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Comparison of Laser and Ultraviolet Techniques Used in the Detection of Body Secretions

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ABSTRACT: Evaluation of the detection capabilities of both laser and ultraviolet light sources was performed. The Spectra-Physics Model 171-19 argon ion laser was used in a comparison with the hand held Mineralight® multiband ultraviolet lamp, Model UVSL-58 and the Fotodyne Foto UV 410. Model 3-4100. Both techniques were evaluated as to their detection limits for various biological stains. A serial dilution was made from semen, saliva, and sweat samples and their corresponding stains were examined under laser and ultraviolet light sources. The techniques were also evaluated as to possible interferences which may arise based on the type of fabric the stains were made on. The advantages and disadvantages of each technique in relationship to their initial costs are discussed.

KEYWORDS: pathology and biology. body fluids, lasers, saliva, semen, sweat, ultraviolet, white light

The detection of various body fluid stains encountered in forensic science casework is one of the primary objectives for a forensic serologist. White light, ultraviolet light, and laser light sources are used for the visualization of body fluid stains on various articles submitted for examination. Because of the fact that a number of body fluid stains, including seminal, saliva, and sweat, fluoresce under ultraviolet and laser light, these sources provide excellent, simple, nondestructive screening techniques [*1-5*]. Chemical methods are also used in the visualization and localization of biological stains. Acid phosphatase mapping is utilized in the presumptive screening for seminal stains [*6*]. Screening tests for possible saliva stains are based on a test paper with procion red-dyed starch [*7*] or on amylase test papers prepared from a blue-dyed starch substrate [*8*]. Based on the capabilities or preference of a laboratory, one or more of these techniques can be used in the screening for body fluid stains.

This project dealt with the detection capabilities of ultraviolet and laser light sources. The techniques were evaluated as to their detection limits on stains made from serial dilutions of semen, saliva, and sweat. Interferences that may arise using these techniques were noted. The advantages and disadvantages of each technique in relationship to their initial cost are discussed.

Equipment

1. White light: room light, LUXO Magnifier and Examiner 10 Spot Light by AMSCO.
2. Mineralight® multiband ultraviolet lamp, Model USVL-58. One shortwave tube, 254 nm and one longwave tube, 366-nm. Cost: $150.00.

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3. Fotodyne Foto UV 410, Model 3-4100. Base contains six 15-W color-corrected white visible bulbs, flashed opal glass diffuser, and a cooling fan. Each of the two sidearm housings contains three 15-W bulbs, one color-corrected white visible, one shortwave ultraviolet (UV), 254 nm, and one longwave UV, 366-nm bulb. Cost: $2300.00.

4. Spectra-Physics Model 171-19 argon ion laser. Continuous wave operation, 454.5 to 514.5 nm, output power 18 W. Cost: $35 000.00.

Method

The semen, saliva, and sweat samples were collected from a laboratory donor. Serial dilutions of neat, 1/2, 1/4, 1/8, and 1/16 were made using fluid semen, saliva, and sweat. Stains were made using 50 uL of each sample and its dilutions on sections cut from 23 different articles including clothes, control cloth, and a sheet. The stained fabrics were examined under white light, ultraviolet light, and with the laser. The stains were examined after complete drying. They were reexamined several times over a 2-month period while being stored at room temperature.

Table 1 lists the different articles used for staining purposes, their color, and whether the items were washed before application of the samples. The articles used in testing were donated by laboratory members and the actual laundry histories were not known. Table 2 lists both the weave/knit and the fiber composition of the different articles tested.

The results on Table 3 were obtained by screening Items 1 through 23 that had a serial dilution series of semen and Item 24, a casework exhibit. Seventeen of Items 1 through 23 had seminal stains visible using the laser. Twelve of these seventeen stains were also visible using the Fotodyne UV light source. A thirteenth stain was visible with the Fotodyne unit, however, this stain was not detected with the laser. Eleven of the seventeen stains visible with

TABLE 1—*Items used in testing.*

Item	Article	Color	Times Washed
1	standard cloth	white	one
2	pants	blue	many
3	bed sheet	white	many
4	panties	white	many
5	shirt	blue/brown	many
6	shirt	white/blue	many
7	shirt	cream/red	many
8	shirt	white	many
9	shirt	yellow/orange	many
10	shirt	white/blue	many
11	shirt	yellow/brown	many
12	shirt	brown	many
13	sweater	gray/black	many
14	sweater	navy/blue	many
15	sock	burgundy	many
16	sweater	charcoal/gray	many
17	shirt	tan	many
18	sock	charcoal/gray	many
19	sock	brown	many
20	sock	gray	many
21	sock	white	many
22	sock	burgundy	many
23	sock	brown	many
24	nightgown	yellow	many

TABLE 2—*Fiber composition of items tested.*

Item	Weave/ Knit	Fiber Composition
1	weave	cotton
2	weave	cotton/polyester
3	weave	cotton
4	knit	nylon continuous filament
5	weave	cotton/polyester
6	weave	polyester & polyester/cotton
7	knit	acetate-continuous filament
8	knit	cotton
9	weave	cotton/polyester
10	weave	cotton/polyester
11	knit	polyester continuous filament and cotton
12	knit	polyester continuous filament and cotton
13	knit	polyester continuous filament and cotton/rayon
14	knit	nylon continuous filament and acrylic
15	knit	nylon continuous filament and acrylic
16	knit	nylon continuous filament and acrylic
17	knit	polyester continuous filament and cotton
18	knit	nylon continuous filament and acrylic
19	knit	nylon continuous filament and acrylic
20	knit	polyester continuous/filament and cotton/rayon
21	knit	acrylic
22	knit	nylon continuous filament and acrylic
23	knit	nylon continuous filament and acrylic
24	knit	cotton

the laser were also visible with the hand held UV unit. A twelfth stain was visible with the hand held UV light, but it was not detected with the laser. The neat stain on Item 22 was detected with both UV light sources, however, it was not visible with the laser. The nightgown, Item 24, had seminal stains detected on the front left side using the laser. These stains were not visible with white light or either UV light sources. Seminal stains were not visible with the laser on six items out of the twenty-four. Three of these items, 2, 15, and 22, had strong fluorescence which masked the presence of the stains. The other three items did not fluoresce, however, the stains were not visible. Items 10, 11, and 13 had strong fluorescence under UV light from both the Fotodyne and hand held units, and as a result, the seminal stains were not visible. The items that had strong fluorescence with the laser or UV light sources made it impossible to visualize the seminal, saliva, or sweat stains on them. Seven of the stains that were visible with the laser and UV light were also visible under white light.

The results on Table 4 were obtained from screening Items 1 through 23 that had serial dilutions of saliva. The nightgown, Item 24, was a casework exhibit that had been screened for the presence of semen and no other stains were visible. Saliva or sweat stains or both may have been originally present on the nightgown, however, no stains other than seminal were found. Seven of the Items 1 through 23 had saliva stains detected with the laser. Five of these seven stains were visible with the Fotodyne unit. Three of these five stains were also visible with the hand held UV light. Three stains that were detected with the laser and UV light sources were also visible with white light.

Table 5 shows the results of the screening of Items 1 through 23 that had a serial dilution of sweat. Five of the twenty-three items had sweat stains detected with the laser. Three of these stains were visible using both the Fotodyne and hand held UV light sources. Item 22 contained a fourth stain that was visible with both UV light sources, but not with the laser. No sweat stains were visible under white light.

TABLE 3—*Screening results for seminal stains.*

Item	White Light[a]	UV-Fotodyne	UV-Hand Held	Laser
1	1/2s[b]	1/16w	1/16w	1/16w
2	NV[c]	NV	NV	NV
3	1/4w	1/8w	1/4w	1/16s
4	1/4w	1/2w	1/2w	1/4w
5	NV	NV	NV	1/2w
6	NV	NV	NV	1/4w
7	1/2	1/16w	1/16w	1/16s
8	1/2w	1/2w	neat w	1/16w
9	NV	neat w	NV	1/2w
10	NV	NV	NV	1/4w
11	NV	NV	NV	1/2s
12	NV	1/4w	1/4w	1/4w
13	NV	NV	NV	1/4w
14	neat w	1/2w	1/2w	1/2w
15	NV	NV	NV	NV
16	NV	NV	NV	NV
17	NV	1/4w	1/2w	1/4w
18	NV	NV	NV	NV
19	NV	1/4w	1/4w	1/4w
20	NV	1/4w	1/4w	1/16w
21	1/2w	1/8w	1/4w	1/8w
22	NV	neat w	neat w	NV
23	NV	NV	NV	NV
24	NV	NV	NV	neat

[a]White light sources were room light, Luxo Magnifier, and Examiner 10 by AMSCO.

[b]Dilution series was neat, 1/2, 1/4, 1/8, and 1/16 (s = strong, w = weak).

[c]NV = not visible.

Discussion

Ultraviolet and laser light sources are being used as simple and nondestructive screening techniques for the presence of various body fluid stains. Semen, saliva, sweat, and other body fluids, because of their inherent luminescence, fluoresce under UV and laser light.

The laser, in comparison with UV light, was shown to be more effective as a screening tool for the detection of body fluid stains. The laser's intensity of radiation and the fact that it is monochromatic results in fluorescence or phosphorescence being excited and easily recorded in very small traces of various substances [*9*]. The amount of luminescence created by the laser that can be seen, measured, or photographed is directly proportional to the average power of laser illumination [*10*]. The average power is the power available during a given period of time for illuminating a surface. Average power is the value used for argon ion laser output energy (which is continuous wave 454.5 to 541.5 nm).

Figures 1 through 4 show the results of screening a piece of commercially purchased standard white cotton cloth, Item 1, that was washed one time after purchase and then the semen was applied. Figures 2 and 3 show that the stains are only weakly visible at a dilution of 1/16 using two different UV light sources. The stains were easily observed through a 1/16 dilution using the laser.

Figures 5 through 8 show the results of screening sections of a white cotton sheet, Item 3, for the presence of semen. The sheet was old and had been washed numerous times before the samples were added. In this case, the seminal stains were visible through a dilution of

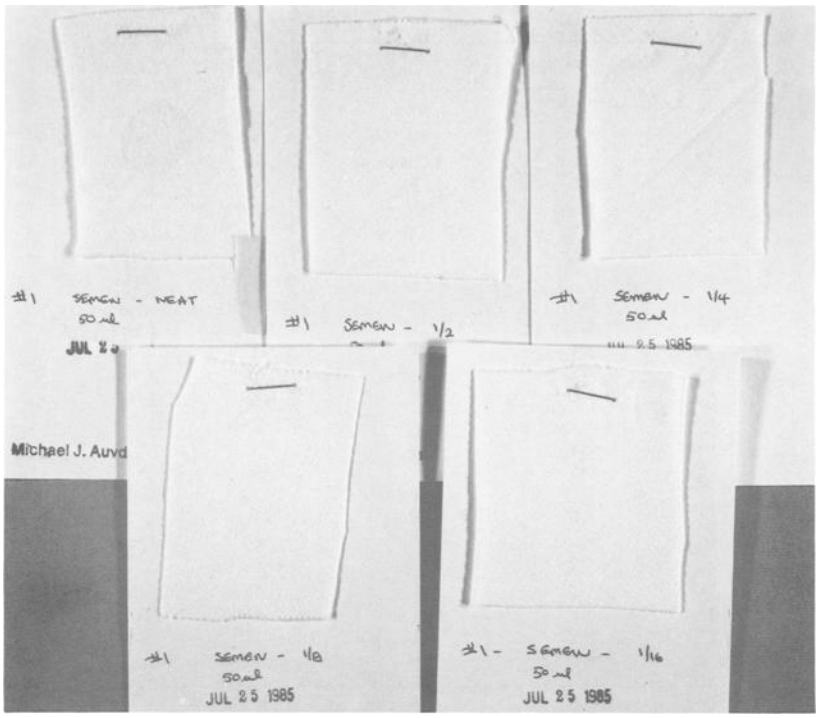

FIG. 1—*Screening of a piece of commercially purchased standard white cotton cloth, Item 1, with white light that was washed one time after purchase and then the semen was applied.*

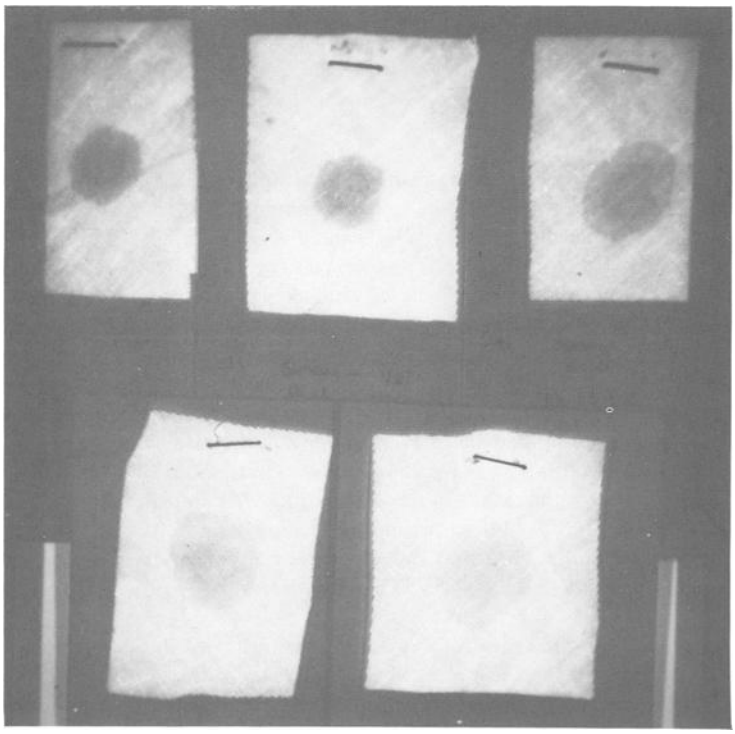

FIG. 2—*Same conditions and cloth as Fig. 1, except screened with UV-Fotodyne.*

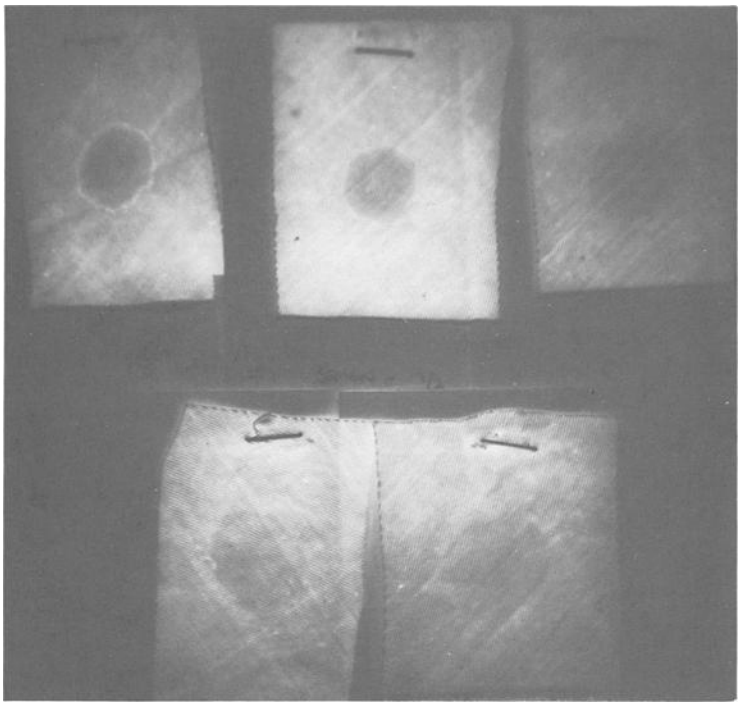

FIG. 3—*Same conditions and cloth as Fig. 1, except screened with UV-hand held.*

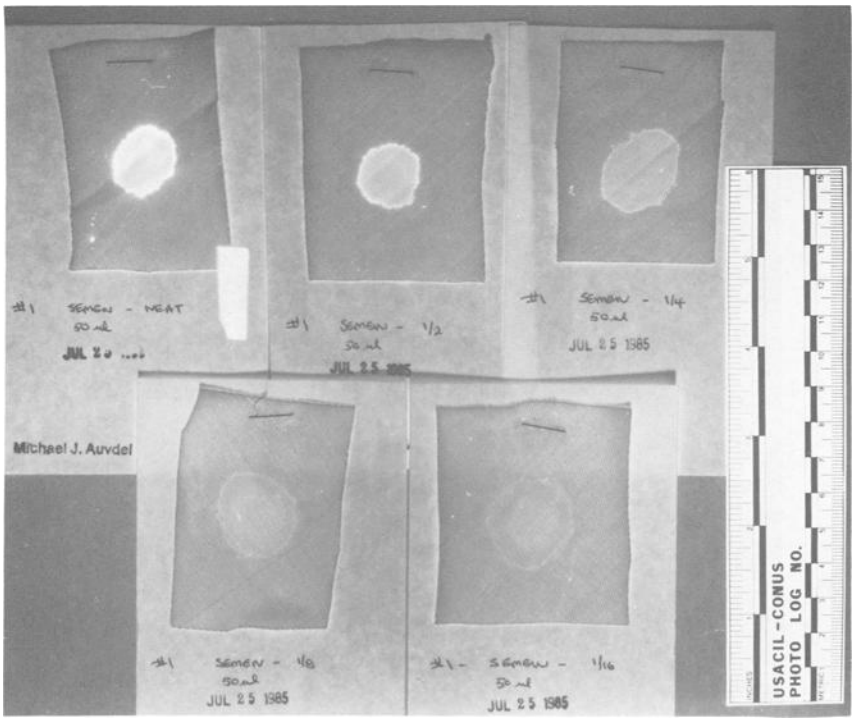

FIG. 4—*Same conditions and cloth as Fig. 1, except screened with laser.*

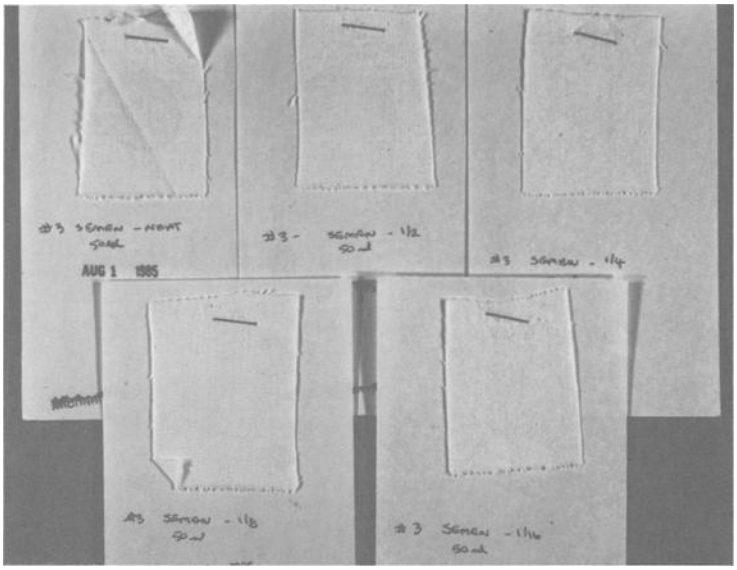

FIG. 5—*Screening with a white light of a white cotton sheet, Item 3, that had been washed numerous times before semen applied.*

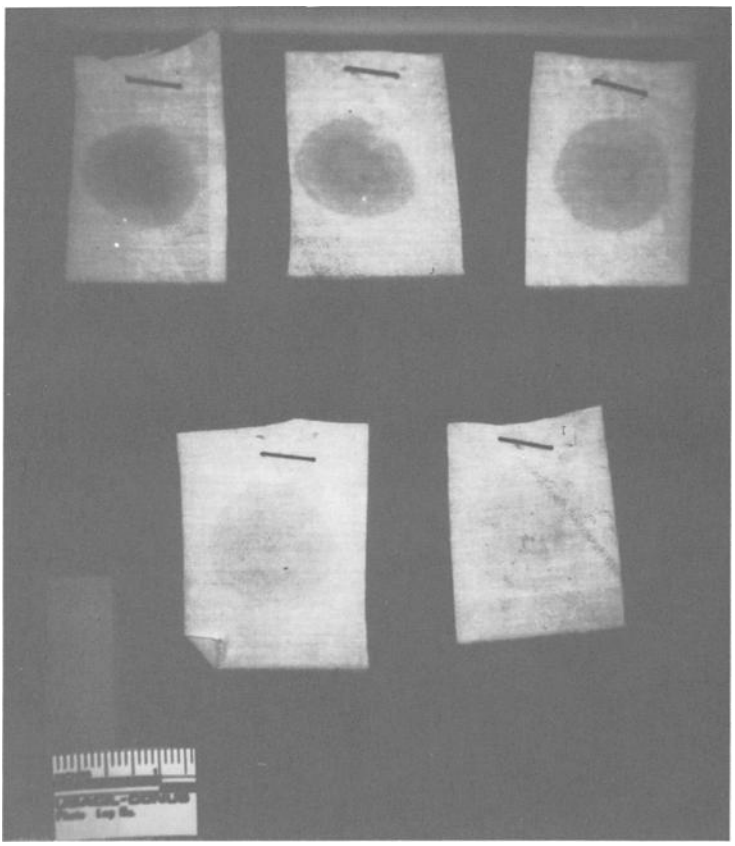

FIG. 6—*Same conditions and sheet as Fig. 5, except screened with UV-Fotodyne.*

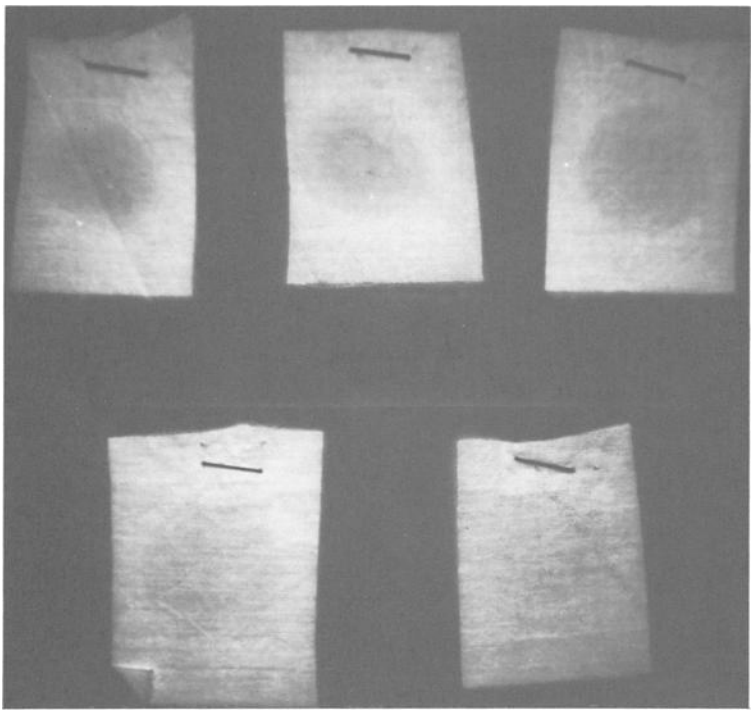

FIG. 7—*Same conditions and sheet as Fig. 5, except screened with UV-hand held.*

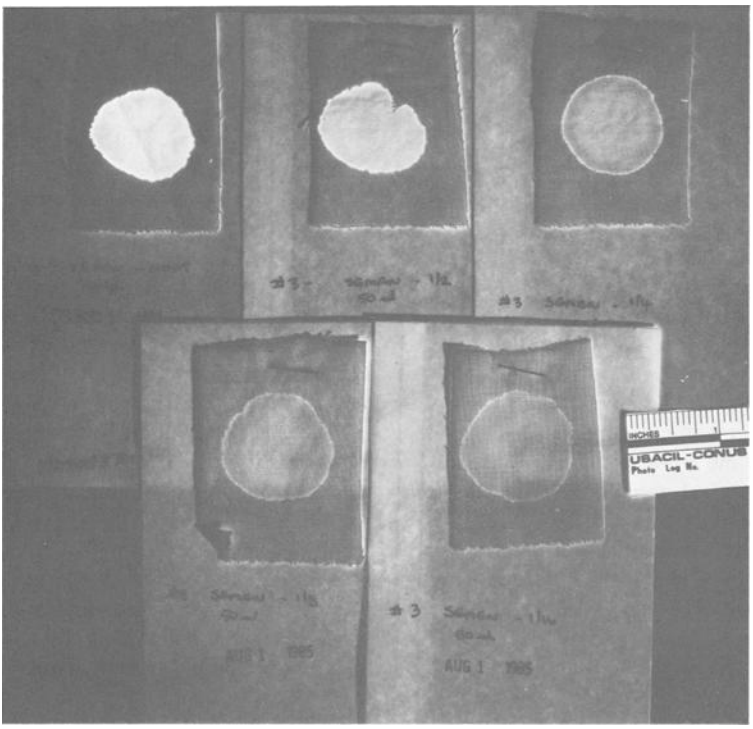

FIG. 8—*Same conditions and sheet as Fig. 5, except screened with laser.*

TABLE 4—*Screening results for saliva stains.*

Item	White Light[a]	UV-Fotodyne	UV-Hand Held	Laser
1	1/2w[b]	1/8w	1/8w	1/8ws
2	NV[c]	NV	NV	NV
3	1/2w	1/16w	1/8w	1/16s
4	NV	NV	NV	neat w
5	NV	NV	NV	NV
6	NV	NV	NV	NV
7	NV	NV	NV	1/2w
8	NV	neat w	NV	neat w
9	NV	NV	NV	NV
10	NV	neat w	NV	1/4w
11	NV	NV	NV	NV
12	NV	NV	NV	NV
13	NV	NV	NV	NV
14	neat w	neat w	neat w	neat w
15	NV	NV	NV	NV
16	NV	NV	NV	NV
17	NV	NV	NV	NV
18	NV	NV	NV	NV
19	NV	NV	NV	NV
20	NV	NV	NV	NV
21	NV	NV	NV	NV
22	NV	NV	NV	NV
23	NV	NV	NV	NV
24[d]	NV	NV	NV	NV

[a]White light sources were room light, Luxo Magnifier, and Examiner 10 by AMSCO.

[b]Dilution series was neat, 1/2, 1/4, 1/8, and 1/16 (s = strong, w = weak).

[c]NV = not visible.

[d]Saliva stains may have originally been present. None were visible.

1/8 with the Fotodyne unit and through 1/4 with the hand held UV. The same stains when viewed under the laser were easily observed through a 1/16 dilution.

Figures 9 through 12 show the results of screening a nightgown from laboratory casework, Item 24, for the presence of semen. Seminal stains were not visible under white or UV light. Seminal stains were detected with the laser (Fig. 12).

Seminal stains were detected on 75% of the items screened with the laser. Only 50% of the seminal stains were detected using both UV light sources. The use of the laser in conjunction with UV light sources increased the detection rate to 79%. The stains not detected using either the laser or UV light were detected using the acid phosphatase (AP) mapping technique [6]. I believe that the AP mapping technique should only be employed as the last step in the screening of articles for the presence of semen. This technique results in the loss of some of the seminal stain when it is transferred to the filter paper, and it can cause dilution of the original stain and may also increase further degradation of the stain with the addition of moisture.

Figures 13 through 16 show the results of screening sections of a white sheet, Item 3, for the presence of saliva. These stains were weakly visible at a dilution of 1/16 using both UV light sources. The stains were easily observed through a 1/16 dilution using the laser. Saliva stains were detected on 30% of the items examined with the laser. Of the stains, 21% were detected using the UV light sources. Use of the laser and the UV did not increase the detec-

TABLE 5—*Screening results for sweat stains.*

Item	White Light[a]	UV-Fotodyne	UV-Hand Held	Laser
1	NV[b]	1/16w[c]	1/16w	1/16w
2	NV	NV	NV	NV
3	NV	1/2w	1/2w	1/16w
4	NV	NV	NV	1/16s
5	NV	NV	NV	NV
6	NV	NV	NV	NV
7	NV	NV	NV	1/8w
8	NV	neat w	neat w	neat w
9	NV	NV	NV	NV
10	NV	NV	NV	NV
11	NV	NV	NV	NV
12	NV	NV	NV	NV
13	NV	NV	NV	NV
14	NV	NV	NV	NV
15	NV	NV	NV	NV
16	NV	NV	NV	NV
17	NV	NV	NV	NV
18	NV	NV	NV	NV
19	NV	NV	NV	NV
20	NV	NV	NV	NV
21	NV	NV	NV	NV
22	NV	neat w	neat w	NV
23	NV	NV	NV	NV
24	NV	NV	NV	NV

[a]White light sources were room light, Luxo Magnifier, and Examiner 10 by AMSCO.

[b]NV = not visible.

[c]Dilution series was neat, 1/2, 1/4, 1/8, and 1/16 (s = strong, w = weak).

[d]Sweat stains may have originally been present. None were visible.

tion rate above 30%. Chemical tests could be employed for the screening purpose of possible saliva stains, but the results would be very limited when no initial staining is visible.

Figures 17 through 20 show the results of screening sections of the white sheet, Item 3, for the presence of sweat. The stains were weakly visible at a 1/2 dilution using the UV light sources. The stains were weakly visible at a 1/16 dilution using the laser. Sweat stains were detected on 21% of the items screened with the laser. Of the stains, 17% were detected using the UV light sources. The use of the laser with the UV light would have only increased the detection rate to 26%. There are no chemical tests presently used for screening articles for the possible presence of sweat stains when no stains are visibly detected.

When employing laser or UV light sources for screening various articles for the presence of body fluid stains, one relies on their ability to fluoresce. Interference problems arise when the article to be screened has its own inherent luminescence, and as a result, fluoresces under laser or UV light. Certain organic compounds possess this property of fluorescence. These fluorescent brighteners (also referred to as fluorescent whiteners and optical brightening agents) are present as deliberate additions in the manufacture of various textiles, washing powders, and fabric conditioners [*11*]. These substances are used to increase the apparent brightness or whiteness of the textile material.

Items 2, 15, and 22 were strongly fluorescent under the laser, and as a result, no stains

FIG. 9—*Screening of a nightgown, Item 24, for semen with white light.*

FIG. 10—*Screening of a nightgown, Item 24, for semen with UV-Fotodyne.*

FIG. 11—*Screening of a nightgown, Item 24, for semen with UV-hand held.*

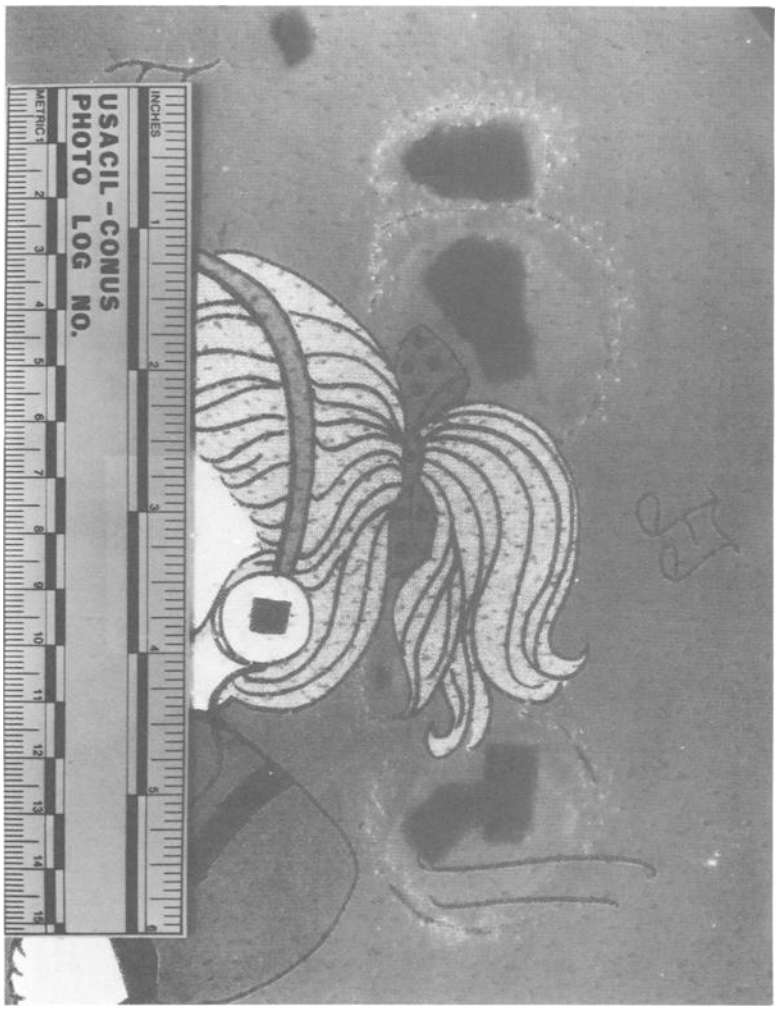

FIG. 12—*Screening of a nightgown, Item 24, for semen with laser.*

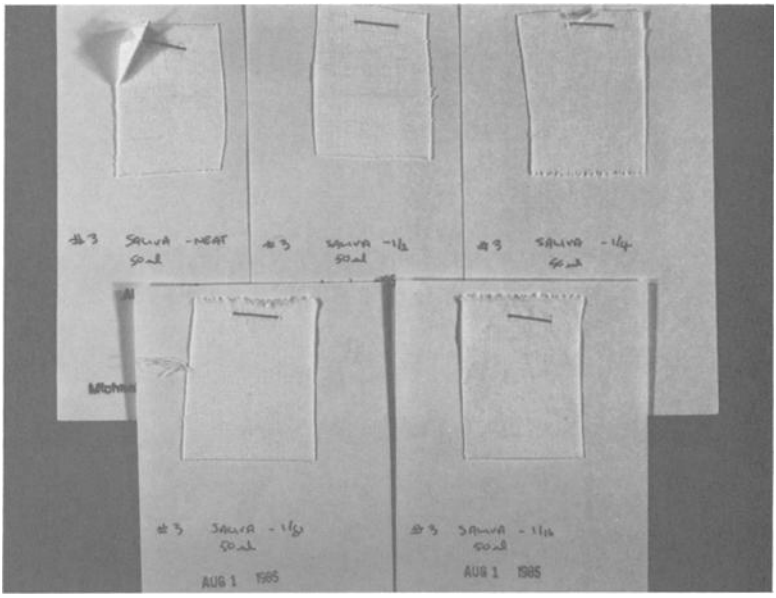

FIG. 13—*Screening sections of a white sheet, Item 3, for the presence of saliva with white light.*

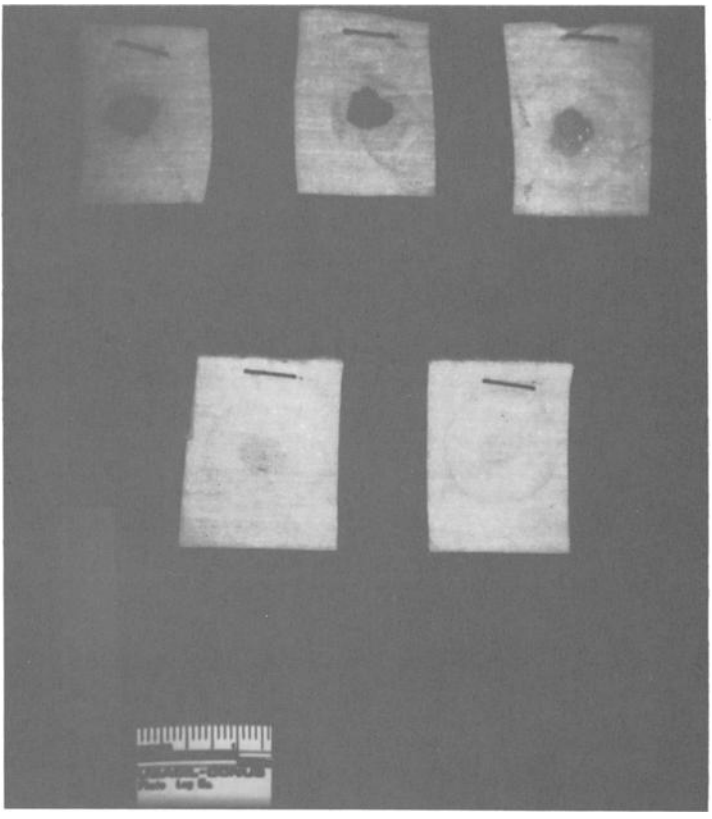

FIG. 14—*Screening sections of a white sheet, Item 3, for the presence of saliva with UV-Fotodyne.*

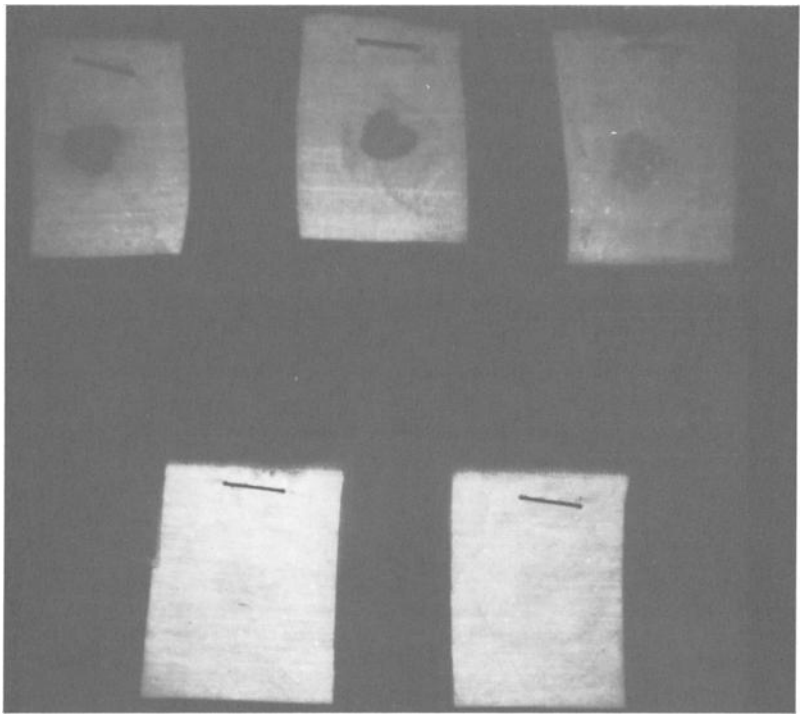

FIG. 15—*Screening sections of a white sheet. Item 3, for the presence of saliva with UV-hand held.*

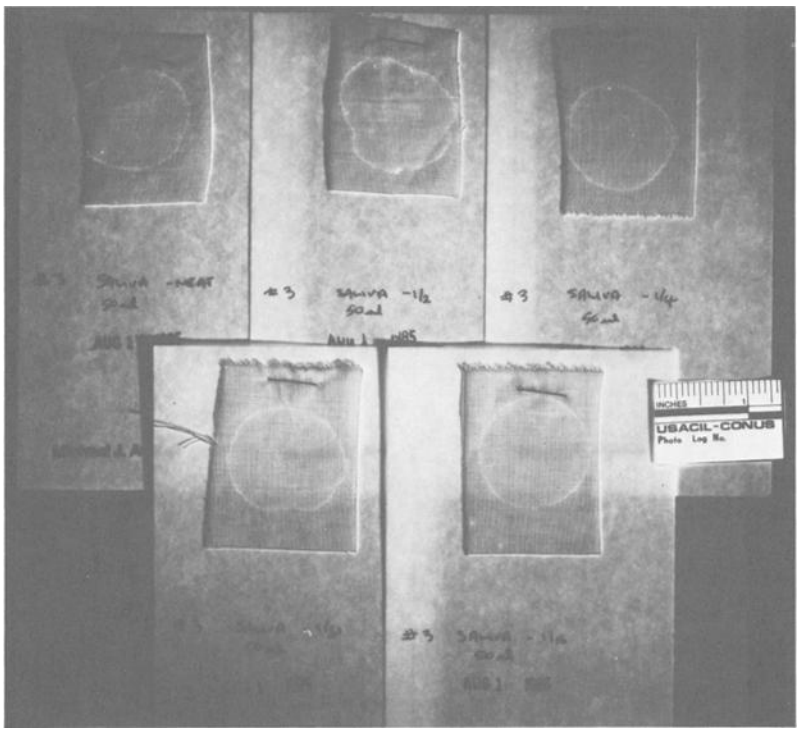

FIG. 16—*Screening sections of a white sheet, Item 3. for the presence of saliva with laser.*

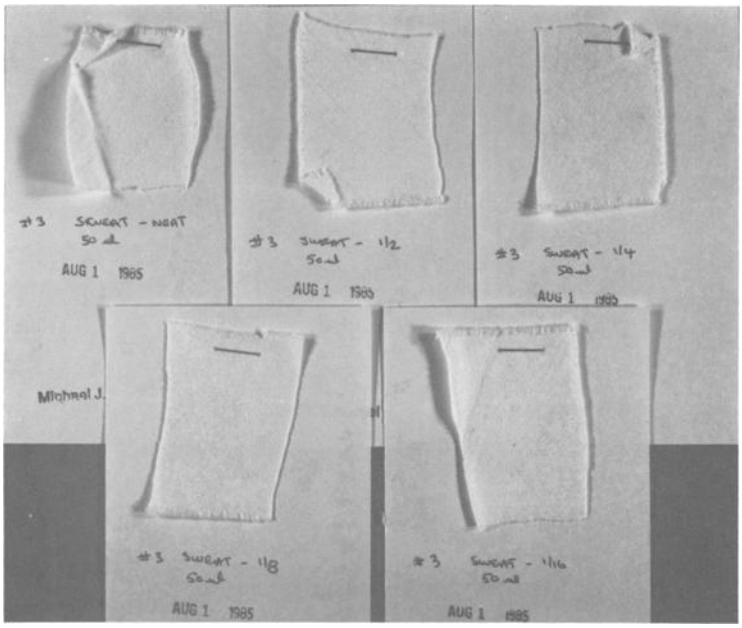

FIG. 17—*Screening sections of a white sheet, Item 3, for the presence of sweat with white light.*

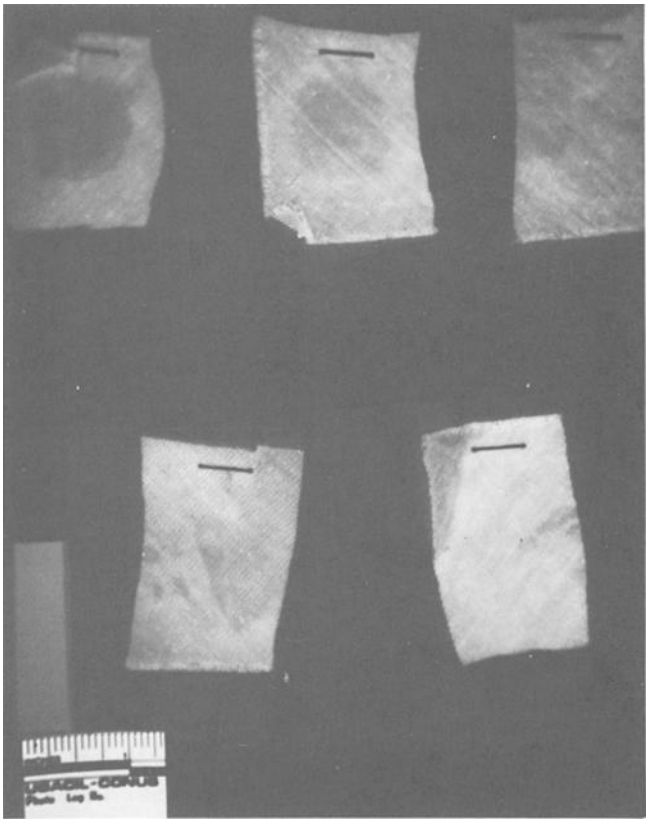

FIG. 18—*Screening sections of a white sheet, Item 3, for the presence of sweat with UV-Fotodyne.*

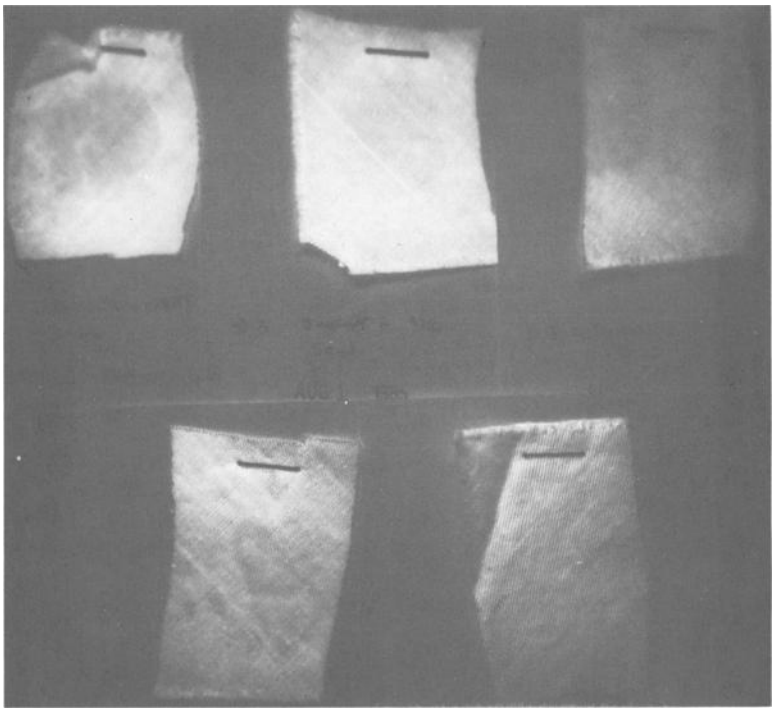

FIG. 19—*Screening sections of a white sheet. Item 3, for the presence of sweat with UV-hand held.*

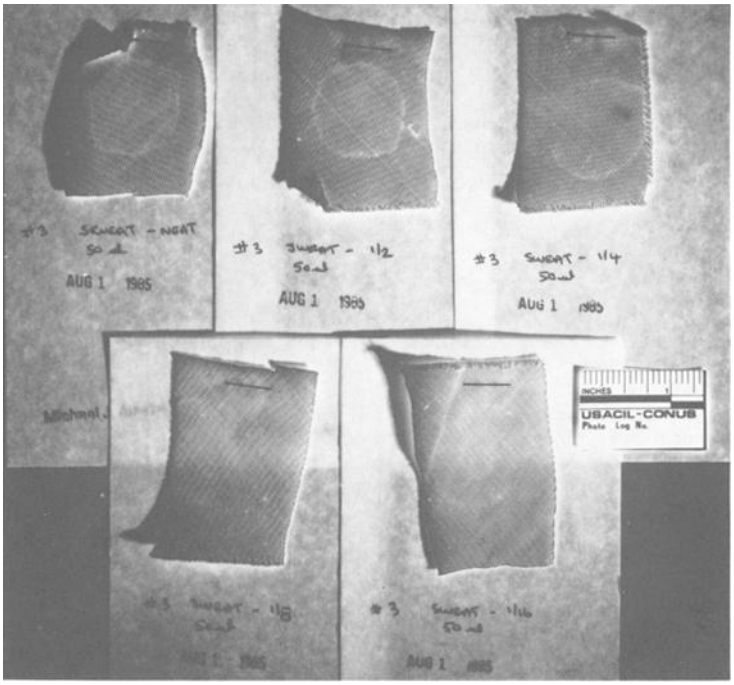

FIG. 20—*Screening sections of a white sheet, Item 3, for the presence of sweat with laser.*

were visible. The articles in Items 10, 11, and 13 produced strong fluorescent interference under UV light.

In some cases with UV fluorescence, body fluid stains appear as darkened areas on the garment. However, in the case where the garment to be screened fluoresces brightly under UV or laser light then alternate chemical screening techniques must be used for the detection of body fluid stains. The majority of the items examined in this project did not have fluorescent interferences to the screening techniques.

Conclusion

The main advantage to using the laser or UV over chemical screening techniques is the fact that they are simple, nondestructive screening techniques. The laser is a more effective screening method than UV light because of its more intense radiation and monochromatic light. The major disadvantage or prohibited factor of the laser when compared to UV light sources is its cost. The approximate cost of $35 000 for the 18-W argon ion laser as compared to $2 300 for the Fotodyne unit and $150 for the hand held UV unit places the laser out of the budget range for most crime laboratories. Other disadvantages to the high power argon ion laser are its power consumption, lack of mobility, and the plasma tube life.

However, the field of laser research has produced lower power, portable units which can fit into the operating budgets of most laboratories. These lower power lasers still provide excellent screening capabilities for body fluid stains both in the laboratory and crime scene environment. The stains that were found with the 18-W argon ion laser were also detected with a 100-mW air-cooled portable argon laser. The overall intensity was less, but the stains were still visible. The stains were reexamined several times over a two-month period and there was no noticeable difference in the intensities detected. The 100-mW air-cooled portable argon laser and fiberoptics systems would place it in the $15 000 price range.

Continuing laser research will produce more portable and lower cost units. This cost reduction will make the laser more affordable, and coupled with its superiority as a screening tool, will make the laser a dominant force in the forensic science field for years to come.

Acknowledgments

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