



Forensic Biology Section

Microscopic Examination of Cell Debris

1. **Scope:**

- 1.1. An aliquot of cellular debris is placed on a slide, differentially stained with Christmas Tree Stain and observed under a light microscope in order to visually identify spermatozoa, epithelial cells, and leukocytes.
- 1.2. Microscopic identification of sperm indicates the presence of semen. Nuclear material stains red and cytoplasm stains green.

2. **Safety:**

- 2.1. Treat all biological specimens as potentially infectious. Gloves and a laboratory coat must be worn at all times. Follow Universal Precautions.
- 2.2. D.P.X. slide mounting media should be used in total-exhaust biosafety hoods or fume hoods when possible.

3. **Specimen:**

- 3.1. Any stain, swab, or cell pellet that is suspected of containing sperm cells.

4. **Reagents and Special Supplies:**

- XMAS Stain-A (Nuclear Fast Red)
- XMAS Stain-B (Picroindigocarmine)
- Molecular biology grade water
- Ethanol
- D.P.X. mountant
- Glass slides
- Cover slips

5. **Christmas Tree Stain:**

- 5.1. The pellet from cell debris extraction is re-suspended in a small volume (e.g. 50 microliters) and a one-tenth volume aliquot is transferred to a properly labeled glass microscope slide for staining and microscopic examination.
- 5.2. Fix cells to a microscope slide on a hot plate until the slide is very warm to the touch. Alternatively, simply allow the slide to thoroughly air dry.
 - 5.2.1. **NOTE:** Etching the underside of the slide to indicate the location of the spotted material can make it easier to locate the general vicinity of cells on the slide.
- 5.3. Gently rinse the slide with molecular biology grade water to remove salts but avoid washing cells off the slide.



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- 5.4. Flood slide with Xmas Stain-A where cells were spotted and incubate for at least 5 minutes. Re-wet slide with additional Xmas Stain-A if necessary.
- 5.5. Gently rinse the slide again with molecular biology grade water.
- 5.6. Flood slide where cells were spotted with Xmas Stain-B for at least 1-3 minutes.
- 5.7. Gently rinse the slide with ethanol.
- 5.8. Allow slide to completely air dry.
- 5.9. Apply one drop of D.P.X. mountant to stained debris and place a cover slip over area.
- 5.10. Observe at 400 X on light microscope and record observations in terms of approximate number of sperm cells and epithelial cells "per high power field" or "on slide".

6. **Interpretation:**

- 6.1. Human sperm cells have a distinctive size and morphology and will stain differentially in a characteristic fashion. While there is slight variation, sperm cells appear clear or green at the tip of the head, intensely red stained at the base of the head, and green at the tail (tail may not be attached).
- 6.2. Epithelial cells have a large green cytoplasm surrounding a faintly red nucleus.
- 6.3. Leukocytes (white blood cells) appear pink with a single, large nuclei or multiple, small nuclei.
- 6.4. Yeast cells stain a uniform red throughout the cell and extend into polyp-like structures, which may be observed occasionally.

7. **Storage:**

- 7.1. Slides mounted with D.P.X. mountant are protected from damage and/or alteration of the sample.
- 7.2. Slides should be stored in a commercially available slide holder at room temperature.
- 7.3. Slide holders should be properly labeled with the case numbers they contain.