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Forensic Ultraviolet Lights in Clinical Practice: Evidence for the Evidence

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Ultraviolet lights are used in clinical forensic practice, particularly with sexual assault victims. Despite their widespread use, there is little data on either the best wavelengths of light for detecting semen stains, or on the sensitivity and specificity of the wavelengths to semen. In the descriptive literature there are varying examples of the colour of stains under UV lights, however this is not linked to particular wavelengths or circumstances. Much of the related research has been conducted on fabric samples rather than skin or with different types of lights. In this article a review of the literature on ultraviolet lights is provided, along with the results from two preliminary studies.

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Cindy is an 18 year-old female who came to Emergency for examination and treatment following a sexual assault. Her treatment options were reviewed and she chose to have a full physical examination, but did not wish the police to be involved. She cited shame and embarrassment, mainly because she had been quite intoxicated at the time and had little recall of the event. She did recall the male ejaculating on her thigh at one point and again on her chest. During the examination it was noted that she had fluorescence in both these areas, but they were not swabbed for DNA analysis given her request that police not be involved. The following day, Cindy reconsidered and reported to police about the incident. Her case went forward to court based on the results of the examination and the interviews. The sexual assault nurse examiner was asked to testify about her findings, including the stains she saw. A critical question, however, involves what the examiner knows about the ability of the light to detect or rule out certain stains, particularly semen. How confident could she be regarding the type of stain she saw?

Unfortunately, the "evidence for the evidence" in sexual assault practices has not always been established beyond the laboratory. Ultraviolet lights and alternate light sources have been used in forensic laboratories and at crime scenes for years to look for blood and body fluid stains. Recently, more portable versions of these lights have been used with varying degrees of success in clinical practice with victims of violence. Some forensic laboratory scientists have expressed frustration with their use by clinical staff, citing high rates of unnecessary swabs being taken which turn out to be negative for semen or DNA, or perhaps other fluids which are not of interest (e.g., hair gel) when sent for analysis.

Clinically, some lights allow many substances to fluoresce including hair gel, lubricant and other innocuous substances, while other lights only make semen and sometimes saliva fluoresce. In the clinical literature there is confusion with terminology between ultraviolet lights, Woods' lamps and alternate light sources. Furthermore, the descriptive literature is inconsistent regarding what positive fluorescence looks like, ranging from greenish yellow or blue to white. There is very little clinical literature as to the recommended wavelength of lights for stains on skin, nor what the stain would look like under various wavelengths. This information is vital in order to educate practitioners appropriately. The purpose of the present research is to attempt to clarify the use of ultraviolet lights for sexual assault teams prior to purchasing new lights. It is also hoped that the results will inform and guide further understanding of what it is we see when fluorescence is noted.

WHAT IS THE CURRENT EVIDENCE?

In order to understand the need for further research we first describe fluorescence of stains and the role wavelengths play in the ability to view fluorescence, including prior research on the forensic identification of various stains.

Ultraviolet Wavelengths

In clinical practice we have come to call every ultraviolet light either a "Wood's Lamp" or an alternate light source (ALS). These two terms describe very different wavelengths of light and are not interchangeable. Ultraviolet (UV) light, also known as ultraviolet radiation, is an energy emitted in various wavelengths within the light spectrum. This causes the substance being viewed to emit light, making it appear fluorescent. UV lights may be short, medium or long wavelengths (Royal Canadian Mounted Police, 2003). The ranges of lights and their utility are shown in Table 1.

Table 1: ***Wavelengths of Light***

Term	Wavelength (nm)	Sources	Comments
Short	80-280 nm	UVC radiation- sun, mercury vapour lamps	Severe burns with prolonged exposure, corneal damage if <300 nm
Medium	280-320 nm	UVB radiation – sun, sun lamps, early UV lights	Corneal damage possible at lower end of spectrum, burns if exposure >15 min
Long	320-400	-also seen in earlier UV lights (e.g. Woods)	Many substances fluoresce besides semen/saliva
ALS	> 400 nm	Meant to refer to laser light source but now commonly to refer to UV lights > 400 nm	Require goggles to filter additional fluorescence (yellow, orange or red depending on stain, nm level), more specific to stains of interest

Short wavelengths are seen in UVC radiation, which emits wavelengths between 80 and 280 nanometres (nm). Examples of sources of UVC include the sun and mercury vapour lamps. The ozone layer around the earth generally serves to protect us from UVC light. Unprotected skin and eyes can sustain severe burns with prolonged exposure to UVC sources. The cornea absorbs wavelengths of less than 300 nm so is susceptible to exposure. Medium wavelengths are between 280 and 320 nm, and are considered UVB radiation. Burns can be serious if skin or eyes are unprotected. The sun emits UVB light as well as UVC. Other sources include sun lamps, and some of the earlier UV lights used in practice, such as the Mineralite.

Long wavelength ultraviolet light is known as UVA radiation and is at least 320 nm to over 400 nm. This longer wavelength, particularly over 400 nm, is the recommended minimum for forensic use. The commonly used "Wood's lamp" typically had a wavelength of approximately 320 nm whereas the newer lights in use are over 400 nm. There remain some health and safety concerns related to the cornea and lens of the eye with direct exposure, at least for lights with a wavelength of closer to 300 nm VA light. Lights of up to 400 nm may cause injury with prolonged exposure such as more than 15 min in an 8 hour period (Muller, Clydesdale, & Muller, 2001). Lights of 400 nm or over are less likely to cause damage. Filters or goggles are required with 400 nm in order to more easily view the fluorescence. At this level, the amount of excitation causes bright light from surfaces surrounding the stain to illuminate and this illumination light needs to be blocked to reveal only the area actually fluorescing (Melles, 2002). High wavelengths are also more likely able to penetrate the skin deep enough to reveal injuries under the surface such as deep bruises not yet visible on the surface. The colour of the goggle/filter varies by wavelength and what stain is of interest. At the 450 nm level, if attempting to view semen, an orange goggle is recommended (Sirchie, n.d.).

Another term often used in the literature is an "alternate light source" or ALS. This term was originally used to refer to argon ion laser lights, such as the Luma-lite. These are more powerful lights that have a wider range of visibility than ultraviolet lights. The use of laser instead of ultraviolet radiation made it an "alternate source". Although technically incorrect, it has now become common to use the term ALS to refer to any light over 400-450 nm. Clinical experience with ultraviolet lights has shown that we can detect various stains on skin. Depending on the light, we may also be able to detect deep bruises before they are visible on the skin. Some skin conditions such as subtle forms of vitiligo (lightened skin patches) may also become very evident with some lights.

This variability of light capabilities has lead to a proliferation of types of lights. Vendors are attempting to sell us "bruise lights" and "semen lights" and "saliva lights" when in fact it is not the light per se but the wavelength of light provided by the device. Unfortunately, practitioners often do not know the wavelength of the light they are using or the particular types of injuries or stains it is capable of detecting.

It is recognized that many substances other than semen can fluoresce. This is dependent upon the wavelength of light and the properties of the substance. In the sexual assault literature, new clinicians are advised that they may recognize semen stains in ultraviolet light by their colour when the stain fluoresces. Unfortunately, there is little agreement in the sexual assault literature as to what to expect. Semen stains are sometimes described as whitish blue, as orange or as green. There is no mention of rationale for the variation in colour such as the wavelength or type of light used, which is likely to affect colour.

Detection of Stains

When body fluid stains are on surfaces such as skin or fabric they may not be visible to the naked eye. The use of fluorescent techniques such as ultraviolet lights and laser lights allows us to see stains not otherwise seen. Use of these lights to fluoresce fingerprints has been described as significantly more sensitive than even using older staining techniques (Dalrymple, 2001). When the ultraviolet light or alternate light source is directed at an area, the light from the substance/fluid is absorbed and another two types of light are produced, making the area fluoresce. These two new lights are known as excitation light and emission light. In medium to long ultraviolet wavelengths (e.g., 320 nm with a Woods lamp), the excitation light is not visible to the naked eye, and only the emission light is seen, making the stain visible (Marshall, Bennett & Fraval, 2001). When the excitation and emission light are both seen, it is difficult to differentiate the stain from surrounding tissue which also shows excitation light. This occurs at very long wavelengths, in greater than 400 nm seen with alternate light sources. When both lights are visible it is known as the visible fluorescence spectrum. The only way to view the stain is by blocking out the excitation light within that fluorescent region. Barrier filters in the form of goggles or camera filters are used for this reason. The colour of the barrier filter differs with the range of light and the body fluid to be seen. As an example, if looking for semen using a 450 nm ultraviolet light, orange goggles are needed to filter out everything but the emission light from the stain and make the stain visible (Sirchie, n.d.).

The first report of UV lights being used to identify semen was in 1919 by Dr. Wood, then subsequently by Dr. Ito in 1927 (Marshall et al., 2001; Santucci & Nelson, 1999). Most of the work involving UV lights has been conducted on stains found on fabric, either at the crime scene or in the laboratory. In sexual assault cases, the presence of semen is particularly important to be able to detect. One of the earlier studies involved comparison of a Mineralite (254 nm) with an argon laser light to detect saliva, semen and sweat stains on fabric in different dilutions (Auvdel, 1987). It was concluded that the Mineralite was able to detect each of the stains and was found to be less expensive when compared to laser.

In contrast, results from a subsequent study on a Wood's lamp with a wavelength of 320-400 nm were disappointing. The Wood's lamp was used in an attempt to detect a wide variety of substances on fabric, including semen and ointment (Santucci & Nelson, 1999). None of the 29 semen samples, wet or dried, became fluorescent under the Woods Lamp, resulting in limited detection (sensitivity) and specificity to stains. This would mean that many stains may be missed, and when a stain is present it may not necessarily be semen, resulting in a high number of false positive swabs being forwarded by the clinician to the forensic laboratory. Their findings were supported by earlier work on detection of dried semen samples, in which it was recommended that semen required a wavelength at least in the 350-450 nm range in order to be visible (Stoilovic, 1991).

Subsequent research using higher wavelengths was more successful in detecting semen. A Sirchie Bluemaxx BM 500 with a wavelength of 450 nm was used by a group of physician volunteers to identify semen stains (Nelson & Santucci, 2002). The physicians were able to detect the semen stains 100% of the time with the higher wavelength, and 83% of the time they could be differentiated from other stains after training. Unfortunately, it was difficult to find studies which incorporated comparisons of wavelengths in the same study for their utility in detecting various stains.

As noted, most of the research available has involved stains on fabric. There is a limited amount of research available regarding detection of stains on human skin. When an ultraviolet light was used on 17 victims of assault in another study, six patients showed fluorescence (Lynnerup, Hjalgrim, & Eriksen, 1995). Four of these areas were from lesions not normally seen in ordinary light and two were saliva and semen. While limited in number, these findings provide some indication of the value of UV lights in clinical use. Another small study was conducted in which 11 adult females had dried semen as well as urine placed on their arms (Gabby, Winkleby, Boyce, Fisher, Lancaster & Sensabaugh, 1992). The stains were sampled with three tests: acid phosphatase, and 2 types of prostatic protein30 tests. The stains were also examined with the Wood's lamp (254 nm). They found that the Wood's lamp was generally not sensitive to the semen, especially after 28 hours or more, and suggested that caution be exercised in the use of these lights.

Detection of Injuries

Some limited work is also available in which the lights have been used to detect various types of injuries. In a study of photographing of injuries, it was found that old injuries visible at 450 nm with fluorescence photography (Barsley, West, & Fair, 1990). Skin trauma and deep bruises were noted in addition to stains in the study by Lynnerup's team (1995). Bitemarks, not yet visible on the surface, were also described as visible when using 450 nm ultraviolet lights (Golden, 1994). The lights have also been helpful in detecting distinctive details of tattoos not seen with ambient light on post-mortem subjects (Bennett & Rockhold, 1999). The ability to detect deep bruising is helpful in the early stages. These marks may take 12-24 hours to appear on the surface on living victims and may not appear post-mortem. By the next day, the living victim has usually returned home and evidence is lost which may support their history of assault if they do not choose to return for photographs.

It may be seen from the above that a number of questions remain unanswered in the ultraviolet light literature. While it appears that higher wavelengths of ultraviolet radiation are better for detecting semen, many of the studies have been conducted on fabric rather than skin. Furthermore, sufficient data is unavailable regarding the sensitivity and specificity of certain wavelengths of light for detecting semen. There is also relatively little comparative data in which the sensitivity and specificity of lights is compared across different types of substances that may be found on a sexual assault victim's skin. These questions led us to conduct two preliminary studies on the identification of semen.

RESEARCH STUDIES

Prior to purchasing new lights for the sexual assault team, it was decided to conduct a small pilot study. The purpose of the study was to compare three different wavelengths of ultraviolet lights in terms of their ability to detect the presence of semen (sensitivity) and their ability to correctly conclude that semen was absent from a sample (specificity). While the ability of the lights to note other substances is of interest, it is the presence of semen, which is of utmost interest for sexual assault victims.

Pilot Study

In order to answer the above questions, we obtained four lights with different types of wavelengths. These are shown in Figures 1and 2, and included: the Mineralite (254 nm); the Evident (365 nm); and two different versions of the Sirchie Bluemaxx lights, each at 450 nm. The Bluemaxx BM500 was previously studied by Nelson and Santucci (2002), but as seen in the photo, it is somewhat large and cumbersome for a team that travels frequently. It is like a very large, heavy flashlight (large enough to hold 3 "D" cell batteries) and several staff commented that their wrists fatigued when using it for more than a few minutes of holding it over a stain. The Bluemaxx Mini was more like a mini-flashlight (7 inches long, 1 inch wide) and therefore lighter to hold and carry.

Figure 1: ***Short Wavelength Light - Mineralite (254 nm)***

Figure 2: ***Long and Alternate Light Sources***

****Note: From top to bottom: Bluemaxx BM500 (450 nm), Bluemaxx Mini (450 nm), Evident CE (365 nm).***

To begin with, we applied saliva, semen and urine to one researcher's forearm. We showed volunteers how these different fluids would look under each of the four lights. We then applied a sample of each of the following substances to numbered locations on the inner aspect of the forearms of the other researcher: lotion; saliva; semen (2 sites); condom lubricant; hair gel; talc powder; urine; Muko (water soluble lubricant); Polysporin antibiotic ointment; and liquid soap. A blank spot was also left in one of the numbered sites as a control. Each substance was placed within a numbered box drawn on the arm with fluorescent marker. All liquids and gels were applied using 0.1 ml from a tuberculin syringe. The powder and condom lubricant were smeared to fill the assigned observation area, covering approximately the same size surface area as the 0.1 ml fluid.

Five sexual assault nurse examiners (SANE's), one non-SANE RN and a student RN were asked to use each of the lights to view the substances within 1 hour after applying the substances. A police identification officer then photographed each of the stains using time exposure techniques and using an orange filter for the 450 nm lights (similar to using the goggles to reduce extraneous light). The results of the sensitivity and specificity for semen with each of the lights are shown in Table 2. The sensitivity of the lights to the other samples is shown in Table 3. It should be noted that the Mineralite was sensitive to most samples, and the Evident CE was sensitive to almost half of them. In comparison, both the Bluemaxx lights were positive for fluorescence with fewer fluids. The Bluemaxx mini, with the exception of 1 rater who saw the polysporin stain as positive, only fluoresced for semen. This would make it the most specific to semen of all the lights.

Table 2: *Pilot Study Results (N=7)*

Light	Sensitivity	Specificity	Colour
Mineralight (254 nm)	92.9	47.1	Blue
Evident Products CE (365 nm)	78.6	57.1	Blue, blue-white, light green
Sirchie Bluemaxx BM 500 (450 nm)	86.7	90	White, yellow-white, green
Sirchie Bluemaxx Mini (450 nm)	100	98.5	White, yellow-white, light green

Table 3: *Sensitivity of Lights to Different Stains (%)*

Light	Lotion	Saliva	Condom lubricant	Body gel	Powder	Urine	Muko	Poly-sporin	Soap	Blank
Mineralight (254 nm)	29	100	14	100	43	71	57	86	57	14
Evident Products CE (365 nm)	0	100	0	100	0	0	29	71	43	0
Sirchie Bluemaxx BM 500 (450 nm)	0	14	0	14	29	14	14	0	0	14
Sirchie Bluemaxx Mini (450 nm)	0	0	0	0	0	0	0	14	0	0

Note: N = 7; 14% represents 1 rater who considered the area "positive", most often the same rater (a junior nurse with no SANE experience and limited clinical experience).

Replication of Pilot

Based on the results of the pilot study, ethical approval was received from the Health Region to replicate the study on a larger scale at a conference for sexual assault examiners and response teams in the United States during May of 2003. It was anticipated that we would be able to involve at least 100 sexual assault examiners to participate at this large international symposium. The study stains were replicated using the same substances as the pilot. As before, two of these stains were semen and one was a blank spot for control purposes. Study participants were asked to view the stains with all three lights representing 3 different wavelengths (the Mineralite, the Evident CE and the Bluemaxx Mini). They were asked to rate any stain they saw by location, speculate whether it was positive or negative for semen, and to note the colour of the stain.

We had a few challenges in implementing the study due to difficulties in transporting and storing the semen sample during travel, affecting the quality of the sample. Even immediately after application it was difficult (although possible) for the researchers to see one of the two semen stains but the second was not visible at all with any of the lights. There were further delays between stain application and viewing of greater than 4 hours compared to less than 1 hour in the pilot. The final complication was related to participation and location of the study room. The room was difficult to find and the study was scheduled at the end of the day, perhaps accounting for only obtaining 27 participants from the potential 280 attendees. This included 18 sexual assault nurses, 4 patient advocates, 3 police, 1 police officer, and 1 person of unknown occupation.

The final results of the sensitivity and specificity to semen are shown in Table 4 for the one semen stain visible to researchers. It may be noted that the Mineralite picked up the semen most consistently, although it also gave a very high false positive rate. In contrast, although the semen stain was missed more often with the Bluemaxx Mini, there was a higher rate of specificity. These findings need to be interpreted cautiously in light of the study complications noted above. A positive finding was that, despite the small samples, the advocates and police were also able to detect the one visible semen stain despite having had no prior training with these lights.

Table 4: *Replication Study Results (N=27)*

Light	Sensitivity	Specificity	Colour
Mineralight (254 nm)	85%	81%	White (6), yellow (3), green (2), blue (1)
Evident Products CE (365 nm)	4	11	Green (1), blue (1)
Sirchie Bluemaxx Mini (450 nm)	60	95	White (3), Orange (1), yellow (1)

DISCUSSION

It was seen in both studies that many of the substances other than semen were fluorescent using the lights with wavelengths below 450 nm. In contrast, the 450 nm lights almost exclusively only fluoresced when the stain was semen. Interestingly the smaller light was seen to be more discriminate (i.e., greater sensitivity and specificity). In the second study the comments from participants were initially quite negative toward the 450 nm lights. They expressed dislike at not seeing very many stains with the longer wavelength lights, making comments such as, "but it doesn't show much". These comments indicate the need for education of staff used to medium or short wavelengths of light who switch to longer wavelengths. Specifically, if the intent is to be more confident that what one see is a particular substance (e.g., semen or saliva), then one would want it to show fewer non-relevant stains and be more selective. There is no clinical benefit to seeing fluorescent stains, which were not important to the case such as hair gel. The inclination is to swab these stains if not sure of their origin and this places an unnecessary burden on forensic laboratories. We can also see that the colour of the stains varied, although with some consistency. The more bluish or white-blue stains were seen with lower wavelengths while the white or white-green was seen with the higher wavelengths. This is consistent with the spectrum of light emitted by the higher wavelengths.

There were a number of limitations with this research. First, the sample size of both studies was small. The only benefit with the first study was the use of photographs, which provided a permanent record of what was seen by participants and have been validated informally by many clinicians subsequent to the study. The attempt to replicate in a follow-up study was fraught with challenges, most notably the storage of the semen sample. Unfortunately both the semen stains were difficult to see, especially one of the stains, perhaps due to the storage issues, the location on the arm (the one not visible was near the bend of the elbow), and the longer period of before the stain was viewed.

We have seen that there are limited data for clinical versus laboratory applications of ultraviolet lights. It is important that we know what we are likely to be detecting when fluorescence is noted, and that we can properly incorporate this knowledge into training of new sexual assault examiners and clinicians. Further questions also emerge from these studies and from clinical practice. Examples include the effects of different coloured skin on visibility of semen stains, the point at which semen becomes visible or stops being visible, the characteristics of deep bruises or skin markings that may determine its visibility, the recommended distance between skin and light source for maximum visibility of stains, or the impact of stains present on different body parts such as those covered by clothing or areas washed frequently. Work on inter-rater reliability would also be desirable.

It is recognized that these two studies are preliminary in nature and require further replication. Plans are currently in progress for a third study to answer some of these questions. We had hoped with this preliminary work to add to the body of "evidence for the evidence" as it relates to ultraviolet light use. In Cindy's case, we can still not be certain of the origin of the stain. We need more research on the sensitivity and specificity of various long wavelength lights on various skin types and at various timeframes after application of stains. We noted the difficulty in viewing stains in the second study, which was longer post-application. It would be important to look at the role of time and normal "wear and tear" on stains since many sexual assault victims come in at least 12-24 hours post-assault. Stains could be altered with clothing rubbing on them or with showering or attempts at hygiene. Ultimately it is hoped through this work, further replication and related studies that forensic clinical examiners will have a greater degree of confidence in their interpretation of their findings with the ultraviolet light. It is also hoped that such research would lead to better discrimination of samples prior to being sent to forensic laboratories for testing.

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