My Desk
Clinical Pathology in Practice

- Ask the audience
- Sample handling
- Cytology
- Hematology
Ask the Audience

- From your smart phone, tablet, or laptop

Respond at PollEv.com/nicolefernann414

Text NICOLEFERNAN414 to 37607 once to join, then A, B, or C
<table>
<thead>
<tr>
<th>Practice Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exclusively small animal</td>
</tr>
<tr>
<td>Exclusively food animal</td>
</tr>
<tr>
<td>Exclusively equine</td>
</tr>
<tr>
<td>Exclusively companion animal (small and equine)</td>
</tr>
<tr>
<td>Exclusively large animal (food animal and equine)</td>
</tr>
<tr>
<td>Other</td>
</tr>
</tbody>
</table>
Why did you decide to attend this session?

Clin path is AWESOME

I am comfortable with cytology and hematology but could use a review

I have forgotten all my cytology and hematology from vet school
Sample Handling

Resources
Cytology samples
Hematology samples
Sample Handling

- PDS Protocol Manual is online
- Search “pds protocol manual”
Sample Handling

PDS lab techs recommend:

- Separate bag for each patient
- Label containers with patient name, date, sample type
  - Is this serum or urine?
- Wipe off outside of containers
- More padding than you think
Sample Handling

Cytology smears

- Air dry slides
- Adequate padding/proper containers
- Pack separately from histopath samples
  - Applies to blood smears too
Sample Handling

Fluid samples

- Submit in EDTA
- Make direct smears
- Keep cool
Sample Handling

Hematology samples

- CBC- EDTA tube
- Make blood smears
- Chemistry- red top tube
- Separate serum from cells if possible
- Keep cool
Cytology

Sample collection
Approach to the slide
A few lumps and bumps
Sample Collection

- FNA and/or FNNA
  - Videos in PDS Protocol Manual
- Make smears quickly
- Air dry
- Stain with Diff-Quik
<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Do you collect cytology samples in-house?</strong></td>
<td></td>
</tr>
<tr>
<td>Yes, all the time</td>
<td></td>
</tr>
<tr>
<td>Yes, occasionally</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

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Do you read your own cytology samples?

<table>
<thead>
<tr>
<th>Option</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes, most of the time</td>
</tr>
<tr>
<td>Yes, I look at them but usually submit them to a diagnostic lab</td>
</tr>
<tr>
<td>No, I submit them all</td>
</tr>
</tbody>
</table>
Approach to the Slide

1. Gross Examination
   - How cellular is the slide?
   - Where on the slide is the material distributed?
   - Is blood present?

2. 4x objective
   - Scan entire smear
   - Find areas to evaluate at higher magnification
     - Intact cells spread in a thin layer

3. 10x-40x objective
   - Evaluate several areas identified at 4x
   - Identify cell types present
   - Classify the lesion (see Figure 1)

4. 100x objective (oil)
   - Confirm identification of cells
   - Evaluate cellular details
   - Identify bacteria
About the images

- Cases from WCVM and UCVM
- Many taken by Dr Galezowski
Cells needed...
Intact cells needed...
In a thin layer...
Without too much blood
Sample quality

Non-diagnostic (collect new sample)

Diagnostic

Submit or evaluate

Inflammatory ---
Cell type?

Septic

Non-septic

Epithelial

Hyperplasia, metaplasia, neoplasia

Mesenchymal (spindle or round cell)

Hyperplasia, metaplasia, neoplasia
In-house Cytology: The Short List

- Melanoma
- Lipoma
- Follicular Cyst
- Abscess
- Mast Cell Tumor
- Sebaceous adenoma
- Septic Inflammation
- Lipoma
- Large bubble-like cells
- Smear appears greasy
- May be poorly cellular
What is your diagnosis?

- Lipoma
- Sebaceous adenoma
- Mast cell tumor
- Follicular cyst
▪ Sebaceous adenoma
▪ Clusters of uniform cells
▪ Vacuolated cytoplasm
What is your diagnosis?

- Septic inflammation
- Mast cell tumor
- Follicular cyst
- Lipoma
- Follicular cyst
- Superficial squamous
- Usually anucleate
- Keratin debris
What is your diagnosis?

- Mast cell tumor
- Septic inflammation
- Non-diagnostic
- Melanoma

200 μm
- Melanoma
- Black-brown pigment
- Any cell shape
- Can be tricky
- Location important
<table>
<thead>
<tr>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mast cell tumor</td>
</tr>
<tr>
<td>Melanoma</td>
</tr>
<tr>
<td>Septic inflammation</td>
</tr>
<tr>
<td>Follicular cyst</td>
</tr>
</tbody>
</table>

What is your diagnosis?
- Mast cell tumor
- Purple granules
- Round cells
- Can be tricky
- May see eosinophils
<table>
<thead>
<tr>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Septic inflammation</td>
</tr>
<tr>
<td>Follicular cyst</td>
</tr>
<tr>
<td>Abscess</td>
</tr>
<tr>
<td>Melanoma</td>
</tr>
</tbody>
</table>
- Abscess
- Numerous neutrophils
- May be degenerate
- May be septic
- Other inflammatory cells may be present
- Septic inflammation
- Numerous neutrophils
- Many degenerate
- Intracellular bacteria
Sub-mandibular mass

Lymph node

Salivary gland
Artifacts

US gel

Stain precipitate
Questions?

Stay tuned for hematology...
Part 2: Hematology

Red cells
White cells
Platelets
Part 2: Hematology

- Ask the audience:

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<tr>
<td>Yes, I run all my CBCs on it</td>
</tr>
<tr>
<td>Yes, I run presurgical and STAT CBCs on it</td>
</tr>
<tr>
<td>No, I send CBCs to a diagnostic lab</td>
</tr>
<tr>
<td>No</td>
</tr>
</tbody>
</table>
Do you routinely examine blood smears from in-house CBCs?

Yes, on almost all patients

Yes, if there is something weird on the CBC

No
Hematology

- Your analyzer can’t tell you everything
- RBC morphology
  - Regeneration
  - Causes of anemia
- WBC morphology
  - Left shift and toxic change
  - Leukemia
- Platelet clumping
The Monolayer

Feathered Edge

Monolayer

Body
Is this anemia regenerative?

Yes

No
What is the evidence in support of regeneration?

- Respond at PollEv.com/nicolefernan414
- Text NICOLEFERNAN414 to 37607 once to join, then A, B, or C
- Regenerative anemia
- Polychromasia
- Macrocytic
- (Hypochromic)
RBC Case 2
RBC Case 2

- Iron deficiency anemia
- Microcytic
- Hypochromic
- Inadequate regeneration
<table>
<thead>
<tr>
<th>Cause of Anemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidative damage</td>
</tr>
<tr>
<td>Iron deficiency</td>
</tr>
<tr>
<td>Blood loss</td>
</tr>
<tr>
<td>IMHA</td>
</tr>
<tr>
<td>DIC</td>
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Text **NICOLEFERNAN414** to **37607** once to join, then **A, B, or C**
Oxidative Damage
Agglutination

Rouleaux
Questions?

WBC coming up...
Leukocyte Review

Dog

Cat

Horse

Cow
Leukocyte Review

Dog

Cat

Horse

Cow
Leukocyte Review

Dog

Cat

Horse

Cow
Leukocyte Review

Dog

Cat

Horse

Cow
Leukocyte Review

Dog

Cat

Horse

Cow
<table>
<thead>
<tr>
<th>Decreased</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within reference intervals</td>
</tr>
<tr>
<td>Increased</td>
</tr>
</tbody>
</table>
WBC Estimate 10x

- Count the leukocytes in at least 3 fields in the monolayer
- Divide the total number of leukocytes counted by the number of fields to obtain the average number per field
- Divide the average number of leukocytes counted by 4 in order to approximate the number of leukocytes x10^9/L
<table>
<thead>
<tr>
<th>What does the WBC morphology indicate?</th>
</tr>
</thead>
<tbody>
<tr>
<td>There is a left shift</td>
</tr>
<tr>
<td>There is toxic change</td>
</tr>
<tr>
<td>There is both a left shift and toxic change</td>
</tr>
<tr>
<td>There is neither a left shift nor toxic change</td>
</tr>
</tbody>
</table>
WBC Case 1
Left Shift
Widest part of nucleus

Constriction <1/3 widest part of nucleus

Constriction >1/3 widest part of nucleus

Mature seg

Band
Source of Confusion

**Toxic change**
- In blood (blood smears)
- Cytoplasmic changes
  - Basophilia
  - Foaminess
  - Dohle bodies
  - Toxic granulation

**Degenerate neutrophils**
- In tissues (cytology smears)
- Nuclear changes
  - Swelling
  - Pale-staining
  - Lysis
  - Hunt for bacteria
Source of Confusion

Nucleated RBC

Lymphocyte
<table>
<thead>
<tr>
<th>Diagnosis</th>
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<tbody>
<tr>
<td>Reactive lymphocytosis</td>
</tr>
<tr>
<td>Lymphocytic leukemia</td>
</tr>
</tbody>
</table>
Inclusion Confusion

Barr Bodies

Bacteria
Inclusion Confusion

*Mycoplasma hemofelis*  Stain precipitate

http://www.eclinpath.com/hematology/sample-collection-heme/stain-precipitate/
Inclusion Confusion

Anaplasma phagocytophilum

Overlapping platelet

Jacob EA. American Journal of Laboratory Medicine Volume 1, Issue 3, November 2016, Pages: 34-57
Platelets

- Feathered edge
- 10x
Platelet Estimate

- 10x Scan the feathered edge for platelet clumps
- 100x Count the number of platelets in at least 5 fields of the monolayer and calculate the average for 1 field. Multiply the average for 1 field by $20 \times 10^9$/L.
- If platelets are clumped the formula will underestimate the platelet count.
- Less than 3 - 4 per 100x field (60 - 80 x $10^9$/L) represents a significant thrombocytopenia
Digital Resources

- Veterinary Clinical Pathology ebook
  - iBooks
  - Google drive link

- Cornell e-ClinPath
  - http://www.eclinpath.com/
Digital Resources

Cells and Smears

VETERINARY CLINICAL PATHOLOGY DIGITAL DATABASE

- https://vetclinpathimages.com/
Digital Resources

- **PDS Protocol Manual**
  - Search “pds protocol manual” for most recent version

- **Videos**
  - FNA & smear making: [https://youtu.be/DkXDBom_3JI](https://youtu.be/DkXDBom_3JI)
  - Impression smear making: [https://youtu.be/prggfKrNlbI](https://youtu.be/prggfKrNlbI)
  - Cytology fluid handling: [https://youtu.be/DI_Ads7mpc](https://youtu.be/DI_Ads7mpc)
  - Cytology microscopy: [https://youtu.be/vWSD7bjskjs](https://youtu.be/vWSD7bjskjs)
  - Urinalysis: [https://www.youtube.com/playlist?list=PLm-jtvx5oGMTmznz0GJvPDzK0O1_AIFz5z](https://www.youtube.com/playlist?list=PLm-jtvx5oGMTmznz0GJvPDzK0O1_AIFz5z)
Digital Resources

- Hematology Videos
  - Manual WBC count: https://youtu.be/WwHL1d5pqSc
  - PCV and total protein: https://youtu.be/cau5wWe4Uds
  - Making/staining blood smears: https://youtu.be/nbRUiWI2Qrs
  - Blood smear evaluation: https://youtu.be/wlZtvTGJL6M