

Protocol 6

Preparation of a Peripheral Blood Film

This protocol describes how to prepare a slide film of peripheral blood for microscopic examination. It is good practice to prepare two slides for each sample. This way, each slide may be used to spread the film of the other, and duplicate slides will be of value if something happens to one of them in the process.

MATERIALS

CAUTION: See Appendix for proper handling of materials marked with <!.>.

Reagents/Animals

Chloroform (*optional*; see Steps 9–10) <!.>

Compound light microscope with high-power oil immersion objectives

Immersion oil (*optional*; see Step 8)

Mounting medium (e.g., Permount)

Dilute before use (two parts mounting medium to one part xylene <!.>)
(*optional*; see Steps 9–10).

Mouse from which a blood sample is to be collected

Silver nitrate powder (e.g., Kwik-Stop Styptic Powder) or gel (e.g., Kwik-Stop Styptic Gel Formula) <!.>

Equipment

Coverslips (glass) (*optional*; see Steps 9–10)

Glass slides, washed and prepared as described in Protocol 5

This procedure requires two slides per animal under study; each slide is used as a “draw” slide to prepare the film on the other slide.

Mouse restrainer

Pencil to label slides

Do not use a pen or other marker material, because these may be dissolved by solvents during the staining process (i.e., all labeling will be lost).

Sealed box containing desiccant (e.g., Drierite) (*optional*; see Step 7)

Sterile scalpel, straight-edged razor blade, or sharp dissection scissors
(per IACUC and/or veterinarian approval)

Swabs containing 70% isopropyl alcohol <!.> or 70% ethyl alcohol <!.>

Alternatively, spray 2 × 2-inch squares of gauze with a spray bottle containing 70% isopropyl alcohol or 70% ethyl alcohol.

METHOD

1. Use a pencil to prelabel all slides on the frosted (textured) or colored surface. Label two slides per animal. Do not attempt to label the slides during bleeding; always label the slides in advance.
2. Set out the mice by caged groups so that they can be retrieved in any order.
3. Place a single drop of blood on the surface of the first labeled slide, near the frosted end (see Video 3, Blood Film).
4. Place the second labeled slide in contact with the first, so that the drop of blood is inside an acute angle between the two slides. If the drop of blood is scant, adjust the slide so that the angle between the two is as acute as possible. If the drop is particularly copious, increase the angle.
 - ▶ *See Discussion.*
5. Working as quickly as possible, make the spread using a single, steady motion, never losing contact between the two slides (see Video 3, Blood Film).

The blood will begin to clot, especially as it contacts the glass slide, so work as quickly as possible. If the blood is initially collected by vascular access using a syringe with an anticoagulant (e.g., EDTA, citrate, or heparin), timing is not as critical. However, there is a greater likelihood that the droplet will be larger, requiring more skill to make a well-distributed smear.

▶ *See Troubleshooting.*

6. Prepare a second smear by repeating Steps 3–5, using the edge of the first slide to spread a drop of blood on the second slide.

It is not appropriate to use the same edge of a slide for more than a single film.

7. Air-dry the slides.

The slides should be dried as quickly as possible to prevent hypertonic shrinkage of the cells, but the drying time is a function of ambient humidity, i.e., the higher the relative humidity, the longer the dry time for blood smears. Indeed, very humid conditions may necessitate the use of a sealed box containing desiccant (e.g., Drierite) to allow the smears to dry sufficiently for subsequent staining, fixation, and mounting as described in Protocol 7. It will be apparent that they are dry once the “wet” appearance of the spread disappears. Under normal circumstances, we recommend that staining be performed (as described in Protocol 7 and Video 4, Staining with Coplin Jar or Carriages) as soon as the smears are thoroughly dry (i.e., 15–30 min postpreparation). Alternatively, for a quick differential or visual exam, proceed to Step 8.

8. If necessary or desired, examine the stained blood films immediately after drying, without coverslipping, using high-power oil immersion objectives.

Although this step is quick and convenient, the blood films are vulnerable to damage and cannot be archived without mounting. Mounting medium and a coverslip provide protection for the film from environmental damage and decomposition.

▶ *See Troubleshooting.*

9. To coverslip the slide examined in Step 8, remove the immersion oil by dipping the slide in chloroform several times until the oil is obviously removed from the surface of the slide.

Although immersion oil is very soluble in other less toxic solvents such as methanol, ethanol, or acetone, each of these quickly removes stain from the cells of the blood film, rendering the slide useless for cell differential analyses.

10. After removing the immersion oil, air-dry the slide and add a coverslip with mounting medium as described in Video 5, Coverslip.

Slides coverslipped in this way will be well preserved for decades maintained at room temperatures in light-tight boxes.

TROUBLESHOOTING

Problem (Step 5): Blood clots before film preparation.

Solution: Consider the following:

- The interval between obtaining the drop of blood (Step 3) and spreading it on the slide (Step 5) may be too long. As skill develops, it will be possible to spread the film more rapidly.
- There may be clotting mechanism pathology in the mice under study. Although not common, it may occur and require collecting the blood initially in an anticoagulant such as EDTA or heparin to permit an acceptable spread.

Problem (Step 8): Blood films are too thick.

Solution: Blood sample size may have been too large. For large drops of blood, increase the angle between the top (spreading) slide and the slide containing the blood film as the spread is made. Alternatively, simply repeat the collection and take a smaller drop of blood.

Problem (Step 8): Blood films are too thin.

Solution: The blood sample size may have been too small. For small drops of blood, decrease the angle between the top (spreading) slide and the slide containing the blood film. In addition, begin the spread before the drop covers the entire distance of the contact between the two slides. Consider shortening the spreading stroke as well concentrating the smaller than average (i.e., limited) blood sample.

Problem (Step 8): Leukocytes are shriveled.

Solution: The blood film may have been air-dried for too long. Apply gentle heat and/or facilitate airflow over the slide to dry the film faster. Alternatively, reduce the volume of the blood sample used.

DISCUSSION

Visually inspecting a peripheral blood film provides important insights as to the quality of the blood film preparation. A suitably sized droplet and a steady motion with the top (spreading) slide are essential for good blood film preparation, creating a defined and extensive “feathered” area. Useful smears are shown in Figure 3, A and B, even though one of the smears (Fig. 3B) was initiated with a larger sample of blood. By comparison, other smears in Figure 3 were made with samples that were either too large (Fig. 3G) or too slight (Fig. 3H). This will make examination of the samples difficult due to cell crowding or the lack of available cells for examination, respectively. The blood smear in Figure 3C results when a slight amount of the blood droplet is ahead of the leading edge of the moving (top) slide. This occurs when the initial placement of the drawn slide is incorrect. Instead of being drawn back to the drop, the slide is placed at the drop’s edge, inadvertently intersecting it such that a fraction of the blood is in front of the slide. The smear in Figure 3D results when the smear movement is halting and interrupted. Similarly, the smear in Figure 3E results from an irregularly paced motion with the upper slide. The smear in Figure 3F may simply be from too scant a drop, one in which there is an excess of interstitial fluid, or a smear from an anemic or otherwise sick animal.

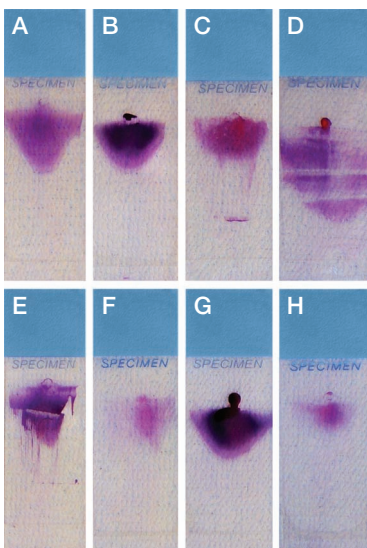


FIGURE 3. Successful preparations (A,B) and representatives of various unsatisfactory results (C–H) of blood film preparation. C occurs as a consequence of the top (spreading) slide “catching” a portion of the blood droplet ahead of the slide’s leading edge. D results when there is a halting motion, interrupting the smooth extension of the droplet. E results from an irregular shift during the spreading stroke of the top slide. F occurs if the initial droplet is too slight, if the blood is diluted with interstitial fluid or serum, or if the animal is anemic. G is an example of starting with too large of a droplet of blood. H is an example of starting with too small of a droplet of blood.