Trypan blue dye for anterior segment surgeries

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Abstract

Use of vital dyes in ophthalmic surgery has gained increased importance in the past few years. Trypan blue (TB) has been a popular choice among anterior segment surgeons mainly due to its safety, ease of availability, and remarkable ability to enable an easy surgery in difficult situations mostly related to visibility of the targeted tissue. It is being used in cataract surgery since nearly a decade and its utilization has been extended to other anterior segment surgeries like trabeculectomy and corneal transplantation. This review will discuss the techniques and outcome of TB dye-assisted anterior segment surgeries.

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Introduction

In ophthalmology, vital dyes have become very effective and useful surgical tools for ocular tissue identification. They have been used in cataract, vitreoretinal, corneal, glaucoma, orbital, strabismus, and conjunctival surgery. Dye-enhanced surgery is a preferred method in cases of cataract surgery, especially with compromised red fundus reflex. Several types of dyes, including fluorescein, indocyanine green (ICG), autologous blood, and gentian violet have been proposed and tested.^{1–3} By far, trypan blue (TB) dye has been the most frequently used agent in cataract, as well as other anterior segment, surgeries. This review will discuss the evolution of use of TB dye in various types of anterior segment ocular surgery. Applications of TB dye and safety of its use would be presented.

History of trypan blue (TB) dye staining in anterior segment surgery

Use of TB in ophthalmology dates back to the 1970s, when it was used to stain the corneal endothelium preoperatively.⁴ It emerged in the late 1990s as one of the known staining material used intraoperatively to aid in visualization of the anterior lens capsule.⁵ Melles *et al*⁵ reported the first experience with intraoperative application of TB to stain the anterior capsule and to facilitate capsulorhexis for surgery for mature cataract.

Concentration of TB

Chang *et al*⁶ assessed the concentration of TB required to effectively stain rabbit anterior capsule sufficient for capsulorhexis. After staining at concentrations of 0.001, 0.01, 0.1, and 0.4% for 1 min, a minimum of 0.1% concentration was thought to be necessary for staining. In contrast, Yetik *et al*⁷ found that a concentration as low as 0.0125% was adequate for staining the capsule. Overall, in the published literature, concentrations used for anterior capsule staining have varied, and have included 0.6,⁸ 0.4,⁹ 0.1,² 0.06,¹⁰ and 0.0125%.¹¹ The choice of concentration in these instances appears to be related to the availability of various formulations of TB. In terms of the duration of staining, *in vitro* testing using human anterior capsules suggests an exposure time of 60 s is adequate for visualization,¹² whereas clinically, the duration of staining is usually 'a few seconds'.^{5,10}

In corneal surgery, TB 0.02% has been used to aid deep lamellar keratoplasty dissection¹³ and penetrating keratoplasty.¹⁴ A concentration of 0.1% was used by Sinha *et al*¹⁵ to stain retained Descemet's membranes after penetrating keratoplasty.

The most commonly used commercially available TB was available as Vision Blue (TB 0.1%, DORC International BV, Zuidland,

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The Netherlands) for use in anterior segment surgery. It is now manufactured at the lower concentration of 0.06%. Vision Blue is widely used in an undiluted form by most surgeons. It is also noteworthy that as there is a reported difference in the purity of TB used in different commercial products, a different percentage of TB may be required for the same efficacy and may account for the differences noted in the literature.

Dye-assisted cataract surgery

Phacoemulsification has become the standard surgical technique of management of cataract and creation of a continuous curvilinear capsulorhexis (CCC) is a critical step of this procedure. The visibility required to create an optimal capsulorhexis depends on red reflex coming from the posterior segment of the eye. This red reflex and view of anterior capsule can be compromised in eyes with white or dense cataract, corneal haze, and vitreous hemorrhage. Poor visualization of the capsule may be associated with a challenging CCC that is increasing the risk for radial tears toward or beyond the lens equator and other complications such as capsule rupture, vitreous loss, nuclear drop, and intraocular lens displacement.

TB provides several advantages in cataract surgery. The rate of conversion to an extracapsular cataract extraction in white cataract as the result of an incomplete CCC has been as low as 3.85% when TB is used compared with 28.3%, when no dye has been used.^{8,16,17} TB use has also been described to stain the leading edge of a lost capsulorhexis.¹⁰

Techniques for capsule staining

Different methodologies for injecting the dye have been described, including beneath air,^{5,18} beneath ophthalmic surgical device (OVD),^{19,20} beneath OVD and BSS,^{21,22} and mixing with an OVD.^{9,23,16} Wong *et al*²⁴ found both methods equivalent.

Air bubble injection

Melles *et al*⁵ initiated the use of TB to stain the anterior capsule under air. This allowed the formation of a dye lake and prevented the dilution of dye by aqueous. An additional benefit of this technique is better staining of the peripheral anterior capsule rim and lack of contact of dye with endothelium. However, this technique has been criticized as time consuming, and it may be difficult to reform the anterior chamber with a single-air bubble.⁷ Any instrument entering the eye will cause small air to escape, raising the lens–iris plane. A small amount of

high-density viscoelastic material placed near the incision site minimizes the risk of sudden collapse.

Intracameral subcapsular injection

In this technique, aqueous is replaced with viscoelastic before injecting dye beneath the anterior capsule. There might be a slight leakage of dye from subcapsular space during this step. The stained viscoelastic is replaced with clear viscoelastic before starting CCC. This technique was originally proposed for fluorescein injection.^{1,25} When the capsular flap is inverted, the stained posterior surface of the anterior capsule enhances visualization and thus facilitates tearing during CCC. It has the advantage of trapping the dye in subcapsular space, mostly in the centre and midperiphery. It gives the surgeon sufficient time for any maneuver until the CCC releases it. It is technically more invasive and anterior capsule may tear if excessive dye is injected.

Injection under viscoelastic agent

An alternative staining technique without the use of air bubble was suggested by Yetik *et al*⁷ and involves injection of dispersive viscoelastics. TB solution is painted onto the anterior capsule. The anterior chamber is irrigated with balanced salt solution (BSS) and excessive TB-viscoelastic mixture is aspirated out. After capsular staining, the anterior chamber is refilled with viscoelastic before starting CCC. The advantages of the procedure include a well-maintained anterior chamber depth and minimal contact of dye with corneal endothelium. The main drawback of staining under viscoelastic is the potential for the TB dye at the viscoelastic–anterior capsule interface to obscure the visibility of CCC. Also, this can potentially increase the cost of surgery. Arshinoff¹⁹ has described the ultimate soft-shell technique, in which the anterior chamber is compartmentalized, and TB with Healon 5 or sodium hyaluronate 1% is used when performing the capsulorhexis in eyes with white, mature, or hypermature cataract. In another variation, Khokar *et al*²⁰ has used a special painting cannula under a viscoelastic agent.

Mixing with viscoelastic injection

Kayikicioglu *et al*⁹ mixed TB with sodium hyaluronate and injected onto the anterior capsule under an air bubble. Thereafter, the colored solution is completely washed out, and the anterior chamber is refilled with clear viscoelastic. Dada *et al*²³ recommended mixing TB with sodium chondroitin sulfate-sodium hyaluronate, suggesting that a more dispersive OVD may enhance endothelial protection.

Use of viscoelastic device and balanced salt solution

Marques *et al*²¹ have described a three-step technique for TB capsular staining. The anterior chamber is filled with high molecular weight ophthalmic viscoelastic device. Using a 27-gauge cannula, balanced salt solution is injected directly onto the anterior capsule. Thereafter, the dye is slowly introduced through a 27-gauge cannula, which has a curved shaft with a single hole on the posterior surface, onto the lens surface through a tuberculin syringe. As the dye mixes with the BSS, the anterior capsule can be painted approximately according to the size of the intended CCC. This technique allows for better control without excessive staining of other intraocular structures. Arshinoff²² has recommended the use of TB and BSS in reverse order.

Intracameral one-step injection

In this technique, the dye is instilled via a paracentesis at the beginning of cataract surgery.²⁶ Aqueous humor is allowed to exit the anterior chamber, which becomes shallow, and the resulting pupil block confines the coloring agent to the anterior chamber. An OVD is used to flush dye-stained aqueous from the anterior chamber. This method requires no additional instruments and materials and is faster.

Types of cataract and TB dye

White cataract

Intumescent, mature, and hypermature cataracts constitute a significant proportion of cases undergoing cataract surgery in developing countries. Creating a CCC may pose a challenge to the surgeon in these eyes. TB dye provides predictable and uniform staining of the anterior capsule and thus allows good visualization of the peripheral anterior rim of the CCC.

The reported success rates of performing CCC in mature cataracts with TB dye staining are as high as 100%.⁵ Jacob *et al*¹⁸ could complete CCC uneventfully in 96.15% eyes with white cataract using TB. Kothari *et al*²⁷ had 100% success in performing CCC in 25 cases of mature or hypermature cataract, including one traumatic cataract. One case had peripheral extension of the capsulorhexis margin but the stained edge of the anterior capsule helped in identification and redirection of the CCC.

Traumatic cataract

Trauma to the crystalline lens may cause cataractous change irrespective of the integrity of the capsule. Selective staining of the anterior capsule enables surgeon

to perform a safe and successful cataract surgery. TB dye helps to identify the presence, extent, and direction of any preexisting capsular tear. Kazem *et al*²⁸ has described three cases of traumatic cataract surgery with use of TB as an adjuvant.

Pediatric cataract

A complete anterior continuous curvilinear capsulorhexis and posterior CCC facilitates intraocular lens (IOL) placement in the bag with or without posterior optic capture during pediatric cataract surgery. The anterior and posterior CCC can be difficult to perform because of elasticity and tension of the capsule in children. Capsular staining dyes have been used to improve the visibility and increase the rate of complete anterior and posterior CCC.²⁹ Saini *et al*³⁰ conducted a prospective randomized trial to create anterior and posterior CCC in pediatric cataract surgery with and without using TB dye. The majority (91.3%) of the eyes had complete anterior CCC and 82.6% eyes had complete posterior CCC in the eyes where TB was used to stain the capsule, in comparison with 73.6 and 52.6% of anterior and posterior CCC, respectively, in eyes without TB. The difference was significant between both groups. Sharma *et al*³¹ had evaluated the efficacy of TB in posterior capsulorhexis with optic capture in pediatric cataracts in a prospective randomized study. Optic capture was possible in 17 (out of 18) eyes in TB-assisted surgery and 11 (out of 17) eyes where no dye was used ($P = 0.04$).

Cataract with corneal opacity

A combined penetrating keratoplasty with cataract surgery is the method of choice for patients with corneal opacity and cataract. However, factors like risk of graft rejection, graft infection, and lack of frequent and meticulous follow-up decreases the chances of success of penetrating keratoplasty. Patients with nebulomacular corneal opacities and a visually debilitating cataract may become ambulatory following cataract surgery. Bhartiya *et al*³² have reported phacoemulsification in 11 eyes with nebulomacular corneal opacities. A complete capsulorhexis could be performed in all cases successfully. Titiyal *et al*³³ had described small incision cataract surgery in cases with corneal opacities using TB dye for capsulorhexis.

Wet lab training of phacoemulsification

It is important for surgeons to practice phacoemulsification in a wet lab setting to reduce the learning curve and enhance safety margin before they operate on an actual patient. Visualization during each

step can be enhanced by the use of adjuvant dyes. Werner *et al*³⁴ had used 0.5% ICG and TB 0.1% to enhance visualization for performing critical steps of phacoemulsification in eight human eyes obtained post mortem and compared the results in eight eyes without using dye (control group). Both dyes enhanced visualization during phacoemulsification compared with control group. Dada *et al*³⁵ evaluated TB dye-assisted CCC in phacoemulsification of immature cataracts by trainee surgeons. Trainee surgeons performed 10 cases each with and without the use of TB dye. A complete CCC was achieved independently in all cases with TB as compared with only 30% cases in the other group.

A report from the American Academy of Ophthalmology in 2006 showed level III evidence that TB dye along with ICG and fluorescein are each effective in staining the lens capsule. There was level II evidence that staining the capsule is helpful in completing capsulorhexis and that it is helpful for pediatric patients <5 years and in cases of white cataract. There are substantial data indicating that TB 0.1% is not toxic to the cornea. The report concluded that it may be reasonable to use dye when inadequate capsule visualization may compromise the outcome in cataract surgery.³⁶

TB in glaucoma surgery

TB has also been used in glaucoma filtration surgery to visualize the treatment area of antimetabolites and to confirm the patency of drainage blebs and tubes. In their observational case series, Healey and Crowston³⁷ mixed 0.1% TB with mitomycin C (MMC) or 5-fluorouracil (5-FU), for a final concentration of TB between 0.01 and 0.05% with MMC or 0.01% with 5-FU. The mixture of antimetabolite and TB was used as per usual clinical practice with sponges or by subconjunctival injection during trabeculectomy surgery. This helped the authors to identify the antimetabolite treatment area, which is important for both the success of the bleb and risk of antimetabolite toxicity from inadvertent treatment of surrounding tissue and leakage of 5-FU from subconjunctival injection sites. Other useful findings included the observation that there was a relatively poor absorption of antimetabolite in posterior sponges when the sponges were pre-placed dry, but not when pre-soaked sponges were used. Use of TB in this study also resulted in easier visualization of the surgical sponges, which aided in their removal. *In vitro* studies with human Tenon's capsule fibroblasts confirmed that the TB did not affect the antimetabolite function of MMC or 5-FU.

It has also been observed that intracameral injection of TB during cataract surgery leads to diffusion into a functioning, but not a flat bleb.^{38,39} Grigg *et al*⁴⁰ describe

the use of intracameral 0.06% TB injected directly through the opening of a Baerveldt tube with a cannula to confirm its patency. The dye was seen to flow into the tube and onto the plate. They report that they have used this method successfully in six out of six cases.

Confirmation of the patency of filtration surgery is important in the management of glaucoma patients.

TB in conjunctival surgery

TB has had some limited usefulness in conjunctival surgery. Kobayashi *et al*⁴¹ describe the successful removal of a conjunctival cyst after initial staining with a 50:50 mixture of Healon V (Pfizer Inc., New York, NY, USA) and 0.06% TB. The staining of the capsule of the cyst facilitated its complete removal, and thus reducing the risk of recurrence. The use of sodium hyaluronate helped to prevent cyst collapse during its excision.

TB for keratoplasty

The concept of use of TB during penetrating (PKP), as well as lamellar keratoplasty, mainly stems from its property of staining the Descemet's membrane. TB is commonly used to stain the anterior lens capsule for capsulorhexis in cataract surgery. As both lens capsule and Descemet's membrane are basement membranes, TB highlights the Descemet's membrane and enhances its visibility during the surgery. Sinha *et al*¹⁵ used TB employing this theory for excision of inadvertently retained Descemet's membrane after penetrating keratoplasty. The technique called 'descemetorhexis' was used in two cases with retained Descemet's membrane after undergoing penetrating keratoplasty for congenital hereditary endothelial dystrophy.

Roos *et al*¹⁴ described the use of TB dye during PKP. They injected 0.02% TB to stain the internal and external cut edges of the cornea, as well as the ophthalmic viscosurgical device, enabling the surgeon to improve visualization of the incision and suture depth therefore improving the alignment of host and donor tissues. This technique also helps in visualization of the OVD allowing its complete removal toward the end of the surgery.

TB has been used in lamellar corneal transplantation surgery. Balestrazzi *et al*¹³ described a surgical technique of deep lamellar keratoplasty using 0.02% TB to stain the intrastromal bed, allowing good visualization of the dissection depth and decreasing the risk of perforation of Descemet's and endothelial layers. After a two-third trephination of the cornea, 0.02% TB solution is injected intrastromally in four quadrants through a 30-gauge cannula for superficial dissection. Additional dye is injected for deep dissection to stain the stromal fibers.

The authors reported that their technique facilitated a safe and effective dissection of the posterior corneal stroma and a successful deep lamellar keratoplasty. TB disappeared from the corneal stroma in early postoperative period.

More recently, Sharma *et al*⁴² described the use of TB during conversion of deep anterior lamellar keratoplasty to penetrating keratoplasty in cases with intraoperative perforation of Descemet's membrane. The presence of extensive corneal emphysema in cases with intraoperative Descemet's membrane perforation results in poor visualization of trephined corneal edges that can make wound apposition difficult during suturing. TB staining of corneal stroma as described in this technique was useful for excising the corneal shelf close to the trephined corneal edge, providing a near-vertical wound profile at the end of surgery. An additional advantage of using the dye is the easy visualization of partially excised Descemet's membrane that can be identified with TB staining.

New techniques for corneal transplantation surgery, such as anterior lamellar keratoplasty, deep lamellar endothelial keratoplasty (DLEK), or Descemet's stripping endothelial keratoplasty (DSEK), are leading to better surgical outcomes, although these techniques require accurate identification of the layers of the cornea. Vital dyes may improve identification of these layers by staining specific structures. TB dye is being utilized by corneal surgeons in different steps of endothelial keratoplasty including identification of diseased corneal endothelial layer.⁴³ Although TB dye is being used for specific steps in the various keratoplasty techniques, there is as of yet no standardization.

TB in other anterior segment surgeries

Visualization of clear corneal incisions and side ports in anterior segment surgery can often be difficult, particularly in the presence of significant corneal edema, scarring, or arcus senilis. Kayikcioglu⁴⁴ described a technique whereby a surgical blade was coated with 0.4% TB 15 min before making a corneal incision. This led to staining of the corneal wound stroma that persisted for only a few days post-operatively. Although such a technique is not routinely used, it has its advantages to reduce unnecessary epithelial trauma when trying to find elusive side ports.

Staining of the anterior vitreous can also be achieved with TB.⁴⁵ TB 0.06% injected under air intracamerally has been shown to stain vitreous within the anterior chamber after 60 s, allowing for its complete removal with a vitrector. Although the use of intracameral triamcinolone⁴⁶ has become more popular, the use of TB

potentially avoids problems with preservatives and post-operative pressure-related complications.

Safety

Since the original description of using TB to facilitate capsulorhexis in cataract surgery in 1999,⁵ the dye has been used widely, with relatively few reports of complications in the literature. *In vitro* testing of TB⁶ on cultured rabbit corneal endothelial cells did not reveal any significant toxicity at concentrations of up to 0.4% for 1 min. However, van Dooren *et al*⁴⁷ found toxicity to cultured human corneal fibroblasts at concentrations of 0.01% or greater, but only after exposure for at least 6 h.

The findings from laboratory studies are supported by clinical studies, where TB has not been reported to cause a significant rise in intraocular pressure, increase in intraocular inflammation, thickening of the cornea or a decrease in endothelial cell counts in a case series report.^{2,5,48,49}

The effect of TB on lens epithelial cells has been tested using different techniques.^{50,51} Although *in vitro* studies found no effect on the viability of LECs at concentrations ranging from 0.025 to 5.0 mg/ml,⁵² Nanavaty *et al*¹¹ reported a significant decrease in LEC density covering the anterior lens capsule after the use of 0.0125% TB for 30 s compared with that of untreated eyes. Portes *et al*⁵³ found LEC death with 0.1% TB which supports that staining with TB can help reduce the incidence of posterior capsule opacification.

TB affects the biomechanical properties of the human lens capsule and leads to a significant reduction in elasticity and increase in stiffness.⁵⁴ The impairment of elastic properties of the lens capsule could increase the risk for capsular tears and may result in a higher incidence of intraoperative complications. A reduction in the elasticity of the anterior capsule has been reported in studies using human anterior capsule,⁵⁴ with a suggestion that this is more significant in diabetic, compared with non-diabetic patients,⁵⁵ in a process that appears to be light-exposure dependent.⁵⁶

TB has also been reported to stain hydrophilic acrylic IOLs in digital image analysis, but not poly methyl-methacrylate or silicone lenses¹² after prolonged exposure of 180 min in *in vitro* testing. Clinical reports confirm permanent staining of the Acqua hydrophilic expandable acrylic IOL (Mediphacos, Minas Gerais, Brazil),⁵⁷ causing a reduction in glare at a mean of 2.6 months post-operatively.⁵⁸ Jhanji *et al*⁵⁹ have reported a case, in which the corneal stroma was inadvertently stained with TB during cataract surgery.

Diffusion of TB has been reported to cause inadvertent staining of the posterior capsule, resulting in a poor red reflex intraoperatively. This was thought to be the result

of zonular dehiscence and separation of the anterior hyaloid face from the posterior lens surface in a vitrectomised eye from previous retinal detachment surgery. Similarly, there have been several cases of inadvertent vitreous staining following intracameral use of TB, each related to traumatic cataracts and thought again, to be related to zonular dehiscence.^{60–62}

The effect of TB on the retina has revealed contrasting results. An *ex vivo* study of porcine retina suggested that exposure of 0.15% TB for 1 min did not cause any histological damage.⁶³ Using cultured human retinal pigment epithelial cells, no increase in cell death was found in concentrations up to 0.5% for 5 min,^{64,65} however, prolonged culture for 6 days at even 0.005% resulted in toxicity.⁶⁵ Luke *et al*⁶⁶ found that exposure of bovine retina to 0.15% TB for more than 15 s caused a reduction in b-wave amplitude, which was only partially reversible after 115 min. In animal studies, intravitreal injection of 0.2 and 0.06% TB into rabbit eyes that had undergone vitrectomy and gas injection showed sectoral damage to multiple retinal layers histologically, although no full-field ERG changes were detected.⁶⁷

There has been one retrospective study suggesting that the use of TB increases the rate of post-operative cystoid macular edema. The TB group had a worse pre-operative visual acuity, suggesting that these cataracts were denser, and hence an accurate conclusion cannot be made.⁶⁸

Perhaps the most serious reported complications of TB have been a report of two cases of toxic anterior segment syndrome (TASS) leading to irreversible endothelial damage.⁶⁹ TB has also been reported to cause a sterile endophthalmitis in 3 out of 17 cases where a single batch of TB 0.6 mg/ml was used.⁷⁰ It was commented that the dye was from an unknown commercial manufacturer, with a contaminant suspected to be the causative agent. These cases highlight the importance of recognizing that the use of generic formulations or unfamiliar commercial products for intracameral use should be carried out with caution.

Finally, it is worth noting that TB use in the pediatric population has been less well studied. Saini *et al*³⁰ found no increase in intraocular inflammation post-operatively when 0.1% TB was used in 42 children with a mean age of 4.13 years, although no other parameters were assessed.

Conclusions

TB is the most commonly used dye in cataract surgery. The prevalence of its use is increasing due to the introduction of wet lab training in almost all the teaching hospitals around the world. Besides, its safety profile allows for expanding indications for its use in corneal, conjunctival, and glaucoma surgeries. The recent

ophthalmic technology assessment report from the American Academy of Ophthalmology for the use of TB in cataract surgery provides further evidence of its use and safety profile.³⁶

Conflict of interest

The authors declare no conflict of interest.

Method of Literature Search

Pubmed was queried with combinations not limited to the following search terms: trypan blue, ocular surgery, cataract, corneal transplantation, keratoplasty, trabeculectomy, cataract surgery training, and complications. A review of the search results was performed and relevant articles to the topics of clinical manifestations and treatment were included. Relevant articles to the management of corneal in various conditions were also included. Case reports without additional value over another report of the same condition were not included.

References

- 1 Fritz WL. Fluorescein blue, light-assisted capsulorhexis for mature or hypermature cataract. *J Cataract Refract Surg* 1998; **24**: 19–20.
- 2 Dada VK, Sharma N, Sudan R, Sethi H, Dada T, Pangtey MS. Anterior capsule staining for capsulorhexis in cases of white cataract: comparative clinical study. *J Cataract Refract Surg* 2004; **30**: 326–333.
- 3 Pandey SK, Werner L, Escobar-Gomez M, Roig-Melo EA, Apple DJ. Dye-enhanced cataract surgery. Part 1: anterior capsule staining for capsulorhexis in advanced/white cataract. *J Cataract Refract Surg* 2000; **26**: 1052–1059.
- 4 Norn MS. Per operative trypan blue vital staining of corneal endothelium. Eight years' follow up. *Acta Ophthalmol (Copenh)* 1980; **58**: 550–555.
- 5 Melles GR, de Waard PW, Pameyer JH, Houdijn Beekhuis W. Trypan blue capsule staining to visualize the capsulorhexis in cataract surgery. *J Cataract Refract Surg* 1999; **25**: 7–9.
- 6 Chang YS, Tseng SY, Tseng SH. Comparison of dyes for cataract surgery. Part 2: efficacy of capsule staining in a rabbit model. *J Cataract Refract Surg* 2005; **31**: 799–804.
- 7 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.
- 8 Jacob S, Agarwal A, Agarwal S, Chowdhary S, Chowdhary R, Bagmar AA. Trypan blue as an adjunct for safe phacoemulsification in eyes with white cataract. *J Cataract Refract Surg* 2002; **28**: 1819–1825.
- 9 Kayikcioglu O, Erakgun T, Guler C. Trypan blue mixed with sodium hyaluronate for capsulorhexis. *J Cataract Refract Surg* 2001; **27**: 970.
- 10 de Waard PW, Budo CJ, Melles GR. Trypan blue capsular staining to 'find' the leading edge of a 'lost' capsulorhexis. *Am J Ophthalmol* 2002; **134**: 271–272.
- 11 Nanavaty MA, Johar K, Sivasankaran MA, Vasavada AR, Praveen MR, Zetterstrom C. Effect of trypan blue staining

- on the density and viability of lens epithelial cells in white cataract. *J Cataract Refract Surg* 2006; **32**: 1483–1488.
- 12 Fritz WL. Digital image analysis of trypan blue and fluorescein staining of anterior lens capsules and intraocular lenses. *J Cataract Refract Surg* 2002; **28**: 1034–1038.
- 13 Balestrazzi E, Balestrazzi A, Mosca L. Deep lamellar keratoplasty with trypan blue intrastromal staining. *J Cataract Refract Surg* 2002; **28**: 929–931.
- 14 Roos JC, Kerr Muir MG. Use of trypan blue for penetrating keratoplasty. *J Cataract Refract Surg* 2005; **31**: 1867–1869.
- 15 Sinha R, Vajpayee RB, Sharma N, Titiyal JS, Tandon R. Trypan blue assisted descemetorhexis for inadvertently retained Descemet's membranes after penetrating keratoplasty. *Br J Ophthalmol* 2003; **87**: 654–655.
- 16 Pandey SK, Werner L, Apple DJ. Staining the anterior capsule. *J Cataract Refract Surg* 2001; **27**: 647–648.
- 17 Rossiter J, Morris A. Trypan blue vital staining of the anterior lens capsule in the management of cataract in true exfoliation of the lens capsule. *Eye* 2005; **19**: 809–810.
- 18 Horiguchi M, Miyake K, Ohta I, Ito Y. Staining of the lens capsule for circular continuous capsulorhexis in eyes with white cataract. *Arch Ophthalmol* 1998; **116**: 535–537.
- 19 Arshinoff SA. Using BSS with viscoadaptives in the ultimate soft-shell technique. *J Cataract Refract Surg* 2002; **28**: 1509–1514.
- 20 Khokhar S, Pangtey MS, Panda A, Sethi HS. Painting technique for staining the anterior lens capsule. *J Cataract Refract Surg* 2003; **29**: 435–436.
- 21 Marques DM, Marques FF, Osher RH. Three-step technique for staining the anterior lens capsule with indocyanine green or trypan blue. *J Cataract Refract Surg* 2004; **30**: 13–16.
- 22 Arshinoff S. Capsule dyes and the USST. *J Cataract Refract Surg* 2005; **31**: 259–260.
- 23 Dada VK, Sudan R, Sharma N, Dada T. Trypan blue with a viscoelastic agent. *J Cataract Refract Surg* 2002; **28**: 205–206.
- 24 Wong VW, Lai TY, Lee GK, Lam PT, Lam DS. A prospective study on trypan blue capsule staining under air vs under viscoelastic. *Eye* 2006; **20**: 820–825.
- 25 Hoffer KJ, McFarland JE. Intracamerular subcapsular fluorescein staining for improved visualization during capsulorhexis in mature cataracts. *J Cataract Refract Surg* 1993; **19**: 566.
- 26 Laureano JS, Coroneo MT. Crystalline lens capsule staining with trypan blue. *J Cataract Refract Surg* 2004; **30**: 2046–2049.
- 27 Kothari K, Jain SS, Shah NJ. Anterior capsular staining with trypan blue for capsulorhexis in mature and hypermature cataracts. A preliminary study. *Indian J Ophthalmol* 2001; **49**: 177–180.
- 28 Kazem MA, Behbehani JH, Uboweja AK, Paramasivam RB. Traumatic cataract surgery assisted by trypan blue. *Ophthalmic Surg Lasers Imaging* 2007; **38**: 160–163.
- 29 Pandey SK, Werner L, Escobar-Gomez M, Werner LP, Apple DJ. Dye-enhanced cataract surgery. Part 3: posterior capsule staining to learn posterior continuous curvilinear capsulorhexis. *J Cataract Refract Surg* 2000; **26**: 1066–1071.
- 30 Saini JS, Jain AK, Sukhija J, Gupta P, Saroha V. Anterior and posterior capsulorhexis in pediatric cataract surgery with or without trypan blue dye: randomized prospective clinical study. *J Cataract Refract Surg* 2003; **29**: 1733–1737.
- 31 Sharma N, Balasubramanya R, Dada VK, Vajpayee RB. Efficacy of trypan blue in posterior capsulorhexis with optic capture in pediatric cataracts (ISRCTN48221688). *BMC Ophthalmol* 2006; **6**: 12.
- 32 Bhartiya P, Sharma N, Ray M, Sinha R, Vajpayee RB. Trypan blue assisted phacoemulsification in corneal opacities. *Br J Ophthalmol* 2002; **86**: 857–859.
- 33 Titiyal JS, Sinha R, Sharma N, Vajpayee RB. Dye-assisted small incision cataract surgery in eyes with cataract and coexisting corneal opacity. *Eye (Lond)* 2006; **20**: 386–388.
- 34 Werner L, Pandey SK, Escobar-Gomez M, Hoddinott DS, Apple DJ. Dye-enhanced cataract surgery. Part 2: learning critical steps of phacoemulsification. *J Cataract Refract Surg* 2000; **26**: 1060–1065.
- 35 Dada T, Ray M, Bhartiya P, Vajpayee RB. Trypan-blue-assisted capsulorhexis for trainee phacoemulsification surgeons. *J Cataract Refract Surg* 2002; **28**: 575–576.
- 36 Jacobs DS, Cox TA, Wagoner MD, Ariyasu RG, Karp CL, American Academy of Ophthalmology; Ophthalmic Technology Assessment Committee Anterior Segment Panel. Capsule staining as an adjunct to cataract surgery: a report from the American Academy of Ophthalmology. *Ophthalmology* 2006; **113**: 707–713.
- 37 Healey PR, Crowston JG. Trypan blue identifies antimetabolite treatment area in trabeculectomy. *Br J Ophthalmol* 2005; **89**: 1152–1156.
- 38 Agrawal S, Agrawal J, Agrawal TP. Use of trypan blue to confirm the patency of filtering surgery. *J Cataract Refract Surg* 2005; **31**: 235–237.
- 39 Dada T, Muralidhar R, Sethi HS. Staining of filtering bleb with trypan blue during phacoemulsification. *Eye (Lond)* 2006; **20**: 858–859.
- 40 Grigg J, Jang JD, Fung AT, Hunyor AP, Wilson T. Trypan blue to assess Baerveldt tube patency after repair of its obstruction. *J Glaucoma* 2010; e-pub ahead of print 16 December 2010; doi: 10.1097/IJG.0b013e3181fa0ea9.
- 41 Kobayashi A, Sugiyama K. Visualization of conjunctival cyst using Healon V and trypan blue. *Cornea* 2005; **24**: 759–776.
- 42 Sharma N, Jhanji V, Titiyal JS, Amiel H, Vajpayee RB. Use of trypan blue dye during conversion of deep anterior lamellar keratoplasty to penetrating keratoplasty. *J Cataract Refract Surg* 2008; **34**: 1242–1245.
- 43 Jhanji V, Greenrod E, Sharma N, Vajpayee RB. Modifications in the surgical technique of Descemet stripping automated endothelial keratoplasty. *Br J Ophthalmol* 2008; **92**: 1311, 1368.
- 44 Kayikcioglu O. Clear corneal incision with trypan-blue-coated blades. *J Cataract Refract Surg* 2007; **33**: 351–352.
- 45 Cacciatori M, Chadha V, Bennett HG, Singh J. Trypan blue to aid visualization of the vitreous during anterior segment surgery. *J Cataract Refract Surg* 2006; **32**: 389–391.
- 46 Burk SE, Da Mata AP, Snyder ME, Schneider S, Osher RH, Cionni RJ. Visualizing vitreous using Kenalog suspension. *J Cataract Refract Surg* 2003; **29**: 645–651.
- 47 van Dooren BT, Beekhuis WH, Pels E. Biocompatibility of trypan blue with human corneal cells. *Arch Ophthalmol* 2004; **122**: 736–742.
- 48 Chung CF, Liang CC, Lai JS, Lo ES, Lam DS. Safety of trypan blue 1% and indocyanine green 0.5% in assisting visualization of anterior capsule during phacoemulsification in mature cataract. *J Cataract Refract Surg* 2005; **31**: 938–942.
- 49 van Dooren BT, de Waard PW, Poort-van Nouhuys H, Beekhuis WH, Melles GR. Corneal endothelial cell density after trypan blue capsule staining in cataract surgery. *J Cataract Refract Surg* 2002; **28**: 574–575.

- 50 Singh AJ, Sarodia UA, Brown L, Jagjivan R, Sampath R. A histological analysis of lens capsules stained with trypan blue for capsulorrhexis in phacoemulsification cataract surgery. *Eye (Lond)* 2003; **17**: 567–570.
- 51 Rangaraj NR, Ariga M, Thomas J. Comparison of anterior capsule electron microscopy findings with and without trypan blue stain. *J Cataract Refract Surg* 2004; **30**: 2241–2242.
- 52 Melendez RF, Kumar N, Maswadi SM, Zaslow K, Glickmank RD. Photodynamic actions of indocyanine green and trypan blue on human lens epithelial cells *in vitro*. *Am J Ophthalmol* 2005; **140**: 132–134.
- 53 Portes AL, Almeida AC, Allodi S, Monteiro ML, Miguel NC. Trypan blue staining for capsulorrhexis: ultrastructural effect on lens epithelial cells and capsules. *J Cataract Refract Surg* 2010; **36**: 582–587.
- 54 Dick HB, Aliyeva SE, Hengerer F. Effect of trypan blue on the elasticity of the human anterior lens capsule. *J Cataract Refract Surg* 2008; **34**: 1367–1373.
- 55 Jardeleza MS, Daly MK, Kaufman JD, Klapperich C, Legutko PA. Effect of trypan blue staining on the elastic modulus of anterior lens capsules of diabetic and nondiabetic patients. *J Cataract Refract Surg* 2009; **35**: 318–323.
- 56 Wollensak G, Sporn E, Pham DT. Biomechanical changes in the anterior lens capsule after trypan blue staining. *J Cataract Refract Surg* 2004; **30**: 1526–1530.
- 57 Werner L, Apple DJ, Crema AS, Izak AM, Pandey SK, Trivedi RH et al. Permanent blue discoloration of a hydrogel intraocular lens by intraoperative trypan blue. *J Cataract Refract Surg* 2002; **28**: 1279–1286.
- 58 Bisol T, Rezende RA, Guedes J, Dantas AM. Effect of blue staining of expandable hydrophilic intraocular lenses on contrast sensitivity and glare vision. *J Cataract Refract Surg* 2004; **30**: 1732–1735.
- 59 Jhanji V, Agarwal T, Titiyal JS. Inadvertent corneal stromal staining by trypan blue during cataract surgery. *J Cataract Refract Surg* 2008; **34**: 161–162.
- 60 Gaur A, Kayarkar VV. Inadvertent vitreous staining. *J Cataract Refract Surg* 2005; **31**: 649.
- 61 Chowdhury PK, Raj SM, Vasavada AR. Inadvertent staining of the vitreous with trypan blue. *J Cataract Refract Surg* 2004; **30**: 274–276.
- 62 Bacsal KM, Chee SP. Trypan blue-associated retinal toxicity post complicated cataract surgery. *Eye (Lond)* 2006; **20**: 1310–1311.
- 63 Grisanti S, Szurman P, Tatar O, Gelissen F, Aisenbrey S, Oficjalska-Mlynczak J et al. Histopathological analysis in experimental macular surgery with trypan blue. *Br J Ophthalmol* 2004; **88**: 1206–1208.
- 64 Stalmans P, Van Aken EH, Melles G, Veckeneer M, Feron EJ, Stalmans I. Trypan blue not toxic for retinal pigment epithelium *in vitro*. *Am J Ophthalmol* 2003; **135**: 234–236.
- 65 Kodjikian L, Richter T, Halberstadt M, Beby F, Flueckiger F, Boehnke M et al. Toxic effects of indocyanine green, infracyanine green, and trypan blue on the human retinal pigmented epithelium. *Graefes Arch Clin Exp Ophthalmol* 2005; **243**: 917–925.
- 66 Luke C, Luke M, Dietlein TS, Hueber A, Jordan J, Sickel W et al. Retinal tolerance to dyes. *Br J Ophthalmol* 2005; **89**: 1188–1191.
- 67 Veckeneer M, van Overdam K, Monzer J, Kobuch K, van Marle W, Spekrijse H 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.
- 68 Gouws P, Merriman M, Goethals S, Simcock PR, Greenwood RJ, Wright G. Cystoid macular oedema with trypan blue use. *Br J Ophthalmol* 2004; **88**: 1348–1349.
- 69 Buzard K, Zhang JR, Thumann G, Stripecke R, Sunalp M. Two cases of toxic anterior segment syndrome from generic trypan blue. *J Cataract Refract Surg* 2010; **36**: 2195–2199.
- 70 Wu L, Velasquez R, Montoya O. Non-infectious endophthalmitis associated with trypan blue use in cataract surgery. *Int Ophthalmol* 2008; **28**: 89–93.