

# **TECHNIQUES OF PREPARING SMEARS**

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## **Techniques of Preparing Smears**

Formal laboratory training is not an absolute necessity for preparing smears and making microscopic examination of the slides. Follow the instructions listed below and generally reliable results can be expected.

1. Scrupulously clean all microscope slides and be sure that these are free of
2. grease or oily material. Wash the slides, submerge in alcohol and wipe dry with a lint-free cloth or paper tissue.
3. Maintain the slides and stain in an environment having a temperature of 95° to 100° F. Cooler temperature of stain will cause sperm to die, the percentage of loss being directly related to the lower temperature of the stain at the time of mixing. Collected ejaculate should be held in the same environment for a few minutes to allow temperatures to equalize.
4. Mix the semen sample well. Invert the collection tube and then return it to an upright position; remove the stopper and transfer a drop of semen from the edge of the stopper to one end of a warm slide. If possible, avoid trapping air bubbles in the drop. If bubbles should form, prick them with a sharp corner of another slide.
5. Observe the drop under low power magnification (100x). The warm slide should be placed on a warm stage with entire instrument being in a warm room. A rough estimate of the live/dead ratio can be made by appraising the “degree of vigor” of the sample.
6. Place a drop of warm stain, comparable in size to the semen on the slide adjacent to but not in contact with the drop of semen. Hold this slide in the left hand, with thumb and forefinger applied to the edges. The second finger can be extended beneath the slide to serve as a support. The end of the slide, opposite the drops, should be pointed away from the hand.
7. A second slide, to be used for smearing, should be held between the thumb and forefinger of the right hand in a position about a 45° angle to the slide in the left hand. Move the edge of the smearing slide in to the drop of stain. Rock the slide until the stain is evenly distributed across the leading edge. Semen should not be contacted at this point.
7. Now move the leading edge of the smearing slide, with evenly distributed stain thereon, toward the drop slide several times (8 to 10) to assure uniform mixing of the stain and semen. Draw the smearing slide backward away from the left hand, moving the smearing edge away, rapidly and evenly, along the length of the other slide to leave a uniform thin film covering the entire slide.
8. Heat and dry the slide immediately. It may be necessary to blow softly across the smear to assure rapid even drying.
9. When the smear is dry, scan the slide under low power objective, with dimmed light to determine the effectiveness of results.

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# INTERPRETATION AND EVALUATION

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## **Interpretation and Evaluation**

In order to minimize the error of interpretation of the Live-Dead reaction, the following characteristics have been designated as guidelines. Each must be visible in each of at least ten (10) microscopic fields before the stained slide can be considered to be a valid indicator of live or dead cells.

1. There must be definite homogeneous colored background over the slide.
2. There must be a distinct color contrast among the sperm cells to indicate that some were dead and some were alive at the time of ejaculation.  
Microscopic fields showing all of the sperm cells colored or all free of color are not representative of the sample and should not be used for tabulation.
3. The living cells must show an area of refraction, delineating the cell with a distinct halo. Those cells that absorb the stain may show a faint indistinct halo. In most instances, the leading semicircle of the head (galea capitus) of necrosing sperm will present a fuzzy, indistinct surface, which seems to merge insidiously with the background color. In some instances, only the posterior half of the dying sperm's head absorbs the stain. If the cell takes any stain at all, it is considered to be dead.
4. At least 100 sperm cells, representing a minimum of ten (10) different microscopic fields observed under the high dry objective (400x) should be counted. Each sperm cell that even partially appears within the area under observation should be tabulated. The percent alive is readily computed as a fraction of the total sperm cells counted.