Bloodfilm Preparation

Pendl Lab
DIAGNOSTIC MICROSCOPY

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Shipment

- Prepare four slides per patient
- Air dry quickly in dust free environment (or use the cold setting of a hairdryer)
- Leave two films native
- Dip two films for 10x into methanol (e.g. light blue solution of Diff Quik® stain)
    - IMPORTANT: Apply highly concentrated methanol (*absolute, free of acetone)* and avoid evaporation by using screw top jars as storage containers. A drop of alcohol concentration below 70% will result in insufficient membrane fixation with progressive blurring and dissolution of subcellular structures in the stained sample.
- Again air dry in dust free environment (see above)
- Send two (1 fix., 1 non-fix.) to Pendl Lab, keep two as a reserve

Technique

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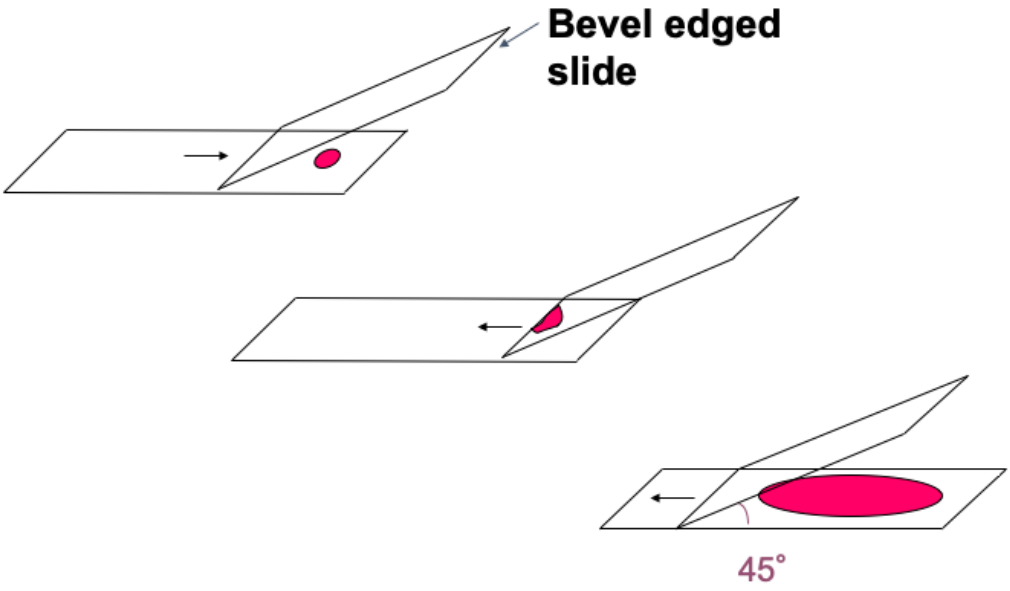
Wedge smear technique



Bloodfilm preparation

- Ideally, the film is made directly from the last drop out of the needle without contact with an anticoagulant.
- Technically at least two people are needed to handle both the patient and the sample.
- Alternatively, the bloodfilm can be made from an EDTA/Heparin sample shortly after venipuncture. Cave: EDTA causes artificial hemolysis in *Corvidae, Struthionidae, Gruidae, Alcedinidae*, and order *Testudinata/Chelonia*
- Typical artifacts due to long inadequate storage include demixing of cells, hemolysis, leukolysis, and bacterial growth. Dusty environment will lead to dark blue preciptations on the stained film, which in the worst case will make the sample unreadable.
- The use of a bevel edged slide for bloodfilm preparation will decrease the number of smudged cells due to sharp slide edges.
- The blood drop should be „drawn“ instead of „pushed“, as soon as it has dispersed two thirds along the length of the edge.
- Some training will be necessary to achieve an even distribution of the blood film. An imagination which comes close to the right technique is *inline skating* or *spreading peanut butter on a slice of bread*.....

Blood Film

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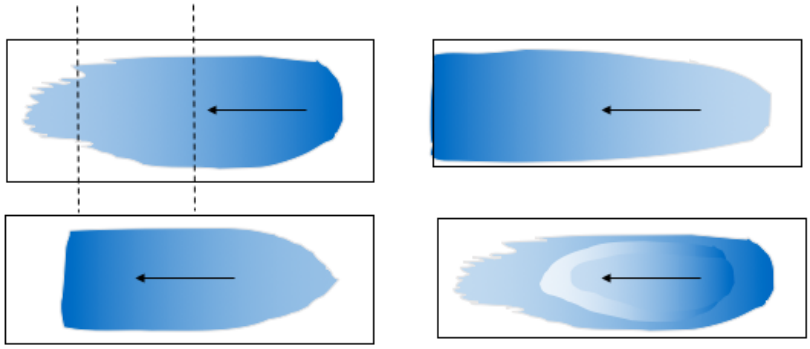
Mistakes



- Left above: ideal smear, even dilution of the blood film with a feathered vane at the end; contains the monolayer area (within the dotted lines), which is the area of optimal cell flattening and distribution for examination.
- Right above: drop too thick, drawn over the slide
- Left below: drop too thick, spreading movement stopped – identical with line concentration technique for hypocellular fluids in cytology
- Right below: Drop too thin or severely anemic patient
- Problem with the last three blood films: Monolayer missing, correct evaluation hampered.
- Immersion of a glass, plastic, or wooden stick into the sample and blotting the adherent amount gently onto 1-3 slides will give the perfectly sized blood drops for spreading.