

## SER: Spermatozoa - Microscopic Examination

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### 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.

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### 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 records.

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## SER: Spermatozoa - Microscopic Examination, Continued

### 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"><li>• 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"><li>– Epithelial cells stain green, with the nuclei inside the epithelial cells appearing purple.</li></ul></li><li>• The sperm tail, if present, appears green.<ul style="list-style-type: none"><li>– With phase contrast microscopy, the acrosomal region appears darker than the rest of the sperm head.</li></ul></li></ul> <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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## SER: Spermatozoa - Microscopic Examination, Continued

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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.

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