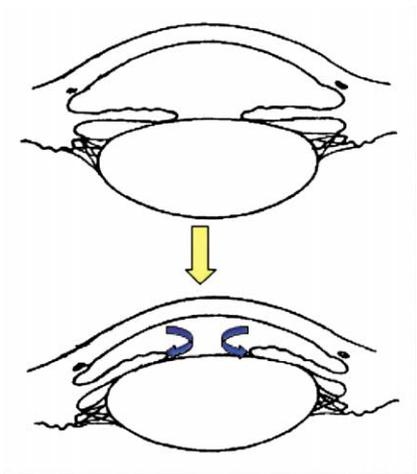
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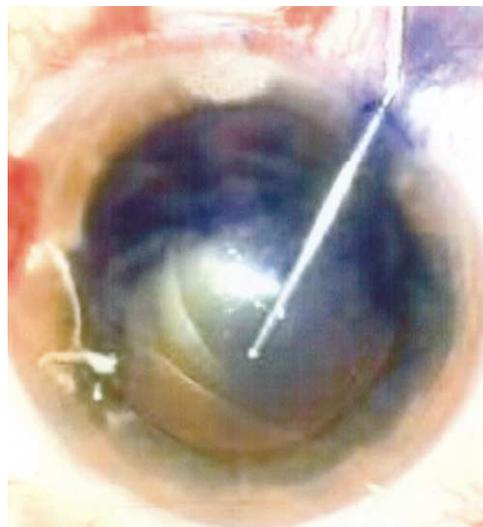
*Dr. Coroneo holds United States patent 6 367 480 and U.S. patent 6 372 449 relevant to ocular use of trypan blue. Dr. San Laureano does not have a financial or proprietary interest in any material or method mentioned.*

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**Figure 1.** (San Laureano) *Top:* A pupil block induced by deliberate shallowing of the AC confines trypan blue to the AC. *Bottom:* Trypan blue is injected into the AC with egress of aqueous humor, allowing shallowing of the AC.

places dyed aqueous humor from the AC through the port site (Figure 2). The dye is further diluted by continued secretion of aqueous humor (Figures 3 and 4). Care is taken to inject a small amount of OVD just inside the paracentesis to minimize damage to the anterior capsule by the cannula tip, since visualization may be momentarily impeded by the dye. Because the dye is kept mostly within the pupil using this method, minimal contact is made with the iris. After the dilution steps, the amount of dye in the OVD is barely visible, minimizing the dye's access to the trabecular meshwork and vascular system. Compared with irrigating dye from the AC, injecting an OVD with aqueous egress is a low-pressure maneuver, minimizing the potential access of dye to the angle.

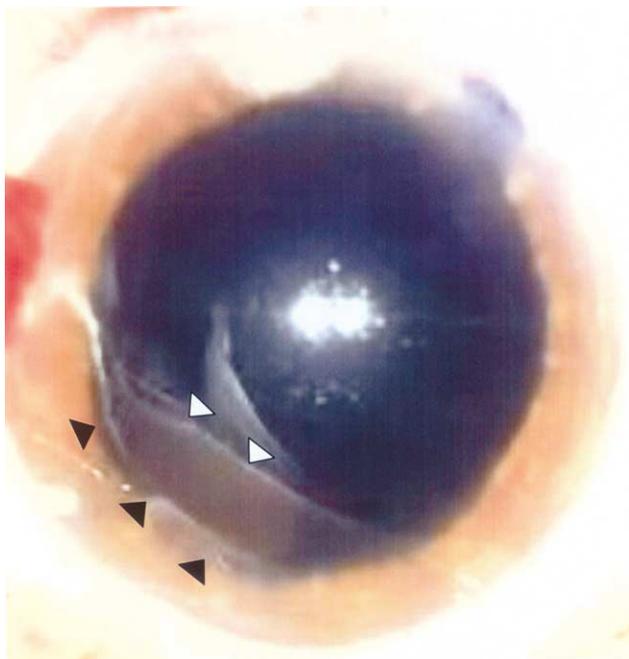


**Figure 2.** (San Laureano) Ophthalmic viscosurgical device expression of dyed aqueous humor from the AC. Note the posterior lip of the paracentesis wound depressed by the injection cannula to facilitate egress of dyed aqueous humor.



**Figure 3.** (San Laureano) Capsulorhexis is started with a clear view through the partially stained OVD.

The corneal or scleral incision is then completed, and additional OVD can be inserted via the incision, further diluting the AC dye. Removing dye or OVD from the AC is unnecessary. Capsulorhexis is then performed (Figure 3) with excellent visualization of the capsule through the trypan-blue-tinged OVD. In our hospital pharmacy, trypan blue was dissolved in a 0.1% balanced salt solution (filtered through a 0.22  $\mu\text{m}$  filter) and used on the day of manufacturing.<sup>2,3</sup> This preparation is preservative free.



**Figure 4.** (San Laureano) Aqueous humor at the pupil margin, evident by dilution of the dyed aqueous (black arrowheads). The freshly secreted aqueous front is delineated with white arrowheads. This phenomenon occasionally requires reinjection of trypan blue into the AC to ensure more uniform capsule staining.

## Results

The technique was used in 50 patients in whom capsulorhexis was expected to be difficult (eg, patients with white or very brunescant cataracts, corneal scarring or haze, or a poor red reflex from retinal hyperpigmentation or vitreous opacity). A single application of the dye was made in 48 cases; in 2 cases, a second application was applied because of aqueous humor at the pupil edge (Figure 4) that diluted the dye at the pupil margin. All cases showed adequate central staining of the anterior capsule with no apparent posterior capsule staining. A successful anterior capsulorhexis was performed in all cases.

## Discussion

This technique results in precise and rapid anterior capsule staining without the need to wash out excess dye, as required by the 2 most common methods of dye application. It is cost effective, as only 1 type of OVD is required, and does not require the use of air, which may damage the corneal endothelium. We have seen little evidence of the toxic effects of trypan blue

on the corneal endothelium, consistent with earlier reports<sup>1</sup> of long-term safety and the routine use of this dye in eye banks as a vital stain for the endothelium.

To date, we have not observed staining of the posterior capsule. This may be important as there is evidence that substances applied or injected into the AC may find their way to the retina.<sup>11,12</sup> Recently, toxic effects of indocyanine green (ICG) for capsule and retinal membrane staining have been reported when the dye was used for macular surgery<sup>13,14</sup>; under certain experimental conditions, trypan blue may also display retinal toxicity.<sup>15</sup> Although the pathophysiology is unclear,<sup>16,17</sup> it is not surprising since the retinotoxic effects of ICG in the rabbit were reported in 1976.<sup>18</sup> Of concern is that ICG appears to bind to the retina for a long time and may be a photosensitizer.<sup>19,20</sup> Our method may reduce the risk for posterior segment access if ICG is used as a capsule dye.

In our method, it appears that as the AC shallows with some loss of aqueous as the dye is injected (with pressure applied by the injection cannula on the posterior lip of the paracentesis port), there is closer iris–lenticular apposition (pupil block). This effectively confines the dye to the anterior capsule and AC. Our method minimizes the risk for dye injection into the posterior segment and thus the risk for posterior segment toxicity.

Of the 2-step techniques, we agree with a previous report that injecting dye under an OVD is faster and safer than the air-bubble technique, given the potentially injurious effect of air on the endothelium.<sup>9,10</sup> It is the third<sup>5,7</sup> 1-step technique described but has the advantage of not requiring a special cannula to paint dye on the capsule. Compared with another 1-step technique, our method does not require lower dilutions of trypan blue.<sup>5</sup> These techniques are quicker than methods in which the OVD is exchanged or in which the dye is mixed with an OVD. The technique we describe does not add appreciably to the operating time, requires no additional instruments or materials, and is safe while allowing the considerable advantages of capsule staining.

## References

1. Norn MS. Per operative trypan blue vital staining of corneal endothelium; eight years' follow up. *Acta Ophthalmol* 1980; 58:550–555
2. Coroneo MT, inventor. Methods for visualizing the anterior lens capsule of the human eye. US patent 6 367 480. April 9, 2002

3. Coroneo MT, inventor. Ophthalmic methods and uses. US patent 6 372 449. April 16, 2002
4. Melles GRJ, de Waard PWT, Pameyer JH, Beekhuis WH. Trypan blue capsule staining to visualize the capsulorhexis in cataract surgery. *J Cataract Refract Surg* 1999; 25:7–9
5. Yetik H, Devranoglu K, Ozkan S. Determining the lowest trypan blue concentration that satisfactorily stains the anterior capsule. *J Cataract Refract Surg* 2002; 28:988–991
6. Lanzl IM, Mertz MM. Why mix trypan blue with viscoelastic agents [letter]? *J Cataract Refract Surg* 2003; 29:237
7. Khokhar S, Pangtey MS, Panda A, Selthi HS. Painting technique for staining the anterior lens capsule. *J Cataract Refract Surg* 2003; 29:435–436
8. Birchall W, Raynor MK, Turner GS. Inadvertent staining of the posterior lens capsule with trypan blue dye during phacoemulsification [photo essay]. *Arch Ophthalmol* 2001; 119:1082–1083
9. Kerr Muir MG, Sherrard ES, Andrews V, Steele ADMcG. Air, methylcellulose, sodium hyaluronate and the corneal endothelium; endothelial protective agents. *Eye* 1987; 1:480–486
10. Çekiç O, Ohji M, Zheng Y, et al. Experimental study of viscoelastic in the prevention of corneal endothelial desiccation injury from vitreal fluid-air exchange. *Am J Ophthalmol* 2003; 135:641–647
11. Wheeler LA, Gil DW, WoldeMussie E. Role of alpha-2 adrenergic receptors in neoprotection and glaucoma. *Surv Ophthalmol* 2001; 45(suppl 3):S290–S294
12. Kanter ED, Brucker AJ. Aminoglycoside macular infarction in association with gentamicin-soaked collagen corneal shield. *Arch Ophthalmol* 1995; 113:1359–1360
13. Engelbrecht NE, Freeman J, Sternberg P Jr, et al. Retinal pigment epithelial changes after macular hole surgery with indocyanine green-assisted internal limited membrane peeling. *Am J Ophthalmol* 2002; 133:89–94
14. Haritoglou C, Gandorfer A, Gass CA, et al. Indocyanine green-assisted peeling of the internal limiting membrane in macular hole surgery affects visual outcome: a clinicopathologic correlation. *Am J Ophthalmol* 2002; 134:836–841
15. Veckeneer M, van Overdam K, Monzer J, et al. Ocular toxicity study of trypan blue injected into the vitreous cavity of rabbit eyes. *Graefes Arch Clin Exp Ophthalmol* 2001; 239:698–704
16. Dietz FB, Jaffe RA. Indocyanine green; evidence of neurotoxicity in spinal root axons. *Anesthesiology* 2003; 98:516–520
17. Enaida H, Sakamoto T, Hisatomi T, et al. Morphological and functional damage of the retina caused by intravitreal indocyanine green in rat eyes. *Graefes Arch Clin Exp Ophthalmol* 2002; 240:209–213
18. Kobayashi N, Tamaki R, Horiuchi T, et al. [Studies on aqueous outlets. Part II. Histopathological investigation on the influence of indocyanine green dye to the ocular tissues]. [Japanese] *Nippon Ganka Gakkai Zasshi* 1976; 80:1526–1531
19. Tadayoni R, Paques M, Girmens JF, et al. Persistence of fundus fluorescence after use of indocyanine green for macular surgery. *Ophthalmology* 2003; 110:604–608
20. Haritoglou C, Gandorfer A, Schaumberger M, et al. Light-absorbing properties and osmolarity of indocyanine-green depending on concentration and solvent medium. *Invest Ophthalmol Vis Sci* 2003; 44:2722–2729