

**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
- G. PC: Phase Contrast

**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<sub>2</sub>O
  - K. Blades
  - L. Christmas Tree Stain
    - 1. KS stain\* (Stain A)
    - 2. PICS stain\* (Stain B)
    - 3. dH<sub>2</sub>O
    - 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<sub>2</sub>O.
- 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<sub>2</sub>O 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. Agitate dilute spermic semen and using a micropipet place approximately 3µl onto the smear.
    - 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 the 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. epithelial cells) according to section 14.5.7 below, using Phase Contrast, FITC fluorescence and DAPI fluorescence. When necessary, capture images according to section 14.5.8.
- 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

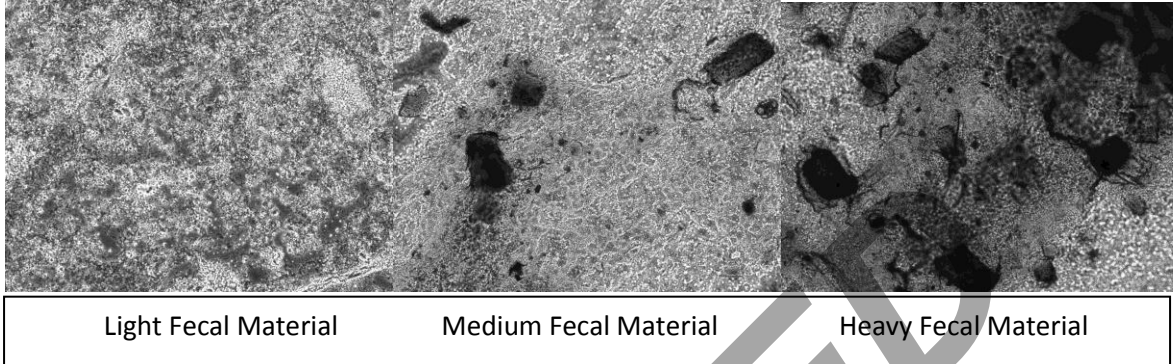
Light Fecal

Medium Fecal

Heavy Fecal

*Approved by Director: Dr. Guy Vallaro*

Microscopic:



- 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.

#### **14.5.7: Conducting Sperm Searches Using the Sperm Hy-Liter Systems**

- A. Sperm searches using the Sperm Hy-Liter Olympus microscope
  1. Turn on the light source, microscope, computer, and Proscan II controller (note that the light source should be on for 15 minutes before turning off - this will conserve bulb life and ensure proper wavelength is optimized).



2. Open the Spermtelligence software. Click on the Fluorescence and the Phase contrast icons as needed to switch between microscope settings (the transmitted light may also be turned on/off using the button on the top of the joystick - by this method both PC and fluorescence may be shuttered at the same time).
  3. Load smear onto stage.
  4. Using the 200x objective and the phase contrast setting (fluorescent shutter closed, filter in #1 position, transmitted light source uncovered, setting with 1 dot on the condenser) check that the smear is in focus.
  5. Search the smear using the fluorescent settings (transmitted light source covered, fluorescent shutter open, filter in the #2 position for FITC, #3 position for dual, and #4 position for DAPI) at 200x magnification (setting with 1 dot on the condenser).
  6. If a magnification of 400x is used, the condenser setting with the 2 dots should be used.
- B. Sperm searches using the Sperm Hy-Liter Zeiss microscope
1. Turn on the light source, microscope, and computer (note that the light source should be left on for at least 5 minutes before turning off - this will conserve bulb life and ensure proper wavelength is optimized).
  2. Load smear onto stage.
  3. Using the 200x objective and the phase contrast setting (fluorescent shutter closed, filter in #1 position, transmitted light source uncovered, setting 2 on the condenser) check that the smear is in focus.
  4. Search the smear using the fluorescent settings (transmitted light source covered, fluorescent shutter open, filter in the #2 position for FITC, #3 position for dual, and #4 position for DAPI) at 200x magnification (setting 2 on the condenser).
  5. If a magnification of 400x is used, no adjustments to the condenser are needed.

- C. For additional information, see appropriate Sperm Hy-Liter user manual.

#### **14.5.8: Capturing Images Using the Sperm Hy-Liter Systems**

A. Capturing images using the Sperm Hy-Liter Olympus microscope system

1. On the microscope, slide the metal rod (light path selector rod) fully out to the camera only setting.
2. On the computer, select the “setting” button. From the list, select the appropriate filter setting (FITC Full, DAPI, PC). Refocus as necessary.
3. Select the “capture” button to take photo.
4. 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).
5. 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.
6. 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).
7. Once finished, save by selecting the tan floppy disc icon.
8. A word document containing the images is automatically created as the images are captured.
9. Resize the images as necessary and include the case #, item #, magnification, filter set, and coordinates by each image.
10. Save this document to the desktop folder labeled “SH case photos”, create a folder

for the case, then save the document to that folder.

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

**B. Capturing images using the Sperm Hy-Liter Zeiss microscope system**

1. Open the Sperm Capture software.
2. On the microscope, slide the metal rod (beam splitter) fully out to the camera only setting. Refocus image as necessary.
3. On the computer, select the “file” menu. Select “load camera settings”. From the list, select the appropriate filter setting (FITC, DAPI, Dual, PC - if using PC, perform white balance by clicking the global white balance icon). Refocus as necessary.
4. Capture image by selecting the “capture and display image” icon (camera icon). Captured image will appear on desktop.
5. Once the image has been captured, close fluorescence shutter to prevent photo-bleaching of smear.
6. Save image to an appropriate folder (click file, then save as to name and save image. If the close option is selected prior to saving, there will be no dialogue box warning that the image has not been saved).
7. Folders may be created as needed for images.
8. Although there are pre-set capture settings for each filter choice, manual adjustments may be made to any settings as needed (this includes but is not limited to brightness, contrast, exposure, gain (color correction), aperture size, etc.)
9. A printed copy of these images should be maintained in the case jacket.

**C. For additional information, see appropriate Sperm Hy-Liter user manual.**

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**14.5.9: Results and Suggested Report Statements**

**State of Connecticut Department of Emergency Services and Public Protection  
Division of Scientific Services**

*Documents outside of Qualtrax are considered uncontrolled.*

- 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 sperm - head portion or intact (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.
- 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 <sub>2</sub> O	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 <sub>2</sub> O	0.89ml	8.9ml	89ml
D. pH paper			
E. Centrifuge tubes			

### Procedure

- A. Combine DTT, 1M KOH and dH<sub>2</sub>O (see chart below).

For 1ml 1M DTT	For 10ml 1M DTT	For 100ml 1M DTT
Add 0.77ml dH <sub>2</sub> O	Add 7.7ml dH <sub>2</sub> O	Add 77ml dH <sub>2</sub> O

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

- C. Add dH<sub>2</sub>O (see chart below) to final solution volume.

For 1ml 1M DTT	For 10ml 1M DTT	For 100ml 1M DTT
Add 0.12ml dH <sub>2</sub> O	Add 1.2ml dH <sub>2</sub> O	Add 12ml dH <sub>2</sub> O

- 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<sub>2</sub>O 9 parts
- C. Bottle (stock)

##### **Procedure**

- A. Dilute the 10X wash buffer 1:10 in dH<sub>2</sub>O 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**

*Approved by Director: Dr. Guy Vallaro*

- 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, Developmental Validation of Sperm Hy-Liter™ A Specific, Sensitive and Confirmatory Microscopic Screening 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 Express™, 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