Cytology Sample Preparation Guide

This booklet contains recommended techniques for routine cytological sampling and staining. These methods are appropriate for preparing and viewing slides traditionally or if submitting samples via digital cytology.

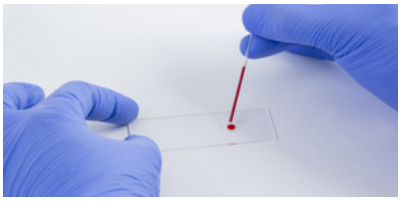
Digital cytology sample preparation is similar in many ways to traditional cytology sample preparation. Using common stains in your clinic (*e.g.*, Diff Quik), you will obtain, smear, and stain samples as you would if you were sending the slides to an outside reference laboratory.

Contents

Whole Blood Film Technique

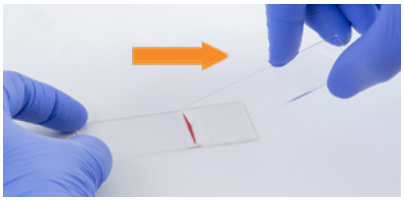
1. Place one slide on counter. A second slide will serve as the pusher slide. Use a plain capillary tube or wooden applicator to dispense EDTA anticoagulated whole blood from a purple top tube.

2. Place a small drop of blood on the slide. The drop should be about this size ●.



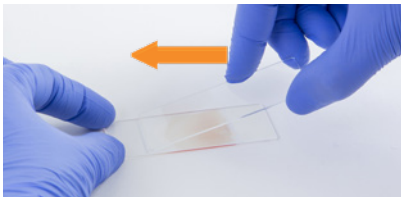
3. Hold the slide in place on the counter as the smear is made. Place the edge of the pusher slide in front of the drop of blood and form an angle of about 30 degrees.

4. Back the pusher slide into the drop of blood and allow the blood to spread along the edge of the slide.

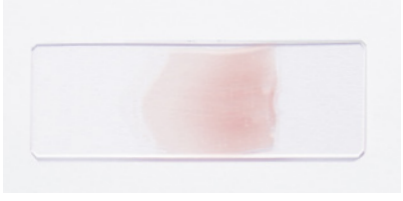


5. Just as the blood spreads close to the slide edge, immediately push the angled slide forward. Use gentle pressure and a quick push.

   The smear should cover 1/2 to 3/4 of the clear glass area of the slide. The end should be rounded and have a feathered edge, creating a thin area that has a rainbow-like appearance when reflected in light. The area just behind the feathered edge contains the monolayer of cells necessary to perform an accurate differential and assess cell morphology.



6. Once satisfied with sample obtained, stain as normal.



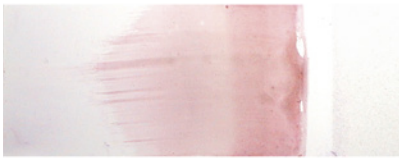
Common Problems in Blood Film Preparation

1. **Film too thin**
   Problem: Abnormal distribution of white blood cells or erythrocyte artifacts in the monolayer.
   Cause: Motion of pusher slide too slow.
   Solution: Increase speed of pusher slide.

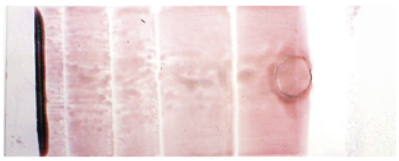
2. **Film too short**
   Problem: May be difficult to stain and examine.
   Causes: Drop of blood too small; angle of pusher slide is too upright.
   Solutions: Use a drop about this size: ●; decrease angle of pusher slide.

3. **Film runs off the end of slide**
   Problem: No monolayer present, too thick for viewing.
   Causes: Drop of blood too large; angle of pusher slide is too flat (too low).
   Solutions: Use smaller drop of blood; increase angle of pusher slide (more upright).

4. **Streaked, irregular film**
   Problem: Monolayer uneven, difficult to examine.
   Causes: Pusher slide had uneven or pitted edge; bottom slide is dirty; platelet clumps present in sample, especially a problem in cat samples; drop of blood allowed to partially dry before smear is made.
   Solutions: Use new slide; verify clumps by looking at stained film; make film immediately after placing drop of blood on slide.

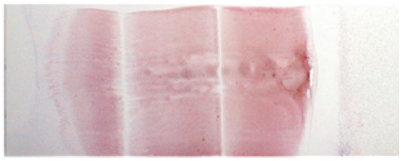


5. **Marked skipping and variation in thickness**
   Problem: Monolayer irregular, difficult to examine.
   Causes: Too much pressure applied to pusher slide; slide pushed too slowly (pusher slide skips along bottom slide).
   Solutions: Apply gentle pressure while pushing forward. Pusher slide should rest on bottom slide; the pressure should be very gentle; push faster.



6. **Additional problems in blood smear preparation**
   Problem: Fixed smear and unable to apply appropriate differential staining.
   Causes: Exposure of slide to formalin vapors.

   Problem: Unknown slide sample.
   Causes: Failure to label slide with patient name and date.



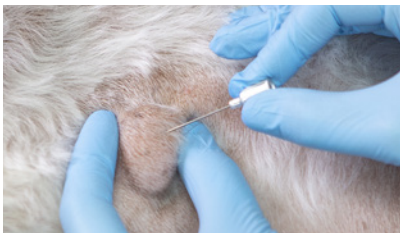
Fine Needle Aspirates

Best used for:

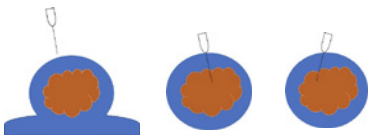
- Masses
- Lymph nodes
- Other solid tissues

Needle Only Technique

1. Aseptically remove 22 gauge needle from package (handle with gloves).

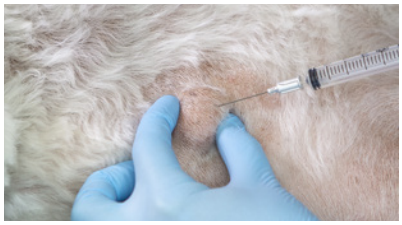


2. Insert needle into lesion, and redirect several times to sample approximately 2/3 of the lesion.
3. Remove needle from lesion.
4. Fill a 3–5 ml syringe with air.
5. Attach syringe to needle.
6. Hold syringe and needle close to a glass slide with the bevel pointing down.
7. Quickly expel the air through the needle, forcing contents onto slide.
8. Smear if needed.
9. Once satisfied with sample obtained, stain as normal.

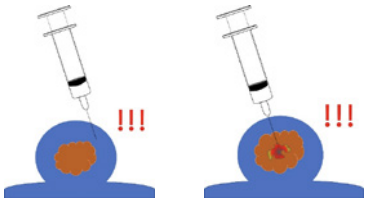


Syringe and Needle Technique (poorly exfoliating masses)

1. Attach a sterile 22–25 gauge needle to a 3–6 ml syringe.
2. Insert needle into lesion, pull back on plunger.
3. Redirect needle several times to sample approximately 2/3 of lesion.
4. Release pressure on plunger while withdrawing needle from lesion.
5. Detach needle, pull air into the syringe (or do this before starting procedure).
6. Reattach syringe to needle and follow steps 6-9 as listed above.



NOTE: Caution should be taken to ensure proper sampling of subdermal masses. Take care not to puncture too far to the side of firm masses.



Additionally, if fluid is obtained upon aspiration of what appears to be a solid tissue mass, the necrotic center may have been sampled, and aspirates of the more viable, solid areas of the mass may yield more viable sample. Preparation from the solid area, as well as any fluid obtained, is recommended.

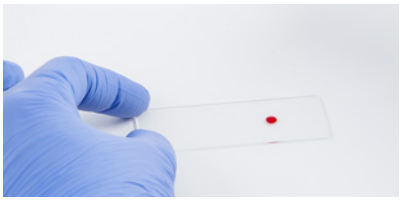
Make smears of the expelled material using the slide-over-slide technique on page 5. If material is fluid, the recommendation is to prepare using the line smear technique on page 4, making sure to separate out any solid or flocculent material.

Smear Preparation Techniques

Line Smear Technique

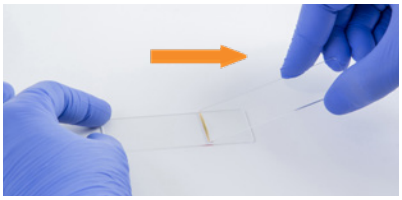
- Best used for thoracic, abdominal and pericardial effusions. Can also be used with fluid material collected from fine needle aspirates.
- Synovial fluid is often too viscous for this technique and may be more easily prepared with slide over slide technique.
- If solid material can be seen floating within the sample, use the Slide-Over-Slide technique described below.

1. Place sample close to frosted edge of glass slide.

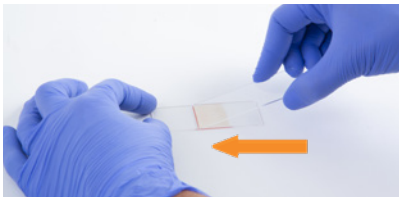


2. Use a second glass slide to form a 30 degree angle dorsal to sample slide. Gradually pull second glass slide toward sample.

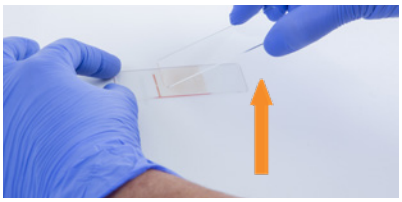
3. A "line" of sample should form at the edge of the second slide once it is in proper position.



4. As this line forms, advance the second slide forward with gentle but steady pressure.



5. Prior to creating a feathered edge, while there is still material across the entire edge of the second slide, quickly lift the second slide straight up from the smear, abruptly stopping the flow of the fluid after the smear covers about 1/2 to 2/3 of the slide. A line of concentrated cells will form at the end of the smear.

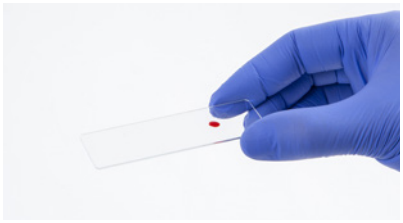


6. Once satisfied with sample obtained, stain as normal.

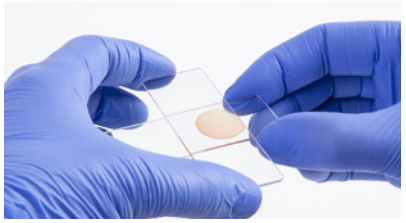
Slide-Over-Slide Technique

- Best used for fine needle aspirates and synovial fluid.
- If solid or flocculent material is present within a sample, smear the fluid portion separate from the more solid material.

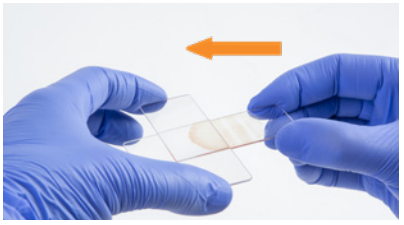
1. Place sample close to frosted edge of glass slide.



2. With a second glass slide perpendicular to the sample slide, gently place the second slide on the sample as shown in image to the right. Do NOT place additional force on sample slide.



3. Once the second slide is on top of the sample slide, move the second slide away from the frosted edge. When around 2/3 of the slide is smeared with sample, carefully lift the second slide up while finishing the direction of the movement.



4. Once satisfied with sample obtained, stain as normal.

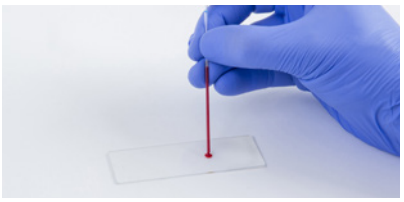
Fluid Cytology

Best used for:

- Thoracic or abdominal fluid
- Synovial fluid
- Bronchoalveolar lavage fluid

Direct Smear

If sufficient sample obtained, prepare a smear of well mixed fluid prior to any centrifugation.



Place 1 drop of sample from an EDTA tube onto a glass microscope slide and follow directions for the line smear technique on page 4.

Concentrated Smear

After preparation of direct smears from well mixed fluid sample, sediment the fluid by centrifugation for 5–10 minutes at approximately 450–500 G. This is a low speed setting, similar to the speed used for urine sediment centrifugation.

If your centrifuge does not have variable speeds, experiment with the time spun to achieve the best preparation. Once spun, remove the supernatant except for a few drops and resuspend the specimen in the remaining fluid, then follow directions on page 4 for the line smear technique.

Impression Smears

Best used for:

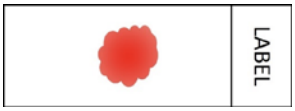
- Exudative lesions
- Excisional/incisional biopsies

Exudative Lesion Impression Smear

1. Gently press the slide on the surface of the lesion.

2. Depending on lesion location and type, clean the surface of the lesion with a saline moistened surgical sponge and repeat the impression smear on a second slide. Alternatively, the surface of some lesions may be scarified prior to imprinting on the slide.

3. Once satisfied with sample obtained, stain as normal.



NOTE: Many cutaneous masses have an ulcerated, inflamed surface. Impression smears of these lesions are often not diagnostic of the underlying mass, instead representing only the superficial inflammatory reaction. In these cases, fine needle aspirate of the underlying, solid mass is necessary to obtain a diagnostic sample.

Swab Preparation

Swabs are not commonly used except in the case of evaluation of ear exudates or vaginal smears.

1. Pre-moisten the swab with sterile saline. This may lessen cell distortion of dry samples.

2. After sample is obtained, roll the swab gently across the slide.

3. Once satisfied with sample obtained, stain as normal.

Staining Techniques

Diff Quik and other Romanowsky quick stains are the most common stains used in practice. The staining procedure is similar for these stains, however manufacturer's guidelines should be followed.

With 3-step stains, the first fluid is a blue alcohol fixative, followed by a red, and then a purple stain.

Keep in mind the amount of time or 'dips' needed to stain thick smears can sometimes be more than the amount needed for thin smears.

Please do not forget about or leave slides in the stain containers for lengthy periods of time, as this can over-stain samples.

1. Dip slide into blue fixative for approximately 20 'dips'

2. Dip slide into red stain for approximately 10 'dips'

3. Dip slide into purple stain for approximately 10 'dips'
4. Rinse slide under a gentle stream of water.

5. Allow slide to dry.

Avoiding Common Staining Problems

1. Use only new, clean slides.
2. To avoid stain contamination, use fresh or newly filtered stain and change stain containers as frequently as needed (typically once per week).
3. Ensure slides are completely dry to prevent loss of sample.
4. Insufficient stain time and/or old stain can lead to weak staining.

Cytology Techniques Poster Order Information

The Cytological Techniques topics found in this document are available in poster size for your lab. Please contact your Heska Representative and refer to the following order #'s.

Order #	Poster Name
US21LT1105	Cytology Poster Whole Blood Film Technique
US21LT1106	Cytology Poster Common Problems in Blood Film Preparation
US21LT1107	Cytology Poster Fine Needle Aspirates
US21LT1108	Cytology Poster Smear Preparation Techniques
US21LT1109	Cytology Poster Fluid Cytology
US21LT1110	Cytology Poster Impression Smears
US21LT1111	Cytology Poster Swab Preparation
US21LT1112	Cytology Poster Staining Techniques

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