# CHRISTMAS TREE STAINING OF SLIDES

## *Background*

This protocol outlines a confirmatory test for sperm. The presence of semen in questioned specimens can be confirmed by the identification of one or more spermatozoa and a positive ABAcard P30 test. Spermatozoa are identified microscopically using a standard or phase-contrast microscope.

## *Summary of the Procedure*

Slides may be stained using the Christmas Tree Staining Method so that spermatozoa may be more easily visualized under the microscope. Extracts of samples are prepared and heat fixed to a microscope slide. The slide is covered with red stain and then placed in a humidity chamber. The slide is removed from the humidity chamber, rinsed with water, and then covered in green stain. The slide is again rinsed, this time with ethyl alcohol and air dried. The identified spermatozoa can be photographed.

## *Sample Handling*

**Note: Forensic samples may be in limited supply. Retain sufficient sample for replicate analysis.**

Label all samples with complete identifying information.

All biological samples and DNA must be treated as potentially infectious. Appropriate sample handling and disposal techniques should be followed. See:

- ***Safety Manual***, *Universal Precautions*
- ***Quality Assurance Manual***, *General Sample Control, Forensic Sample Control, and Forensic Sample Preservation Policy*
- ***Analytical Procedures Manual***, *Forensic Evidence Handling*

## *Reagents and Materials*

MBG Water
Phosphate Buffered Saline (PBS)
Nuclear Fast Red Stain
Green Picroindigocarmine Stain
100% ethanol
Cytoseal

Lab Coat
Kimwipes
Gloves
Protective Eyewear
Sample Handling Tools (Scissors, forceps, sterile wood applicators)
Clear microcentrifuge tubes
Microcentrifuge
Pipette
Nutator
Glass microscope slide
Glass cover slip
Humidity chamber (devised by placing wet paper towels in a tightly closed plastic container)
Microscope

## *Reagents and Materials – Storage and Handling*

All reagents and materials are to be kept under sterile conditions. Store all reagents according to manufacturer's recommendations.

Do not use reagents beyond the listed expiration dates. Date and initial all reagents when put in use. Record in **Reagent Log.**

## *Quality Control*

Use of the **Evidence Examination Worksheet** or other appropriate worksheet is required for documentation. All information must be completed.

All results must be verified by a second qualified analyst. If a second qualified analyst is unavailable photo documentation of the result is acceptable

## *Procedure*

Sexual assault evidence specimens may be collected as physiological fluids on swabs or as stains on fabric.

### *Preparing Extracts*

1. Allow the sample to warm to room temperature.

2. If sample has been extracted for p30 then proceed to step #3. To extract a specimen from a stain or a swab:
    a. Make a cutting of the stained material.
    b. Soak the cutting for at least 30minutes in ~300µl of PBS (or Molecular Biology Grade Water can be used with approval) at RT on a nutator.
    c. Twirl the swab or fabric with a sterile applicator stick for several seconds or a light vortex to agitate the cells off the substrate. Transfer the cutting into a spin basket. Centrifuge the sample for 5 minutes at 13,200 rpm.
    d. Without disturbing the pellet, remove all but ~50µL of the supernatant using a sterile pipette.
    e. Lightly vortex (several seconds) the 50uL to resuspend the pellet in solution.

**Note: The pellet may contain both epithelial cells and sperm cells. An ABAcard® p30 test may be done on the supernatant prior to discard.**

3. Remove 5-10µL (or about 10%) of the resuspended sample using a sterile disposable pipette tip and spot the sample in a pre-marked area of the slide.

4. Label the slide with the sample ID/date/initials.

### *Christmas Tree Staining*

1. Fix cells to the microscope slide by incubating on a slide warmer until dry.

2. Let the slide cool to room temperature. Stain cells with 1 to 2 drops of Nuclear Fast Red stain in a humidity chamber for 5-15 minutes. Wash the slide gently with deionized water until the Nuclear Fast Red stain washes off, about 5 seconds.

    (A humidity chamber may be devised by placing wet paper towels in a tightly closed plastic container. Set the slide on top of the wet paper towels.)

3. Stain the slide with 1 drop of Picroindigocarmine Stain (green) for 5-15 seconds.

4. Rinse the slide with ethanol then dry the slide.

5. Add 1 drop of Cytoseal and cover with a cover slip.

## *Results and Conclusions*

Report conclusions related to the identification or lack of identification of sperm.

The epithelial cells will stain green with red nuclei. The sperm cells will stain red with green tails. The sperm head stains differentially such that the acrosomal cap stains pink and the sperm base stains red. If epithelial cells are detected, continue on with the **Differential Extraction of Semen Stains** procedure.

Positive identification of spermatozoa requires the identification of one or more spermatozoa at 400x or 1000x.

Below is a grading scale for spermatozoa and epithelial cells:

0: None
1: Few (≤5 cells) [Note if only one sperm or Ecell]
2: Some (~10 cells))
3: Moderate
4: Many (Sperm or Ecell in every field)
INC: Inconclusive

## *Comments on Storage*

Store the slide at room temperature in a suitable container.

## *References*

Criminalistics An Introduction to Forensic Science; Edition 6; Saferstein, Richard (p. 389-393)
Techniques of Crime Scene Investigation; Edition 5; Fisher, Barry (p. 346-352)