

## A LIVE-DEAD STAIN TO TEST POULTRY SEMEN QUALITY

The authors are R. A. Ernst, Extension Poultry Specialist and  
F. X. Ogasawara, Associate Professor, Department of Avian Sciences, Davis.

Poultry males can be evaluated as potential breeders at the beginning of the season by visual examination of their semen. Thick creamy semen of good volume usually means the semen has a high fertilizing capacity and the male should be retained. Males with thin watery semen should be culled. Microscopic examination of semen prepared with a live-dead stain can help identify low fertility males.

### PROCEDURE

Step 1. Mix the following proportions of stain and semen at room temperature in a small (10 x 77 mm) culture tube.

#### Turkey

20 drops stain (Pasteur pipette)

Coat inside tip of Pasteur pipette with semen and rinse it in stain.

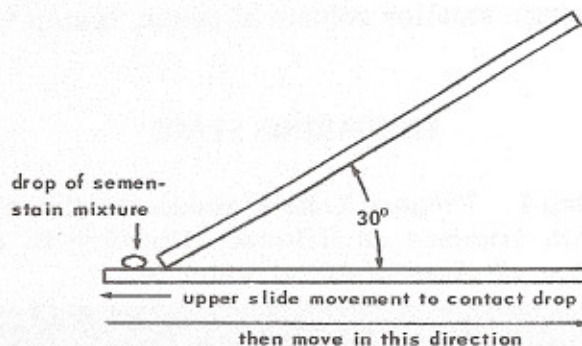
#### Chicken

12 drops stain (Pasteur pipette)

1 drop semen (Pasteur pipette)

Step 2. Agitate the mixture and let it stand - 1 minute for chicken semen, 2 minutes for turkey semen.

Step 3. Place 1 large drop (Pasteur pipette) on one end of an oil-free slide; make contact with the second slide (see diagram) until the stain is uniformly spread; now draw the mixture across the bottom slide (i. e., bloodsmear technique) making certain that the smear is uniform and not too light in color. This technique minimizes mechanical damage to the spermatozoa.



Step 4. Dry quickly with warm air from a small hair dryer or some other heat source. After the slide is completely dry, you should examine it within three days.

Step 5. Differentiate a total of 200 or 300 spermatozoa into **NORMALS** (morphologically normal, unstained); **ABNORMALS** (change in morphology: coiled, twisted, broken, bent, etc.); and **DEAD** (stained, partially or totally) under the oil immersion lens of a standard microscope. A laboratory counter with a totalizer key is useful for counting the individual spermatozoa.

## O N E - S H E E T   A N S W E R S

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Example of average counts for semen of good quality.

	number	percent
NORMALS	156	78
ABNORMALS	36	18
DEAD	<u>8</u>	<u>4</u>
	200	100

Greater numbers of dead or abnormal sperm indicate a male with lower fertilizing capacity. CAUTION. 1) If most sperm stain pink but appear normal the slide may not have been properly dried or the stain used may have deteriorated. 2) If sperm cells are too closely packed for easy reading, prepare a new slide using a smaller volume of semen in step 1.

### PREPARING STAIN

Step 1. Prepare Lake's glutamate diluent with fructose as follows: Dissolve in 1 liter of glass-distilled water -

17.350 grams sodium glutamate (mono),  
 $C_5H_8N \cdot NaO_4$   
 1.280 grams potassium citrate,  
 $K_3C_6H_5O_7 \cdot H_2O$   
 8.511 grams sodium acetate,  
 $CH_3COONa \cdot 3H_2O$   
 0.676 grams magnesium chloride,  
 $MgCl_2 \cdot 6H_2O$

The pH of this solution should be approximately 7.0. This can be checked with litmus paper or a pH meter.

Step 2. Dissolve 5 grams of water-soluble Nigrosin and 1 gram of water-and-alcohol-soluble Eosin Bluish in 100 ml Lake's diluent.

### Precautions

- . Prepare stain at least 1 day before use.
- . Always store stain and Lake's diluent in a refrigerator (4-5 C) in a closed container.
- . Always use clean glassware when preparing stain or diluent.

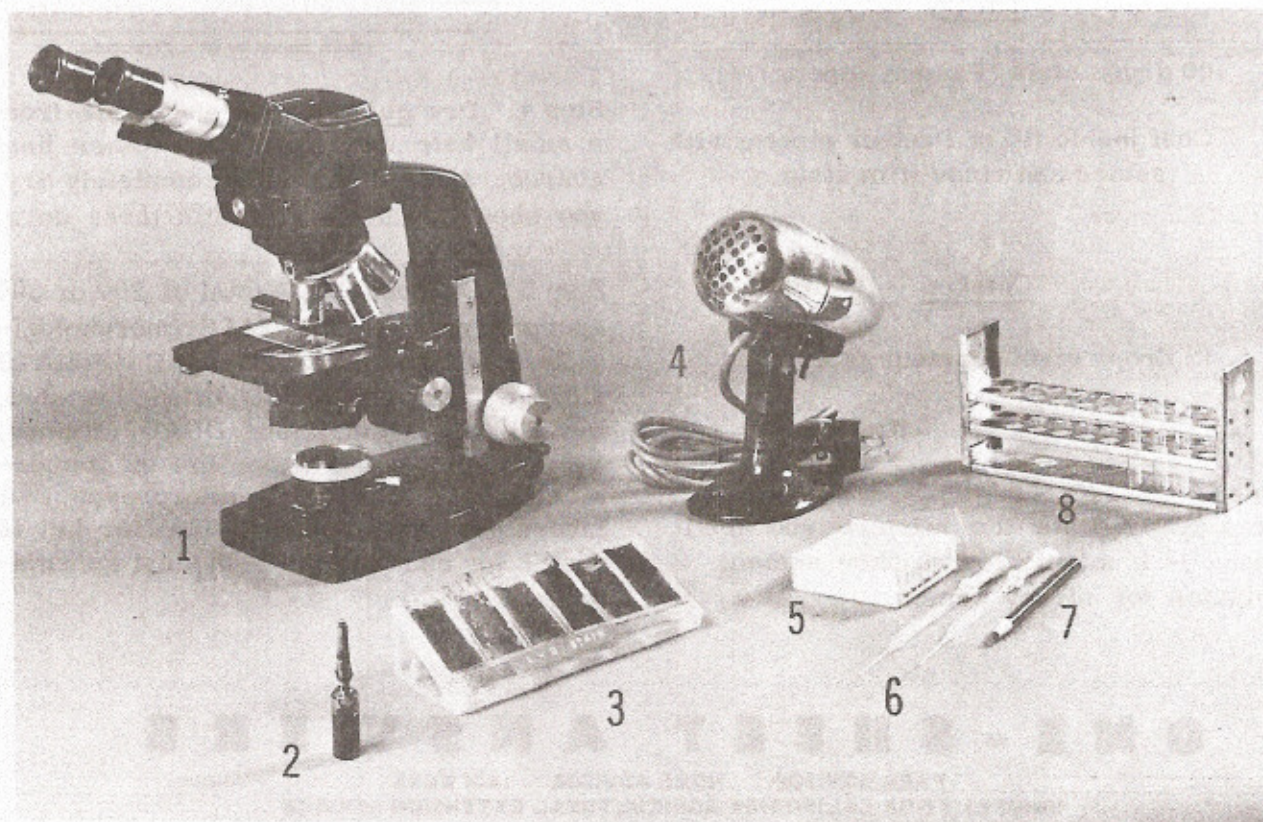


Figure 1. Equipment - 1) Microscope; 2) Stain; 3) Slide Drying Rack; 4) Electric Hair Dryer; 5) Clean Glass Slides; 6) Pasteur Pipette; 7) Wax Pencil; 8) Staining Tubes in Holding Rack.