Hancock Stain User Guide

Introduction:

Hancock Stain (P/N HCS-101) is an Eosin-Nigrosin stain used to determine the number of live-dead spermatozoa in an ejaculate and to analyze morphological characteristics. Spermatozoa with intact cell membranes will not absorb the stain while those with damaged cell membranes will absorb the stain and appear darker.

This stain is used as an aid for reproductive evaluation. More information on the morphological examination of equine spermatozoa can be found in the book Equine Reproduction by McKinnon and Voss and/or the Colorado State University Bulletin #5, Management of the Stallion for Maximum Reproductive Efficiency, II.

Slide Preparation:

The following instructions show the steps necessary to prepare a slide for morphological evaluation.

1. In order to prevent morphological abnormalities due to cold shock, pre-warm a slide to 37°C using a slide warmer (P/N 581-MOD1). Once slide is warm, place 1 drop of Hancock Stain solution on one end of the slide (Figure 1).

   CAUTION: *Placing semen on the slide before the stain will contaminate the Hancock Stain.*

2. Using a disposable pasteur pipette (P/N 537-503), place 1 drop of fresh semen next to the stain (Figure 2) and use the tip of the pipette to gently mix (Figure 3).

Figure 1. Placement of stain on slide.

Figure 2. Placement of semen on slide.

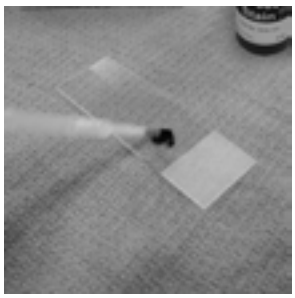

Figure 3. Mixing stain and semen.

ARS™ ANIMAL REPRODUCTION SYSTEMS
14395 Ramona Ave. Chino, CA 91710
www.arssales.com ❖ sales@arssales.com ❖ (800) 300-5143 ❖ FAX: (909) 597-3043

3. Use the edge of another slide, held at a 45 degree angle (Figure 4), to touch the mixture and allow the stain to collect along the bottom edge of the second slide. Push the second slide forward to create a smear (Figure 5).

4. Quickly dry the slide by waving it back and forth, setting it on a slide warmer, or using a hair dryer.

5. Using a 400x or greater magnification microscope, observe the sperm cells located in the feathered region of the slide at the edge furthest from where the initial droplets were deposited (Figure 6).

6. Determine the percent live cells by counting 100 cells throughout the slide for each of two slides and recording the average number of live (unstained) versus dead (stained) cells.

7. Determine the percent normal cells by counting 100 cells throughout the slide for each of two slides and recording the average number of normal versus abnormal sperm observed.

Figure 4. Using another slide to spread mixture.

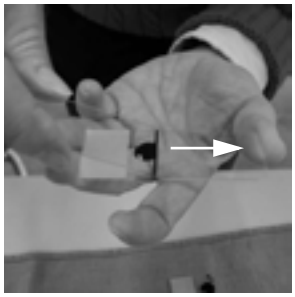

Figure 5. Push second slide forward to create smear.

Figure 6. View feathered edge under microscope.

Storage:

Store bottle in a cool location.

LIMITED LIABILITY STATEMENT

DUPREE, INC., a corporation (“SELLER” or “DUPREE”), warrants to the original purchaser only that the products sold by its Animal Reproduction Systems Division shall be free from defects in material and/or workmanship WHEN DELIVERED BUT NOT THEREAFTER. The purchaser must examine such products immediately upon receipt thereof and determine whether the same conform to the description thereof as set forth in the product data sheet accompanying this Limited Warranty Statement. If any such product does not conform to any of the data sheet descriptive criteria provided by SELLER with this statement, or if any such product appears to be defective in any respect, such product shall be immediately returned to SELLER for replacement and/or repairs as appropriate to the product. Such inspection and return of any allegedly defective product shall be made within five (5) days from the date of delivery thereof to the original purchaser and, if not so returned, this WARRANTY shall be void and of no effect whatsoever. Any allegedly defective product returned for repair or replacement within said five day period will be repaired or replaced and reshipped to the original purchaser within ten (10) days after return of the defective product to SELLER.

The restrictive and limited nature of SELLER’S warranty obligations as herein set forth is required by reason of the character of the products and the circumstances and conditions under which the same are likely to be used. The warranty obligations of SELLER shall be limited to repair or replacement of any allegedly defective product as set forth above and DUPREE shall not be liable or responsible under any circumstances or in any amount for incidental or consequential damages or for injury or damages to persons or property using or used in connection with any of such products, whether or not defective, or for loss of profits or other costs, expenses or charges of any kind whatsoever. SELLER’S warranty obligations as set forth above shall be absolutely void if the allegedly defective product is not returned to seller within the five day inspection period set forth above, or if the product is misused, neglected or damaged, or if the instructions for its use as set forth in the accompanying data sheet have not been strictly followed, or if anyone other than SELLER has made any attempt whatsoever to repair or to alter the product in any respect.

There are no warranties or representations by SELLER other than as set forth herein, either express or implied, as to any of the products or the origin thereof or any other matter relating thereto, and no person, firm or corporation is authorized to make any representation or to incur any obligation in the name or on the behalf of DUPREE, contrary to any of the provisions or conditions of this statement.