Vetscan Imagyst® Digital Cytology

THE BASICS

Staining and Submission

Keys to Excellent Stain Quality

- Always stain samples using a quick stain (e.g., Diff Quik®, quick aqueous or other Romanowsky-type stain) according to manufacturer's protocol
- A dip method is preferred to flooding the slides with stain to ensure uniform staining
- Stains should be replenished regularly to avoid depletion and build up of stain precipitate
- Avoid heating, freezing, or refrigerating cytology slides, which may distort cells
- Ensure unstained cytology slides are not collected in the vicinity of formalin or formalin vapors. Exposure of cytology specimens to formalin prior to staining may make the slides uninterpretable as formalin interferes with cell staining
- **Alternative staining techniques such as Gram staining or urine sediment (supravital) stain are not acceptable for submission**

Coverslipping

- Use Zoetis issued or similar coverslip (24 x 60 mm; 0.13-0.17 mm thickness)
- Ensure that only one coverslip is used as coverslips can easily stick together
- Always use a coverslip

1. Place stained slide on flat surface.
2. Add 2 drops of immersion oil to surface of sample. Use only enough to cover sample. Excess oil can contaminate scanner lens.
3. Handle coverslip by its edge to avoid fingerprints.
4. Place edge of coverslip onto sample and roll over sample, avoiding formation of air bubbles.
5. Blot excess oil with a delicate task wipe or lotion free tissue.

Scanning

Slide

- Place sample side up, label to the right
- Flat on stage, stage clips flush with edges of the slide
- Slide lock engaged

Mask

- Masked area(s) completely within bounds of coverslip
- Avoid masking large areas that do not contain much stained material

Scanner

- Lens and stage are clean
- No objects nearby scanner that may impede movement
- No nearby centrifuges in operation during scan

BLOOD SMEAR

Keys To Excellent Stain Quality

Brief blood smear preparation instructions below. More detailed instructions available in the Vetscan Imagyst® AI Blood Smear User Guide: https://files.helpdocs.io/la8tfkiodj/other/1657821731340/vetscan-imagyst-blood-smear-user-guideglobal.pdf

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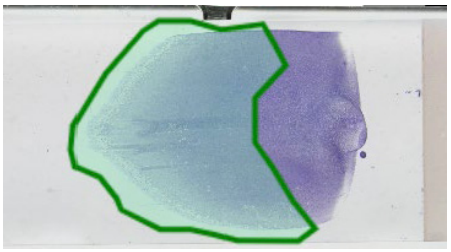
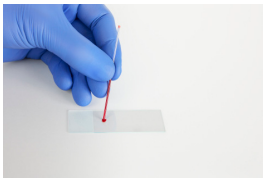
Key Components for Submission

- Stained blood smear
- CBC data



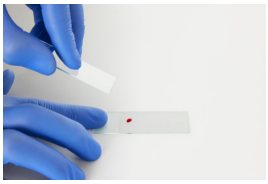
The feathered edge, monolayer, and at least a portion of the body should be included in the scan area.

Blood smear preparation



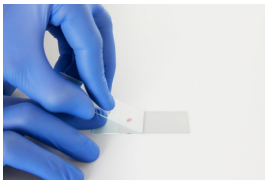
Prepare The Slide

- Mix the EDTA anticoagulated blood
- Use a microhematocrit capillary tube or precision pipette to draw blood from the tube that has just been mixed, and gently place a drop onto the labeled slide
- Care should be taken not to tap the tube against the slide
- A wooden stick should not be used for this blood transfer - platelets and white blood cells tend to adhere to the stick



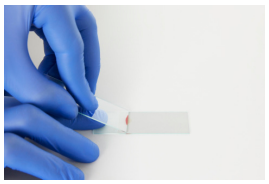
Place Spreader Slide

- Place the spreader slide on top of the labeled slide in front of the blood droplet and hold at a 30° to 45° angle



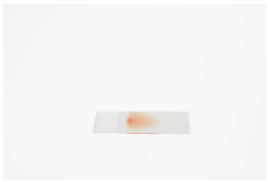
Spread The Blood

- Draw the spreader slide back until it makes contact with the blood droplet
- Capillary action will draw the sample toward the edges of the slide
- Do not allow the blood to reach the edges of the slide



Complete The Spread

- Before the blood reaches the edges of the slide, with a smooth, stable and fluid motion, push the spreader slide away from the sample blood drop across the bottom slide. Maintain the same angle throughout the motion, and do not apply downward pressure. This should produce a uniform blood film covering approximately 1/2 to 2/3 of the slide
- Let the slide air-dry to avoid air-drying artifacts (DO NOT HEAT FIX THE SLIDE)



Inspect Slide

- After the blood smear is made, visually inspect the slide to ensure that 1/2 to 2/3 of the slide is covered
- The smear should look like a thumbprint and exhibit a visible feathered edge at its end

TISSUE CYTOLOGY
(Fine Needle Biopsy/Aspiration)

Key Components for Submission

- Up to four slides with well-stained, thinly spread samples
- A maximum of two different tissues sites/sources
- Relevant history/lesion description

Fine needle aspiration (FNA)

Fine needle biopsy (without aspiration) is preferred for most lesions.

Fine needle aspiration may be considered if there is concern that the lesion will exfoliate poorly.

Impression Smears and Swabs

Can be used for making imprints from a biopsy specimen or from superficial/draining lesions not amenable to FNA, but interpretation may be limited.

May not adequately sample cell populations or organisms deeper in tissue.

Maximize Cellular Spread and Integrity

1. Expel the sample onto a clean glass slide.
2. Place a second slide gently over the sample and **without applying pressure**, pull the two slides apart in a **smooth horizontal motion.**

PRO TIP: Holding the sample slide and spreader slide above the table can help to ensure no pressure is applied during the spreading process.

PRO TIP: Avoid pulling the sample slide and spreader slide apart in a vertical fashion. This will create a sandwich preparation where cells are often not spread thinly enough for optimal assessment.

Staining and Submission

1. After smears are prepared, they should be rapidly air-dried to avoid drying artifact. A hair dryer on cool setting can help facilitate drying.
2. Ensure slides are labeled with site and patient name.
3. Stain slide using a Romanowksy-type stain according to manufacturer's protocol.
4. Apply immersion oil and coverslip (see The Basics).

When Scanning, Make Sure That

- Slide is sample side up, pointing toward scanner lens
- Slide lock is engaged
- There are no objects preventing movement of scanner (including no operating centrifuges)

FLUID – BODY CAVITY
(Pleural, Peritoneal)

Key Components for Submission

- Stained direct (unconcentrated) preparation
- Stained sediment (concentrated) preparation

Highly Recommended for Submission

- Total protein measurement
- Cell count

Fluid Sample Collection

- The fluid sample should be promptly placed into an EDTA tube. If enough fluid remains, place a portion into a red top tube
- Fluid in the EDTA tube should be used for slide preparation. Fluid in the red top tube may be needed for additional testing

Total Protein Measurement

- Use a refractometer
- It is best to use the fluid supernatant for measurement, but the unspun fluid can be used for measurement if the fluid is clear
- EDTA can cause overestimation of protein. Red top tube is best

Cell Count

- A CBC analyzer can be used to obtain a WBC count on the fluid if the fluid does not contain particulate material
- Please see analyzer manufacturer's directions for fluid analysis

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Direct (Unconcentrated) Preparation

1. Label the slide direct with a pencil.
2. Gently invert the tube of EDTA fluid several times to ensure it is well mixed.
3. Place a drop of fluid near the label end of the slide and use the blood smear technique to spread the fluid, making sure to leave a feathered edge.
4. Rapidly dry the slide (a hair dryer on cool setting can be used). Do not heat fix.
5. Stain the slide and allow to dry.
6. Place immersion oil and coverslip prior to submission.

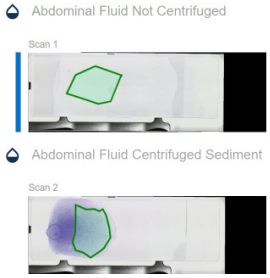
Sediment (Concentrated) Preparation

1. Label the slide sediment with a pencil.
2. Aliquot a portion of well-mixed fluid into a separate tube for centrifugation.
3. Spin down the fluid, decant the supernatant, and gently resuspend the pellet in the small amount of remaining fluid (similar to preparation of urine sediment).
4. Place a drop of the sediment near the label end of the slide and use the blood smear technique to spread the fluid, making sure to leave a feathered edge.

Continue with Steps 4-6 under Direct Preparation

Submission

Make sure it is clear which slide is the direct preparation and which slide is the sediment preparation.



When Scanning Make Sure That

- Slide is sample side up, pointing toward scanner lens
- Slide lock is engaged
- There are no objects preventing movement of scanner (including no operating centrifuges)

FLUID – CSF
(Cerebrospinal Fluid)

REQUIRES SPECIAL HANDLING

CSFs Will Not Be Accepted Without The Following

- Cytocentrifuge/Cytospin preparation
- Hemacytometer cell count (performed manually by trained individual)

Please send CSF samples to a reference laboratory if hemacytometer cell count and cytocentrifuge preparations are not available.

Fluid Sample Collection

The CSF sample should be promptly aliquoted into an EDTA tube and into a red top tube if enough fluid is available.

Cytocentrifuge Preparations

A cytocentrifuge (or 'cytospin') is a specialized centrifuge that is used in the reference lab setting to concentrate very low cellularity fluids, such as washes, onto a small circular area of the slide. This preparation technique helps to **preserve cell integrity** and **ensure there are cells available for evaluation** by the pathologist.

Cytocentrifuge preparation

1. Label the slide CSF with a pencil.
2. Prepare the funnel, slide, and clip apparatus according to the cytocentrifuge manufacturer's instructions.
3. Carefully pipette 200 uL of CSF into the funnel (minimum 100 uL).
4. Centrifuge the sample according to manufacturer's instructions. Remove the slide from the centrifuge and unclip from the funnel apparatus.
5. Using a slide marking pen, **draw a circle on the *underside of the slide* surrounding the sample area. Do NOT draw on the sample side of the slide.** Even concentrated CSF samples can be of very low cellularity and difficult to visualize on the slide once stained. This step will ensure the area of interest can be easily visualized and included in the scanned field.
6. Stain and coverslip slide.

Submission

Please Include

- Relevant history
- Description of fluid
- Hemacytometer manual cell counts (RBC and WBC)
- Cytocentrifuge slide

When Scanning, Make Sure That

- Slide is sample side up, pointing toward scanner lens
- Slide lock is engaged
- There are no objects preventing movement of scanner (including no operating centrifuges)

FLUID – SYNOVIAL/JOINT

Key Components for Submission

- Stained direct (unconcentrated) preparation

Highly Recommended for Submission

- Visual fluid parameters

Fluid Sample Collection

If only a small amount of fluid is collected, slides may be made directly from needle/syringe. Otherwise, place an aliquot of fluid in an EDTA tube and mix well. If enough fluid remains, place a portion into a sterile tube without additive in case culture is needed.

Visual fluid parameters

- Color: Straw/yellow or blood contaminated?
- Clarity: Clear or cloudy?
- Viscosity: Stringy and viscous or watery?

Direct (unconcentrated) preparation

1. Label the slide(s) with the sample source and patient name.
2. If only a small amount of fluid is aspirated, transfer directly onto the slide from the needle syringe. If fluid was placed into an EDTA tube, gently invert the tube of fluid several times to ensure it is well mixed.
3. Place a drop of fluid near the label end of the slide and use the blood smear technique to spread the fluid, making sure to leave a feathered edge.
   - Alternatively, if the sample is highly viscous, use the squash preparation technique. Place a second slide gently over the sample and without applying pressure, pull the two slides apart in a smooth horizontal motion.
4. Rapidly dry the slide (a hair dryer on cool setting can be used). Do not heat fix.
5. Stain the slide and allow to dry.
6. Apply immersion oil and coverslip (see The Basics).

When scanning make sure that

- Slide is sample side up, pointing toward scanner lens
- Slide lock is engaged
- There are no objects preventing movement of scanner (including no operating centrifuges)

FLUID – BODY CAVITY
(Pericardial)

Key Components for Submission

- Stained direct (unconcentrated) preparation, similar to a blood smear

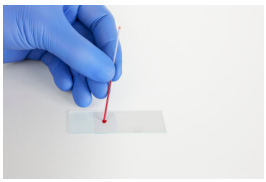
Fluid Sample Collection

The fluid sample should be promptly placed into an EDTA tube.

A Note on Pericardial Fluid

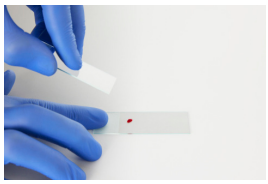
Pericardial fluid often has an appearance similar to peripheral blood. If the pericardial fluid sample obtained **does not** appear to be blood, please refer to Body Cavity Fluids (peritoneal, pleural) for sample preparation recommendations.

Direct (Unconcentrated) Preparation:



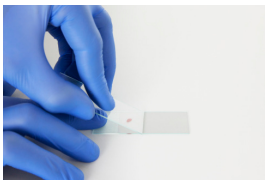
Prepare The Slide

- Mix the EDTA anticoagulated blood
- Use a microhematocrit capillary tube or precision pipette to draw blood from the tube that has just been mixed, and gently place a drop onto the labeled slide
- Care should be taken not to tap the tube against the slide
- A wooden stick should not be used for this blood transfer - platelets and white blood cells tend to adhere to the stick



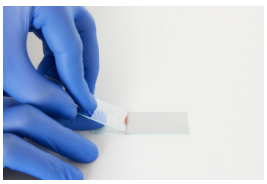
Place Spreader Slide

- Place the spreader slide on top of the labeled slide in front of the blood droplet and hold at a 30° to 45° angle



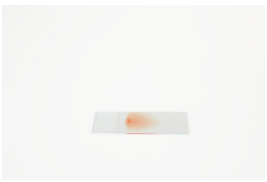
Spread The Blood

- Draw the spreader slide back until it makes contact with the blood droplet
- Capillary action will draw the sample toward the edges of the slide
- Do not allow the blood to reach the edges of the slide



Complete The Spread

- Before the blood reaches the edges of the slide, with a smooth, stable and fluid motion, push the spreader slide away from the sample blood drop across the bottom slide. Maintain the same angle throughout the motion, and do not apply downward pressure. This should produce a uniform blood film covering approximately 1/2 to 2/3 of the slide
- Let the slide air-dry to avoid air-drying artifacts (DO NOT HEAT FIX THE SLIDE)



Inspect Slide

- After the blood smear is made, visually inspect the slide to ensure that 1/2 to 2/3 of the slide is covered
- The smear should look like a thumbprint and exhibit a visible feathered edge at its end

FLUID – WASHES

(TTW, BAL, Nasal Flush, Urinary Tract Wash, Etc.)

Key Components for Submission

- Stained direct (unconcentrated) preparation
- Stained sediment (concentrated) preparation

Highly Recommended If Available

- Cytocentrifuge/Cytospin preparation

Fluid sample collection

The fluid sample should be promptly placed into an EDTA tube. If enough fluid remains, place a portion into a sterile tube without additive.

Fluid in the EDTA tube should be used for slide preparation. Fluid in the sterile tube may be needed for additional testing, such as culture.

Cytocentrifuge Preparations

A cytocentrifuge (or 'cytospin') is a specialized centrifuge that is used in the reference lab setting to concentrate very low cellularity fluids, such as washes, onto a small circular area of the slide. This preparation technique helps to **preserve cell integrity and ensure there are cells available for evaluation** by the pathologist. *Scan time is also significantly reduced.*

Small versions of cytocentrifuges are available for in-clinic use.

Direct (Unconcentrated) Preparation

1. Label the slide direct with a pencil.
2. Gently invert the tube of EDTA fluid several times to ensure it is well mixed.
3. Place a drop of fluid near the label end of the slide and use the blood smear technique to spread the fluid, making sure to leave a feathered edge.
4. Rapidly dry the slide (a hair dryer on cool setting can be used). Do not heat fix.
5. Stain the slide and allow to dry.
6. Place immersion oil and coverslip prior to submission.

Sediment (Concentrated) Preparation

1. Label the slide sediment with a pencil.
2. Aliquot a portion of well-mixed fluid into a separate tube for centrifugation.
3. Spin down the fluid, decant the supernatant, and gently resuspend the pellet in the small amount of remaining fluid (similar to preparation of urine sediment).
4. Place a drop of the sediment near the label end of the slide and use the blood smear technique to spread the fluid, making sure to leave a feathered edge.

Continue with Steps 4-6 under Direct Prepartion

Submission

Make sure it is clear which slide is the direct preparation and which slide is the sediment preparation.

When Scanning Make Sure That

- Slide is sample side up, pointing toward scanner lens
- Slide lock is engaged
- There are no objects preventing movement of scanner (including no operating centrifuges)

OTHER SAMPLE TYPES

Urine Sediment

- ✓ Air-dried urine sediment smear/concentrated preparation (Diff Quik or similar)
- ✓ Wet prep/unstained: accepted as 'Add-On Expert Review' following AI Urine Sediment evaluation
- ✗ Sedi-stain (not accepted)

Tape Preps

- ✗ Generally too thick/poor clarity for adequate evaluation

Fecal Cytology

- ✓ Stained slide (Diff Quik or similar)
- ✗ Gram stain (not accepted)

Information Provided

- ✓ Inflammatory or other nucleated cell types
- ✓ General description of bacteria/yeast or other organisms

References

Image: istock 450726311