To: hematology

Bloodsmear preparation

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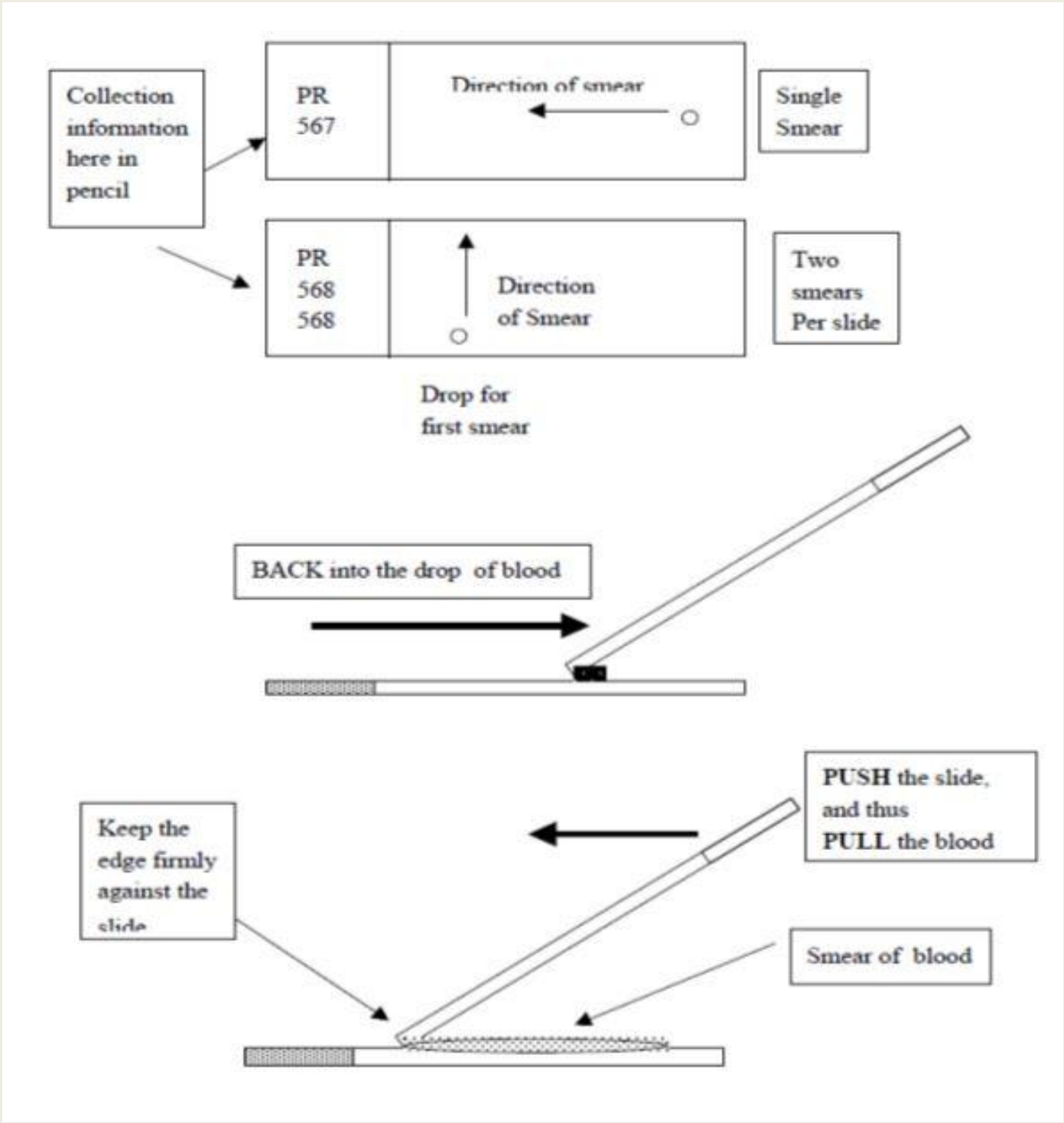
Materials
Blood filled capillary tube or EDTA (purple top) tube
Applicator sticks
Lens cleaner and lens paper
Glass microscope slides (clean in 70% alcohol and air dry)
Dip-quick stains
Pencil to mark slides
Immersion oil

Blood smears should be prepared as soon as possible after collection. Delay can result in changes in parasite morphology and staining characteristics. Thick smears are used to search for blood parasites; thin smears are used for differential white blood cell counts. Under field conditions, if slides are scarce, you can prepare both a thick and a thin smear on the same slide just make sure that only the thin smear is fixed.

Thin smears

1. In thin smears the blood is spread in a layer such that the thickness decreases progressively toward the feathered edge. In the feathered edge, the cells should be a monolayer, not touching one another.
2. Clean the slide and label with the elephant's ID and the date
3. Gently tap the end of the capillary tube or invert the EDTA tube several times and use an applicator stick to place a drop of blood (2-4 mm in diameter) on the slide near its frosted end.
4. Spread the drop by using another slide (called here the "spreader"), placing the spreader at a 30 - 45° angle and BACKING into the drop of blood.
5. As soon as the blood has spread along the edge of the spreader slide quickly push the spreader slide forward. This action pulls the blood.
6. Make sure that the smears have a good feathered edge. This is achieved by using the correct amount of blood and spreading technique.
7. Allow the smears to air dry.
8. Fix the smears by dipping them in absolute (100%) methanol being careful not to dip the frosted end with the ID information.
9. If you are preparing one smear per slide, the spreader then becomes the next slide to receive a smear. Each slide thus serves two duties, as a spreader, then as a slide to receive a smear. If two smears are made per slide, be sure to flip over the spreader to use the other edge for the second smear produced. The spreader then is used to receive the next two smears. If there is surplus blood on the spreader, wipe it off carefully before flipping it over to make the second smear on the slide.



Common causes of a poor blood smear

1. Drop of blood too large or too small.
2. Spreader slide pushed across the slide in a jerky manner.
3. Failure to keep the entire edge of the spreader slide against the slide while making the smear.
4. Failure to keep the spreader slide at a 30° angle with the slide.
5. Failure to push the spreader slide completely across the slide.
6. Waiting too long after placing the drop of blood on the slide.

Staining the Slide

The Jorvet Dip Quick Stain is a quick and easy stain that gives comparable results to the Wright-Giesma method. These polychromic stains will color acid groups blue (DNA/RNA), basic groups orange (protein eosinophil granules), and metachromic substances violet (mast cell and basophil granules). It is a valuable stain for blood cell differential count and evaluations. It also works well for general diagnostic cytology.

- The slide is allowed to air dry.
- After air drying, dip the slide repeatedly in the wide mouth bottle marked #1 fixative for a total of 5 seconds. Hold the slide up over the bottle and allow the excess fixative to drain off.
- Dip the slide repeatedly in the bottle with component #2 for a total of 5 seconds. Hold the slide up over the bottle and allow the excess stain to drain off.
- Gently rinse with distilled water and gently blot dry with a paper towel.
- Always close the lid tightly in the bottles to avoid evaporation loss.

Examining the smear

Use a systematic approach:

1. Scan the smear at low power (10 X objective) to find the optimal area for examination at higher power and to evaluate the distribution of WBCs
2. Perform the differential (see below)
3. Estimate platelet numbers (100 X)
4. Assess morphology (100 X) See appendix C. White Blood Cell Morphology

Thick smears for blood parasites

Thick smears consist of a thick layer of lysed red blood cells (RBCs). The blood elements (including parasites, if any) are ~ 30 times more concentrated than in an equal area of a thin smear. Thick smears are better for detecting some parasites. However, they do not permit an optimal review of parasite morphology. A thin smear may be needed for species identification.

1. Place a small drop of blood in the center of the pre-cleaned, labeled slide.
2. Using the corner of another slide or an applicator stick, spread the drop in a circular pattern until it forms a small circle.
3. A thick smear of proper density is one which, if placed (wet) over newsprint, allows you to barely read the words.
4. Lay the slides flat and allow the smears to dry thoroughly; protect from dust and insects. Insufficiently dried smears (and/or smears that are too thick) can detach from the slides during staining. The risk is increased in smears made with anticoagulated blood. At room temperature, drying can take several hours; 30 minutes is the minimum; in the latter case, handle the smear very delicately during staining. Protect thick smears from hot environments to prevent heat-fixing the smear.
5. Do not fix thick smears with methanol or heat. If there will be a delay in staining smears, dip the thick smear briefly in water to hemolyze the RBCs.

Knott's Techniques for Detecting Microfilariae

1. Prepare 2% formaldehyde (2 ml of 37% formaldehyde + 98 ml H_2O).
2. Mix 9 ml of the 2% formaldehyde with 1 ml of EDTA blood.

3. Centrifuge at 500 × g for 10 minutes; discard supernatant. Sediment is composed of WBCs and microfilariae (if present).
4. Examine as temporary wet mounts.
5. Prepare thick and thin smears; allow to dry; stain and examine

References:

http://www.dpd.cdc.gov/dpdx/HTML/Frames/DiagnosticProcedures/body_dp_bloodprocess.htm
www.uvm.edu/~jschall/pdfs/techniques/bloodsmears.pdf
http://www.austincc.edu/mlt/phb/phb_unit9Lab10PreparationOfBloodSmearsFall2010.pdf