



Pathology Biology Section – 2007

G22 To Dye or Magnify! A Proposal to Study the Efficacy of Toluidine Blue Dye vs. Colposcopy in the Postmortem Anogenital Examination

Sharon R. Crowley, RN, MN*, 122 Emeline Avenue, Santa Cruz, CA 95060

The goals of this presentation is to determine if toluidine blue is a reliable or useful adjunct in the postmortem genital examination; to describe a baseline study protocol that compares results from photocolposcopy at 7.5X and 15X magnification vs. photocolposcopy plus the addition of toluidine blue dye; and to better understand the nature and appearance of the postmortem anogenital anatomy.

This presentation will impact the forensic community and/or humanity by demonstrating an increase in the reliability and consistency

of postmortem anogenital examination techniques; and increasing in the diagnostic acumen of the forensic examiner.

Recent studies have focused on the application of a 1% solution of toluidine blue dye, a general nuclear stain, as a practice standard for the medical-legal examination of *living* sexual assault victims. Specific recommendations, e.g., the sequence of the dye application during the pelvic examination, have been delineated and advocated. Some authors and numerous practitioners recommend application of the nuclear stain *prior* to insertion of the speculum. This is based on the assumption that that the nuclear stain will delineate iatrogenic injury from pre-existing traumatic findings due to a sexual assault. In one study of antemortem sexual assault cases, Jones, Dunnuck, Rossman, et al described a 3.7% incidence (1/27 cases) where one additional genital injury was delineated via toluidine blue, after speculum insertion and removal by the examiner. This injury was located on the posterior labia minora.

In a study by Hochmeister, Whelan, et al., (JFS, 1997), there was no effect on either PCR or RFLP recovery when vaginal swabs were exposed to the dye. However, the sample size was limited to only five women and postcoital swabs were collected within six hours of coitus. In California, the medical-legal protocol recommends that dye application be deferred until *after* collection of biological specimens.

A review of the original methodologies from Richart (1963), Collins (1966), Lauber & Souma (1982), and McCauley (1987) was done by Crowley and Peterson (*To Dye or Not to Dye*, AAFS, 2005). Variability in interpretation of results in antemortem patients may be due to *many* factors. Toluidine blue is specific for zones of parakeratosis; thus positive results can be due to inflammatory, benign, or malignant vulvovaginal diseases. Twenty-three different benign vulvovaginal conditions, in addition to the presence of cervical mucous, will yield false positive results with application of this dye *in vivo*.

A paucity of data exists on the "normal" appearance of the anogenital tissues during the postmortem interval. Detailed observation and baseline studies are ideally done with colposcopy and documentation via magnified photos, to facilitate peer review. Colposcopy has been thoroughly utilized by numerous authors to enable the study of both normal and abnormal findings in both child and adult sexual assault victims since the late 1980s. The protocol for detailed postmortem inspection and the methodology for an evidentiary anogenital examination have been previously described (Crowley, JFS, 2004). The correct application and interpretation of results/findings were thoroughly described by the earlier authors (Richart, Collins, Lauber & Souma, and McCauley). The intensity of the stain is correlated to the nuclear density of the tissues. Most of the earlier authors reiterated that the proper decolorization of the dye was the most important part of the methodology. Conventional methodologies vary widely, as do the post-assault time intervals for application of the dye. Another salient factor in any discussion of the efficacy of a nuclear stain vs. colposcopy is the anatomic site to which the dye can be appropriately applied. Although used by the earliest authors for diagnosis of cervical and vulvar neoplasias, Lauber and Souma, in 1982, first described its use for evaluation of sexual assault victims and comparison to a control group of consenting women. It is important to remember that at that time, colposcopy was not widely available for examination of this population. These authors also limited the application of the dye to the posterior fourchette, an area that is histologically comprised of skin-like stratified squamous epithelium.

As mentioned previously, subtle findings may be an examiner issue (Slaughter, personal communication, 2004). Many programs do not routinely include follow-up examinations in their protocol. Without incorporation of follow-up examinations, it may be extremely difficult to evaluate findings such as localized redness and swelling. In all cases, both antemortem and postmortem, it is essential to employ the highest standards, in order to differentiate traumatic findings from either preexisting benign vulvovaginal conditions or postmortem artifact. Even experienced sexual assault examiners, whose expertise is confined to antemortem cases, may confuse normal postmortem changes or findings secondary to the cause and manner of death with traumatic lesions that are consistent with penetrating injury, i.e., sexual assault.



Pathology Biology Section – 2007

Materials and Methods: Using the mobile system of technology described by Crowley (*JFS*, 2004), the author proposes a baseline study of postmortem genital examinations with concomitant application of a 1% solution of toluidine blue dye. Utilizing colposcopy at 15X magnification, no injuries were noted in living sexual assault victims via the nuclear stain that had not already been visualized with colposcopy (Slaughter, Brown, Crowley, & Peck, *Amer. J of Ob Gyn.*, 1997).

Careful scrutiny and photo documentation with colposcopy at 7.5X and 15X should be done *prior* to speculum insertion and anoscopy. After the speculum (and anoscopic) examination, the dye can be applied to the following sites: lateral aspects of the **labia majora**, **posterior fourchette**, **fossa navicularis**, **perineum**, and the **perianal** area up to, but not beyond the area of the anal verge. The labia minora are moist and the epithelium is similar to mucous membrane. Only the lateral aspects are covered by skin. Thus, any positive findings at this anatomic site must be interpreted with caution. Application of the dye and scrupulous decolorization should then be done in the manner described by the original authors. These anatomic sites are selected based on histological composition and applicability to the techniques that were thoroughly described by previous authors, Richart (1963), Collins (1966), Lauber & Souma (1982), and McCauley (1987).

Another salient factor that requires further study and comparison to photocolposcopy is the interval of applicability for both antemortem and postmortem cases. Currently, a wide variation exists in standards. In non-genital sites, toluidine blue has been shown to yield positive uptake in granulation tissue. The great variety of normal artifact often present in postmortem cases presents many additional challenges. It is crucial that examiners avoid working in a vacuum. Often forensic nurse examiners are called to examine postmortem cases because of their expertise in living sexual assault cases. It is imperative that they consult and collaborate with the forensic pathologist, especially if their expertise has been previously confined only to the antemortem arena. Normal postmortem artifact has been misinterpreted as trauma and the additional use of toluidine blue will likely only complicate interpretation until it has been sufficiently studied. In equivocal cases, the forensic pathologist can remove the relevant tissues en bloc for dissection and microscopic evaluation. Prior to this, it is useful to have an initial in situ examination via colposcopy of the anogenital tissues.

The ultimate goal is to better visualize the anogenital tissue in the postmortem patient, in order to improve the understanding of what is normal, and what is not, at various postmortem intervals. It is possible that the application of toluidine blue dye may then enhance, for pictorial documentation.

Colposcopy, Toluidine Blue Dye, Forensic Nurse Death Investigator