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| Section F | Dichlorofluorescein | Subsection 4 |
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Name of Procedure:

Dichlorofluorescein

Suggested Uses:

Dichlorofluorescein is used to enhance bloody impressions on porous and nonporous items of evidence. This process oxidizes the hemoglobin in the blood to produce a highly fluorescent compound; therefore, it must be used with the appropriate laser and/or alternate light source.

Equipment Needed to Perform Procedures:

- A - Rubber apron and rubber gloves
- B - Magnetic stirrer, magnetic follower and magnetic retriever
- C - Glass beakers (100 ml)
- D - Four (4) glass trays
- E - Brown shatter proof storage container (four (4) liter)
- F - Three (3) glass measuring cylinders (various volumes)
- G - Camera (35 mm, 2 1/4, MP-4, CU5, TC III)
- H - Fume hood
- I - Refluxing kit
- J - Mist sprayer

Chemicals Needed For Preparation of Chemical Solution(s):

- A - 0.1 gram of 2,7 Dichlorofluorescein
- B - Ten (10) grams of Zinc Metal (mossy)
- C - Seventy (75) ml of Methanol

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D - Twenty-five (25) ml of Formic Acid

E - Three (3) ml of 30 % Hydrogen Peroxide

F - Twenty (27) ml of Ethyl Ether or Acetone

G - Twenty (20) ml of Acetone

Formula/Directions for Preparation of Chemical Solution(s):

Stock Solution:

1. Reflux the following chemical for four (4) to five (5) hours until the solution becomes colorless:
 - a. 0.1 grams of 2,7 Dichlorofluorescein
 - b. Ten (10) grams of Zinc Metal (mossy)
 - c. Seventy-five (75) ml of Methanol
 - d. Twenty-five (25) ml of Formic Acid
2. Decant the solution and store in a covered container in the refrigerator until needed.

Hydrogen Peroxide Solution:

1. Place twenty-seven (27) ml of Ethyl Ether or Acetone in a clean one-hundred (100) ml beaker with a magnetic stirrer.
2. Add three (3) ml of 30 % Hydrogen Peroxide to the Ethyl Ether or Acetone. Stir the solution until thoroughly mixed.
3. Place the solution in a mist sprayer until needed.

Working Solution:

1. Place twenty (20) ml of Acetone in a clean one-hundred (100) ml glass beaker with a magnetic stirrer.
2. Add ten (10) ml of Stock solution to the Acetone and stir until thoroughly mixed.

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3. Place the solution in a mist sprayer until needed.

Processing Procedures for Application to Item(s) of Evidence:

Prior to spraying the item of evidence with any of the solutions, the bloody impression must be dried or cured. This procedure is necessary to keep the print from dissolving when the solution is applied.

1. Carefully spray the item of evidence with the working solution. Allow the area to completely dry. Spray the item a second time again allowing the area to completely dry prior to proceeding. A fine mist is highly recommended when spraying the item of evidence.
2. After the second application of the working solution has completely dried, carefully spray the item with the Hydrogen Peroxide solution. Spray the item a second time again allowing the area to completely dry prior to proceeding. A fine mist is a **must** when spraying the item of evidence with the Hydrogen Peroxide solution.
3. Examine the item of evidence with the appropriate laser and/or alternate light source to locate the impressions (See Laser/Alternate Light Sources).

Steps to Preserve Developed Impressions:

The most appropriate methods to preserve developed impressions is through photography, using the proper techniques (See Photographic Equipment/Procedures) and/or electronically recording the impressions (See Image Processing).

The latent impressions developed with Dichlorofluorescein must be photographed immediately as exposure to air will slowly cause the development of a fluorescent background which will mask the latent impression.

Safety Concerns:

Ethyl Ether is explosive and should be substituted with Acetone whenever possible.

The toxic and carcinogenic properties of Dichlorofluorescein have not been thoroughly

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investigated. The solutions should be handled with extreme care.

All mixtures should be conducted in a fume hood with rubber gloves and aprons.

Hydrogen Peroxide will irritate the skin and eyes especially when used in conjunction with other solvents (Acetone, etc.)

Storage and Location of Chemicals and Solutions:

2,7 Dichlorofluorescein and Zinc Metal should be stored in the original shipping container until needed.

30 % Hydrogen Peroxide should be stored in the original shipping container and refrigerated until needed.

Formic Acid and Acetone should be stored in a flammable cabinet until needed.

Stock solutions should be stored in dark shatterproof containers or bottles.

Shelf Life:

Dichlorofluorescein, Zinc Metal - Indefinite

Formic Acid, Hydrogen Peroxide and Ethyl Ether - Indefinite if stored properly

Stock and Hydrogen Peroxide solutions - Indefinite if stored properly

Working Solution - Immediate use only

Other Information:

Dichlorofluorescein will fluoresce yellow in color and should not be used on items which fluoresce the same color.

Great care should be taken when applying the solutions as blood prints could be easily destroyed in this procedure.