IDENTIFICATION OF SPERMATOZOA

14.1 PURPOSE

14.1.1: To identify spermatozoa in Forensic samples.

14.1.2: To perform quality control on new stain lots.

14.2 RESPONSIBILITY

14.2.1: Forensic Science Examiners from the Division of Scientific Services who have been trained in the discipline of identifying spermatozoa according to FB SOP-26 (Training Manual and Checklist).

14.2.2: Forensic Science Examiners in the Forensic Biology Unit. Ordering information is maintained in a log book in the Forensic Biology Unit. New chemicals, reagents and kits are purchased according to GL-6 (Purchasing). For additional information, refer to the Biological Inventory in Appendix 2.

14.3 SAFETY

Use appropriate measures for the proper handling of biohazardous materials and of Picroindigocarmine, potassium hydroxide, DTT according to GL-2 (Safety Manual) and the Safety Data Sheets.

14.4 DEFINITIONS

A. KS: Kernechtrot solution
B. PICS: Picroindigocarmine solution
C. OCME: Office of the Chief Medical Examiner
D. AP: Acid Phosphatase
E. DTT: Dithiothreitol
F. RSID™: Rapid Stain Identification

14.5 TEST PROCEDURES

These staining procedures will be performed at the discretion of the examiner, with input from the Unit Lead(s), based on the submitting agency requests, case information and condition of the evidence.

A. Typically, Sperm Hy-Liter staining is used for Hospital/OCME prepared smears and Christmas Tree staining is used for extract smears.

B. Sperm searches may be conducted unstained according to FB SOP-02 (Sexual Assault Evidence Collection Kit Examination, section 2.6.F).

14.5.1: Materials

A. Hospital/OCME prepared smears
B. Extracted Samples
C. Positive AP swab
D. Micropipet and tips
E. Centrifuge tubes and spin baskets

F. Centrifuge
G. Glass slides
H. Acetone
I. Swabs
J. dH_2O
K. Blades
L. Christmas Tree Stain
    1. KS stain* (Stain A)
    2. PICS stain* (Stain B)
    3. dH_2O
    4. Ethanol
M. Microscopes
N. Sperm Hy-Liter Stain
    1. RSID™-Universal Buffer
    2. Aliquot of diluted spermic semen
    3. Swabs
    4. Known spermic semen/epithelial cell swab
    5. Known spermic semen swab
    6. Known epithelial cell swab
    7. Fixative Solution*
    8. Sample Preparation Solution*
    9. 1M DTT solution
    10. Blocking Solution*
    11. Sperm Head Staining Solution*
    12. Mounting Media*
    13. 1X Wash Buffer (from 10X Wash Buffer*)
    14. ImmEdge Pen*
    15. Sperm Hy-Liter masked slides*
    16. Cover slips
    17. Clear nail polish
O. Sperm Hy-Liter Microscope System

* provided by the manufacturer

14.5.2: Christmas Tree Stain Smear Preparation Procedure

A. Hospital/OCME prepared smears, no additional preparation needed.

B. Directly from pellet:
    1. Remove 3µl of the pellet with a micropipet onto a glass slide and form a smear.
    2. Allow the smear to dry at room temperature or 37°C.

C. Liquid sample suspected to contain semen:
   1. Place the sample into centrifuge tube(s) and centrifuge for 10 minutes. The centrifuge will be set at approximately 13,000 rpm. If necessary, decant excess liquid (supernatant) back into the original container.
   2. Remove 3µl of the pellet with a micropipet onto a glass slide and form a smear.
   3. Allow the smear to dry at room temperature or 37°C.

D. Directly from a positive acid phosphatase sample:
   1. The swab/cutting may be used to form a smear directly onto a glass slide.
   2. Allow the smear to dry at room temperature or 37°C.

14.5.3: Christmas Tree Staining Procedure

A. Cover the smear with KS stain (Stain A) and allow to stand for 20 minutes.

B. Gently rinse KS off slide with dH_2O.

C. Cover the smear with PICS stain (Stain B) and allow to stand for 15-20 seconds.

D. Gently rinse PICS off slide with ethanol* and blot dry or air dry at room temperature.
*Wash off with dH_2O if Sperm Hy-Liter procedure may be needed.

E. Examine the smear for spermatozoa and other cells under the microscope at 200X, verify under 400X if needed.

F. Record the staining procedure used on the appropriate Quality Record Worksheet.

G. Record the lot numbers of reagent(s) and solutions used on FBQR-09 (General Reagent Sheet).

H. Record the results of the samples on the appropriate Quality Record Worksheet.
   1. If an AP positive sample was used to make the smear, then record on the appropriate Quality Record Worksheet along with the result ("positive" or "no sperm noted/ identified").

   2. If no spermatozoa were noted/identified, then the sample will be extracted according to FB SOP-13 (Extraction of Samples).

14.5.4: Sperm Hy-Liter Smear Preparation Procedure

A. Hospital/OCME prepared smears

1. Prepare a sperm/epithelial cell control for Hospital/OCME prepared smear:
   a. Collect an epithelial cell sample on a swab and form a smear onto a glass slide.
   b. With a micropipet place approximately 3µl of thawed diluted spermic semen onto the smear. Re-freeze the remaining semen aliquot.
   c. Dry the control smear at room temperature or 37°C (do not apply open flame heat).
   d. Control smears may be made in advance. Store at room temperature and replace as needed. Record appropriate information on the Body Fluid Standard Reagent Log Sheet.
2. Shake the ImmEdge Pen. Then depress the tip of the ImmEdge Pen onto the inside wall of a centrifuge tube to release reagent (need ~50-100µl per smear). Pipette the reagent around the control smear and questioned smear(s), then allow to dry at room temperature or 37°C.
3. Stain the sperm/epithelial cell control smear concurrently with the questioned smears.

B. Smears made directly from the pellet

1. Prepare the extracted sample smear:
   a. Remove 3µl of the pellet with a micropipet to a glass slide (standard or Sperm Hy-Liter) and form a smear.
   b. Allow the smear to dry at room temperature or 37°C (do not apply open flame heat).
2. Prepare the sperm/epithelial cell control for smears made directly from pellet:
   a. Extract a known spermic semen/epithelial cell swab in a centrifuge tube with 350µl of RSID™-Universal Buffer.
   b. Incubate for 30 minutes in the ultrasonic bath.
   c. Place the sample into a spin basket and place back into the tube. Centrifuge for 10 minutes. The centrifuge will be set at approximately 13,000 rpm.
   d. Remove 3µl of the pellet with a micropipet to a glass slide (standard or Sperm Hy-Liter) and form a smear.
   e. Allow the smear to dry at room temperature or 37°C (do not apply open flame heat).

3. If made on a standard glass slide: Shake the ImmEdge Pen, then depress the tip of the ImmEdge Pen onto the inside wall of a centrifuge tube to release reagent (need ~50-100µl per smear). Pipette the reagent around the positive control smear and questioned smear(s), then allow to dry at room temperature or 37°C.

4. Stain the sperm/epithelial cell control smear concurrently with the questioned smears.

5. Record the type of extract smear made on the appropriate Quality Record Worksheet.

C. Smears made from re-suspended pellet

1. Prepare extracted sample smear:
   a. Remove 300µl of the supernatant, place into a new centrifuge tube and set aside (do not discard).
   b. Re-suspend the pellet in the remaining supernatant.
   c. Place 10µl of the re-suspended pellet onto the well of the Sperm Hy-Liter slide.
   d. Allow the smear to dry at room temperature or 37°C (do not apply open flame heat).

2. Prepare the sperm/epithelial cell control for smears made from re-suspended pellet:
   a. Place a known spermic semen swab into a centrifuge tube.
   b. Place a known epithelial cell swab into another centrifuge tube.
   c. Add 350µl of RSID™-Universal Buffer to each tube.
   d. Incubate for 30 minutes in the ultrasonic bath.
   e. Place the samples into spin baskets and place back into the tubes. Centrifuge for 10 minutes. The centrifuge will be set at approximately 13,000 rpm.
   f. Remove 300µl of the supernatant from each tube and discard.
   g. Re-suspend the pellets in the remaining supernatant.
   h. Place 8µl of the epithelial cell re-suspended pellet onto one (1) well of the slide and 2µl of the positive semen re-suspended pellet into the same well.
   i. Allow the smear to dry at room temperature or 37°C (do not apply open flame heat).
   j. Any remaining portion of the re-suspended pellets may be re-used. Store in the refrigerator for one (1) week and then discard.

3. Stain the sperm/epithelial cell control smear concurrently with the questioned smears.

4. Record the type of extract smear made on the appropriate Quality Record Worksheet.

14.5.5: Sperm Hy-Liter Staining Procedure

A. Record test(s) used on the appropriate Quality Record Worksheet.

B. Record reagent(s) and solutions used on FBQR-09 (General Reagent Sheet).

Type of smear	Extract smears	Hospital/OCME Prepared Smear
# of drops of reagent per smear	2-3 drops	6 – 12 drops

1. Use sufficient volume of reagent to cover sample.
2. Once the number of drops has been determined in the first step with the Fixative Solution, the same number of drops will be used for each subsequent solution (Sample Preparation Solution, Blocking Solution and Sperm Head Staining Solution).

C. Add the Fixative Solution to each smear. Incubate at room temperature for five (5) minutes.

For dense/thick smears: The smear may be incubated for ten (10) minutes with the Fixative Solution.

D. During Fixative incubation: Thaw an aliquot of 1M DTT solution. For each smear to be stained, add 5µl of 1M DTT solution per one (1) drop of Sample Preparation Solution into a centrifuge tube according to FBQR-14 (Sperm Hy-Liter Calculation Worksheet). For Anal Smears, see section 14.5.6 below.

E. Use 1X wash buffer to gently rinse each smear. Remove excess wash buffer with the corner of a paper towel.

F. Add the Sample Preparation Solution + 1M DTT solution to each smear. Incubate at room temperature for fifteen (15) minutes.

Type of smear	Extract smear	Hospital/OCME Prepared Smear
Volume of Sample Preparation Solution + 1M DTT solution	75µl - 100µl	200µl – 320µl

Use sufficient volume of Sample Preparation Solution + 1M DTT solution to cover sample. The above calculations are used as an approximation.

G. Use 1X wash buffer to gently rinse each smear. Remove excess wash buffer with the corner of a paper towel.

H. Add the Blocking Solution to each smear. Incubate at room temperature for fifteen (15) minutes.

I. Use 1X wash buffer to gently rinse each smear. Remove excess wash buffer with the corner of a paper towel.

J. Add Sperm Head Staining Solution to each smear. Incubate at room temperature for fifteen (15) minutes.

K. Use 1X wash buffer to gently rinse each smear. Remove excess wash buffer with the corner of a paper towel.

L. Add one (1) to three (3) drops of Mounting Media to each smear and cover each smear with a cover slip. Remove excess Mounting Media by placing slide between two (2) paper towels and gently pressing down to blot. Outline edge of cover slips with clear nail polish to seal.

M. Examine the smear for spermatozoa and other cells (i.e. epithial cells) with the Sperm Hy-Liter microscope manual search instructions below, using Phase Contrast, FITC fluorescence and DAPI fluorescence.

N. Place a photocopy of FBQR-14 (Sperm Hy-Liter Calculation Worksheet) into the appropriate case jacket(s). File the original in the designated notebook.

O. Record the staining procedure used on the appropriate Quality Record Worksheet.

P. Record the lot numbers of reagent(s) and solutions used on FBQR-09 (General Reagent Sheet).

Q. Record the results of the sperm/epithelial cell controls (Sperm Hy-Liter) and samples on the appropriate Quality Record Worksheet.

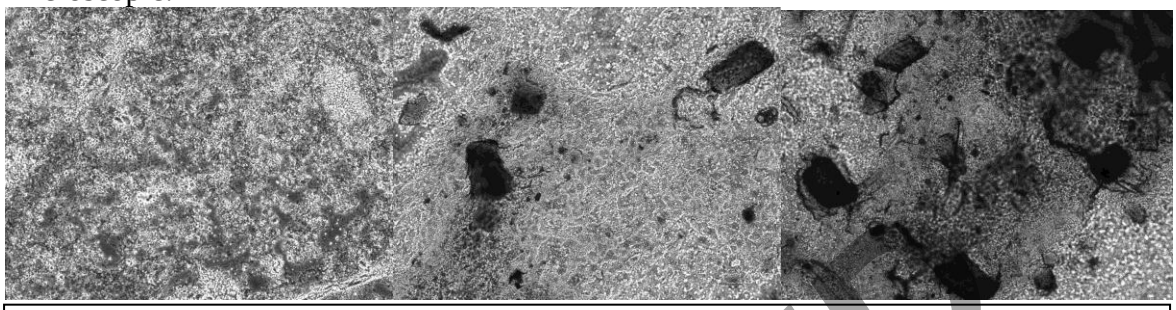
14.5.6: Sperm Hy-Liter Anal smears (Fecal Material)

A. The photos below may be used to determine the amount of fecal material on the anal smear to be examined.

Macroscopic:

No Fecal	Light Fecal	Medium Fecal	Heavy Fecal

Microscopic:



Light Fecal Material	Medium Fecal Material	Heavy Fecal Material

B. For an anal smear with light fecal material, add 50µl of 1M DTT solution (10X concentration) per one (1) drop of Sample Preparation Solution according to FBQR-14 (Sperm Hy-Liter Calculation Worksheet).

C. For an anal smear with medium fecal material, add 100µl of 1M DTT solution (20X concentration) per one (1) drop of Sample Preparation Solution according to FBQR-14 (Sperm Hy-Liter Calculation Worksheet).

D. For an anal smear with heavy fecal material, add 150µl of 1M DTT solution (30X concentration) per one (1) drop of Sample Preparation Solution according to FBQR-14 (Sperm Hy-Liter Calculation Worksheet).

E. If unable to determine which concentration of 1M DTT to use, it is suggested to use the higher concentration.

F. If the Hospital/OCME prepared anal smear is very heavy/thick with fecal material, the examiner may choose to proceed to extracting an anal swab and staining the extract smear according to the protocol for anal smears.

For additional instruction, refer to sections 14.5.7 (Manual Search for Spermatozoa) and 14.5.8 (Work Instruction for Capturing Images Using the Sperm Hy-Liter System) below.

14.5.7: Manual Search for Spermatozoa

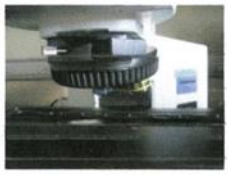
Instructions on how to use the Automated System for Manual Analysis

There are times when the user may want to quickly view a slide without performing an automated analysis. The SPERMTELLIGENCE System was designed to be used both automatically and manually.

1. Turn on Olympus BX51 microscope. Light source LED should be kept at 6-8V.

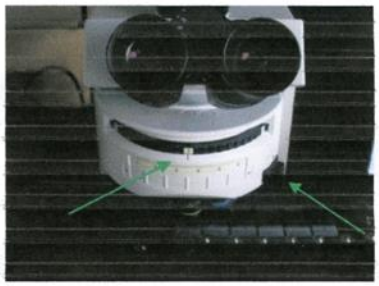
2. Turn on Proscan II controller.

3. Turn on Lumen Pro illuminator. Note: Illuminator must be on for a minimum of 15 minutes before turning off. This will save on bulb life and insure proper wavelength is optimized. Bulb is guaranteed for 2000 hours.



4. Load microscope slides. Using knurled ring on objective holder, rotate objectives toward back of microscope to allow more working room for loading slides. Slides can be loaded in any convenient position. Once slides are loaded, rotate 20X objective back into place.

5. Use joystick control to move stage and joystick focus to focus slides

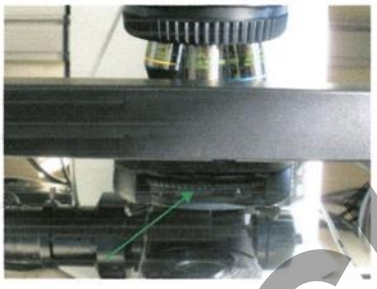


6. Rotate fluorescence turret to cube #1 for phase contrast viewing.

7. Open/close shutter for fluorescence light as needed.

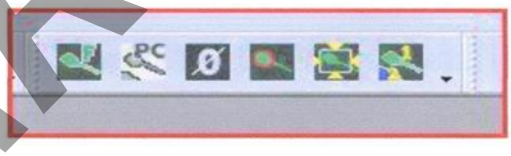
SPERMTELLIGENCE™
AUTOMATED SPERM HY-LITER™

8. Rotate bottom condenser to PH1 position which has bright fluorescent green dot for easier identification.



9. Turn on computer and monitor.

10. Double click on **SPERMTELLIGENCE** Automated Sperm Hy-liter icon to open up software.



Software Action Buttons

11. Turn on transmitted light shutter for phase contrast by clicking top button on joystick. This is the ideal way to turn on the light, because both phase contrast and fluorescence can be shuttered on at the same time. User can toggle this button on and off as needed. Clicking phase contrast icon will also shutter on the light.

12. Turn on fluorescence light by clicking fluorescence shutter icon. Verify fluorescence is working by rotating fluorescence turret to postion #2 for FITC, opening fluorescence shutter, and viewing blue fluorescence light. User can switch back to phase by rotating fluorescence turret to position #1. Fluorescence can be toggled on and off as needed using this icon. Note: this will open/close the shutter only. The Lumen Pro light source stays on until power is turned off.

13. Turn off shutters by clicking on Turn Off Light Sources icon.
14. Using joystick to move stage and joystick focus control, user can now use microscope to view slides.

15. View live images on the monitor and capture them by clicking on camera icon.

EASY 5 STEP SPERM HY-LITER Procedure for Quick Sperm Detection:

1. **Turn on microscope power/Turn on fluorescence power supply/Rotate to cube position 1.**
2. **Engage 10X or 20X phase contrast objective and PH1 position of condenser – focus on specimen.**
3. **Cover bottom transmitted light source with black dust cover (this saves on bulb life).**
4. **Rotate to cube position 2 or FITC, open shutter, refocus if necessary, scan slide to view if SPERM HEADS are present.**
5. **OPTIONAL: To confirm SPERM HEADS are present, double check by rotating to DUAL or DAPI cube, view fluorescence and phase at same time, or view specimen in phase contrast.**

14.5.8: Work Instruction for Capturing Images Using the Sperm Hy-Liter System

A. On the microscope, slide the metal rod (light path selector rod) fully out to the camera only setting.

B. On the computer, select the "setting" button. From the list, select the appropriate filter setting (FITC Full, DAPI, PC). Refocus as necessary.

C. Select the "capture" button to take photo.

D. Repeat steps 2 and 3 for each filter setting (when capturing the image in phase contrast reset the white balance by selecting the "WB" button and lighten or darken as needed with the light adjustment knob).

E. To save the images, select the "x" in the upper right hand corner of the image (this will close the image). In the window that appears, select the "pick folder" option, and in the next window select the "hard drive" option. The cabinet will appear, select the appropriate file cabinet with current cases, save the photos to the next available file folder by clicking "save" in the window that appears.

F. Go to the file cabinet, select the drawer and then the file folder with the case images. From this window you can label the file with the case # by selecting the image of the file folder with a pencil, and label the photos with the appropriate information (Case#, Sample# (Item) Investigator, Technician (if different from Investigator), Lot #, Optics, Coordinates).

G. Once finished, save by selecting the tan floppy disc icon.

H. A word document containing the images is automatically created as the images are captured.

I. Resize the images as necessary and include the case #, item #, magnification, filter set, and coordinates by each image.

J. Save this document to the desktop folder labeled "SH case photos", create a folder for the case, then save the document to that folder.

K. A printed copy of these images should be maintained in the case jacket.

14.5.9: Results and *Suggested Report Statements*

A. Christmas Tree

A stained, intact spermatozoon will have a red head and a greenish tail and neck. A stained, non-intact spermatozoon will have a red head and no tail. Epithelial cells will be green or bluish with purplish-red nuclei. Some bacteria or yeast may also stain red.

B. Sperm Hy-Liter

A stained spermatozoon will have a green head under FITC fluorescence. Epithelial cell nuclei and spermatozoon will be blue under DAPI fluorescence. Bacteria and yeast will also stain blue under DAPI fluorescence.

C. Positive

The identification of a stained, intact spermatozoon or the head portion of a non-intact spermatozoon. Sperm search results are rated as follows and recorded on the appropriate Quality Record Worksheet:

4+ - numerous sperm in every field
3+ - a few sperm in every field
2+ - sperm not in every field but easy to locate
1+ - a few sperm (coordinates are needed to relocate)

1. Kit/Extract Smears

Spermatozoa were identified upon microscopical examination of [ ].
The head portion of one (1) spermatozoon was identified upon microscopical examination of [ ].

2. AP smears

Spermatozoa were identified upon microscopical examination of a sample/portion of [ ].
The head portion of one (1) spermatozoon was identified upon microscopical examination of a sample/portion of [ ].

3. Red Blood Cells

Red blood cells were identified upon microscopical examination of [ ].

D. Negative

The absence of identifiable stained spermatozoa.

1. Kit/Extract Smears

Spermatozoa were not identified upon microscopical examination of [ ].

2. Red Blood Cells

Red blood cells were not identified upon preliminary microscopical examination of [ ].

14.6 QC Procedure

Manufacturer's expiration dates with only month and year indicated (i.e. 04/2014) expire the last day of the month noted.

14.6.1: Christmas Tree Stain

This reagent is purchased from an approved outside vendor.

Materials

A. KS stain (provided)
B. PICS stain (provided)
C. Known diluted spermic semen aliquot (thawed)
D. Swab
E. Glass slide

Procedure

A. Collect a buccal sample on a swab and form a smear onto a glass slide.

B. Place approximately 3µl of the known diluted semen onto the smear. Re-freeze the remaining semen aliquot.

C. Dry the control smear at 37°C or over an alcohol burner for staining.

D. 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.

E. Test each new lot of reagent before use with the control smear according to the test procedure and the Christmas Tree Stain Reagent Log Sheet. Record the required information.

F. If the appropriate results are not obtained, review the procedure and repeat the test. If the reagent still does not yield the appropriate results, then inform the Unit Lead, determine the root cause and correct.

G. If the lot is suitable for use, record the date received, date opened and examiner's initials on each bottle.

H. Store and discard according to the manufacturer's instructions.

14.6.2: Sperm Hy-Liter 1M Potassium Hydroxide Solution

Materials

	1ml 1M KOH	2ml 1M KOH	12ml 1M KOH
A. Potassium Hydroxide (KOH)	0.056g	0.112g	0.672g
B. dH_2O	1ml	2ml	12ml

Procedure

A. Mix all materials together and use immediately for 1M DTT solution.

B. Discard excess potassium hydroxide solution after preparing 1M DTT solution.

14.6.3: Sperm Hy-Liter 1M DTT Solution

Materials

	1ml 1M DTT	10ml 1M DTT	100ml 1M DTT
A. DTT	0.154g	1.54g	15.4g
B. 1M Potassium Hydroxide (KOH)	0.11ml	1.1ml	11ml
C. Total volume of dH_2O	0.89ml	8.9ml	89ml

D. pH paper

E. Centrifuge tubes

Procedure

A. Combine DTT, 1M KOH and dH_2O (see chart below).

For 1ml 1M DTT	For 10ml 1M DTT	For 100ml 1M DTT
Add 0.77ml dH_2O	Add 7.7ml dH_2O	Add 77ml dH_2O

B. Test pH, should be approximately pH 8.

C. Add dH_2O (see chart below) to final solution volume.

For 1ml 1M DTT	For 10ml 1M DTT	For 100ml 1M DTT
Add 0.12ml dH_2O	Add 1.2ml dH_2O	Add 12ml dH_2O

D. Test each new lot before use according to the test procedure and the Sperm Hy-Liter Stain Reagent Log Sheet. Record the required information.

E. If the appropriate results are not obtained, review the procedure and repeat the test. If the solution still does not yield the appropriate results, then inform the Unit Lead, determine the root cause and correct.

F. If the lot is suitable for use, aliquot into centrifuge tubes labeled with the reagent and quantity.

G. 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.

H. Discard after two (2) years.

I. Any unused portion may be re-frozen one (1) additional time.

J. Discard any unused portion after being thawed a second time.

14.6.4: Sperm Hy-Liter 1X Wash Buffer

Materials

A. 10X wash buffer (provided) 1 part
B. dH_2O 9 parts
C. Bottle (stock)

Procedure

A. Dilute the 10X wash buffer 1:10 in dH_2O and place into a stock bottle.

B. 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.

C. Store and discard according to the manufacturer's instructions.

14.6.5: Sperm Hy-Liter Stain Kits

A. Test each new lot before use according to the test procedure and the Sperm Hy-Liter Stain Reagent Log Sheet. Record the required information.

B. If the appropriate results are not obtained, review the procedure and repeat the test. If the reagent still does not yield the appropriate results, then inform the Unit Lead, determine the root cause and correct.

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.

14.7 REFERENCES

A. Independent Forensics, Sperm Hy-Liter™ Technical Information and Protocol sheets.
B. Independent Forensics, Sperm Hy-Liter™ PLUS Technical Information and Protocol sheets.
C. Independent Forensics, Sperm Hy-Liter™ Express Technical Information and Protocol sheets.
D. Independent Forensics, Sperm Hy-Liter™ Recommended Laboratory Recipes and Procedures, p 1-12.
E. DNA Analyst Training, Laboratory Training Manual, Protocol 2.05, Semen Stain Identification: Kernechtrot Picroindigocarmine Stain (KPIC), President's DNA Initiative, Illinois State Police.
F. Jennifer Old Ph.D., Brett A. Schweers Ph.D., Pravat Boonalangoor Ph.D. and Karl Reich Ph.D, Developemental Validation of Sperm Hy-Liter™ A Specific, Sensitive and Confirmatory Microscopic Sceening Method for the Detection of Human Sperm from Sexual Assault Evidence, p 34-51.
G. Jennifer Old Ph.D., Dina Mattes, Pravat Boonalangoor Ph.D. and Karl Reich Ph.D, Development Validation of Spermtelligence™, Software Aided Identification of Human Sperm using Sperm Hy- Liter™ Stained Slides, p 2-13.
H. Jennifer Old, Chris Martersteck and Anna Kalinina, Developmental Validation of Sperm Hy-Liter ExpressTM, p 1-11.
I. Connecticut State Forensic Science Laboratory, Sperm Hy-Liter Internal Validation, 2010.
J. Connecticut State Forensic Science Laboratory, Sperm Hy-Liter Express and Fecal Material Internal Validation, 2012.
K. Gaensslen, R.E., Ph.D., Sourcebook in Forensic Serology, Immunology, and Biochemistry, Detection and Identification of Spermatozoa, 1983, p 150-152.
L. GL-2 (Safety Manual)
M. GL-6 (Purchasing)
N. Safety Data Sheets