Toluidine Blue Dye Procedural Guideline with Sample Policy & Competency 2025

Consent obtained from all subjects used in photos.

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Introduction

Toluidine blue (TB) dye, a nuclear stain, has been widely recommended and adopted as a practice standard. TB dye is often recommended in local, regional, national, and international guidelines for sexual assault victims, including the Department of Justice (DOJ), Agency for Healthcare Research & Quality (AHRQ), American College of Emergency Physicians (ACEP), New England Journal of Medicine (NEJM), and World Health Organization (WHO).
The use of this guideline will help promote the continuing education and development of the highly specialized skills necessary for effective anogenital assessment and evaluation utilizing TB dye.

What is Toluidine Blue (TB) Dye?
TB dye is a basic thiazine metachromatic dye, which has a high affinity for acidic tissue components, thereby staining tissues rich in DNA and RNA.[1-2] The epithelium of the external genitalia (for either biological male or female genitalia) does not have nucleated cells and therefore prevents contact of stain with nuclei. However, when the epithelium is damaged and the underlying nucleated cells are exposed, TB dye is absorbed by the nuclei of damaged epithelial cells to identify breaks in epithelial tissue and thus aids in the visualization of microscopic injuries.

TB dye History

Toluidine blue has been known for various medical applications since its discovery by William Henry Perkin in 1856, after which it was primarily used by the dye industry. There are two techniques of vital staining, namely, intravital staining in the living body (*in vivo*) and supravital staining outside the body usually applied to slide preparation of detached cell. TB was first applied for *in vivo* staining by Reichart in 1963 for uterine cervical carcinoma *in situ*. During 1960s, suggestion was made that TB dye may stain malignant epithelia of the mucous membrane *in vivo*, whereas normal tissue failed to retain the dye.[2]

Vital staining of the oral epithelium has been suggested as a means of surveillance in patients who are at risk of developing oral cancer and for those who had confirmed neoplasms of other parts of aerodigestive tract. TB has been used as a vital stain to highlight potentially malignant oral lesions and may identify early lesions, which could be missed out on clinical examination. Moreover, it can outline the full extent of dysplastic epithelium or carcinoma prior to excisions and can detect multicentric or second tumors and can help in the follow up of patients with oral cancer. It is useful in obtaining the marginal control of carcinoma and in selecting the biopsy sample site in premalignant lesions.[2]

The dye is sometimes used by surgeons to help highlight areas of mucosal dysplasia (which preferentially take up the dye compared to normal tissue) in premalignant lesions (e.g. leukoplakia). This can be used to choose the best site of the lesion to biopsy, or during surgery to remove the lesion to decide whether to remove more tissue from the margins of the excision defect or leave it behind. It is also commonly used to identify mast calls.[2]

Does TB Dye interfere with evidence collection?
While it has been shown that TB dye does not interfere with DNA detection and identification,[3] it's generally recommended to collect forensic biological evidence (e.g., swabs) before dye application to avoid any potential complications or ambiguity.

Is TB Dye safe?
It is safe for use on humans. Although a slight stinging sensation may occur on open/broken tissue, it does not have any long-term adverse effects.[4] Contraindications include allergies to TBD and patient declination. TB dye should also not be used on patients with G6PD deficiency.[5]

Where do most injuries occur?
The most likely genital structures on females to be injured during genital penetration are the posterior fourchette, the fossa navicularis, the hymen, and the labia minora. The genital and anal injuries found during clinical exams are generally subtle, shallow, and do not typically show blood on the surface. Peri-anal injuries (in both biological males and females) are generally in the form of multiple linear lacerations of the perianal area inside and outside the midline and extending into the anus. The color of the normal tissue in these structures is typically similar to that of injured tissue, which can make visibility challenging. Injuries from genital penetration can show a distinctive distribution, with lacerations appearing most often on the posterior fourchette and fossa and abrasions appearing more often on the labia minora.[6-10]

Can you have injuries with consensual intercourse?
Yes. Injuries due to cooperative, consensual genital penetration are generally limited to only one injury to one genital anatomical site. If more than one genital anatomical site is injured, or multiple injury types are identified (i.e. laceration, contusion, abrasion), the likelihood that the penetration took place with consent and cooperation may decrease.[6]

Does TB Dye increase injury identification?
Yes, multiple studies have reported increased identification of injury findings after TB dye application.[4,7,11]

Can TB dye cause false positives?
Yes, TB dye stains *all* exposed nucleated cells, therefore could yield positive results in all conditions that could result in broken skin, such as vulvitis, herpes, and other ulcerations, self-inflicted injury (i.e., scratching), dermatological conditions, and more. In cases where the cause of the disruption is not clear, the medical history should be used to assess for differential diagnoses, which should be considered as indicated. The speculum exam should occur after applying TB dye, as the speculum can cause small injuries to the tissue from insertion and could create a false positive with TB dye uptake.[12]

Can TB dye cause false negatives?
Yes. Injuries may be missed if there is a barrier between the tissue and the stain, such as lubricating gel or oozing blood. Therefore, the tissue should be dry prior to TB dye application. Healing can also interfere with the staining of injuries if there are scabs or tissue remodeling/scar tissue formation.

Distinguishing various causes of positive TBD uptake

The distribution and nature of the damaged area differs between the different causes of epithelial damage as follows:

1. In herpetic and other ulcerative conditions, the stain will show up in the typical minor breaks in the muco-cutaneous epithelium.
2. Vulvitis presents as a patchy uptake of dye and is unlikely to be symmetrical.
3. Self-inflicted injury due to scratching may be present in the presence of a history of itching. It will likely present as multiple visible abrasions and will have an irregular appearance. Dermatological conditions or infections causing itching should be excluded.
4. The distribution of lacerations is linear and generally occurs at the fossa navicularis and the posterior fourchette in the area of the 3-9 o'clock location.
5. Abrasion due to genital penetration will likely appear as a diffuse uptake of dye in a symmetrical distribution in the fossa navicularis.

Errors with TB Dye Interpretation

When applied poorly, or excess not removed properly, there is a concern that injury may be overcalled because the pooling of dye creates artifact. Dye should be applied meticulously and with precision and excess dye should be removed accordingly. TB dye should be used like a highlighter, not a searchlight.

Court Considerations

The presence of anogenital injuries in SA cases is of major importance in legal proceedings. The general (albeit inaccurate) perception is that SA should result in injuries. With the use of TB dye, injuries may be more likely to be visualized. The defense is generally that the sexual act was consensual, and the regular question is, "Can you rule out consensual intercourse on grounds of the medical examination?". A Medical Forensic Exam will not determine whether sexual activities were consensual or not. Many times, a sexual assault will leave no injuries. Injuries discovered through a medical forensic exam can also result from consensual sexual activity. Utilization of the research, as well as clinical experience, should be used when formulating a response.

Process for applying TB dye

1. Perform visual examination, capture photodocumentation, and collect all external anogenital biological evidence as indicated prior to application of the dye.
2. If both the anal and vaginal areas are to be examined, the dye should first be applied to the anal area to prevent cross-contamination.
3. Before speculum examination, apply 1% toluidine blue dye to the vulva, with focus on structures based on medical history. The anus may also be stained. Do not use the TB dye in the vaginal vault or mucous membranes (i.e. hymen). TB dye may be used on labia majora, labia minora, posterior fourchette, fossa navicularis, perineum, and perianal area. (Focus on the areas between 3 o'clock and 9 o'clock as this is where most injuries occur if patient was in supine position during assault).
4. Allow to dry for 30-60 seconds.
5. Photograph the area(s) that TB dye was applied to, prior to removal.

6. Remove excess dye with a water-soluble, sterile lubricant jelly by gently blotting the area with sterile gauze pads until the excess dye is removed. Do NOT rub the area as this may cause abraded tissue. This may take several gauze pads with lubricant. Alternatively, a 1% acetic acid solution or baby wipes may be used.
7. Photograph the area and interpret the results.
8. Provide patient education that traces of TB dye may shed onto their clothes for 1-3 days after the exam.

Pictures courtesy of Diana Faugno

Before TB dye application

After TB dye application and removal of excess

Description of injury: Laceration to 6 o'clock of the posterior fourchette extending superiorly into the fossa navicularis. Diffuse abrasions to 8-9 o'clock of the inner right labia minora. Positive dye uptake.

SAMPLE POLICY

Forensic Nurse Examiner (FNE): Toluidine Blue Dye (TBD) Usage	
Effective Date:	**Policy Owner:**

Introduction:
The purpose of this policy is to outline the TBD procedure. TBD aids in the detection and visualization of minor injury to the anogenital area that may not be readily identified on visual inspection and may be used on the external anogenital structures of males and females. TBD does not stain the intact epithelial tissue but will dye nucleated squamous cells exposed by injury such as lacerations and abrasions.

Scope:
Applies to (insert department) patient care areas.

Policy Details:

I. Procedure:
- A. Examine and photograph the anogenital area before using TBD.
- B. Collect all external anogenital evidentiary specimens prior to TBD application.
- C. Explain to the patient that the TBD is not permanent on the skin, but may stain undergarments as it sheds off, for up to 3 days. The application may feel cold. Mild stinging may occur.
  - i. Supplies (including but not limited to):
    - a. 1% TBD aqueous solution (or commercial TBD applicator swabs) https://www.tedpella.com/SDS_html/19451_sds.pdf
    - b. Water-soluble lubricant (or squirt bottle containing 1% acetic acid or baby wipes) https://www.nano.pitt.edu/sites/default/files/Acetic_Acid.pdf
    - c. Cotton swabs
    - d. Gloves
    - e. 2x2 gauze
- D. Prior to speculum examination or instrumentation to area, apply TBD by using a cotton swab (or apply using commercial TBD applicator) to indicated anogenital area.
  - a. TBD may be used on labia majora, labia minora, posterior fourchette, fossa navicularis, perineum, and external anal area. Avoid hymen and vagina.
- E. Allow to dry for 30-60 seconds and capture photodocumentation.
- F. Apply water-based lubricant to a gauze pad and blot away excess dye. It is important not to rub the area to avoid irritation and diffuse staining
  - a. Alternatively, may use a gentle, broad spray of of 1% acetic acid and flood the area of excess TBD. Use gauze or cotton swabs to remove excess TBD. Caution as this technique may cause stinging on open skin. May also consider baby wipes to remove excess.
- G. Photograph after removal, regardless of injuries revealed.
- H. Document use of TBD, and findings, in health record.
- I. After speculum exam, the area may be further cleansed if needed.

Definitions:
Forensic Nurse Examiner**:** A forensic nurse is a nurse who provides specialized care for patients who are victims and/or perpetrators of trauma (both intentional and unintentional). Forensic nurses have a specialized knowledge of the legal system and skills in injury identification, evaluation, and documentation

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References:
List applicable references here

Toluidine Blue Dye Competency
Forensic Nurse Examiner Team
(Insert agency/organization here)

Forensic Examiner: _____

SKILL	YES	NO	COMMENTS
Properly gathers the correct supplies needed for the procedure			
Verbalizes the purpose and rationale for using TBD, including its ability to identify and highlight injuries and improve photographic documentation			
Verbalizes potential side effects or contraindications, as well as patient education regarding potential skin and clothing staining.			
Verbalizes how to interpret TBD results, including examples of false positives and false negatives, and reasons for artifact			
Demonstrates knowledge of the correct application techniques, including the types of swabs, dye concentration, drying time, and areas/structures to apply and avoid			
Verbalizes photography process for TBD (before application, after application but before removal, and after removal of excess)			
Demonstrates knowledge of correct removal techniques			
Describes how to document TBD findings and description of uptake			

Employee Signature: _____

Preceptor Signature: _____ **Date:** _____

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