



## Pathology & Biology Section – 2005

### G83 To Dye or Not to Dye: A Tale of the Blues

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After attending this presentation, attendees will be able to evaluate the reliability of toluidine blue dye application as part of the postmortem genital examination; be able to compare the results from photo-colposcopy at various magnifications vs. photo-colposcopy plus toluidine blue dye application; and be able to better understand the nature and appearance of postmortem anogenital anatomy at various intervals.

This presentation will impact the forensic community and/or humanity by helping to promote consistency and reliability among examiners; improving diagnostic acumen of examiners; and enhancing both antemortem and postmortem investigations and genital examinations.

**Living with the Blues:** Richart (1963) reported the use of 1% toluidine blue in more than 200 women as an in vivo staining method to delineate areas of neoplastic epithelium on the cervix. The intensity of the stain was closely related to the number of nuclei per unit area. Collins *et al.* (1966) studied 242 patients to determine the efficacy of the dye for outlining neoplastic areas on the vulva. Lauber and Souma (1982) utilized the dye as an adjunct in the evaluation of traumatic intercourse. Results were compared between a group of 22 rape victims and 22 controls that had engaged in consensual coitus. Both groups were examined < 48 hours. Further investigation of toluidine blue by McCauley *et al.* (1987) evaluated the influence of race, parity, age, and other factors in 24 rape victims and 48 controls, all examined within 48 hours.

**Dying with the Blues:** In 1992, Bays and Lewman described 4 case studies of children, ages 3 months to 4 years, where toluidine blue was used at autopsy to aid in the detection of genital and anal injuries due to child sexual abuse. In 3 of the cases, the dye uptake revealed previously undetected lacerations and a patterned injury due to a foreign object. Use of colposcopy was documented in only one case; magnification was not specified. This autopsy was done 3 days after the disappearance of the child.

**When DNA does the Blues:** One study, by Hochmeister, Whelan, *et al.* (1997), studied vaginal swabs from women after consensual intercourse. The postcoital swabs were directly exposed to toluidine blue and other destaining agents in order to determine if the dye had an adverse effect on recovery of DNA. Although there was no effect on either PCR or RFLP recovery, the sample size consisted of only 5 women and the collection time was only 6 hours post-coitus.

**Rhythm of the Blues:** As a general nuclear stain, toluidine blue, when used in vivo, depends on the presence or absence of a nucleated cell population at the exposed surface. Because many current protocols stem from the earlier studies, salient recommendations from those **methodologies** should be considered:

- Richart (1963), described proper decolorization as the most important part of the method. In very mild dysplasia with very small lesions, application of the acetic acid destaining agent in *too liberal* or *too vigorous* a manner might rapidly remove all the stain, even from areas of dysplasia.
- Lauber and Souma (1982) used lubricating jelly to decolorize. Like Richart, they stressed that it was essential to ensure that the tested area was wiped repeatedly with cotton balls until completely dry. They also described the use of finer stroking with a dry cotton tip applicator to differentiate lacerations from dye trapped in crevices.
- In addition to cervical mucous, columnar epithelium, and areas of inflammation, the concomitant presence of 23 categories of benign diseases will cause a **false positive** dye uptake in living subjects (Collins, 1966).
- **Application interval:** a great deal of variability exists in the application of this nuclear stain for documentation of traumatic intercourse in the living. Original studies were done on subjects who were examined within 48 hours. The effects of wound healing on dye application have not been studied. Programs that employ toluidine blue during extended intervals after reported sexual assault must consider the possibility of false positive dye uptake in areas of granulation tissue.
- Lauber and Souma recommended application of the dye **before** speculum insertion to avoid the possibility of findings due to iatrogenic trauma and to circumvent the known spermicidal effect of the dye in vitro. However, they also recommended procuring a *hanging drop vaginal specimen*, prior to application of the dye, in order to compare with a subsequent sample. Recent protocols do not recommend this step.
- Programs that use this nuclear stain vary significantly in their methodology, i.e., **timing** of dye application *before* or *after* speculum insertion. The Office of Criminal Justice Planning (OCJP) protocol in California recommends dye application at the *conclusion* of biological evidence



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collection.

- Early studies were done before colposcopy with magnified photographs was incorporated into sexual assault examinations. **Slaughter, Brown, Crowley, and Peck** (1997) saw no injuries with toluidine blue that were not already seen via colposcopy. However, 15X magnification was routinely used for inspection and photographs. Visualization at lesser magnifications may not allow the same level of scrutiny of the anogenital tissues. Photos taken *prior* to speculum insertion can establish the presence or absence of pre-existing injury. Likewise, when and if iatrogenic injury occurs, it can be documented as such.
- Subtle findings are an examiner issue. Follow-up exams are needed to understand those findings that may mimic trauma *and* to appreciate changes that occur with healing (Slaughter, personal communication).
- Antemortem use of the **victim as his/her own control**: when patients who present with acute genital injury are brought back for a follow-up examination, the resolution of injury and course of healing can be documented. For the rape-homicide victim, comparisons are best drawn from a baseline group of cases where the cause of death has a non-sexual etiology.
- **Postmortem artifact**: in addition to all of the conditions that affect dye uptake in the living, factors such as skin slip, mucosal autolysis, blood, and other secretions, may cause a false positive uptake during the postmortem interval.

Further study is needed to assess the efficacy and reliability of this nuclear stain as an adjunct to the postmortem genital examination. A prospective study of postmortem cases drawn from various causes of death will allow a comparison of toluidine blue and colposcopy. This subgroup will be part of a larger baseline study on the nature and appearance of the anogenital tissues at various postmortem intervals. When the understanding of what is normal and what is not in the postmortem interval is improved, the application of any staining adjuncts may then enhance, for pictorial purposes, what is already known to be present.

### **Colposcopy, Toluidine Blue Dye, Postmortem Genital Examinations**