Making and Staining a Blood Smear

A well-made blood smear is a beauty to behold, and likely to yield interesting and significant information for a research project. A poor slide is a torment. The extra time and care taken during the field season will be rewarded later when the smears must be scanned, and parasites identified and counted. Here, the methods for making and staining smears are given, as well as a list of sources for high quality slides, stain, and chemicals. Photographs showing well-made smears are shown on the website.

Dried blood samples for genetic studies should always be made at the same time as the smears. The method is very easy and modern research must combine studies of morphology under the microscope with molecular methods. The technique for making and storing dried blood samples is given in the section "Dried Blood Samples".

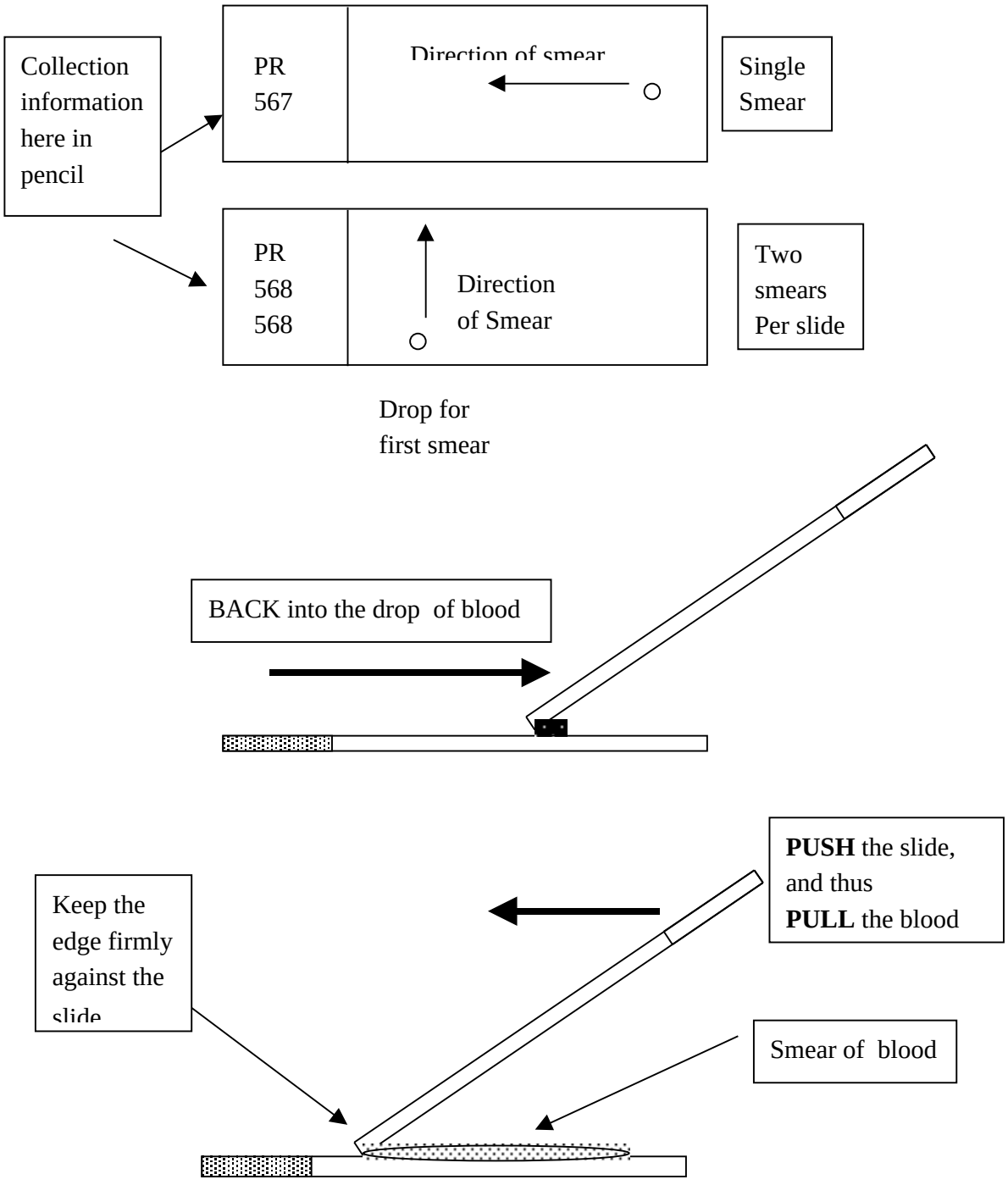
Making a smear

1. A single smear can be made per slide (smear running the length of the slide) or two (or even three) smears can share a slide, with the smears running the width of the slide. Putting two smears per slide saves on weight (glass is heavy) for field trips, and storage space. A picture showing both versions is included on the website.

2. It is easiest to use microscope slides with a frosted end, so that identifying information can be written there with pencil. Warning: Compare different pencils to find one that does not yield labels that rub off or wash off in the methanol dip.

3. Place a drop of blood approximately 4 mm in diameter on the slide (near the end if one smear is to be made, or at the proper location if two smears are to share a slide). See the drawing below.

4. Spread the drop by using another slide (called here the "spreader"), placing the spreader at a 45° angle and BACKING into the drop of blood. The spreader catches the drop and it spreads by capillary action along its edge. To make a short smear, hold the spreader at a steeper angle, and to make a longer smear, hold it closer to the drop. Now, push the spreader across the slide; this PULLS the blood across to make the smear. Do not push the blood by having it ahead of the smearing slide! It should take about one second to smear the drop. A smooth action is required, with the edge of the spreader held against the slide. This will yield a nice, even smear.


Collection information here in pencil
PR 567
Direction of smear
Single Smear
PR 568 568
Direction of Smear
Two smears Per slide
Drop for first smear
BACK into the drop of blood
PUSH the slide, and thus PULL the blood
Keep the edge firmly against the slide
Smear of blood

5. If doing one smear per slide, the spreader then becomes the next slide to receive a smear. Thus, each slide 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. Warning: If there is surplus blood on the spreader, wipe it off carefully before flipping it over to make the second smear on the slide.

6. Photographs are shown in the website.

7. For blood taken from mammals, a THICK blood film can also be made, but this is not possible with blood from birds or reptiles. Only mammals have erythrocytes that lack a nucleus. Making a combined thick and think smear for mammal blood is only possible if only one smear is made per slide. Make the thin smear starting about 1/3 from the nonfrosted end of the slide. Then, place another drop of blood at the clear end and use the edge of the smearing slide to spread the drop out to about a 1 cm circle. The thick smear will take longer to dry. Because the erythrocytes of mammals lack a nucleus, thousands of cells can be stacked, and parasites still seen (not for identification, but simply to detect an infected animal).

8. Smears should be air-dried, and then dipped into 100% methanol. A coplin jar with a screw top is best for this. We use a plastic version, which won't break in the field, but has a poorly sealing top. Slides can be stored while drying in a small plastic slide box (holds 25 slides). Then, they are placed, two at a time, back-to-back, into the slots in the coplin jar. Thus, ten slides can be dipped at once. Be sure the alcohol does not reach the frosted end of the slide. After one minute, the slides are removed and placed on end to drain the alcohol. They can then be placed into a plastic slide box for complete drying.

9. In the field, we place the plastic slide box or boxes into a zip-lock bag with silica gel, and they are allowed to dry overnight.

10. To store slides during long field trips, and where many slides are to be made, they can be placed back into their original cardboard boxes, with a piece of index card or other clean paper between each slide.

Field vs. lab preparation of smears (wild caught animals)

For our work with lizard malaria parasites, we always bring the lizards back into the lab in the evening for processing (even if the "lab" is a hotel room!), so the smears can be made in a somewhat controlled environment. For the work on bird parasites, smears must be made at the site of capture (usually when mist-netting in the early morning, and often in web environments). Very good quality smears are still produced by working on the tailgate of a pick-up truck, or on a field table (a piece of stiff plastic placed on the ground). Smears are kept after dipping in alcohol in a bag with silica gel. Smears made in the field in hot and dry climates often are of very poor quality, probably because they dry too rapidly. Smears made in the veterinary clinic should be of very high quality because of the uniform and clean environmental conditions.

Staining smears

1. First prepare the buffer. The stock buffer should be kept in the refrigerator, but if not possible, can be stored at room temperature for several weeks. Make working buffer which can be stored at room temperature for a few days. Buffer should be pH 7.0 to 7.2. Although this is a higher pH than normally used to stain blood cells, the parasites will stain darker and be more visible under the microscope.

2. A high-quality Giemsa should be used. Not all Giemsa stains are equal in quality. We place a layer of stain in the bottom of a glass coplin jar (about 3 mL), then add buffer to a level that will just cover the slides (except for frosted ends!) when they are in the jar. A little practice will tell the amount of buffer to add. Place the slides, back-to-back into the slots of the jar, and stain at room temperature for about 50 minutes.

3. Remove slides, rinse by dipping a few times into plain buffer, then stand on end to dry. Some workers prefer to run a thin stream of tap water over the slide to remove all the remaining stain; we have not found this necessary. Be sure to wash out the coplin jars after each use. If not properly washed, stain builds up inside the jar and reduces the quality of staining.

4. There is no need to cover-ship the slides. Immersion oil can be placed directly on the smear for observing under 1000x.

Preparing staining buffer

Stock buffers (two)

The alkaline stock is Sodium phosphate, dibasic anhydrous, N_2HPO_4, Sigma Chemical S-0879. Mix 9.5 gm with distilled water to make 1000 mL.

The acid stock is Potassium phosphate monobasic anhydrous, KH_2PO_4, Sigma P5379, mix 9.07 gm with distilled water to make 1000 mL

Working buffer: Mix 39 mL of acid stock with 61 mL of the alkaline stock, and 900 mL of distilled water. Check pH, and adjust to ph 7 or 7.2 by adding the acid buffer stock to lower pH or alkaline to raise pH. Just a very few mL should be necessary to reach the required pH.

Other supplies

Microscope slides. Good-quality slides seldom will retain any oil from machines used in their manufacture, so cleaning should not be required. We use slides with frosted end from VWR (#48311-950).

Giemsa. Not all Giemsa stains are equal in quality. We use Baker obtained from VWR No. JTM708-1, a 500 mL bottle. This plastic bottle has a pour spout that ALWAYS leaks. So, we store the bottle in a plastic bag and always handle the bottle through the bag. Giemsa stain will color skin for several days!

Slide boxes. In the field we use blue plastic slide boxes that hold 25 slides. These are obtained from Carolina Biological Supply (Carolina Blue Boxes, #HT-63-4200)). For permanent storage, we use wooden boxes from VWR (#48450-006).

Coplin jars. The plastic jar used in the field for dipping into methanol is obtained from Carolina (#HT-74-2155). Staining jars are available from many sources (Carolina has them #HT-74-2160). Most of ours were hand-me-downs from retiring faculty over the years.

Silica gel is from Sigma (S7500) that we buy in the 1 kg can.
Zip-lock plastic bags should be the ones used for freezer storage.