

Document Title: Smear Staining Reagents QC

Controlled: Yes, with red stamp present

Controlled By: Quality Manager

Prepared By: \_\_\_\_\_

Date: \_\_\_\_\_

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Date: \_\_\_\_\_

**A. PURPOSE:**

To prepare reagents for smear staining and to perform quality control on prepared reagents and new kit lots.

**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 nuclear fast red, indigo carmine, picric acid, potassium hydroxide and DTT according to SOP-GL-2 (Safety Manual) and the Material Safety Data Sheets.

**D. DEFINITIONS:**

1. DTT: Dithiothreitol
2. KS: Kernechtrot solution
3. PICS: Picroindigocarmine solution

**E. PROCEDURE:**

1. Christmas Tree Stain

Nuclear Fast Red (KS) Solution

a. Materials:

- |     |   |       |
|-----|---|-------|
| aa. | Aluminum Sulfate Hydrated Crystal         | 15g   |
| bb. | Distilled water – hot (dH <sub>2</sub> O) | 300ml |
| cc. | Nuclear Fast Red                          | 0.3g  |
| dd. | Filter paper (P8 15.0 cm)                 |       |
| ee. | Glass bottle (stock)                      |       |
| ff. | Brown dropper bottles (30ml)              |       |

b. Procedure:

- aa. Dissolve aluminum sulfate in hot distilled water.
- bb. Add Nuclear Fast Red and stir.

- E. 1. b. cc. Allow to cool and then filter the solution twice.
- dd. Place stock solution into a glass bottle.
- ee. Place working solution into dropper bottles.

Picroindigocarmine (PICS) Solution

- a. Materials:
  - aa. Indigo Carmine 1.0g
  - bb. Picric Acid (saturated solution)\* 300ml
  - cc. Filter paper
  - dd. Glass bottle (stock)
  - ee. Brown dropper bottles (30ml)  
\*purchased from outside vendor
- b. Procedure:
  - aa. Dissolve Indigo Carmine in saturated picric acid solution.
  - bb. Filter the solution.
  - cc. Place stock solution into a glass bottle.
  - dd. Place working solution into dropper bottles.

Christmas Tree Stain Reagent

- a. Materials:
  - aa. KS solution
  - bb. PICS solution
  - cc. Known 1:500 spermic semen aliquot (thawed)
  - dd. Swab
  - ee. Glass slide
- b. Procedure:
  - aa. Collect a buccal sample on a swab and form a smear onto a glass slide.
  - bb. Place approximately 3 $\mu$ l of the 1:500 diluted semen onto the smear. Re-freeze the remaining semen aliquot.
  - cc. Dry the control smear at 37°C or over an alcohol burner for staining.
  - dd. Test each new batch of reagent before use with the control smear according to SOP-FB-13 (Identification of Spermatozoa) and the Christmas Tree Stain Reagent Log Sheet. Record the required information.

- E. 1. b. ee. If the appropriate results are not obtained, discard the reagent, review the procedure and make new reagent.
- ff. If the reagent is suitable for use, record the reagent, lot # (date of preparation), expiration date and examiner's initials on the stock bottles and the dropper bottles.
- gg. Store the stock solution in the refrigerator and the dropper bottles at room temperature.
- hh. Discard unused reagent after one (1) year.

2. Sperm Hy-Liter Stain

1X Wash Buffer

a. Materials:

- aa. 10X wash buffer (provided) 1 part
- bb. Distilled water (dH<sub>2</sub>O) 9 parts
- cc. Bottle (stock)

b. Procedure:

- aa. Dilute the 10X wash buffer 1:10 in dH<sub>2</sub>O and place into a stock bottle.
- bb. Label the stock bottle with the buffer, lot # (date of preparation), expiration date and examiner's initials. Record the required information on the Sperm Hy-Liter Stain Reagent Log Sheet.
- cc. Store and discard according to the manufacturer's instructions.

1M Potassium Hydroxide Solution

a. Materials:

- aa. Potassium Hydroxide 0.056g
- bb. Distilled water (dH<sub>2</sub>O) 1ml

b. Procedure:

- aa. Mix all materials together and use immediately for 1M DTT solution.
- bb. Discard excess potassium hydroxide solution after preparing 1M DTT solution.

E. 2. 1M DTT Solution

- a. Materials:
  - aa. DTT 0.154g
  - bb. 1M Potassium Hydroxide 0.11ml
  - cc. Distilled water 0.89ml
  - dd. pH paper (1-12 pH)
  - ee. Microcentrifuge tubes
  
- b. Procedure:
  - aa. Combine DTT, 1M Potassium Hydroxide and 0.77ml of distilled water.
  - bb. Test pH, should be approximately pH 8.
  - cc. Add 0.12mL of distilled water to final solution volume of 1ml.
  - dd. Aliquot 40µl volumes into microcentrifuge tubes labeled with the reagent.
  - ee. Store in the freezer in a zip lock bag labeled with the solution, lot # (date of preparation) and examiner's initials. Record the required information on the DTT Reagent Log Sheet.
  - ff. Discard after two (2) years.
  - hh. Any unused portion may be re-frozen one (1) additional time.
  - ii. Discard any unused portion after being thawed a second time.

#### Sperm Hy-Liter Stain Kits

- a. Test each new lot before use according to SOP-FB-13 (Identification of spermatozoa) and the Sperm Hy-Liter Stain Reagent Log Sheet. Record the required information.
- b. If the appropriate results are not obtained, review the procedure, repeat the test and replace the lot if necessary.
- b. If the lot is suitable for use, record the date received, date opened and examiner's initials on each box and the provided reagent dropper bottles and buffer bottle. Store according to the manufacturer's instructions.
- c. Kits beyond the Manufacturer's suggested expiration date may be used as long as a positive and negative control are run concurrently with the questioned sample and are working properly. If the controls do not work as expected, the lot will be removed from service.

#### **F. REFERENCES:**

1. Independent Forensics, Sperm Hy-Liter™ Technical Information and Protocol sheets.
2. Independent Forensics, Sperm Hy-Liter™ *PLUS* Technical Information and Protocol sheets.
3. Independent Forensics, Sperm Hy-Liter™ Recommended Laboratory Recipes and Procedures, p 1-12.
4. SOP-GL-2 (Safety Manual).
5. Material Safety Data Sheets.