VITICUSGROUP™
WVC ANNUAL CONFERENCE
MARCH 2 - 5, 2025 | LAS VEGAS, NV

Blood Morphology Matters

Common Clinically Significant Findings You Might Miss If You Don’t Smear

Financial Disclosure

I have a direct or indirect relationship with IDEXX Labs, Inc. Because of the nature of the relationship, it **will** influence my presentation.

What the medical benefits of doing blood smears or blood morphology assessments?

Platelets

- Evaluate platelet clumping
  - Assess whether reported low platelet count is real
  - Inform clinical decision making

Other

+ Microfilaria
+ Bacteria

RBCs

- Abnormal red cell shape
  - Spherocytes
  - Ghost cells
  - Heinz bodies
  - Schistocytes
  - Etc.
- Hemoparasites
- Agglutination

WBCs

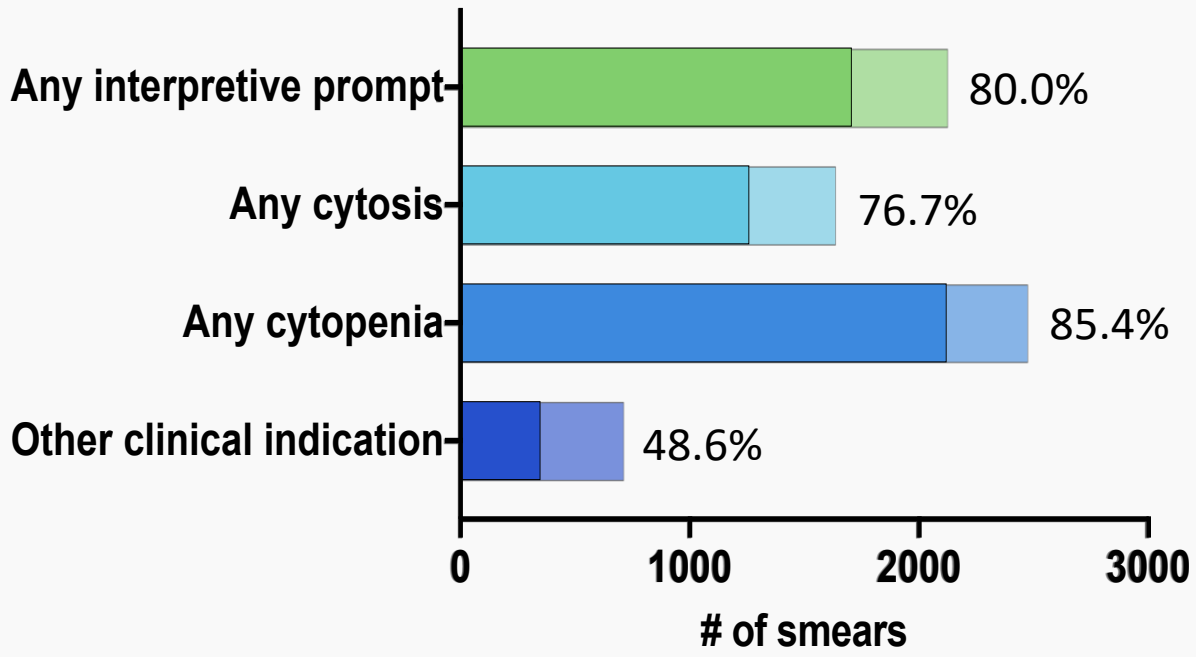
- Identify WBC subsets outside of the 4- or 5-part automated differential
  - Band neutrophils
  - Leukemic cells
- Verify automated WBC differential when there are instrument prompts
- Infectious organisms

High frequency of clinically valuable comments on blood smear reviews ordered after an in-clinic CBC

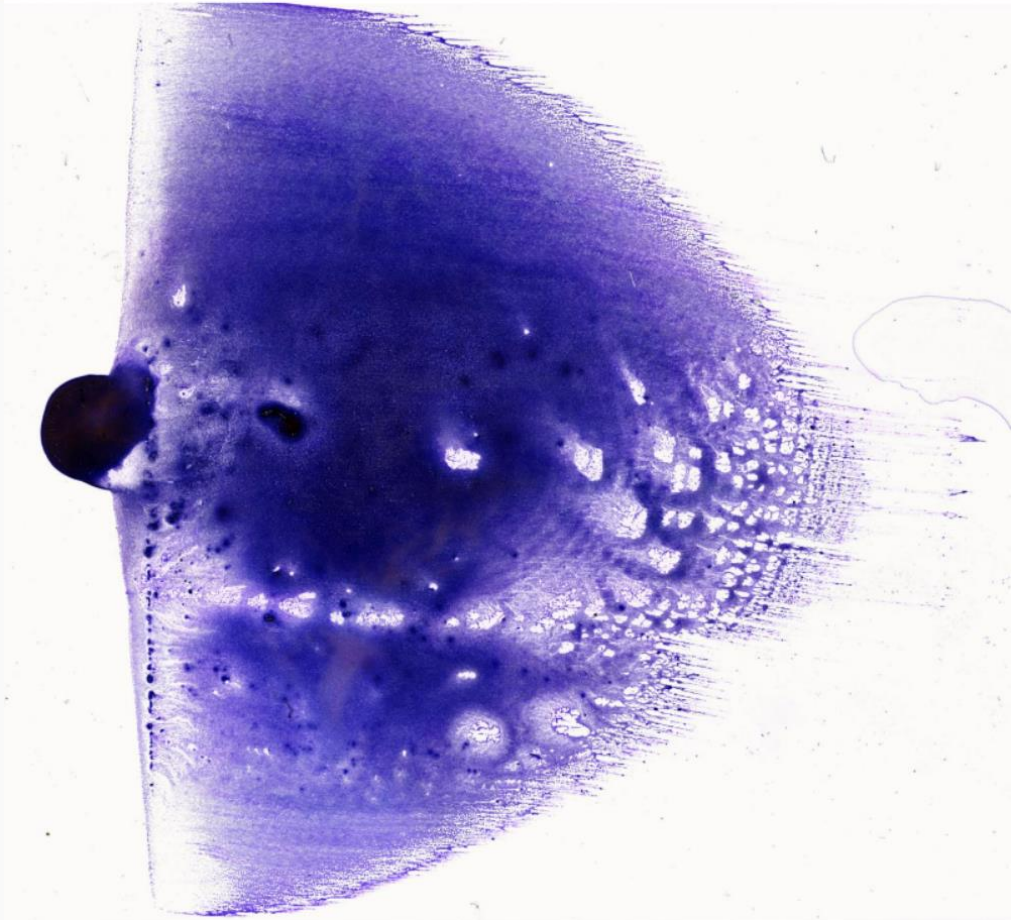
- 4176 blood smears sent to IDEXX reference laboratories for slide review after an in-clinic CBC in 2022

Research questions:

- How many CBCs had abnormalities or interpretive prompts?
- How many slides had clinically relevant findings on blood smear review?

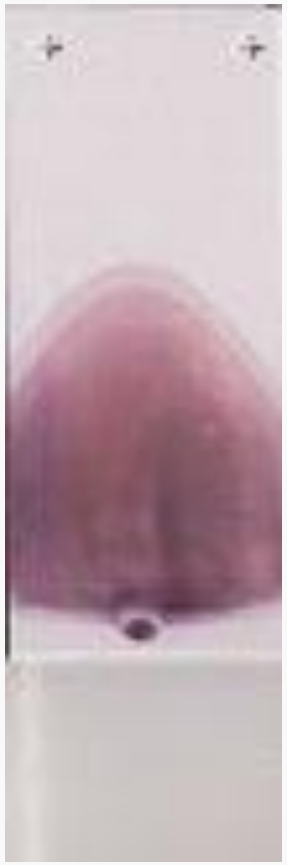


Why this slide is not interpretable



- Disrupted red cell monolayer reading area
- Disrupted feathered edge
- Overstained – too purple

Areas of a blood smear



Feathered edge

Too Thick

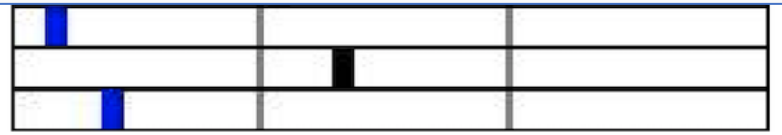
- Feathered edge
  - Evaluate for platelet clumps
  - Microfilaria
  - Unusual large WBCs (with care – might be disrupted!)
- Red cell monolayer
  - Red cell morphology
  - Platelet estimate
  - WBC estimate
  - WBC differential and evaluation
- Thick area of the smear
  - Hemoparasite detection (concentrated but difficult to ID)

Platelets

Patient Case Example: Artifactual versus real thrombocytopenia?

- Platelet aggregates: a common cause of false thrombocytopenia (artifact)
- Patient causes of thrombocytopenia:
  - Consumption
  - Immune-mediated thrombocytopenia (ITP):
    - Primary
    - Secondary, e.g., infection or neoplasia
  - Other: bone marrow failure, idiopathic/multifactorial, or sequestration

PLT	* 49 K/µL	151 - 600	LOW
MPV	14.8 fL	11.4 - 21.6	
PCT	0.07 %	0.17 - 0.86	LOW

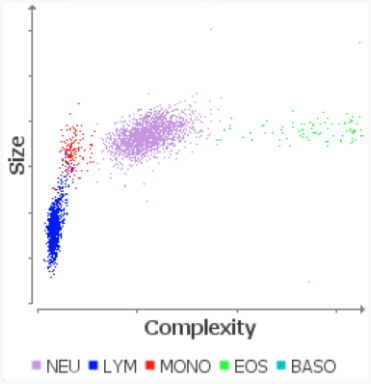


* Confirm with dot plot and/or blood film review.

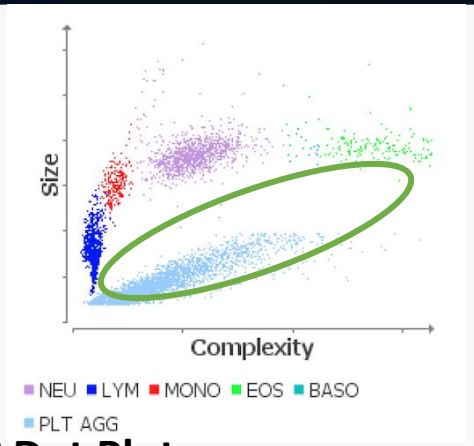
PLT Aggregates Detected

Platelet clumps are visible on dot plots and on blood film review

Normal Reference

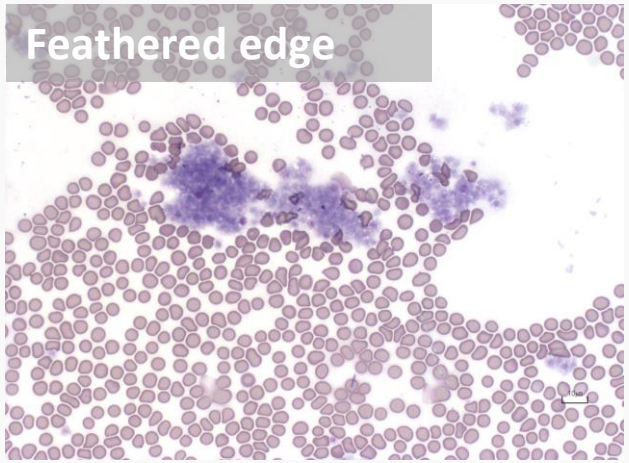


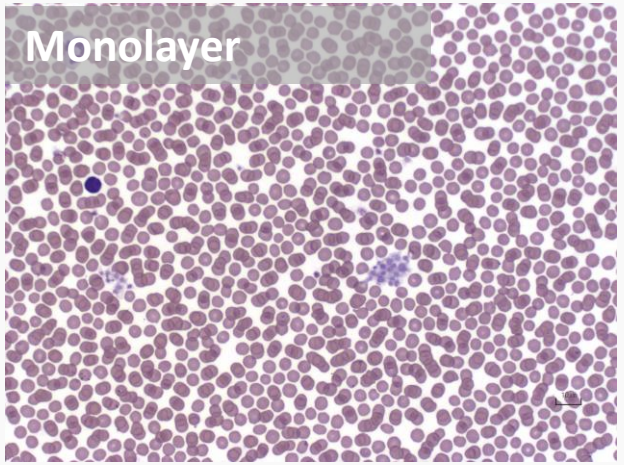
Patient

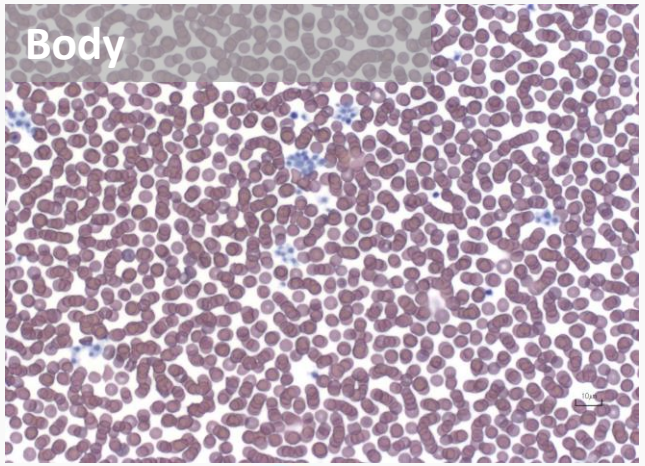


- Presence of platelet clumps indicates reported PLT is an artifact
- Likely adequate platelet count

ProCyte One™ Feline WBC Dot Plots





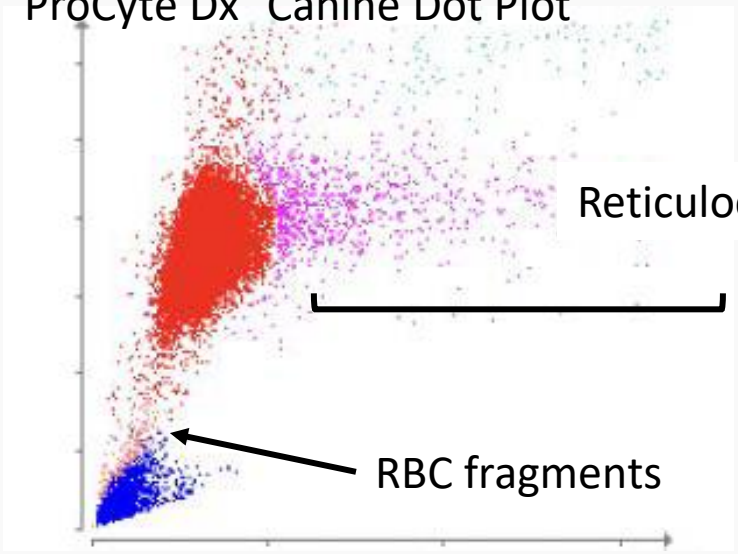


RBC morphology

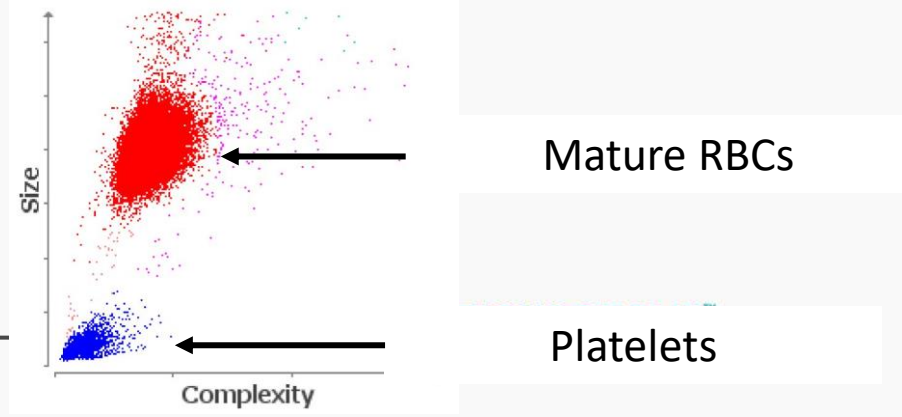
What can hematology analyzers tell us about RBCs?

- RBC density
- Reticulocytes
  - Best indicator of red cell regeneration
  - Does not always correlate with polychromasia on blood film
- Red cell fragments
  - May show up in dot plots as small RBCs

ProCyte Dx® Canine Dot Plot



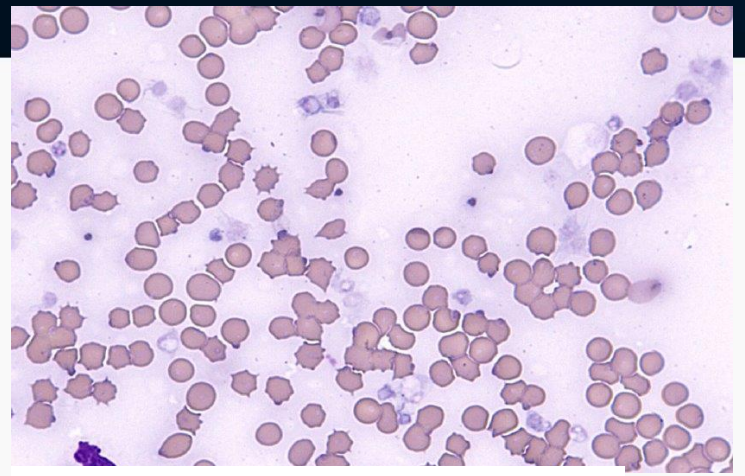
Normal dog



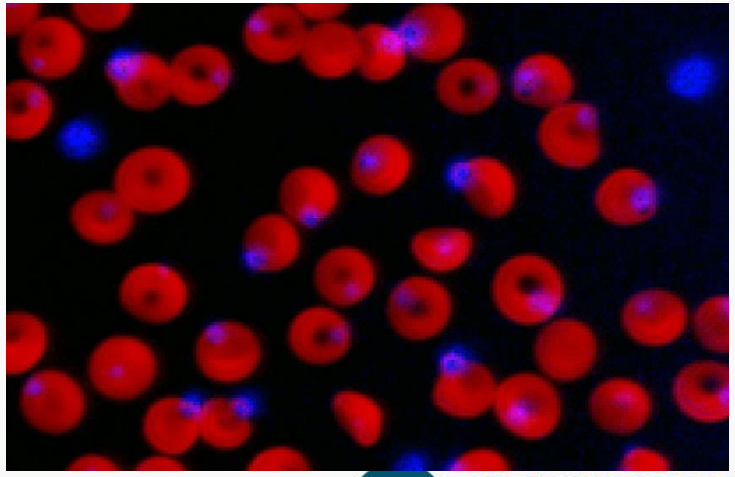
Options for evaluating RBC morphology changes

- Blood smear on slide
  - Review on microscope or using digital microscopy system
  - Alcohol-based Romanowski or Wright's stains
- inVue Dx™
  - 3D fluid system
  - Avoids some artifacts
  - Fluorescent and brightfield microscopy

Traditional Blood Film

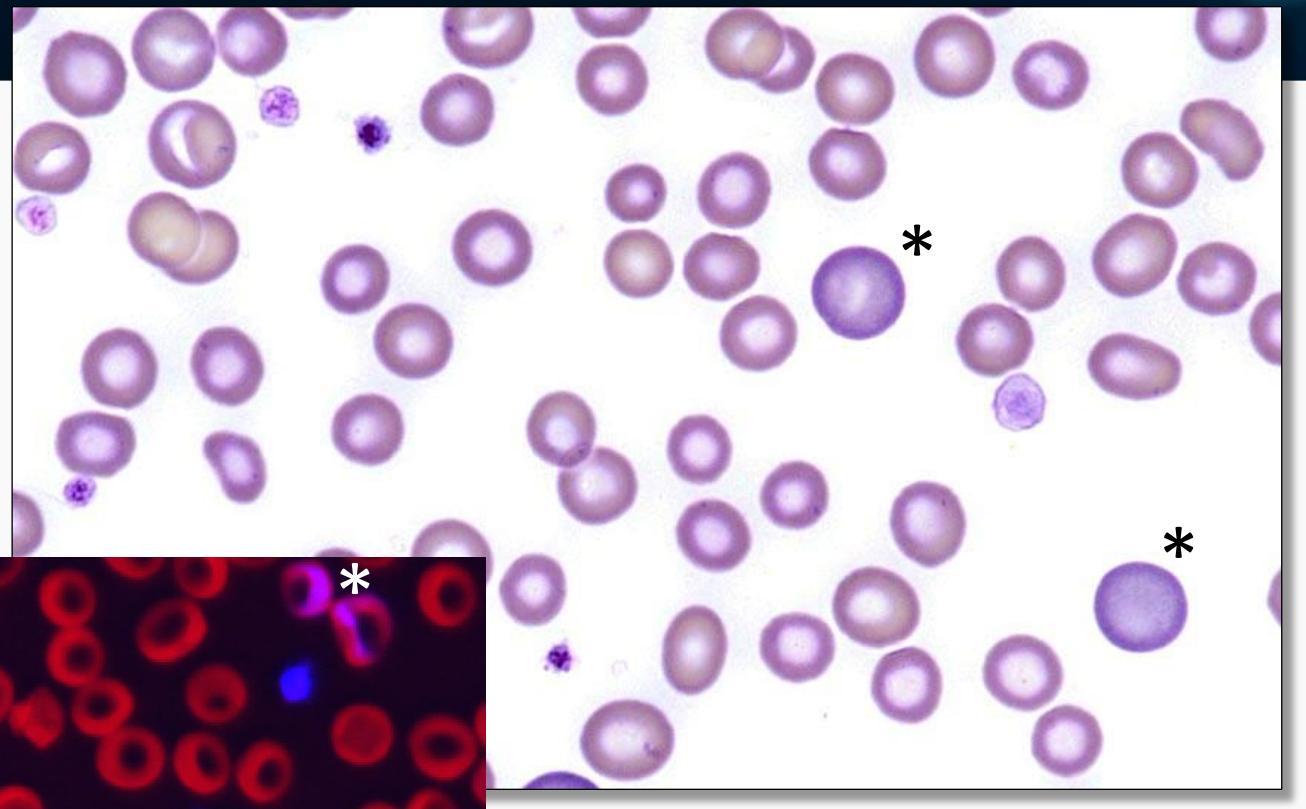


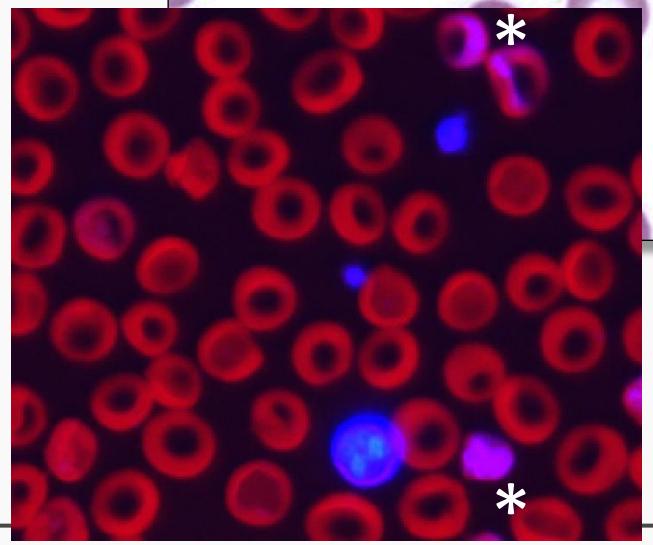
inVue Dx composite image



Are reticulocytes and polychromasia the same thing?

- Polychromasia is an insensitive marker of regeneration
- Polychromatophilic cells appear larger and more purple
- New Methylene Blue stain is needed to definitely identify reticulocytes on blood smears

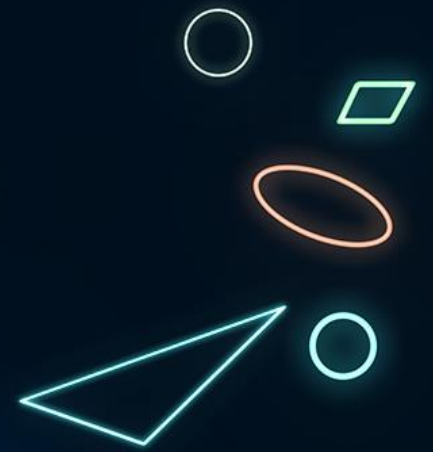




Common RBC morphology changes and findings

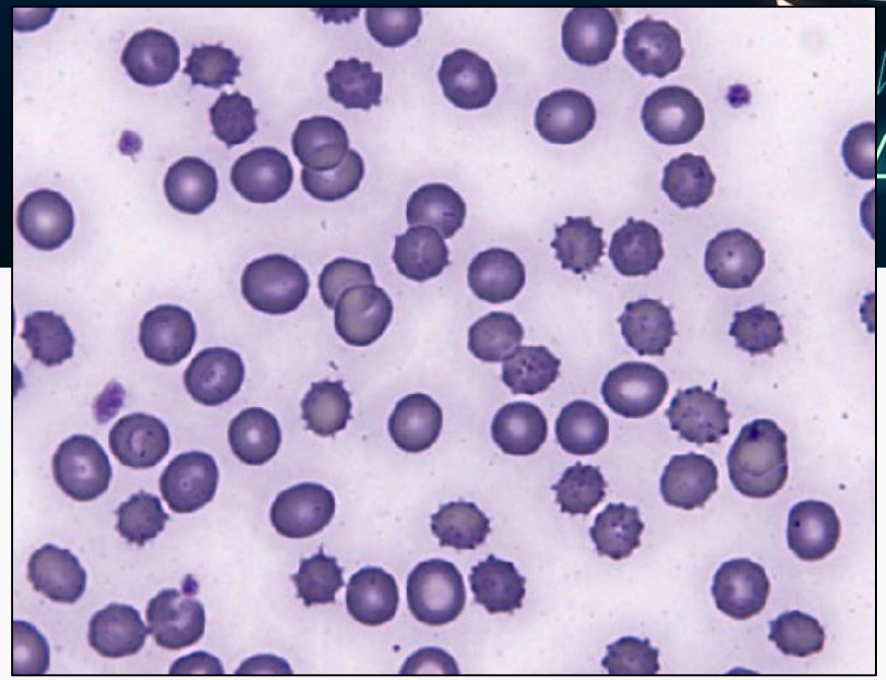
- Shapes with spicules
  - Echinocytes
  - Acanthocytes
  - Keratocytes/Blister cells
- Weird shapes
  - Schistocytes
  - Poikilocytes
- Abnormal central pallor
  - Target cells
  - Stomatocytes
  - Spherocytes
  - Increased central pallor
- Heinz bodies
- Retained nuclear material
  - Howell-Jolly bodies
  - Basophilic stippling
  - Nucleated red cells

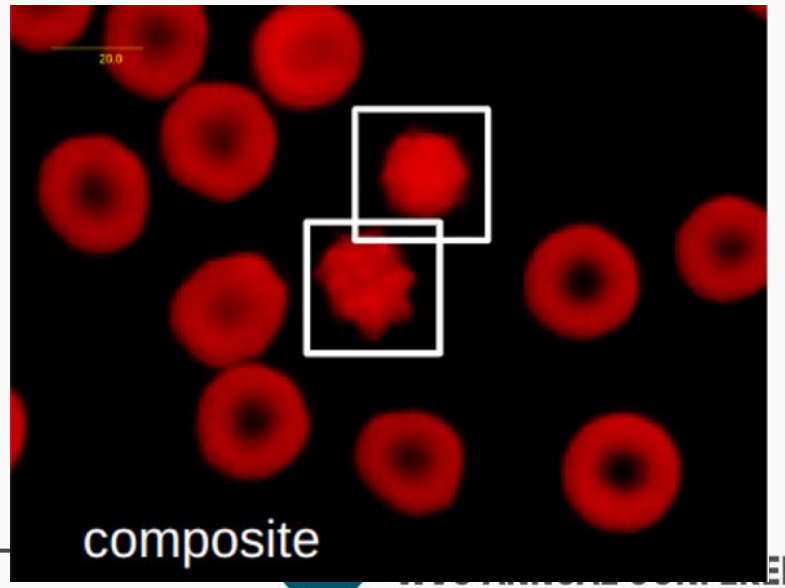
Shapes with spicules



Echinocytes

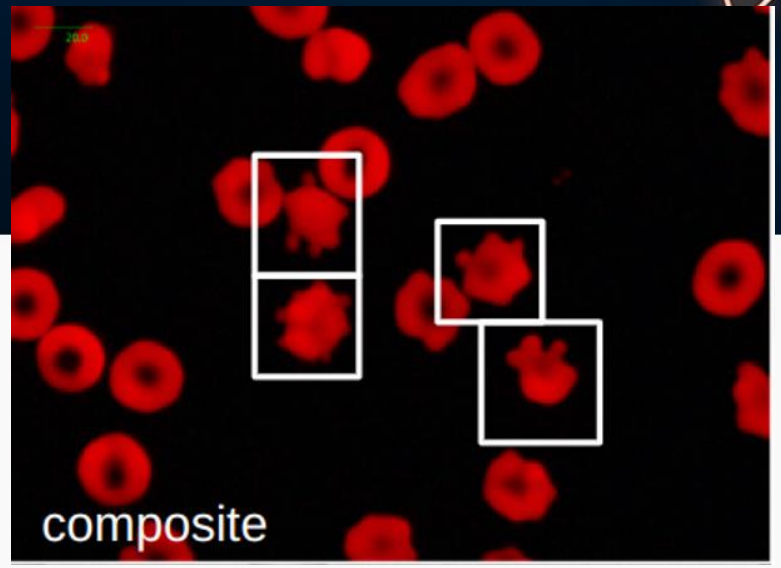
- Regular short spicules – hedgehog appearance
- Often artifacts of blood collection or storage
  - High EDTA concentrations
  - Sample aging
- Toxin exposure
  - Snake bite – (rattlesnake, coral snake, vipers)
  - Bee stings
- Total body electrolyte depletion (particularly horses)
  - Heavy sweating or diarrhea
- Renal disease (dogs)
- Pyruvate kinase deficiency (dogs)

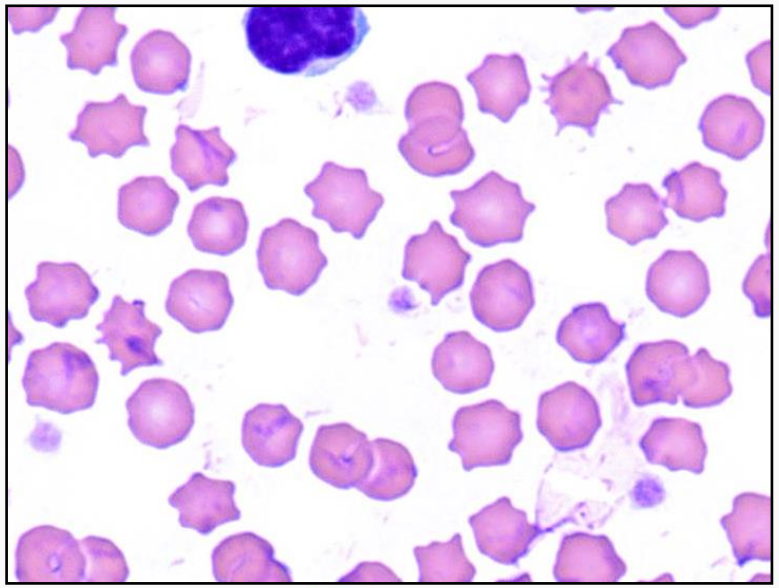




Acanthocytes

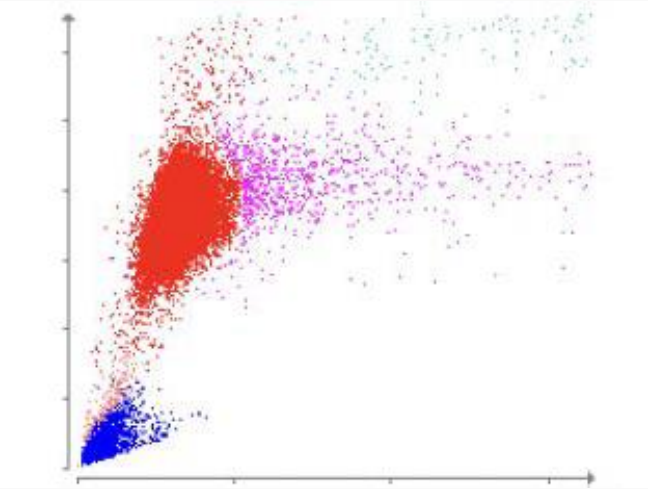
- Irregular spicules
- Caused by changes in membrane phospholipids
- Microangiopathic damage is a possible cause
- Occur with a variety of diseases
  - Common in dogs with hemangiosarcoma

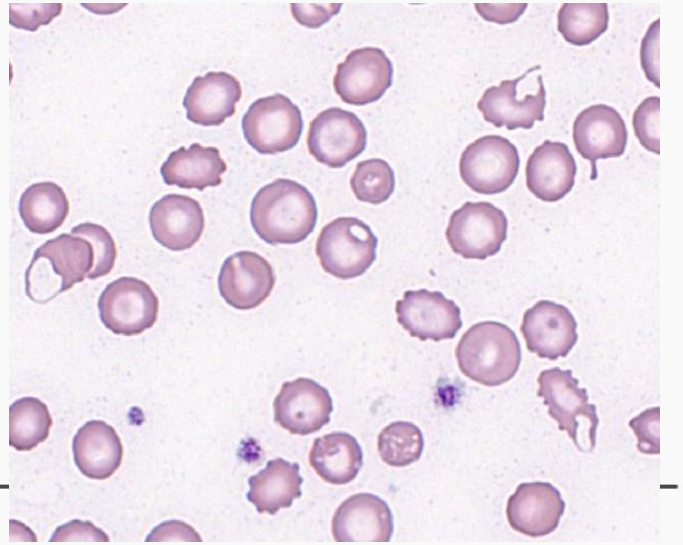


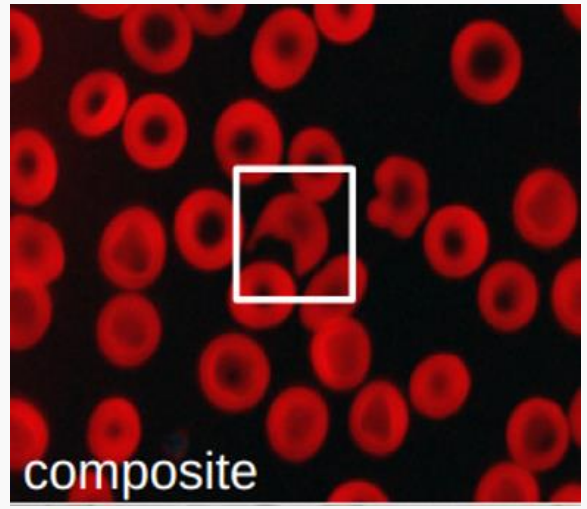


Keratocytes/blister cells

- Appear initially like a “blister on the cell margin (arrow)
- Rupture to create “horn-like” projections (star) or “bite”
- Fragmentation injury (microangiopathic)
  - Often co-occur with schistocytes and acanthocytes
- Commonly part of poikilocytosis



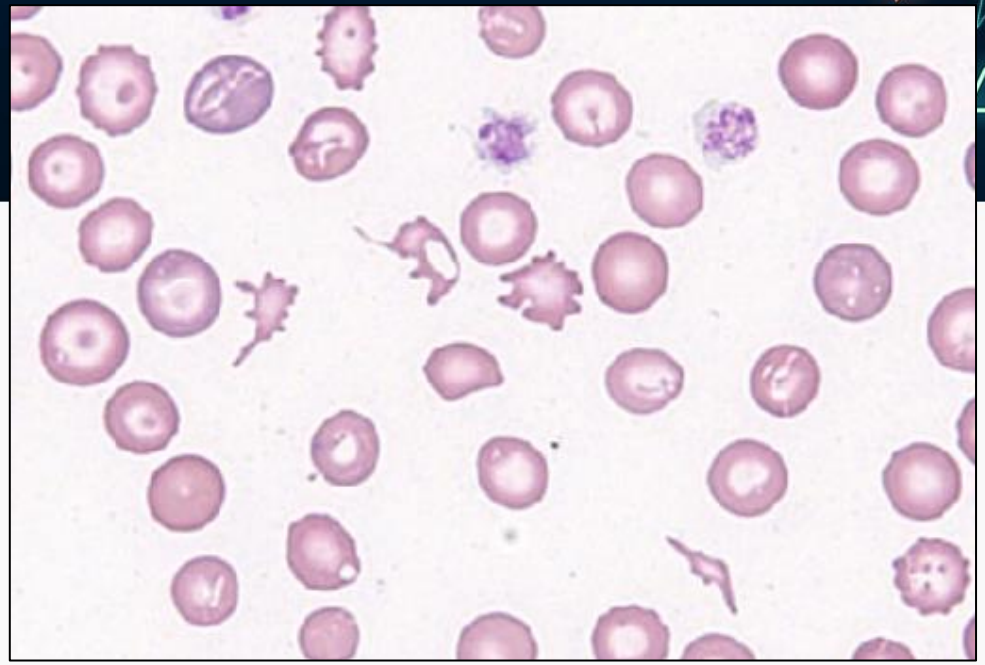


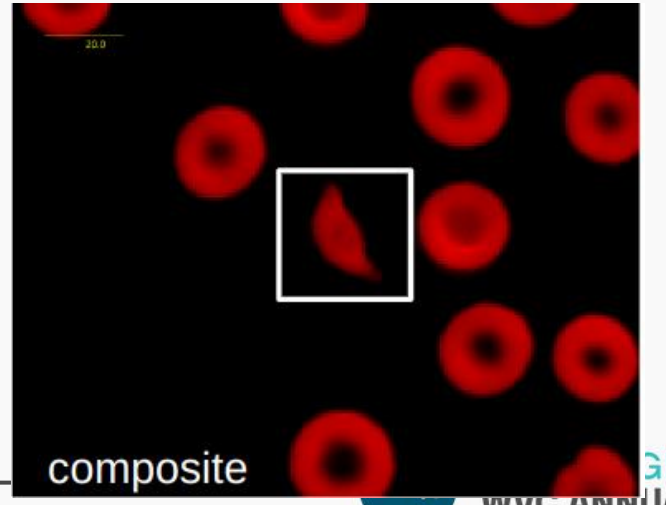


Weird shapes

Schistocytes

- Irregular RBC fragments
- Believed to form through microangiopathic damage
- Can occur in a number of diseases

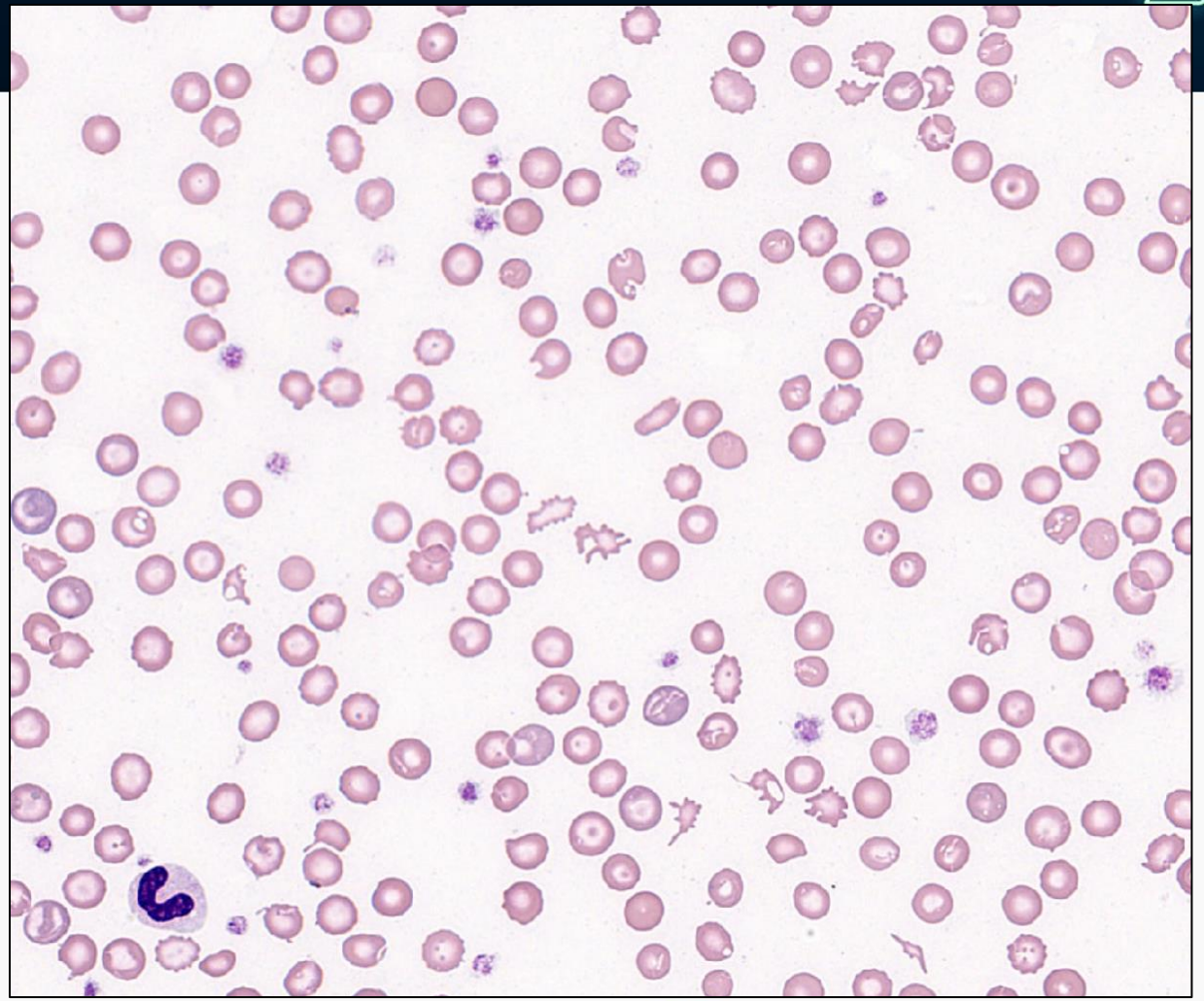




Poikilocytosis

- Mixture of red cell shape changes
- Often includes acanthocytes, schistocytes, keratinocytes, etc.

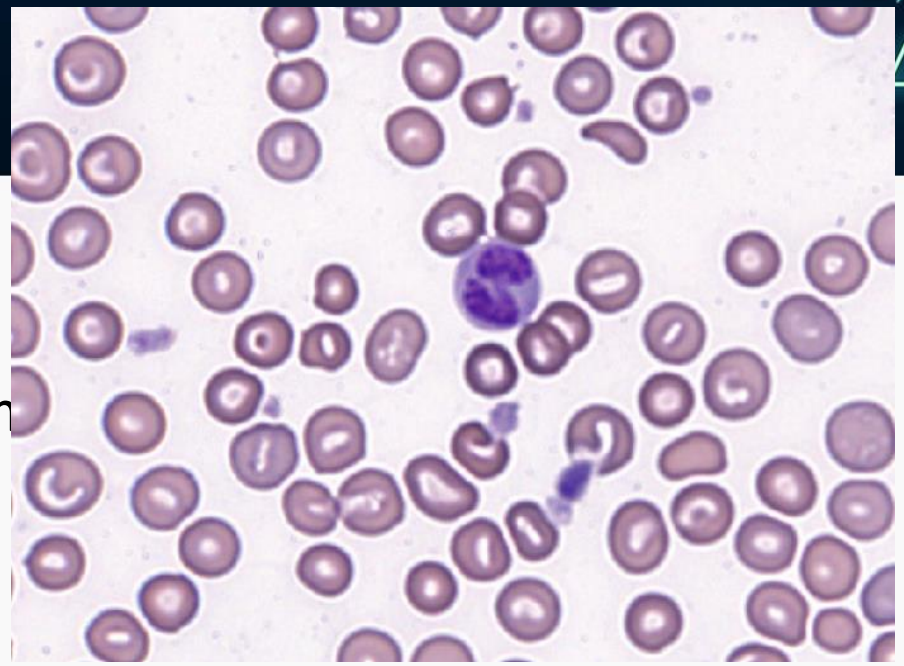
- Non-specific finding – occurs with a variety of processes
  - Cats – Common
    - Liver disease
  - Goats and kids – Common
    - potentially associated with iron deficiency
  - Dogs
    - Congenital ventricular outflow obstruction

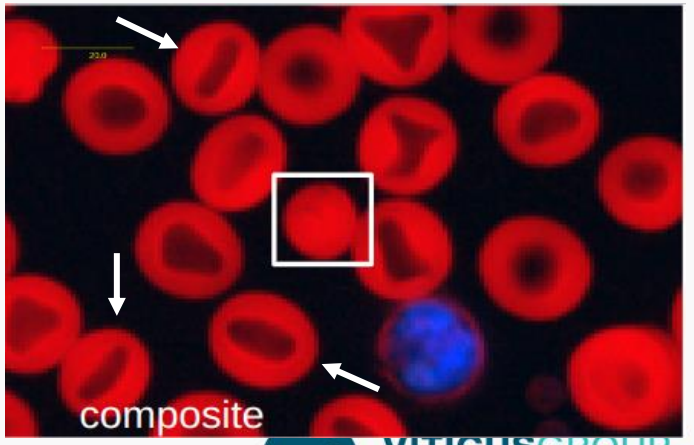


Abnormal central pallor

Stomatocytes

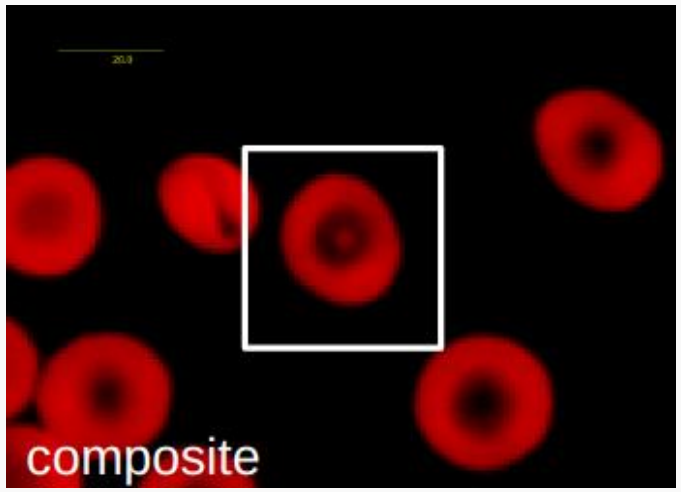
- Stomatocytes
  - Oval to rectangular area of central pallor
  - Can be associated with osmotic fagility and hem
  - Genetic disorders
    - Chondrodysplastic Alaskan Malamutes
    - Drentse Partrijshonds
    - Schnauzers
    - Pomeranians

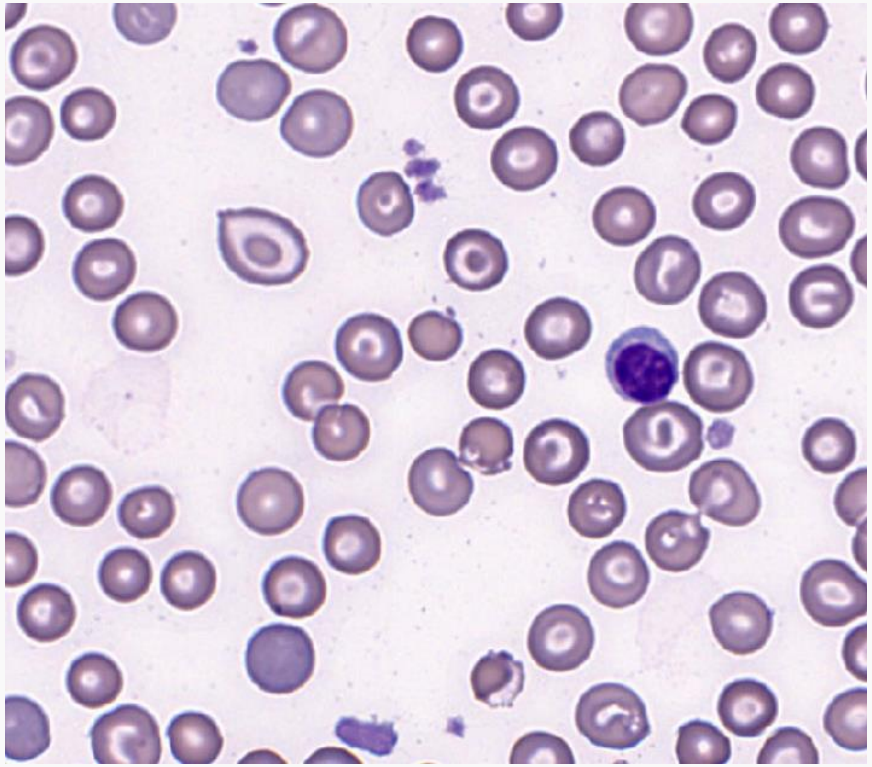




Codocytes

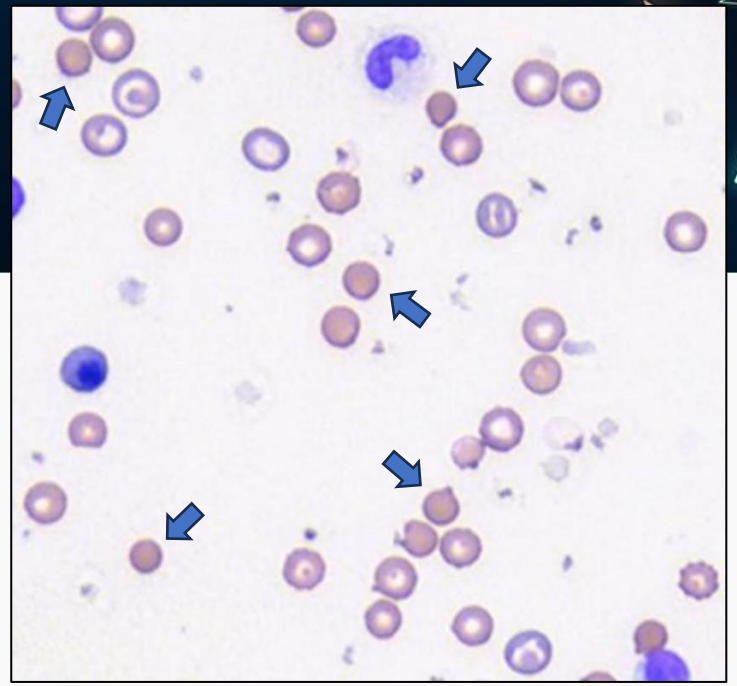
- Target or bullseye appearance to central clear area
- Often interpreted as extra membrane in the transition from polychromatophilic cell to a mature RBC

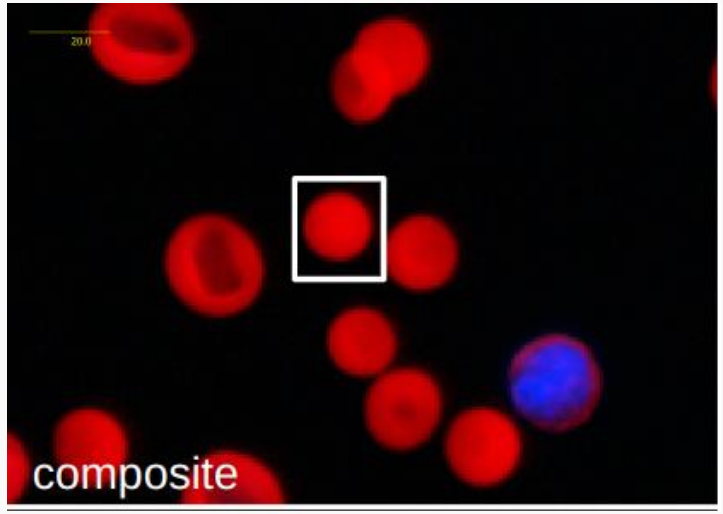




Spherocytes

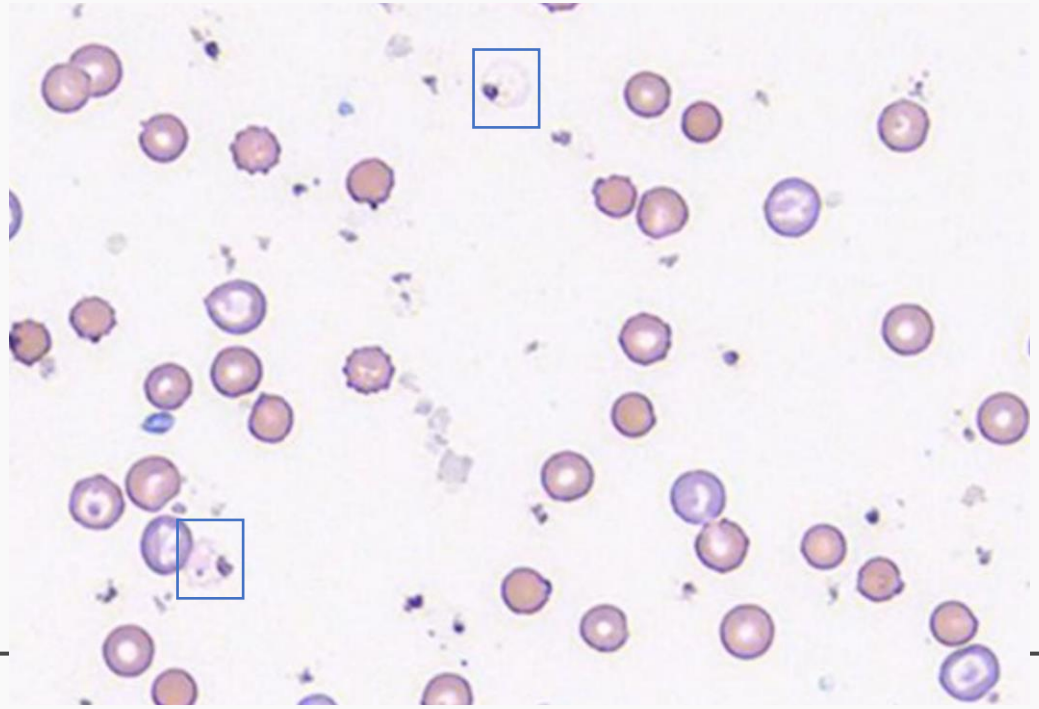
- Sphere-shaped RBCs with no central pallor
- Require evaluation in the correct reading area
  - Artifactual “spherocytes” along feathered edge
- Low numbers of spherocytes can occur in a variety of conditions
  - Fragmentation anemia, oxidative damage, coral snake venom, bee venom, aberrant macrophage function, prolonged storage
  - Pyruvate kinase deficiency in Basenjis (spheroechinocytes)
- Marked spherocytosis is consistent with IMHA
  - Ab bound to RBCs leads to phagocytosis by macrophages
  - Can confirm with Coomb’s test or agglutination

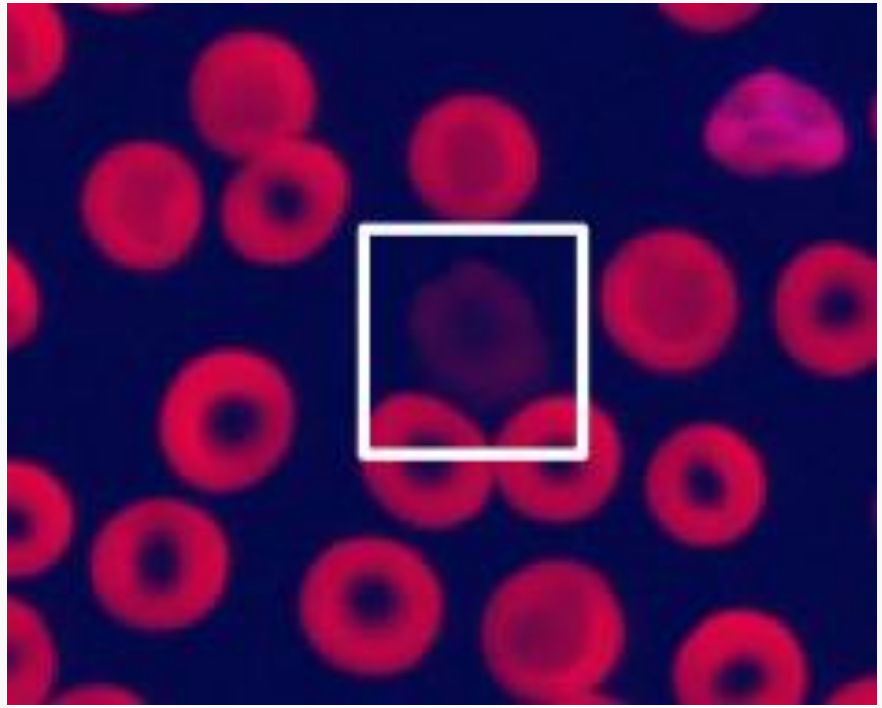




Ghost cells

- Lysed red cells with only membrane remaining
- Indicates intravascular hemolysis

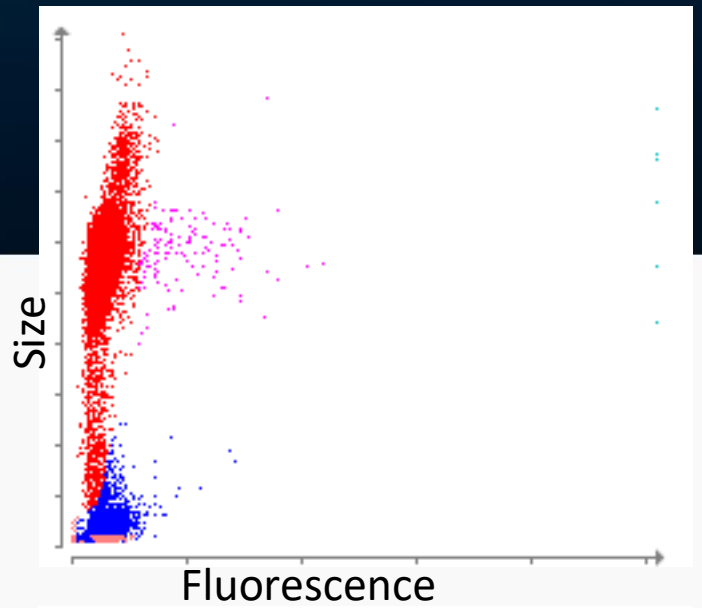




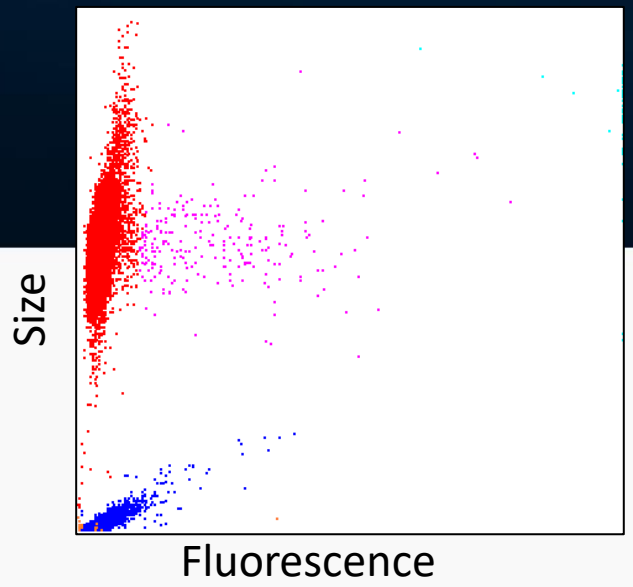
Case spotlight

- 4-year-old, spayed female Australian shepherd dog
- Presents for vomiting, diarrhea, lethargy following eating something on a walk
- Exam findings: T 104.8F, mildly icteric mmbr, markedly injected sclera and mild hyphema OU, port-colored urine, melena

Canine Patient dot plot



Normal ProCyte Dx



Case spotlight

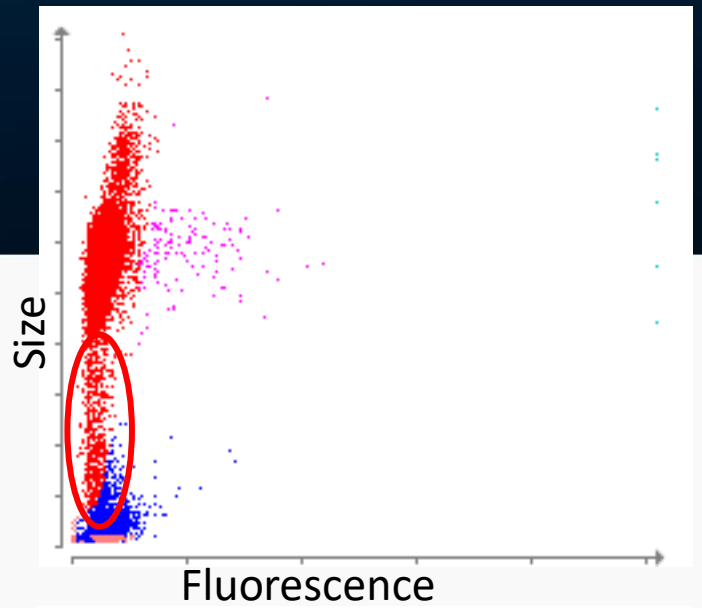
Dot plots provide a sensitive indicator of a change in blood cell morpholgy

- Pathologic RBCs

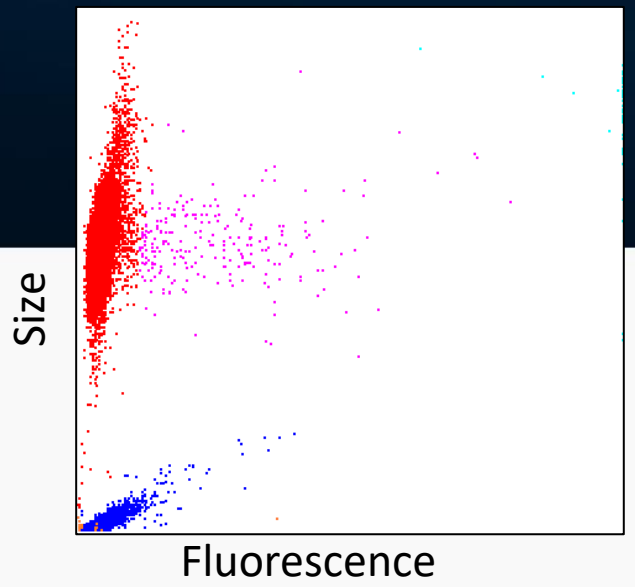
Graphical representation of the CBC that supports the numeric data

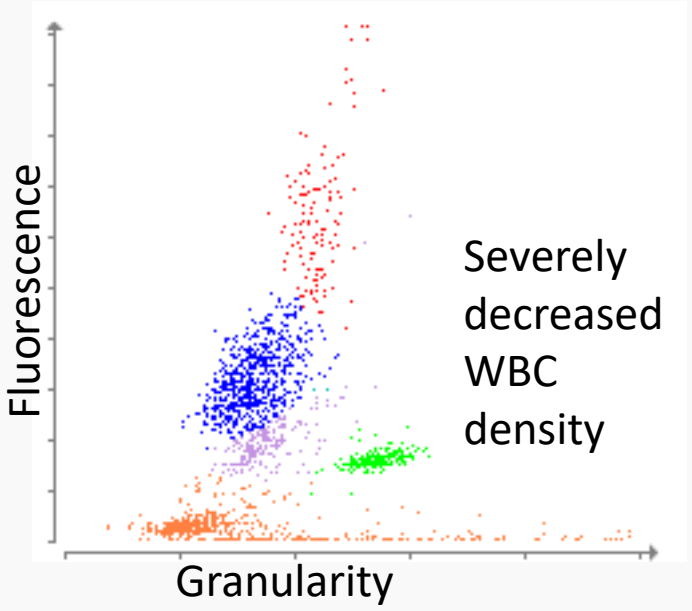
Abnormal dot plots indicate the need to assess blood morphology

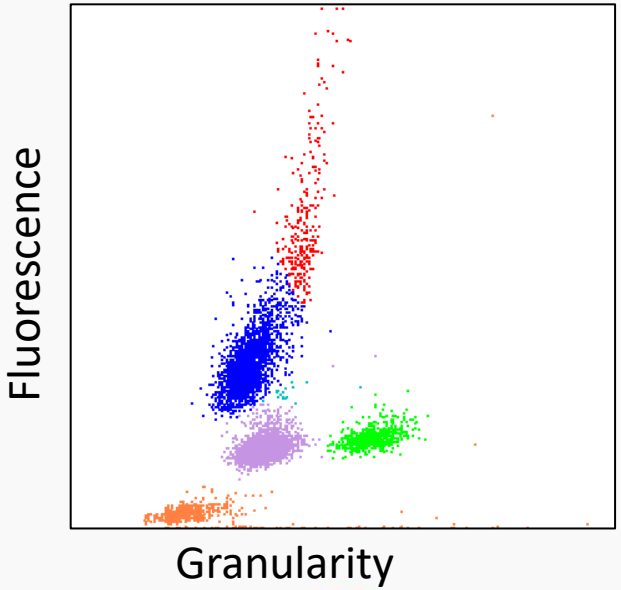
Canine Patient dot plot



Normal ProCyte Dx

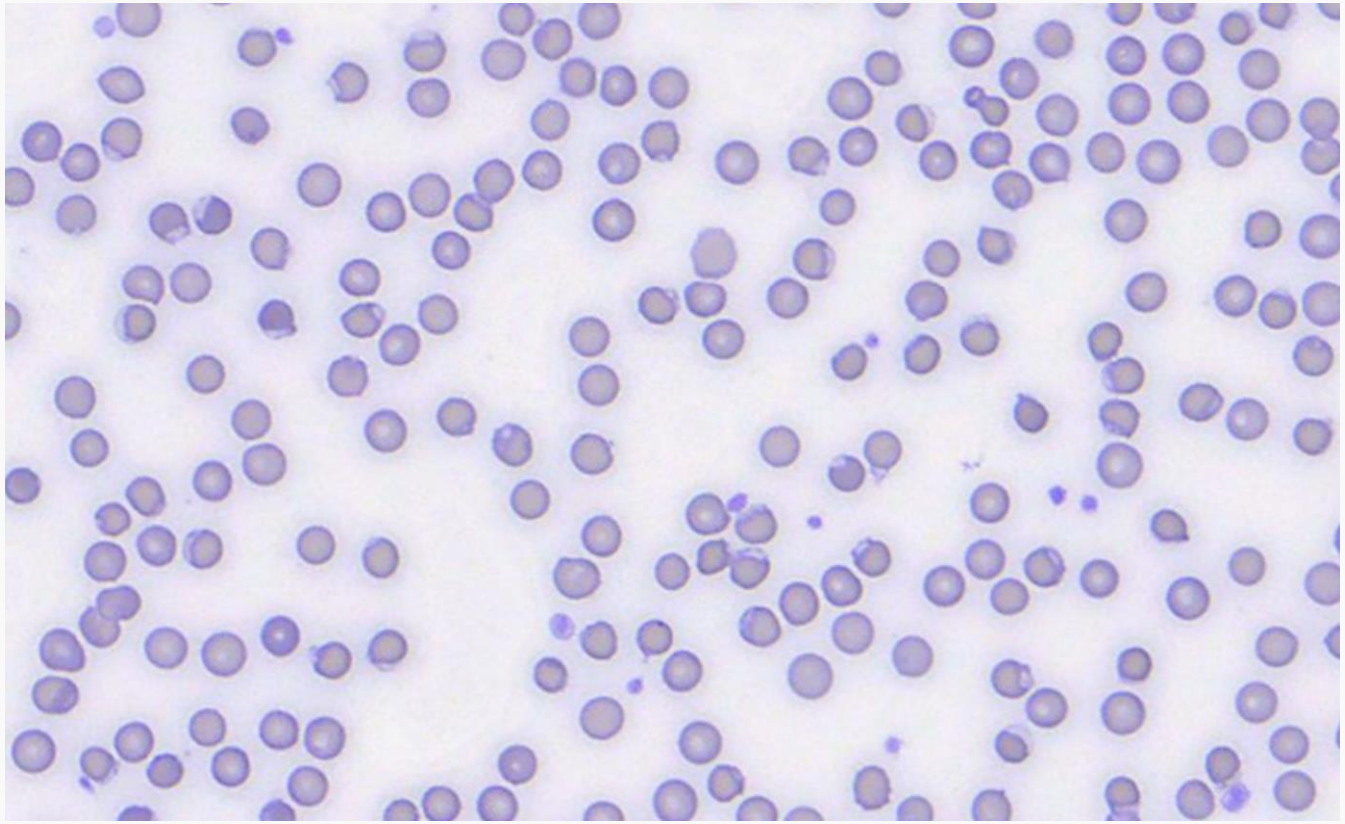


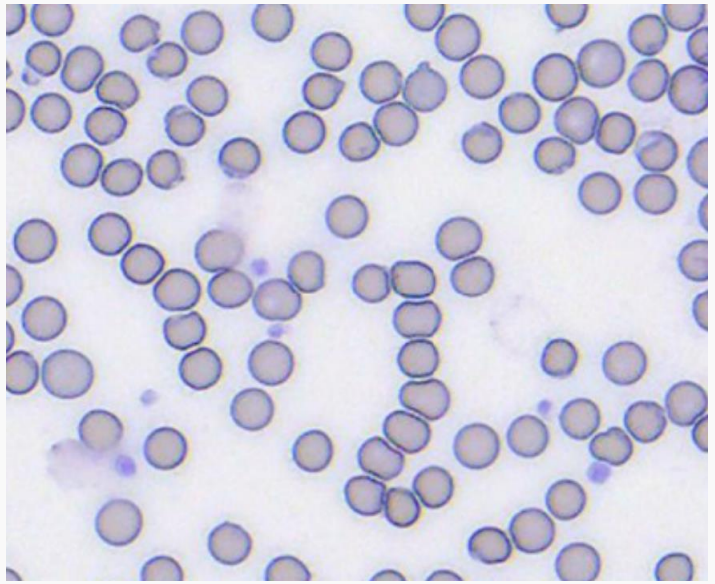




Blood Morphology

- Few Heinz bodies
- Ghost cells
- Eccentrocytes

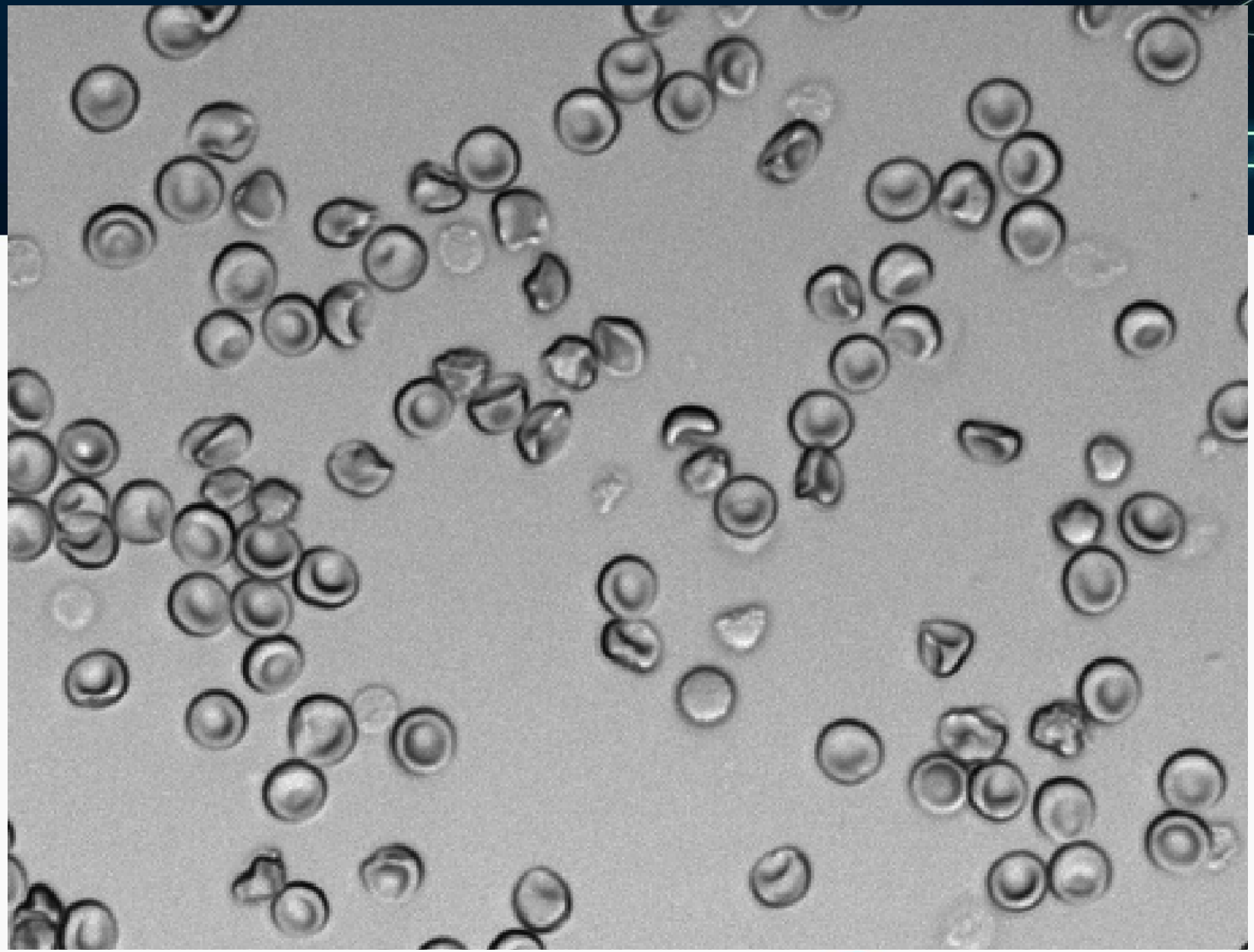




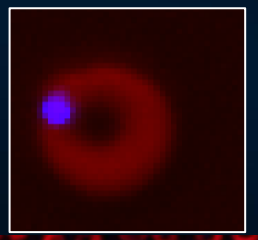
IDEXX inVue Dx results

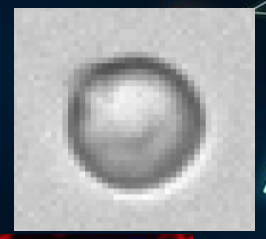
Eccentrocytes

Ghost Cells



IDEXX inVue Dx results
10 days later

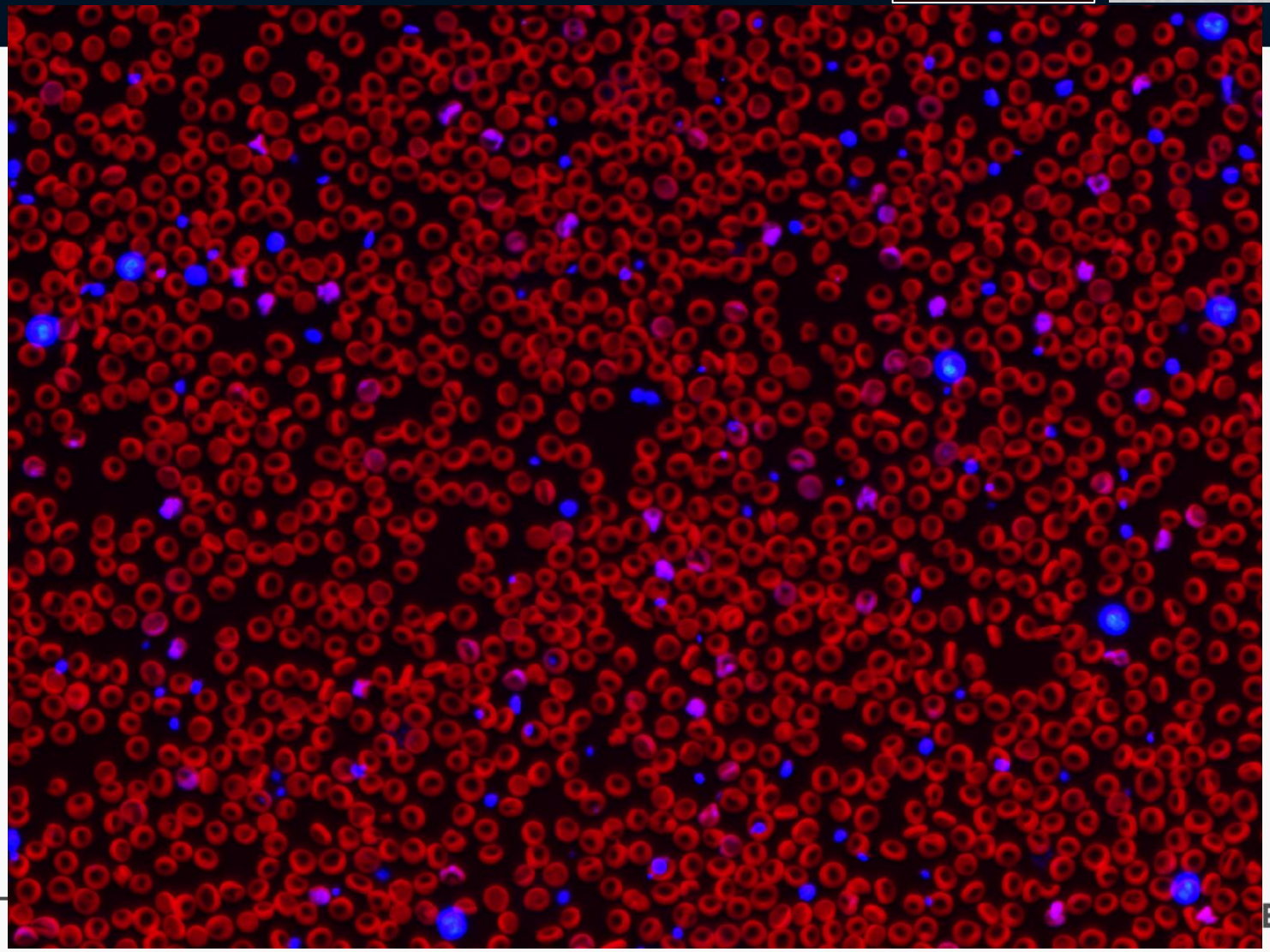




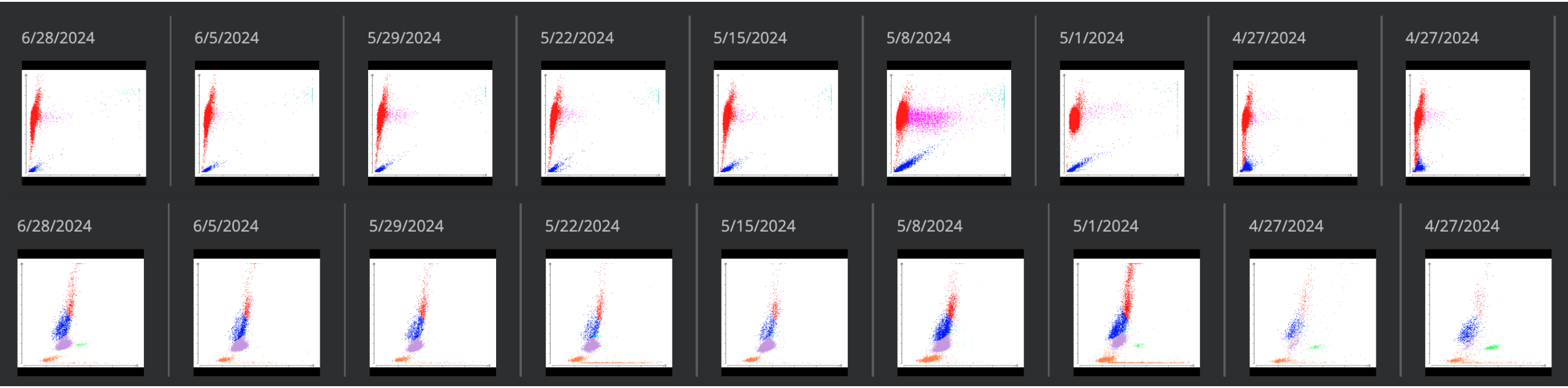
Heinz Bodies

Polychromasia

Composite increased fluorescence



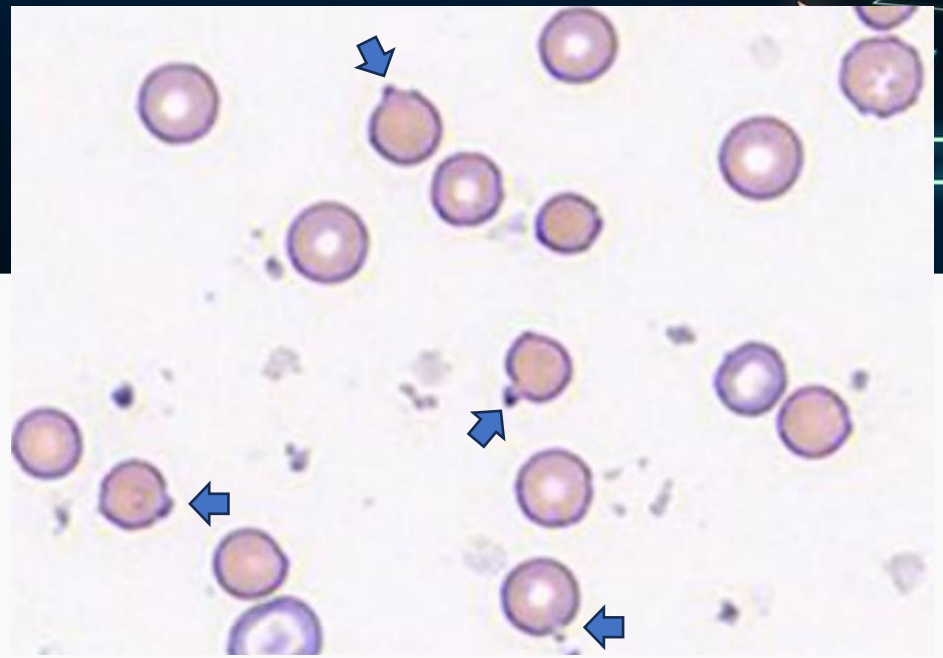
Serial Dot Plots on Vet Connect Plus

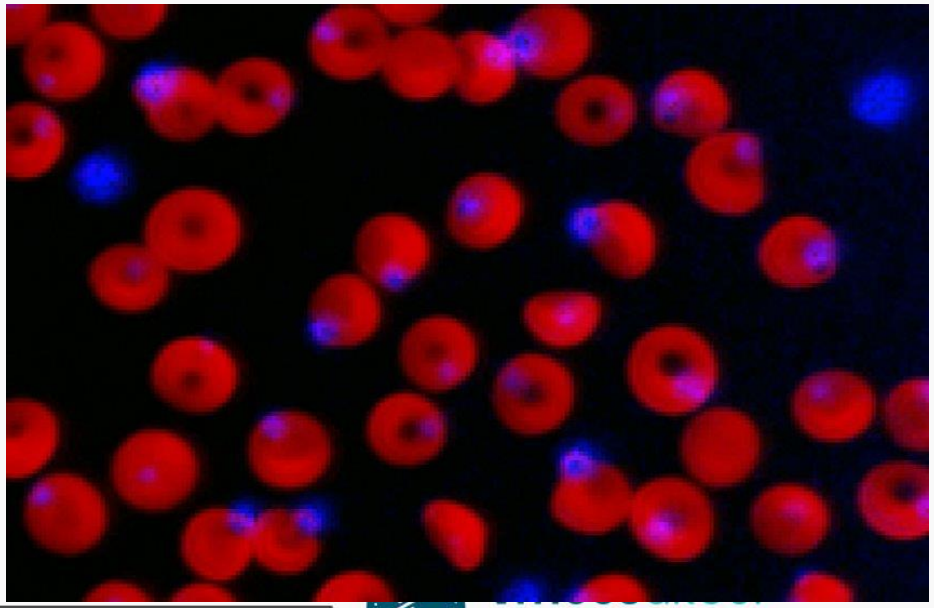


Things in RBCs

Heinz bodies

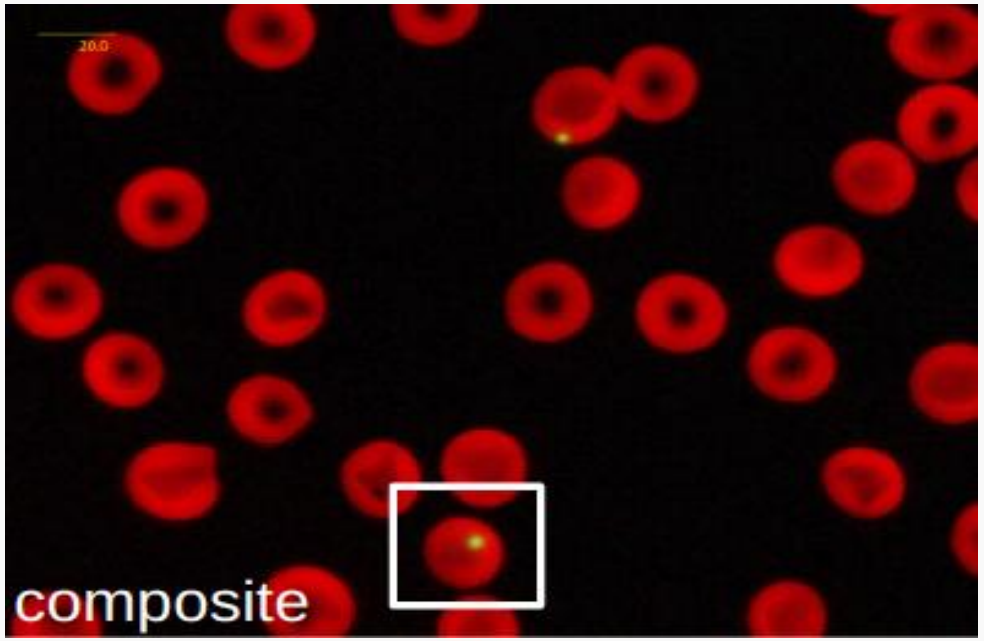
- Appear as blunt projections on glass slides
- New methylene blue allows better visualization
- Occur due to oxidative damage distorting the tertiary structure of Hgb
- Small Heinz bodies are common (normal) finding in cats
- Large Heinz bodies are abnormal in all species
  - Can cause hemolysis
  - Allium ingestion
  - Acetominophen, propofol, propylene glycol
  - Zinc toxicity
  - Red maple leaves (horses), Brassica ingestion (ruminants)





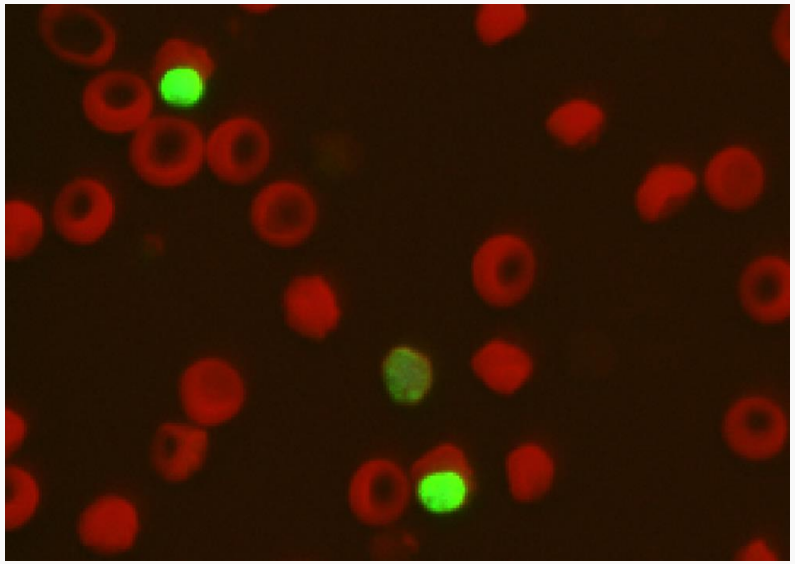
Howell-Jolly bodies

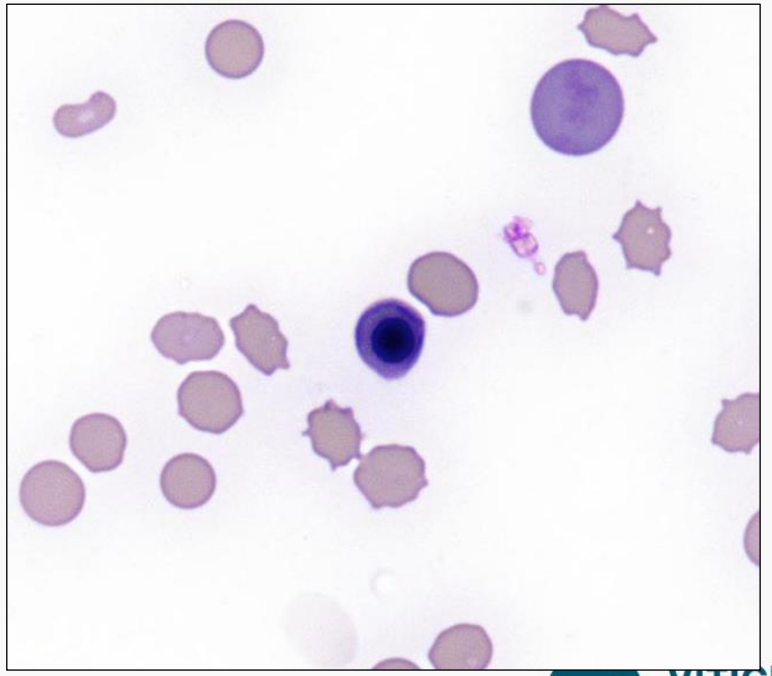
- Small nuclear remnants in RBCs
  - Usually removed in spleen
- Low numbers can be normal in horses and cats
- Splenectomy or splenic dysfunction
- Regenerative anemia
- Erythroid dysplasia
  - Myelodysplastic syndrome
  - Hereditary macrocytosis in toy and miniature poodles



Nucleated red blood cells

- Earlier stage than reticulocytes
  - Most commonly metarubricyte stage
  - Can occasionally be earlier RBC precursors
- Rare to see in blood in healthy dogs

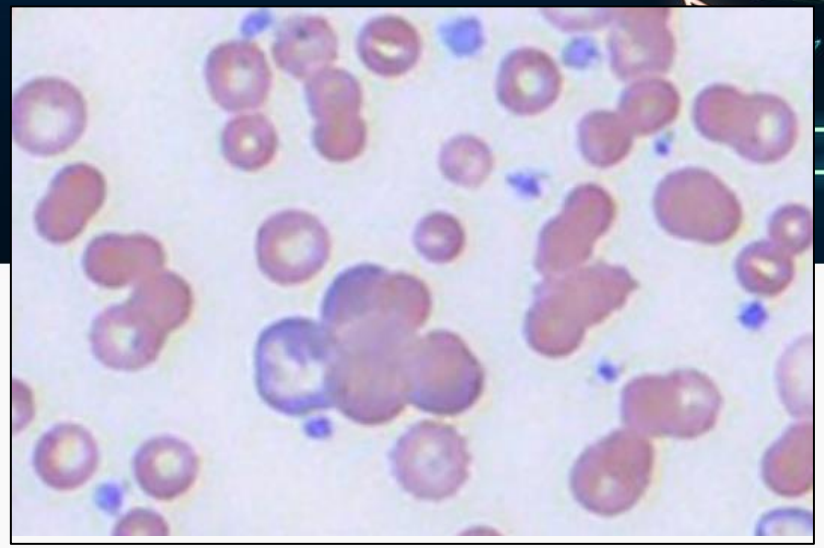


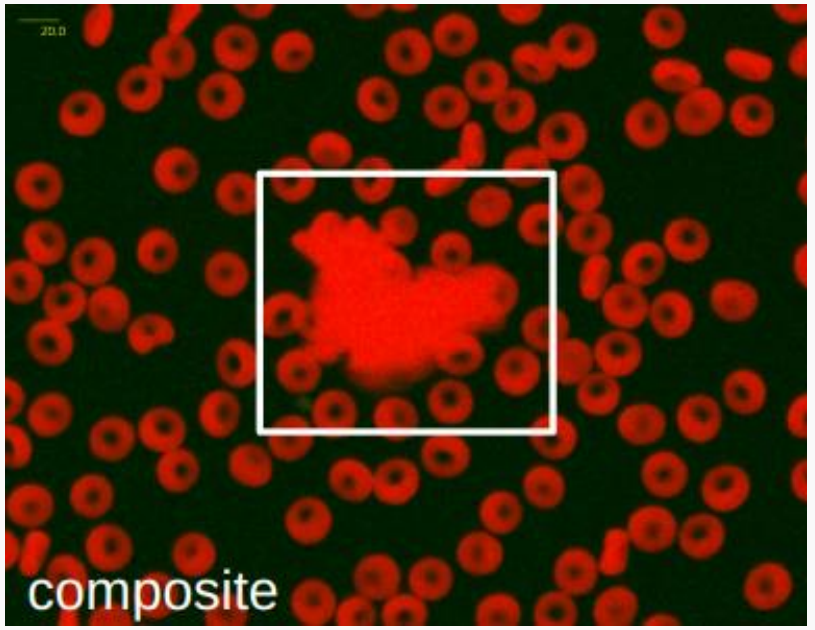


Agglutination vs rouleaux

Agglutination

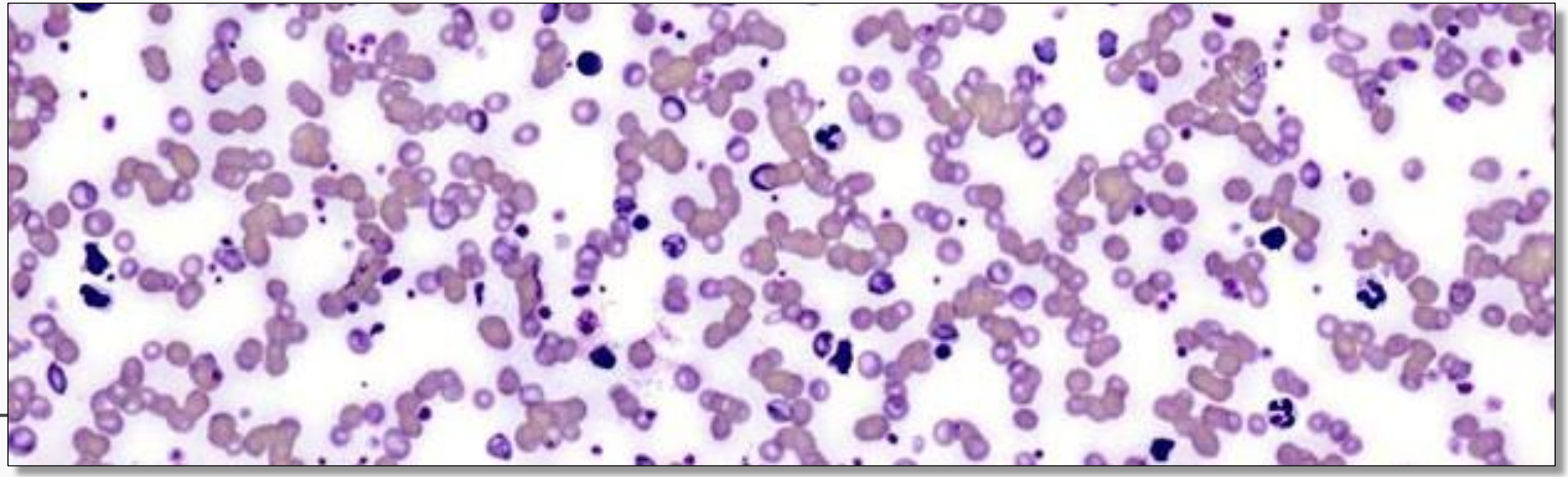
- RBCs are bound together by antibodies
- Usually indicates IMHA
- Cold agglutinins
  - Rare condition causing agglutination and hemolysis in cold temperatures
    - Can cause *in vitro* agglutination at <37 C and disperses with warming
    - May cause intermittent or localized clinical signs
  - Auto-immune disease
  - Can be secondary to infections, neoplasia, lead toxicity



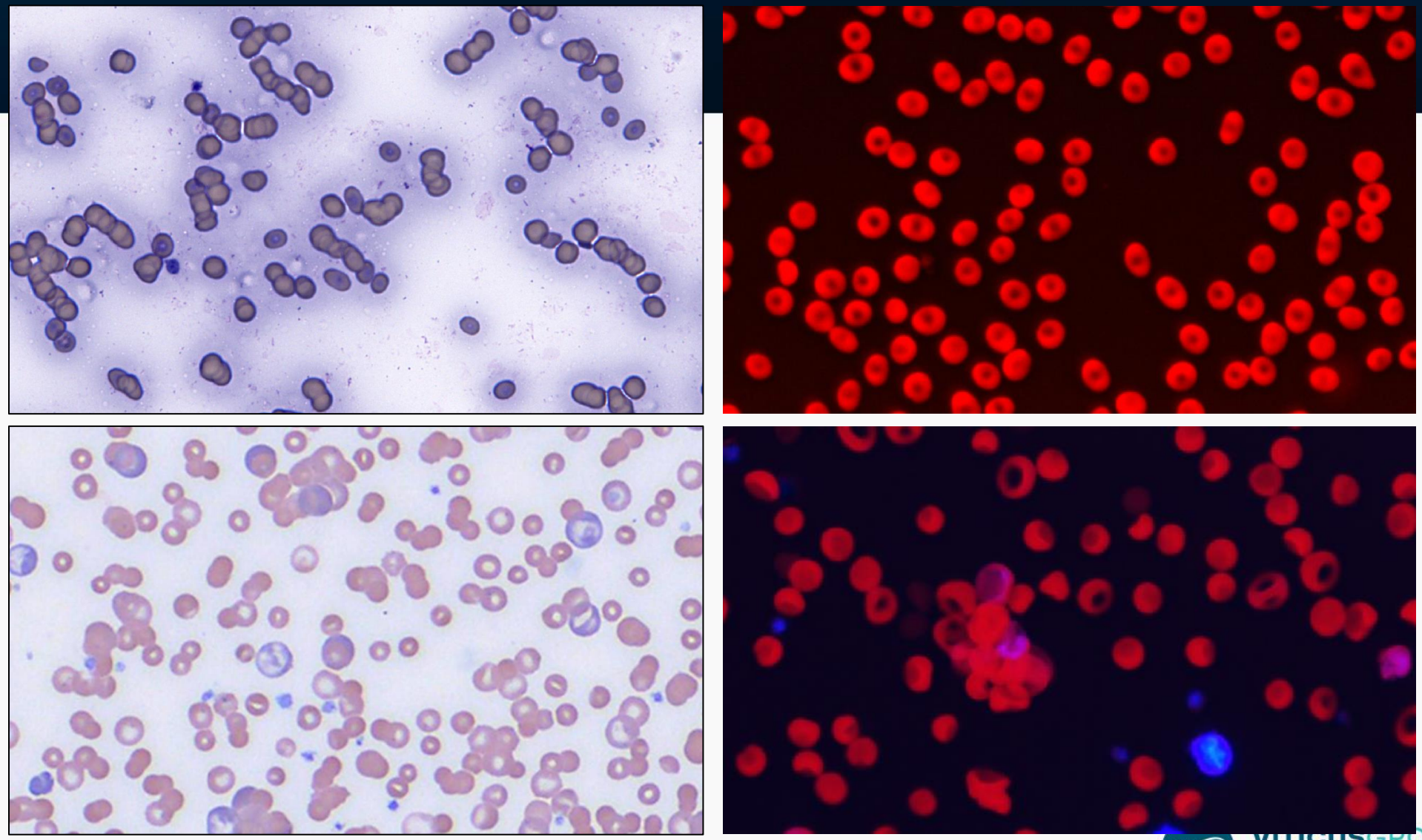


Rouleaux

- RBCs appear grouped or like a stack of coins
- Can be difficult to differentiate from agglutination in some cases
- Often occurs with high serum protein
- Seen most often in the body of the smear



inVue Dx™ solves clinical confusion of rouleaux and agglutination



WBC review

White blood cell information

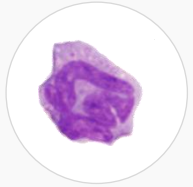
- Five types of white blood cells = “5-part differential”

Neutrophils

Lymphocytes

Monocytes

Eosinophils

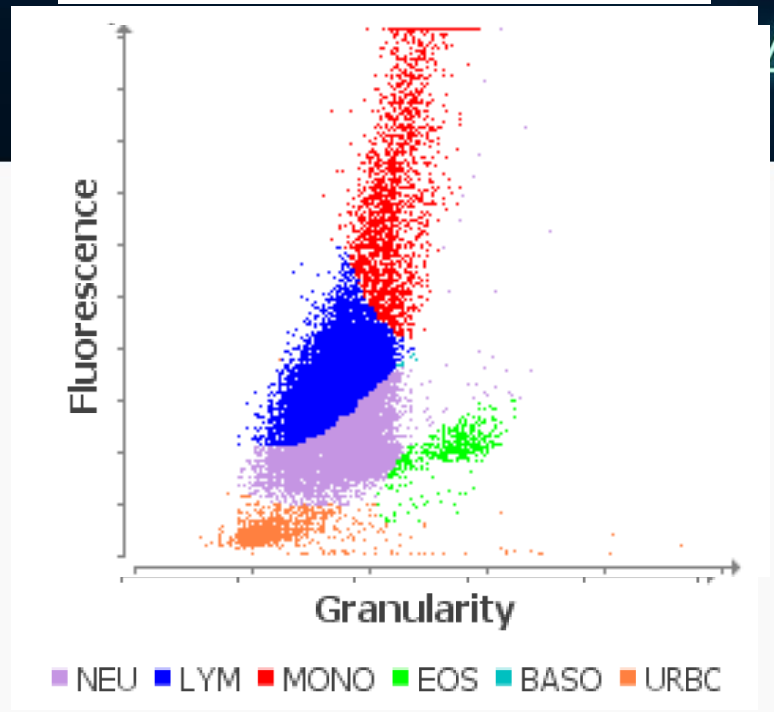


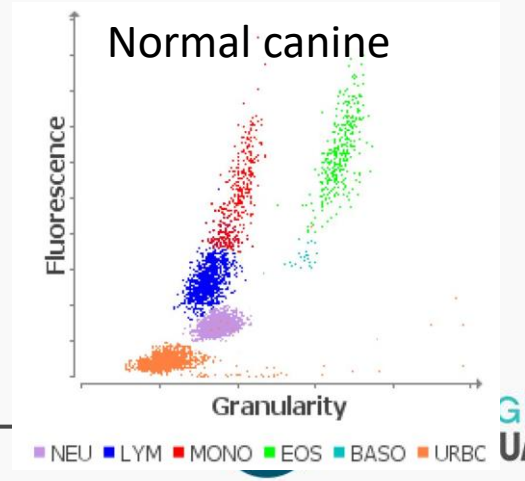
Basophils

When do you need a blood morphology evaluation of WBCs?

- Hematology analyzers provide excellent WBC differentials in normal samples
- Only directly identify 4–5 cell types in differential
  - Neutrophils
  - Lymphocytes
  - Monocytes
  - Eosinophils
  - +/- Basophils
- Unusual cell types may cause inaccurate automated differentials
  - Left shift or toxic change
  - Reactive lymphocytes or monocytes
  - Leukemia or circulating tumor cells
  - Abnormal red cells – nucleated RBCs, unlysed RBCs, etc.

ProCyte Dx® Patient Sample





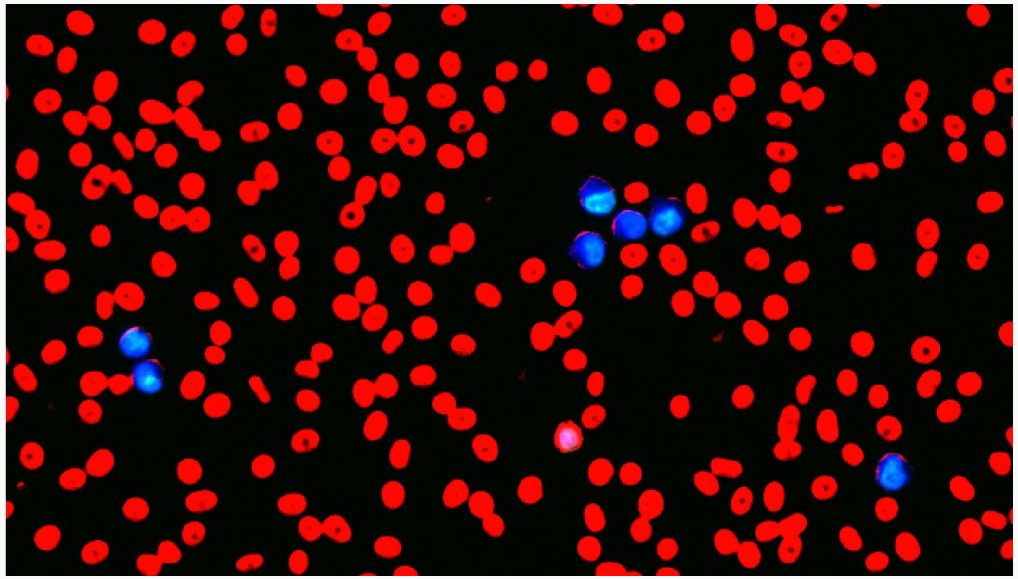
GROUP™
UAL CONFERENCE

Cassie

- 10-year-old, female spayed, DSH cat

Presents for:

Ongoing issue D+, V+ Occasionally, lethargic, hair loss, weight loss. Primary thinks cancer was waiting on u/s but worsened tonight - not eating, hardly moving, isolating



- Exam findings:
  - QDR
  - Pale mmbr

Chemistry Results

Total protein within normal limits

ALP elevated

T. bili within normal limits

fPL Abnormal

SDMA mildly elevated

D/T DRAWN: 04/30/24 : BY: SG D/T ENTERED: 04/30/24 12:15
ACCESSION: 994 COMMENT:

TEST	RESULT	NORMAL RANGE	LL[NORMAL]HH	UNITS
NA	153.3	146.0 - 160.0	[*]	mmol/L
K	4.15	3.30 - 5.40	[*]	mmol/L
CL	121.9	110.0 - 123.0	[*]	mmol/L
NAK	36.9	30.0 - 100.0	[*]	
CHOL	128.	71. - 218.	[*]	mg/dL
ALT	114.	26. - 128.	[*]	U/L
AMYL	2274. HD	422. - 1328.	[ ]*	U/L
ALP	735. H	14. - 102.	[ ]*	U/L
TBILI	0.3	0.2 - 0.3	[*]	mg/dL
GLU	116.	56. - 153.	[*]	mg/dL
PHOS	5.6	2.7 - 7.5	[*]	mg/dL
TP	6.7	5.9 - 8.4	[*]	g/dL
ALB	2.2 L	2.3 - 3.9	*[ ]	g/dL
GLOB	4.5	2.0 - 5.0	[*]	g/dL
AGR	0.49	0.35 - 1.50	[*]	
BUN	16. L	18. - 36.	*[ ]	mg/dL
CREAT	1.04	0.80 - 2.00	[*]	mg/dL
BCR	15.	3. - 40.	[*]	
CA	9.8	8.7 - 11.7	[*]	mg/dL

L=LOW H=HIGH D=DILUTED

Chemistry

4/30/24 12:22 PM

TEST	RESULT	REFERENCE VALUE
IDEXX SDMA	[a] 15	0 - 14 µg/dL H

[a] SDMA:

SDMA is increased, no CREA result: likely impaired GFR and kidney function. Recommended next step: evaluation of other renal function tests, and complete urinalysis. For information on recommended actions visit: www.idexx.com/sdmaalgorithm.

SNAP fPL Abnormal

CBC results

Severe nonregenerative anemia

TEST	RESULT	REFERENCE VALUE	
RBC	**2.46**	**6.54 - 12.20 M/µL**	L
Hematocrit	**10.4**	**30.3 - 52.3 %**	L
Hemoglobin	**3.6**	**9.8 - 16.2 g/dL**	L
MCV	42.3	35.9 - 53.1 fL	
MCH	14.6	11.8 - 17.3 pg	
MCHC	34.6	28.1 - 35.8 g/dL	
RDW	21.4	15.0 - 27.0 %	
% Reticulocytes	0.4	%	
Reticulocytes	10.3	3.0 - 50.0 K/µL	
Reticulocyte Hemoglobin	19.4	13.2 - 20.8 pg	

CBC results

Severe nonregenerative anemia

Bands suspected

Severe monocytosis

Mild eosinophilia

Thrombocytopenia

WBC	*11.86	2.87 - 17.02 K/µL	
% Neutrophils	*24.3	%	
% Lymphocytes	*26.6	%	
% Monocytes	*48.1	%	
% Eosinophils	*0.5	%	
% Basophils	*0.5	%	
Neutrophils	*2.88	2.30 - 10.29 K/µL	
Bands	*Suspected		
Lymphocytes	*3.15	0.92 - 6.88 K/µL	
Monocytes	***5.71**	**0.05 - 0.67 K/µL**	H
Eosinophils	***0.06**	**0.17 - 1.57 K/µL**	L
Basophils	*0.06	0.01 - 0.26 K/µL	
Platelets	**34**	**151 - 600 K/µL**	L
MPV	20.6	11.4 - 21.6 fL	
Plateletcrit	**0.07**	**0.17 - 0.86 %**	L

CBC results

Severe nonregenerative anemia

Bands suspected

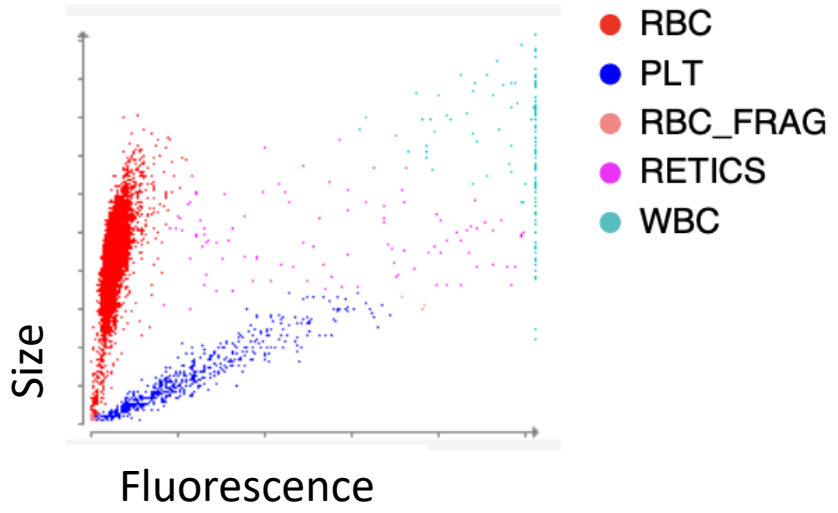
Severe monocytosis

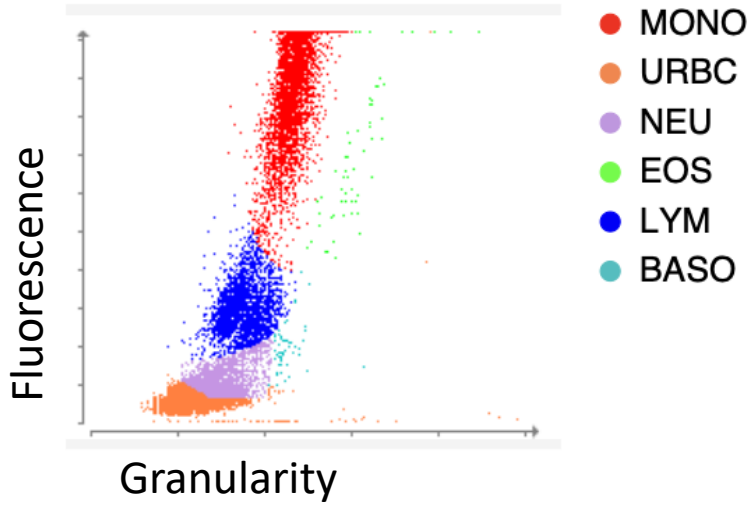
Mild eosinophilia

Thrombocytopenia

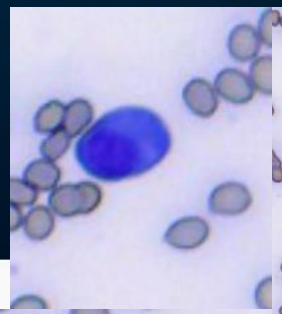
Abnormal dot plots

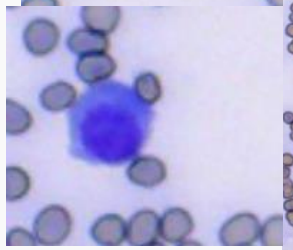
- Monocyte cloud abnormal
- Left shift?

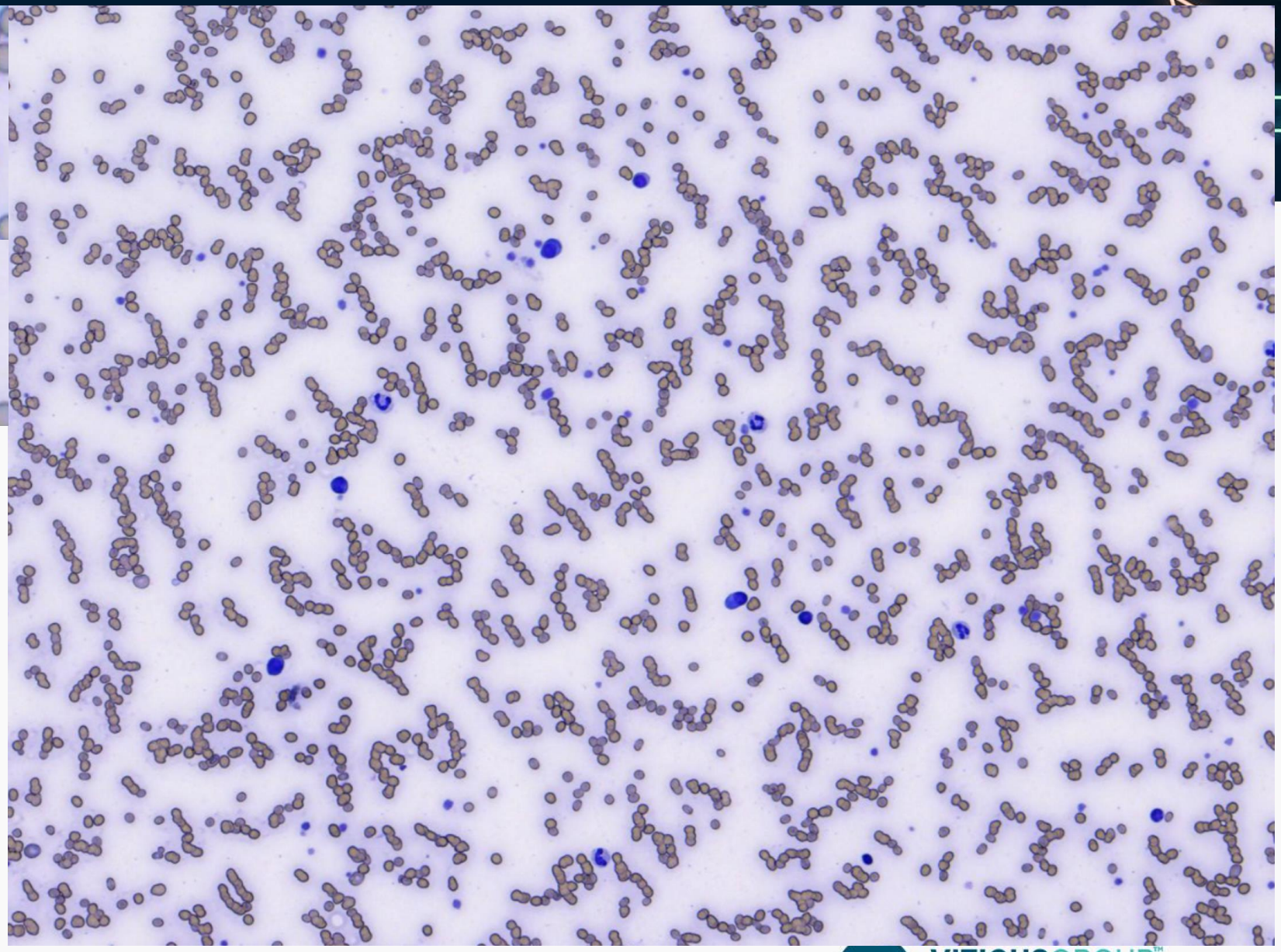




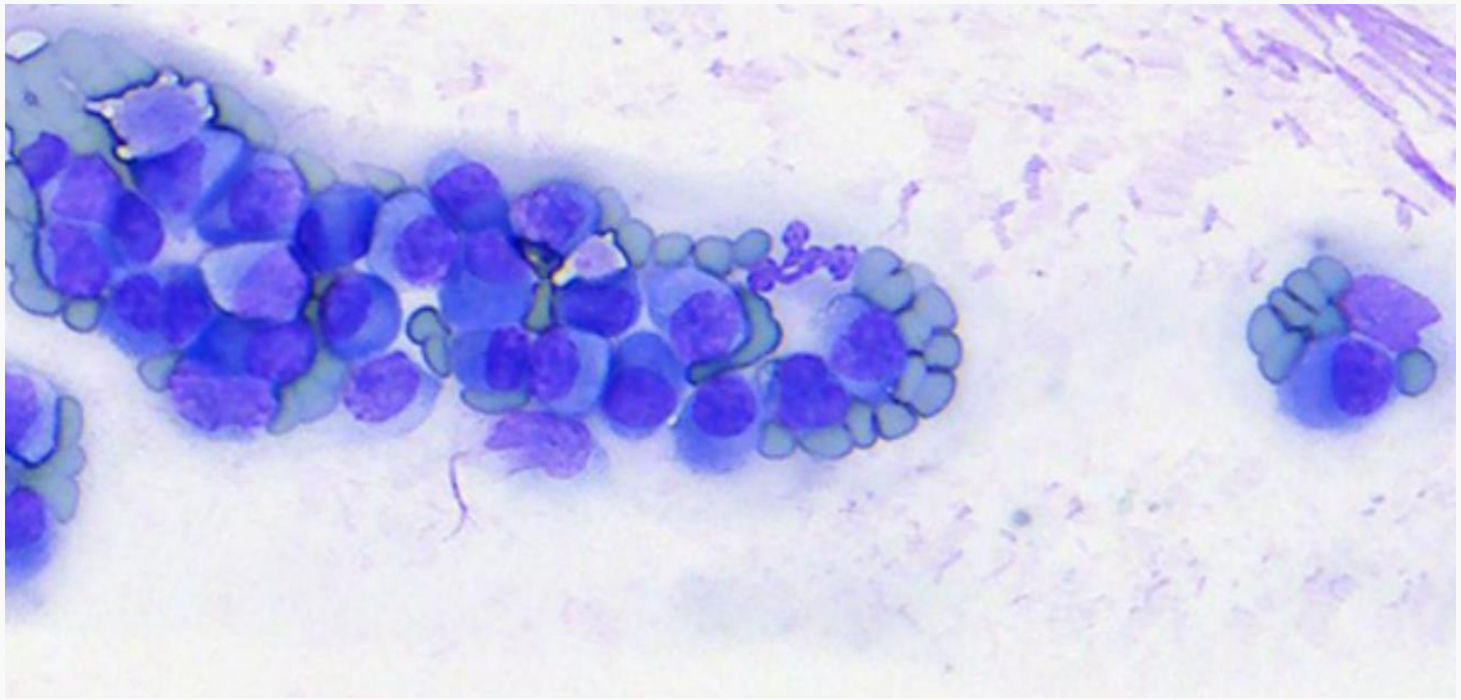
Blood film results



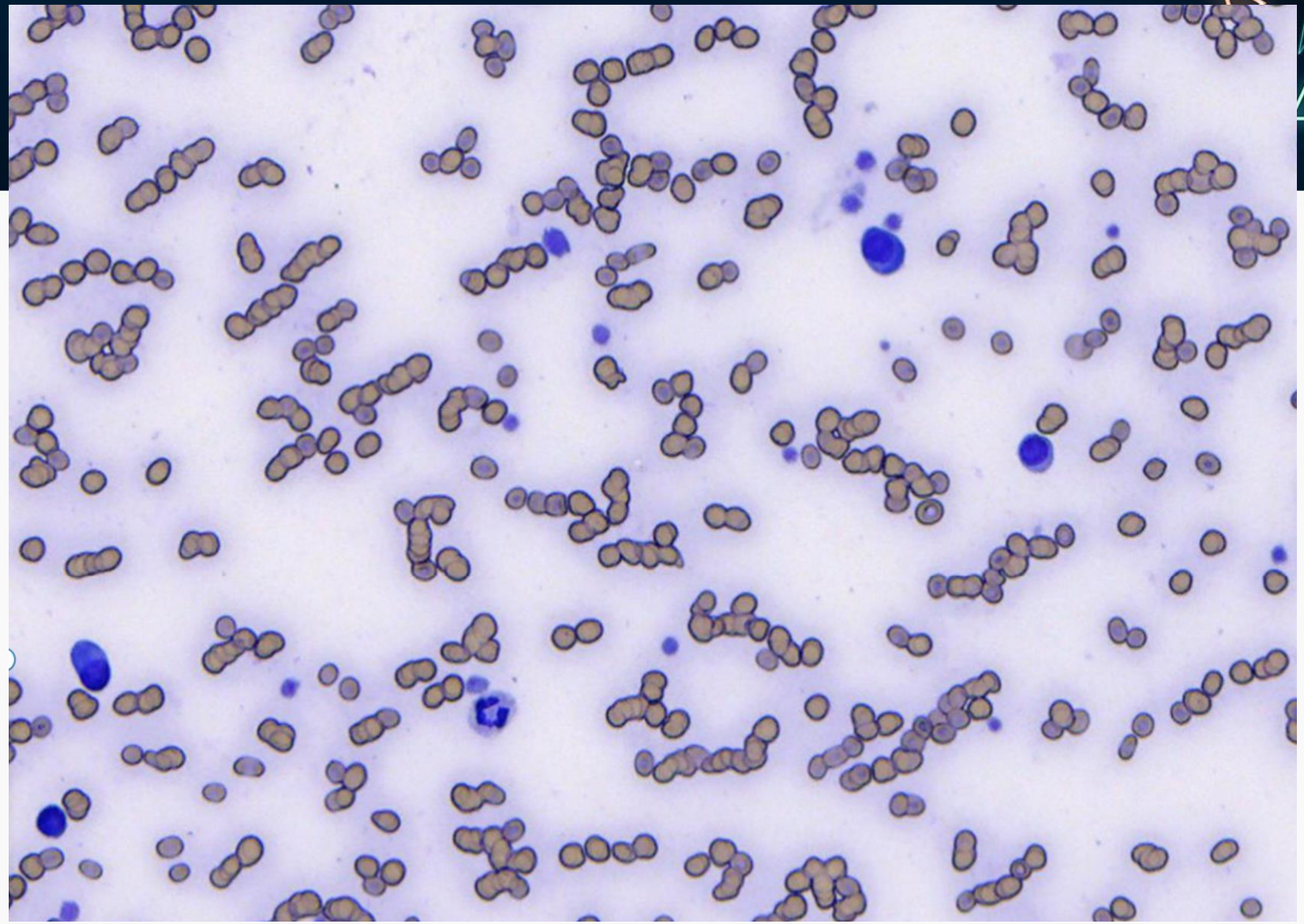




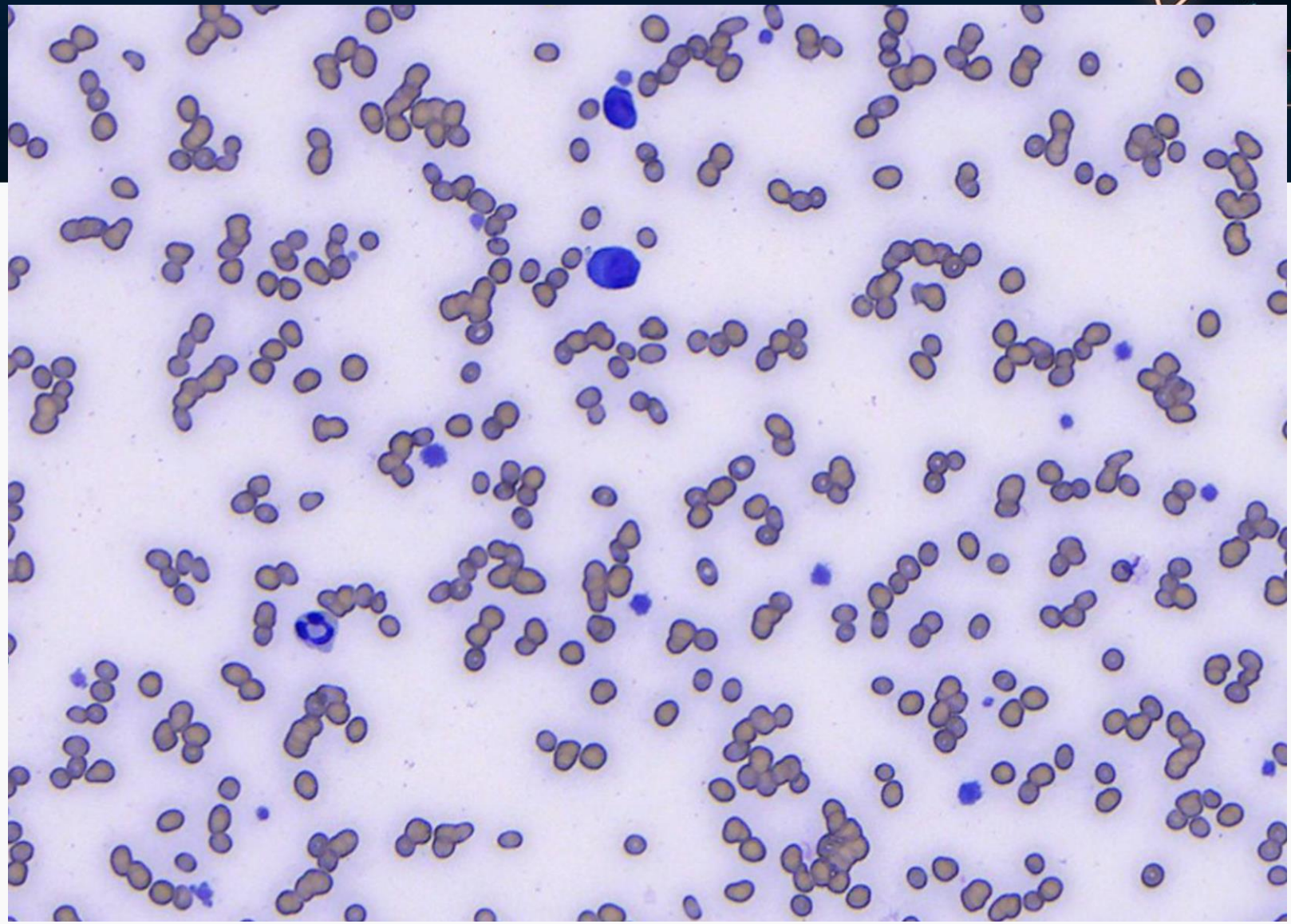
Blood film results



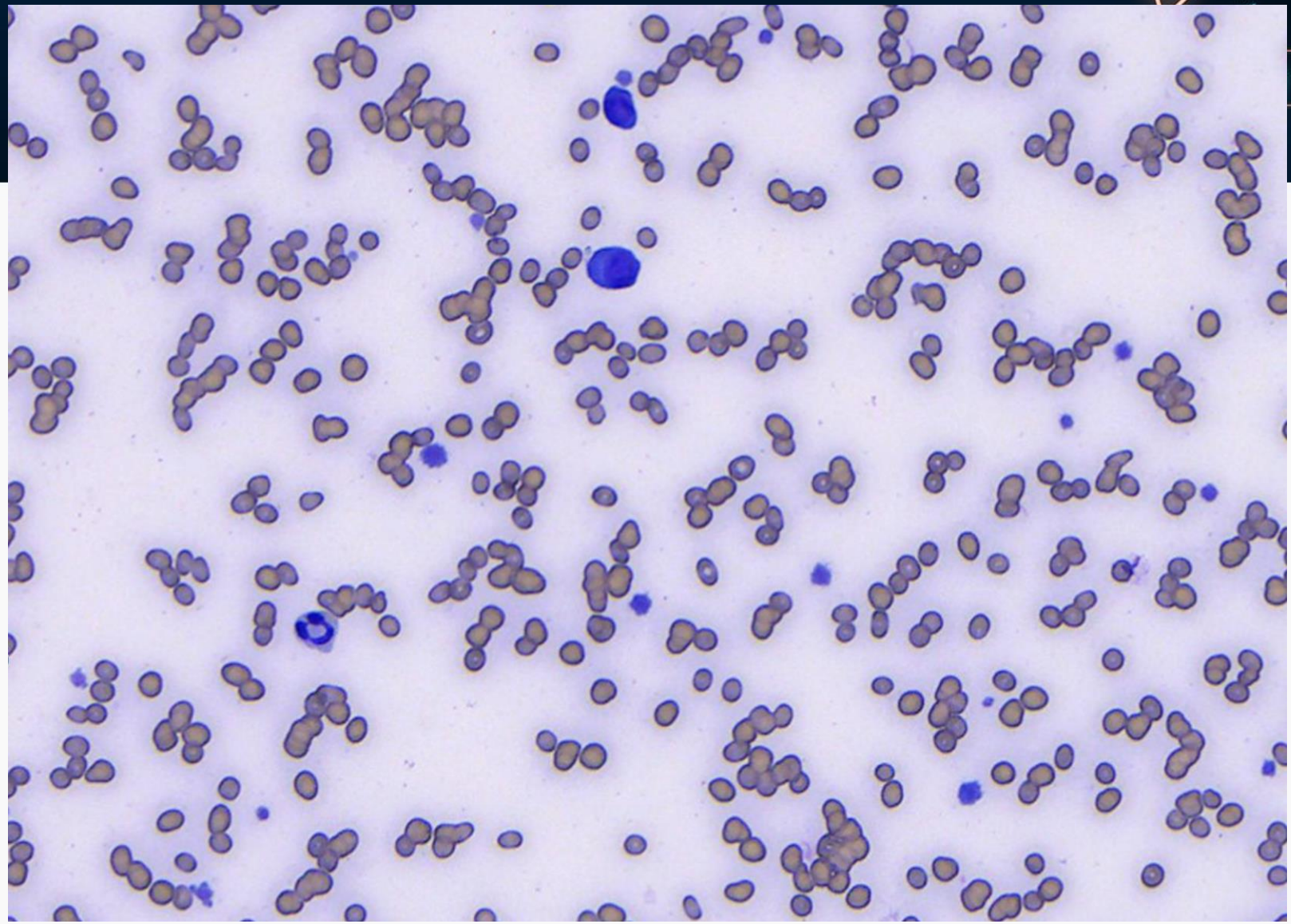
Blood film results



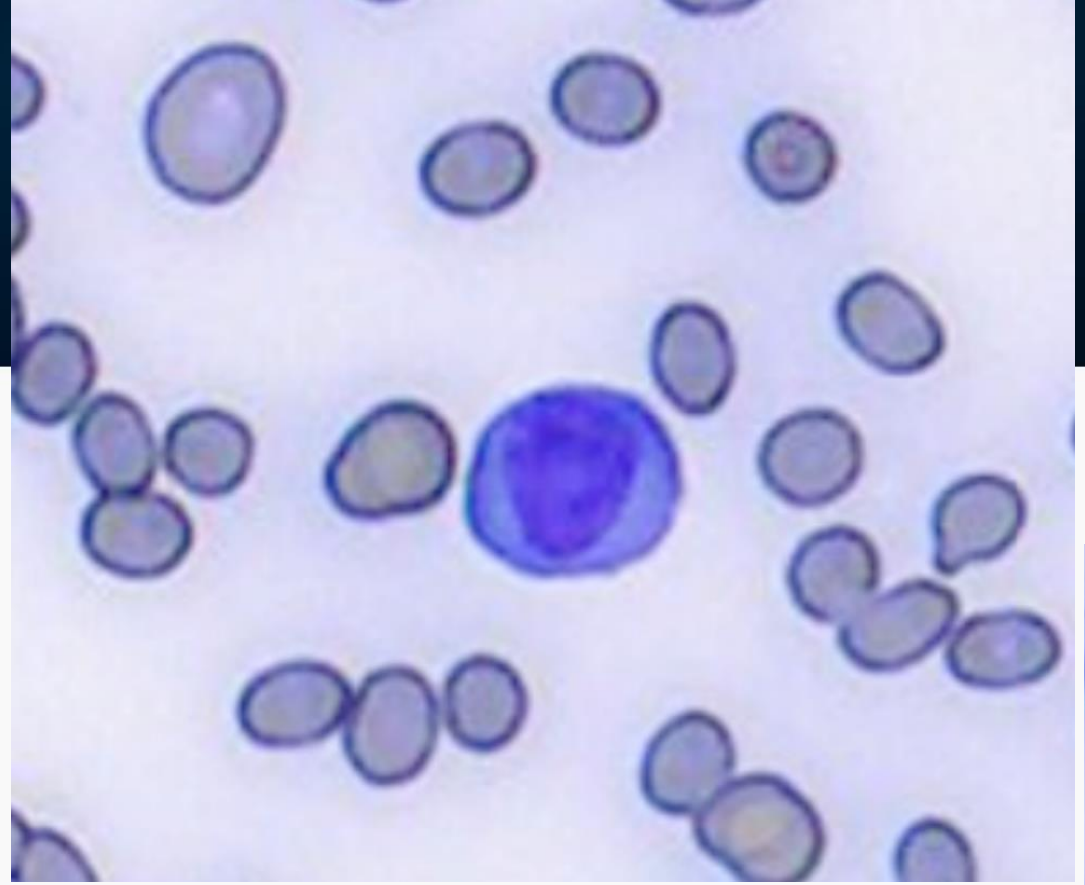
Blood film results

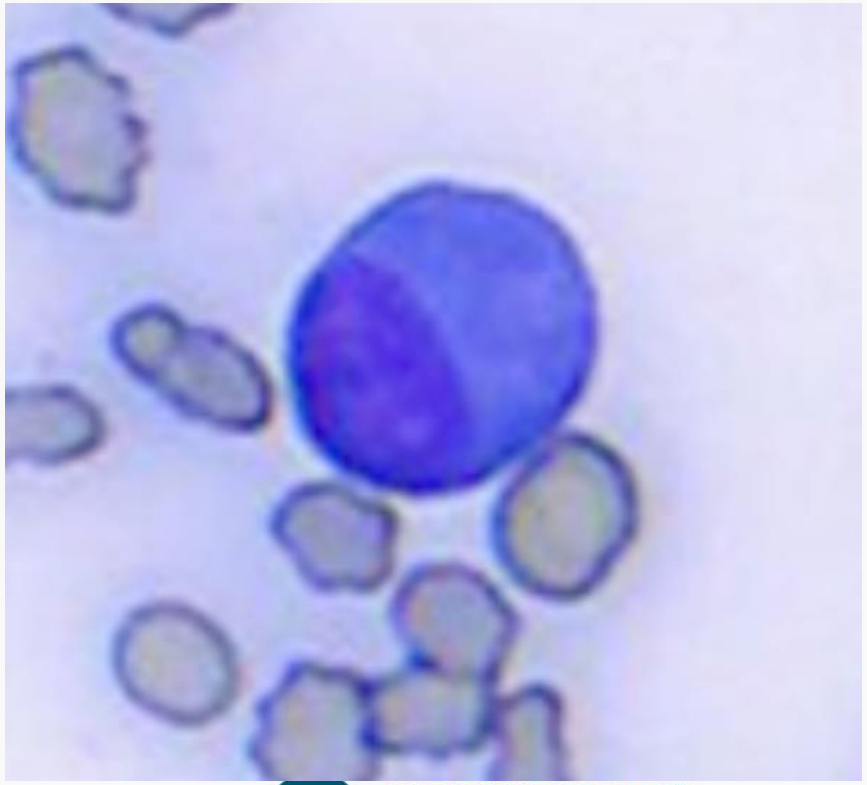


Blood film results



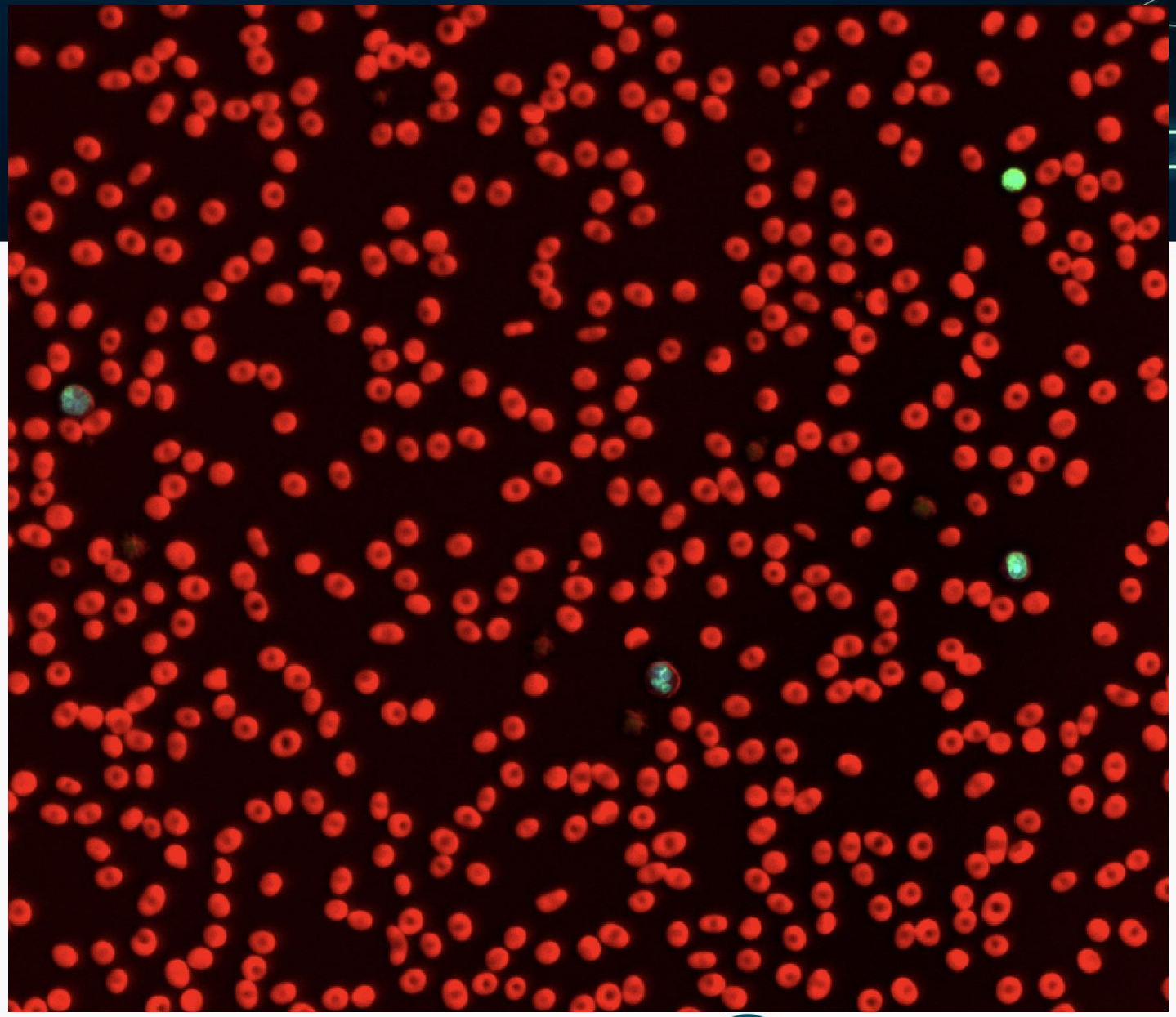
Blood film results



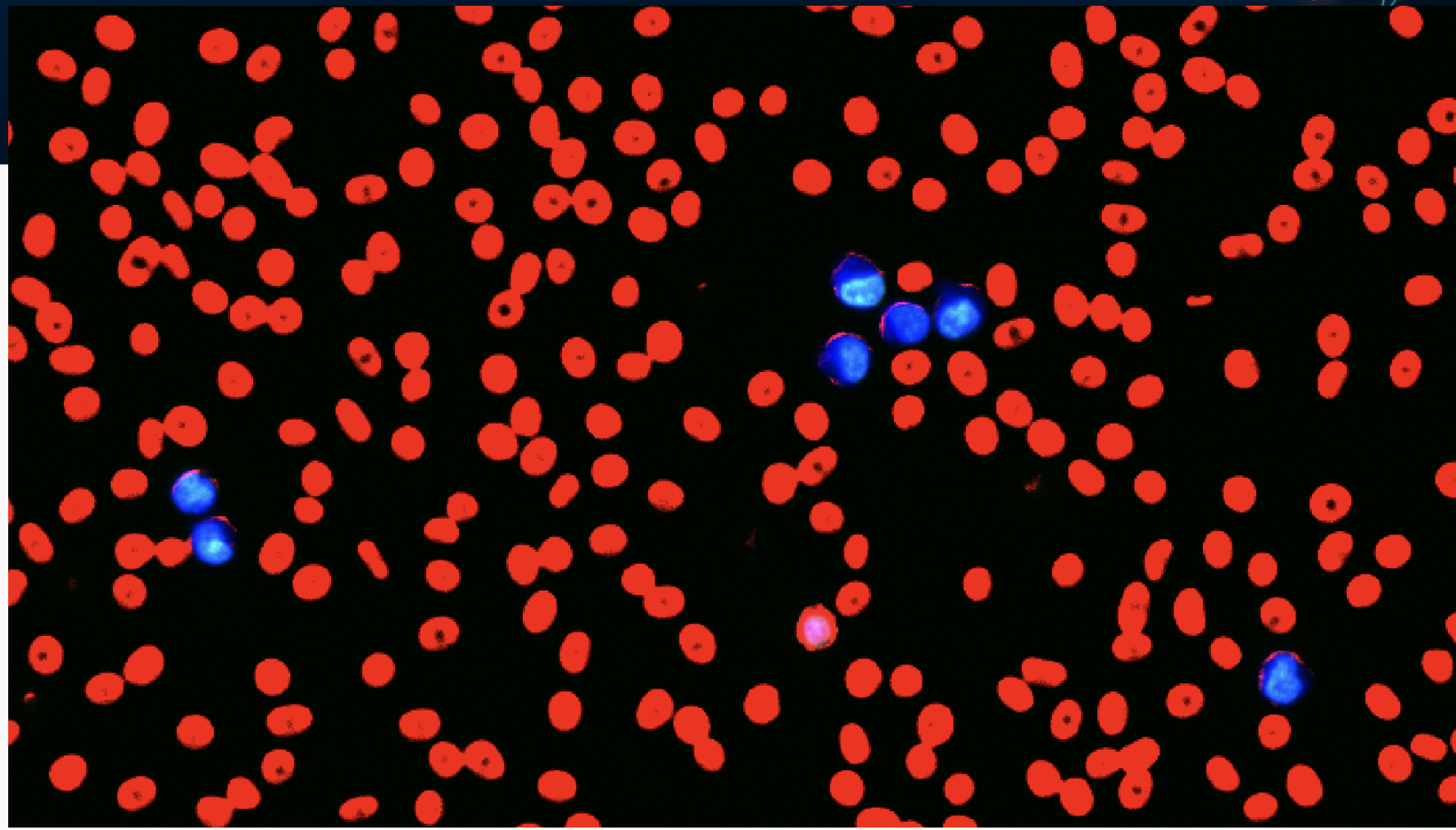


IDEXX inVue Dx results

No platelet clumps seen to explain thrombocytopenia

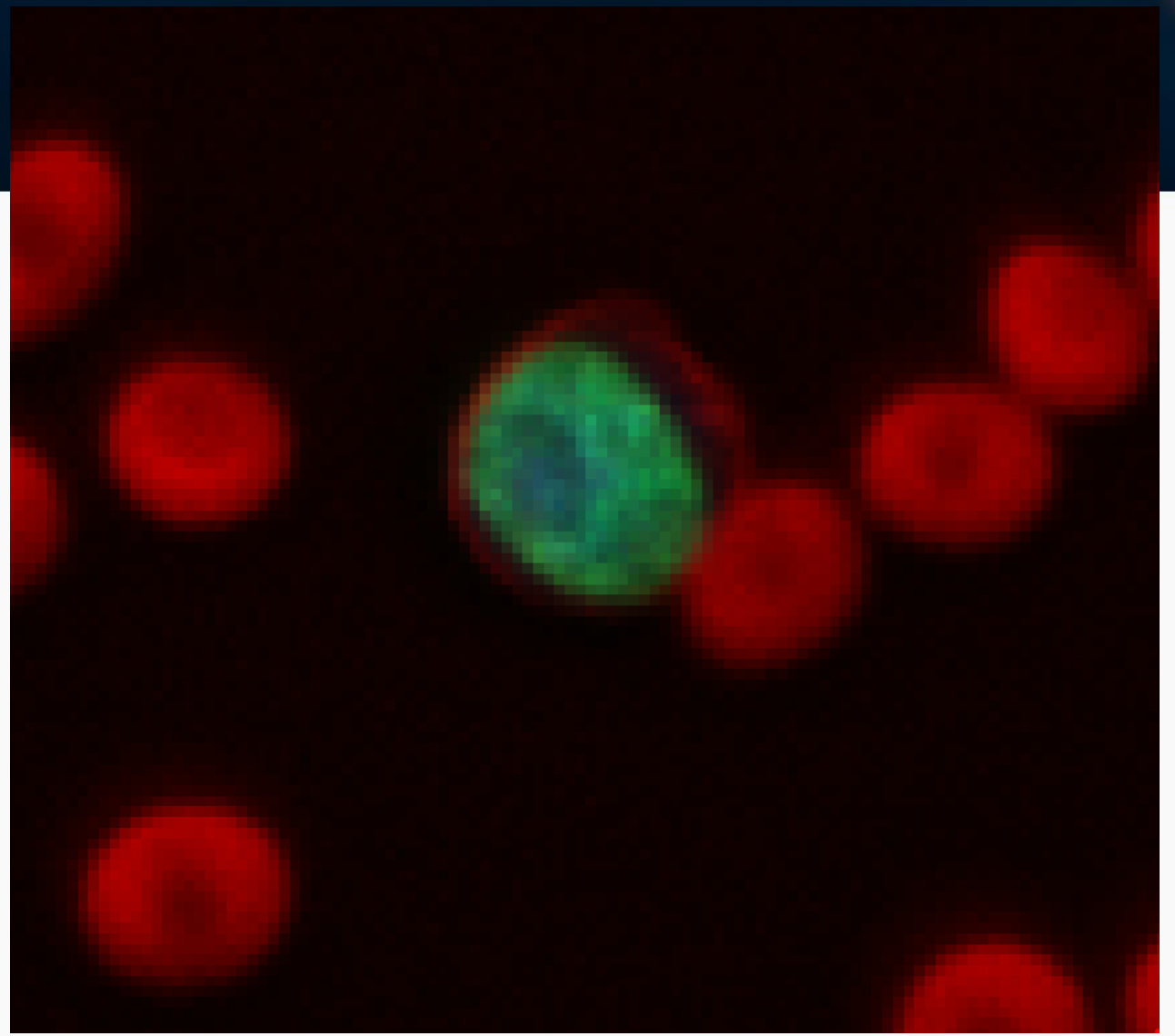


IDEXX inVue Dx results

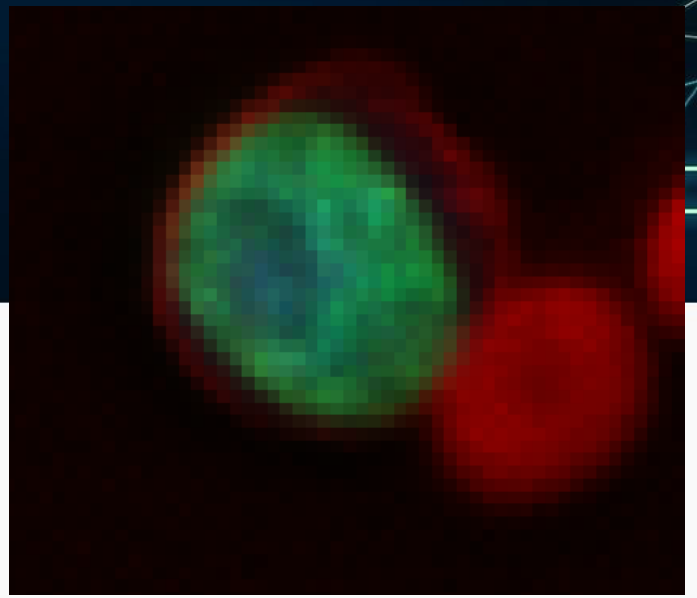


IDEXX inVue Dx results

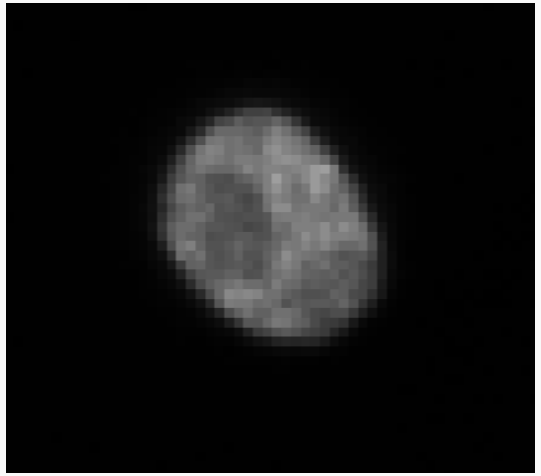
Composite: Large immature mononuclear cell

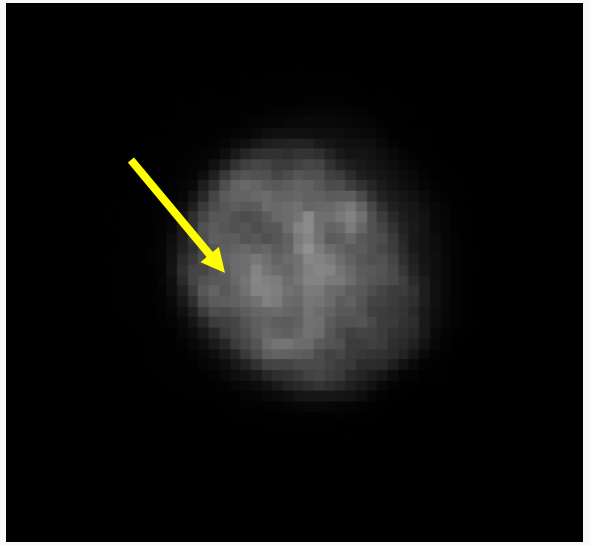


IDEXX inVue Dx
Different light channels



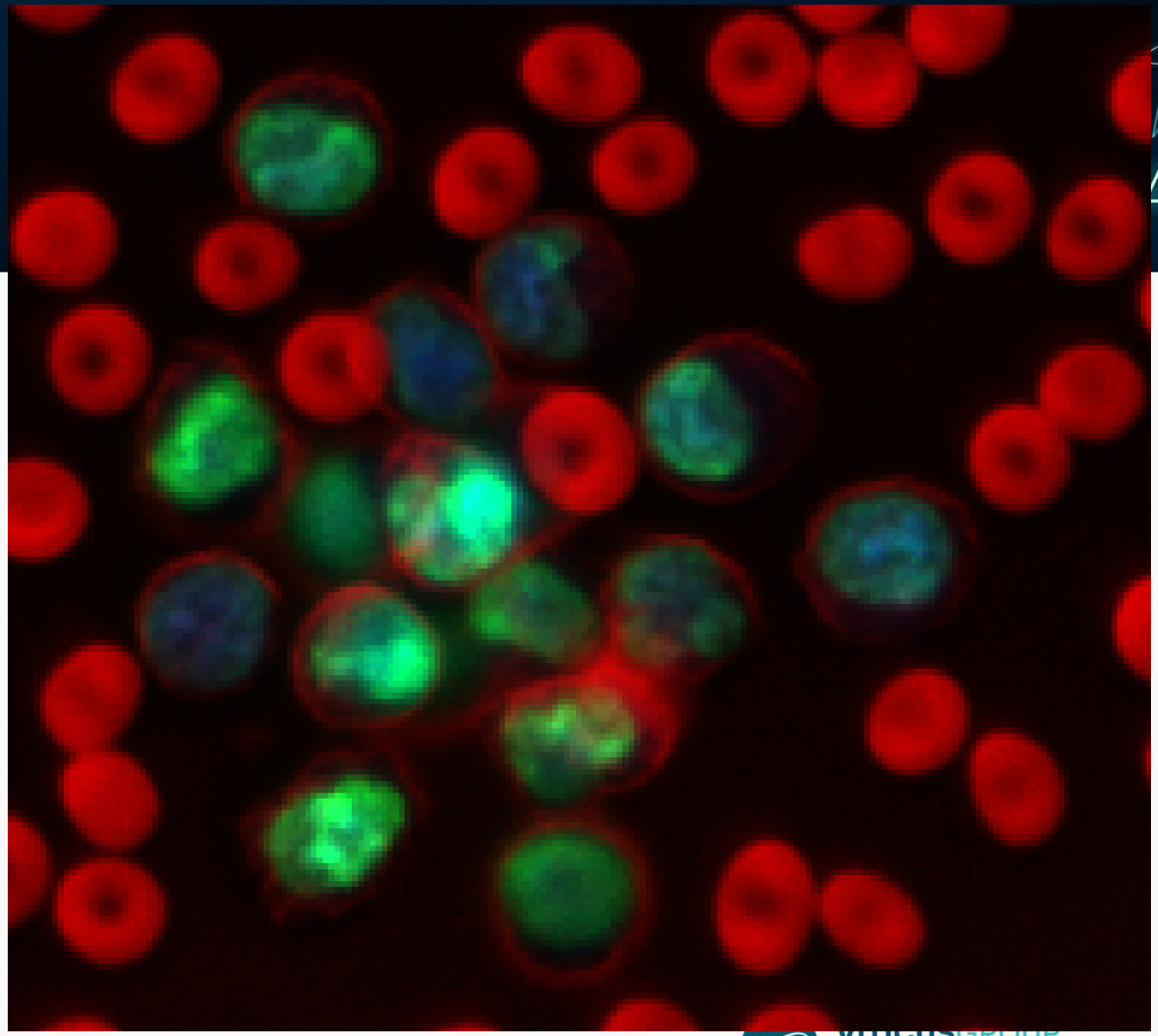
- Large immature mononuclear cell
- Bottom left: see nuclear chromatin features
- Bottom right: arrow points to nucleolus.





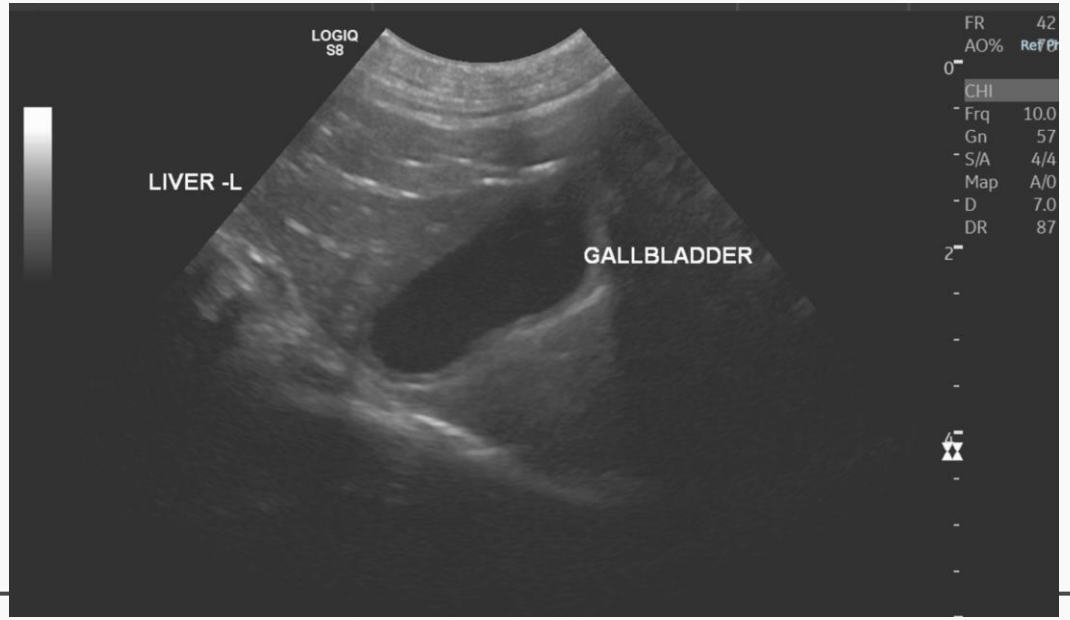
IDEXX inVue Dx results

Composite: Cluster of large immature mononuclear cells



Cassie

- Ultrasound exam findings:
  - Unremarkable
- Diagnosed with leukemia



What the medical benefits of doing blood smears or blood morphology assessments?

Platelets

- Evaluate platelet clumping
  - Assess whether reported low platelet count is real
  - Inform clinical decision making

Other

+ Microfilaria
+ Bacteria

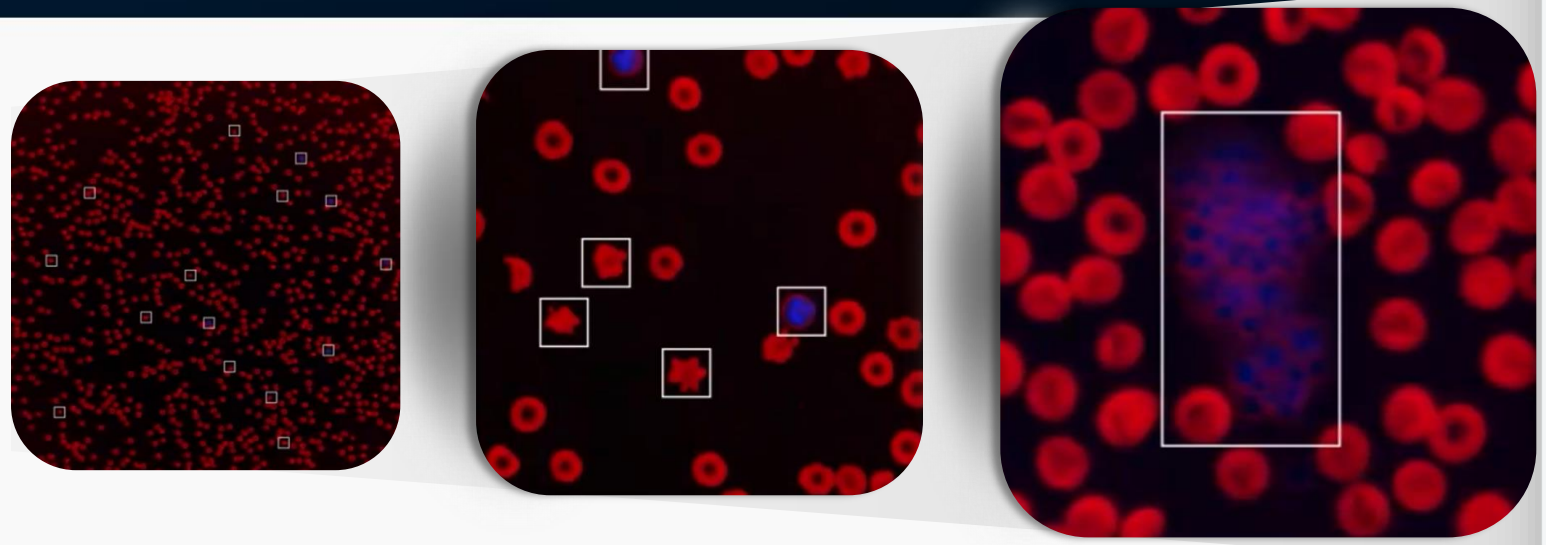
RBCs

- Abnormal red cell shape
  - Spherocytes
  - Ghost cells
  - Heinz bodies
  - Schistocytes
  - Etc.
- Hemoparasites
- Agglutination

WBCs

- Identify WBC subsets outside of the 4- or 5-part automated differential
  - Band neutrophils
  - Leukemic cells
- Verify automated WBC differential when there are instrument prompts
- Infectious organisms

inVue Dx Provides: automated quantification, classification, and interpretation of blood morphology



Delivering reference lab-quality results in a real-time environment

IDEXX VetConnect PLUS | Home | Directory of Services | Imaging | Telemedicine | James Herriot, All Creatures Animal Hospital | Sign out

ZOE CLARK 203AB Patient Management
Canine | Brussels Griffon | Female | 8y

Order New Diagnostics

2024 | Jan 27 | Jan 27

Result Details | Add to Order

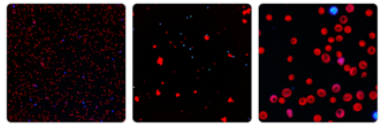
Hematology	1/27/24 9:43 AM			1/27/24 9:43 AM
RBC	a. 1.09	5.65 - 8.87 M/µL		1.09
Hematocrit	b. 9.8	37.3 - 61.7 %		9.8
Spherocytes	60% (Marked)			
Agglutination	Present			
% Reticulocyte	17.0	%		17.0
Reticulocytes	184.8	10.0 - 110.0 K/µL		184.8
WBC	c. 43.20	5.05 - 16.76 K/µL		43.20
% Neutrophils	69.5	%		*69.2
% Immature Neutrophils	18.5	%		
% Lymphocytes	1.9	%		*21.6
% Monocytes	9.7	%		*8.9
% Eosinophils	0.2	%		0.2
% Basophils	0.1	%		0.1
Neutrophils	30.02	2.95 - 11.64 K/µL		*29.89
Immature Neutrophils	7.99	K/µL		
Lymphocytes	0.84	1.05 - 5.10 K/µL		*9.34
Monocytes	4.20	0.16 - 1.12 K/µL		*3.85
Eosinophils	0.09	0.06 - 1.23 K/µL		0.09
Basophils	0.03	0.00 - 0.10 K/µL		0.03
Platelet Estimate	50-100 K/µL (Moderately decreased)			

Diagnostic Considerations

The presence of regenerative anemia, spherocytosis, and RBC agglutination are strongly suggestive of immune-mediated hemolytic anemia. Other clinical features include icterus, hyperbilirubinemia/bilirubinuria (in the absence of liver dysfunction), or hemoglobinemia/uria. Investigate for underlying causes such as infection, neoplasia, concurrent inflammatory conditions, or history of recent drugs/vaccines.

This platelet estimate incorporates enumeration of individual platelets and platelets within clumps. Moderately decreased platelets may be seen with platelet consumption, immune-mediated destruction, decreased production from the bone marrow, and sequestration in the spleen. If this finding is unexpected, please redraw a new sample to rule out artifactual thrombocytopenia (e.g., clot in the blood tube).

Images



a. RBC results imported from ProCyte.
b. HCT results imported from ProCyte.
c. WBC results imported from ProCyte. The white blood cell differential has been updated based on cytologic evaluation.

Thank You