Clinical Pathology Tidbits: *Answering some commonly encountered (and sometimes frustrating) "what the…" and "why does…" questions (which seems to occur in clinic when you can't call a clinical pathologist to help you!)*

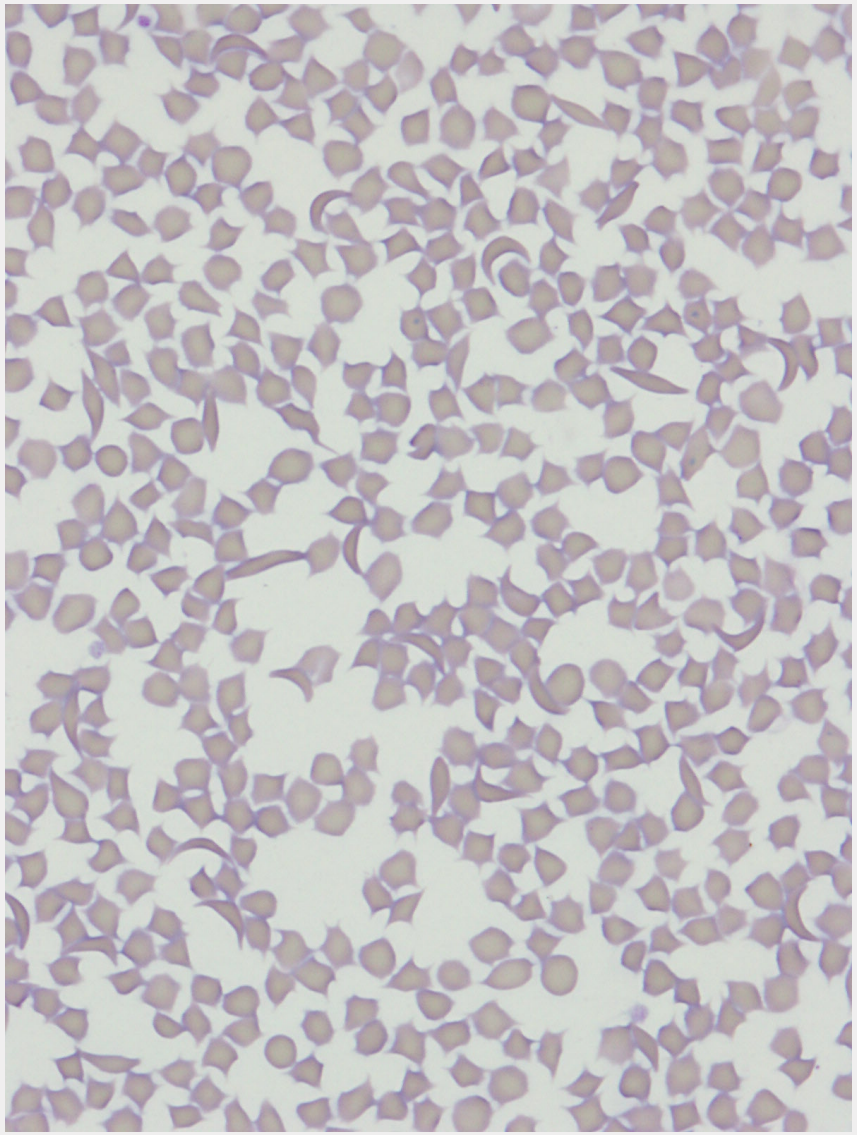
Dr. Angelica Galezowski

BSc, DVM, MVetSc, DACVP (Clinical Pathology)
Assistant Professor
Veterinary Clinical and Diagnostic Sciences

UNIVERSITY OF CALGARY
FACULTY OF VETERINARY MEDICINE

Overview

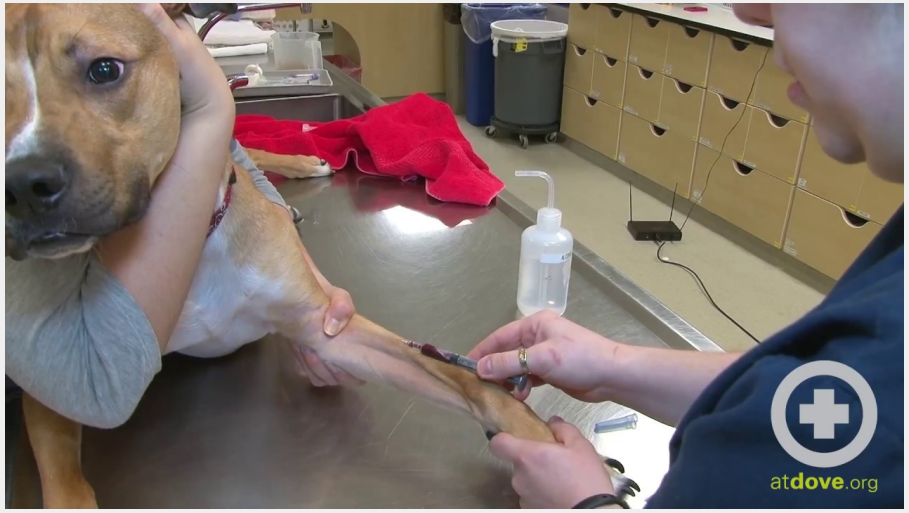
- Sample collection
- Sample preparation
- Storage artifacts
- Staining artifacts
- Fun Facts
- Blood smear evaluation



This RBC morphology is “normal” for which species?

Sample Collection

- Clean venipuncture is essential!
  - To minimize artifactual changes in blood results
  - Ex) Hemolysis:
    1. Needle too small
    2. Pulling too hard on plunger of syringe
    3. Expelling blood vigorously into a tube
    4. Shaking or mixing specimen in tube too vigorously
    5. Not allowing alcohol to dry before drawing blood



Anticoagulant Type

- **Hematology:**
  - Preferred sample in most domestic species: **EDTA/Lavender**
    - How it works: chelates calcium, thus prevents clotting
  - Ensure tubes are adequately filled with blood
    - *Underfilling can result in:*
      - Falsely ↓ PCV (especially if EDTA is liquid)
      - Falsely ↓ MCV
      - Falsely ↑ MCHC
      - Poorly stained cells
  - EDTA = hypertonic (versus RBCs):
    - Small blood sample (0.5mL) placed in a 5 ml purple top tube, RBCs will shrink!
  - *"EDTA samples should ideally be more than half full" – Cornell University*

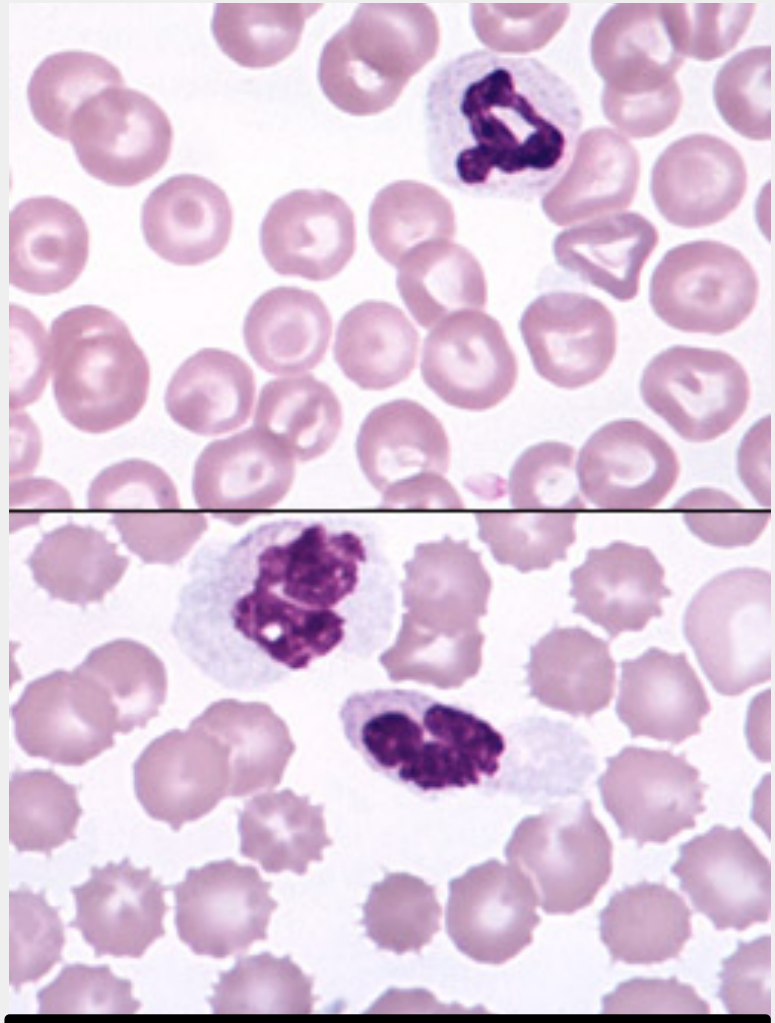
Anticoagulant Type

- **Hematology:**
  - Other sample types:
  - **Heparin (green top):**
    - *Typically not recommended*
      - Platelet and WBC clumping
        - Leads to erroneous counts
    - **EXCEPTION:**
      - Select Avian and other exotic species
        - EDTA may lyse WBC in select species
        - Sample size
          - Small animal = small volume = one tube only for chem and CBC

  - **Citrate (light blue top tube):**
    - *Typically not recommended for CBC analysis*
    - Chelates calcium like EDTA (but "gentler")
    - Sample is diluted, thus need to to mathematical gymnastics to correct CBC generated value

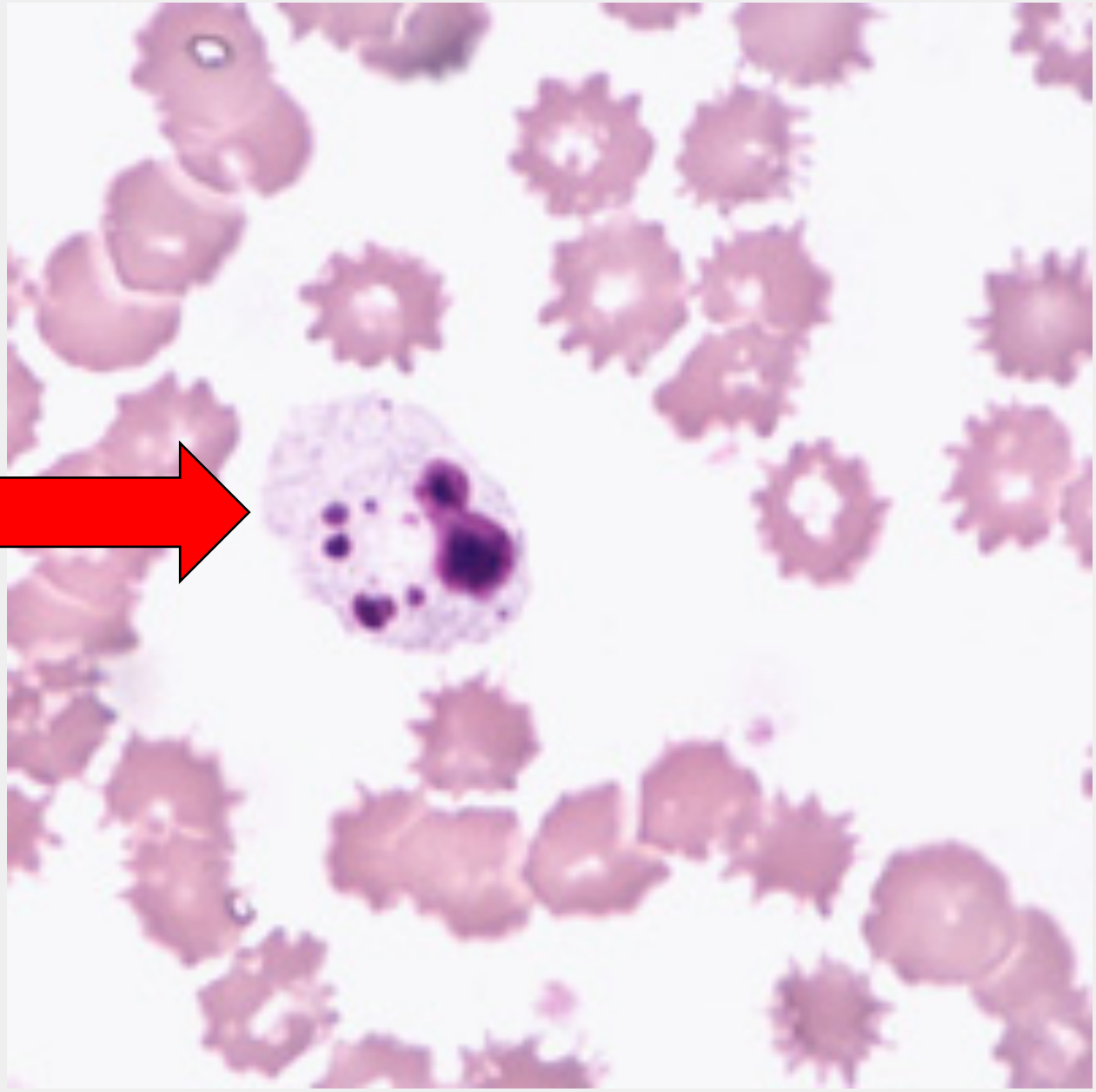
Sample preparation

- Ensure tube is mixed thoroughly, but gently (3-5 times) after blood sample is collected
  - Inadequate mixing will result in sample clotting, which may not be visible to the naked eye (microclots)
- Check blood tube for clots:
  - If present, can affect WBC, RBC and platelet counts → need to recollect
- **MAKE BLOOD SMEARS!!! DON'T WAIT! ☺ ☺ ☺**
  - Delay in blood smear preparation results in artifactual changes that presents a diagnostic challenge

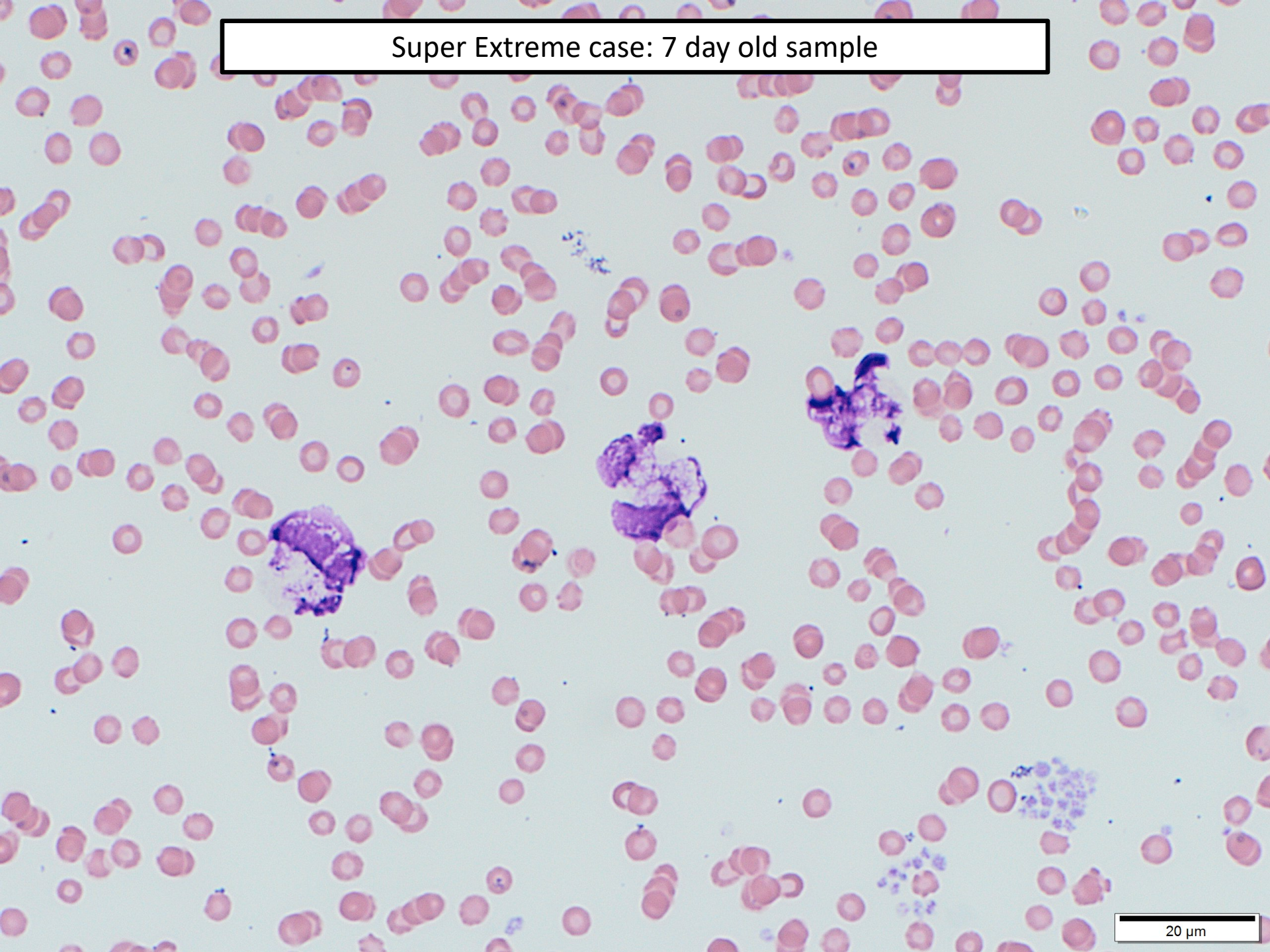


Fresh (top) and overnight (bottom) smears made from the same blood sample. *Eclinpath.com*

?



Extreme case: >48 hour old sample *(eclinpath.com)*

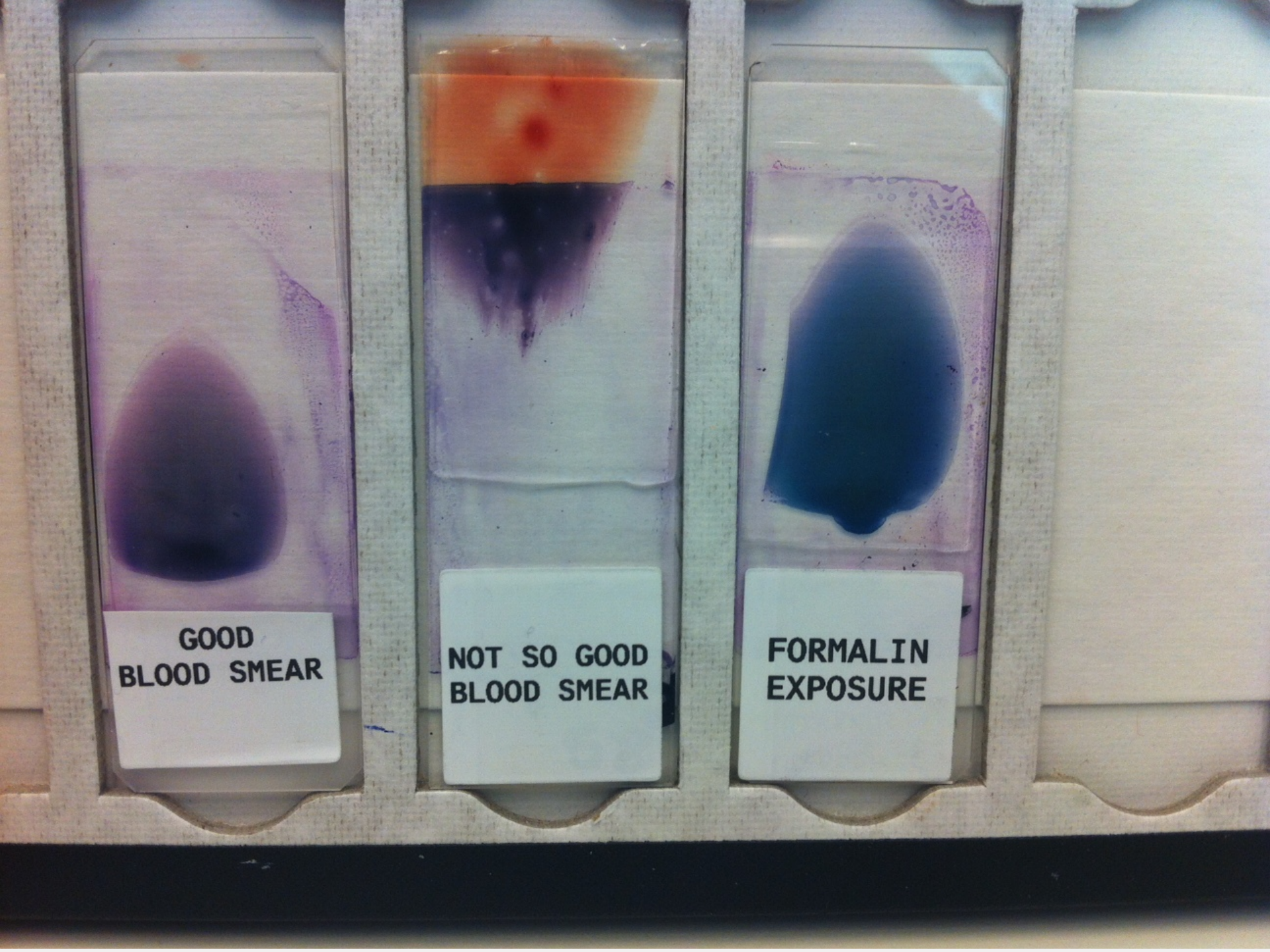

Super Extreme case: 7 day old sample
20 µm

Sample preparation

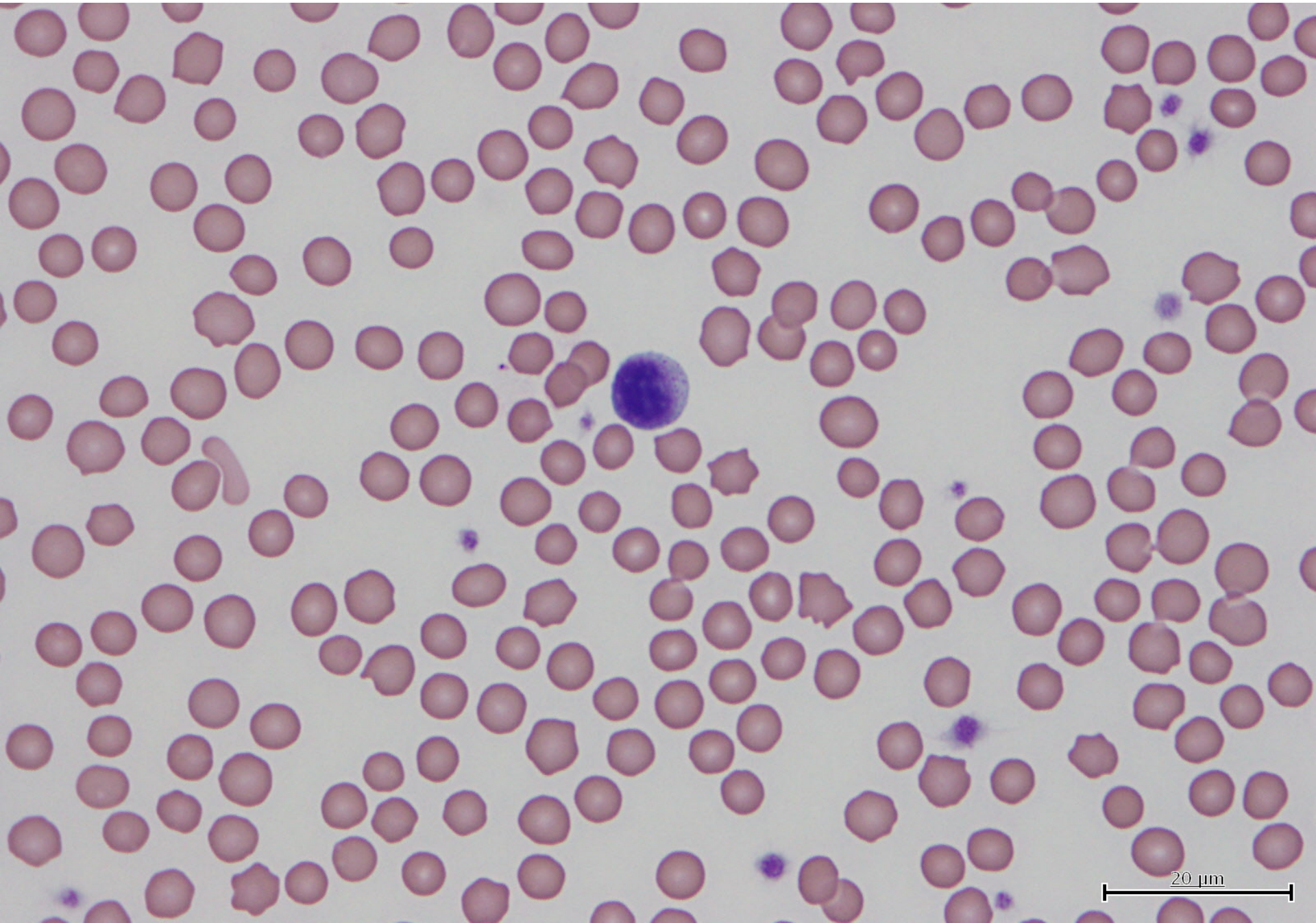
- Labelling:
  - Use pencil for slides; permanent marker/pen for tubes
  - Provide animal and/or owner name or some type of identifier → DON'T SUBMIT BLANK/UNLABELLED SLIDES!

- Storage:
  - Blood tubes: ***Refrigerated*** upon submission to lab
  - Blood smears: Placed in a slide holder at ***room temperature***

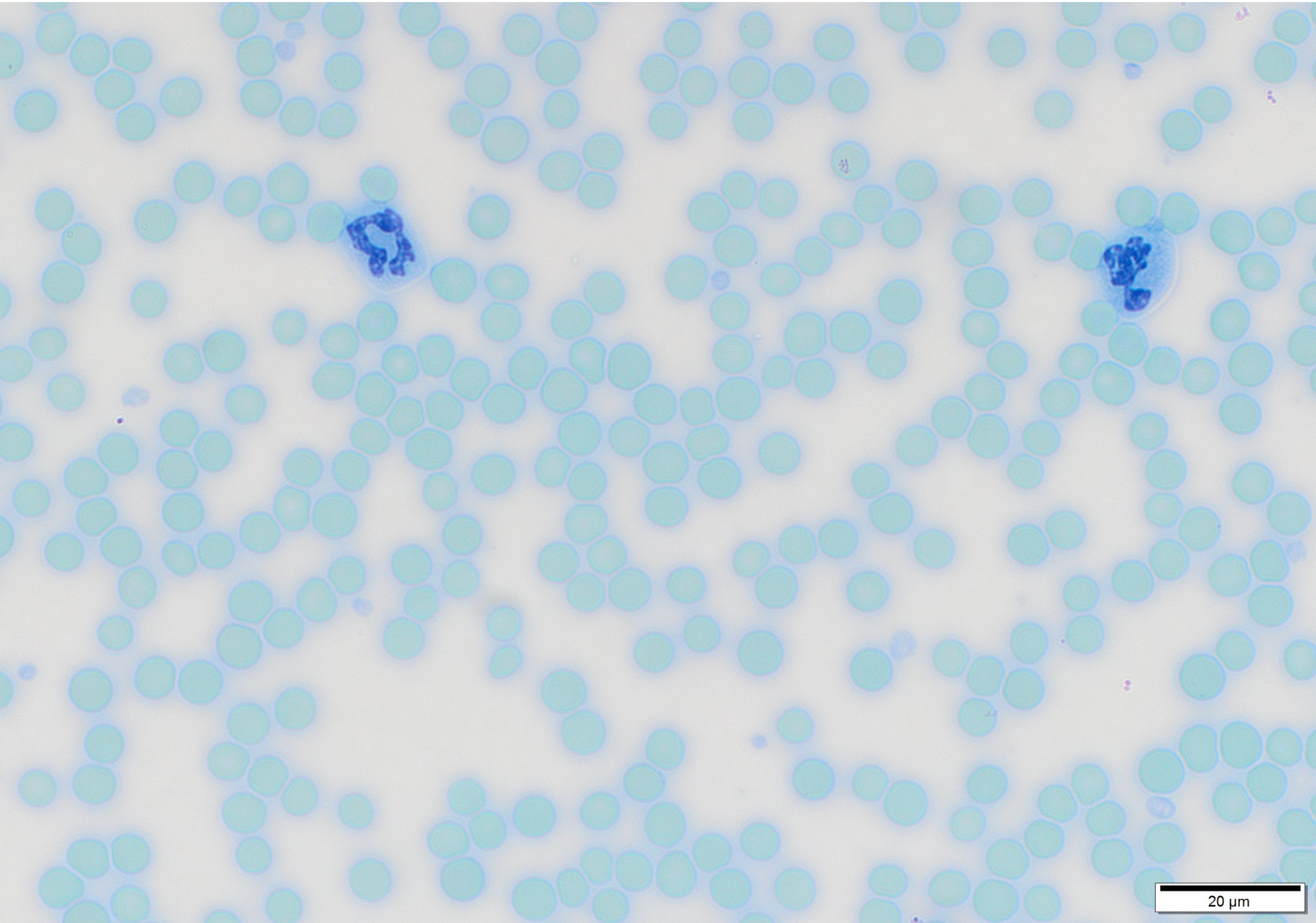
- Submission:
  - Submit with ice packs; wrap blood samples in tissue; ensure slides are in a holder and are not in contact with the ice packs
    - Slides may freeze or become moist → ruptured cells
  - Do not submit blood smears in same bag as surgical biopsy/histo samples


GOOD
BLOOD SMEAR
NOT SO GOOD
BLOOD SMEAR
FORMALIN
EXPOSURE

Blood smear stained with Modified Wright's



Blood smear exposed to formalin



Storage Artifacts

- **Hemolysis:**
  - Usually in vitro due to poor venipuncture technique, freezing of whole blood samples, delayed separation of serum or plasma from cells, delayed submission, etc.
  - **Expected CBC changes:**
    - ↓ Hct, RBC:
      - Results will be falsely low because the lysed RBCs are NOT included in the count/measurement
    - Total Protein (by refractometry):
      - Hemolysis blurs the line, making it difficult to read
    - ↑ Platelet count:
      - Ghost RBCs may be counted by analyzer, falsely increasing the count
    - Hemoglobin parameters:
      - *Most accurate measurement in such cases*
        - Automated analyzers deliberately lyse RBCs to measure Hgb

Storage Artifacts

- **Hemolysis:**
  - **Expected Chemistry changes:**
    - ↑ $K^{+:}$
      - Horses, select dog and cattle breeds, pigs and sheep RBCs contain high K^{+}
    - ↑ AST
    - ↑ Iron
    - ↑ Phosphate
    - ↑ LDH
    - ↑ Mg
    - ↑CK
    - ↓ Amylase
    - ↓ GGT
    - ↓ ALP

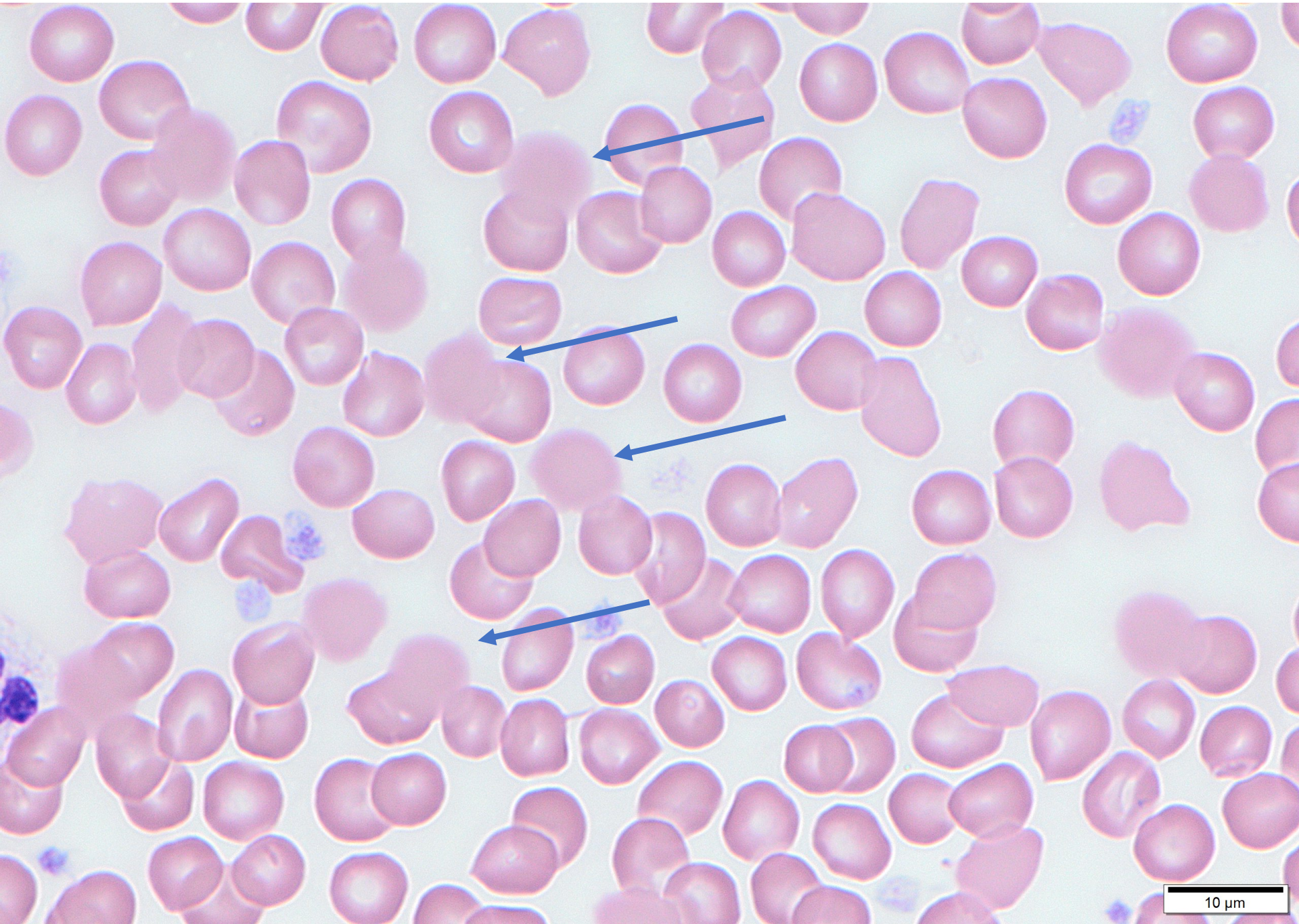
Due to a variety of different methods that you don't have to know, but just be aware this happens!!!

Typically, hemolysis is more problematic when using bench top analyzers; reference labs can usually render a result (unless hemolysis is severe)

Storage Artifacts

- **Lipemia:**
  - Caused by ↑ triglycerides
  - Either a post prandial effect (non-fasted patient) or due to disease (diabetes mellitus, pancreatitis, etc.)
  - **Expected CBC changes:**
    - ↑ Hgb (and Hgb related indices):
      - Falsely high readings due to increased absorbance from lipemia
        - Reference chemistry analyzers overcome this issue as it is DIRECTLY measured
    - Total Protein (by refractometer):
      - Lipids refract light, falsely increased TP
    - ↑ Platelet count:
      - Severe lipemia, large lipid molecules may be counter as platelets
  - **Blood smears changes**
    - Results in “fuzzy” appearing RBCs, can further distort RBC morphology

Lipemic Blood



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  - **Blood smears changes**
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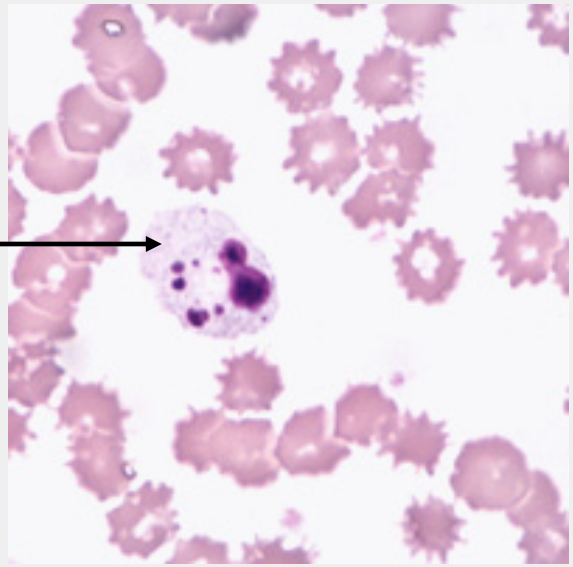
  - **Expected chemistry changes:**
    - Variable effect depending on degree
      - ↓ Na^+, ↓ Cl^-, ↓ HCO_3^- , ↓ LDH, ↑ Mg

Storage Artifacts

- **Delayed processing of sample and/or storage:**
  - **Expected CBC changes:**
    - ↑ MCV, ↓ MCHC:
      - Due to cell swelling
    - **↓ WBC and inaccurate differential count**
      - If submitting to a reference laboratory, ensure a fresh blood smear is concurrently submitted with the whole blood to minimize this issue
      - ↓ platelet count
      - Neutrophil nuclear swelling
      - RBCs swelling:
        - Macrocytic, hypochromic RBCs in old samples
  - **Expected Chemistry changes:**
    - ↑ K^+ (see hemolysis section)
    - ↓ glucose:
      - Consumption by RBCs and WBCs



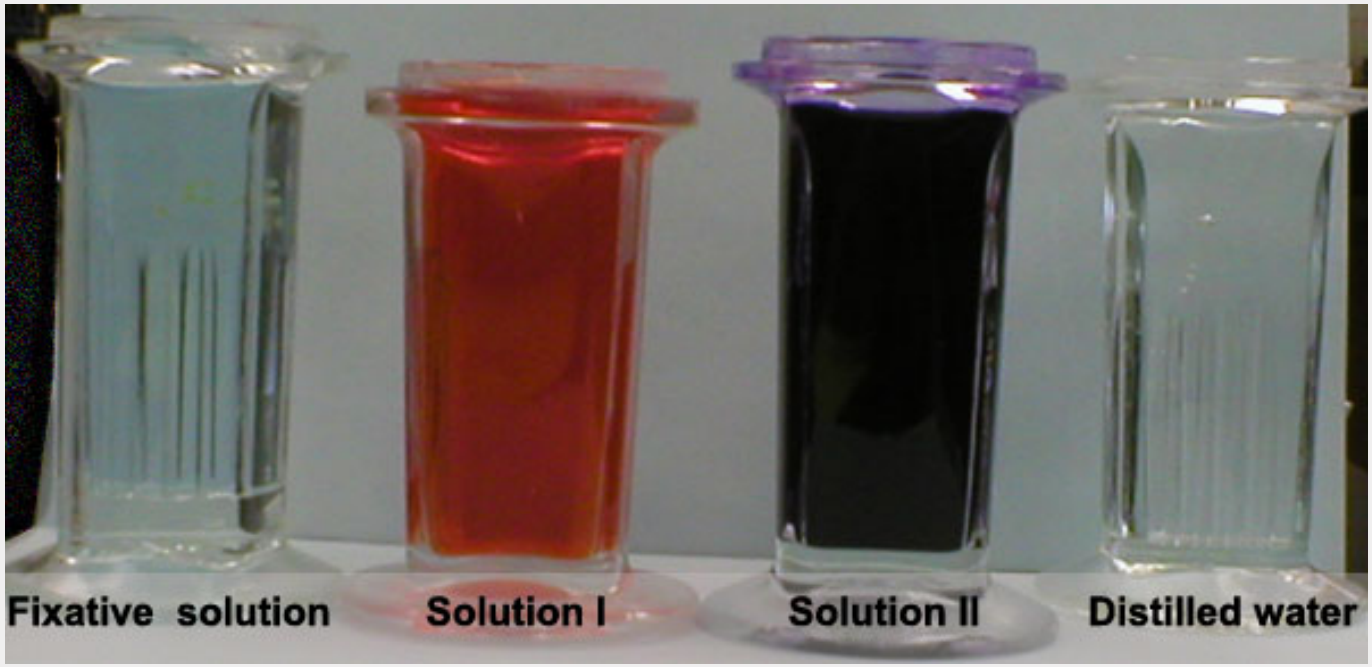
Blood Smears

- Make after blood collection
- Practice always makes perfect!!
- Good blood smear: Nice “thumbnail” shape with an apparent feathered edge

Not so good blood smear examples

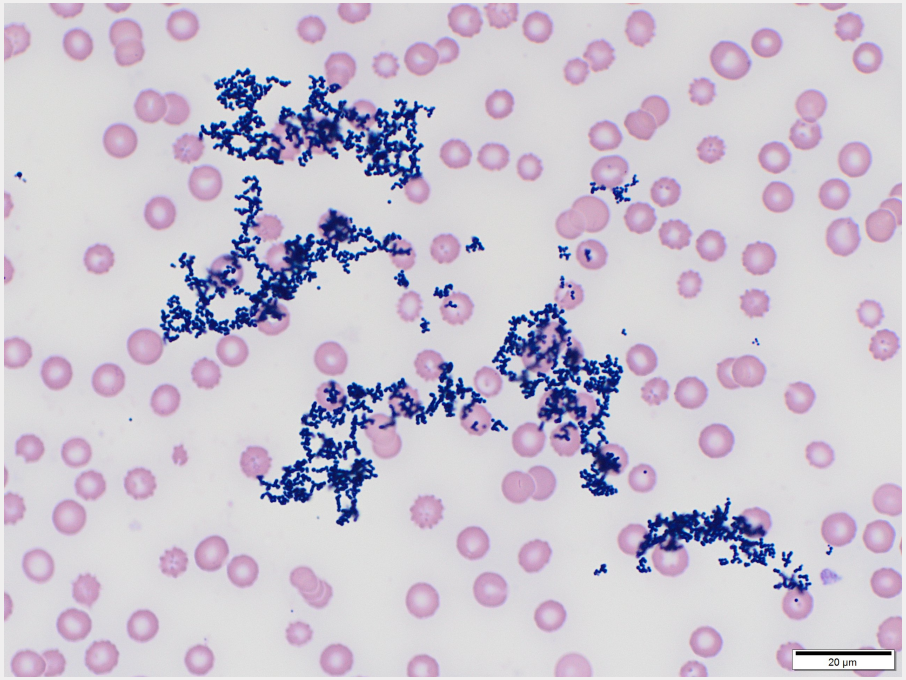
Staining blood smears

- Diff quik → Most popular type of Wright's stain
- Key Points:
  - Seal solutions after use
  - Don't "top up" solutions
  - Beware of artifacts! ☺

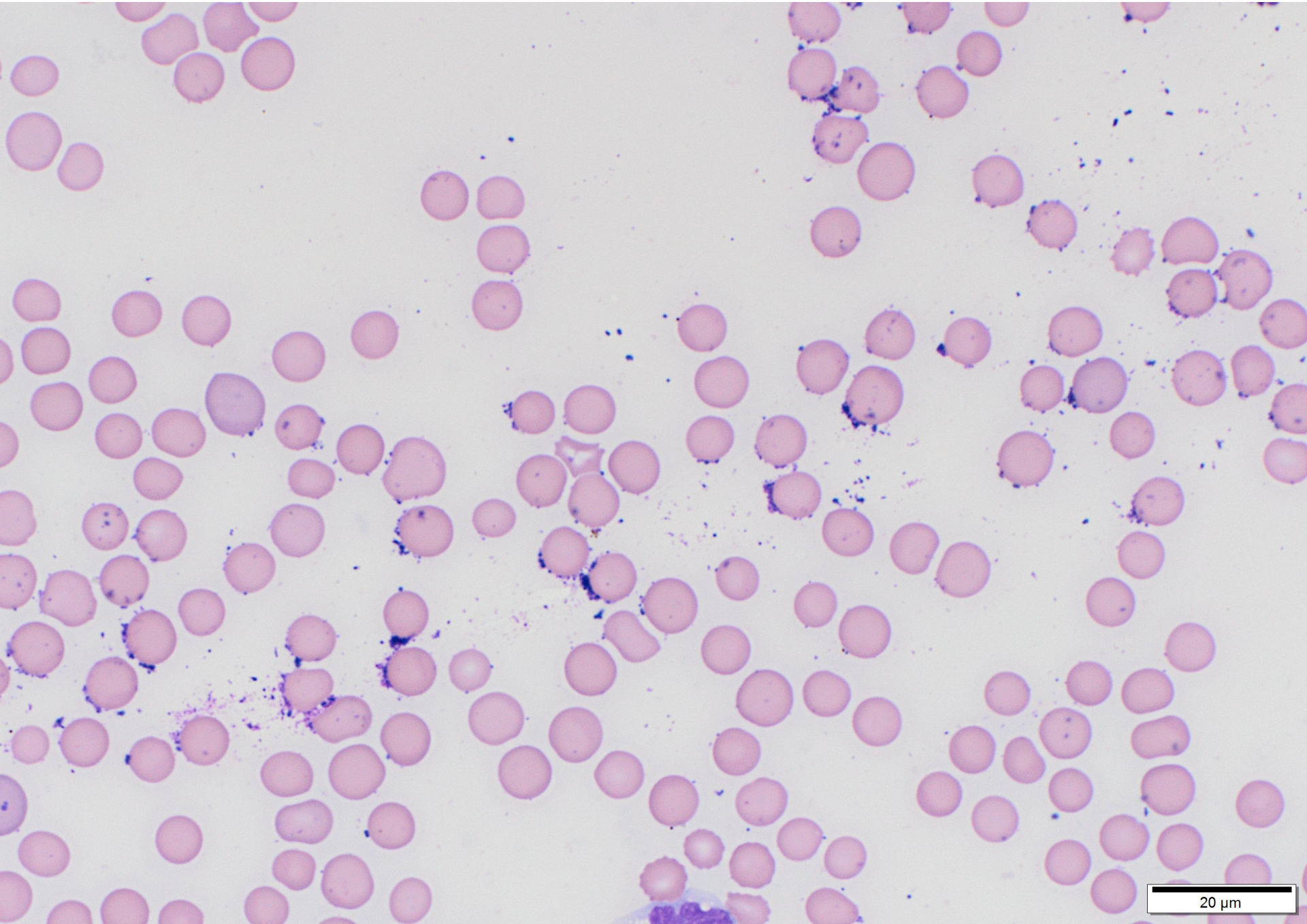


Staining artifacts

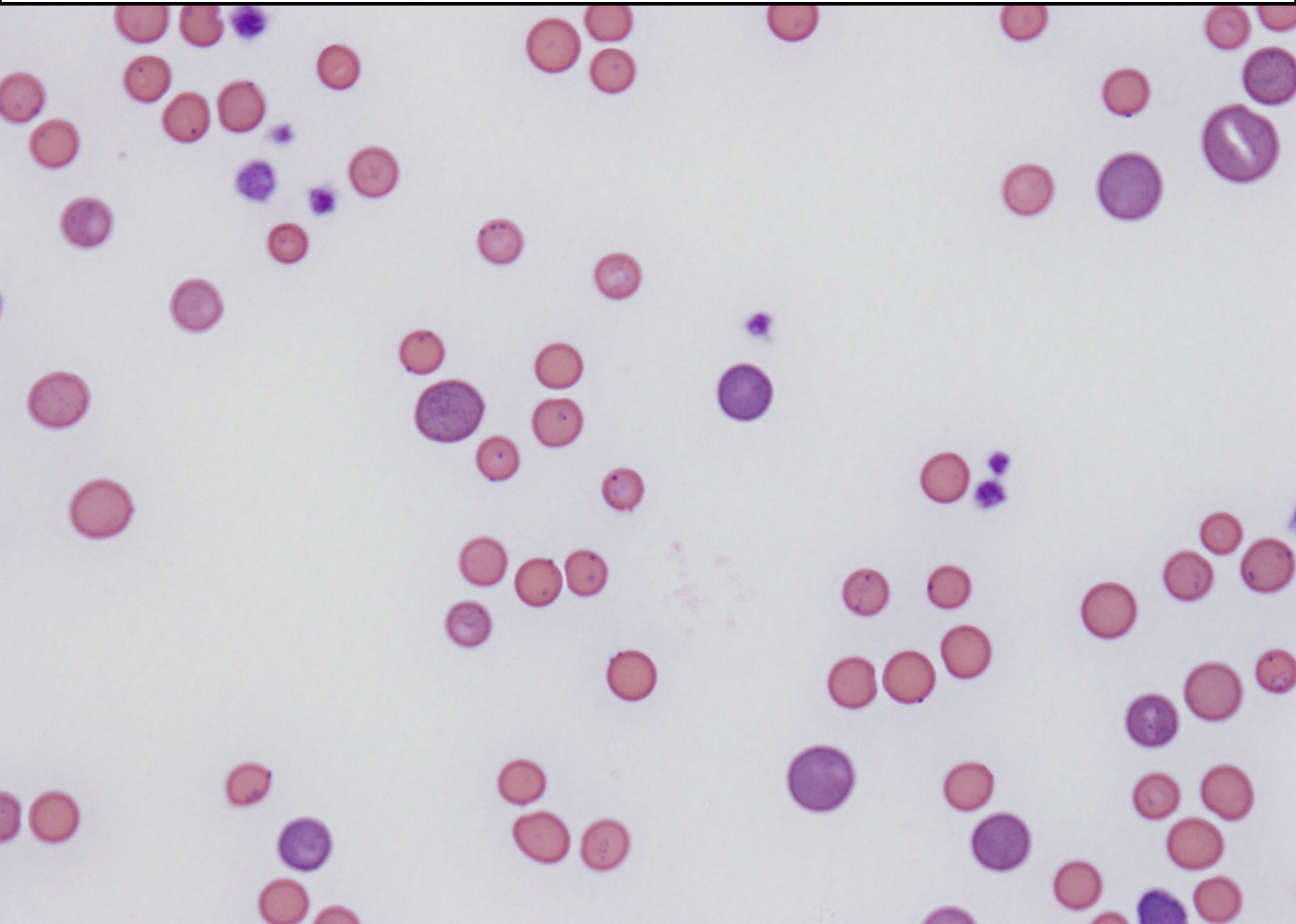
- Stain precipitate:
  - Seen with aged/old stains
  - Samples poorly rinsed after being stained
  - Variably sized clumps of deep purple, round, irregularly shaped extracellular material
- How to differentiate from organisms i.e.) Mycoplasma:
  - Focus in and out of the field:
    - Stain will be out of focus when RBC would be in focus
    - Stain is irregularly shaped whereas bacteria would be consist in size and tinctorial properties



Stain precipitate

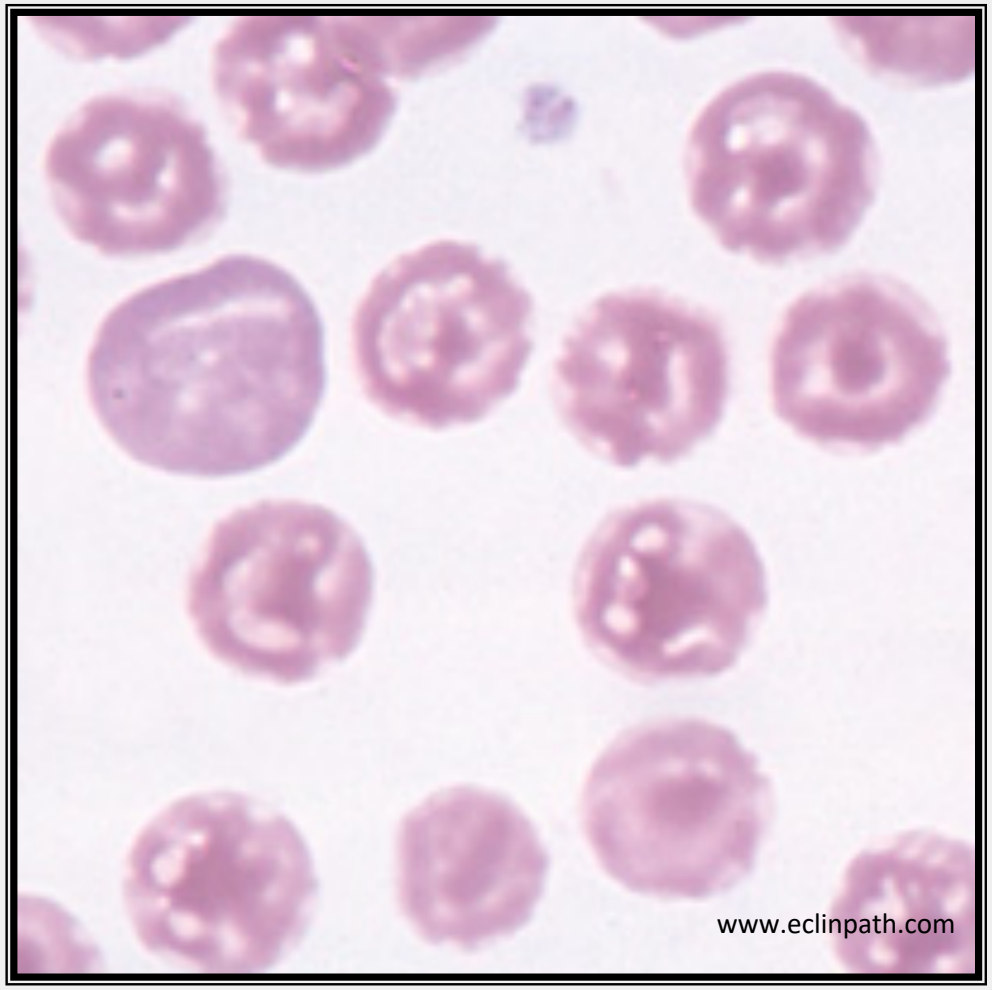


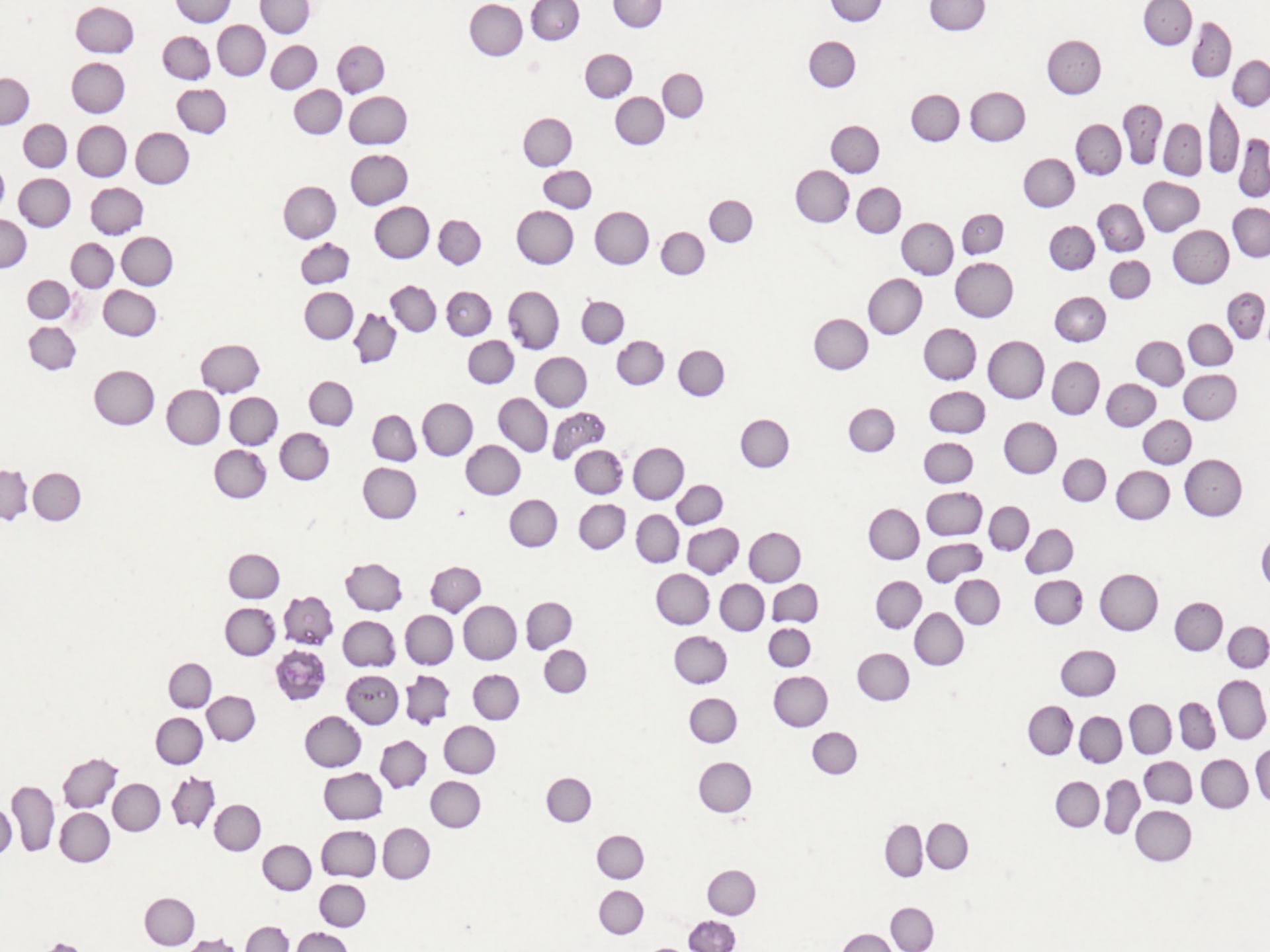
Is this stain precipitate? Or bacterial organisms?? How can you tell the difference?

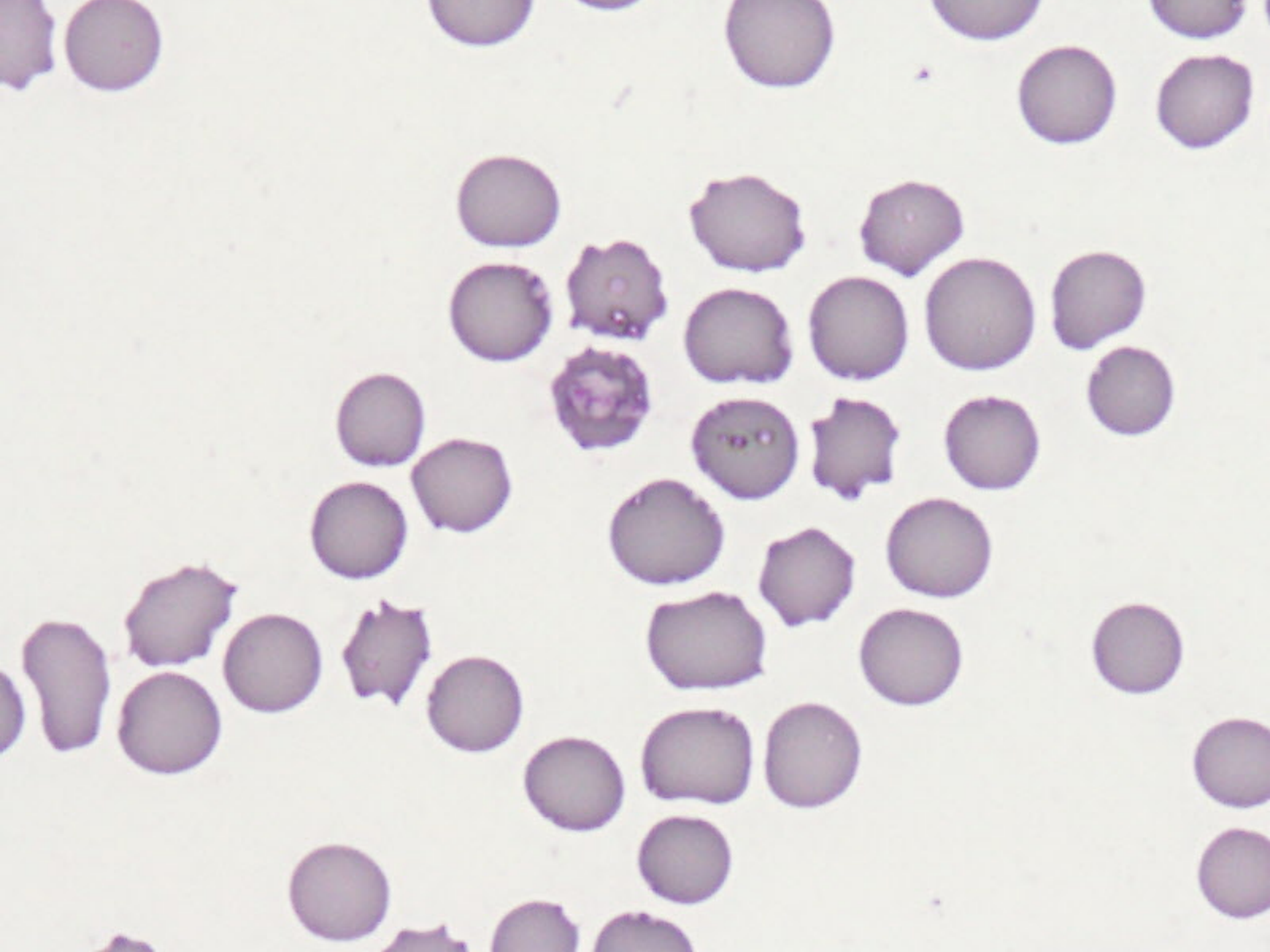


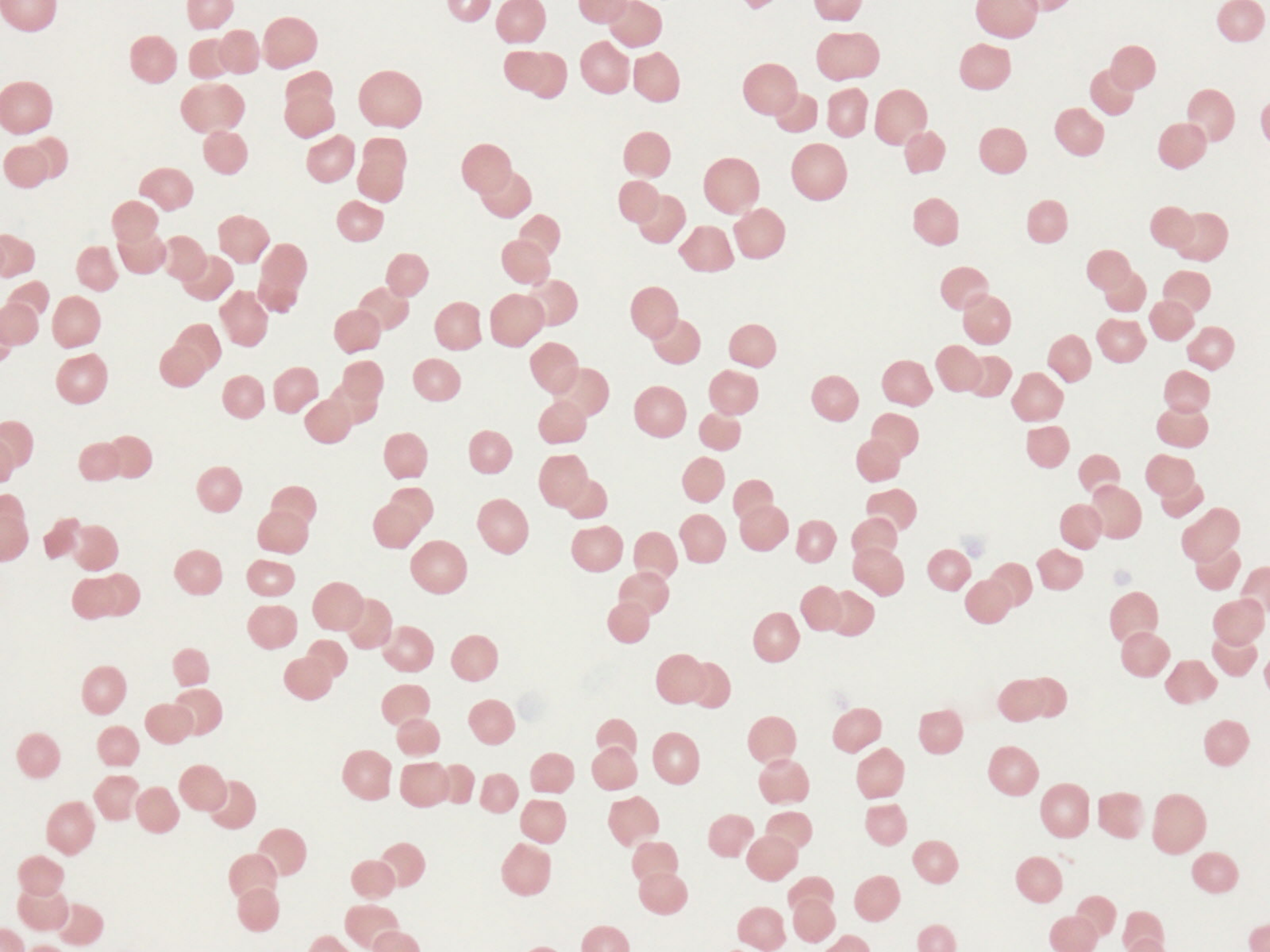
Other Artifacts

- Water artifact:
  - Due to water in the staining solutions
    - Especially seen in high humidity areas
- Appearance:
  - “Bubbly” or moth eaten appearance within erythrocytes
  - Can be mistaken for pathologic inclusions









Bottom Line

- There are **MANY** pre-analytical changes that may account for some of the abnormalities you may encounter on CBCs as well as blood smears
- Imperative to rule out these issues prior to making a diagnosis that has significant treatment and/or prognostic implications
- It's the FIRST STEP in interpreting any abnormal parameters

When the bloodwork is abnormal…

- **Ask yourself: Is the blood work abnormal or is the patient abnormal?**
  - Methodologic differences may cause normal patients to have test results that fall outside published reference intervals
  - Specific reference intervals may not be available
    - i.e.) neonatal patients, reproductive status or even veterinary species
  - Any possibility of pre-analytical or analytical changes to consider?
    - Was there a delay in sampling?
    - Evidence of hemolysis, lipemia and/or icterus?
    - Is the patient on any medications?
  - Normal clinical chemistry profile does not rule out disease
    - Ex) BUN and Creatinine → see elevations when 66-75% of renal function is lost

information from Dr. Leslie Sharkey

Fun Fact #1

- Microcytosis (as evidenced by a ↓ MCV) can be seen in healthy dogs of a certain breed.

Japanese breeds: Shiba Inu, Akitas, etc

Important to recognize potential breed related changes, but when else can we see a microcytosis?

Fun Fact #2

- Macrocytosis (as evidenced by a ↑ MCV) can be seen in healthy dogs of a certain breed.

Miniature or Toy Poodles

Fun fact #3

- Inherited macrothombocytopenia has been documented in which dog breeds?

- Most notably:
  - Cavalier King Charles Spaniel
- But also:
  - Norfolk and Cairn Terriers
  - Other breeds:
    - Labrador Retrievers, Poodle, Chihuahua, Maltese Terrier

What do you see:

- Low platelet counts: 30,000 - 150,000 μL with large circulating platelets
- Affected dogs are asymptomatic and have normal platelet crits (measurement of total platelet mass); with IM, it's due to increased platelet size

Fun Fact #4

- **Puppies:**
  - RBC mass varies significantly from that of adults
  - Hct:
    - Approximately <30%
    - Generally reach adult levels by about 6 months to 1 year of age
  - ↑ ALP, ↑ P
    - Seen in growing animals

- **Foals:**
  - Microcytosis:
    - Up to 4 months of age
  - ↑ ALP, ↑GGT, ↑bilirubin
    - First couple of weeks to 2-3 months of age

Need to be aware that potential neonatal changes exist before diagnosing a puppy with anemia or foal with liver disease!

Fun Fact #5

- Many clinicians dislike endocrine…
- Especially when results are not black and white

Interpret these findings and give a diagnosis for this 5 year old FS Labrador Retriever:

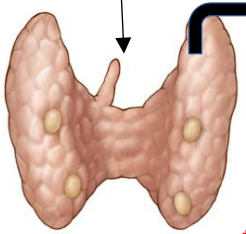
iCa: 2.1 mmol/L (1.27 – 1.51)
Phosphorus: 0.68 mmol/L (0.63 – 2.41)
PTH: 14 pmol/L (0 – 8)
PTHrP: 0

Interpret these findings and give a diagnosis for this 5 year old FS Labrador Retriever:

iCa: 2.1 mmol/L (1.27 – 1.51)
Phosphorus: 0.68 mmol/L (0.63 – 2.41)
PTH: 5 pmol/L (0 – 8)
PTHrP: 0

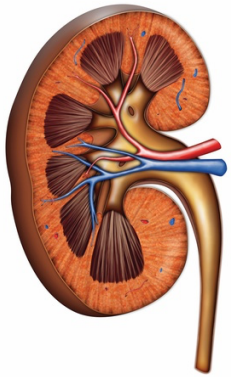
Normal Calcium Homeostasis

↓ blood iCalcium levels



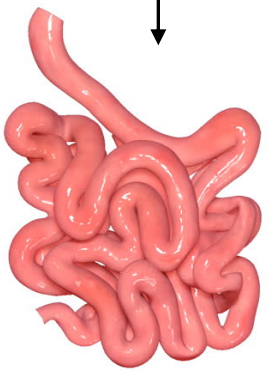
PTH

Parathyroid gl



- ↑ calcium resorption from tubules
- ↑renal excretion of P
- ↑ calcitriol

- ↑ calcium resorption from bone matrix (osteoclasts)



↑ intestinal Ca (and P) absorption

Net Result: 1) ↑ blood iCalcium levels

Primary Hyperparathyroidism

- PTH secreting adenoma or carcinoma
- Keeshonds and German Shepherds
- Clinical signs:
  - PU/PD, urinary incontinence
    - Impaired renal tubular response to ADH and reduced medullary tonicity
    - CNS signs (listlessness, depression, etc) → due to effects of Ca on nervous tissue; suppression of excitability and cell membrane permeability
- Dx:
  - ↑ iCa, ↓ P, N - ↑ PTH, N PTHrp

Diagnosis of 1˚ Hyperparathyroidism based on the PTH assay results relies on the recognition of inappropriate PTH levels in the presence of hypercalcemia" Schaefer et al

TABLE 1 Differential Diagnosis of Hypercalcemia[1,2,5,7,9]

Cause	Comment
Hypercalcemia of malignancy (lymphoma, carcinoma, multiple myeloma, melanoma)	▸ Mediated by PTHrp, which is released by tumor tissue ▸ PTHrp increases osteoblastic bone resorption and renal tubular calcium resorption ▸ ↑ total Ca, ↑ *i*Ca, low-normal to ↓ PTH, normal to ↓ P
Hypoadrenocorticism	▸ Multifactorial pathogenesis ▸ Hyperproteinemia from dehydration and hemoconcentration ▸ Increased plasma protein–binding affinity for calcium ▸ Increased concentration of calcium citrate complexes ▸ Increased renal tubular resorption of calcium ▸ ↑ total Ca, *i*Ca in reference range
Primary hyperparathyroidism	▸ Autonomous secretion of PTH from parathyroid chief cells ▸ ↑ total Ca, ↑ *i*Ca, normal to ↑ PTH, normal to ↓ P
Chronic kidney disease	▸ Complex pathogenesis ▸ Impedance of excretion of PTH and its metabolites ▸ Decreased renal excretion of calcium due to reduction in GFR ▸ Increased concentration of PTH due to excessive secretion and reduced renal tubular hormone degradation ▸ Renal failure or PTH-induced increased concentration of organic cations and complexed calcium ▸ Exaggerated response to vitamin D with increased intestinal absorption of calcium ▸ ↓, normal, or ↑ total Ca; normal to ↓ *i*Ca; normal to ↑ PTH; ↑ P
Vitamin D toxicosis (cholecalciferol rodenticides, human psoriasis medications [calcipotriol, calcipotriene], overzealous dietary supplementation, plants [*Cestrum diurnum, Solanum malacoxylon, Trisetum flavescens*])	▸ ↑ total Ca, ↑ *i*Ca, normal to ↑ P, normal to ↓ PTH
Hemoconcentration (spurious)	▸ Mild hypercalcemia ▸ Fluid volume contraction and secondary hyperproteinemia ▸ Resolves with fluid therapy
Granulomatous disease	▸ Due to alteration of endogenous vitamin D metabolism ▸ Activated macrophages can develop ability to convert 25-hydroxyvitamin D to calcitriol in an unregulated manner ▸ ↑ total Ca, ↑ *i*Ca, low-normal to ↓ PTH, normal to ↑ P

PTHrp = parathyroid hormone–related peptide; *Ca* = calcium; i*Ca* = ionized calcium; *PTH* = parathyroid hormone; *P* = phosphorus; *GFR* = glomerular filtration rate.

- 3 year old Female Saluki in apparently good health
- In for a pre-breeding screen
- Full bloodwork:
  - T4 and Free T4 <7 mmol/L and 10 pmol/L respectively (both are decreased compared to RI)
  - TSH is within normal limits
- Is this dog hypothyroid?

INVITED REVIEW

Clinical pathology of Greyhounds and other sighthounds

S. Zaldívar-López[1,2], L.M. Marín[1], M.C. Iazbik[3], N. Westendorf-Stingle[1,3], S. Hensley[1], C.G. Couto[1,3,4]

[1]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA; [2]Center for Molecular and Human Genetics at The Research Institute at Nationwide Children's Hospital, Columbus, OH, USA; [3]Veterinary Medical Center, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA; and [4]Ohio State University Comprehensive Cancer Center, The Ohio State University, Columbus, OH, USA

Key Words
Adaptation, clinical chemistry, coagulation, hematology, reference interval

Correspondence
Guillermo Couto, Veterinary Medical Center, College of Veterinary Medicine, The Ohio State University, 601 Vernon L. Tharp St., Columbus, OH 43210, USA
E-mail: guillermo.couto@cvm.osu.edu

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Abstract: Owing to the development of Greyhounds as racing sighthounds, these dogs have acquired unique physiologic adaptations that distinguish them from other breeds. Reference intervals for many analytes in retired racing Greyhounds (RRGs) differ from those of other breeds; most of the hematologic differences have also been described in other sighthounds. In this review, we provide a survey of the literature on clinical pathology of Greyhounds and other sighthounds and results of laboratory testing, including analysis of CBCs, biochemical profiles, coagulation tests, and blood gases, in RRGs at The Ohio State University. Major clinicopathologic differences in this breed include higher RBC mass, creatinine concentration, glomerular filtration rate, activities of hepatic enzymes, and concentration of cardiac troponin, as well as lower WBC, neutrophil, and platelet counts, thromboelastographic values, and concentrations of serum haptoglobin, total globulins, and T4.

VCP 2011; 40(4): 414-425

Thyroid hormones

Greyhounds and other sighthounds have basal serum total T_4 (tT_4) concentrations below non-breed-specific reference intervals.[43–51] Free T_4 (fT_4) concentrations may also be low, although not to the same extent as tT_4, with reported mean values ranging from 6.0 to 11.6 pmol/L.[45,47] Trained and racing Greyhounds had lower tT_4 concentrations than retired racers had, and tT_4 concentrations were higher 5 minutes after racing.[47] Highly variable total T_3 concentrations have been reported[52]; fT_3 concentrations in Greyhounds are usually below nonbreed-specific reference intervals.[47,51,53] Young pretraining Greyhounds

Need to have a **concurrently high clinical suspicion for hypothyroidism** and full thyroid panel to appropriately diagnose a true hypothyroid greyhound or sighthound.

Canine hypothyroidism is the most commonly over-diagnosed endocrinopathy

Canine hypothyroidism is the most commonly over-diagnosed endocrinopathy

- Commonly used tests are:
  - T4
  - FT4
  - TSH
- Need to consider entire patient, not just lab results
  - Other causes for low T4:
    - Breed, age, daily fluctuations, concurrent illness, medications
- ***You should have a valid reason to run thyroid assays***

Thyroid hormones

- **T4:**
  - Great first choice; but not in isolation given that other factors can impact T4 levels
- **FT4:**
  - Non-protein bound fraction
    - Should be assessed via equilibrium dialysis
  - Still affected by non-thyroid illness…
    - BUT not as much
    - Can still be influenced by medications
    - NOT affected by auto T4 antibodies
- **TSH:**
  - Should be ↑ with hypothyroidism
  - BUT…approximately 30% of hypothyroid dogs will have normal TSH levels
  - Euthyroid and medications can influence TSH levels
- **Others:**
  - T3, FT3:
    - Not clinically relevant; fluctuate more dramatically than T4
    - Cross react with T3 Autoantibodies (present in many patients)
  - T4aa:
    - Cross reacts with some lab methodology
    - Could increase to normal or HyperT4 range
    - Run if:
      - Clinical suspicion of T4 and normal or elevated T4 values

Case example - Jem

- 9 year old FS Lab X
- History:
  - Hair loss (non pruritic), normal thirst and urination
- PE:
  - Relatively unremarkable (aside from the alopecia)
  - Dry Hair Coat

Differentials?

- Hypothyroidism
- Hyperadrenocorticism

Bloodwork would help narrow it down further:

- Jem only had a marked hypercholesterolemia

HYPOTHYROIDISM

What's your diagnosis?

Test	Result	Flag	Ref Int	Units
TT4	<5	L	12-40	nmol/L
TSH	1.7	H	0.03-0.58	Ng/ml

But these are Jem's ACTUAL results

Test	Result	Flag	Ref Int	Units
T4	42	H	12-40	nmol/L
TSH	1.7	H	0.03-0.58	ng/mL

But what if you had this?

Test	Result	Flag	Ref Int	Units
T4	59		15-67	nmol/L
TSH	61	H	0-37	mU/L
fT4	0	L	6-42	Pmol/L
T4aa	29	H	0-20	%

Courtesy of Dr. McMillan, DACVIM, UCVM

The following demonstrates the importance of testing only with clinical suspicion of disease

T4	Low in 31%
fT4	Low in 22%
TSH	Increased in 8-12%

Population of ill patients undergoing thyroid hormone testing

Courtesy of Dr. McMillan, DACVIM, UCVM

Diagnostic Testing Summary

1) Laboratory results should ***always*** be interpreted in conjunction with clinical signs and history when diagnosing canine hypothyroidism.

2) ↓ TT4 or ↓ FT4 (eqd) + ↑ TSH = Hypothyroidism
Best combination for screening of hypothyroidism.

3) Concurrent illness or medications can result in a similar thyroid hormone profile as a hypothyroid patient, further reinforcing the importance of clinical signs and history when diagnosing hypothyroidism.

- FT4 (eqd) less commonly affected by non-thyroidal illness than T4
- Normal thyroid panel + clinical signs & history: Less likely to be thyroid; need to start looking for other metabolic and/or systemic diseases, **BUT…**
    - If clinical index of suspicion is still high, re-test in 4-6 weeks (ensure animals are off thyroid supplementation for 6-12 weeks)

Urine Storage

- Ideally evaluated within 30 minutes of collection
- According to ASVCP guidelines:
  - Storage for maximum of 24 in the refrigerator (Dr. Osborne suggests 6-8 hours)
- Be aware that urine glucose, bilirubin and pH (especially if bacteria are present) are unstable
- Crystalluria can for *in vitro* during storage at room or refrigerator temp
  - If crystalluria is a concern, fresh sample needs to be evaluated immediately
  - Take Pictures!

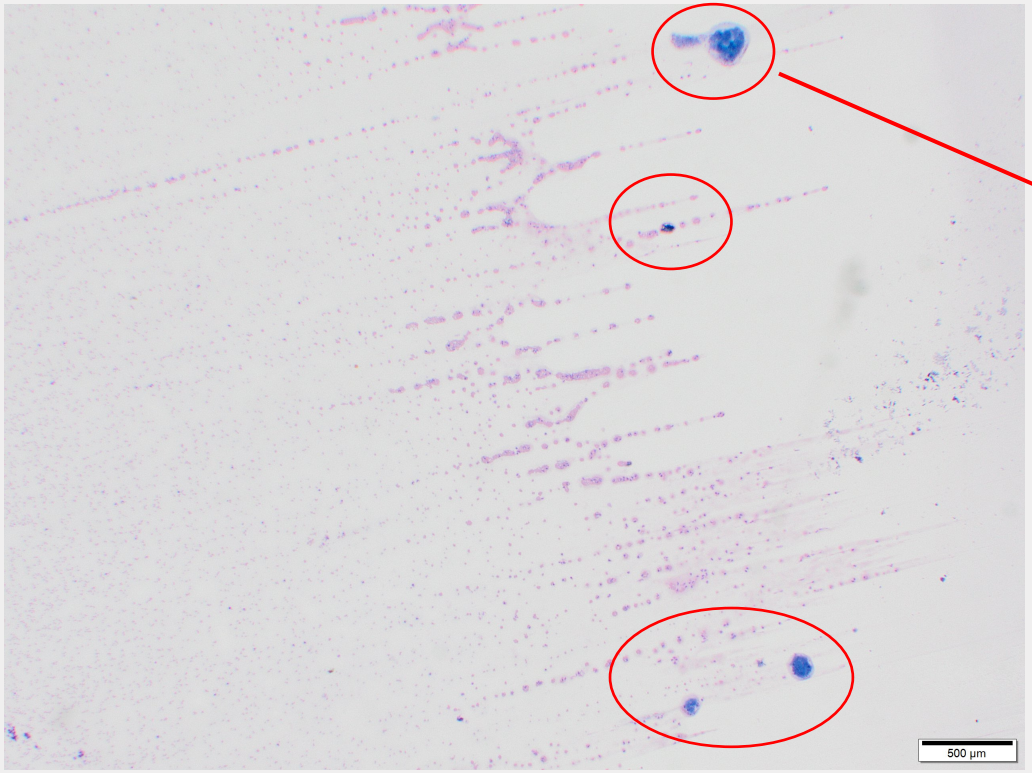
Blood smear evaluation

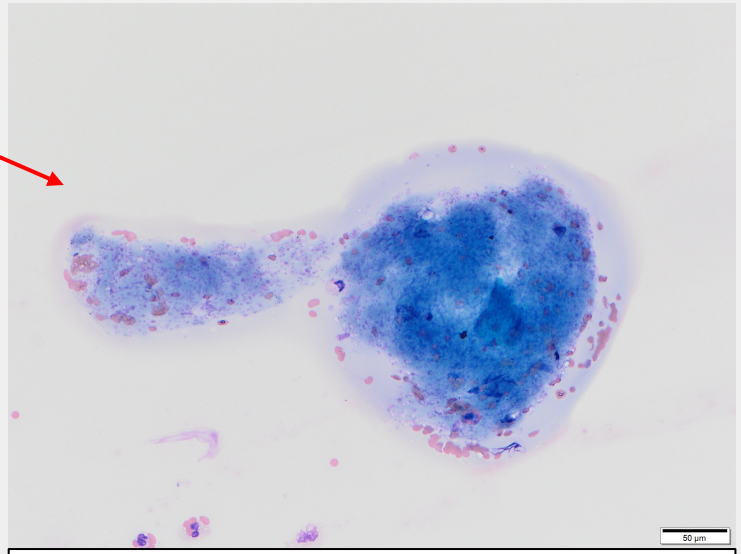
- How to systematically review blood smears will be done tomorrow
- Basics:
  - 10X:
    - WBC Estimate
    - Evaluate for Rouleaux, Agglutination, large parasites (ex) heartworm, *platelet clumps at the feathered edge*
  - 50X (or your first oil immersion):
    - WBC differential count
  - 100X:
    - RBC morphology
    - Platelet count
  - ALL done at the **monolayer** (except for platelet clump evaluation)

Monolayer (1-1.5 fields over from the feathered edge when under 50X)

Never forget about the feathered edge!

- Always need to check for platelet clumping, particularly when your platelet count is low!
- Area where cells and other “things” of interest can be found…





Large platelet clump at 100X

Toni

- 9 year old MC Bernese Mountain Dog
- History:
  - Lethargy for approximately 2-3 weeks
  - Panting all the time
- PE:
  - Pale mucous membranes
  - TPR not provided

CBC

Leukocytes	Value		Flg	Ref. Int. x 10 9/L
WBC				4.80-13.9
Corrected WBC	19.3		H	
NRBC /100 WBC's	8			
Differential (100)	Rel%	Abs	Flg	Ref. Int. x 10 9/L
Segs	71	13.703	H	3.0-10
Bands	2	0.386	H	0.0-0.1
Metamyelo				
Myelo				
Toxic Change				
Eos	1	0.193		0.0-1.1
Basos				rare-
Lymphs	9	1.737		1.2-5.0
Monos	17	3.281	H	0.08-1.0
Other				
ATypicals				

Erythrocytes	Value	Flg	Ref. Int.	Units
RBC	3.79	L	5.20-8.20	x 10 12/L
Hgb	100	L	128-196	g/L
Hct	0.289	L	0.365-0.573	L/L
MCV	76.1	H	65.2-73.6	fL
MCH	26.4	H	22.5-25.5	pg
MCHC	347		335-357	g/L
RDW	14.6		13.8-17.6	%
Retics	8.1		%	%
RPI	2.88			

RBC Morphology
Aniso 1+, Macro 2+, Poly 1+

Platelets	Value	Flg	Ref. Int. x 10 9/L
Clumped (slide)	Yes		
Estimate (slide)	Decreased		
Morph (slide)	Enlarged		
PCT			
MPV			
PDW			
Auto Count (min.)	8.32	L	200-900

Moderately decreased. Manul count na due to clumps present.

Plasma Total Protein by Refractometry	Value	Flg	Ref. Int. g/L
Total Protein	NA	L	56-74
Fibrinogen			
Total Solids: Fib Ratio			

Protein	Hemolysis	Lipemia	Yellow
Plasma Appearance			

Substances that artefactually increase total protein by refractometry include urea, glucose, cholesterol, lipoproteins and excess anticoagulant.

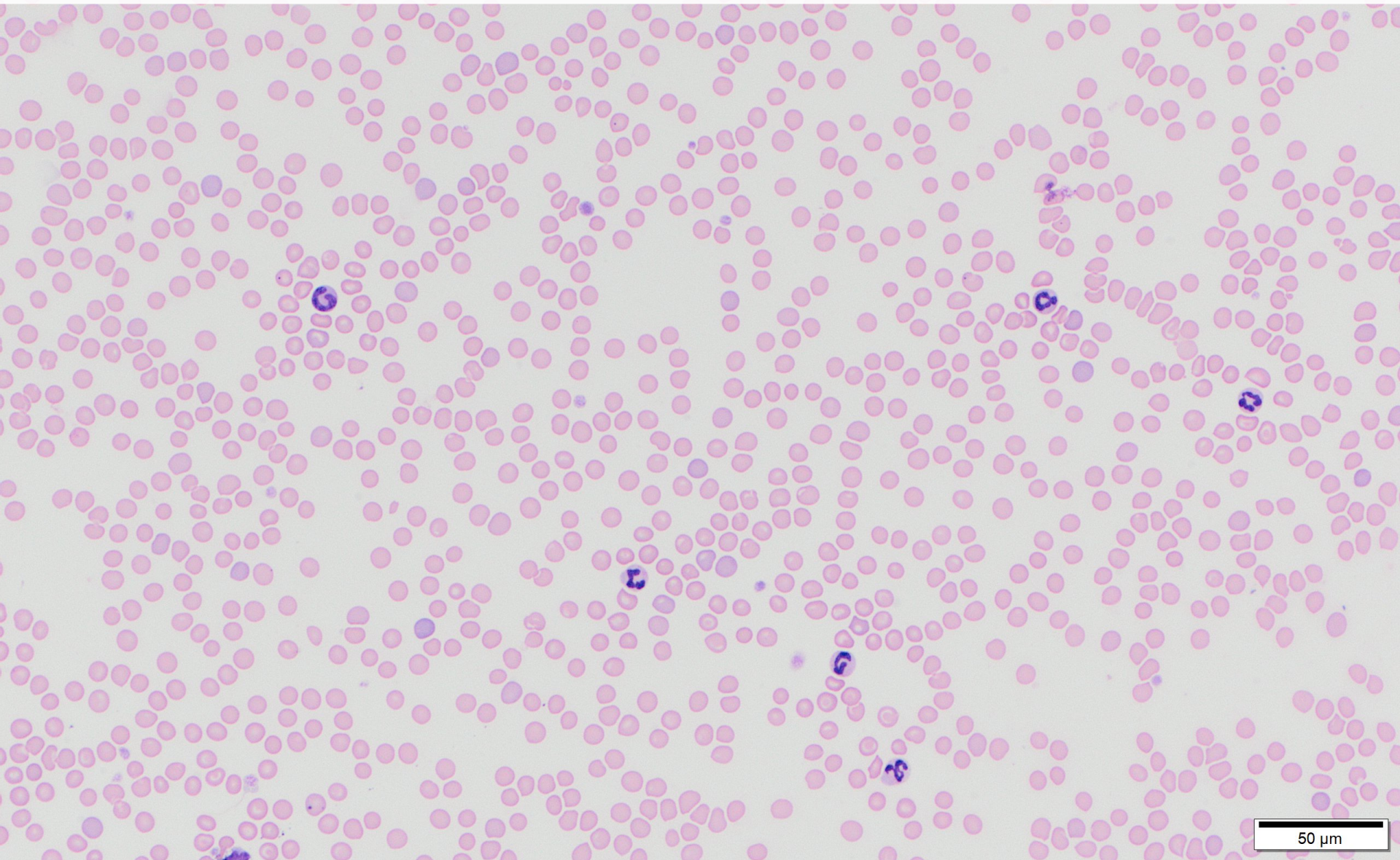
Please see chem panel for total protein.

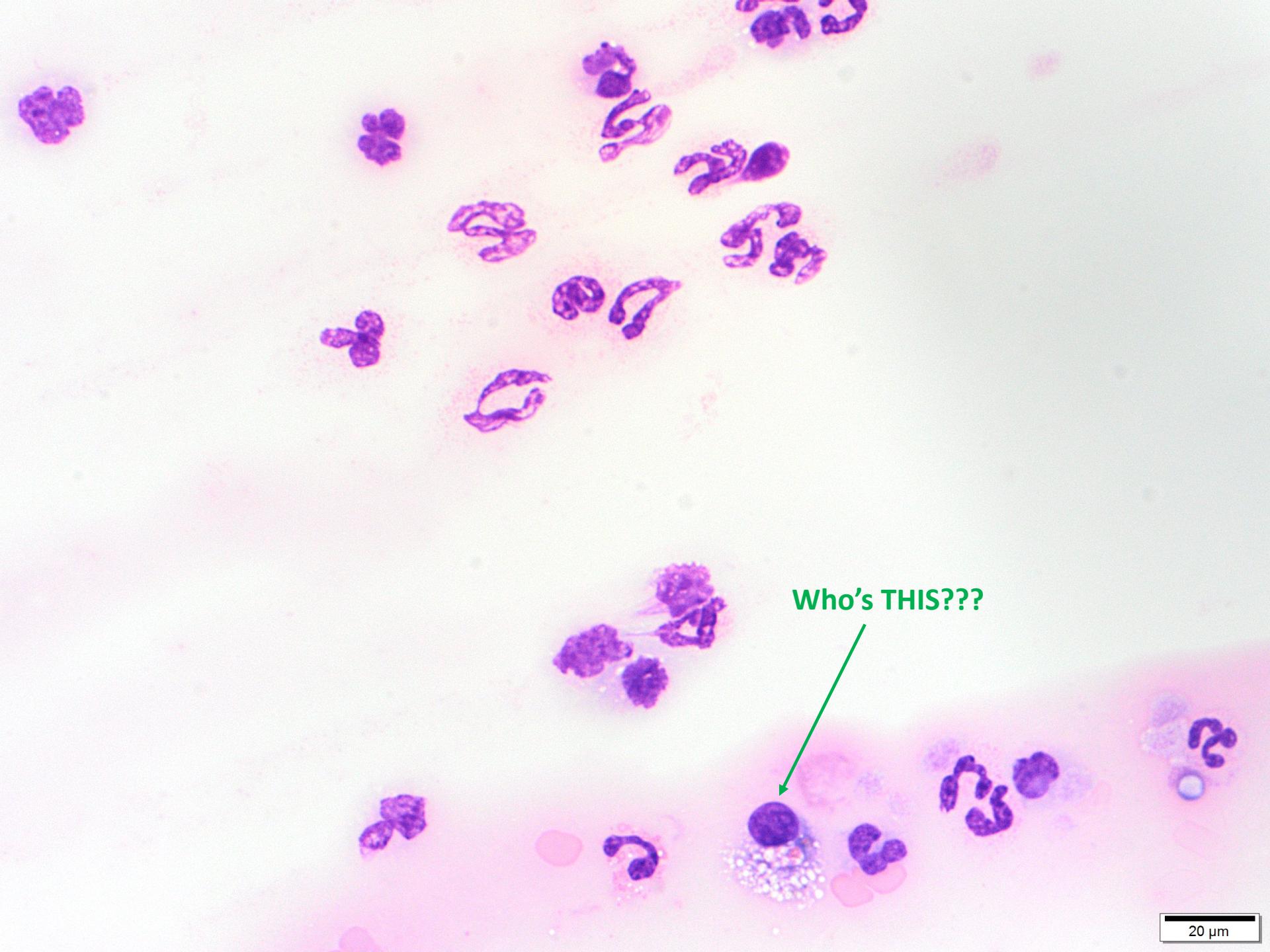
Summary

- Bicytopenia:
  - Thrombocytopenia and a regenerative anemia
  - Worry about the potential for a bone marrow problem; however, smear revealed enlarged platelets, suggesting active thrombopoiesis and the anemia is regenerative (with bone marrow disease, typically these anemias are non-regenerative)
  - Active bleed with DIC?
  - Immune mediated destruction of RBCs and platelets??
- Focus of inflammation – location??
- Other than that, there is not much else to go on based on blood work…OR IS THERE??

🎶Cue mysterious music ☺ 🎶

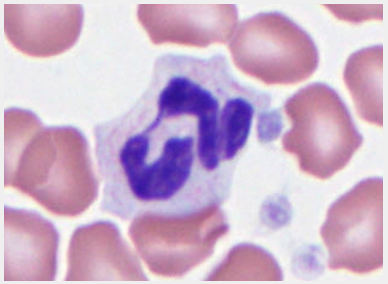
Monolayer (no overt abnormalities noted)

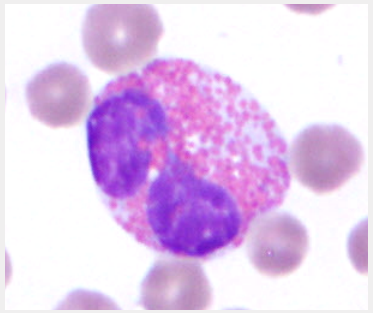


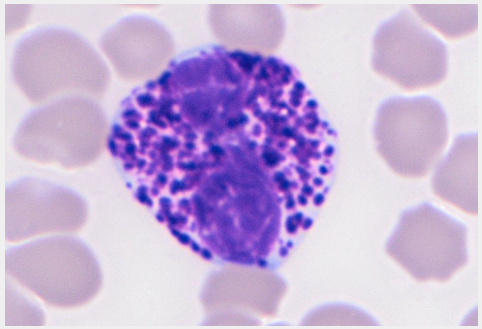

Who’s THIS???
20 µm

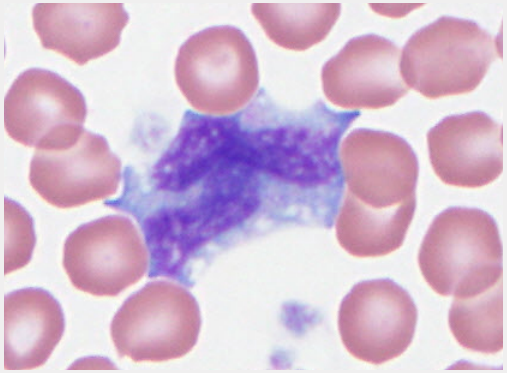
What are the five leukocyte types in circulation?

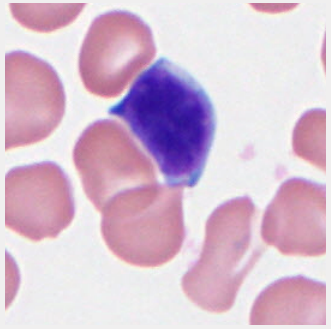
- **Polymorphonuclear cells:**
  - Neutrophils
  - Eosinophils
  - Basophils
- **Mononuclear cells**:
  - Monocytes
  - Lymphocytes

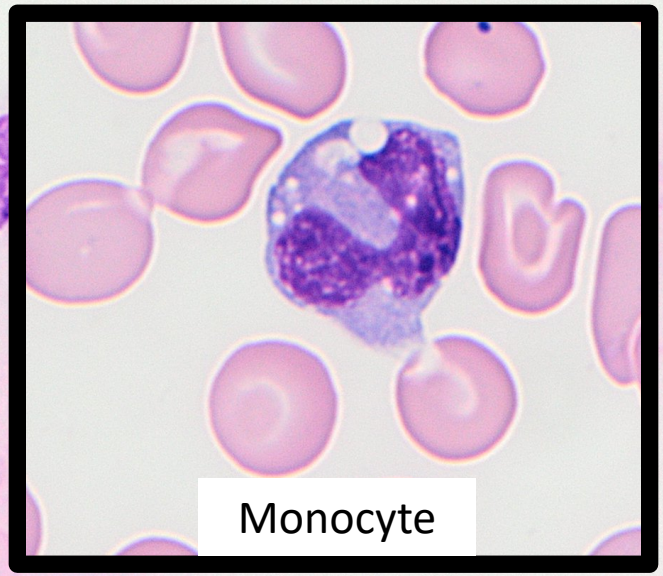








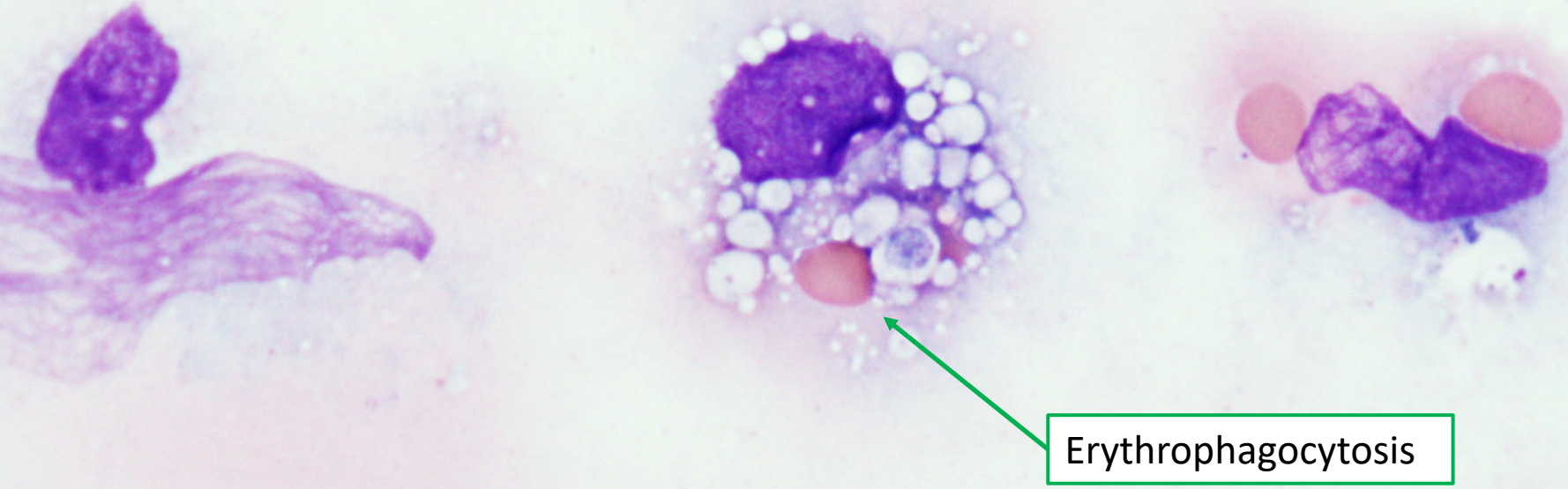




Large atypical mononuclear cell a fine chromatin pattern and indistinct nucleolus, basophilic cytoplasm that contains abundant, variably sized crisp, clear vacuoles.

20 µm

These histiocytes were ***only*** found in the feathered edge and a differential diagnosis of *histiocytic sarcoma* was tentatively reached. Diagnosis was confirmed on BM aspirate and core biopsy.
SO REMEMBER! The feathered edge is your friend!!



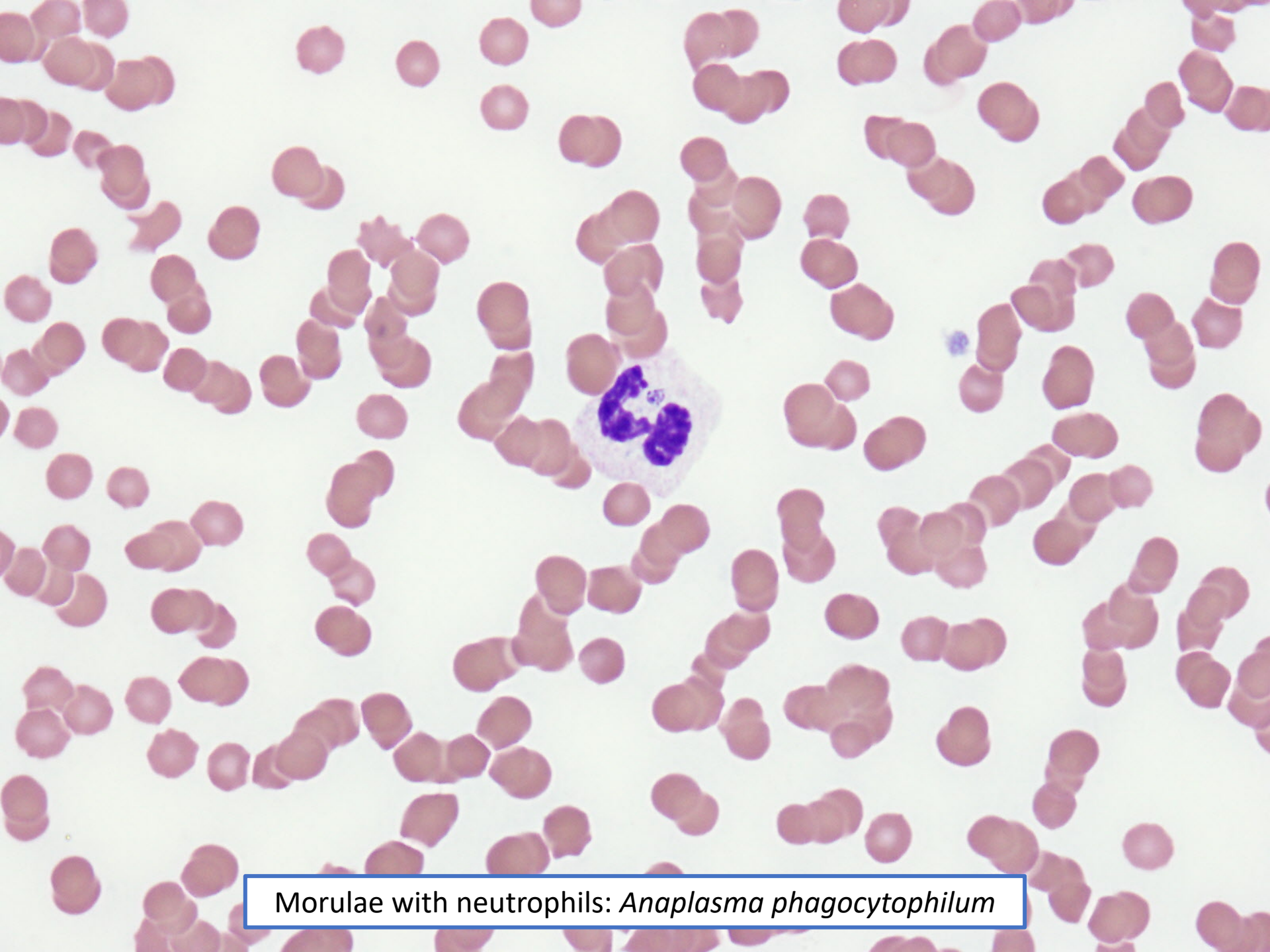
Another reason to always make and evaluate a blood smear…

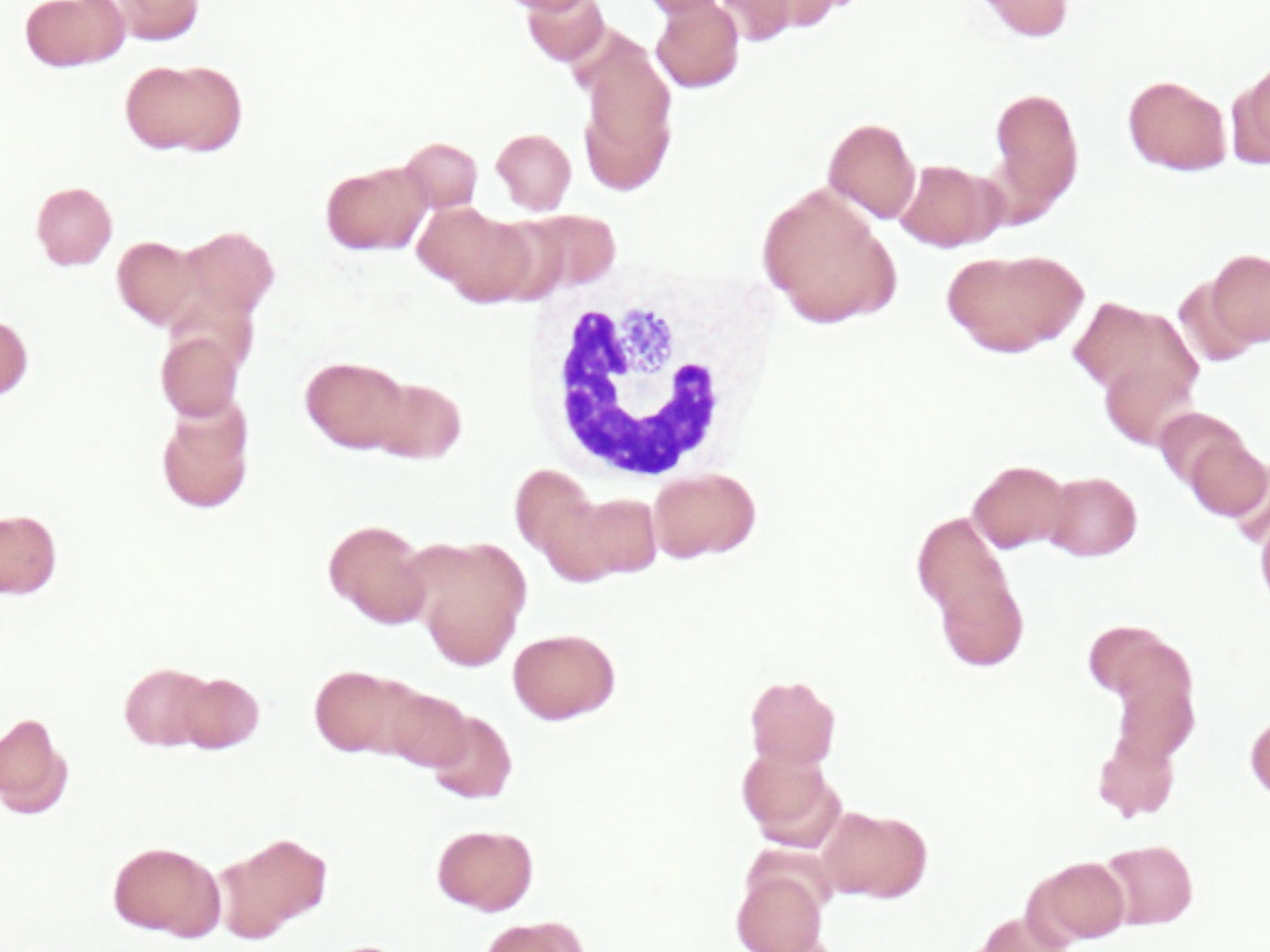
- 3 year old thoroughbred mare with a history of lethargy, and febrile
- CBC:
  - WBC: 6.0 x 10^9/L. (5.1– 11)
  - Hct: 30% (28 – 44%)
  - Platelets: 36 x 10^9/L (100 – 600)

- What should you be asking yourself?

Need to corroborate the platelet count → Evaluate for the presence of platelet clumps that are not detected/counted by the analyzer (which can result in decreased platelet counts)

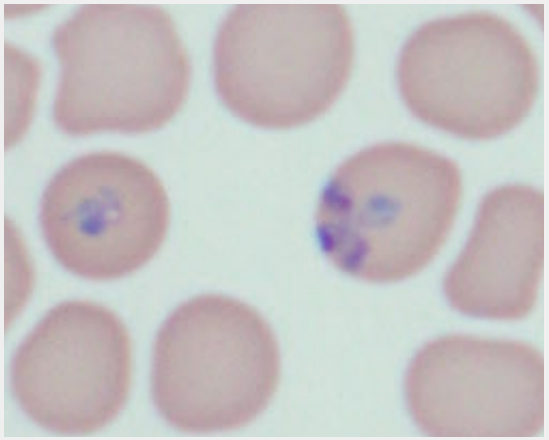
Can the bloodwork explain the clinical signs?


Morulae with neutrophils: *Anaplasma phagocytophilum*

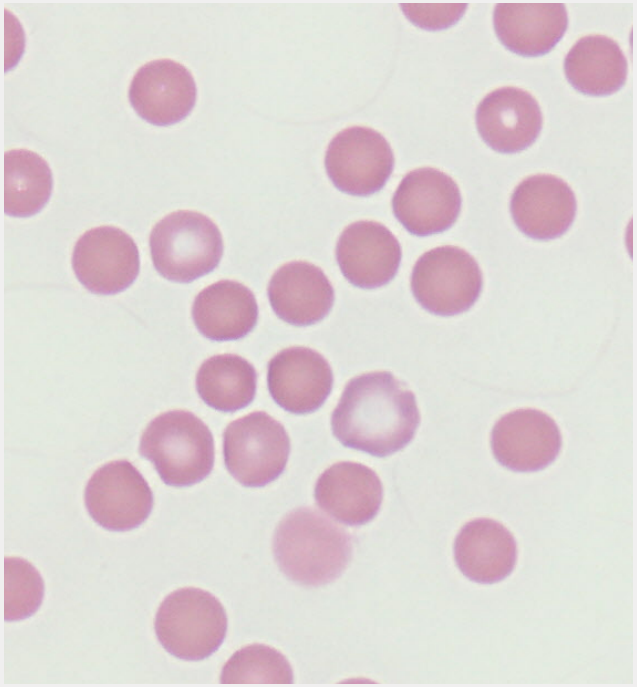


What your hematology analyzer can't tell you…

- **Presence of infectious agents:**
  - Within leukocytes: *Anaplasma phagocytophilum, Ehrlichia sp.*
  - Within erythrocytes: *Mycoplasma, Anaplasma, Babesia, Thelieria, etc.*
  - Within platelets: *Anaplasma platys*

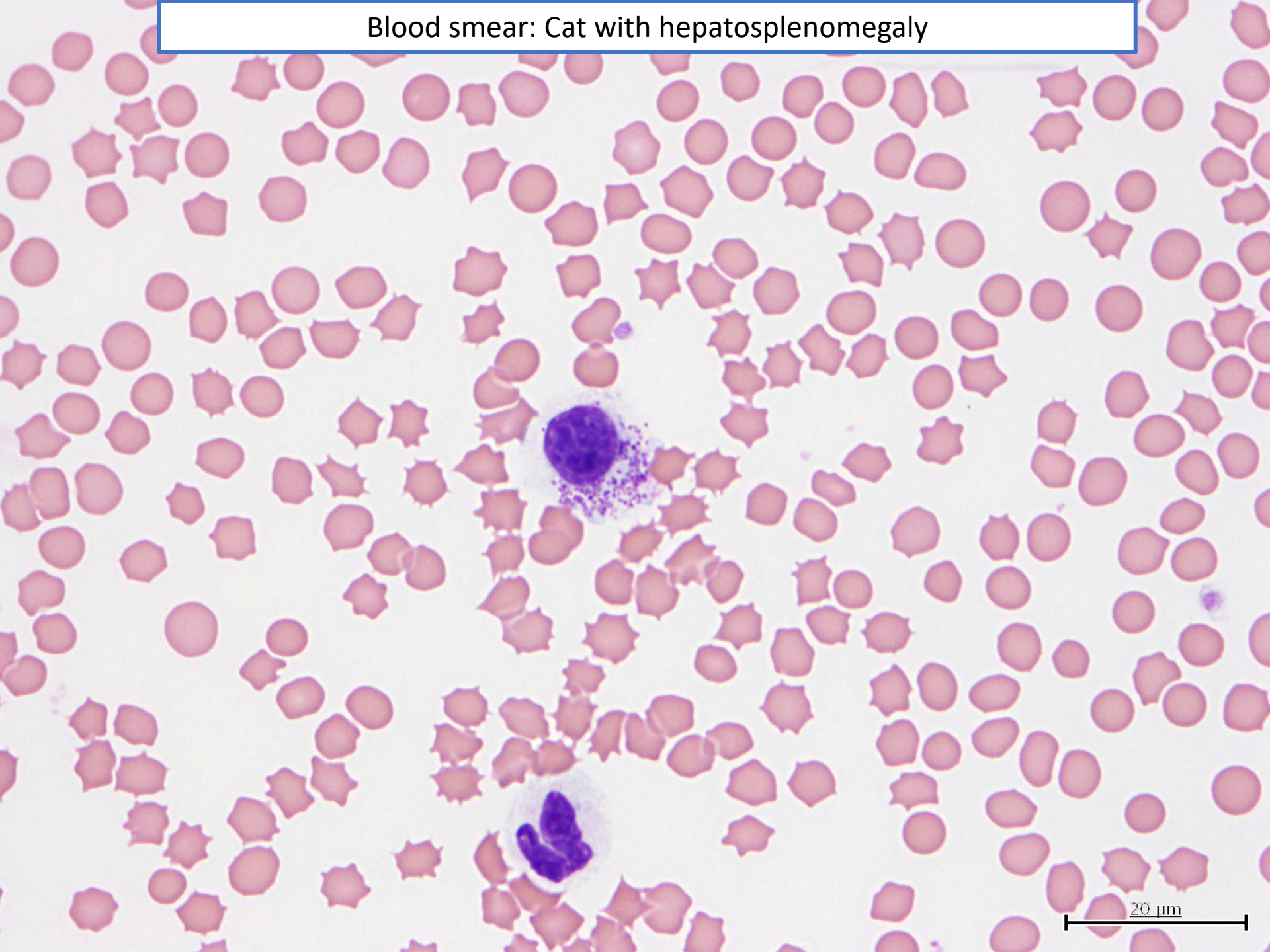


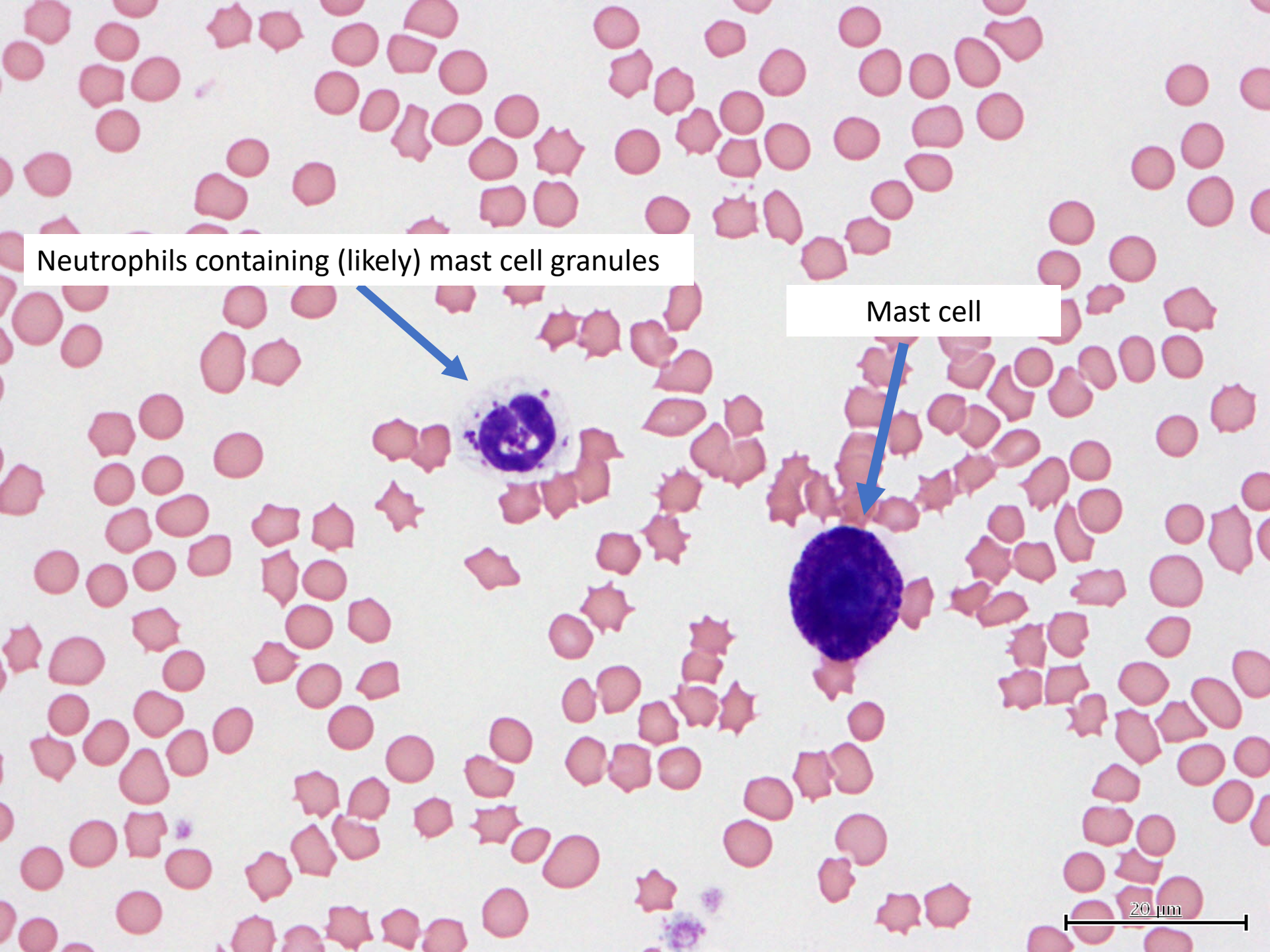
- **Presence of bands (left shift) and toxic change**
- **Erythrocyte morphology**
  - Kinda important when you have an anemia!!
    - Spherocytes, Heinz bodies, acanthocytes, schiztocytes, etc.
    - Direct you to cause, and ultimately treatment!

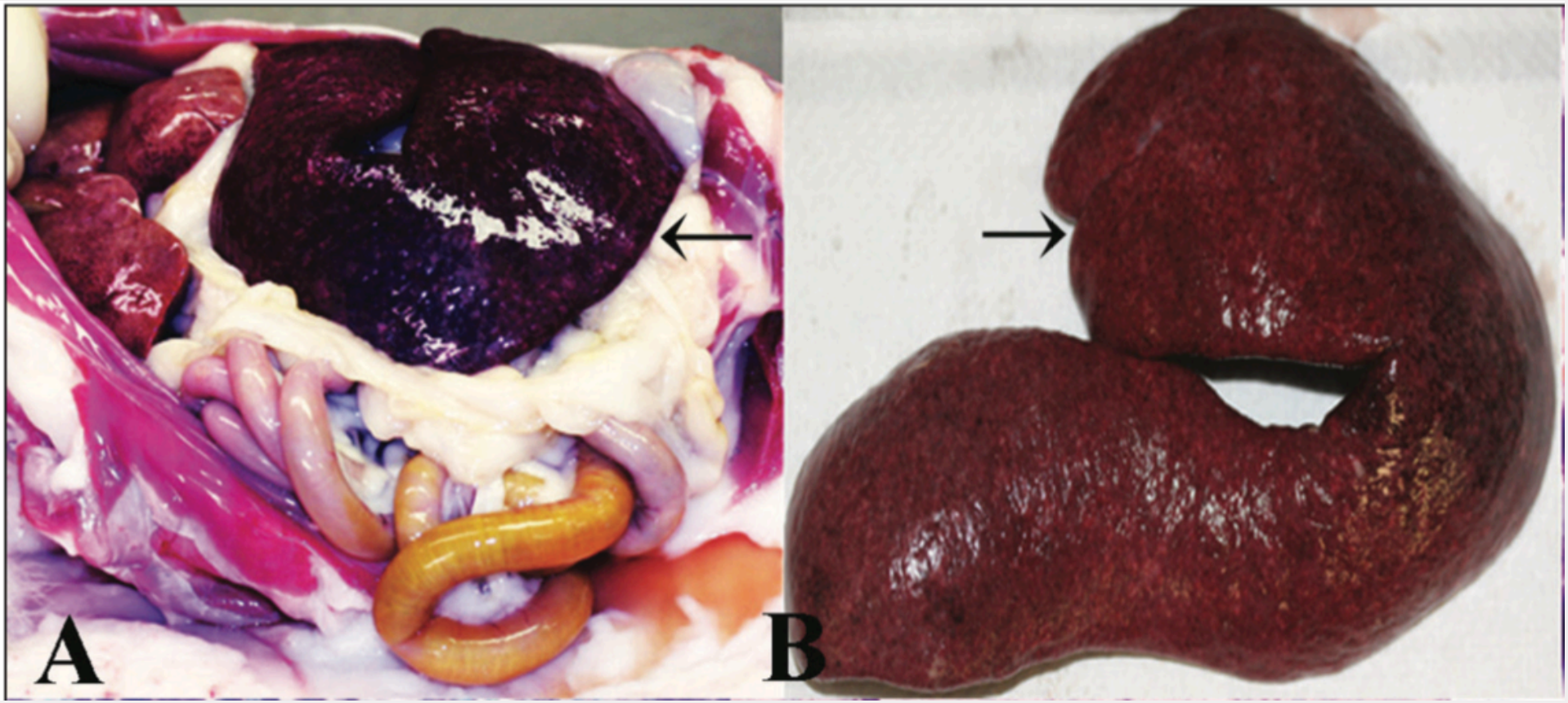


- **Identify atypical cells**

Blood smear: Cat with hepatosplenomegaly



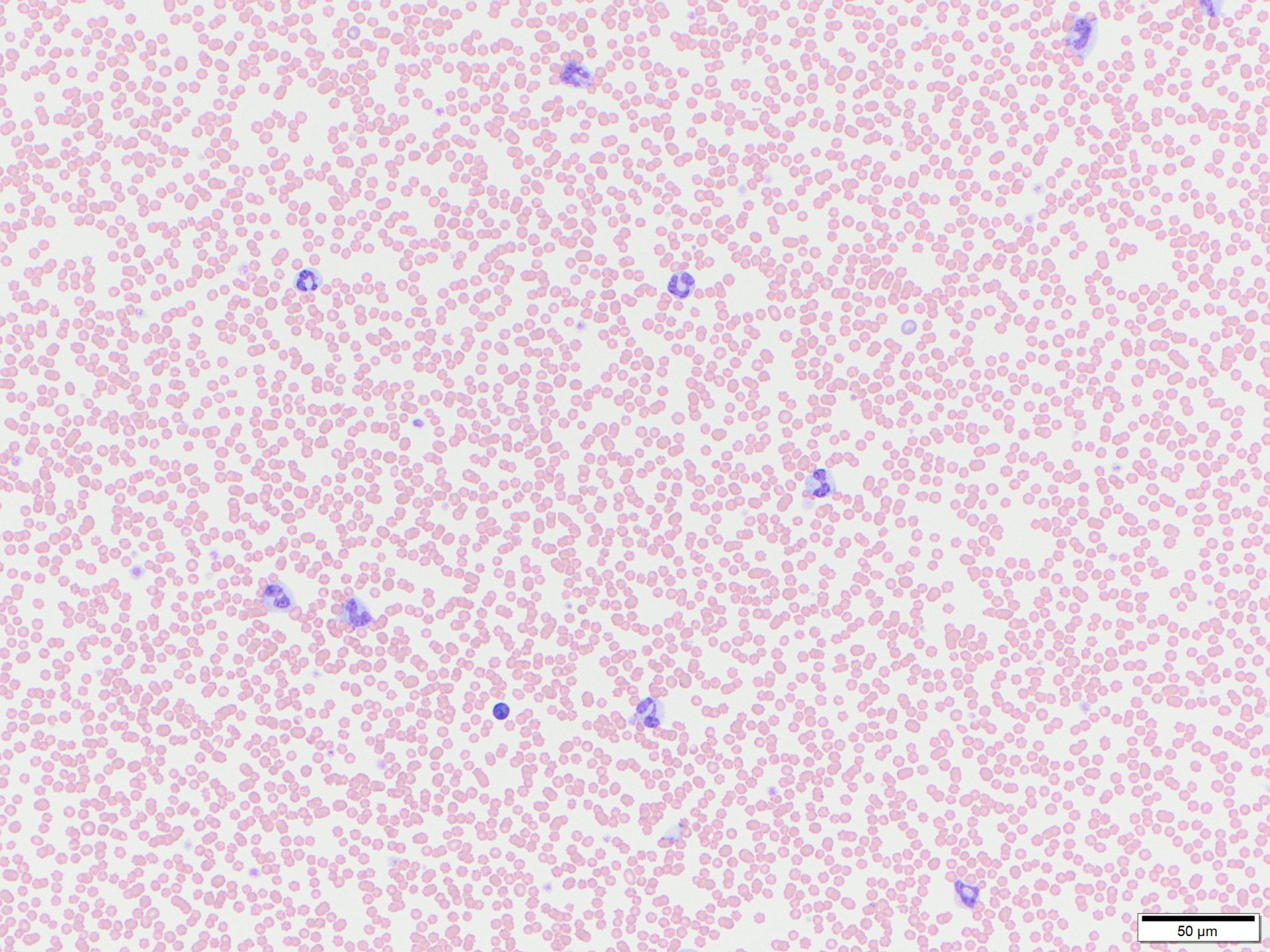

Neutrophils containing (likely) mast cell granules
Mast cell
20 µm



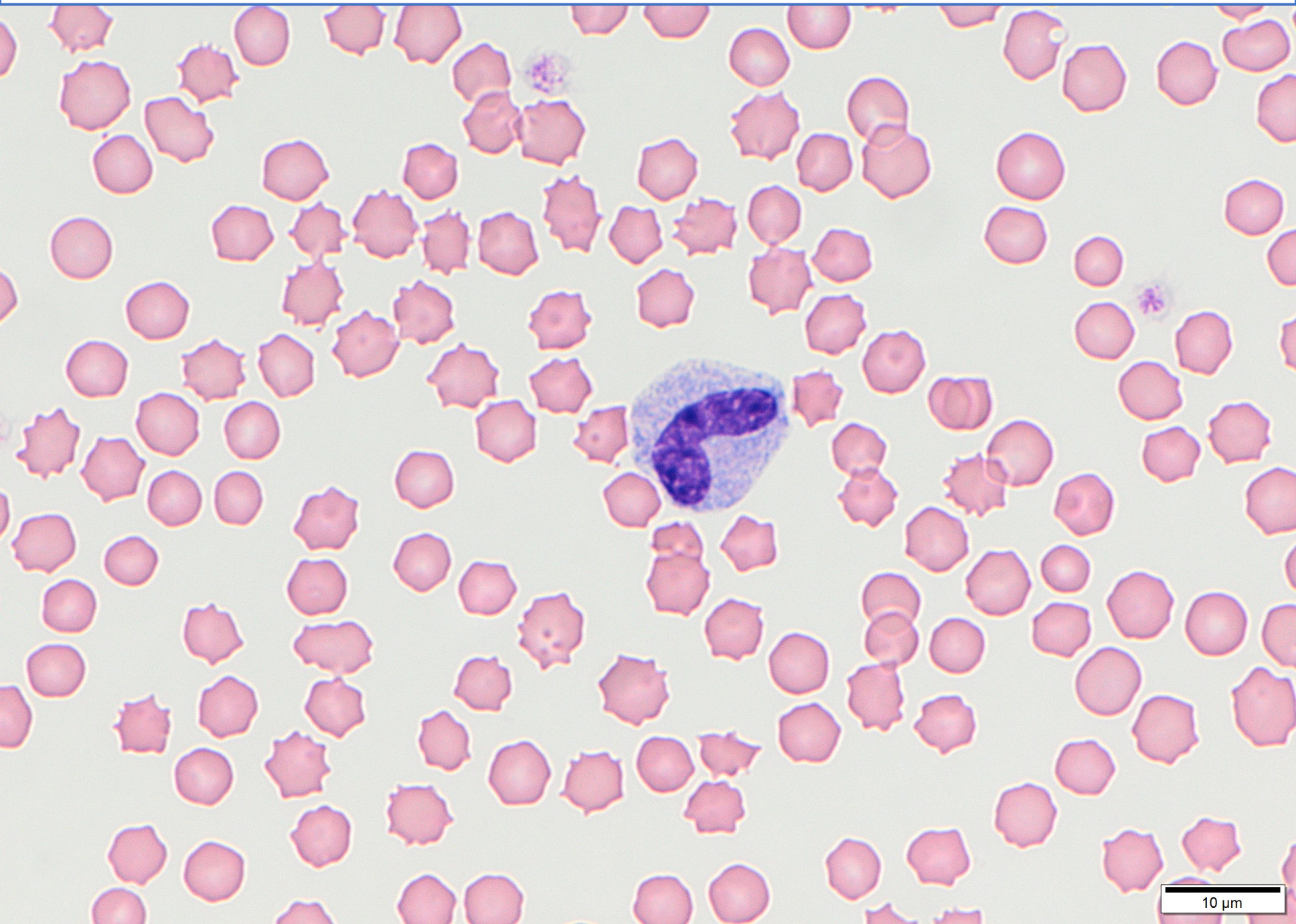
Splenic mast cell tumor with systemic mastocytosis in a cat. A and B — Markedly enlarged spleen (splenomegaly, arrows) at necropsy. *Can Vet J 2017;58:293–295*

Some tips and tricks

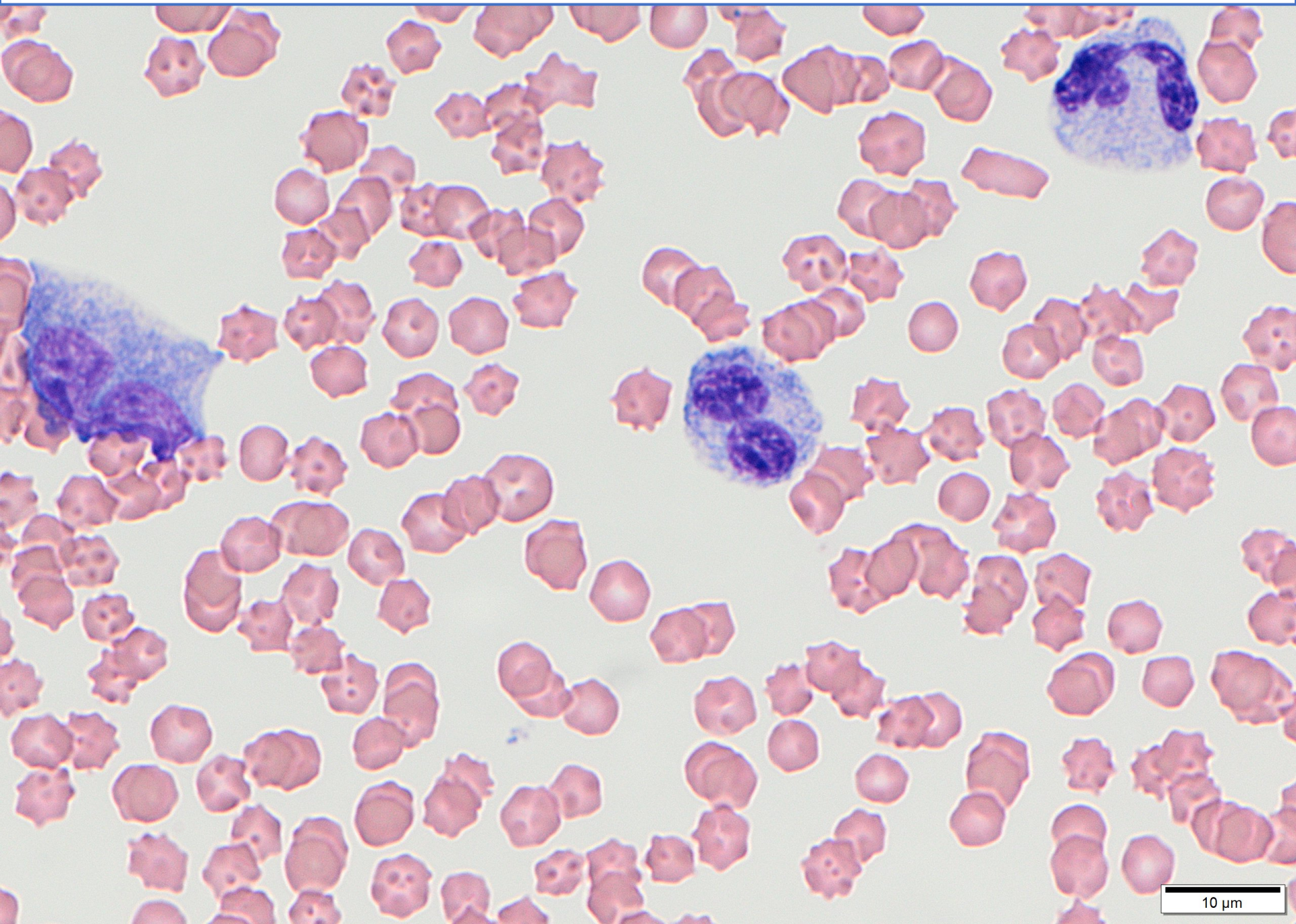
- When performing a differential, it's imperative to evaluate the smear at **10x BEFORE GOING TO OIL!**
  - Low power appreciation of leukocyte types present
- This is particularly important when you have "abnormal" cells in circulation:
  - Band with toxic change
  - Atypical cells
- Allows to better distinguish what cell types are in circulation and allows for greater confidence in identification when performing your differential on high power

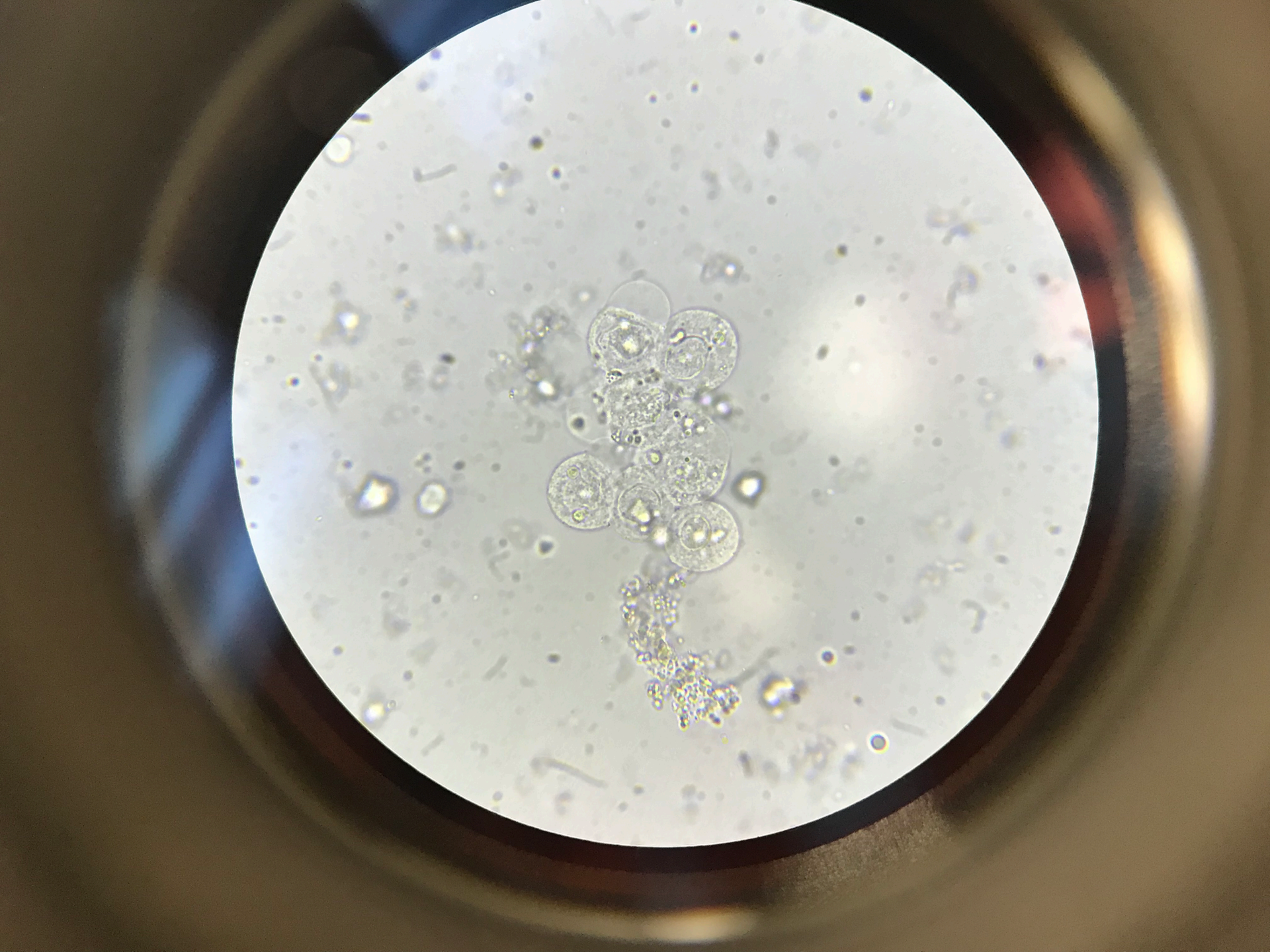

50 µm

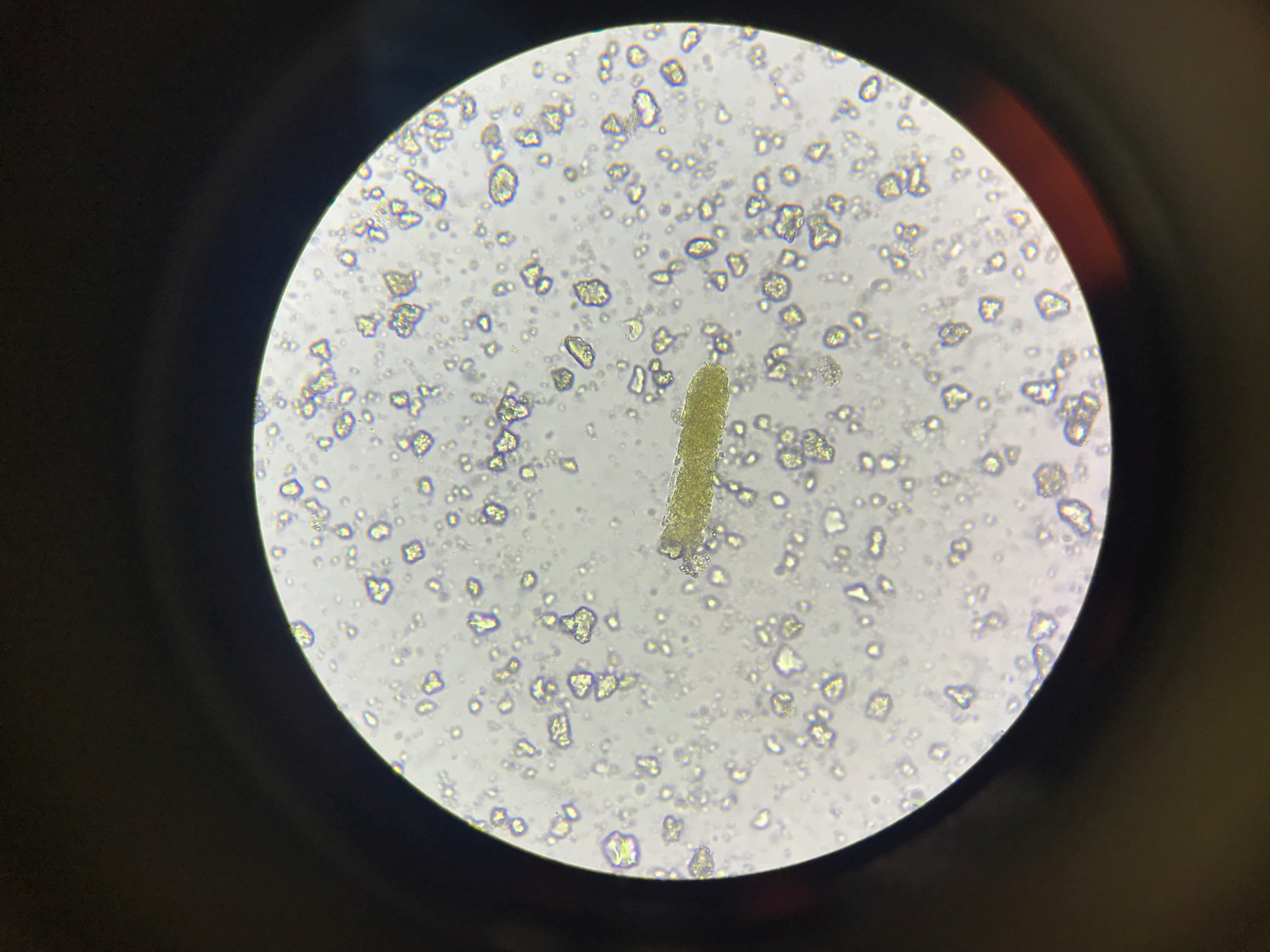
Very toxic band neutrophil

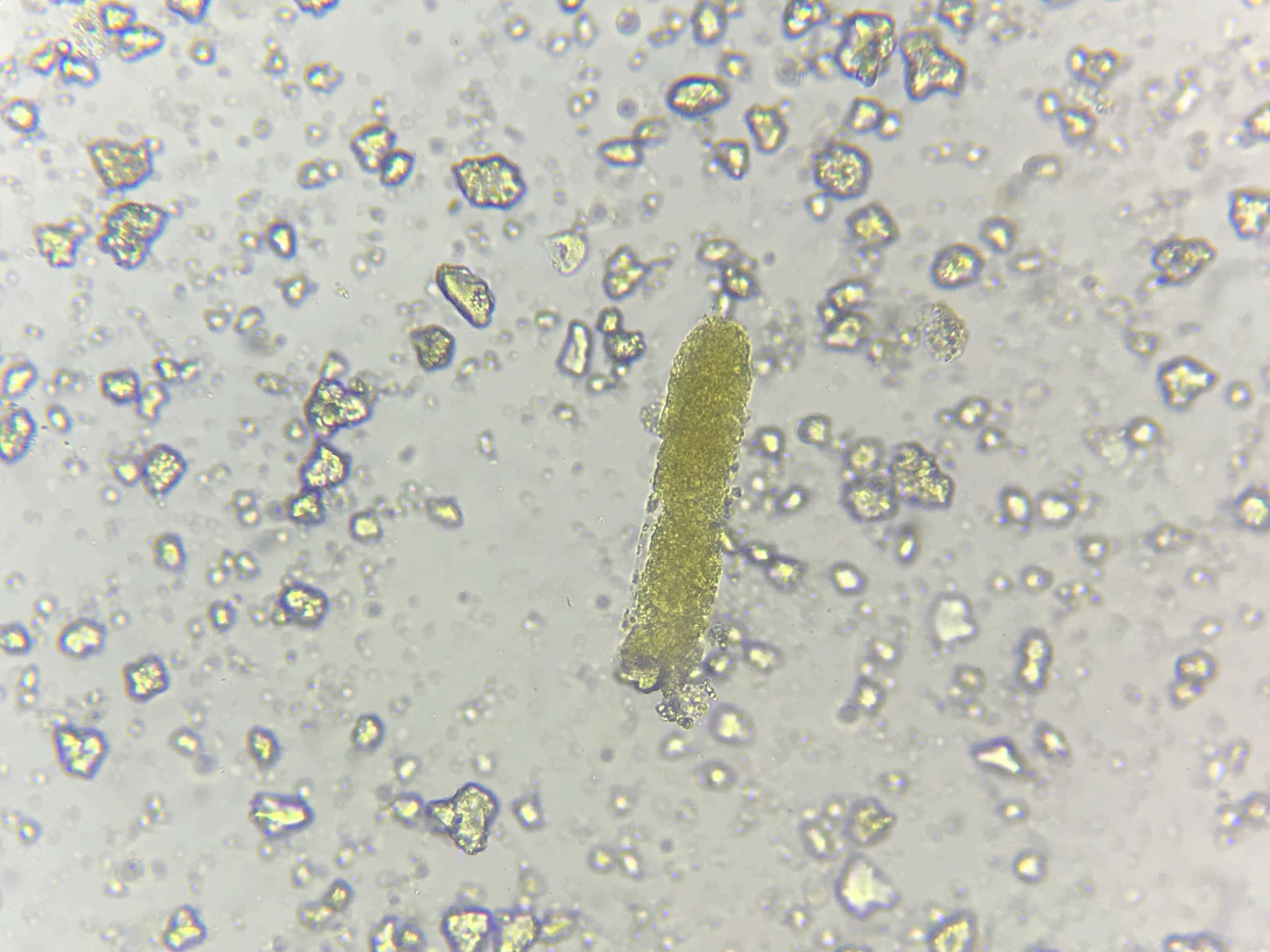


What do you think these cells are?





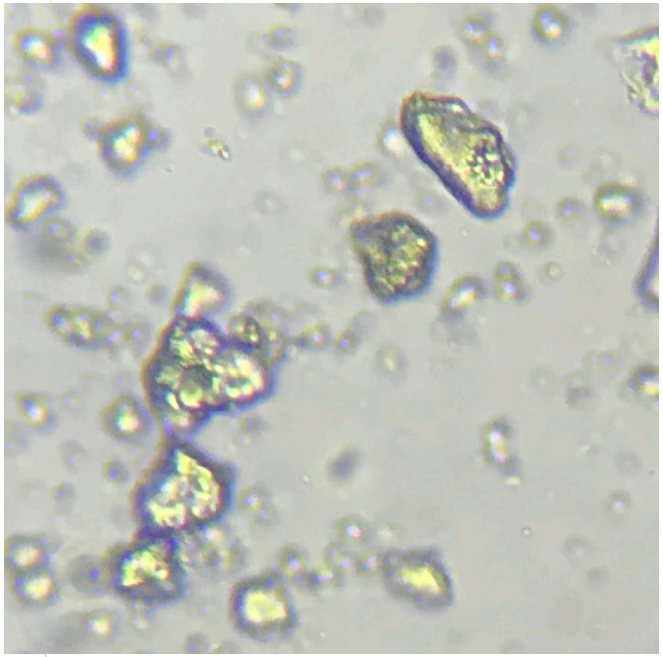





Plain Tube
2.0ml
No Additive
vacutube
150801
201610


acuCyte
Clot
Activator
UR040A
4ml

- Specially coated red top serum tubes with microscopically coated silica particles which activate the coagulation process



- Similar crystalline material was seen in two separate patients with same tube type used for sample collection
- Sample re-collected in plain top tube → no crystalline material seen
- Suspect tube “contaminant”

BLOOD

To avoid hemolysis draw blood as quickly and cleanly as possible into the appropriate tube. Avoid slow collection and repeated jabs with the needle. Fill container to the appropriate level and avoid vigorous mixing. Do not freeze whole blood, unseparated serum, or unseparated plasma.

Red top*
(Clotted blood)

Let sit to allow clot formation (30-60 minutes at room temp or 2-4 hours refrigerated)

Centrifuge after clot forms, transfer supernatant, and discard red cells

Label new tube with owner, animal ID, and as **SERUM**

Submit this sample for chemistry panels, serology, vitamin E, and most endocrinology testing (EXCEPT ACTH endogenous and ACTH and Insulin)

Lavender (purple) top
(EDTA whole blood)

Gently invert to mix

Centrifuge ASAP, transfer supernatant, and discard red cells

Label new tube with owner, animal ID, and as **EDTA PLASMA**

Submit this sample for ACTH endogenous and ACTH & Insulin

Label tube with owner name, animal ID, and as **EDTA WHOLE BLOOD**
(Do not centrifuge)

Submit this sample for a hemogram (and 2 smears), many PCR tests, lead, selenium, Knott's heartworm and for BVDV PI testing in calves or crias less than 61 days of age

Green top
(Heparin whole blood)

Gently invert to mix

Centrifuge ASAP, transfer supernatant, and discard red cells

Label new tube with owner, animal ID, and as **HEPARINIZED PLASMA**

Submit this sample for toxicology testing of copper, iron, nitrate/nitrite, zinc**, and pre-purchase drug screens. This can also be submitted for most clin path chemistry tests.

Label tube with owner name, animal ID, and as **HEPARIN WHOLE BLOOD**
(Do not centrifuge)

Submit this sample for Knott's heartworm and many toxicology tests including selenium and lead.

Blue top
(citrate whole blood)

Gently invert to mix

Centrifuge ASAP, transfer supernatant, and discard red cells

Label new tube with owner, animal ID, and as **CITRATE PLASMA**

Submit this sample for most comparative coagulation tests

*Serum separator tubes (tiger top) can be substituted for red top tubes in some instances but should be avoided for certain endocrinology and clinical pathology tests. Please centrifuge the serum separator tubes after a clot forms, transfer the supernatant to another tube and label the new tube with owner, animal ID, and as SERUM.

Please refer to the Animal Health Diagnostic Center Test and Fee Schedule for specific test sample requirements.

**A trace element tube (Royal Blue), if available, will provide the highest accuracy zinc testing.

VSS-WEB-008-V01 5/21/08

Bottom Line

Be mindful of pre-analytical errors:

Sample collection, storage, artifacts, etc.

Always make a blood smear as soon as possible

Never underestimate the power of a blood film evaluation

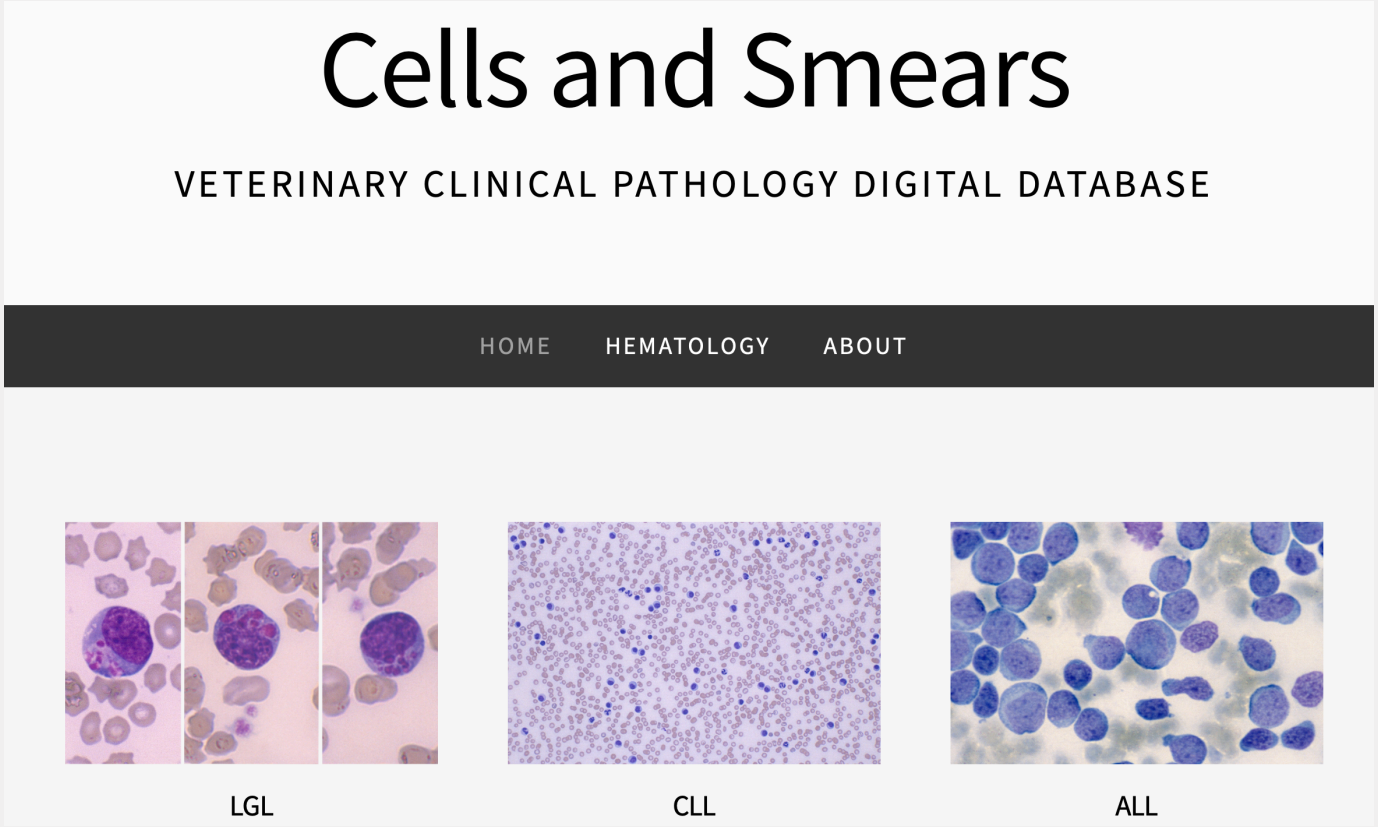
Submit the blood smear you evaluated along with an unstained smear

Remember, evaluation of a CBC/blood smear only represents one moment in time!

Serial blood evaluations may be necessary to determine trends, responsiveness of bone marrow, etc.

Cells & Smears Website

- https://vetclinpathimages.com/about/



Questions?