

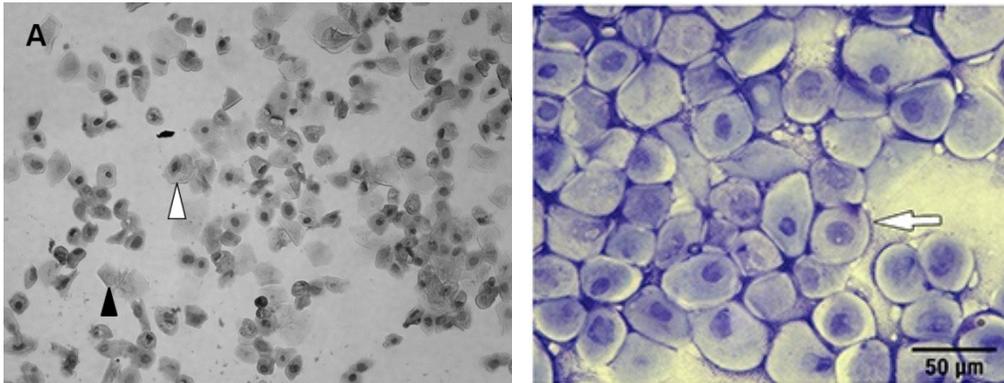
## FLSC Standard Operating Procedure for Staining and Reading Vaginal Cytology

### Purpose

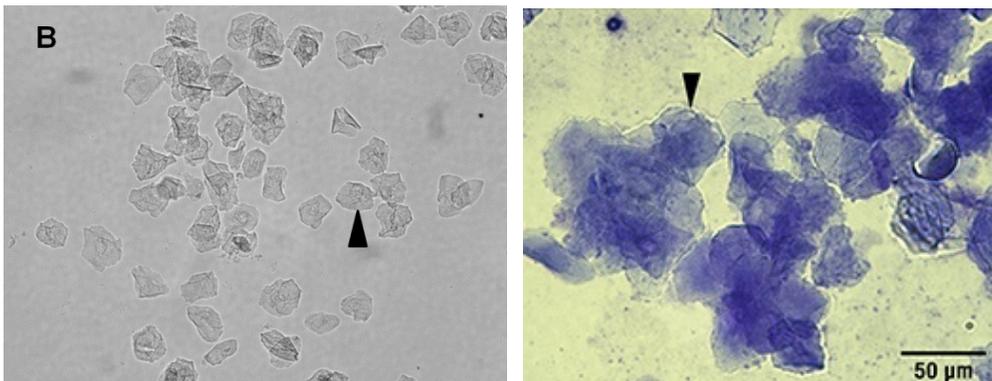
To assess the estrous cycle it is necessary to understand the stages and their cytological appearance. An evaluation of the estrous cycle is helped or hindered by the quality of the slide and the availability of a good quality microscope.

### Estrous Cycle Vaginal Cytology

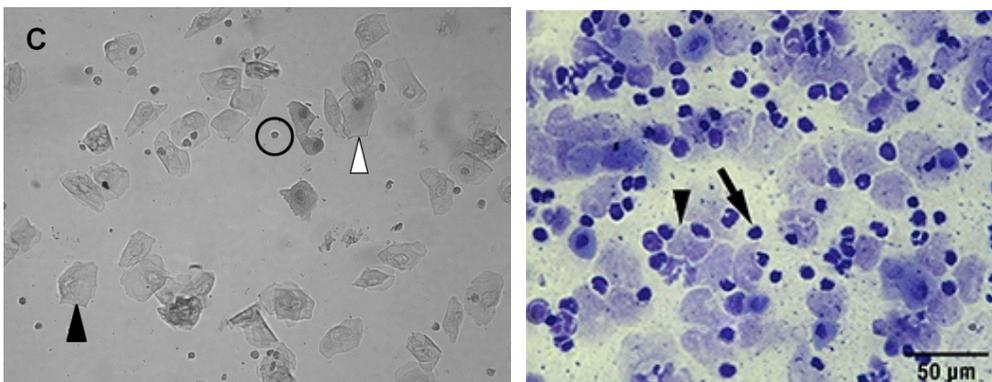
1. Proestrus - Cells are almost exclusively clusters of round, well-formed nucleated epithelial cells. The nucleus will stain darker than the cytoplasm.



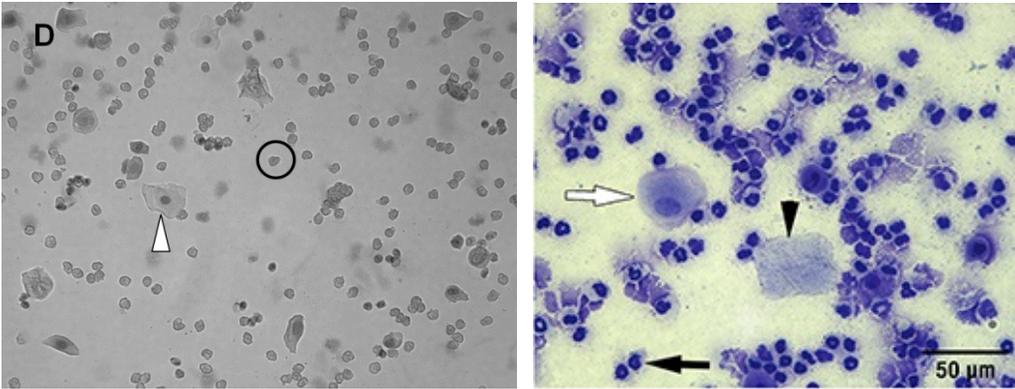
2. Estrus - Cells are predominantly cornified squamous epithelial cells, present in densely packed clusters. These cells lack a nucleus and have an angular appearance.



3. Metestrus - Small darkly stained leukocytes predominate. Cornified squamous epithelial cells may be observed, often in fragments. The leukocytes are generally neutrophils with sausage-link nuclei which stain very dark purple.



4. Diestrus – The predominant cells are leukocytes. Also present will be nucleated epithelial cells and rarely cornified squamous epithelial cells.



### Materials

Microscope with 10X and 40X objectives  
Distilled water  
Glycerol

Coplin jars  
Stain  
coverslips

### Unstained Evaluation

Vaginal cytology evaluation can be done on an unstained specimen however the unstained slides can be difficult for the novice to differentiate the cellular components of the sample.

1. Place the unstained wet slide on the microscope stage and observe unstained material under light microscope with a 10× objective. It is not necessary to use a cover slip.
2. If the drop dries, it is possible to add more saline (10 microliters) with another clean pipet tip to dampen it.
3. To visualize the cells, use low illumination in the microscope, without the use of the condenser lens to assure good contrast. The determination of the estrous cycle phase is based on the proportion among the three cell types, nucleated epithelial cells, cornified epithelial cells, and neutrophils (segmented white blood cells). It is easier to determine the proportion of cells if the 10 × objective is used.
4. Switch to the 40 × objective lens to characterize the cell types.

### Stains

A variety of stains and staining methods can be used. Below are several different stains and their protocols for use.

#### Crystal Violet Stain

1. To prepare stain for cytological assessment, add 0.1 g of crystal violet powder to 100 ml of ddH<sub>2</sub>O. Mix well. Crystal violet stain (0.1%) can be stored in a tightly sealed container at room temperature.
2. Place the dry slide in a coplin jar containing the crystal violet stain for 1 min.
3. Remove to a second coplin jar containing ddH<sub>2</sub>O. Wash the slide with ddH<sub>2</sub>O for 1 min. Repeat.
4. Remove the excess ddH<sub>2</sub>O from the edges of the slide with a tissue, avoiding contact with the stained surface.
5. Pipette approximately 15 µl of glycerol on top of the smear and apply a glass coverslip.
6. Examine the smear under light microscopy to determine cell types present. Microscopic examination should be done immediately after staining as the crystal violet will diffuse from the cells over time when using glycerol for cover slipping.

## **Wright-Giemsa Stain**

1. This stain is available commercially as a one-step stain that does not requiring fixation of the slide to prevent cells from washing off during the staining process. There are many manufacturers and suppliers.
2. Place the slide in a coplin jar containing the Wright-Giemsa stain for 45 seconds – 1 minute depending on the manufacturer’s instructions.
3. Remove to a second coplin jar containing ddH<sub>2</sub>O or RO H<sub>2</sub>O. Agitate the slide to rinse for 30 seconds - 1 min.
4. Remove excess water from the back and edges of the slide with a tissue, avoiding contact with the stained surface.
5. Drying can be hastened by gently blotting with bibulous paper.
6. Place a coverslip over the stained material and view immediately with a microscope.

## **References**

Identification, Ashleigh C. McLean, Nicolas Valenzuela, Stephen Fai, Steffany A.L. Bennett, J. Vis. Exp. (67), e4389, doi:10.3791/4389 (2012).

2. Mouse Estrous Cycle Identification Tool and Images, Shannon L. Byers, Michael V. Wiles, Sadie L. Dunn, Robert A. Taft, Published: April 13, 2012DOI: 10.1371/journal.pone.0035538

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