



Pathology/Biology Section - 2013

G116 Till Death Do We Dye?

Sharon Rose Crowley, MN*, 122 Emeline Ave, Santa Cruz, CA 95060

The goal of this presentation is to determine a meaningful consensus on reliability and validity of this adjunct, in order to better evaluate current practice.

This presentation will impact the forensic science community by showing: (1) improved outcome in service for victims of gender-based violence, a recognized public health issue (CDC, 2009); (2) improved examination methodology, greater diagnostic acumen, more accurate documentation, and better communication between homicide investigation teams and forensic science and public health communities; and, (3) increased transparency, efficiency, communication, and reduced health disparities for marginalized groups of victims, e.g., prostitutes, victims of human trafficking, and undocumented immigrant women.

The interpretation of genital findings in the deceased is both timely and pivotal. Gender-based violence is both a domestic and global issue. The focus of the present discussion is to discuss the use of 1% aqueous toluidine blue dye as an adjunct to the evaluation of the sexual assault victim. This discussion is based on a review of the literature of toluidine blue dye in *living* sexual assault victims and results of dye application as part of an exploratory study on fatal sexual violence against women, using a cross-sectional, prospective design and a convenience sample of 74 females: 46 from the Body Donation Program at University of California, Davis Medical school; 18 coroner's cases; and ten homicide cases, ranging in age from 24 months to 75 years.

Toluidine blue dye, a nuclear stain, has been widely recommended, adopted as a practice standard, and 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), World Health Organization (WHO), and the International Association of Forensic Nurses (IAFN).

With increasing frequency, new studies on genital injury in *living* sexual assault victims incorporate dye in their methodology. Each new study builds upon the assumption that both reliability and validity of the dye have been recognized. Most of these authors cite original, well-regarded studies, including Richart,¹ Collins,² and Lauber & Souma,³ as foundational cornerstones. As the yield of studies expands, it may be useful to ask questions germane to accurate interpretation: Have the reliability and validity of toluidine blue dye for application on various sites of the anogenital anatomy been sufficiently studied to warrant the current recommendations and practice guidelines for examination of sexual assault victims? In this *Fatal Sexual Violence Against Women* study, most of the 46 cases from the Body Donation Program were examined with both colposcopy at 7.5X and 15X magnification and a 35mm Single Lens Reflex (SLR) digital camera.

Dye was applied to 30/46 subjects, with 100% false-positive dye uptake. Richart¹ and Collins² noted 23 benign conditions that caused false-positive results in their samples. Current studies often question the admissibility of evidence, e.g., reliability of colposcopy, citing *Daubert and Federal Rules of Evidence*,⁴ versus *Kelly-Frye*.⁵ Colposcopy is a modality of binocular microscopy, with the capacity for magnified digital, film, and/or video photographs.

Are colposcopes photographs inherently different types of physical evidence? If reliability and validity is well established for both film and digital imaging, does this transfer to images viewed through colposcopic optics and captured by cameras? Toluidine blue dye is far less costly than colposcopy. As a quality indicator, what level of influence does this have on outcome? Another consideration is an examiner's expertise. Detailed information on sexual anatomy, even in living victims, is still a young field, especially when contrasted with other forensic specialties. Less experienced examiners may benefit from methods that include magnified photographs, whether colposcopy, or other digital systems.

- In a review of the original, core, studies on toluidine blue, the following issues were found to be germane: Dye requires meticulous technique; decolorization is more important than application.
- Interpretation of findings is subjective. Richart,¹ Collins,² and Lauber & Souma,³ all used *qualitative* terms, such as *deep royal blue*, (positive), or *diffuse and/or patchy uptake* (non-specific), to describe patterns of dye uptake; these are *descriptive*, but also non-specific and subjective.
- Original study findings were *confirmed by biopsy*. The majority of subsequent studies on genital injury in adult/adolescent female subjects did not incorporate *follow-up* examinations, even when acute trauma was present.

Follow-up would effectively use the victim or study subjects as their own control.

Much has been learned about sexual anatomy and injury in female sexual assault victims. Concomitantly, the capacity to compare and contrast *normal* with various other cohorts increased. Toluidine blue dye was included in the initial iteration of the *sequential methodology for postmortem genital examinations*.⁶ After further study, recommendations for dye application were removed from later iterations and publication.⁷ A review and consensus by subject matter experts could evaluate the pivotal issues of reliability and validity. Such a panel could also facilitate the comparison and contrast of "cornerstone" results to those of recent studies. Individual anatomic sites could be reviewed for histological similarities/differences, to help determine appropriateness, applicability, and generalizability.



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The ultimate goal of this endeavor is to improve the understanding of what is *normal*, and *what is not*, for the anogenital anatomy during the postmortem interval. The astute examiner must perform these examinations with optimal expertise and chronicle vital data. In this manner, the capacity to understand fatal sexual violence against women will continue to grow.

References:

1. Richart R. A clinical staining test for the *in vivo* delineation of dysplasia and carcinoma *in situ*. *American Journal of Obstetrics & Gynecology* 1963; 86(6):703-712.
2. Collins C, Hansen L & Theriot E. A clinical stain for use in selecting biopsy sites in patients with vulvar disease. *Obstetrics & Gynecology* 1966;28(2): 58-163.
3. Lauber A and Souma M (1982). Use of toluidine blue for documentation of traumatic intercourse *Obstetrics & Gynecology* 1982;60(5): 644-648.
4. Federal Rules of Evidence [Daubert (509 U.S., 592-594, 113 S.Ct. 2786); Testimony by Expert Witnesses (Rule 702); Bases of an Expert's Opinion Testimony (Rule 703)].
5. Kelly-Frye—*Frye v. United States*, 54 App. D.C. 46, 293 F. 1013 [19231].
6. Crowley S R. The genital exam of sexual homicide victims by forensic nurse examiners. *Proceedings of the American Academy of Forensic Sciences; 50th Annual Scientific Meeting; 1998, San Francisco, CA.*
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Gender-Based Violence, Toluidine Blue Dye, Colposcopy