Fluorescein as a Field-worthy Latent Bloodstain Detection System

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Abstract: Fluorescein was considered to be the reagent with the greatest potential in the detection of latent bloodstains in the California Criminalistics Institute study by Maciari and Monk in 1991 [1]. The purpose of this study was to develop and improve the fluorescein technique into a practical field system for the detection of latent bloodstains.

Current Material Safety Data Sheets (MSDS) state fluorescein to be no more hazardous than luminol, presently in use by many investigative organizations. Also, fluorescein has a twenty year history in the medical field of ophthalmology and in Food and Drug Administration (FDA) approved for clinical application in retinal and choroidal angiography [2].

Unfortunately, unlike luminol’s single reagent application, fluorescein requires as applications of itself followed by hydrogen peroxide (H₂O₂). This double reagent application can be problematic, especially on vertical, non-porous surfaces, resulting in bloodstain pattern distortion due to major running. A commercial thickener was used to overcome this problem, allowing crime scene photographers greater opportunity to document the bloodstain patterns as evidence.

Introduction

During the 1980s, the Environmental Protection Agency became stricter with respect to toxic and/or mutagenic reagents utilized in the workplace, including those used as blood enhancement techniques. Many reagents previously used in a cavalier manner have been placed under greater scrutiny, resulting in the enforcement of more stringent

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safety guidelines. Lumino, one of the most commonly used bloodstain detection reagents, has been erronously listed as kosher for use in California in some literature. The current MSDS lists lumino as "possible carcinogenic". Hence, no such ban is in place on this reagent at this time. Nevertheless, finding a safe, reliable alternative bloodstain enhancement technique could be very valuable.

The literature has fluorescein as the reagent with the greatest promise to replace lumino [3]. Although fluorescein is also listed as "possible carcinogenic", it has been used in the clinical setting since the 1940s, and more recently has been the angiography diagnostic mainstay for vascular ophthalmic disorders requiring FDA approval for clinical use [2]. This clinical heritage and FDA sanction may put it to rest the safety issue with respect to the exposure to fluorescein.

The application of the fluorescein technique should be limited to latent stains only. The two factors which most directly bear on the effective use of this technique are either the blood concentration which has been reduced to an amount that can no longer be seen by any other means, and/or the surface on which the stain is located lacks adequate color contrast differential. If any new bloodstains are found, adequate samples should be taken for further serological testing before the fluorescein technique is employed. Due to the high alkaline nature of the fluorescein technique, any bacterial activity (i.e., ABG, anti-bacterial, secretory status) may not be possible afterwards.

The substrate surface texture upon which the stain is located also plays an important role. Porous versus non-porous surfaces, and vertical versus horizontal factors are important to the success of this technique. One proof to be problematic is the case of a non-porous substrate on a vertical surface. To alleviate this problem, a commercial thickener, Keliril RD, or talc, talcum powder, has been used. This was added to the fluorescein diluent which helped to slow the dispersal or distortion of the bloodstain pattern after the fluorescein was applied, preserving the bloodstain pattern for documentation. With the increased viscosity, it became necessary to utilize a power-spray to produce even and consistent coverage for the target area. Previously, a hand spray pump bottle was adequate for this and still is for the application of hydrogen peroxide. However, sufficient pressure is not possible for the more viscous reagent application for the fluorescein. A Low Pressure, High Volume (LPHV) spray gun was attained because of its capacity to deliver the reagent in an even, consistent manner.

In its reduced color form, the literature cites hours to change back to a sufficient color to cause no damage to the scene. For pot to take place, the effective it will be, the technique to be tried format was adopted. Currently, which would not be disapproved. Minimizing fluorescein reagent is stressed that this need interest have been preserved.

Materials and Methods

II. Delivery Device

A. LPHV Spray Gun

III. Documentation

Alternates

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allow gr
Video c
Tips, etc.)
because of its capacity to cope with the higher viscosity and ability to deliver the reagent in a controlled, contained and detailed manner.

In its reduced (colorless) state, fluorescein has a very short shelf life. The literature calls for usage within 48 hours. However, even after 23 hours the change back to its oxidation (colored) state, fluorescein was sufficient to cause problems with background fluorescence [1, 3]. Even though it is possible to prepare fluorescein in the laboratory and transport it to the crime scene, the freshness of the fluorescein reagent is the more effective it will be, hence the need for field preparation. For this technique to be truly field-worthy, a premeasured and aliquotized kit format was adopted. This required a more simplified preparation procedure, which should be within the skill level of crime scene technicians. Minimizing the equipment and skill required to prepare the fluorescein reagent is critical to its success in the field. Again, it must be stressed that this technique is to be utilized only after all other tests of interest have been performed and its only application is on latent bloodstains.

Materials and Methods

I. Reagents and Equipment
   - Fluorescein
   - Hydrogen Peroxide (H₂O₂)
   - NaOH
   - Hot Plate / Stirrer
   - Zinc Powder
   - Gloves
   - Reagent RD

II. Delivery Devices
    - Hand pump spray bottles
    - LPHV power spray gun

III. Documentation Equipment
     - Alternate light source, 450nm filter and hand 35mm SLR camera, orange barrier filter (Nikon #556)
     - Video camera
     - Tripods, etc.

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IV. Personal Safety Equipment
- Particle face mask
- Orange eye goggles
- Latex gloves and lab coat

Reagent Preparation

Fluorescein Reagent

1) A 10% sodium hydroxide solution (10.0 grams sodium hydroxide in 100ml deionized water) is prepared. A stock quantity of this solution may be kept on hand provided it is stored in a nalgene type container. Storage of strong bases in glass containers is not recommended.

2) 1.0 gram of fluorescein is measured and allowed to dissolve in 100ml of the 10% sodium hydroxide stock, using a 250ml Erlenmeyer flask with a stir bar to facilitate dissolving.

3) While the fluorescein is dissolving completely into solution, 10.0 grams of zinc powder is weighed.

4) The Erlenmeyer flask with the fluorescein/sodium hydroxide solution is placed on the hot plate/stirrer, then stirred and heated gently. The 10.0 grams of zinc powder are added to this solution, and heated and stirred to a gentle boil. (Note: the zinc will not dissolve.) This solution will lose most of its color at this time. The solution is allowed to cool and the zinc to settle.

5) The cooled solution is decanted carefully to exclude any of the undissolved zinc. A 1:20 dilution (1 part [50ml] of the decanted solution with 19 parts [950ml] deionized water/dissolved commercial thickener) is made and mixed thoroughly. This will be the fluorescein reagent solution.

6) When the commercial thickener is used, approximately 5.0 grams Kelzol RD per 1000ml DI water in the total volume of the fluorescein solution is adequate. However, the Kelzol RD dissolves slowly and should be rehydrated in advance. This may be stored frozen, then thawed and used in the fluorescein’s diluent.

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Hydrogen Peroxide Solution

1) A 10% hydrogen peroxide bottle is prepared. A stopper is added, and the bottle is shaken to ensure the mix.

2) At the time of use, shake the bottle.

Application Procedure

1) Spray bottle #1 (underneath the subject) and spray the area of uniform spray from the bottle. A few seconds are allowed for the area to become wet.

2) Spray bottle #2 (underneath the subject) to apply a mist in the pattern of the victim.

3) In accordance with light, all personnel:

4) The area of interest (450mm).

Application of the fluorescein solution to the bloodstain will develop the bloodstain pattern. At this time, some background colorization is visible in the background and false stain pattern. Using a near ultraviolet fluorescent light, the false stain pattern is visible.

Quality Control

If possible, a strongly reactions from a negative control should be examined. Also, the diluted or an actual portion should be utilized.
Hydrogen Peroxide Solution

1. A 10% hydrogen peroxide solution (1 part [100ml] 3% hydrogen peroxide with 2 parts [200ml] deionized water) is prepared. A stock solution of this may be kept in an opaque bottle and refrigerated.

2. At the time of use, 300ml is placed in the second spray bottle.

Application Procedure

1. Spray bottle #1 (ths. 1:20 fluorescein dilution) is used to spray the area of interest. The area is misted with a fine, uniform spray from a distance of 12"-18". Two applications are made in this fashion while taking care not to make the misting so heavy as to cause the reactant to run. A few seconds are allowed for the color to develop.

2. Spray bottle #2 (10% hydrogen peroxide solution) is used to apply a mist in the same manner described above.

3. In accordance with standard safety practices for using UV light, all personnel must wear safety goggles.

4. The area of interest is illuminated with near UV light (450nm).

Application of the fluorescein dilution (bottle #1) on the targeted area of bloodstain will develop a yellow colorization within a few seconds if blood is present. At this stage, the bloodstain will be apparent and some background colorization may be apparent. However, the application of the hydrogen peroxide dilution (bottle #2) will help to reduce the background and false positive reaction, thus clarifying the bloodstain pattern. Using a near UV light source the bloodstained areas will fluoresce when illuminated.

Quality Control

If possible, a strongly reactive control, a weakly reactive control and a negative control should be simultaneously performed during routine examination. Also, the substrate of the area of interest should be similar or an actual portion of the area of interest, properly labeled, should be utilized.

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46 (6), 1995/635
Limitations and Precautions

The purpose of this technique is to enhance the appearance of latent bloodstain patterns. It will not differentiate the type or source of the bloodstain, and can yield false positive results with certain substances (e.g., Fe, Cu, or soil [bacterial contamination]).

Two separate spray applications are necessary for this technique. A light and even misting yields the most successful results. However, non-porous vertical surfaces are susceptible to reagent running, which can distort or destroy any bloodstain patterns, and use of the commercial thickeners is recommended in all circumstances.

Two major safety precautions are necessary with this technique. First, 10% sodium hydroxide (strong base pH 13.5) is extremely caustic and dangerous to work with. Also, storage of strong bases in glass containers is not recommended. Second, UV light can be very hazardous, therefore the proper precautions must be implemented and safety goggles must be employed.

Results

The goal of this effort will be to focus on the following areas:

1) To simplify the chemical procedure for reducing fluorescein to fluorescam.
2) To verify aspects of Moak's results with fluorescein respectively sensitive, false positives (specificity) and establish optimum working dilutions.
3) To resolve the reagent running problem, hence expanding the documentation window.

By attaining the aforementioned goals, it is hoped that the fluorescein technique will be appreciated as a truly field worthy and practical procedure remaining within the scope and skill levels of crime scene technicians and investigators, hence resulting in an improvement in documentation.

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1) Simplification pres
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to fluorescein (reduced
gram) in 10% NaOH (i
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would not be conduct
refluxing apparatus at
appear to have reduc
solution. Once equal
technique utilized in d
defined as in "Mate

To determine if any
the simplification proc
Utilizing a common
1:105,000) two robo
received equal portion
bution, fluorescein (1.3)
1) Simplification procedure of fluorescein to fluorescin

In previous literature, fluorescein (oxidized color state) was reduced to fluorescin (reduced colorless state) via heating the fluorescein (1.0 gram) in 10% NaOH (100 ml) with 10.0 grams zinc powder. This was originally accomplished by the use of a refluxing column so as not to boil away the solution. This task may be routine for chemists, but would not be conducive to field production. The elimination of the refluxing apparatus and only bringing the solution to a boil does not appear to have reduced the effectiveness of the resulting fluorescin solution. Once established, this adaptation was used as the standard technique utilized in all subsequent experiments, which is previously delineated in “Materials and Methods”.

To determine if any sensitivity would be lost due to the adoption of the simplification procedure, the following experiment was conducted. Utilizing a common serial blood dilution (ranging from 1:1000 to 1:105,000) two racks of test tubes (12 x 75 mm) were set up. Each tube received equal portions (approximately 30 microliters) of blood dilution, fluorescin (1:3) and H₂O₂ (10%). Rack A utilized fluorescin

![Figure 1](image_url)

"The Kit"

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reduced via the refluxing apparatus, and rack B utilized fluorescein reduced via the adopted simplification technique. Negative controls (blanks) were used with each rack in which the blood dilution was substituted with deionized water. Both racks were illuminated with a Wood’s lamp (long wave UV). A plus/minus system was utilized to grade each tube for fluorescence. Equal results were achieved with each blood dilution and titrations, establishing that the sensitivity was positive at 1:105/900 for both reduction techniques.

2) Specificity (cross reactivity/false positives)

The purpose of this experiment was to determine what other common substrates may react with fluorescein yielding false positive results. All of the tested items are typically found in residential settings and may be mistaken for blood by either coloration (red/brown), or reaction to the fluorescein reagent (fluorescence). Table 1 notes the reactions observed during this test.

<table>
<thead>
<tr>
<th>Stained Items</th>
<th>Fluorescence inherent</th>
<th>UV (450 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control blood</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Saliva</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Coffee</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Tea</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gross stain</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Soil*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chocolate</td>
<td>±</td>
<td>-</td>
</tr>
<tr>
<td>Cola</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Strawberry jelly</td>
<td>±</td>
<td>-</td>
</tr>
<tr>
<td>Ketchup</td>
<td>±</td>
<td>-</td>
</tr>
<tr>
<td>Beet juice</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>Horseradish</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>Cherry-strawberry juice</td>
<td>±</td>
<td>-</td>
</tr>
<tr>
<td>Cherry-cranberry juice</td>
<td>±</td>
<td>-</td>
</tr>
</tbody>
</table>

* Soil is tested as positive in the laboratory; however, this soil yielded a negative result. Soils may vary greatly from one location to another.

J. Forensic Sci.
e88/45 (6), 1985
Also Tested

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Urine</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Steel</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steel (with rust)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Aluminum</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Copper</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

**Work dilutions**

Two separate experiments were conducted during this study to determine the optimum fluorescein dilution to use. The first experiment was similar to the procedure simplification experiment (equal portions in test tubes) of testing varying dilutions of fluorescein ranging from 1:3 to 1:150 against blood dilutions ranging from 1:6000 to 1:105,000, as noted in Table 2. Grading the reaction with a zero to five plus system resulted in the following:

**Table 2**

<table>
<thead>
<tr>
<th>Blood Dilutions x 1000</th>
<th>Prescence</th>
<th>UV (450 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>12</td>
<td></td>
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<tr>
<td>36</td>
<td>8</td>
<td></td>
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<tr>
<td>48</td>
<td>5</td>
<td></td>
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<tr>
<td>75</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>105/Blank</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Phosphorus Dilutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:3</td>
</tr>
<tr>
<td>1:50</td>
</tr>
<tr>
<td>1:100</td>
</tr>
<tr>
<td>1:150</td>
</tr>
</tbody>
</table>

The second experiment utilized varying blood dilutions absorbed into strips of blotter paper. Based on the results of the previous experiment, the fluorescein dilution range was narrowed to a scope of 1:3 to 1:50. Due to the different substrate, the blood dilution was changed to a scope of 1:10 to 1:24,000. These results are listed in Table 3. Due to the difficulty in subjectively judging the reaction of the fluorescein on this substrate, a simple plus/minus grading system was employed.
Table 3

<table>
<thead>
<tr>
<th>Blood Dilutions</th>
<th>1:1000</th>
<th>1:500</th>
<th>2000</th>
<th>2500</th>
<th>3000</th>
<th>4000</th>
<th>6000</th>
<th>12000</th>
<th>24000</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

3) Documentation Improvement

Because of the need to apply two reagents (fluorescein dilution/hydrogen peroxide), reagent running was cited as a significant shortcoming to this technique [1]. If this problem could be overcome, it would improve documentation for court presentation. A commercial thickener, such as that used in "guaranteed one-coat" house paints (Keltrol 3D) was utilized [4].

For this product to be useful in this technique, three criteria must be met: 1) it must be able to tolerate the high pH of the fluorescein without detrimental effects to its thickening properties; 2) it must not cross-react with the fluorescein, or cause any detrimental effects on the fluorescein's properties; and, 3) it must be capable of being applied in an even and consistent manner which is viscous enough to prevent reagent running and establishes bloodstain pattern stabilization long enough for documentation. All horizontal and vertical glass panels were similarly bloodstained. The before (Figure 2) and after depiction (Figure 3) demonstrate the problematic nature of double reagent systems (fluorescein/HzO2), on a non-porous substrate (glass panels). The two glass panels on the right in Figure 3 illustrate the improvement with the Keltrol 3D additive.

Early on, the manufacturer (Keltrol) assured me that pH should not be a problem, since its product was derived via fermentation and endured other rigorous demands in that process.

The cross-reactivity would be known immediately as soon as the Keltrol and fluorescein were mixed together, and a blood sensitivity

J. Forensic Sci. 640 / 46 (8), 1966
Table 1. Fluorescin Dilutions

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Fluorescin Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:10</td>
<td></td>
</tr>
<tr>
<td>1:20</td>
<td></td>
</tr>
<tr>
<td>1:40</td>
<td></td>
</tr>
<tr>
<td>1:50</td>
<td></td>
</tr>
</tbody>
</table>

Three criteria must be met for a successful fluorescein staining: 1) the solution must be kept at a pH of 7; 2) it must not cross-react with other substances; 3) the solution must be applied in an optimal ratio to provide the best sensitivity. A commercial thick-coat house paint (Keltrol RD) was used to achieve the best results. The results showed that the solution was effective and produced a clear image of the glass panes. The procedure was repeated four times to ensure accuracy. After the final image was captured, the panes were reprocessed with fluorescein without the Keltrol RD. The panes showed that the fluorescein solution was still effective without the Keltrol RD.

Figure 2
Glass panes with latent bloodstains

Figure 3
Glass panes after processed with fluorescein without Keltrol RD (left) and with Keltrol RD (right)

gradient test was conducted without any negative side effect. In fact, the sensitivity was slightly greater with the Keltool, probably due to stabilizing the reagents on the bloodstain, allowing the reagents more time to react. However, it was apparent immediately that the hand pump delivery system would not be capable of facilitating any viscosity necessary to alleviate the reagent running problem. Hence, a power spray delivery device would be necessary to satisfy the last (third) criterion. A 1.0% stock solution of Keltool RD was utilized and later diluted to the desired viscosity (0.5%) and used in the fluorescein’s diluent.

<table>
<thead>
<tr>
<th>Keltool RD Concentration</th>
<th>Delivery System</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤0.2%/volume</td>
<td>Hand pump spray bottle</td>
</tr>
<tr>
<td>0.3% - 0.6%/volume</td>
<td>Power spray device</td>
</tr>
</tbody>
</table>

Discussions and Conclusions

Considering that this technique would be one of the last procedures performed at a crime scene, it is possible for the crime scene investigators to notify the laboratory to prepare the fluorescein reagent and transport it to the crime scene. However, protection of the fluorescein reagent via darkened opaque nalgene bottles will be necessary. Exposure of the reduced (colorless state) fluorescein to sunlight or any UV light source may prematurely oxidize the fluorescein back to the oxidized (color state) fluorescein, hence undermining the effectiveness of this technique.

The “Kit” concept yields greater latitude to the investigators with investigative organizations which have geographically large jurisdictions, and where transporting reagent from the laboratory to the crime scene is not practical for time or distance reasons. Also, the simplified reduction procedure requires less equipment and skill level to prepare, hence more personnel will be capable of performing this task, perhaps yielding better utilization of crime scene staff. Conversely, this technique can only be applied once to a target area. Each time a bloodstain is sprayed, to some degree dispersion of the pattern (distortion) will occur. Also, the background fluorescence will be very high on target areas which have been previously treated.

Once it was realized that a power spray device would be necessary to facilitate the increased viscosity of the Keltool RD, a power spray manufacturer was approached. A low pressure power spray would reduce the NaOH fumes emitted from the power spray gun manufacturer and could accommodate approximately 3000 psi pressure to small areas inside a fireplace or smoke chamber. A Low Pressure spray Washington State was suggested, due to its uniformity and its ability to decrease the chance of reducing the chance of all the time the gun wash mask. Due to the cost of destroying evidence, training and experience was important.

The documentation of the Keltool RD (xanthine) reaction was an interesting procedure. A better court presentation of this test will be a matter of fact. The results presented herein will provide a more informative and effective technique. Generally, experience has shown that on various substrates a foot trail may lead to a favorable substrate.

Preliminary research has shown that the “Kit” technique to be at least as effective as the Bonder-Santon technique. The effects of environmental conditions might be exposed by the Bonder-Santon technique and appear to be consistent with the forensic analysis technology. It has been unsuccessful in many cases, and these topics are still under investigation.
negative side effects. In fact, the Keltrol, probably due to the high cost and difficulty of application, was quickly discarded.

The solution to the problem was found in the development of the chloroform based spray device. The sprayer was designed to provide a controlled, even distribution of the fluorescent reagent, allowing for a more consistent and uniform coverage on the surfaces to be examined. The device also incorporated a mechanism to regulate the flow of the reagent, ensuring precise control over the amount applied.

The pump spray bottle system was found to be particularly effective in the application of the fluorescent reagent. The device was compact and portable, allowing for easy transportation to different crime scenes. Additionally, the spray mechanism was simple and user-friendly, enabling even those with minimal experience to apply the reagent effectively.

The pump spray bottle system provided a significant improvement over the previous methods. It allowed for a more controlled application of the fluorescent reagent, resulting in a more consistent and uniform coverage on the surfaces to be examined. The device was found to be particularly effective in the detection of trace evidence, such as breath samples and fingerprints, even under challenging conditions.

The development of the pump spray bottle system was a significant milestone in the field of forensic science. It represented a major advancement in the technology used for the detection and analysis of trace evidence. The device was found to be highly effective in improving the accuracy and reliability of forensic investigations, thereby enhancing the overall effectiveness of the criminal justice system.

The pump spray bottle system has since been adopted by various law enforcement agencies and forensic laboratories, becoming a standard tool in the detection and analysis of trace evidence. Its success has led to further developments in the field, with ongoing research aimed at improving the technology and expanding its applications.

In conclusion, the development of the pump spray bottle system was a significant achievement in the field of forensic science. It has played a crucial role in improving the accuracy and reliability of forensic investigations, and its ongoing development continues to advance the field.
Documentation Guidelines

The fluorescein reaction can be documented with still photography or by video camcorder. The scene should be photographed prior to the fluorescein application. The substrate with the suspected bloodstain may then examined for any inherent fluorescence and any results should be documented by photography.

Prior to application, the fluorescein reagent should be tested on sample blood and checked for the proper reaction. The reagent should be applied on a like substrate, checked for cross-reactivity and the results documented. The reaction can be visualized and photographed with ad without the use of an orange barrier filter. Unlike lumino photog- raphy, the scene does not need to be completely darkened for the reaction to be visualized and photographed. Some ambient light in the scene will aid the photographer/investigator in later orienting the scene and its contents in the resulting documentation, and alleviates the need for fill flash photography.

Photographic documentation of the fluorescein reaction is best accomplished with a tripod-stationed 35mm camera with the aperture set at f/8 using an orange barrier filter and color print film (E1400). The exposure times should be varied bracketing between 5 and 30 seconds, depending upon the lighting conditions at the scene. Photographs may also be taken utilizing the aperture-priority automatic function of the camera, resulting in quality photographs.

The fluorescein reaction can occur for several minutes before the bloodstain pattern begins to degrade and background fluorescence becomes problematic. This allows ample time for the photographer to vary their exposures and document the scene with and without the orange barrier filter. (Note: a yellow barrier filter may also yield favorable results.)

The fluorescein latent bloodstain detection system has been adopted by the San Diego Sheriff's Regional Crime Laboratory, and is currently operational and employed when appropriate circumstances require such technique. It is the authors' hope that this study, and the improvements which it has produced, will add to the arsenal of scientific techniques necessary to discern the facts present at a crime scene.

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