

**Connecticut Department of Public Safety
Division of Scientific Services
Forensic Laboratory**

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Page 1 of 5

Document Title: Blood Enhancement Reagents QC

Controlled: Yes, with red stamp present

Controlled By: Quality Manager

Prepared By: _____

Date: _____

Approved By: _____

Date: _____

A. PURPOSE:

To prepare reagents for blood enhancement tests and to perform quality control on prepared reagents.

B. RESPONSIBILITY:

Forensic Science Examiners 1 and 2 in the Forensic Biology Section. Ordering information is maintained in a log book in the Forensic Biology Section.

C. SAFETY:

Use appropriate measures for the proper handling of glacial acetic acid, ethanol, collodion, diethyl ether and sodium perborate according to SOP-GL-2 (Safety Manual) and the Material Safety Data Sheets.

E. PROCEDURE:

The following reagents are prepared for use by the Connecticut State Police Major Crime Squads at crime scenes.

o-Tolidine/Collodion Reagent

1. Acetate Buffer

a. Materials:

- aa. Sodium acetate (anhydrous) 10g
- bb. Distilled water 100ml
- cc. Glacial acetic acid 86ml

b. Procedure:

- aa. Mix all materials together and place in stock bottle.
- bb. Label the stock bottle with the buffer, lot # (date of preparation), expiration date and examiner's initials.
- cc. Store up to six (6) months at 4°C and then replace.
- dd. Use the acetate buffer in the Sodium Perborate solution below.

E. 2. Sodium Perborate Solution

a. Materials:

- aa. Sodium perborate 0.20g
- bb. Acetate buffer 4ml
- cc. Plastic tubes with screw caps (15ml)

b. Procedure:

- aa. Mix all materials together and place into a plastic tube with cap.
- bb. Label the tube with the solution, lot # (date of preparation) and examiner's initials.
- cc. Make the solution up fresh for each use.
- dd. Refrigerate until needed.

3. o-Tolidine/Collodion Solution

a. Materials:

- aa. Ethanol 6ml
- bb. Collodion 15ml
- cc. Diethyl ether* 60ml
*can be substituted with 60ml of ethanol for use on horizontal surfaces.
- dd. o-tolidine 0.12g
- ee. Sprayer and glass bottle (180ml) with screw cap

b. Procedure:

- aa. Mix all materials together, place into a spray glass bottle, cap and cover with foil.
- bb. Make the solution up fresh for each use.
- cc. Discard the diethyl ether according to the manufacturer's suggested expiration date or after one (1) year from the date opened.

4. Working Spray Reagent:

a. Materials:

- aa. Sodium Perborate Solution
- bb. o-Tolidine/Collodion Solution
- cc. Controls: known blood stain and blank filter paper

b. Procedure:

- aa. Test each new batch of reagent before use according to the o -Tolidine/Collodion Reagent Log Sheet and prior to step 4.b.dd. below. Record the required information. Add a drop of the sodium perborate solution followed by a drop of the o-tolidine/collodion solution to the controls. A positive reaction occurs within seconds resulting in a color change to blue.

- E. 4. b. bb. If the appropriate results are not obtained, discard the reagent, review the procedure and make new reagent.
- cc. If the reagent is suitable for use, include the lot # (date of preparation) and examiner's initials on the spray bottle. Store the spray reagent and sodium perborate solution separately in the refrigerator until use.
- dd. Immediately before use, add 4ml of the sodium perborate solution to the o-tolidine/collodion solution and attach a sprayer.
- ee. Gently swirl the reagent until thoroughly mixed.
- ff. Spray the surface lightly.
- gg. A positive reaction occurs within seconds resulting in a color change to blue.
- hh. Prepare working spray reagent fresh for each use.
- ii. Discard any remaining working spray reagent after use.

Luminol Reagent

1. Solution I

- a. Materials:
- aa. 3-Aminophthalhydrazide ("Luminol") 0.1g
 - bb. Distilled water (dH₂O) 50ml
 - cc. Ethanol 20ml
 - dd. Spray glass bottle (180ml) with screw cap
- b. Procedure:
- aa. Mix all materials together, place into spray glass bottle, cap and cover with foil.
 - bb. Label with the solution, lot # (date of preparation) and examiner's initials.
 - cc. Make the solution fresh for each use.

2. Solution II

- a. Materials:
- aa. Sodium Carbonate 0.5g
 - bb. Sodium Perborate 0.7g
 - cc. Distilled water 30ml
 - dd. Spray glass bottle (180ml) with screw cap

- E. 3. b. Procedure:

- aa. Mix all materials together, place into spray glass bottle, cap and cover with foil.
- bb. Label with the solution, lot # (date of preparation) and examiner's initials.
- cc. Make the solution fresh for each use.

Working Spray Reagent

- a. Materials:
 - aa. Solution I
 - bb. Solution II
 - cc. Spray unit
 - dd. Controls: known blood stain and blank filter paper
- b. Procedures:
 - aa. Test each new batch of reagent before use according to the Luminol Reagent Log Sheet and prior to step 3.b.dd. below. Record the required information. Add a drop of Solution I followed by a drop of Solution II to the controls. Perform the test in complete darkness. A positive fluorescent reaction occurs within seconds and only lasts a very short period of time.
 - bb. If the appropriate results are not obtained, discard the reagent, review the procedure and make new reagent.
 - cc. If the reagent is suitable for use, store the solutions separately in the refrigerator until use.
 - dd. Immediately before use, combine solutions I and II and attach a sprayer.
 - ee. Gently swirl the reagent until thoroughly mixed.
 - ff. Perform the test in complete darkness.
 - gg. Spray surface lightly.
 - hh. A positive fluorescent reaction occurs within seconds and only lasts a very short period of time.
 - ii. Prepare working spray reagents fresh for each use.
 - jj. Discard any remaining working spray reagent after use.

F. REFERENCES:

1. Lee, Dr. Henry C. Crime Scene Investigation. Central Police University Press. Taiwan. 1994.

2. Gaensslen, R. E. , Sourcebook In Forensic Serology, Immunology, and Biochemistry , U.S. Government Printing Office, Washington D.C., 1983.
3. SOP-GL-2 (Safety Manual).
4. Material Safety Data Sheets.