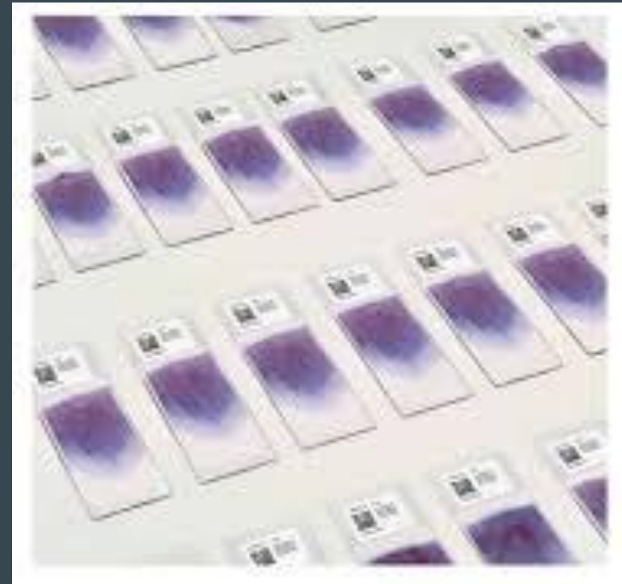
Slide Preparations

Blood smears, urine dry preps, slide agglutinations, urine sediments (wet preps)

Blood Smear Preparation

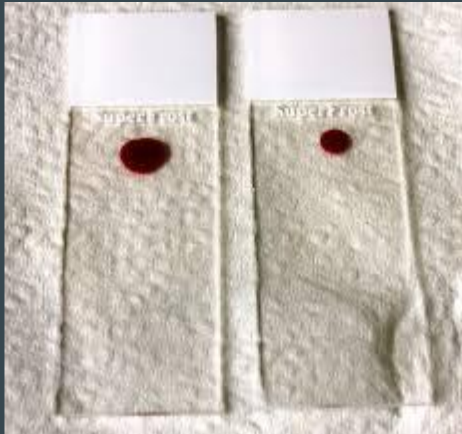
When do we do this?

- With every CBC
  - Idexx machines are generally quite accurate
  - The human eye can be more discerning of abnormalities of cells
- Manual platelet counts



Step 1
Add blood drop

Too big



Good size

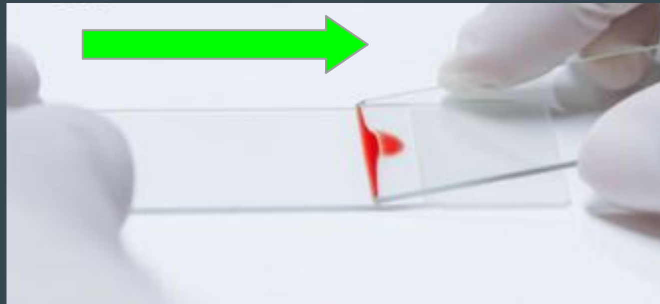
- Get two clean slides
- Place them frosted-side up on a flat surface/counter
- Use needled syringe, pipette, or PCV tube to place a small drop of blood on one slide
  - Make sure your blood is fresh or from a well-mixed EDTA tube (LTT)
  - Center the drop on the midline of the slide, close to the frosted end
  - Practice helps you gauge if a blood drop is too big or small

Step 2

Use spreader slide

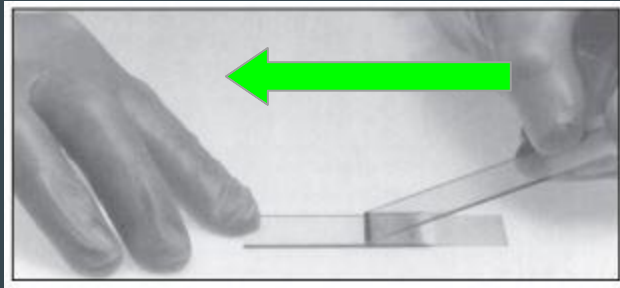
Spreader slide

Sample slide



- Using a new, clean slide (spreader slide), touch its bottom edge to your sample slide
  - Hold the spreader slide firmly by the edges
  - Create a 30°-40° angle
- Slowly drag the slide **back** until it touches your blood droplet
  - Capillary action will cause the blood to cling to the spreader slide
  - Allow the blood to migrate along the juncture of the two slides until it covers most of the width of the sample slide (bottom slide)

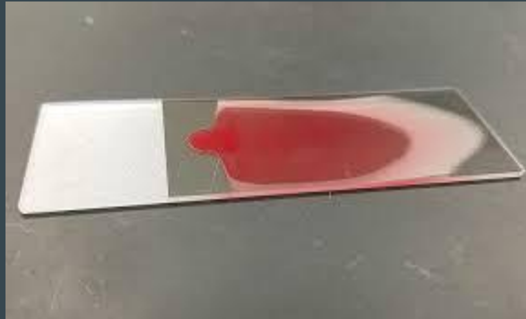
Step 3
Create blood smear



- Using a smooth, forward motion, push the spreader slide forward across the sample slide
  - This drags the blood out in a thin layer on the sample slide
  - You do not need much pressure
  - Maintain contact between the two slides the entire time
- The film of blood should cover approximately ⅔ of the sample slide

Step 4
Evaluate the slide

Unstained Stained



- Does your blood smear cover approximately ⅔ of your slide?
  - If not, you may need to change the angle of your spreader slide
    - Bigger angle = thicker/shorter smear
    - Smaller angle = thinner/longer smear
  - Or change the size of your blood droplet
- Is there a nice, feathered edge?
  - This is the thinnest portion of blood, at the end of your smear (farthest from starting droplet)
  - The **monolayer** by the feathered edge is where you read your diff
- Label your slide
  - Write the patient's name on the frosted section of slide
  - Use PENCIL!

Step 5
Stain the slide

Unstained Stained

- Allow your slide to fully air-dry
- Use the three jars of Diff-Quik stain **(BLOOD STAIN JARS)**
  - Blue - fixative
  - Pink - eosinophilic
  - Purple - basophilic
- Dip your slide sequentially through each stain
  - Approximately 10-15 seconds each
  - Blot excess stain on a paper towel in between colors
  - Use a clothes pin to grasp slide
- Using a *gentle stream* of water, rinse the slide after the last stain
- Allow to air dry

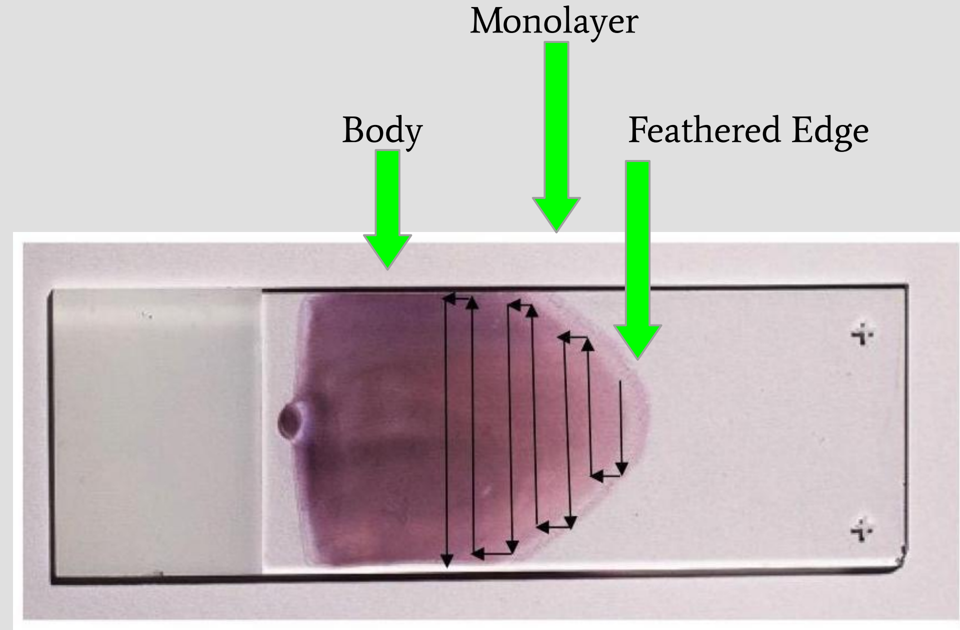
Troubleshooting Blood Slides

A to H : Unacceptable peripheral blood films.
Slide appearances associated with the most common errors.

A Chipped or rough edge on spreader slide.
B Hesitation in forward motion of spreader slide.
C Spreader slide pushed too quickly.
D Drop of blood too small.
E Drop of blood not allowed to spread across the width of the slide.
F Dirt or grease on the slide; may also be PB specimen elevated lipids.
G Uneven pressure on the spreader slide.
H Time delay; drop of blood began to dry prior to spread.

Reading Differentials

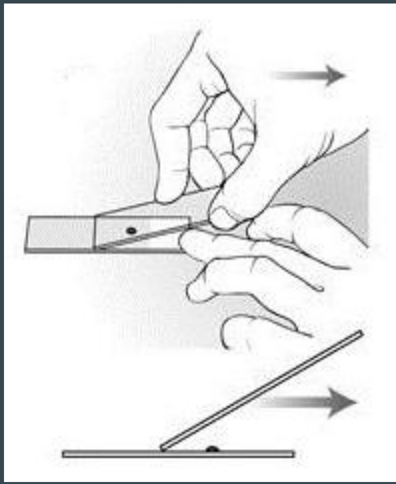
- Move to different fields of the slide using a **scan pattern** as shown to the right
- Check the extreme feathered edge where cells are very sparse
  - Unusually large cells
  - Platelet clumps
  - Abnormalities (i.e. microfilaria)
- Do your differential count in the **cellular monolayer** where the feathered edge meets the body of the smear
- Do not count in the body where cells are overlapping
- Poorly made slides may have
  - White cells accumulating in the thin areas of the feathered edge
  - Platelet clumping
  - Artifact rouleaux
  - Poor staining making platelets or WBCs difficult to identify accurately



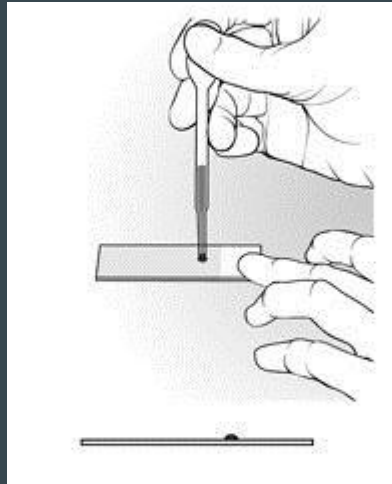
Slide issues

Slide issues

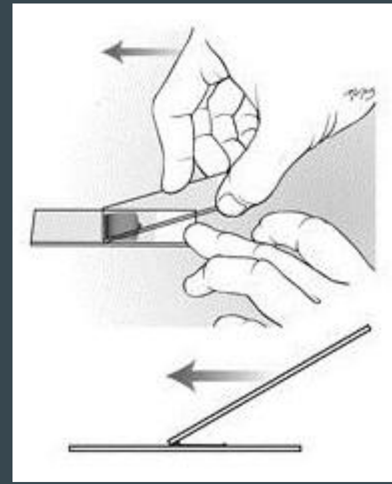
Slide issues



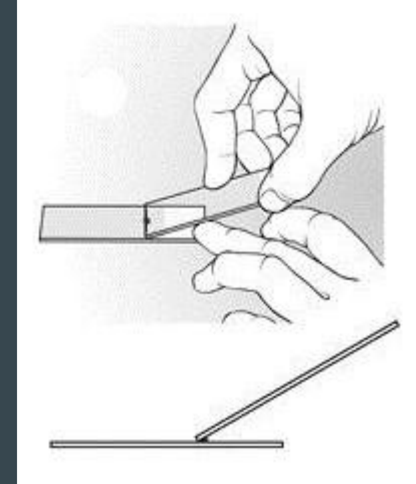
A



B



C



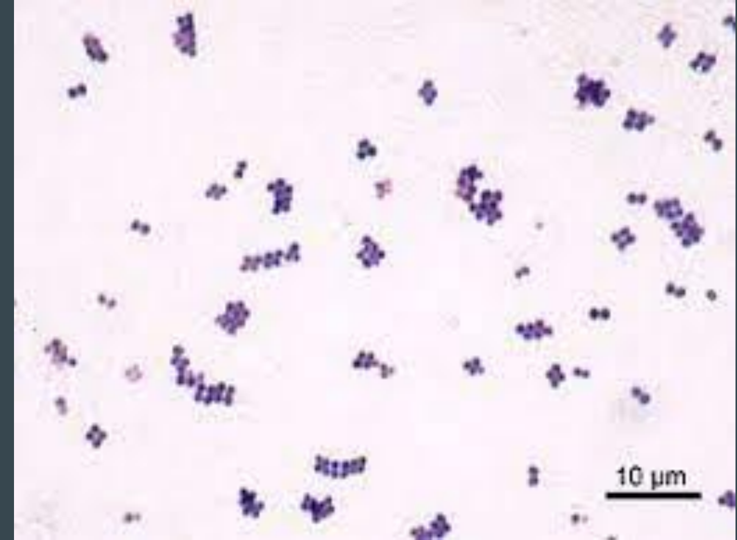
D

1. Arrange these pictures in order.
   B - A - D - C
2. In a good slide, approximately how much of the slide should the blood film cover?
   Approximately ⅔ of the slide
3. What is the area of the smear called where the you will examine cells for the differential?
   The monolayer
4. Where do you label your slide? What do you use to label it?
   The frosted section, with pencil

Urine Dry Preparation

Why do we do this?

- To confirm the presence or absence of bacteria in a urine sample
  - Identify type of bacteria, if present (cocci vs. rods)
- Examine and characterize other cellular elements in urine



Step 1
Spin down urine

Unspun Spun

- Only perform a dry prep urine slide after a SediVue and other lab tests are complete
- Use centrifuge to spin urine down in white top tube
- Carefully remove tube from centrifuge
  - You do not want to disturb the pellet at the bottom
  - Tipping or shaking the tube can resuspend the solids that have formed the pellet
  - If the sample has very few cells, the pellet may be difficult to see

Step 2

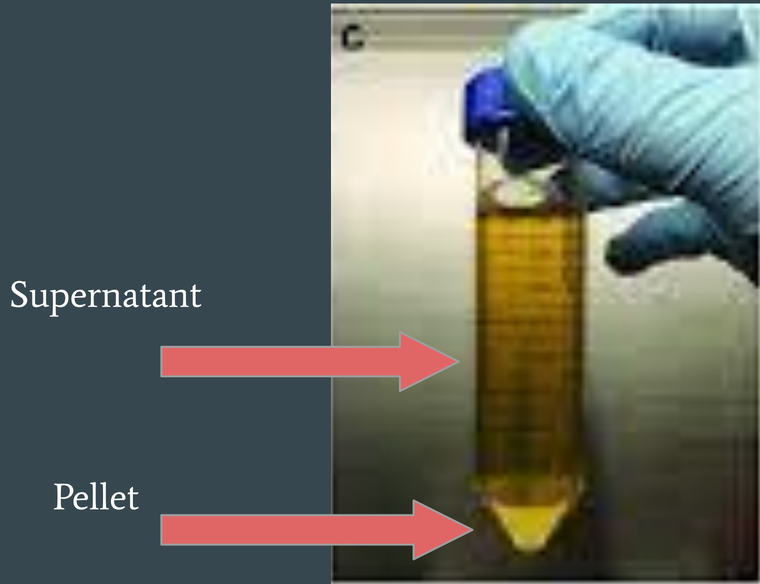
Remove fluid part

Supernatant

Pellet

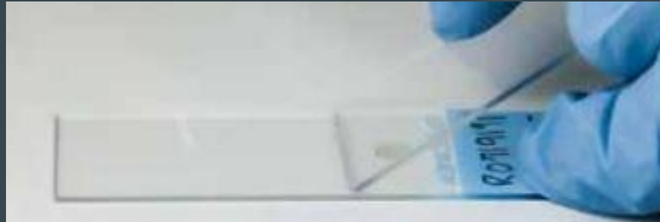
- Using a pipette, gently and carefully remove supernatant (fluid portion)
- Do not disturb the pellet
- Leave a very small amount of fluid in the tube

Step 3
Resuspend solids



- Gently flick the bottom of the tube a few times
- This agitates the pellet and causes it to be redistributed in the remaining fluid
  - This is essentially concentrating all the solid components of the urine to be able to more easily visualized on the slide

Step 4a
Make urine dry prep

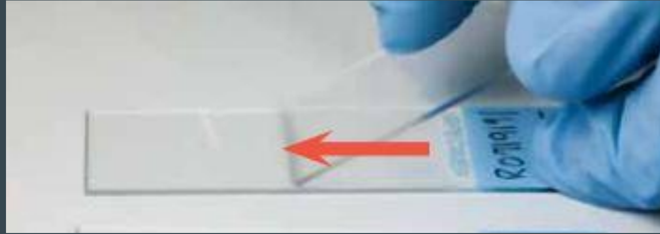


- Using the same technique as a blood smear, make a urine smear using a drop of the urine with the resuspended pellet

- Add a drop to your sample slide
- Using a spreader slide, pull back into the urine droplet

Step 4b

Make urine dry prep



- Push the spreader slide forward, dragging the urine out in a thin film
- ***DIFFERENCE:** do **not** create a feathered edge
    - Instead, **stop in the middle and lift the spreader slide straight up**
    - This creates a straight line of concentrated material to view microscopically

Step 5
Stain the slide

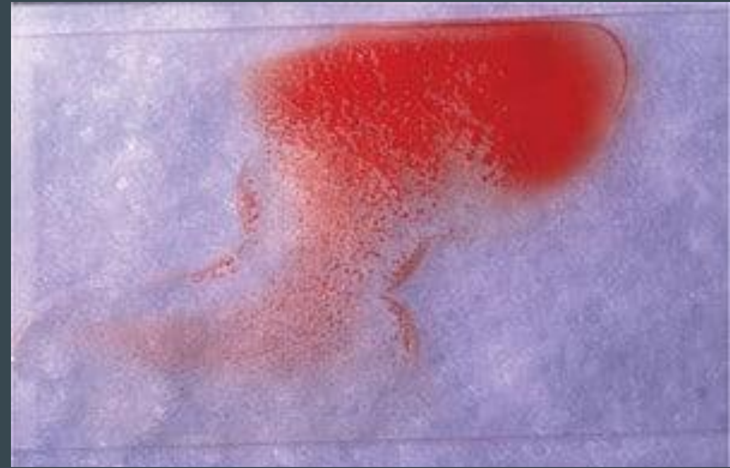
- Allow your slide to fully air-dry
- Use the three jars of Diff-Quik stain **(URINE STAIN JARS)**
  - Blue - fixative
  - Pink - eosinophilic
  - Purple - basophilic
- Dip your slide sequentially through each stain
  - Approximately 10-15 seconds
  - Blot excess stain on a paper towel in between colors
  - Use a clothes pin to grasp slide
- Using a *gentle stream* of water, rinse the slide after the last stain
- Allow to air dry

Slide Agglutination

When do we do this?

- To differentiate between true autoagglutination and rouleaux formation
- In suspected cases of IMHA
- Often before blood transfusions

Only technicians will prepare slide agglutinations



Step 1

Combine blood and saline

+ NaCl

- Get a clean slide
- Add one drop of well-mixed EDTA blood in center of slide
- Add one drop of saline to blood
  - May need 2 drops of saline for cats
    - Feline blood is more likely to form rouleaux

Step 2
Mix by rocking slide

- Using a gentle rocking motion, swirl and mix the blood and saline together on the slide
    - Can rock in back-and-forth motion

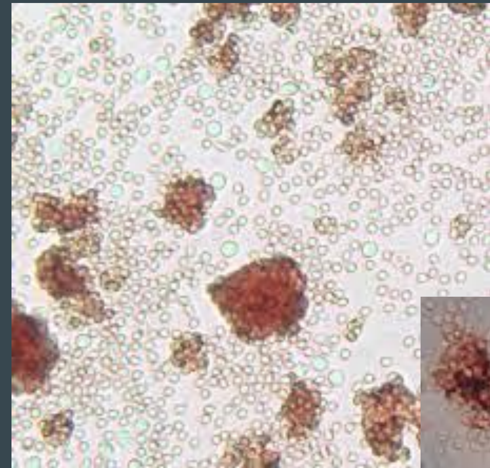
    OR

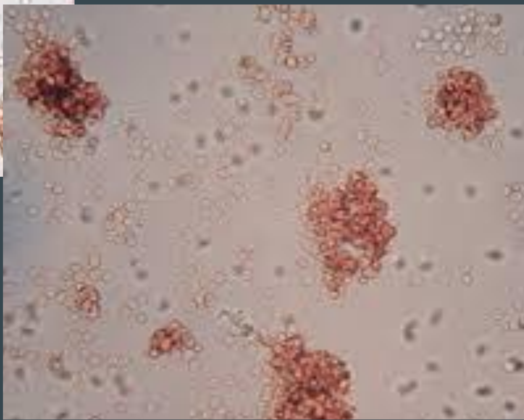
    - Can rock in circular motion
- Usually only takes a few seconds to mix well
- Goal = spread out the blood droplet to be able to visualize any clumping

Step 3
Evaluate slide - MACRO

- Evaluation should be prompt
- Do you see clumps of RBCs?
  - Sometimes as you swirl, you may see transient clusters - do they persist?
- **Yes?** → Macro + (positive)
- **No?** → Macro - (negative)
- Label your slide
  - Write the patient's name on the frosted section of slide
  - Use PENCIL!

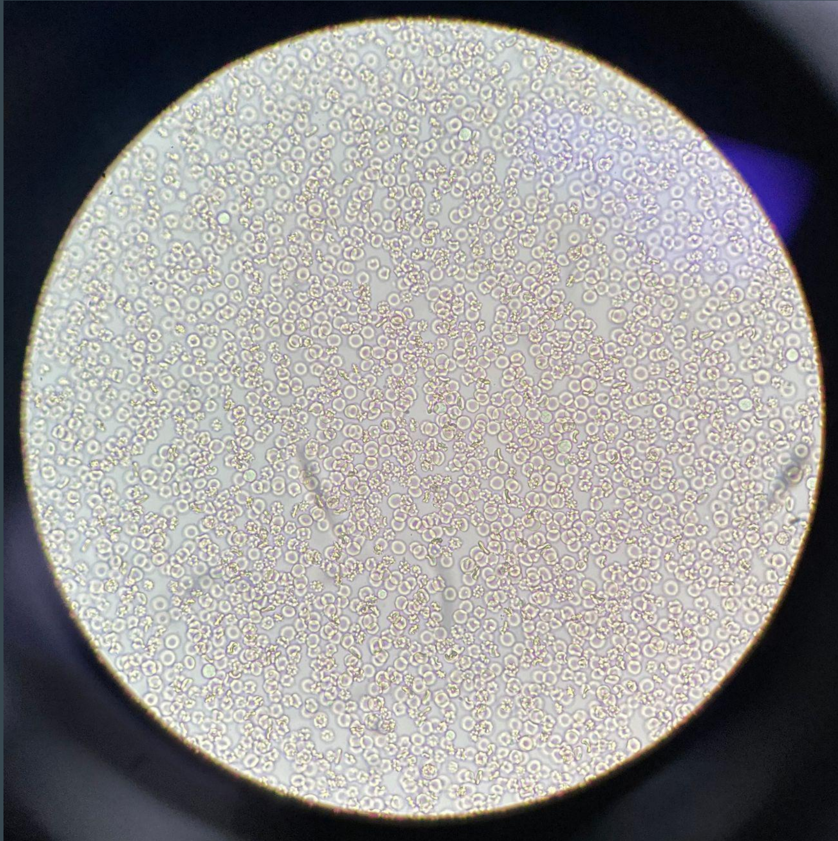
Step 3
Evaluate slide - MICRO



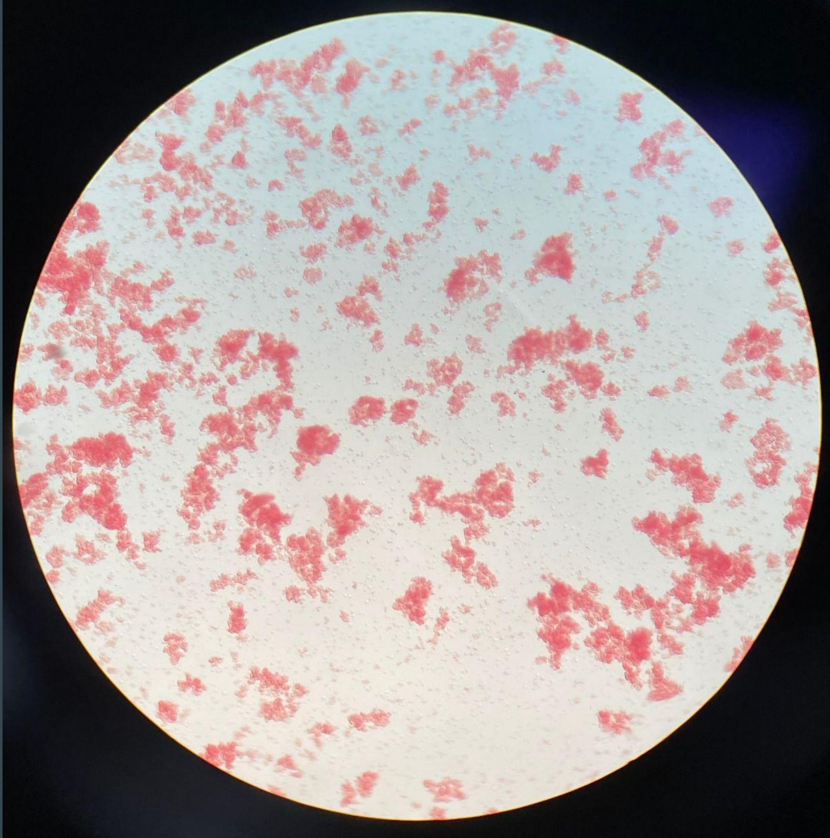


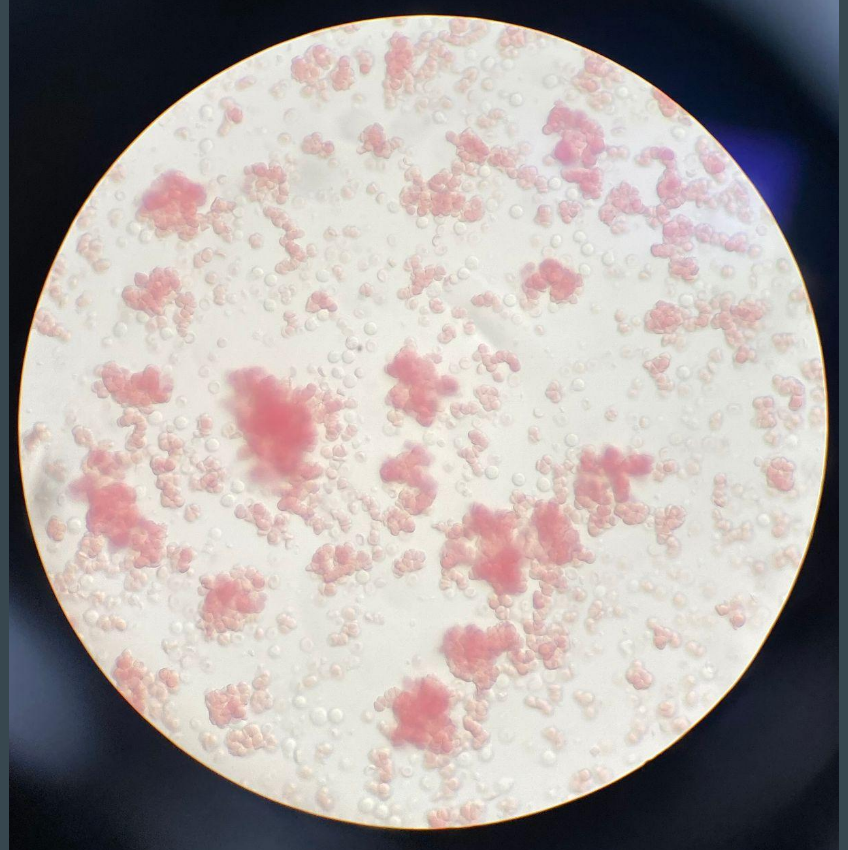
- Place a coverslip on slide
- Examine under microscope
  - 10x or 40x magnification
- Do you see clumps of RBCs?
  - True agglutination will appear as clumps and clusters, not as rouleaux
  - An additional drop of saline can disperse rouleaux but will not affect true autoagglutination
- **Yes?** → Micro + (positive)
- **No?** → Micro - (negative)
- Report results to the DVM
- Record results in patient's record in ezyVet

No Agglutination

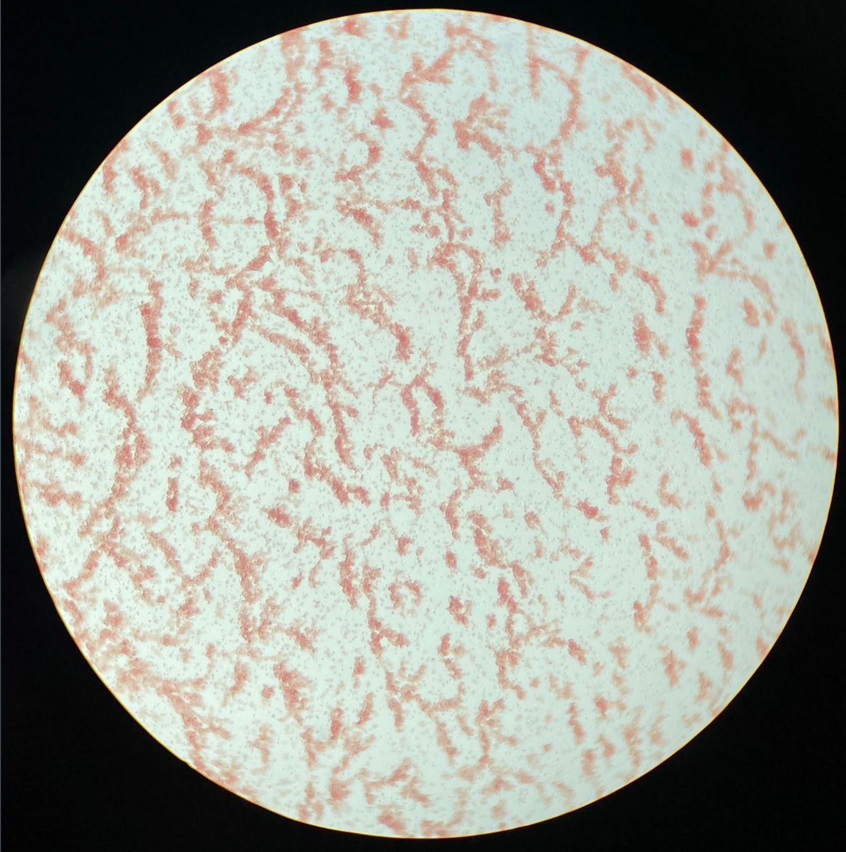


Agglutination





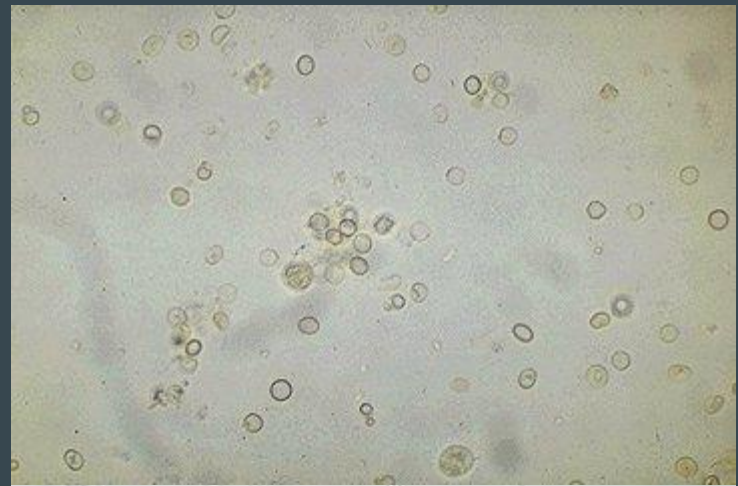
Agglutination



Urine Sediment (Wet Prep)

When do we do this?

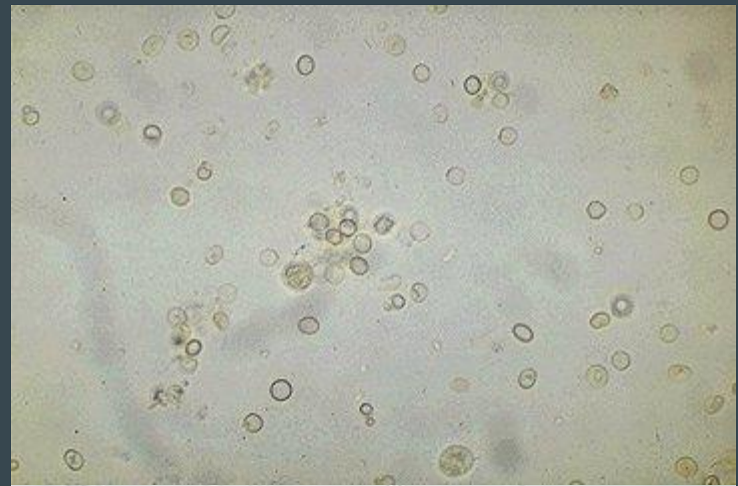
- We used to do this for every urinalysis
- Now, we have the Idexx SediVue
- Will still perform by DVM request



Urine Sediment (Wet Prep)

What are we looking for?

- Blood cells
- Crystals
- Bacteria (not as reliable as dry prep)
- Casts
- Tissue cells
- Other abnormalities
  - Yeast or fungal infection
  - Parasites



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- Carefully remove tube from centrifuge
  - You do not want to disturb the pellet at the bottom
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Remove fluid part

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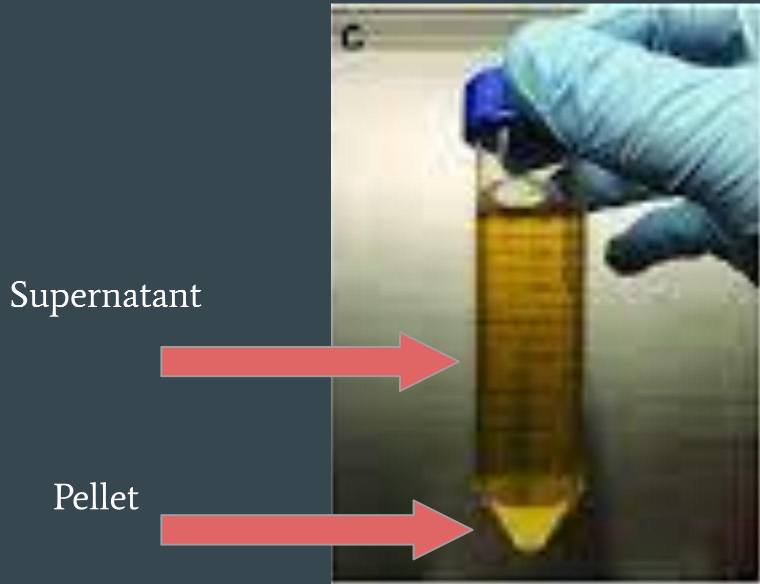
Step 3
Resuspend solids



- Gently flick the bottom of the tube a few times
- This agitates the pellet and causes it to be redistributed in the remaining fluid
    - This is essentially concentrating all the solid components of the urine to be able to more easily visualized on the slide

Step 4

Make urine wet prep

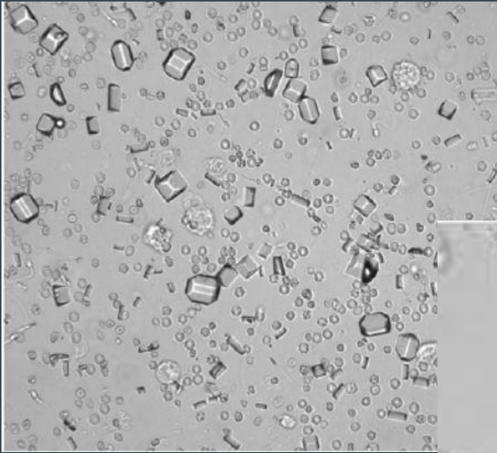
- Add a substantial drop of urine to one end of your sample slide using a pipette

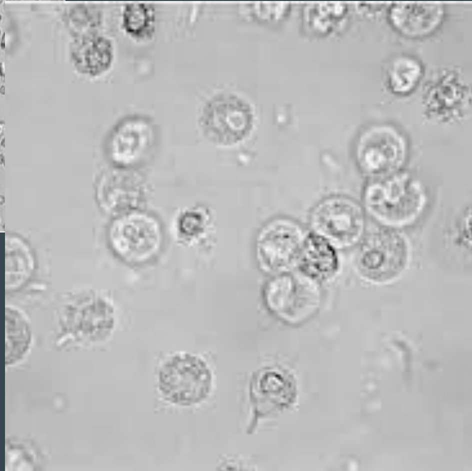
THEN

- Add a **small** drop of the purple Urine Sediment stain to your white top tube
- Mix well by flicking or swirling tube
- Add a second drop of urine (stained this time) to the other end of your sample slide
    - Same pipette is OK

Step 5

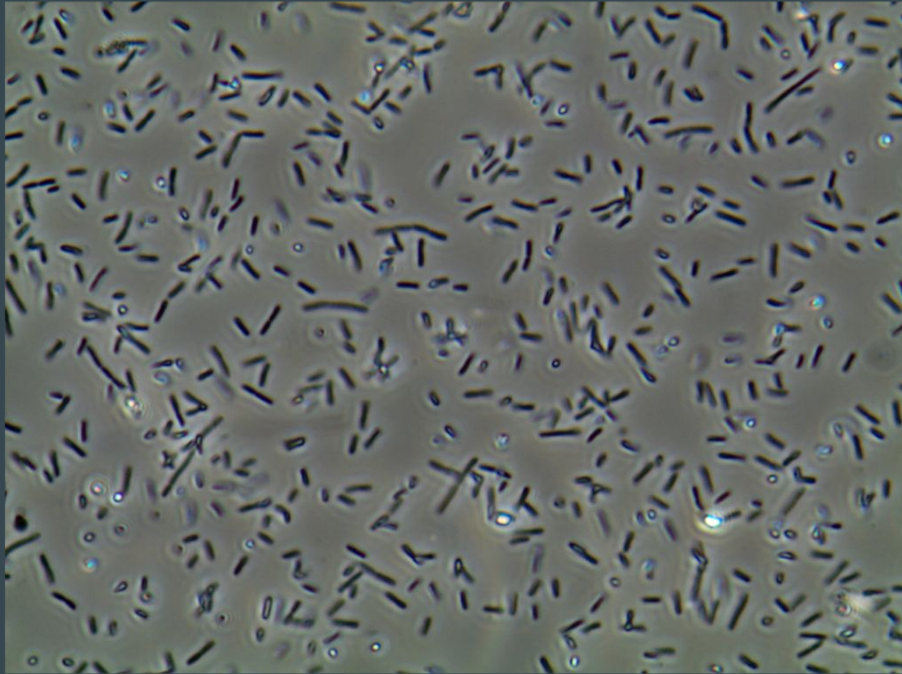
Examine slide





- Add a coverslip to each urine sample population on your slide
- Examine under the microscope
    - Use 10x, then 40x magnification
    - Dimming the light with the diaphragm adjuster makes visualizing the samples easier
- Evaluate both unstained and stained samples

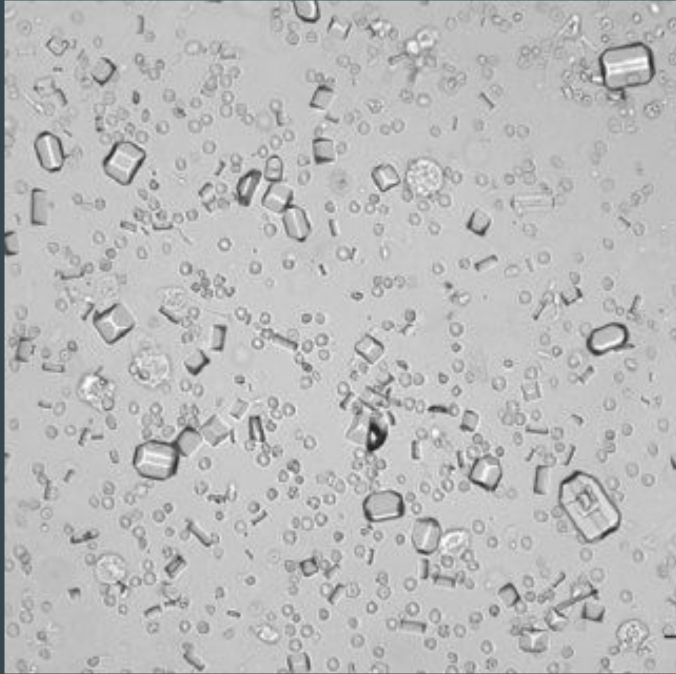
Urine examples



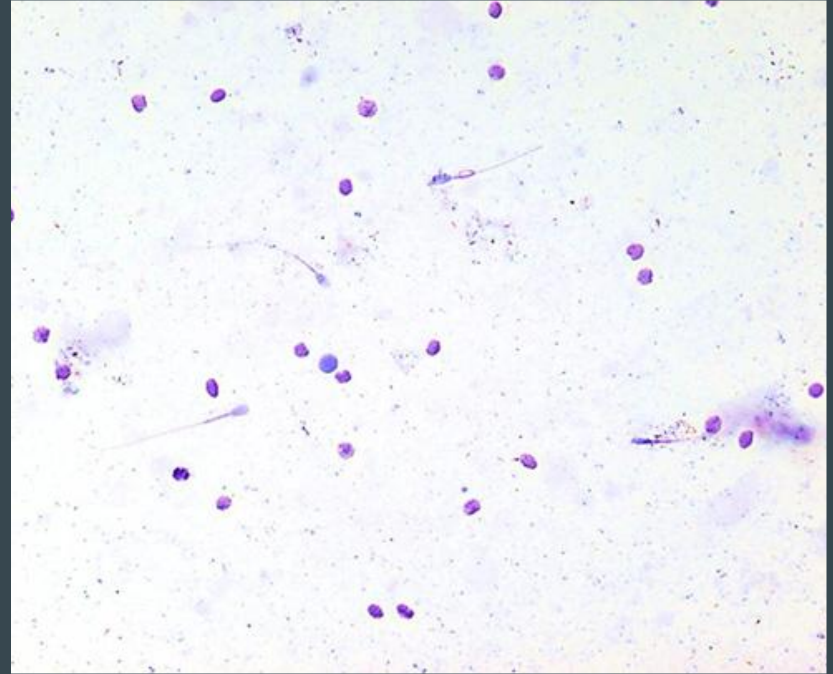
Significant bacteria, rods (400x)

Numerous struvite crystals

Urine examples



Numerous RBCs, struvite crystals, few other cells (WBC? Transitional epithelial?)



Some RBCs, rare WBC, occasional spermatozoa, +/- bacteria vs. stain precipitate

Review

Question	Answer
Urine dry preps are mostly used to identify what?	• Presence of (and classification of) bacteria
Who is responsible for preparing slide agglutinations?	• Technicians
Describe a slide that is slide agglut negative.	• Uniform distribution of RBCs with no clumping or clustering
What is the fluid part called after you spin down a urine sample?	• Supernatant

Review

Question	Answer
What is the color order for staining blood or urine slides?	• Blue → pink → purple
Describe the difference in making blood smears vs. urine dry preps?	• For blood smears, you want a smooth, continuous push forward with your spreader slide. In a urine dry prep, you abruptly stop the push and lift the spreader slide up.
What do you use to label your slides? Why?	• Pencil only. If you use pen or sharpie, it can bleed if stain touches it.
What are four things we may see in a urine sediment (wet prep?)	• RBCs, WBCs, other cells (squamous, transitional), crystals, casts, bacteria, sperm, other (yeast/fungus, parasites)

Thanks!