

## The Nuances of Neutrophils Mastering Interpretation

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## Neutrophil Nuances

- The many faces of neutrophils
- Mechanisms of neutrophilia and neutropenia
- Causes of neutrophilia neutropenia
- Artfactual neutrophilia and neutropenia
- Automated differential pitfalls

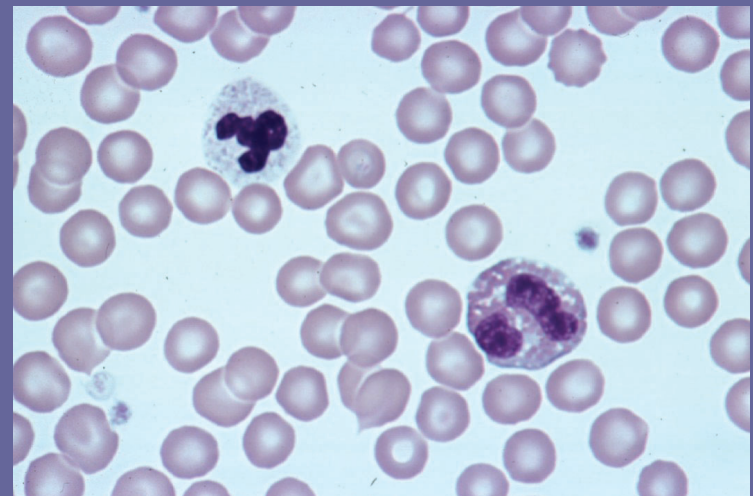
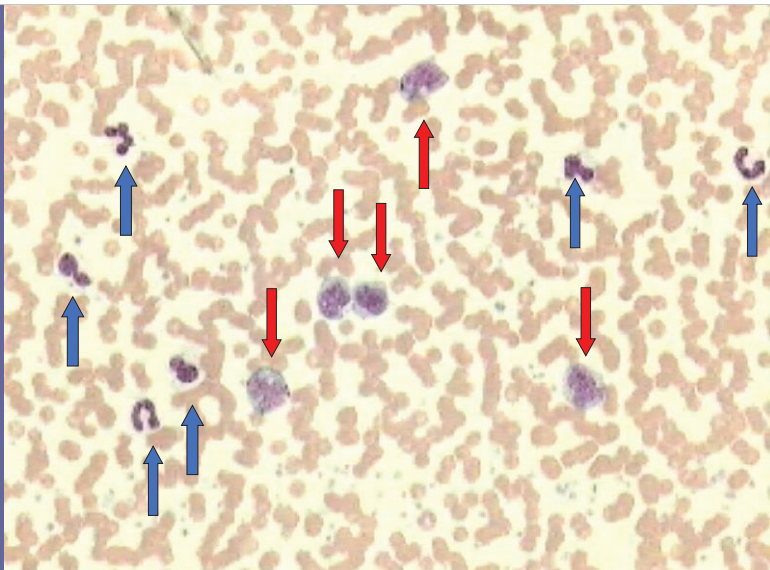


Image Noah's Arkive  
Davis Thompson Foundation

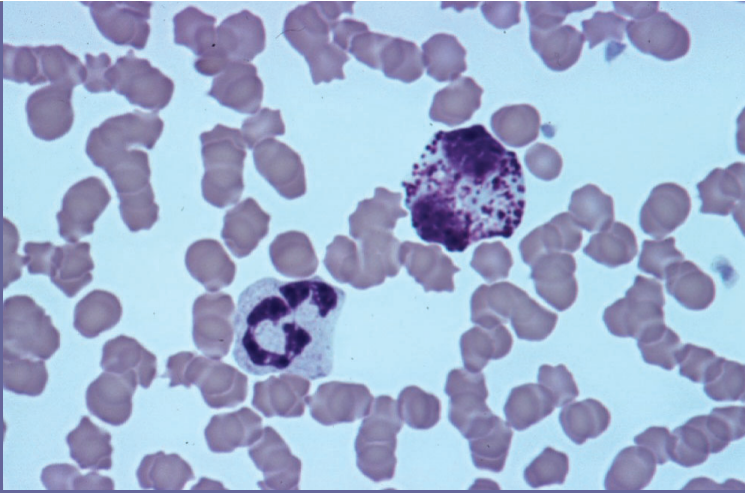


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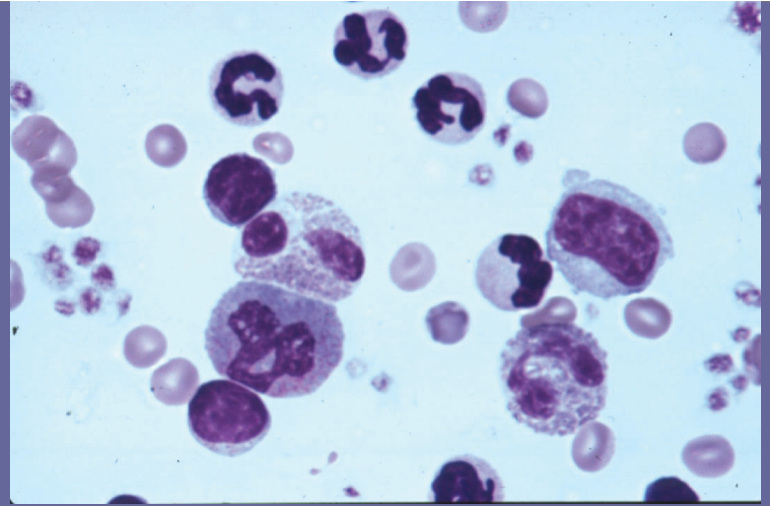


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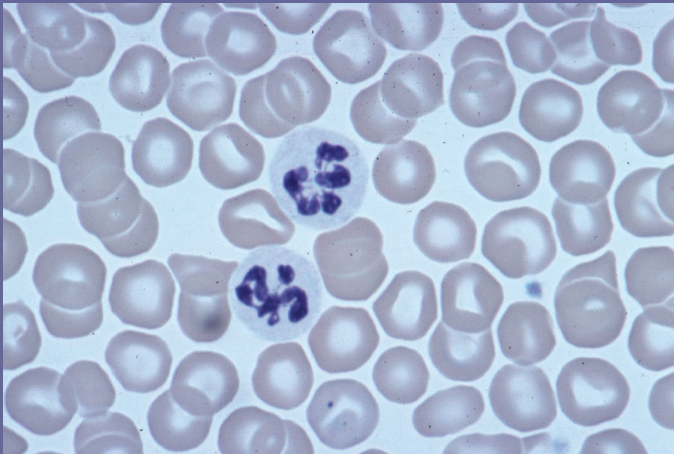
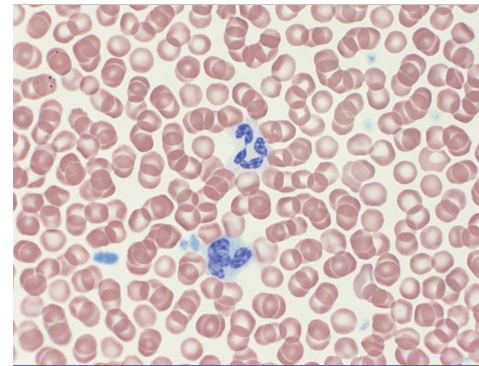
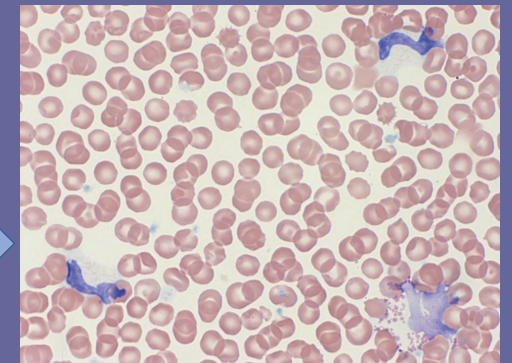


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← NO EDTA

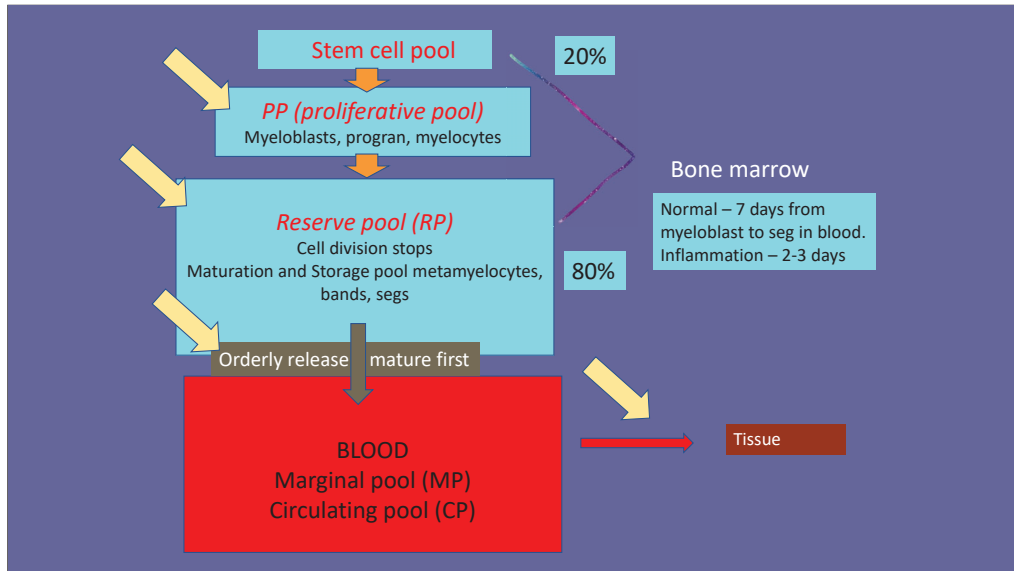
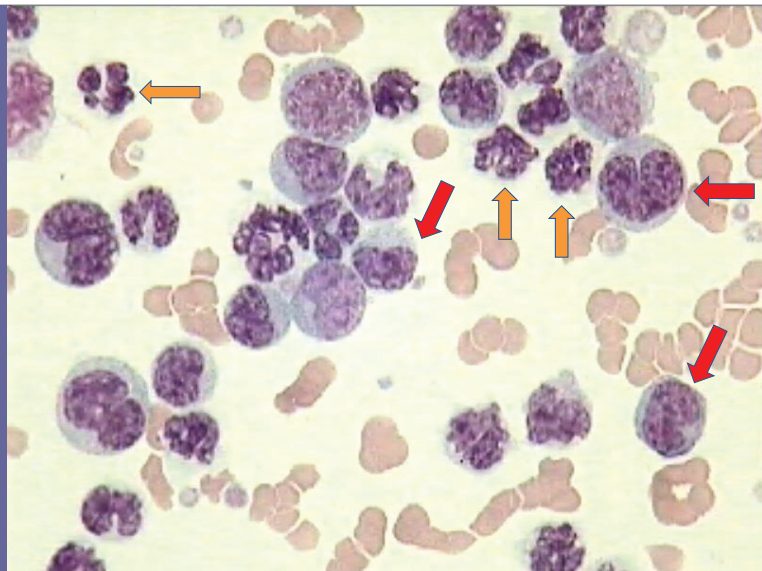
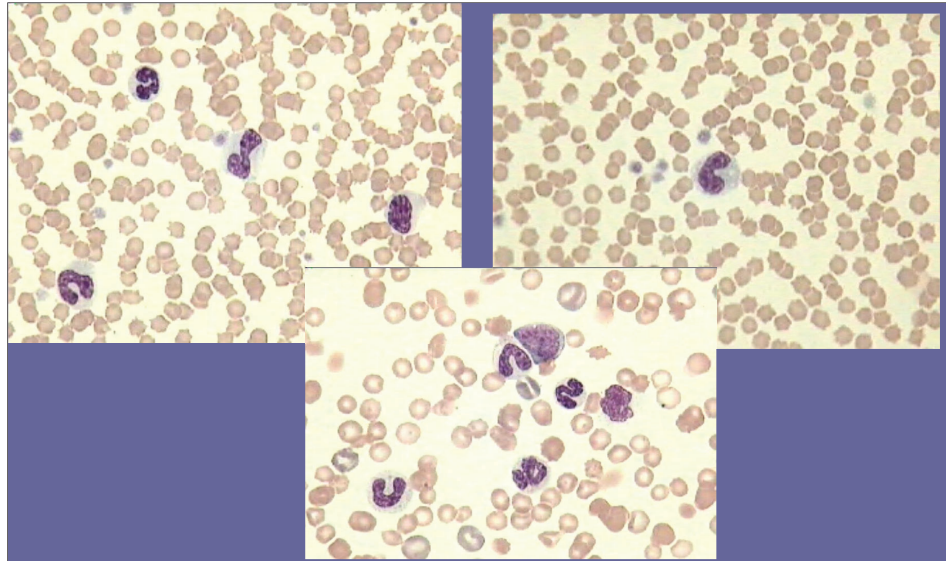
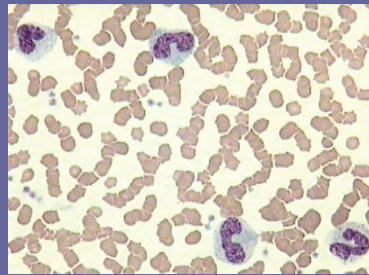
EDTA →

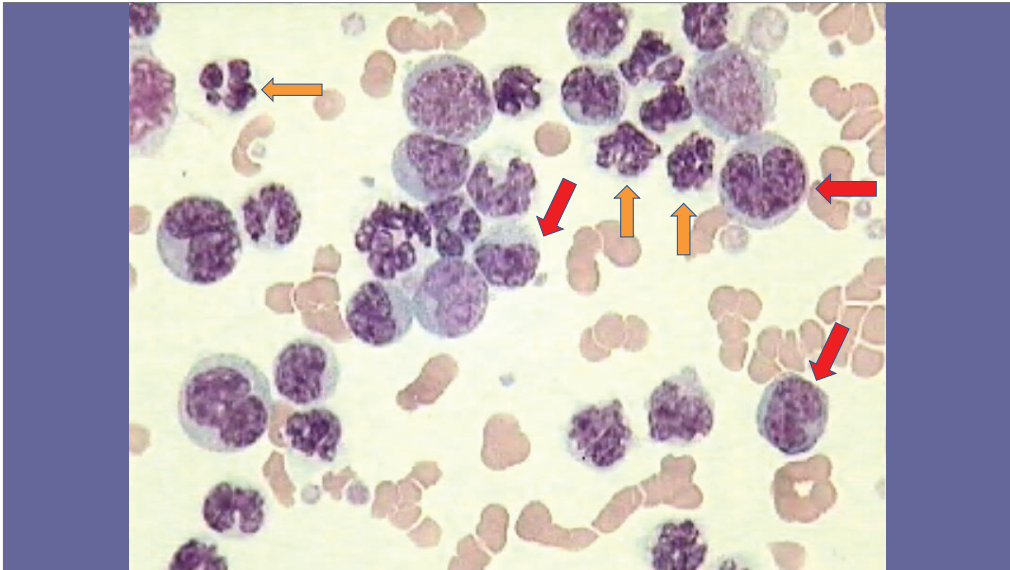




# “Toxic Change”

- Response to marked bone marrow stimulation (cells not toxic)
- Cytoplasmic basophilia
- Dohle bodies (also can be artifact from EDTA storage)
- Cytoplasmic (primary) granules (purple/red)
- Foamy cytoplasm (dispersed organelles)

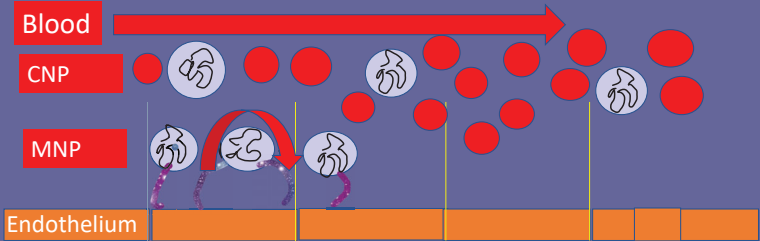




### Neutrophil Kinetics



- Samples of peripheral blood reflect a subset of the neutrophil population – the circulating pool of neutrophils (CNP)



- Neutrophils moving as fast as RBCs = CNP
- MP = Neutrophils moving more slowly than RBCs
  - Adhesion molecules
    - Neutrophils hesitatingly adherent
    - Uneven distribution of cells in blood vessels
    - Greater concentration of neutrophils in postcapillary venules



Marginated pool = Circulating pool dog, horses, calves



Marginated = 3X Circulating pool cats

Marginated pool

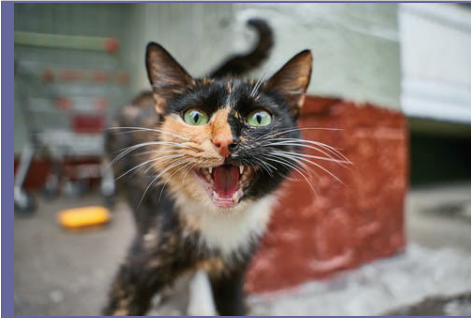
Circulating Pool



## Neutrophilia

## Neutrophilia – What are the causes?

- Physiologic (epinephrine)
- Steroid effect
- Inflammation
  - Neutrophil influx > egress
    - Particularly profound
      - Sequestered inflammation
      - Young animals
      - Certain infectious agents
      - Immune mediated hemolytic anemia
        - May relate to ischemia, thrombosis
- Paraneoplastic neutrophilia
- Inherited conditions (LAD)



## Physiologic neutrophilia (Fear, excitement, etc)

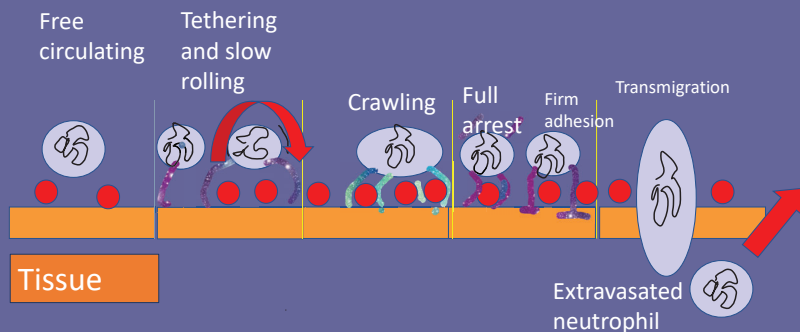
- Catecholeamines (not steroids)
- MNP shift to CNP (TBN normal)
- Neutrophilia and lymphocytosis
  - Lymphocytes < 10,000/uL usually
- 20 – 30 minute duration
- Up to 2 X URL dogs, horses, cows
- UP to 3 – 4 URL cats
- Common in young horses and in cats
- Uncommon in dogs

## Steroids and neutrophilia

- Down regulation of adhesion molecules (decreased stickiness)
  - Shift from MNP to CNP
- Increased bone marrow release
- Decreased migration neutrophils into tissues
- Neutrophilia, **lymphopenia**, eosinopenia, monocytosis
- Peak – 4 – 8 hours after steroid administration
- WBC return to normal 24 hrs after single injection and 2 – 3 days after cessation of long term steroid therapy
- Continuous steroid therapy – Neutrophil count normalize in sev wks
- Common in dogs, not common in cats
- Cows – stress, LDA, milk fever, ketosis, etc
- Horses - exercise

## Steroid leukograms in various species

WBC	Dogs	Cats	Horses	Cows
Total WBC (X 10 <sup>3</sup> )	15 – 35	20 – 30	15 – 20	8 -18
Segs	↑	↑	↑	WRI ↑
Bands	WRI ↑	WRI	WRI	WRI
Lymph	↓	↓	↓	↓
Monos	WRI ↑	WRI ↑	WRI	WRI ↓
Eos	↓	WRI	WRI	WRI ↓

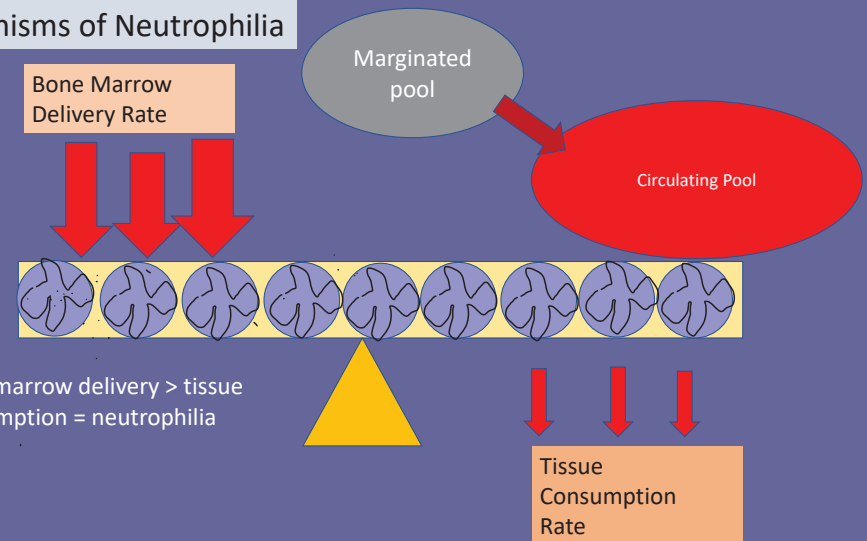


Neutrophil response to inflammatory stimuli

### New information:

1. Neutrophil subsets
2. Interaction with other immune cells: Dend C, B/T lymphs, endothelial cells and influence immune response
3. Reverse migration from tissues

## Mechanisms of Neutrophilia



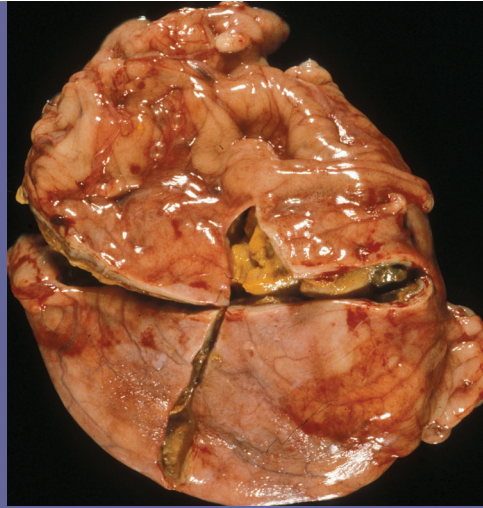
Bone marrow delivery > tissue consumption = neutrophilia

Tissue Consumption Rate



## Inflammation and neutrophilia

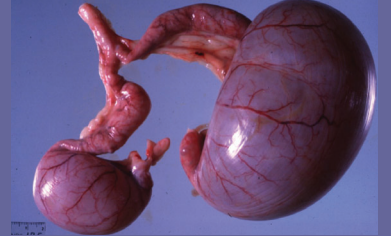
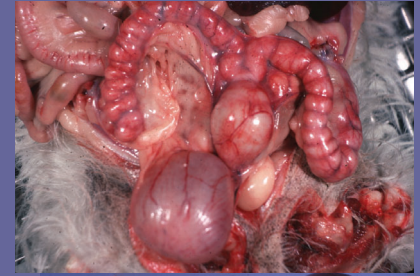
- Neutrophilia with/without left shift
- Toxic change (+/-)
- Monocytosis
- Usually lymphopenia but can be normal or slightly increased



Mesenteric abscess in a dog  
Noah's Arkive/Davis Thompson Fnd

## Sequestered Inflammation and Neutrophilic Leukocytosis

- Pyometra
  - Neutrophilia can be marked >125,000/uL
  - Increases transiently after OHE



Images courtesy of Noah's Arkive Davis Thompson Foundation

## Hepatozoon canis – Queensland Dog, 2018

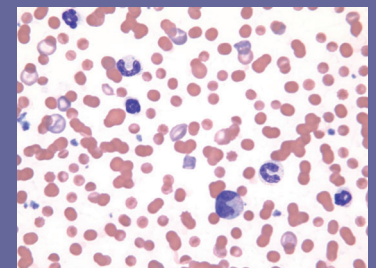
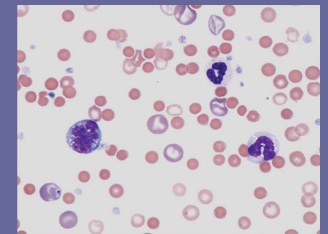


Image courtesy of Davis Thompson foundation

- Asymptomatic to severe disease
  - Lethargy, cachexia, fever, anemia
- Extreme neutrophilia in severe cases (up to 150,000/uL)
- Rhipicephalus sanguineus is main vector
  - Dog ingests infected tick
- Diagnosis: blood smear/buffy coat exam; PCR

## Neutrophilia and anemia

- IMHA
- Rarely - marked leukocytosis (up to 175,000/uL)
- Primary mechanism may be inflammation relating to effects of ischemia and thrombosis
- Can be poor prognostic sign
- Can be difficult to distinguish from septic inflammation



## Paraneoplastic granulocytosis (30 – 40,000 - > 100,000/uL)

- G-CSF production
- Metastatic Fibrosarcoma
- Pulmonary adenocarcinoma
- Rectal adenomatous polyp
- Renal cell carcinoma
- Splenic sarcomas
- Squamous cell carcinoma (cat)
- Mammary carcinoma (cat)



Noah's Arkive; Davis Thompson Foundation

## Leukocyte Adhesion Deficiency

- Irish Setters and Holstein cattle
- Autosomal recessive
- Defect in adhesion glycoprotein
- Decreased neutrophil adhesion, impaired chemotaxis, minimal bactericidal activity
- Gingivitis, oral ulcers, periodontitis, chronic pneumonia, nonhealing wounds
- Marked neutrophilia (up to 100,000/uL)
- Nonregenerative anemia
- Polyclonal gammopathy



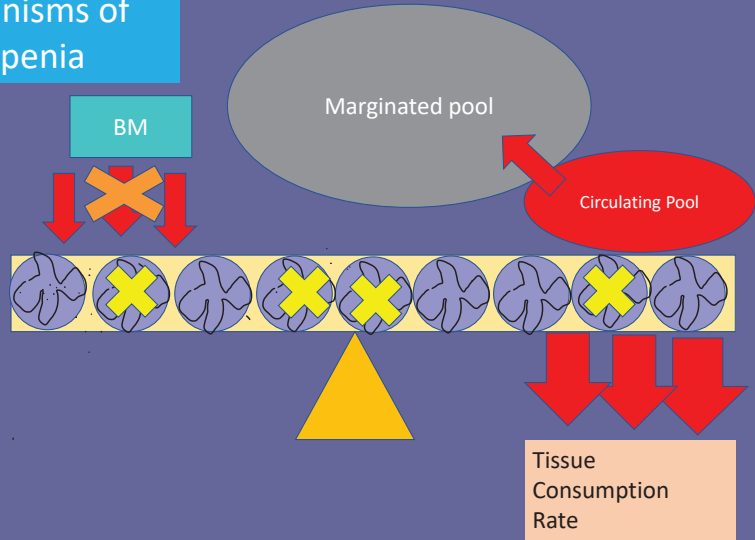
## Neutropenia

## Neutropenia – how does it happen?

- Shift from CNP to MNP
- Overwhelming tissue demand – neutrophils exiting blood into tissue faster than they can be replaced
- Neutrophil destruction in the peripheral blood or marrow
  - Can be a mechanism in viral infection
- Bone marrow lesions
  - Decreased or abnormal neutrophil production in the marrow
    - Can be a mechanism in viral infection
- Breed associated



## Mechanisms of neutropenia



## Examples of Neutropenia

- Shift from CNP to MNP
  - Initial reaction to endotoxin
  - Benign transitory neutropenia
- Excessive tissue demand
  - Acute bacterial infection (more common in cows)



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## • Neutrophil destruction or sequestration

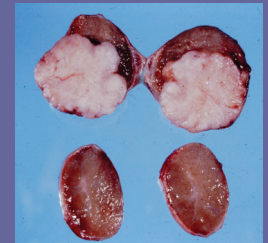
- Hemophagocytic syndrome
- Hypersplenism
- Immune mediated – rare (dogs, horses)
- Drug induced (antibiotics, antithyroid drugs, NSAIDS)
- Unknown (cats)
  - Chronic idiopathic neutropenia?



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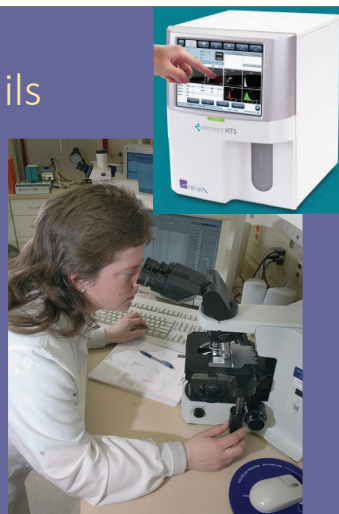
## • Bone marrow disorders

- Decreased neutrophil production
  - Viral diseases, estrogen toxicity, irradiation, chemotherapy, marrow tumor infiltration, hereditary bone marrow disorders
- Increased ineffective granulopoiesis (normally 20% ineffective)
  - FeLV, myelodysplasia, marrow tumor infiltration,



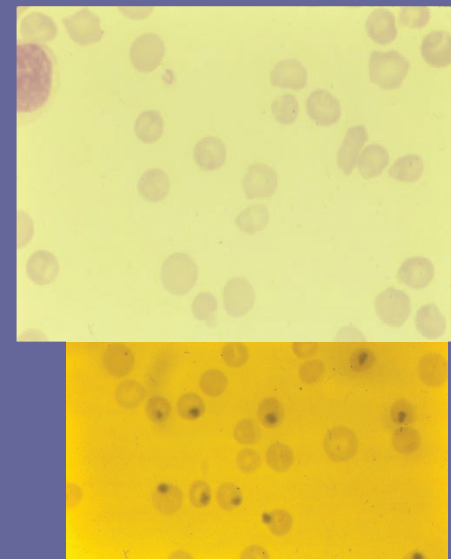
## Counting Neutrophils

- WBC from analyzer
  - Manual differential
  - Automated differential count with analyzer total WBC count
    - Verify on blood smear
    - Estimate WBC count
    - Verify Differential Count
    - Check for inclusions
    - RBC morphology



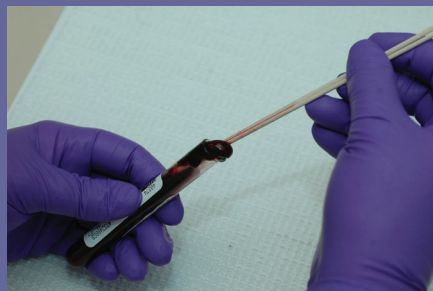
## Causes of falsely increased WBC count

- Incomplete lysis of RBCs
  - Can occur with Heinz bodies
- Large platelets
  - Primarily in cats
- Platelet clumps
- Nucleated RBCs



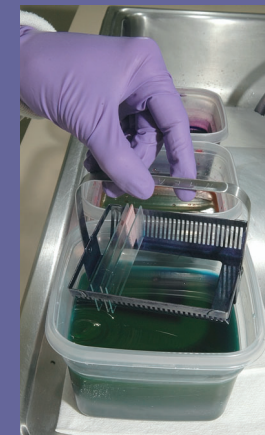
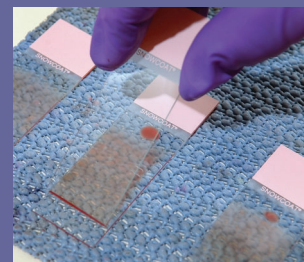
## Causes of false low WBC counts

- Incompletely mixed blood tubes
  - Common problem with microtainer tubes
- Blood clots
  - Also low RBCs and platelets



## How to ensure CBC results are valid

- The blood smear is the best QC



## Manual Differential Count

- Scan slide at low power
- Exam feathered edge for large cells, platelet clumps, parasites
- Find “counting area” where RBC touch but not overlap
- Count and identify 100 leukocytes
  - Or estimate differential to compare with analyzer
- Perform RBC morphology

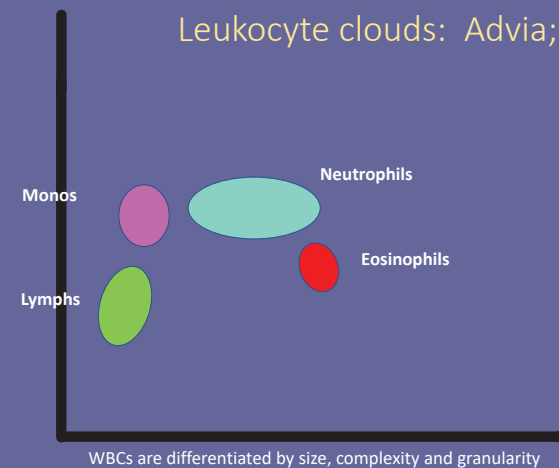
## Automated Differential Counts

- Leukocytes are stained with a fluorescent dye binds to DNA, RNA and cytoplasmic organelles.
- Laser flow cytometry separates cell populations on the basis of fluorescence and light scatter.
- Some analyzers differentiate cells based on staining intensity, light scatter, size and lobularity
- Cytograms display the leukocyte types in clouds
- Species differences in cytogram cell “clouds”.

## Automated differential count does not replace slide evaluation

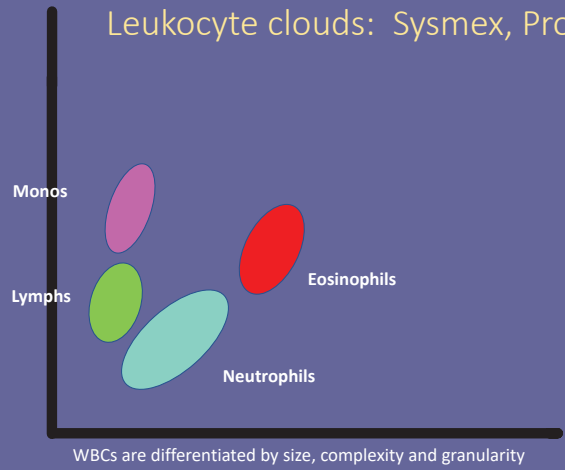
- Reduce the time spent in slide evaluation
- **Verify** the differential count
  - Differentials are not 100% reliable, especially if results are not normal
  - Must verify with slide or risk misdiagnosis!
- Leukocyte morphology
- RBC morphology
- Parasites/viral inclusions

### Leukocyte clouds: Advia; HT5

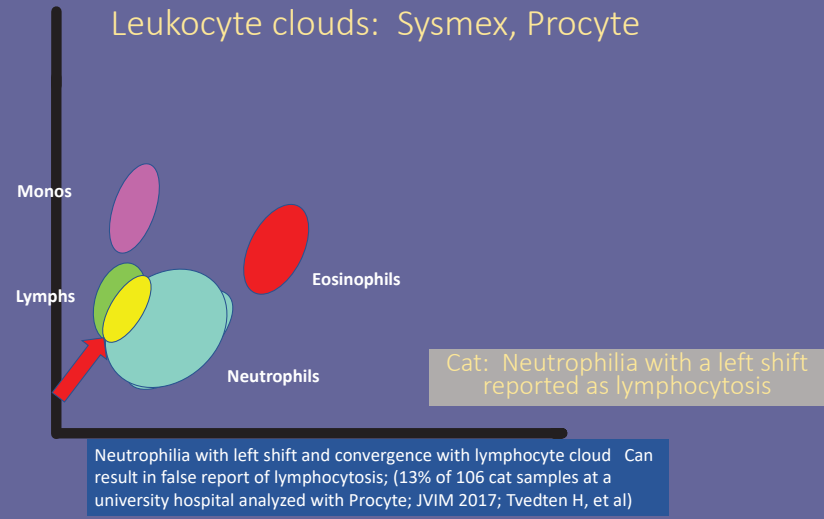




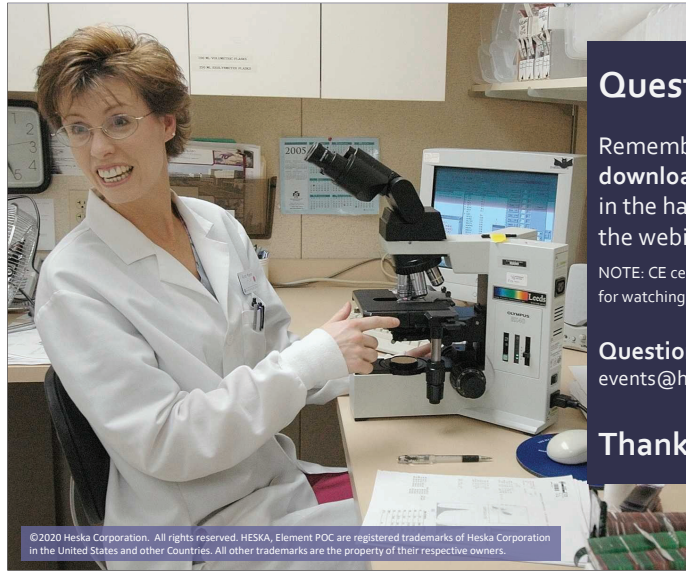
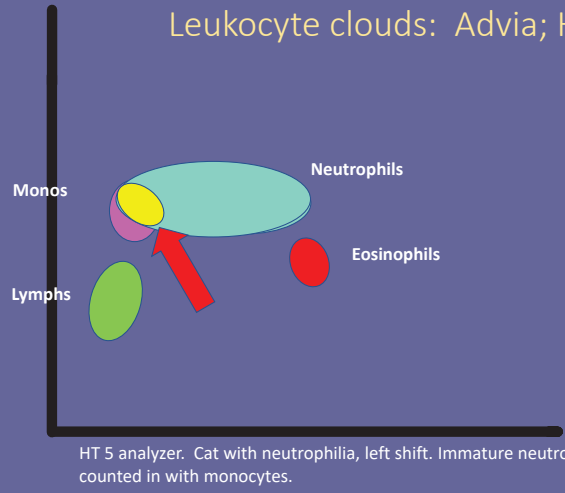
## Leukocyte clouds: Sysmex, Procyte



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## Leukocyte clouds: Advia; HT5



**Questions?**

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events@heska.com

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