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.

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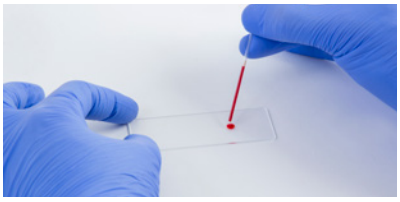
Procedure

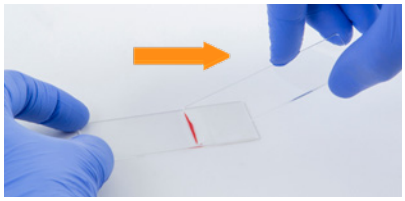
1. Place one slide on counter. A second slide will serve as the pusher slide. Use a plain capillary tube or wooden applicator to dispense EDTA anticoagulated whole blood from a purple-top tube.

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



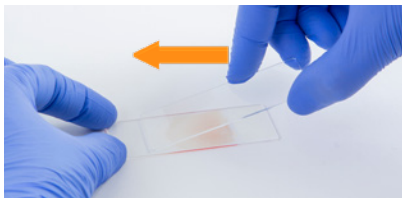
3. Hold the slide in place on the counter as the smear is made. Place the edge of the pusher slide in front of the drop of blood and form an approximately 30° angle.

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.

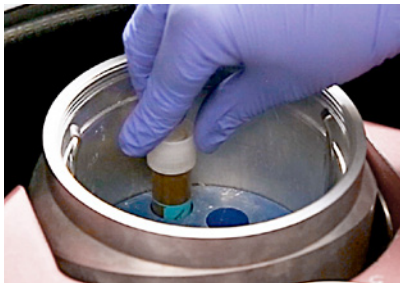
   The smear should cover ½ to ¾ of the clear glass area of the slide. The end should be rounded and have a feathered edge, creating a thin area that has a rainbow-like appearance when reflected in light. The area just behind the feathered edge contains the monolayer of cells necessary to perform an accurate differential and assess cell morphology.



6. Once satisfied with sample obtained, stain as normal. As described in *Staining Technique*.

Concentrated Smear Technique

Best used for fluid with low cellularity for example:
- Abdominal fluid
- BAL/ETW/TTW fluid
- Pericardial fluid
- Peritoneal fluid
- Thoracic fluid



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 as described in *Line Smear Technique*.

Line Smear Technique

Best used for:
- Abdominal, pericardial, peritoneal, or thoracic fluid.
- BAL/ETW/TTW fluid.
- Fluid material collected from fine needle aspirates.
- Synovial fluid

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

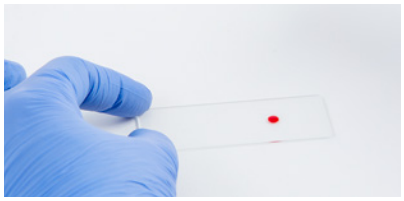
Place 1 drop of sample from an EDTA tube onto a glass microscope slide and follow directions for the line smear technique.

Synovial fluid is often too viscous for this technique and may be more easily prepared with the *Slide-Over-Slide Technique*.

If solid material can be seen floating within the sample, use the *Slide-Over-Slide Technique*.

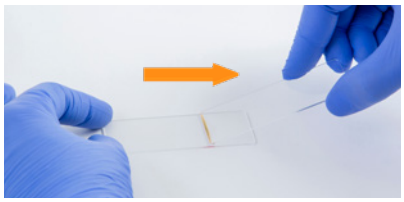
Procedure

1. Place sample close to frosted edge of glass slide. Place 1 drop of sample from an EDTA tube if there is concern for clotting.

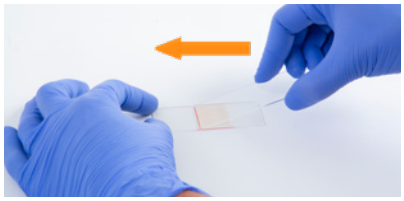


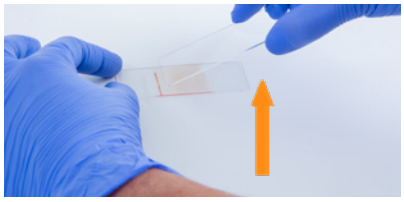
2. Use a second glass slide to form a 30° angle dorsal to sample slide. Gradually pull second glass slide toward sample.
3. A "line" of sample should form at the edge of the second slide once it is in proper position.

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 ½ to ⅔ 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. As described in *Staining Technique*.

Tips and Tricks

- Start with less sample than you think. Too much sample can cause crowding of cells obscuring cytologic results.
- Make the angle of the spreader slide less acute for thinner samples.

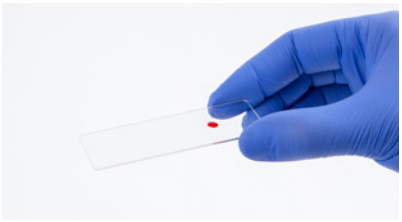
Slide-Over-Slide Technique

Best used for:
- Fine needle aspirates
- Synovial fluid
- Urine sediment dry mount

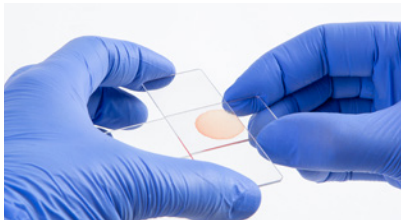
Procedure

If solid or flocculent material is present within a sample, smear the fluid portion separate from the more solid material.

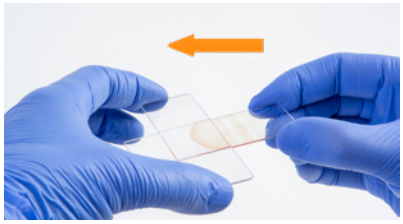
1. Place sample close to frosted edge of glass slide.



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 ⅔ 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, as described in *Staining Technique*.

Urine Sediment Dry Mount

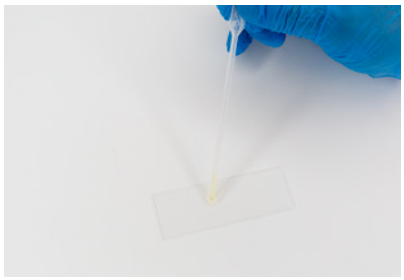
The HeskaView™ Telecytology system is unable to scan wet mount slides. However, air-dried and stained urine sediment smears can be evaluated by pathologists for cancer cells or inflammation.

NOTE: As with all sample types, it is strongly recommended to ensure the object of interest is apparent on urine dry mount and not distorted in clinic prior to submitting for pathology review.

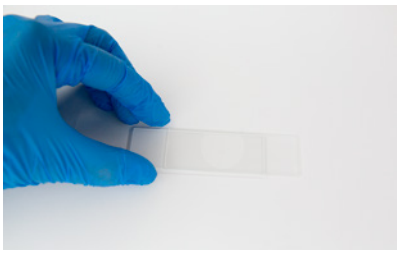
1. Collect sterile urine sample as usual. If there is concern for neoplasia, sterile catheterization is generally recommended. This not only can prevent potential seeding of neoplastic cells but can increase the amount of abnormal cells collected for evaluation.
2. Centrifuge approximately 5 mL of urine at low speed for 5 minutes.
3. Remove all but approximately 0.5 ml of the supernatant (do not discard) with pipette.

4. Resuspend the sediment in the remaining 0.5 ml urine and gently mix.

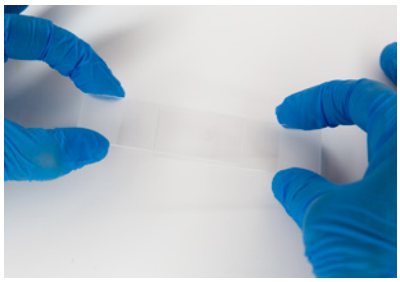
5. Place a drop of mixture on glass slide.



6. Place second glass slide over sample and leave in place to allow sample to spread (2–3 seconds).



7. Keeping slides parallel, pull the slides apart.
8. Ensure the slide is completely dry before staining. This can be done via air drying, hair dryer at low setting or on a heat plate for 30 seconds.
9. Stain as usual as described in *Staining Technique*.



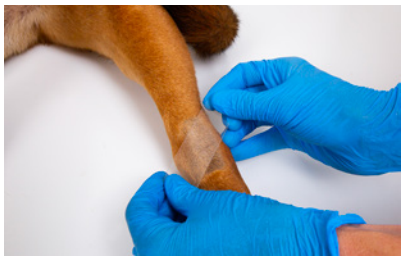
Non-Fluid Techniques

Direct Impression Smears

Best used for:
- Excisional/incisional biopsies
- Exudative lesions

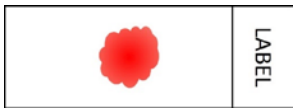
Procedure

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



2. For biopsy samples, clean the surface of the lesion with a saline moistened surgical sponge and repeat the impression smear on a second slide. Alternatively, cleaning the surface of dermatological samples prior to sampling is not recommended.

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

Best used for:
- Ear exudates
- Vaginal smears

Procedure

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. as described in *Staining Technique*.

Additional Information for Fluid Sample Analysis

Depending on the fluid sample type being analyzed, the following information will be helpful for concurrent evaluation with cytologic samples. If submitting cases through the HeskaView Telecytology Patient Portal, users will automatically be prompted to provide this information to aid in pathology review.

Fluid Analysis

Complete fluid analysis by veterinary pathologist will include:

- Gross appearance
- Total protein
- Packed cell volume
- Total nucleated cell count
- Cytologic review

Blood PCV

A measure of the amount of red blood cells present in venous whole blood. Packed cell volume (PCV) and hematocrit (HCT) are measured manually or pulled from results produced by an automated analyzer. This information is crucial when analyzing a blood film.

Fluid Clarity

Fluid clarity aka turbidity can indicate the presence or lack of excessive cells or protein in effusions, synovial fluid and cerebrospinal fluid. This information is used in conjunction with and to aid with cytological analysis.

Method

1. Analyze fluid sample directly from sampling syringe or after being placed into appropriate collection tube type.
2. Notation of fluid clarity is qualitative and based on observation of how clear or opaque a sample appears.

Fluid Chemistry

Blood and fluid triglyceride (+/- cholesterol), creatinine, bilirubin and less commonly lactate and glucose can be compared to aid in the determination of the origin of free fluid.

Methods

Blood triglyceride, cholesterol, creatinine, bilirubin and less commonly lactate, and glucose are generally measured by automatic analyzers and available in a chemistry report. Handheld analyzers can be utilized to measure blood lactate, blood glucose, and blood creatinine. Prior to running body fluid samples, ensure with the manufacturer that your specific analyzer is equipped and calibrated to analyze body fluid.

Serum/Effusion Biochemical Comparisons	
Triglycerides	Chylous effusion has > 2x the Trig concentration of serum.
Bilirubin	Bile peritonitis typically has > 2x the Tbili concentration of serum, but white bile peritonitis related to a ruptured mucocele can break this rule.
Creatinine	Uroabdomen has > 2x the creatinine concentration of serum.
Glucose	Septic peritonitis often has glucose concentration > 20 mg/dL less than serum. Measure on an analyzer and NOT a strip-based hand-held (*e.g.*, Alpha Trak).
Lactate	Septic peritonitis often has a lactate concentration > 2.0 mmol/L higher than serum.

Body Fluid PCV/RBC Count

A measure of red blood cells present in fluid samples and can be indicative of hemorrhage as a component of pathology. Blood and fluid PCV can be performed manually or a hematocrit (HCT) can be generated by an automated analyzer.

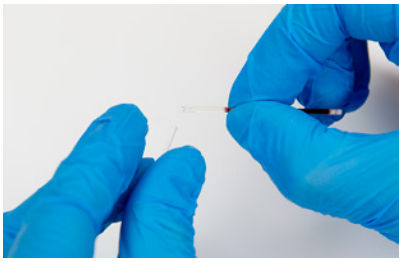
Fluid or Serum Total Solids/Total Protein

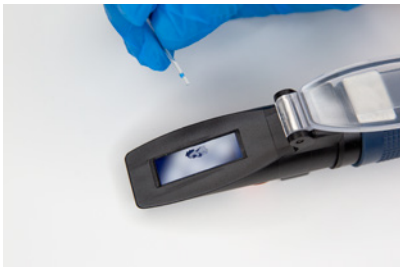
Fluid total solids is a measurement of the amount of proteins within serum or other fluids such as effusions and synovial fluid. Increased or decreased levels of protein can help identify underlying disease processes for abdominal, pericardial, and thoracic effusion as well as synovial fluid.

Fluid and serum total solids are both performed by use of a refractometer. Ideally total solids results require a clear, colorless fluid sample to generate an accurate result. Results for opaque or hemolyzed samples should be considered estimates as they will likely be skewed.

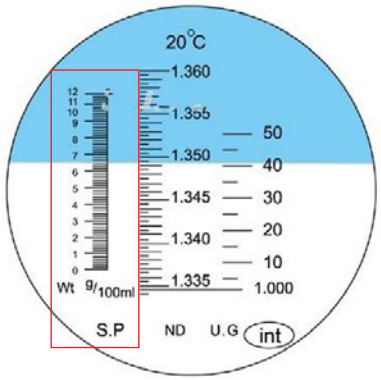
Method

1. Follow steps for preparing and spinning blood PCV. This is the same process used for determining serum total solids as well as fluid total solids.
2. Once complete, break the hematocrit tube above concentrated red blood cell line.





3. Place spun serum or fluid onto refractometer.

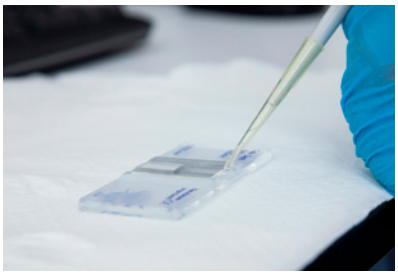


4. Holding the refractometer to the light measure the level of protein expressed in g/dl.
   - For fluid samples (not blood), these do not necessarily have to be spun in centrifuge if results are needed quickly. Fluid can be placed directly on the refractometer and protein level measured. Keep in mind, however, that blue indicator line may be blurry compared to centrifuged samples. Use approximation of best measurement.
   - If protein level is below readable level on refractometer indicate this by utilizing less than symbol (<).

Fluid Total Nucleated Cell Count

Fluid Total Nucleated Cell Count (TNCC) is a measure of the amount of nucleated cells present in a fluid sample. This can be indicative of inflammatory or non-inflammatory disease processes in effusions and synovial fluid. TNCC can be measured in the fluid or estimated from smears.

Cells can be counted with a hematology analyzer or manually with a hemacytometer.



Additional Diagnostic Parameters for Fluid Samples*

Abdominal/Pericardial/Thoracic Fluid	Required	Optional
Blood Lactate		✓
Fluid Bilirubin		✓
Fluid Cholesterol		✓
Fluid Clarity	✓	
Fluid Color	✓	
Fluid Creatinine		✓
Fluid Glucose		✓
Fluid Lactate		✓
Fluid PCV		✓
Fluid Total Nucleated Cell Count (TNCC)	✓	
Fluid Total Solids/Total Protein		✓
Fluid Triglyceride		✓
RBC Count		✓
Blood Film		
Blood PCV	✓	
Serum/Plasma Color	✓	
Cerebrospinal Fluid		
Fluid Clarity	✓	
Fluid Color	✓	
Microprotein Concentration		✓
RBC Count		✓
WBC Count		✓
Synovial Fluid		
Fluid Clarity	✓	
Fluid Color	✓	
RBC Count		✓

*As recommended by HeskaView Telecytology pathologists.

Diff Quick Staining Technique

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.

Procedure

1. Dip slide into blue fixative for approximately 10–15 seconds.

2. Dip slide into red stain for approximately 10–15 seconds.

3. Dip slide into purple stain for approximately 10–15 seconds.
4. Rinse slide under a gentle stream of water.

5. Allow slide to air dry.

Obtaining Samples

Abdominocentesis Technique

Materials

- Sterile gloves, clippers, and aseptic surgical prep supplies
- Needles 16–22 gauge, 1½ to 2 inches
- Syringes of appropriate size
  Consider large syringe (35 cc), three-way stopcock, extension tubing, butterfly catheter and over the needle catheter if attempting to drain larger amount of abdominal fluid.
- EDTA (purple-top) and red-top tubes
- Glass slides

Procedure

1. The patient is typically placed in lateral recumbency. The area surrounding the umbilicus is clipped and surgically prepped.

   The site for needle insertion is generally 1–2 cm caudal to the umbilicus to avoid falciform fat. If scar is present from previous surgical incision inset the needle at least 1.5 cm away to avoid potentially adhered viscera.

2. Ensuring the bladder is empty can help to avoid accidental cystocentesis.
3. Use a 1½ in to 2-in 16 to 22-gauge needle or fenestrated over-the-needle catheter with syringe attached.

   - If fluid appears in the hub and flows freely it can be collected into a Red or purple-top tube.
   - If fluid does not flow freely:
     1. Attach syringe to needle (if not already) and apply gentle negative pressure.
     2. If using an over-the-needle catheter with the needle removed, gentle abdominal compression can be utilized to enhance fluid collection.
     3. Alternatively, ultrasound can be used to localize pockets of fluid and direct the needle there.
     4. Consider four-quadrant paracentesis or diagnostic peritoneal lavage if the above techniques fail to yield abdominal fluid sample.
4. Prepare fluid sample utilizing the fluid preparation method as described in *Concentrated Smear, Line Smear and Slide-Over-Slide Technique* and consider fluid centrifugation, if indicated.

Arthrocentesis Technique

Materials

- Sedation if indicated
- Sterile gloves, clippers, and aseptic surgical prep supplies
- 1 inch, 25-gauge needle (small dogs and cats)
- 1½ inch needle may be required for large dogs, elbow or shoulder samples
- 3 inch spinal needle may be required for shoulder joint
- Sterile 3 mL syringes
- Purple and red-top blood tubes
- Clean glass slides

Procedure

1. Place the patient in lateral recumbency and clip the hair from the site(s). Surgically prep the area.
2. Wearing sterile gloves, palpate the selected joint space with your index finger. Flex or extend the joint to distract the joint surfaces, so that the joint space is maximized.
3 Gently introduce needle attached to syringe.

   Grip the syringe in such a manner that you can easily aspirate without having to reposition your hands on the syringe.

   Insert the needle gently toward and through the joint capsule to avoid damaging articular cartilage.
4. Once the needle is inside the joint, gently aspirate and watch for synovial fluid in the hub. Continue with gentle aspiration until enough sample is obtained (amounts will vary from just a small amount in needle hub to 1 mL).
   - If blood is visualized in the hub of the needle, release pressure and withdraw the needle.
   - Suction should always be released before withdrawing needle to help prevent blood contamination.

Sample Preparation and Storage

Note fluid volume, color, turbidity, and viscosity.

If only a few drops of fluid were collected, place one drop on a slide for cytology. Prepare utilizing the fluid preparation technique, as described in *Concentrated Smear, Line Smear and Slide-Over-Slide* techniques, air-dry and stain, as described in *Staining Technique*.

Make synovial smears as close to collection as possible to prevent cellular degradation.

Another drop of synovial fluid can be saved in a culturette for culture if necessary.

If more than a few drops of fluid are obtained, place some in a purple-top tube and some in a red-top tube.

If fluid is minimal, excess EDTA should be drained from the purple EDTA collection tube to minimize diluting effect.

The specimen in the EDTA purple-top tube is used for cytology and the cell count. The specimen in the red-top tube is sent in for culture or placed in the refrigerator and held for culture until the cytology results are known. Specimens placed in purple-top tubes cannot be used for culture due to the EDTA in the tube.

Cytocentrifugation concentration can be helpful but is not essential to an effective evaluation.

1. Flex the stifle joint.
2. Insert the needle just lateral to the patellar ligament and distal to the patella
3. Direct the needle medially and proximal toward the medial condyle of the femur.

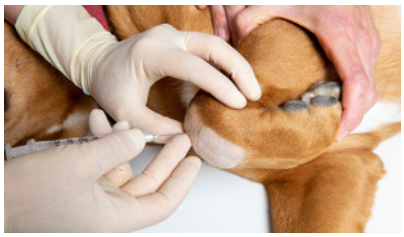
Individual Joints

Carpus

The antebrachiocarpal or middle carpal joints are the most used.

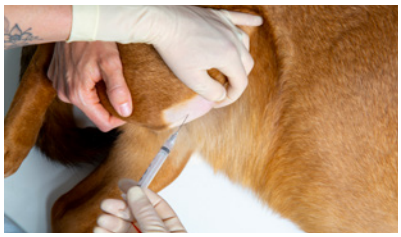
- Antebrachiocarpal joint landmarks: distal radius and proximal radial carpal bone
- Middle carpal joint landmarks: between distal radial carpal bone and the 2nd and 3rd carpal bones.

1. Hold the joint in flexion and palpate any intercarpal joint space during joint motion.
2. Insert the needle from the dorsal aspect just medial of center perpendicular to the skin to avoid the joint surfaces.



Stifle

1. Flex the stifle joint.
2. Insert the needle just lateral to the patellar ligament and distal to the patella
3. Direct the needle medially and proximal toward the medial condyle of the femur.



Elbow

Hyperextend or flex the joint.

Hyperextension

1. Introduce the needle medial to the lateral epicondyle of the humerus and lateral to the olecranon.
2. Once in the joint space, guide the needle cranially toward the humoral condyle.

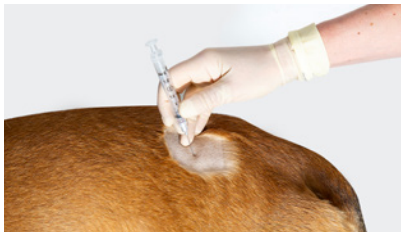
Flexion

1. Introduce the needle just proximal to the olecranon and medial to the lateral epicondylar crest.
2. The needle should be parallel to the olecranon and the long axis of the ulna.

Coxofemoral

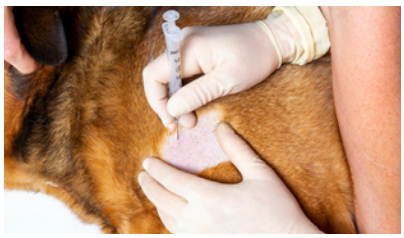
1. Abduct the femur and extend the leg caudally.
2. Introduce the needle cranial to the greater trochanter of the femur.
3. Insert the needle caudal and distal or ventral toward the joint.

NOTE: A longer needle is usually required for this joint and often needs to be introduced to the hub.



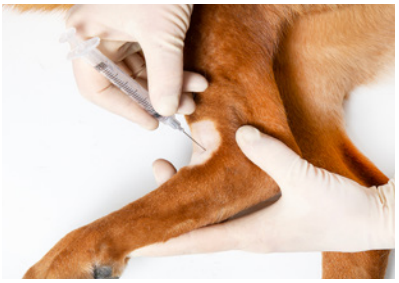
Shoulder

1. Insert the needle distal to the acromion of the scapula and caudal to the greater tubercle of the humerus from the lateral aspect.
2. Direct the needle medially toward the greater tubercle and distal to the supraglenoid tubercle of the scapula. The needle is inserted about 1 cm distal to the acromion process and slightly caudal to it.



Tarsus

The tarsal joint may be approached from either the cranial or caudal aspect.



Cranial approach:
Slightly flex the tarsus. Introduce the needle at the space palpated between the tibia and talus bones just lateral to the tendon bundle.

Caudal approach:
Extend the joint and insert the needle medial or lateral to the calcaneus with a cranial and slightly plantar path.

Bone Marrow Aspiration and Preparation Materials

Materials

- Sedation and/or general anesthesia and monitoring equipment
- Sterile gloves, clippers, and aseptic surgical prep supplies
- 2% lidocaine, <5 mg/kg, (<0.25 mL/kg)
- #11 scalpel blade
- 15 to 18-gauge Jamshidi, Rosenthal or Illinois sternal needle; pre-flushed with EDTA if desired, 1 inch, 20-gauge needle with attached 3 mL syringe for sternebrae collection
- 10–12 mL syringe containing 0.3 mls, 2.5% to 3% EDTA
- EDTA (purple-top) tube
- Clean glass slides

Procedure

1. Clip, clean and aseptically prepare skin overlaying sampling site.
2. Inject 2% lidocaine into skin, subcutis and periosteum.
3. Using a #11 scalpel blade make a small stab incision into the skin just adjacent to sampling site to help prevent infection.
4. Insert sternal needle into the appropriate area of the bone with a twisting/rotating motion until the needle and stylet are seated in the bone.
5. Remove the stylet and attach a 10–12 mL syringe containing EDTA to needle.
6. Apply strong negative pressure to syringe pulling back ⅔ to ¾ volume of the syringe in quick successive pulls.
7. Once red marrow is visualized in the hub of the needle release negative pressure.
8. 0.2 mL to 0.4 mL of bone marrow fluid is sufficient to prepare multiple smears.
9. Withdraw the needle and syringe and apply direct pressure to the skin. Suture if needed.

Troubleshooting

If marrow is not obtained repeat the procedure by repositioning the needle at the same site, advancing, or retracting slightly or angling medially or laterally.

The needle can also be redirected through a different site on the same bone or a new anatomical sampling site.

Aspiration failure may be due to poor technique, occlusion of the needle with skin or bone, marrow fibrosis, hypoplasia, or densely packed marrow.

Sites for Collection

Proximal Humerus

The proximal humerus has its advantages because it is an easily accessible site in dogs and cats of all sizes. There is little overlying tissue, and it provides a large area for placement of the needle.

NOTE: DO NOT utilize this site in young growing animals due to proximity to the growth plate.

1. Place the patient in lateral recumbency.
2. Palpate the greater tubercle. Flex the shoulder and stabilize the limb.
3. Insert the needle into the flat area distal to the greater tubercle and advance caudomedially along the axis of the bone.

Iliac Crest

This location is best for large dogs but may be difficult to access in obese animals.

1. Place in sternal recumbency although siting, standing or lateral recumbency can also be utilized.
2. Palpate the greatest prominence of the iliac crest.
3. Stabilize the ilium by placing a finger on either side of the wing.
4. Insert the needle parallel to the ileum and direct ventromedially remaining parallel to the long axis of the wing of the ileum.
5. The transilial approach can be utilized for small dogs and cats where needle is inserted via lateral approach to the wing of the ilium.

Trochanteric Fossa

The trochanteric fossa is good option for cats and small dogs however cortical bone may be too dense in this location in older patients.

Avoid using this location in obese animals.

Take care to avoid the sciatic nerve located medial and caudal to the greater trochanter.

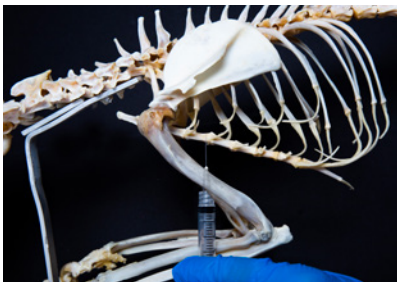
1. Please the patient in lateral recumbency.
2. Locate the greater trochanter of the proximal femur by palpation.
3. Stabilize the femur by grasping the stifle. Slight internal rotation of the stifle may enhance exposure of the fossa.
4. Insert the needle medial to the trochanter with the long axis of the needle parallel to the long axis of the femur.

Sternebrae

NOTE: For aspiration only NOT biopsy.

Take care not to penetrate the thoracic cavity.

This may be performed with only light sedation.



1. Place the patient in sternal recumbency but sitting, standing or lateral recumbency can be used to access sternebrae number 1–4.
2. Locate the 1st sternebra and stabilize with one hand.
3. Insert a 1 inch, 20-gauge needle with attached 3 mL syringe into cortex of the first sternebra and advance carefully until firmly embedded then aspirate. Number 2–4 sternebrae may also be utilized.

Bone Marrow Sample Preparation

Bone marrow clots quickly so it is imperative to prepare the slide rapidly and efficiently even when EDTA is utilized.

Material can either be directly applied from the syringe onto the slides or by expelling the material into a Petri dish or watch glass containing EDTA.

Bone marrow spicules can then be identified and transferred to the slides with a micro-hematocrit tube or pipette.

Spicules are generally clear to slightly opaque, light-gray and irregularly shaped.

1. Once the marrow is on the slides, tilt 45° to 70 degrees to allow the blood to drip off the slide while bone marrow flecks remain adhered.
2. Utilize the slide-over-slide technique by gently placing a second glass slide (or cover slip for samples with very delicate cells) onto the sample oriented at a 90° angle to the original slide. Smoothly separate the slides. See *Slide-Over-Slide technique* for further details.
3. Stain as usual with Diff-Quick, as describe in *Staining Technique,* but leaving slides in each container at least twice as long as other cytologic samples. Allow to air dry (do not blot).

*Always submit CBC results along with any bone marrow slides.

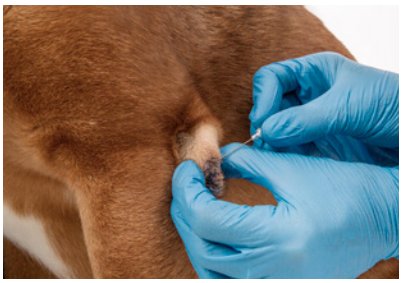
Fine Needle Aspirates

Best used for:

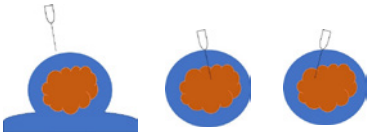
- Lymph nodes
- Masses
- Other solid tissues

Needle Only Technique

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



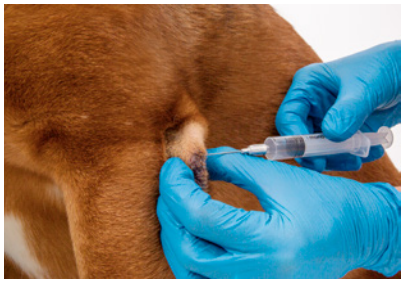
2. Insert needle into lesion, and redirect several times to sample approximately 2/3 of the lesion.
3. Remove needle from lesion.
4. Fill a 3–5 ml syringe with air.
5. Attach syringe to needle.

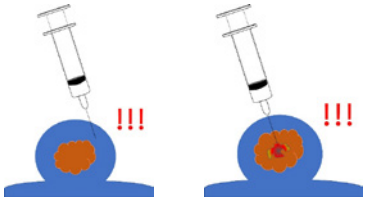


6. Hold syringe and needle close to a glass slide with the bevel pointing down.
7. Quickly expel the air through the needle, forcing contents onto slide.
8. Make smears of the expelled material using the *Slide-Over-Slide Technique*. If material is fluid, the recommendation is to prepare using the *Line Smear Technique*. Make sure to separate out any solid or flocculent material.
9. Once satisfied with sample obtained, stain as normal, as described in *Staining Technique*.

Syringe and Needle Technique (poorly exfoliating masses)

1. Attach a sterile 22 to 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 ⅔ 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. 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. Make smears of the expelled material using the *Slide-Over-Slide Technique*. If material is fluid, the recommendation is to prepare using the *Line Smear Technique*. Make sure to separate out any solid or flocculent material.
9. Once satisfied with sample obtained, stain as normal, as described in *Staining Technique*.

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, a necrotic center may have been sampled. Aspirates of the more viable, solid areas of a mass may yield a more viable sample. Preparation from the solid area, as well as any fluid obtained, is recommended.

Impression Smears for Dermal Samples

Direct Impression Smear

Materials

- #15 scalpel blade (dulled)
- Clean glass slides

Procedure

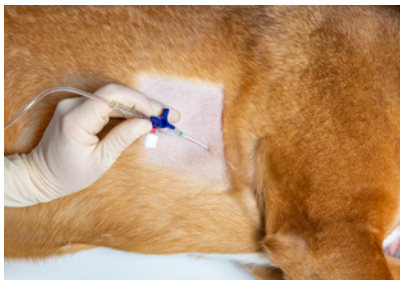
1. Samples are best collected from pustules, erosions, ulcers, draining lesions, crusts, or underneath leading edge of epidermal collarette.
2. Papular lesions can be traumatized by the corner of a glass slide or a needle and then squeezed to express fluid.
3. Lichenified regions can be sampled by sticking of the slide onto the affected area or using a dry scalpel blade to collect material and smear onto dry slide.
4. Allow material to dry and stain.

Pericardiocentesis

Materials

- Sterile gloves
- Clippers and aseptic surgical prep supplies
- ECG monitor
- 2% lidocaine, 3 OR 6 mL syringe with 22 gauge, ¾ inch needle for lidocaine injection
- #11 scalpel blade
- 14 or 16-gauge Abbott large bore over-the-needle catheter
- 30 or 60 mL syringe
- Consider three-way stopcock, IV extension tubing, and bowl if draining large amount of fluid
- Red and purple-top tubes
- Clean glass slides

Procedure



1. Attach ECG monitor to evaluate for cardiac dysrhythmias during procedure.
2. Clip and surgically prepare large area on the right lateral thoracic wall over the 5th to 8th ribs.
3. Insert bleb of lidocaine dorsal to the sternum and just caudal to the point of the elbow or at the 6th intercostal space. Ensure lidocaine is infused into the intercostal muscles. Continue to inject as the needle is drawn out creating a tunnel of anesthetized tissue.
4. Re-scrub the area and drape.
5. Make small stab incision through the anesthetized skin.
6. Insert over-the-needle catheter through the skin incision and body wall through anesthetized tissue watching ECG monitor closely.
7. Advance until flash of blood is noted in the needle hub.
8. Once a flash of blood is observed push the catheter off the stylette and remove stylette.
9. Attach the hub of the catheter to the syringe or length of IV tubing if withdrawing large amount of fluid from pericardial sac.
10. Place a small amount of pericardial fluid in the red and purple-top tubes. Watch for clot formation in red-top tube as this may indicate an active bleed or more commonly that the catheter has been inserted into the heart rather than the pericardium.
11. Prepare slides as described for fluid cytology. See *Concentrated Smear, Line Smear, Slide-Over-Slide Technique*. Stain as usual as described in *Staining Technique*.

*Contraindicated in cases of clotting disorders and/or rodenticide toxicity.

Thoracocentesis

Materials

- Sterile gloves, clippers, and aseptic surgical prep supplies
- 18–20 gauge fenestrated over-the-needle catheter for large dogs
- 19–21-gauge butterfly needle for cats and small dogs
- 20–60 mL syringe
- Purple and red-top tube for sample collection
- Clean glass slides
- Consider a collection bowl, three-way stopcock and IV extension tubing if removing a larger amount of fluid

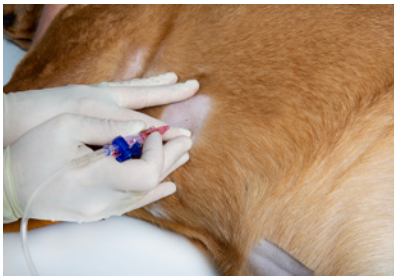
Procedure

Tranquilization and local anesthesia are generally not necessary for collecting a small sample but may be indicated if a large amount of fluid is going to be removed from the chest.

Utilize radiology or ultrasonography to visualize fluid and aid thoracocentesis especially if fluid is compartmentalized.

If fluid is not compartmentalized, thoracocentesis is performed approximately two thirds down the chest near the costochondral junction at the 6th, 7th or 8th intercostal spaces.

1. Place patient in sternal or lateral recumbency.
2. Surgically prepare a 4 to 8 cm area in the middle of the chest.
3. Insert the catheter or butterfly needle (attached to a syringe or IV extension tubing and syringe) next to the cranial edge of the rib avoiding the caudal aspect at the level of the costochondral junction at a 30° angle.



4. Rotate the bevel of the needle toward the center of the patient as the needle is inserted through the skin perpendicular to the body wall.
5. Once the catheter or butterfly needle is in the thoracic cavity, direct the needle parallel with the body wall and ventrally to avoid iatrogenic lung puncture.
6. Advance slowly until there is a "flash" of fluid in the hub.
7. Remove the needle if utilizing a catheter and attach the syringe or three-way stopcock with extension set in order to apply gentle negative pressure.
8. Prepare fluid sample utilizing the fluid preparation method and consider fluid centrifugation, if indicated. See *Concentrated Smear Technique, Line Smear Technique, Slide-Over-Slide Technique)* for further instruction. Stain as usual as described in *Staining Technique*.

Transtracheal Sampling

Materials

- Sterile gloves, clippers, and aseptic surgical prep supplies
- Light sedation drugs if indicated
- Small amount of 1% or 2% lidocaine
- Over-the-needle catheter or IV catheter and 3.5 French polyethylene urinary catheter
- Smaller catheters recommended for cats and small dogs
- 12 ml syringe or larger containing 1–2 ml/kg of non-bacteriostatic, sterile buffered saline
- Purple-top tube
- Clean glass slides

Procedure

1. Surgically prepare the skin over cranioventral larynx.
2. Inject a small amount of lidocaine into subcutaneous tissue.
3. Restrain in sitting position or sternal recumbency with the neck extended.
4. Palpate the small, triangular depression just cranial to the ridge of the cricoid cartilage. This is the location of needle insertion (cricothyroid ligament). Alternatively, the catheter may be inserted between two tracheal rings 1–3 cm below the larynx.
5. Directing the needle slightly caudal insert through the skin, subcutaneous tissue, and cricothyroid ligament of the larynx or in between tracheal rings as described above.
6. Once within the trachea lumen, position the needle parallel to the trachea. Advance the catheter down the trachea to just above the carina. This step usually induces coughing.

   If catheter does not pass easily, it may be embedded in the in the dorsal tracheal wall, failed to enter the trachea and embedded in the peritracheal tissue. In these cases, remove the entire needle and catheter and repeat procedure.
7. Remove the needle once the catheter is in place.
8. Sometimes samples can be obtained simply by aspirating at this point but oftentimes saline must be infused in order to obtain an adequate sample.
9. Inject saline through the catheter until a cough is elicited or all of the saline is injected.
10. Aspirate as soon as coughing starts. Aspirate for only a few seconds to avoid contamination. Only a small amount of fluid will be recovered, and this is normal.

    If cough does not occur, try coupage.
11. Apply gentle pressure to insertion site once catheter is removed. Gauze wrap can be utilized for 12–24 hours to help prevent subcutaneous emphysema.
12. Place sample into purple-top tube and prepare utilizing the fluid preparation method, as described in *Line Smear and Slide-Over-Slide* techniques. Consider fluid centrifugation for a concentrated sample, as described in *Concentrated Smear Technique,* if indicated. Stain as usual, as described in *Staining Technique.*

Transtracheal Wash/Bronchoalveolar Lavage

Materials

- Anesthesia and monitoring equipment
- Sterile gloves
- Sterile endotracheal tube
- 16 French polyvinyl chloride stomach tube for medium to large dogs OR 5 French polypropylene urinary catheter for cats
- Bronchoscope (if available)
- Bronchodilators (optional)
- Non-bacteriostatic, sterile buffered saline. Three 2 ml/kg aliquot for cats OR 3–2 mg/kg aliquots for dogs.
- Purple-top tube
- Glass slides

Procedure

1. Pre-oxygenate patient
2. A T or Y piece can be connected to the endotracheal tube to deliver oxygen and anesthetic gas throughout the procedure.
3. Anesthetize the patient and place the endotracheal tube with as little contact with the oropharynx and larynx as possible to avoid contamination.
4. Place in sternal or lateral recumbency. A foam wedge can be used in lateral recumbency to elevate the cranial thorax above the caudal thorax.

   BAL:
   Prior to inserting through the endotracheal tube, externally measure the catheter, bronchoscope or stomach tube to the level of the 7th and 11th ribs.

   Insert through the endotracheal tube. Advance tube until it stops, withdraw a few centimeters, rotate gently and re-advance until resistance is felt at a consistent level. This can be visualized if utilizing a bronchoscope.

   TTW:
   Externally measure to the level of the 4th intercostal space as sample collection does not occur past the level of the carina. Insert through the endotracheal tube to the measured depth.
5. Introduce appropriate amount of fluid (one aliquot at a time) and an additional 5 mL of air. Repeat aliquots may be administered until sufficient sample is retrieved but no more than 3 aliquots are generally used.
6. Prepare sample smears closely to time of collection. Remove mucous sample with pipette or applicator tip. Aspirate sample when close to mucous to avoid excess saline.
7. Put small amount of mucous sample onto slide, remove excess saline and utilize *Slide-Over-Slide Technique*. Allow to dry and stain as usual. See *Staining Technique*.

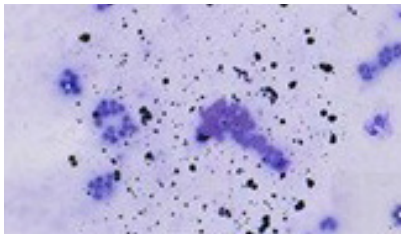
Troubleshooting Sample Preparation Techniques

Common Problems in Blood Film Preparation

Stain Precipitate

Problem

These artifacts tend to have a homogenous deep purple color and vary in shape and size. This is crystallized stain that can be mistaken for bacterial organisms.



Solution

Replace stain every 1–2 weeks and store at room temperature. Stain may need to be replaced more frequently depending on usage. If staining artifacts are noted the stain should be replaced, regardless of the time frame.

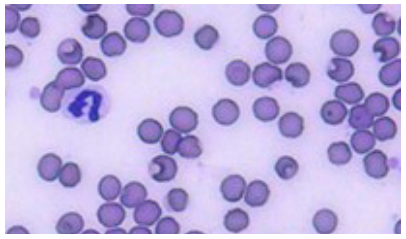
Ensure slide is not sitting in Solution III for too long and is properly rinsed following staining.

Also, double check that stains have not expired prior to refilling stain dishes. Stain containers should be covered to reduce contamination. Stain containers should be cleaned each time the stain is replaced. Do not 'top-off' solutions when fluid levels drop or staining quality declines.

Water Artifact

Problem

Refractile spot in cell due to contamination of stain with water. When severe, cells can appear moth-eaten and can be mistaken for a red blood cell inclusion such as a parasite.



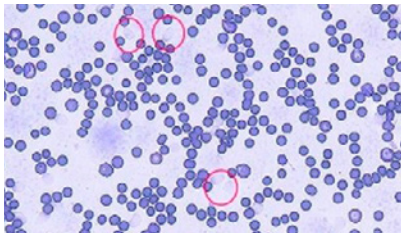
Solution

Dry slides completely before viewing or scanning and keep stain containers closed when not in use. Ensure stains are fresh and replace as needed.

'Ghost' Red Blood Cells

Problem

Cells appear pale and swollen with clear preservation of cellular outline that can often be vacuolated potentially mimicking true intravascular hemolysis.



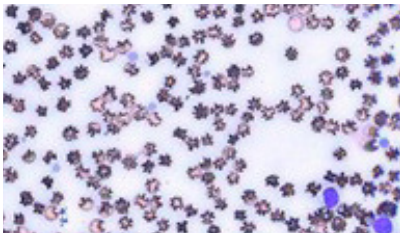
Solution

Evaluate blood collection and storage procedure. Red blood cells can be damaged or 'sheared' if venipuncture is difficult or needle gauge was not large enough. For venipuncture a minimum of a 23 G needle should be used (21–22 G is ideal for small animals and 18–20 G is ideal for large animals). Additionally, ensure blood tubes are delicately inverted rather than shaken and blood is gently expulsed into anti-coagulant tube.

Sample Age Related Changes

Problem

Blood or other fluid samples stored in specimen tubes for too long alters cellular appearance on blood films. Red blood cells can appear crenated or 'spiked', ruptured, or swollen potentially masking pathologic shape changes. Leukocyte nuclei can undergo pyknosis and karyorrhexis making identification very difficult. Platelets can clump and degranulate.



Delay of fixation of sample onto slide (over 24 hours) can lead to cytoplasmic and nuclear vacuoles, patchy staining, loss of cellular architecture, and possible bacterial contamination.

Solution

Fix sample onto slide and stain within 15 minutes of collection. Storage of blood or other fluid within sample tube prior to slide preparation should be no longer than 24 hours in cold storage (refrigerated not frozen).

Cells are Not 'Blue' Enough

Problem

Basophilic staining is weak.

Solution

Solution III (blue, basophilic stain) may be diluted. Try replacing and leaving slide in basophilic stain longer. Also ensure there is adequate amount of basophilic stain in stain dish.

Cells are Too 'Blue'

Problem

Basophilic stain is too strong.

Solution

Decrease time in Solution III (blue, basophilic stain) and ensure smear is not too thick.

Cells are Too 'Pink'

Problem

Eosinophilic stain is too strong.

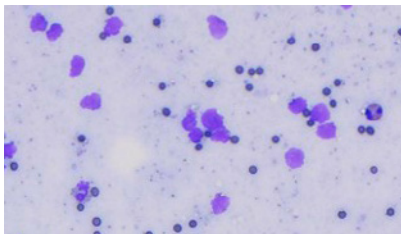
Solution

Decrease time in Solution II (pink, eosin stain) and ensure Solution III (blue, basophilic stain) is fresh and filled completely.

Ruptured Cells

Problem

Broken cells appearing small and irregular with free nucleoli. Typically, smudged cells have enlarged, pale nuclei with more prominent nucleoli. This may occur from trauma from the aspirate or slide making procedure.



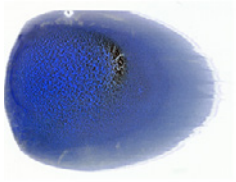
Solution

Using the needle only technique (no negative pressure applied during sampling) to sample lymph nodes or masses may reduce the frequency of artifactually distorted or broken cells. Use only gentle pressure when making slides. When spreading sample pull the spreader slide in a swift motion along the entire length of the bottom slide until it has naturally run off the sample slide. Do not pull the spreader up prematurely or add any extra pressure.

Monolayer is not Present

Problem

Lack of feathered edge on blood smear.



Solution

Refer to *Sample Preparation Techniques, Blood Smear Techniques*.

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.

Tips and Tricks

- Ultrasound gel will obscure nucleated cells and mimic bacterial organisms. Wipe the skin free of ultrasound gel prior to any 'centesis' procedure.
- Save more effusion fluid than you think you will need!
- Never put fluid intended to be analyzed for cytology with a crystalline clot activator (Red-Top with a White Ring or Yellow-Top tube).
- The minimum needed to classify an effusion MUST include total protein and total nucleated cell count.
- Consider an archive of stained, direct preparation effusion slides with known numerical total nucleated cell counts for reference.

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