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/heat block
- microscope slides
- pipette
- pipette tips
- microcentrifuge tubes
- ethanol
- cover slips
- mounting medium

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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Sample preparation

Use the following procedure to prepare the sample for slide examination.

Step	Action
1	Place one whole swab or stain cutting into a sterile microcentrifuge tube.
2	Add 300 µL of TE Buffer and incubate at room temperature for 30-60 minutes on the ThermoMixer.
3	Transfer 270 µl of the supernatant to a new tube.
4	Re-suspend the cellular pellet in the remaining 30 µl of supernatant.
5	Remove 2 µl of the re-suspended sample for slide examination.

Procedure

Use the following procedure to prepare and stain slides.

Step	Action
1	Dry 2 µl of re-suspended sample on a microscope slide for a minimum of 10 minutes in a 60° oven or on a heat block.
2	Stain with *Christmas Tree Stain A* for 5 to 30 minutes. Rinse with deionized water.
3	Stain with *Christmas Tree Stain B* for 5 to 60 seconds. Rinse with ethanol.
4	Allow the slide to dry. Add mounting medium and a coverslip.

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Procedure (continued)

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>5</td><td>Examine the slide with a microscope at 200x magnification. A phase contrast optical accessory can enhance visualization.

• With bright field microscopy, sperm heads appear red; the acrosomal region of the head appears lighter than other portions of the head.
– Epithelial cells stain green, with the nuclei inside the epithelial cells appearing purple.
• The sperm tail, if present, appears green.
– With phase contrast microscopy, the acrosomal region appears darker than the rest of the sperm head.

NOTE: If the presence of debris or other cellular material interferes with the identification of spermatozoa, the analyst may clean up the sample and prepare a new slide.

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Transfer 2 µl of the re-suspended sample to a new tube.</td></tr>
<tr><td>2</td><td>Add 300 µl of Digest Buffer and 15 µl of Proteinase K Solution.</td></tr>
<tr><td>3</td><td>Incubate for approximately 30 minutes at 56±1°C.</td></tr>
<tr><td>4</td><td>Pellet the cellular material by centrifugation.</td></tr>
<tr><td>5</td><td>Transfer the cellular pellet to a new slide, dry, and stain.</td></tr>
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<tr><td>6</td><td>Record how many sperm are present using the grading scale on the Sexual Assault Examination Worksheet.</td></tr>
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