

Approved by Director: Dr. Guy Vallaro

A. PURPOSE:

To perform quality control on new stain 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 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
 - a. This reagent is purchased from an outside vendor and is tested/used as received.
 - b. Materials:
 - aa. KS stain (provided)
 - bb. PICS stain (provided)
 - cc. Known diluted spermic semen aliquot (thawed)
 - dd. Swab
 - ee. Glass slide
 - c. Procedure:
 - aa. Collect a buccal sample on a swab and form a smear onto a glass slide.
 - bb. Place approximately 3 μ l of the known 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.

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- E. 1. c. dd. Control smears may be made in advance. Store in the freezer and replace as needed. Record appropriate information on the Body Fluid Standard Reagent Log Sheet.
- ee. Test each new lot 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.
- ff. If the appropriate results are not obtained, review the procedure, repeat the test and replace the lot if necessary.
- gg. If the lot is suitable for use, record the date received, date opened and examiner's initials on each bottle.
- hh. Store and discard according to the manufacturer's instructions.

2. Sperm Hy-Liter Stain

1X Wash Buffer

a. Materials:

- aa. 10X wash buffer (provided) 1 part
bb. Distilled water (dH₂O) 9 parts
cc. Bottle (stock)

b. Procedure:

- aa. Dilute the 10X wash buffer 1:10 in dH₂O and place into a stock bottle.
- bb. Label the stock bottle with the buffer, lot # (date of preparation), expiration date and examiner's initials. Place diluted buffer into a wash bottle labeled with the buffer, lot # (date of preparation), fill date, 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

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a. Materials:

	1ml 1M KOH	2ml 1M KOH	12ml 1M KOH
Potassium Hydroxide (KOH)	0.056g	0.112g	0.672g
Distilled water (dH ₂ O)	1ml	2ml	12ml

E. 2. 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.

1M DTT Solution

a. Materials:

	1ml 1M DTT	10ml 1M DTT	100ml 1M DTT
DTT	0.154g	1.54g	15.4g
1M Potassium Hydroxide (KOH)	0.11ml	1.1ml	11ml
Total volume of Distilled Water (dH ₂ O)	0.89ml	8.9ml	89ml

pH paper
Microcentrifuge tubes

b. Procedure:

- aa. Combine DTT, 1M KOH and dH₂O (see chart below).

For 1ml 1M DTT	For 10ml 1M DTT	For 100ml 1M DTT
Add 0.77ml dH ₂ O	Add 7.7ml dH ₂ O	Add 77ml dH ₂ O

- bb. Test pH, should be approximately pH 8.

- cc. Add dH₂O (see chart below) to final solution volume.

For 1ml 1M DTT	For 10ml 1M DTT	For 100ml 1M DTT
Add 0.12ml dH ₂ O	Add 1.2ml dH ₂ O	Add 12ml dH ₂ O

- dd. 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.

- ee. If the appropriate results are not obtained, review the procedure, repeat the test and remake the solution if necessary.

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- ff. If the lot is suitable for use, aliquot into microcentrifuge tubes labeled with the reagent and quantity.
- gg. Store in the freezer labeled with the solution, lot # (date of preparation), control date and examiner's initials. Record the required information on the DTT Reagent Log Sheet.
- hh. Discard after two (2) years.
- ii. Any unused portion may be re-frozen one (1) additional time.
- jj. Discard any unused portion after being thawed a second time.

E. 2. 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.
- c. If the lot is suitable for use, record the date received, date opened and examiner's initials on each box.
- d. Store and discard according to the manufacturer's instructions.

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™ Express Technical Information and Protocol sheets.
4. Independent Forensics, Sperm Hy-Liter™ Recommended Laboratory Recipes and Procedures, p 1-12.
5. DNA Analyst Training, Laboratory Training Manual, Protocol 2.05, Semen Stain Identification: Kernechtrot Picroindigocarmine Stain (KPIC), President's DNA Initiative, Illinois State Police.

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6. SOP-GL-2 (Safety Manual).
7. Material Safety Data Sheets.