

SER: Spermatozoa - Microscopic Examination

Principle

The cellular component of semen is spermatozoa. The microscopic identification of spermatozoa indicates the presence of semen.

Christmas Tree stain is a differential biological stain used to differentiate parts of cells and can be used to visualize spermatozoa. The two-reagent procedure stains nuclear material (sperm heads) red and epithelial membranes (sperm tails) green.

Equipment and supplies

This procedure uses the following laboratory equipment and supplies:

- compound microscope
 - centrifuge
 - oven
 - microscope slides
 - pipette
 - pipette tips
 - microcentrifuge tubes
 - ethanol
 - cover slips
 - mounting medium
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Reagents

This procedure uses the following reagents:

- SERI Christmas Tree Stain Solution A
- SERI Christmas Tree Stain Solution B

Reagents can be stored at room temperature.

Record lot number and expiration date in examination documentation.

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Sample preparation

The stain cutting (approximately 5 mm x 5 mm) or swab (approximately one quarter) should be extracted in 50 µl saline for a minimum of 30 minutes and all cellular material pelleted by centrifugation.

Slides provided by the hospital can also be stained using this procedure.

Procedure

Use the following procedure to prepare and stain slides.

Step	Action
1	Dry pelleted cellular material on a microscope slide for approximately 30 minutes in a 60° C oven.
2	Stain with <i>Christmas Tree Stain A</i> for 5 to 30 minutes. Rinse with deionized water.
3	Stain with <i>Christmas Tree Stain B</i> for 5 to 60 seconds. Rinse with ethanol.
4	Allow the slide to dry. Add mounting medium and coverslip.
5	<p>Examine slide with microscope at 200x magnification. A phase contrast optical accessory can enhance visualization.</p> <ul style="list-style-type: none">• With bright field microscopy, sperm heads appear red; the acrosomal region of the head appears lighter than other portions of the head.<ul style="list-style-type: none">– Epithelial cells stain green, with the nuclei inside the epithelial cells appearing purple.• The sperm tail, if present, appears green.<ul style="list-style-type: none">– With phase contrast microscopy, the acrosomal region appears darker than the rest of the sperm head. <p>NOTE: If the presence of debris or other cellular material interferes with the identification of spermatozoa, the <i>DNA: Differential Extraction</i> procedure may be used to clean up the sample. Prepare a new slide from the differentially extracted sample.</p>
6	Record how many sperm are present using the grading scale on the <i>Sexual Assault Examination Worksheet</i> .

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Interpretation If only a single spermatozoon is detected on a slide, the analyst may have another qualified analyst verify the presence of the single spermatozoon.

The detection of a single spermatozoon should be appropriately qualified in the report.
