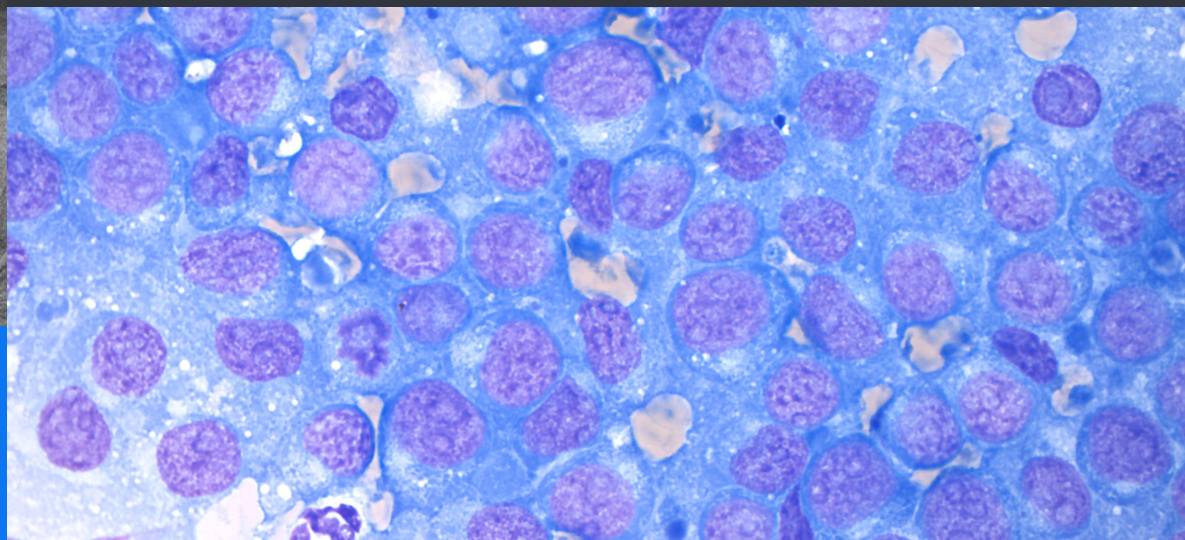
IDEXX

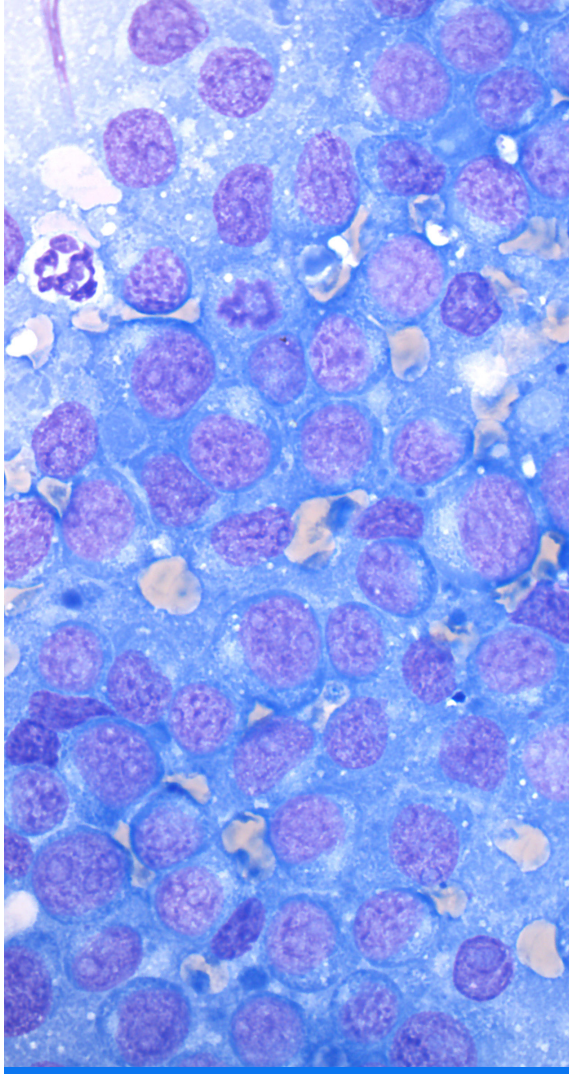
Preparing high-quality cytology slides



CREATING CLARITY + + + + +

One or two high-quality slides are sufficient for your most common cytology submissions.

Specimen type	Slide preparation and workflow required
FNA	+ Prepare 2 direct slides.
Blood	+ Prepare 2 blood films.
Body effusion and joint fluid	+ Prepare 1 direct slide. + Prepare 1 line preparation from unadulterated (neat) specimen.
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 from well-mixed, nonconcentrated fluid.
Urine sediment (concentrated)	+ Prepare 1 direct slide. + Prepare 1 line preparation.



Slide submission tips

When selecting slides to submit to the reference lab, 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 (4x and 10x) and a majority of intact cells when observed at higher magnification (10x–20x).

Note: Glass submission to the IDEXX Reference Laboratories, please submit at least one unstained slide.

Important slide preparation information

When preparing slides, remember to:

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

Need help with specimen preparation?

Watch the videos available at idexxlearningcenter.com.

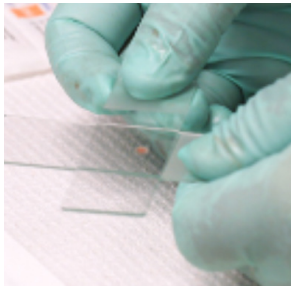
Preparing FNA slides (2 direct slides recommended)

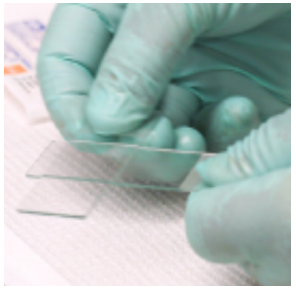
Skin/subcutaneous masses, lymph nodes, and internal organs

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 specimen into the syringe by withdrawing the plunger, creating negative pressure.
3. Release the plunger 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 by pressing the plunger.
6. After specimen is applied to 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 Staining Slides for Cytology.

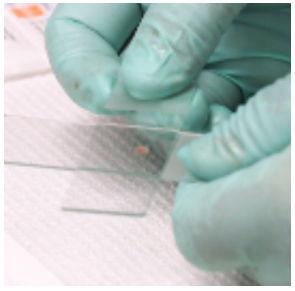


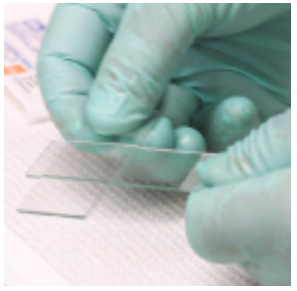


Step 6

Fine needle nonaspiration technique:

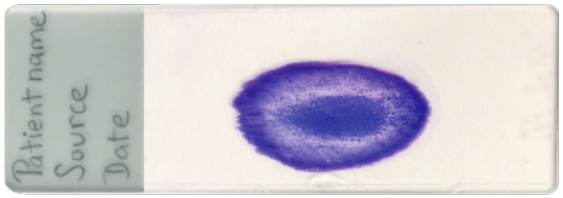
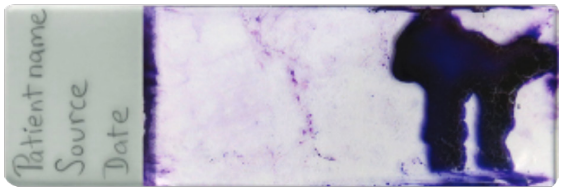
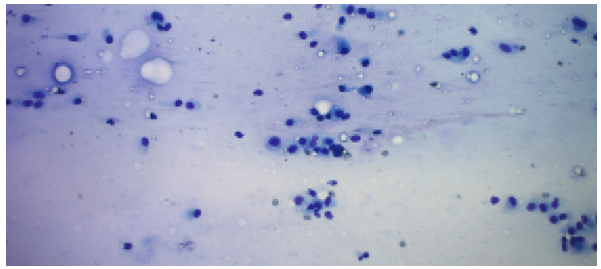
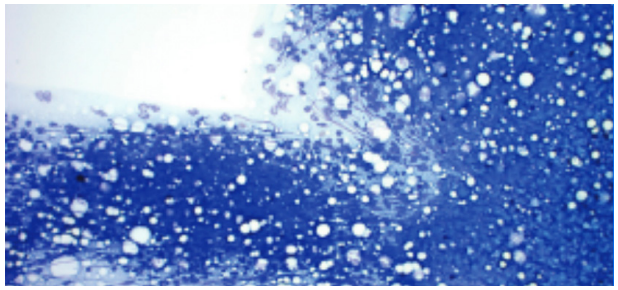
1. Immobilize the lesion with one hand while introducing a needle.
2. Once in the lesion, move the needle up and down several times at an angle and in the same needle track to collect material from the lesion.
3. Withdraw the needle.
4. Aspirate air into a syringe, and then attach the needle to the syringe.
5. Expel material onto 2 slides using the air in the syringe.
6. After the specimen is applied to 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.
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 using the cool setting on a fan. For more information, refer to Staining Slides for Cytology.





Step 6

Selecting FNA slide(s) for submission

	What to look for	What to avoid
Visual slide inspection	 + Slide has visible material (typically stains blue)/specimen is spread on the slide	 + Mostly blood or thick droplets of specimen not evenly or thinly spread (as shown above) + Specimen covering frosted edge, or specimen 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

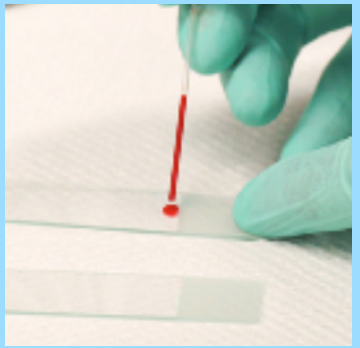
Preparing blood films
(2 blood films recommended)

Prepare a blood film using fresh blood (< 24 hours old). Specimen 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 ¾ 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 Staining Slides for Cytology.

1



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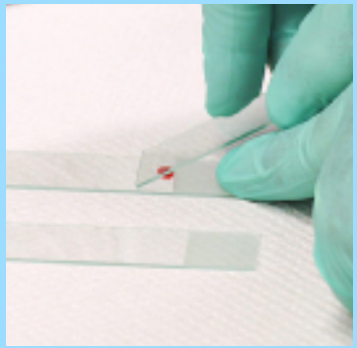

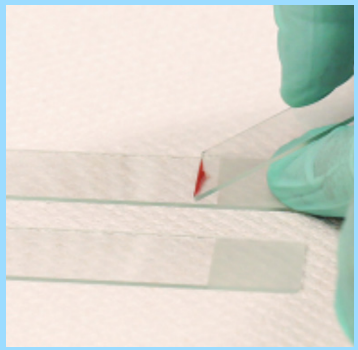

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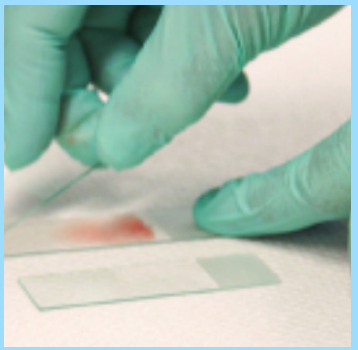
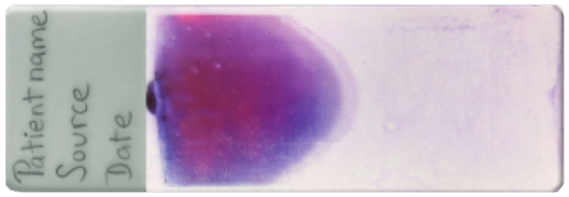
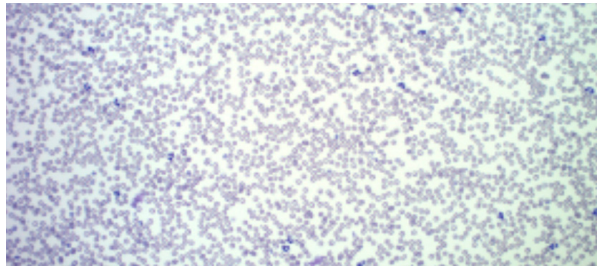
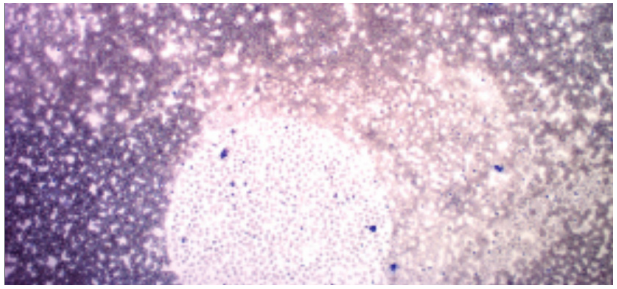


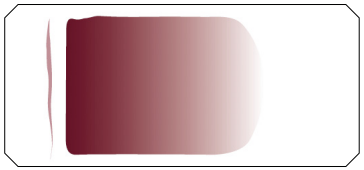
5

Selecting blood films for submission

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

Troubleshooting blood films

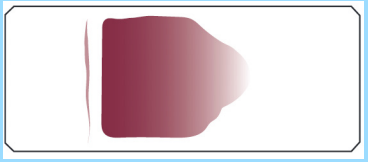
DO



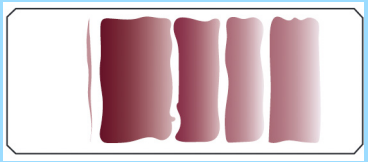
Do make sure there is a feathered edge.

DON'T

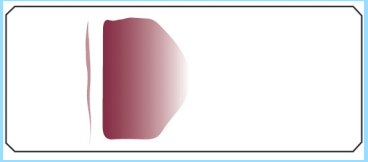
Don't use a dirty or chipped spreader slide.



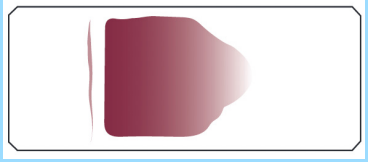
Don't go too fast.



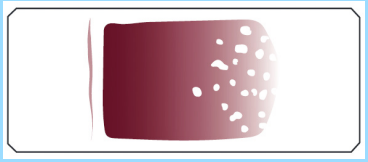
Don't hesitate with the spreader slide.



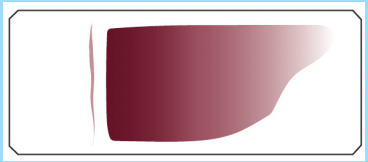
Don't use a drop of blood that is too small.



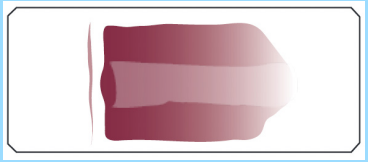
Don't move so quickly that the blood doesn't have a chance to spread across the spreader slide.



Don't use slides contaminated with dirt or grease. Elevated blood lipids may give a similar appearance.



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

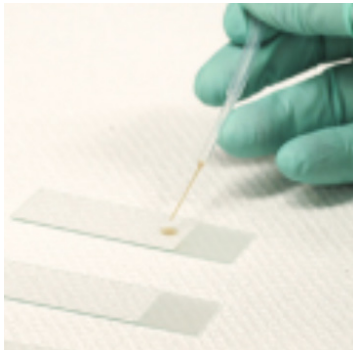
IMPORTANT: Record color, clarity, and protein with refractometer. Run on hematology analyzer using the appropriate setting per the specimens type. Prepare 1 direct slide and 1 line preparation slide. When creating a requisition for fluid specimens, specify color, clarity, and total protein when submitting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

+ Prepare a direct slide. For more information, see the Blood Films section on pages 6–8.
+ If the specimens 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 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 specimens 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 Staining Slides for Cytology.

+ Additional preparations: If flocculent material is present, collect some of the material and prepare an additional slide using the blood film technique (see “Preparing blood films” on page 6).
+ For specimens with large blood content, a buffy coat may be prepared for submitting along with a direct slide.

1



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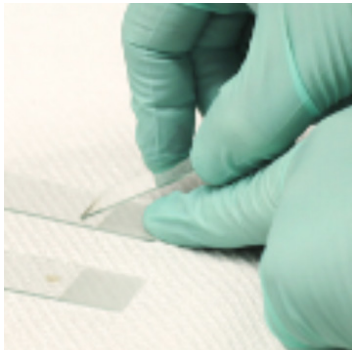

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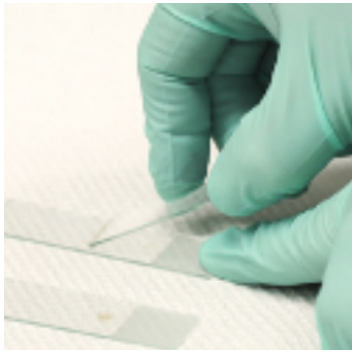

Selecting fluid slides for submission

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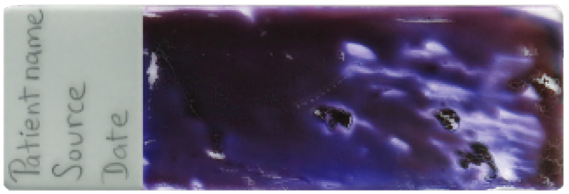
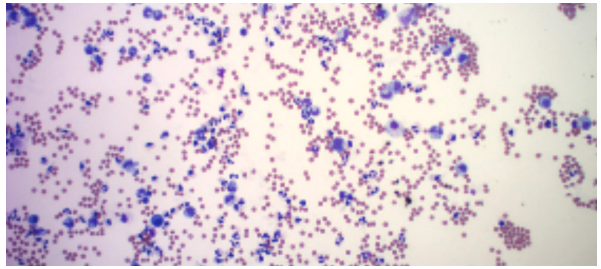
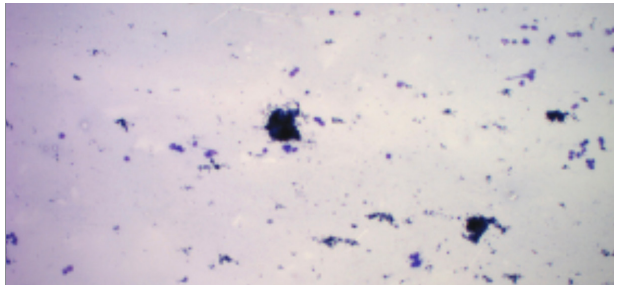
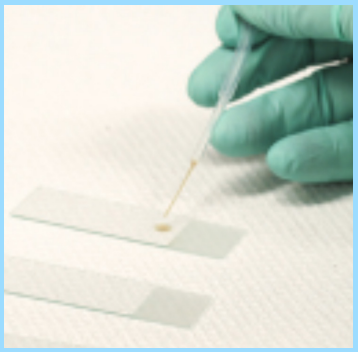
Visual slide inspection		
	+ Thin film of specimen and noticeable, distinct strip of material in line smear	+ Specimen too thick and poor spreading
Microscopic screening (4×, 10x or 20x objective)		
	+ Good cell color contrast, intact cells, even cell distribution (direct slide), and minimal stain precipitate	+ Stain precipitate, air-drying artifacts (fuzzy cells), and lysed cells

Preparing urine sediment slides

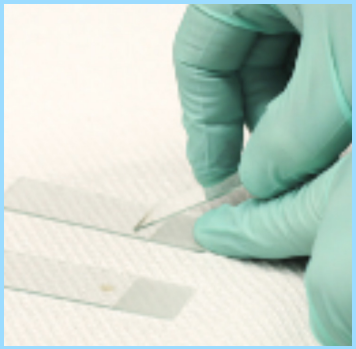
(1 direct and 1 concentrated line preparation)

1. Concentrate a urine sample using these steps:
   a. Fill a centrifuge tube with 5 mL fresh, well-mixed urine and then centrifuge it on the urine setting (or 400 x g) for 5 minutes. If your centrifuge does not have a urine setting, refer to your operator's guide for centrifugation settings and times.
   b. 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.
2. Prepare a direct slide. For more information, see the Blood Films section on pages 6–8.
3. Prepare a line preparation slide using these steps:
   a. Place a drop of well-mixed resuspended urine sediment (obtained via centrifugation) on a clean glass slide.
   b. Place a clean glass spreader slide in front of the drop of fluid at approximately a 30°–40° angle to the smear slide.
   c. Back the spreader slide into the drop, allowing the material to spread along the edge of the spreader slide.
   d. Move the spreader slide toward the end of the specimen slide, keeping the two in contact with each other.
   e. In the middle of the slide, abruptly stop spreading the specimen and lift the spreader slide straight up to form a line of material.
   f. Let the slide air-dry or use a fan (not heat) to avoid air-drying artifacts.
4. Stain the slides and allow to air-dry or use the cool setting on a fan. For more information, refer to Staining Slides for Cytology.

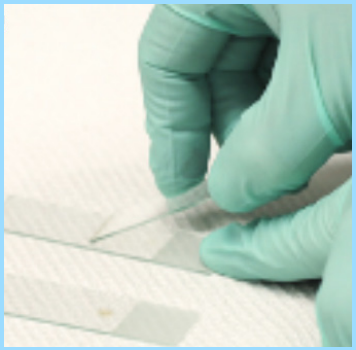
3a



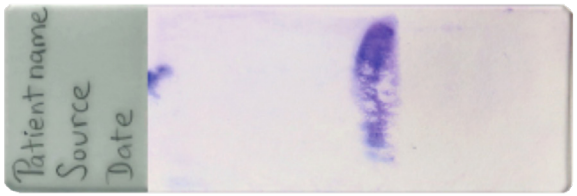
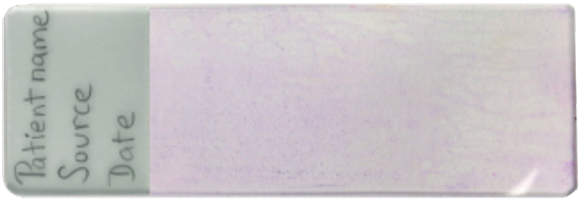
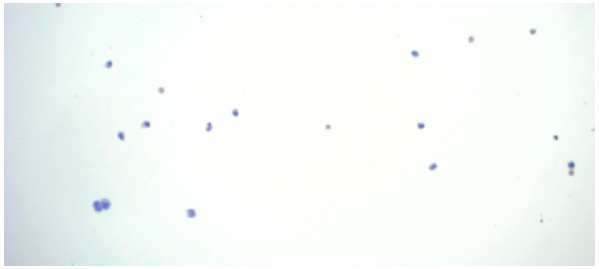
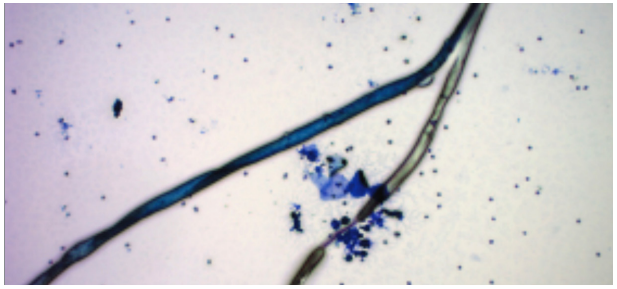
3c

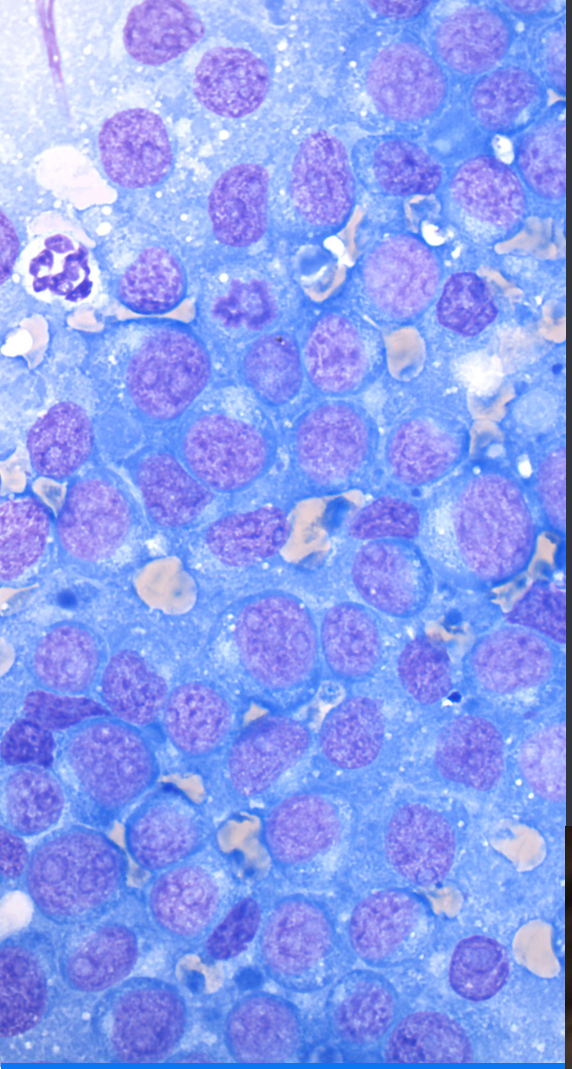


3e



Selecting urine slides for submission

	What to look for	What to avoid
Visual slide inspection	 + Thin film of specimen and noticeable, distinct strip of material in line smear	 + Insufficient drying can lead to washing off the specimen during staining
Microscopic screening (4x, 10x, or 20x objective)	 + Good cell color contrast, intact cells, even cell distribution (direct slide), and minimal stain precipitate	 + Presence of foreign material, overstained slides



IDEXX

For more information on pathology testing, visit idexx.com/pathology.