

Preparing Slides for
IDEXX Digital Cytology*



The number of and types of slides that you'll need to prepare will vary depending on the sample type:

Sample type	Slide preparation and workflow required
FNA	Prepare 2 direct slides.
Blood	Run the sample on an IDEXX in-house hematology analyzer. Prepare 2 blood smears.
Body effusion and joint fluid	Record protein with refractometer and run on an IDEXX in-house hematology analyzer as the specified sample type. Prepare 1 direct slide. Prepare 1 line preparation (unconcentrated).
Tracheal wash or bronchoalveolar lavage	If flocculant material is present, prepare 1 direct slide and 1 direct squash of material. If flocculant material is not present, prepare 1 direct slide and 1 line preparation (unconcentrated).
Urine sediment	Prepare 1 direct slide. Prepare 1 line preparation (concentrated).

Digital slide submission tips

When selecting slides to scan, be sure to:

- Visually inspect the slides in good light, without the microscope.
- Evaluate the stained slides under the microscope. The best slides contain visible cellular material on low power (4× and 10×) and a majority of intact cells when observed at higher magnification (10×–20×).

Important: When preparing slides, remember to:

- Label each with the name, date, source, and slide preparation type.
- Save the sample and any stained slides submitted digitally for 2 weeks.
- Save 1–2 additional unstained slides in case additional testing is recommended.

Need help with sample preparation?

Watch the videos available at

[idexxlearningcenter.com](https://www.idexxlearningcenter.com).

Preparing FNA slides (2 direct slides recommended)

Skin/subcutaneous masses, lymph nodes, and internal organs

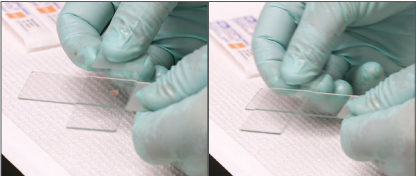
Note: Selecting the optimal needle gauge is important as small gauge needles may cause increased cell lysis and large gauge needles may introduce excessive blood. 22-gauge needles work well for most tissue types.

Fine needle aspiration technique:

1. Immobilize the lesion with one hand while introducing a needle with a syringe attached.
2. Draw the sample into the syringe by withdrawing the plunger with negative pressure.
3. Release negative pressure and then withdraw the needle.
4. Remove the needle from the syringe, aspirate air into the syringe, and then reattach the needle.
5. Expel the cellular material onto 2 slides.
6. After sample is applied on one slide, place another slide on top so that they are perpendicular to each other. Without adding pressure, pull the top slide down the length of the bottom slide with even pressure to make a smear.
7. Let the slide air-dry or use a fan (not heat) to avoid air-drying artifacts.
8. Stain the slides and allow to air-dry or use the cool setting on a fan. For more information, refer to the *Staining Slides for IDEXX Digital Cytology* mat.



Fine needle nonaspiration technique:

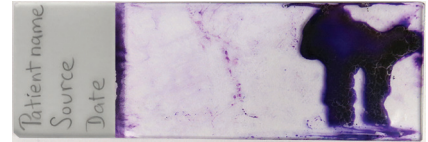
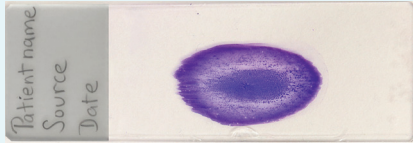
1. Immobilize the lesion with one hand while introducing a needle.
 2. Once in the lesion, move the needle up and down several times at an angle and in the same needle track to collect material from the lesion.
 3. Aspirate air into the syringe, and then attach the needle to the syringe.
 4. Expel material onto 2 slides using the air in a syringe.
 5. After the sample is applied on one slide, place another slide on top so that they are perpendicular to each other. With light, even pressure, pull the top slide down the length of the bottom slide to make a smear.
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6. Let the slide air-dry or use a fan (not heat) to avoid air-drying artifacts.
 7. Stain the slides and allow to air-dry using the cool setting on a fan. For more information, refer to the *Staining Slides for IDEXX Digital Cytology* mat.

Selecting FNA slide(s) to scan

What to look for

What to avoid

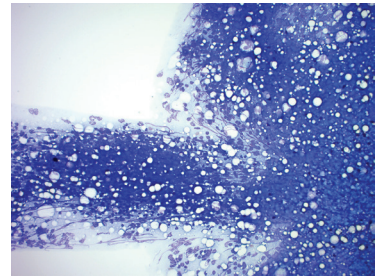
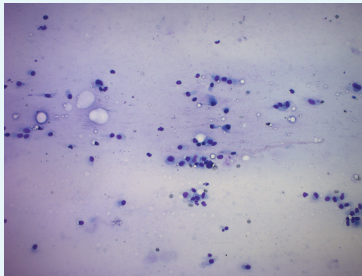
Visual slide inspection



Slide has visible material (typically stains blue)/ sample is spread on the slide

Mostly blood or thick sample droplets, sample covering frosted edge, or sample material on the opposite side of the frosted edge

Microscopic screening (4x, 10x or 20x objective)



Intact cells and layers of cells that are one cell thick with good cell color contrast

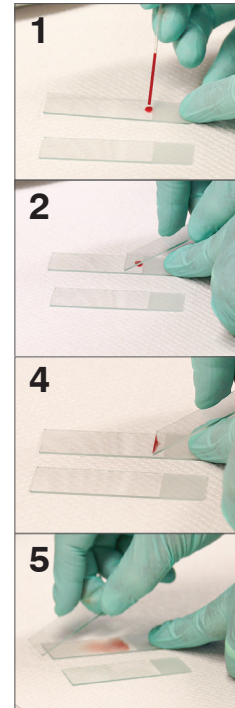
Predominance of lysed, overly pink, or pale cells

Preparing blood film slides (2 blood smears recommended)

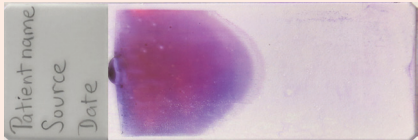
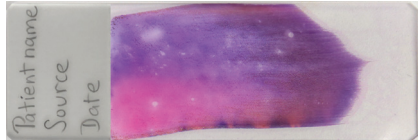
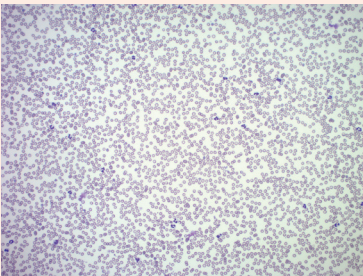
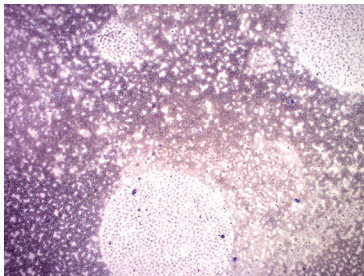
Prepare a blood film using fresh blood (<24 hours old). Sample deterioration occurs with prolonged storage.

Blood film technique

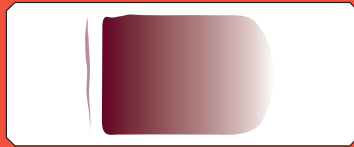
1. Place a small drop of fresh, well-mixed anticoagulated blood on a clean glass slide approximately 2 cm from one end of the slide.
2. Place a clean glass spreader slide in front of the drop of blood at approximately a 30° angle to the film slide.
3. Back the spreader slide into the drop of blood.
4. Let the blood spread along the contact line between the two slides until it covers $\frac{3}{4}$ of the width of the slide (this should take place quickly).
5. With a steady and seamless movement, move the spreader slide down the entire blood film slide, maintaining the angle without lifting the spreader slide. Blood from the drop will follow the spreader slide, placing a thin film on the other slide. The blood film should be 3–4 cm in length and the shape of a thumb print.
6. Let the slide air-dry or use a fan (not heat) to avoid air-drying artifacts.
7. Stain the slides and allow to air-dry or use the cool setting on a fan. For more information, refer to the *Staining Slides for IDEXX Digital Cytology* mat.



Selecting blood film slides to scan

	What to look for	What to avoid
Visual slide inspection	 <p>A thumbprint appearance and presence of a feathered edge</p>	 <p>Uneven staining, incomplete and asymmetrical feathered edge</p>
Microscopic screening (4x, 10x or 20x objective)	 <p>Presence of monolayer, minimal to no stain precipitate</p>	 <p>Sample over 48 hours old, uneven film, lysed cells</p>

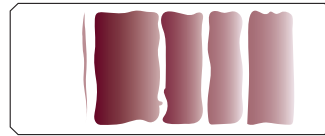
Troubleshooting blood smears



Do make sure there is a feathered edge.



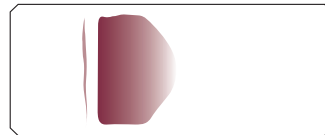
Don't use a dirty or chipped spreader slide.



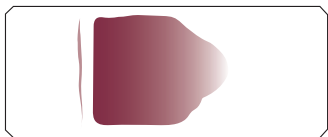
Don't hesitate with the spreader slide.



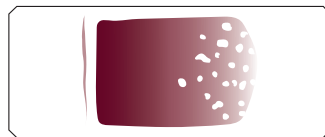
Don't go too fast.



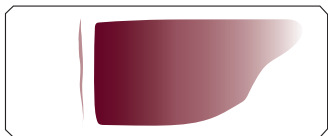
Don't use a drop of blood that is too small.



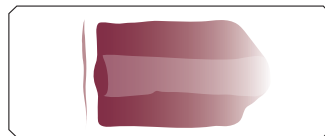
Don't move so quickly that the blood doesn't have a chance to spread across the spreader slide.



Don't use slides contaminated with dirt or grease, or with elevated lipids in the sample.



Don't use uneven pressure on the spreader slide.



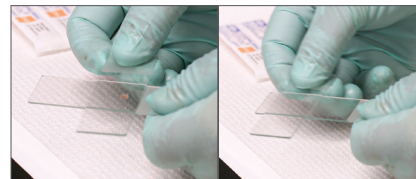
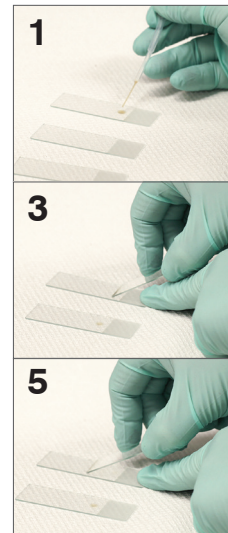
Don't allow the drop of blood to begin to dry (avoid time delays).

Preparing fluid slides (1 direct and 1 concentrated line prep recommended)


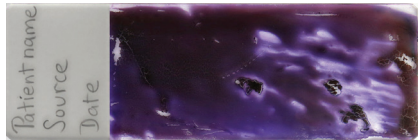
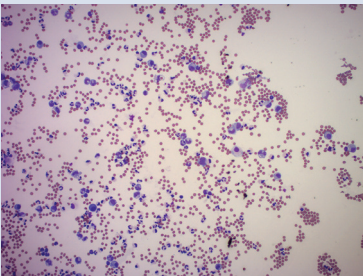
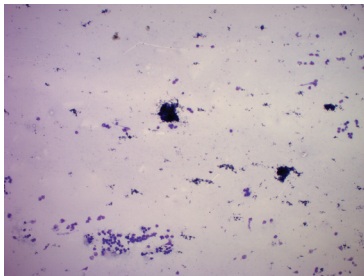
Body cavity effusions, bronchoalveolar lavage (BAL)/ tracheal lavage, or joint fluid:

IMPORTANT: Record protein with refractometer and run on hematology analyzer using the appropriate setting per the sample type. Prepare 1 direct slide and 1 line preparation slide (unconcentrated). When creating a requisition for fluid samples, specify color, clarity, and total protein when submitting the test.

- If the sample has low cellularity or when infectious agents are suspected, an additional line smear may be prepared to enhance cytologic evaluation:
 1. Place a drop of well-mixed, nonconcentrated fluid on a clean glass slide.
 2. Place a clean glass spreader slide in front of the drop of fluid/urine at approximately a 30°–40° angle to the smear slide.
 3. Back the spreader slide into the drop, allowing the material to spread along the edge of the spreader slide.
 4. Move the spreader slide toward the end of the specimen slide, keeping the two in contact with each other.
 5. In the middle of the slide, abruptly stop spreading the sample and lift the spreader slide straight up to form a line of material.
 6. Let the slide air-dry or use a fan (not heat) to avoid air-drying artifacts.
 7. Stain the slides and allow to air-dry or use the cool setting on a fan. For more information, refer to the *Staining Slides for IDEXX Digital Cytology* mat.
- Additional preparations: If flocculent material is present, collect some of the material and prepare an additional slide using the smear technique described for cytology samples.
- For samples with large blood content, a buffy coat may be prepared for submitting along with a direct film.

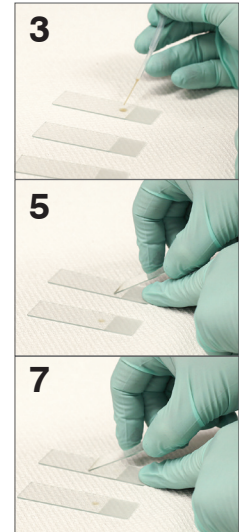


Selecting fluid slides to scan

	What to look for	What to avoid
<p>Visual slide inspection</p>	 <p>Thin film of sample and noticeable, distinct strip of material in line smear</p>	 <p>Sample droplets (poor spreading)</p>
<p>Microscopic screening (4x, 10x or 20x objective)</p>	 <p>Good cell color contrast, intact cells, even cell distribution (direct smear), and minimal stain precipitate</p>	 <p>Stain precipitate, air-drying artifacts (fuzzy cells), and lysed cells</p>

Preparing urine sediment slides (1 direct and 1 concentrated line preparation)

1. Fill a centrifuge tube with 5 mL fresh, well-mixed urine and then centrifuge it on the urine setting (or 400 x g). If your centrifuge does not have a urine setting, refer to your operator's guide for centrifugation settings and times.
2. Gently aspirate the supernatant down to the pellet, leaving an extremely small amount of urine in which to resuspend the pellet. Then lightly flick the bottom of the tube multiple times with your finger to gently resuspend the formed elements.
3. Place a drop of well-mixed, nonconcentrated fluid or resuspended urine sediment (obtained via centrifugation) on a clean glass slide.
4. Place a clean glass spreader slide in front of the drop of fluid/urine at approximately a 30°–40° angle to the smear slide.
5. Back the spreader slide into the drop, allowing the material to spread along the edge of the spreader slide.
6. Move the spreader slide toward the end of the specimen slide, keeping the two in contact with each other.
7. In the middle of the slide, abruptly stop spreading the sample and lift the spreader slide straight up to form a line of material.
8. Let the slide air-dry or use a fan (not heat) to avoid air-drying artifacts.
9. Stain the slides and allow to air-dry or use the cool setting on a fan. For more information, refer to the *Staining Slides for IDEXX Digital Cytology* mat.

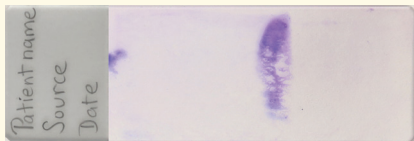


Selecting urine slides to scan

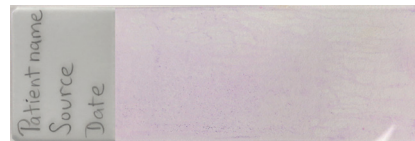
What to look for

What to avoid

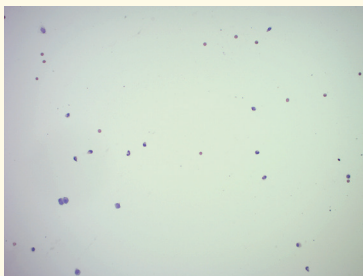
Visual slide inspection



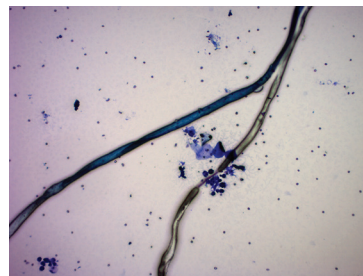
Thin film of sample and noticeable, distinct strip of material in line smear



Insufficient drying can lead to washing off the sample during staining

Microscopic screening
(4×, 10× or
20× objective)

Good cell color contrast, intact cells, even cell distribution (direct smear), and minimal stain precipitate



Presence of foreign material, overstained slides

For more information on pathology testing, visit [idexx.com/pathology](https://www.idexx.com/pathology).

