Blood Smear Evaluation: The good, the bad, the ugly

Marta Costa

DVM, MSc, FRCPath, DipECVCP

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Disclosure:
Full-time Employee of IDEXX

Blood smear evaluation:
so much ground to cover…

1. Review the “*good*” (what is normal)
2. Recognise the “*bad*” (what is abnormal)
3. Acknowledge the “*ugly*” (what should ideally be reviewed by a referral laboratory or needs auxiliary testing)

In this session:

Smear quality	ID a good quality smear
Areas to examine	ID best areas to evaluate in the smear
Normal patient	Recognize what is expected from a healthy patient and the importance of flags
Common changes	Evaluate and interpret common pathological changes that have clinical impact on patient care and can be readily identified on blood smear analysis
Planning referral	Know how to select changes that are best appraised by a referral laboratory and specialist and/or need additional auxiliary testing

ID Good Quality Smears

Good quality smears

- Fresh is best…
- Feathered edge is a must…
- At the end of the rainbow….

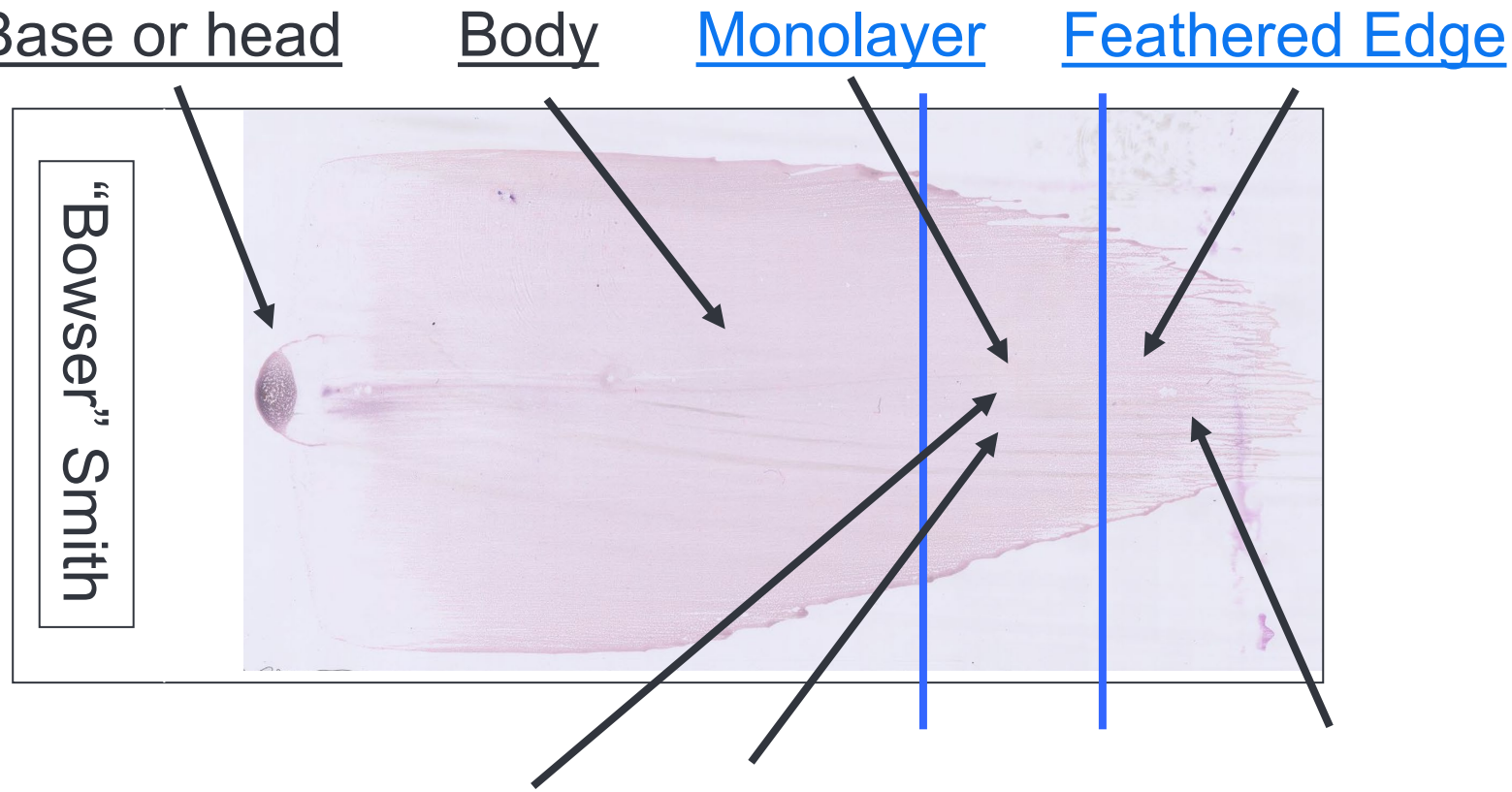
- Kept safe
  - Away from formalin
  - Away from moisture
  - At room temperature

- Stain clean and fresh

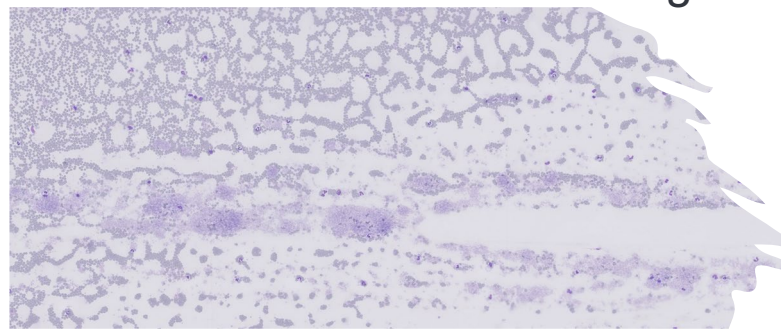
ID areas of interest

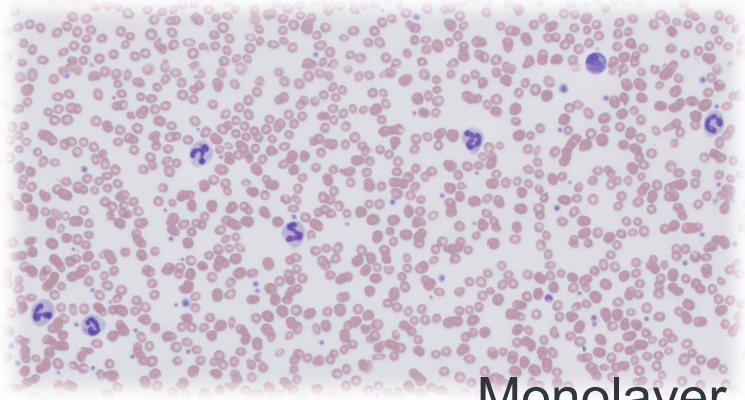
+ Feathered edge
+ Body of the smear
+ Monolayer

Anatomy of the Peripheral Blood Film



Feathered Edge





Monolayer

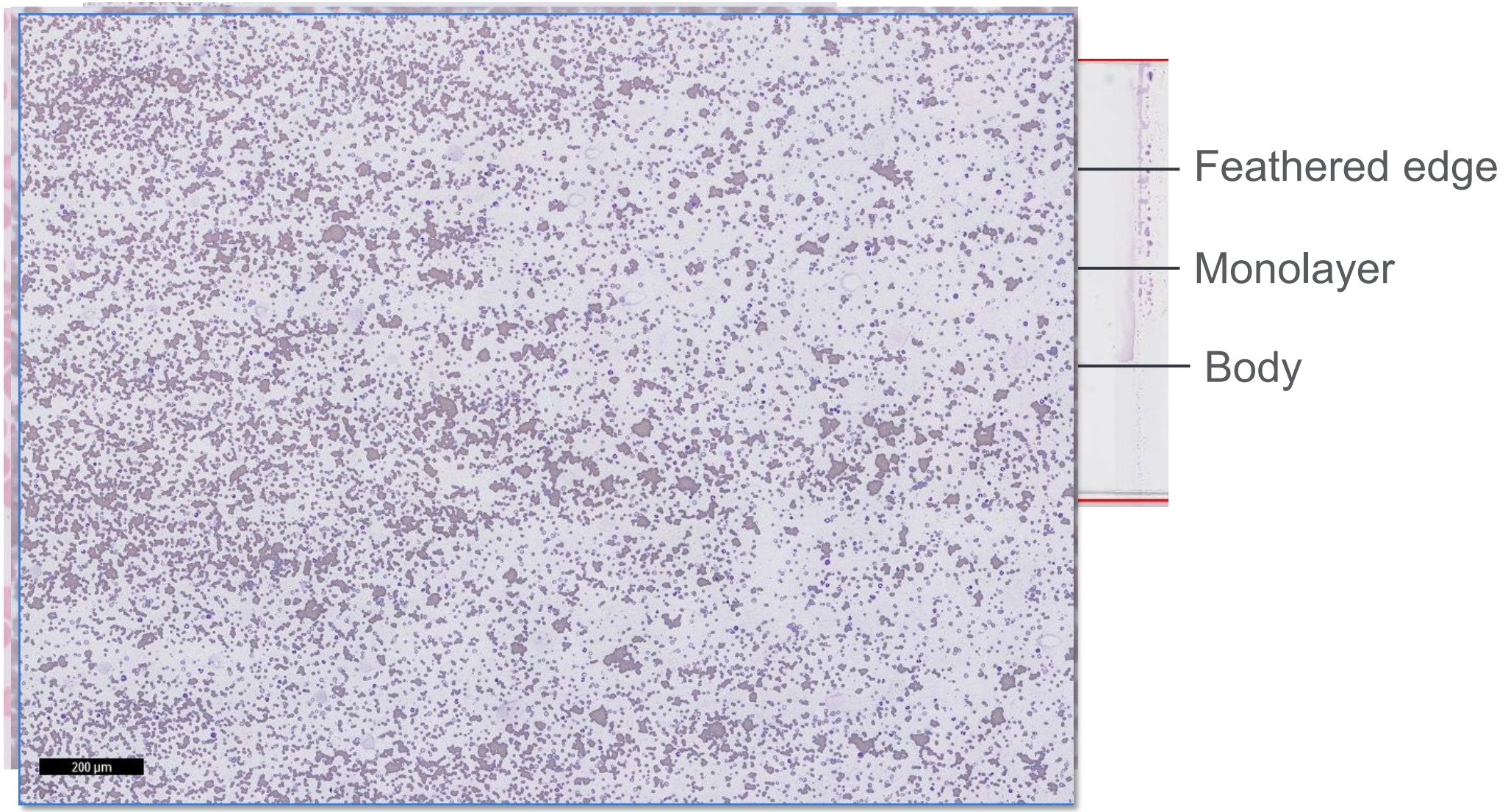
- Rouleaux
- Agglutination

- Estimate numbers
- Morphology

- Platelet clumps
- Large cells
- Microfilaria

IDEXX

Film examination: structure of a blood smear



Systematic stepwise approach

1. Start small – with low magnification and at the feathered edge of the smear

2. Go deeper - Go two to three fields back to the body of the smear into the monolayer

3. Go bigger – Increase to oil and evaluate morphology

4. Finish to the side – go to lateral edge and count 100 leucocytes into types, looking for abnormal forms as you go

Blood smear evaluation steps - Systematic stepwise approach

Cell distribution

For all cell types:

Agglutination/ aggregation

Platelets

Estimate plt numbers

Examine morphology

Red Blood cells

Evaluate:

+ size,
+ shape,
+ color,
+ inclusions.

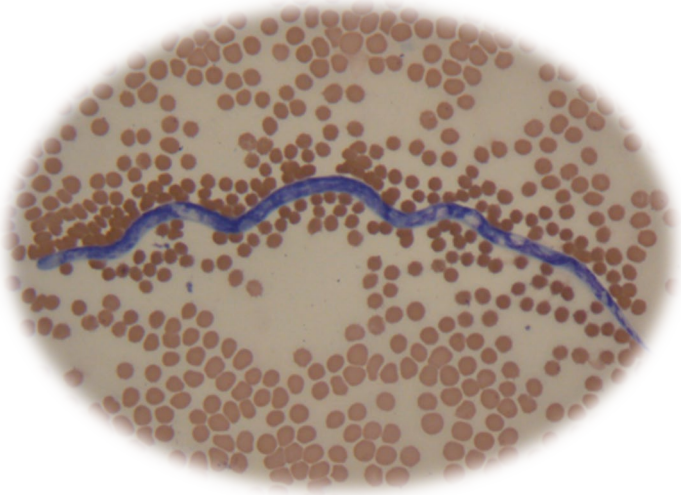
White blood cells

Do or verify differential cell count

Evaluate morphology changes and inclusions

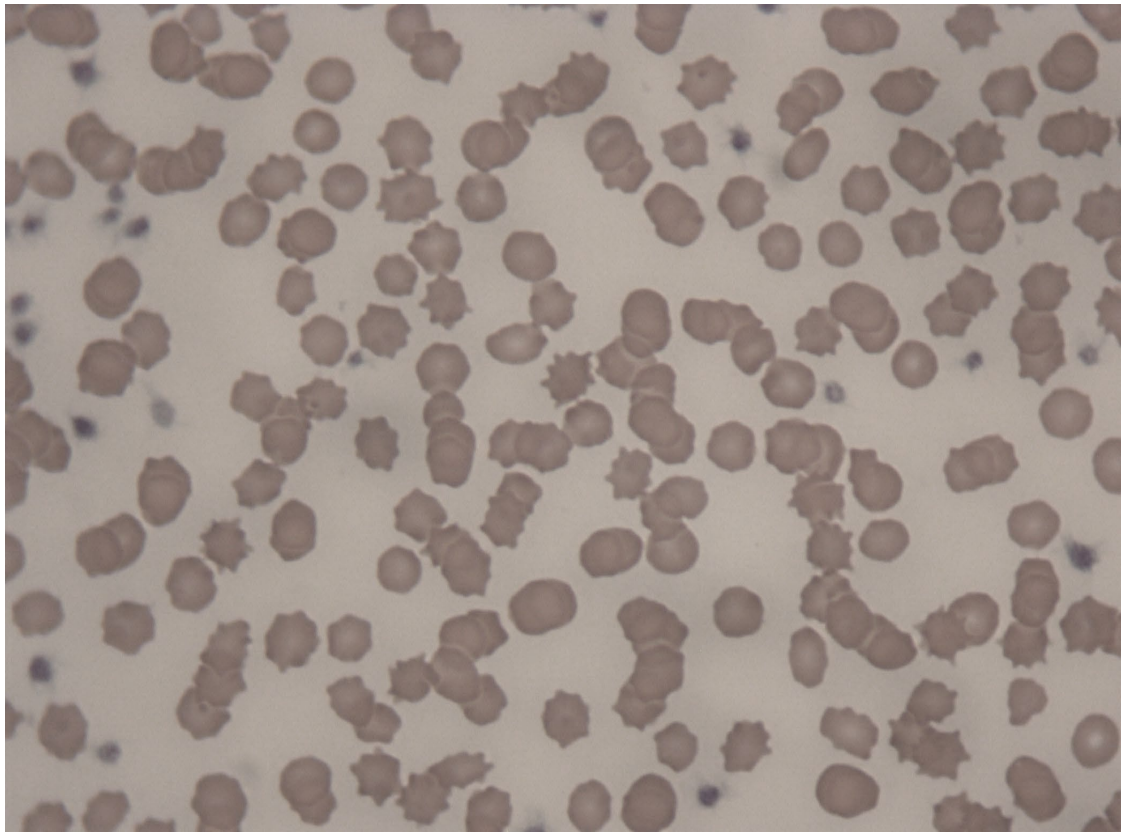
At low magnification:
In the feathered edge

- Cell distribution
- Platelet clumps
- Parasites/organisms
- “Big Blue Blobs”
- Neoplastic cells

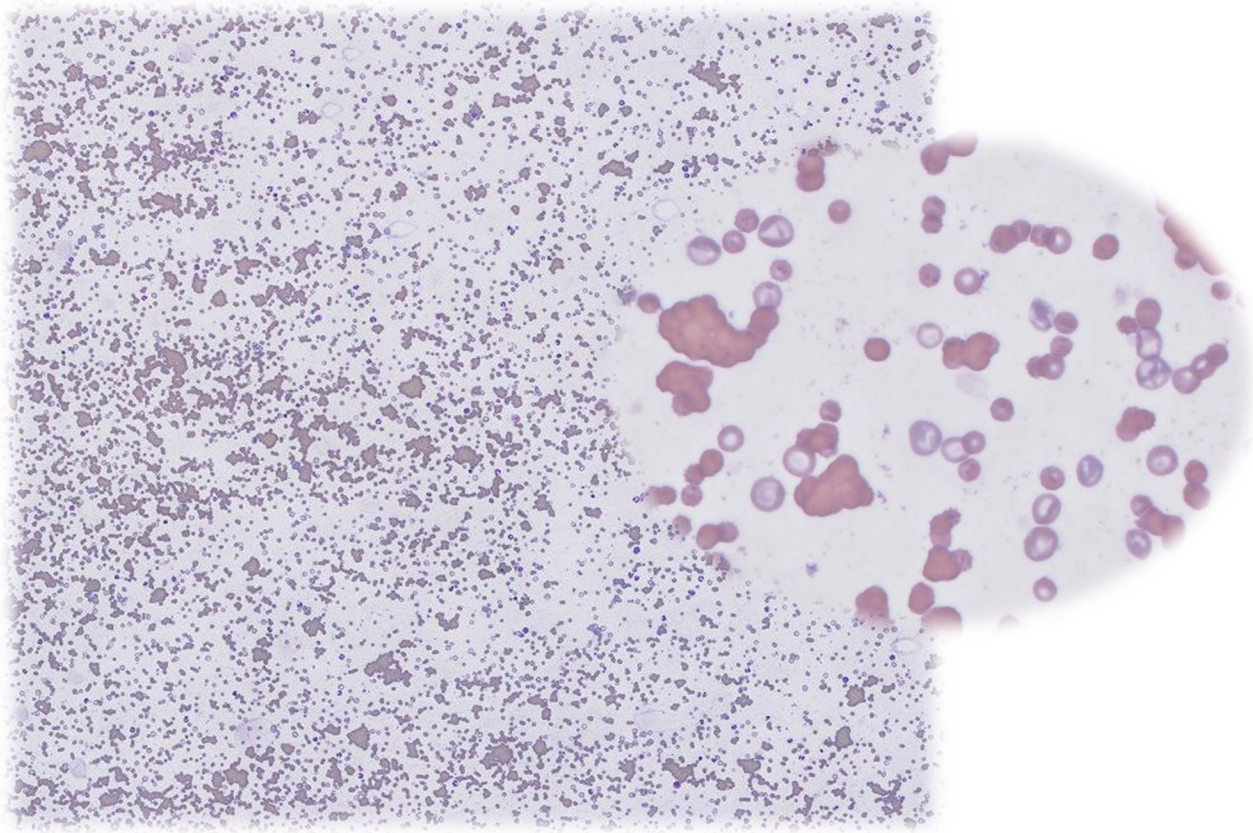


Cell distribution

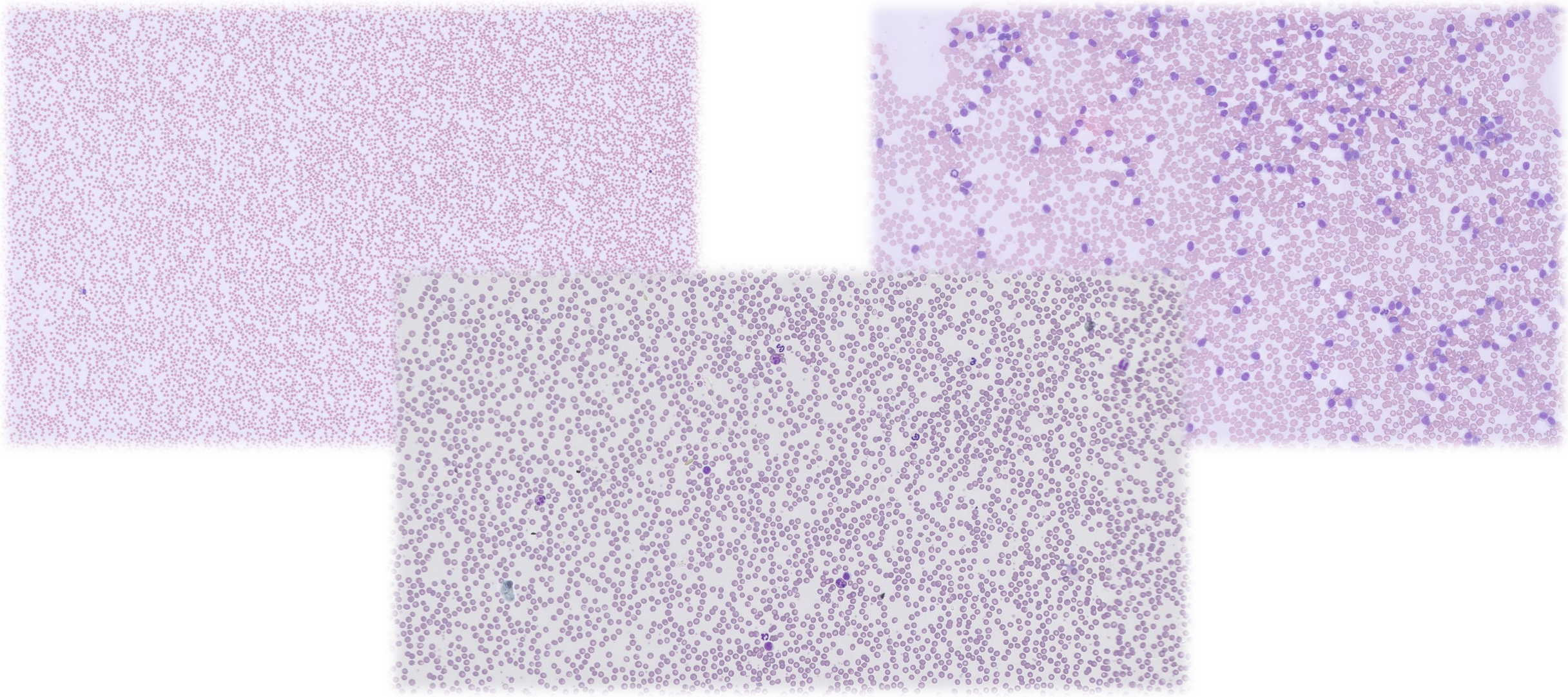
Rouleaux



Agglutination



Cell estimates



Platelet estimates

- ✓ Assuming NO PLT CLUMPS in feathered edge and NO CLOTS!

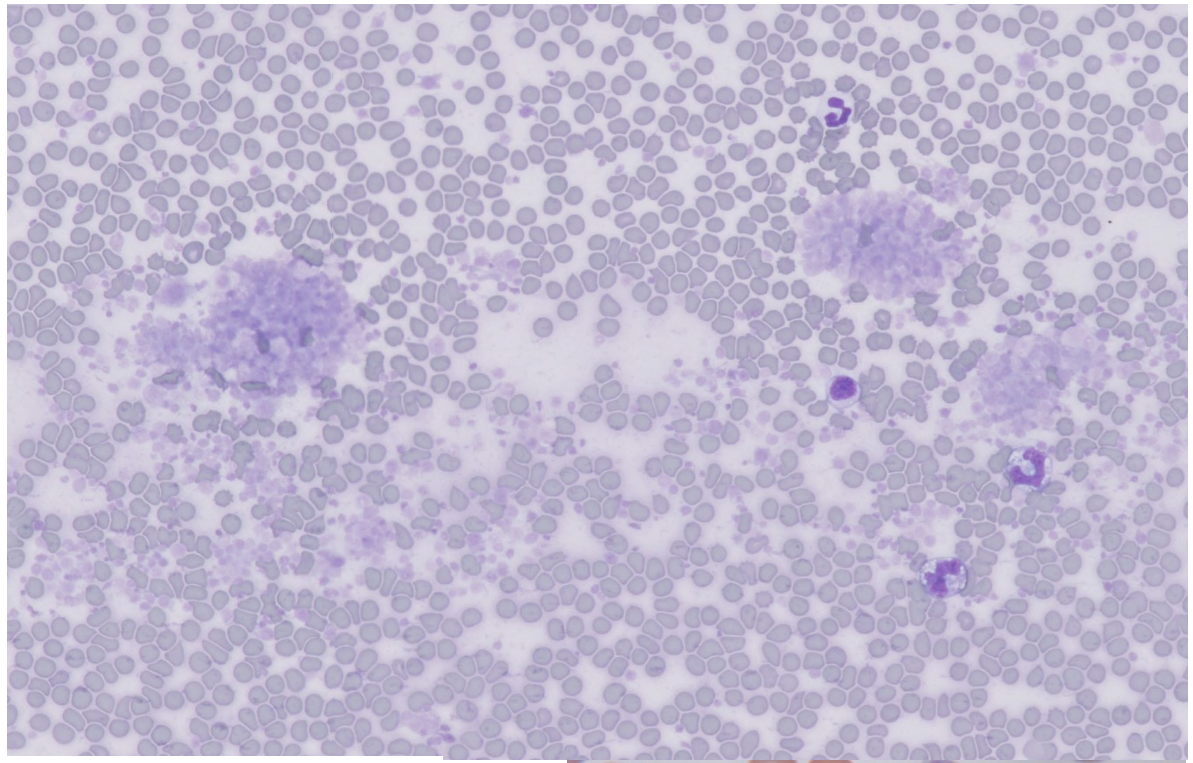
1. Count number of platelets in 10 fields oil immersion in monolayer

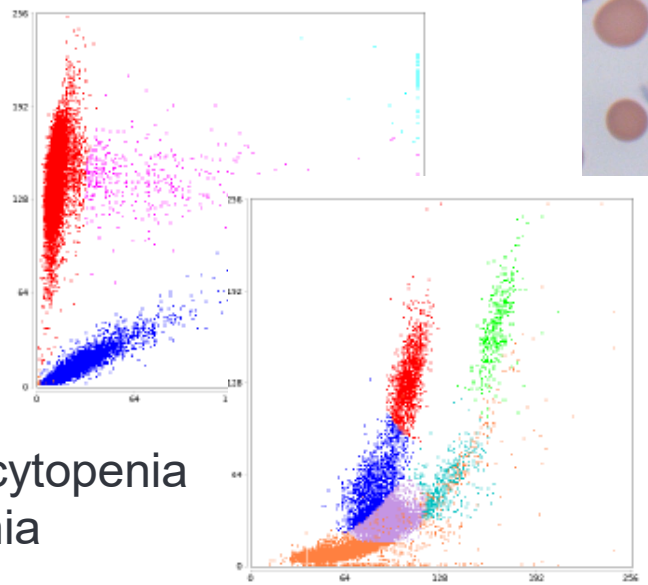
2. Do the average (Σ /10)

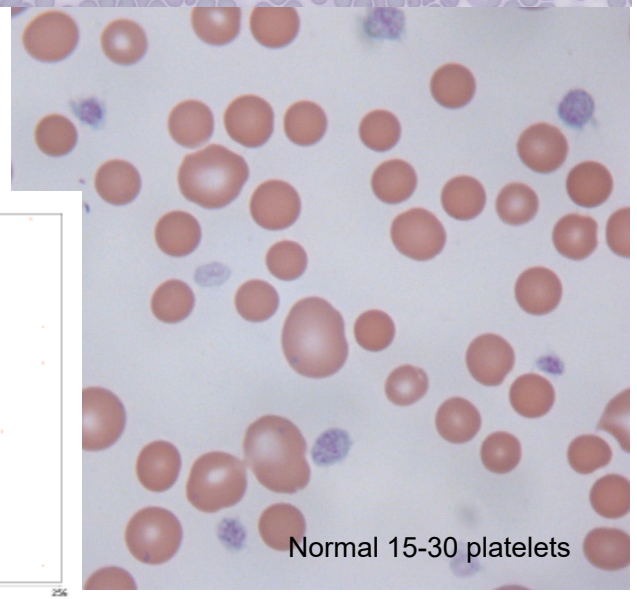
3. Multiply by 15 or 20

4. This is the ***estimated*** number

   (x 10^9/L)





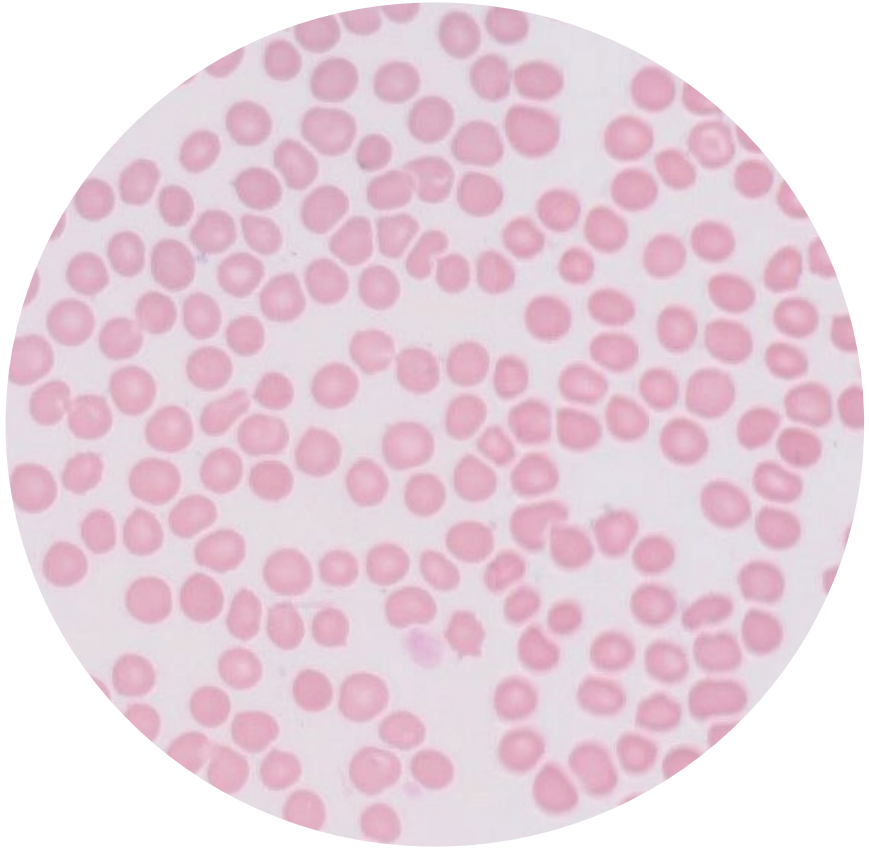


Normal 15-30 platelets

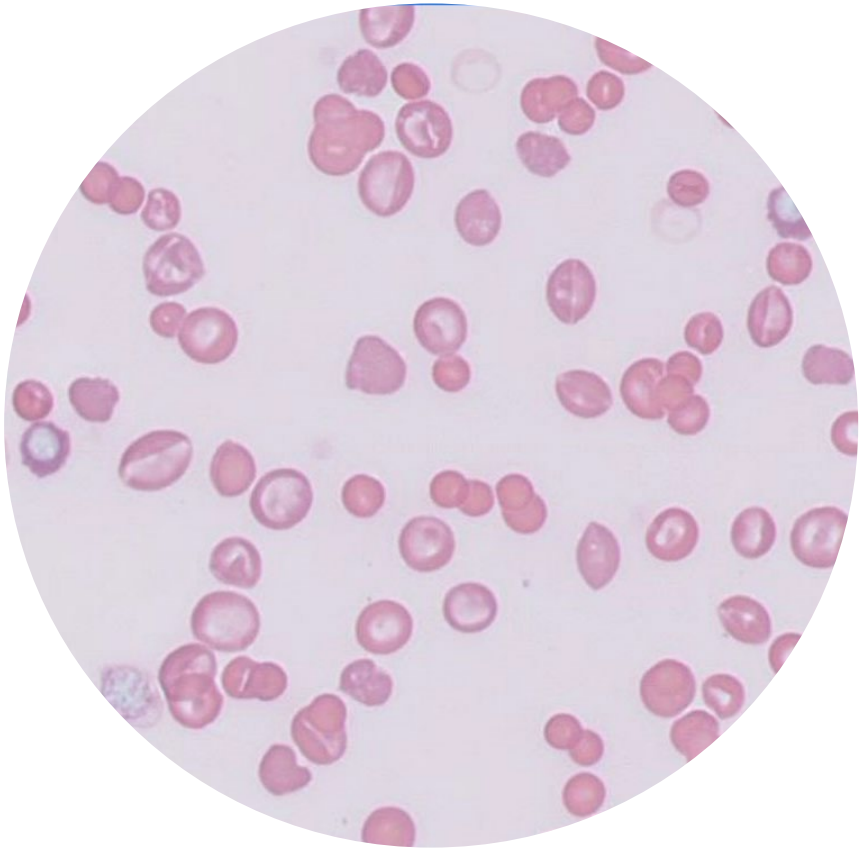
>10 per HPF = no significant thrombocytopenia
<2 per HPF = marked thrombocytopenia

IDEXX

Film examination: Finding the monolayer



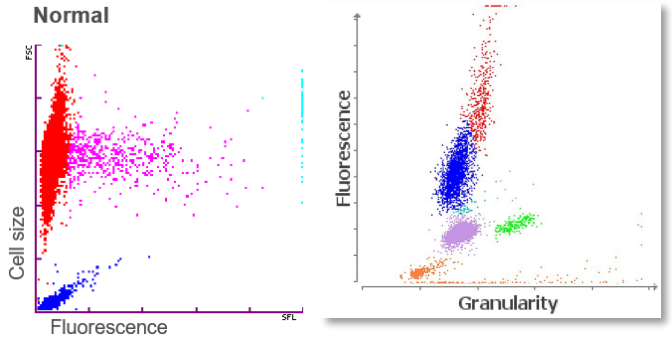
PCV 40%

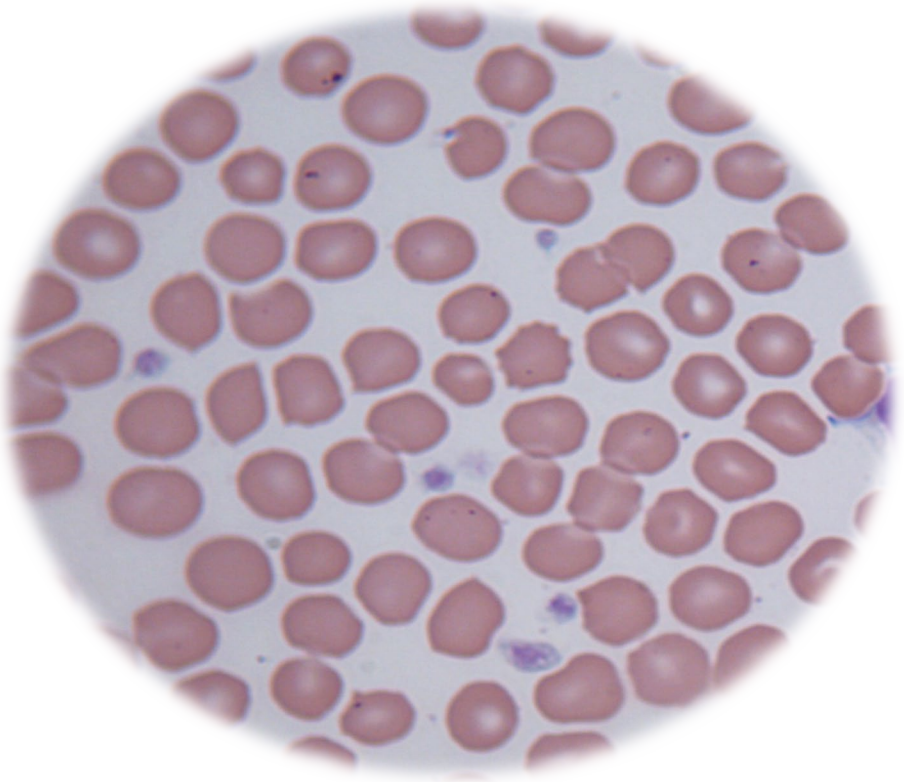


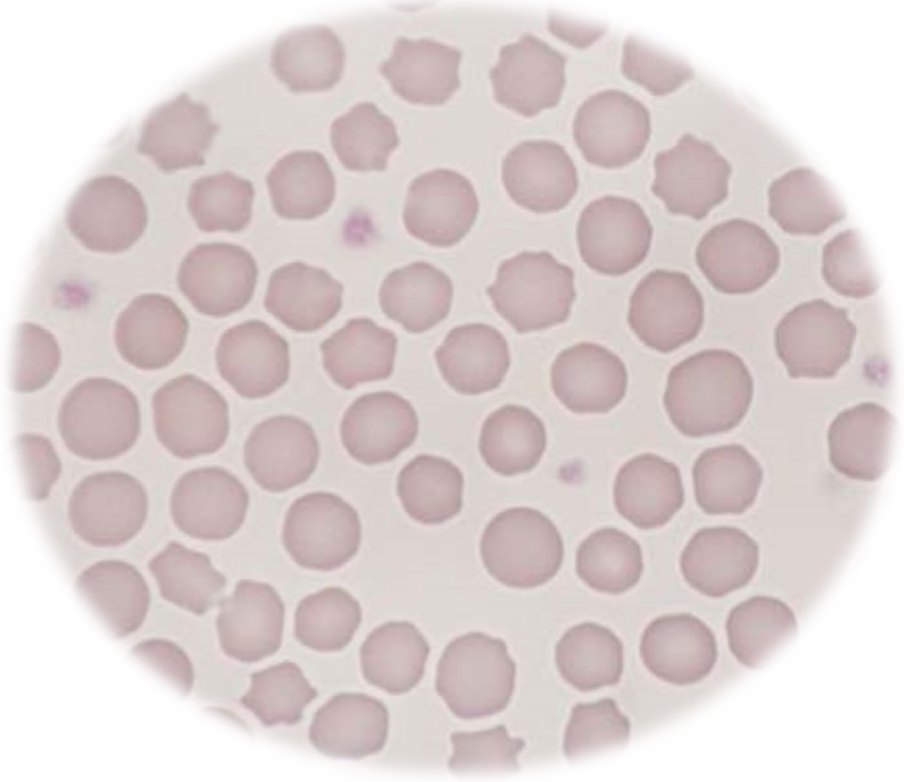
PCV 16%


RECOGNIZE NORMAL

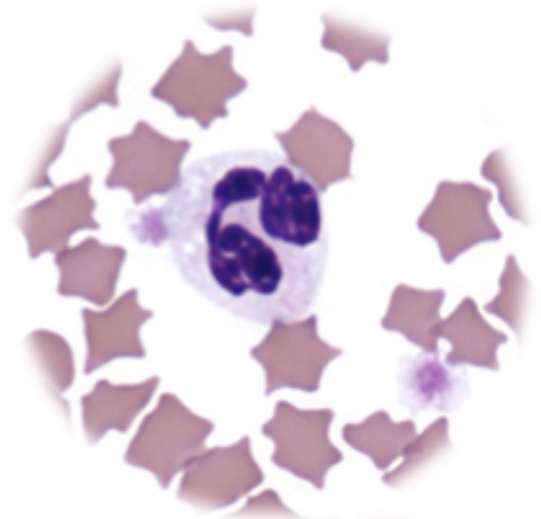
What is normal?

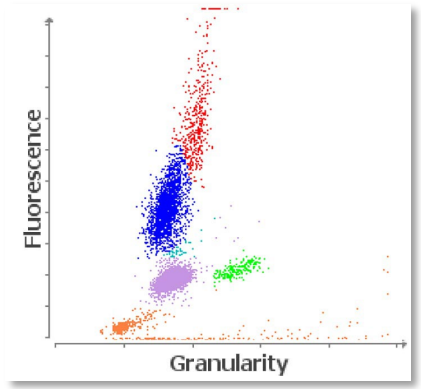


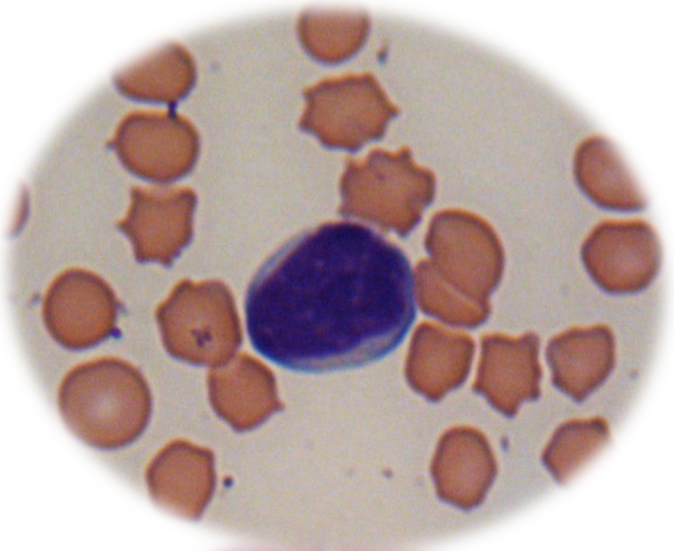


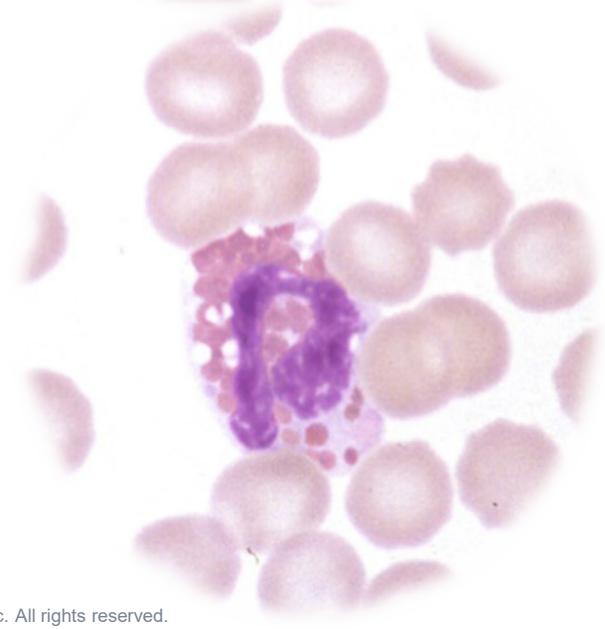


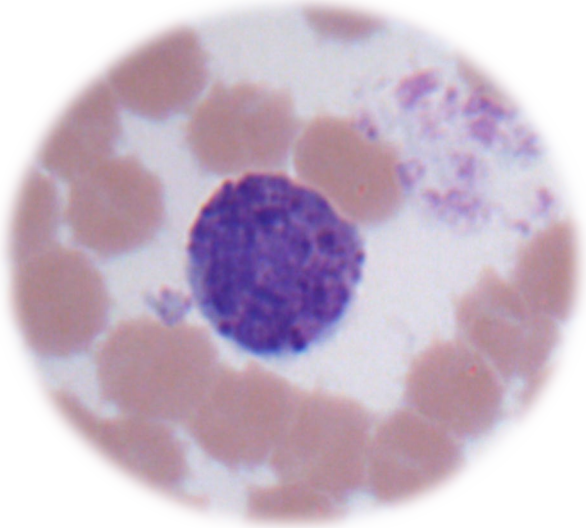
What is normal?

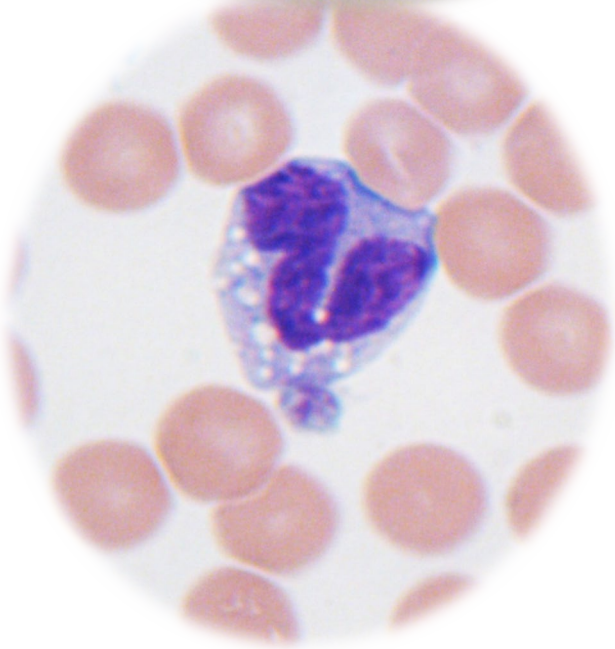












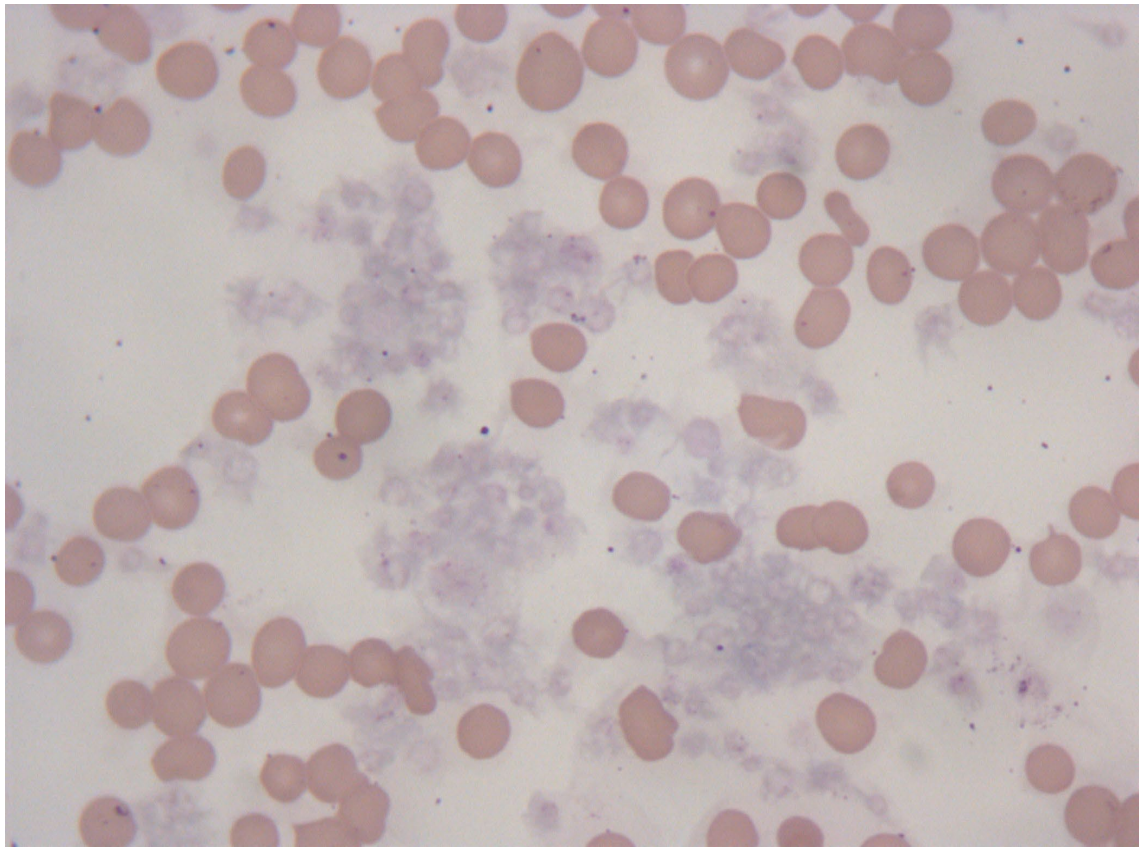
Common changes

Common sample features that interfere with automated CBCs

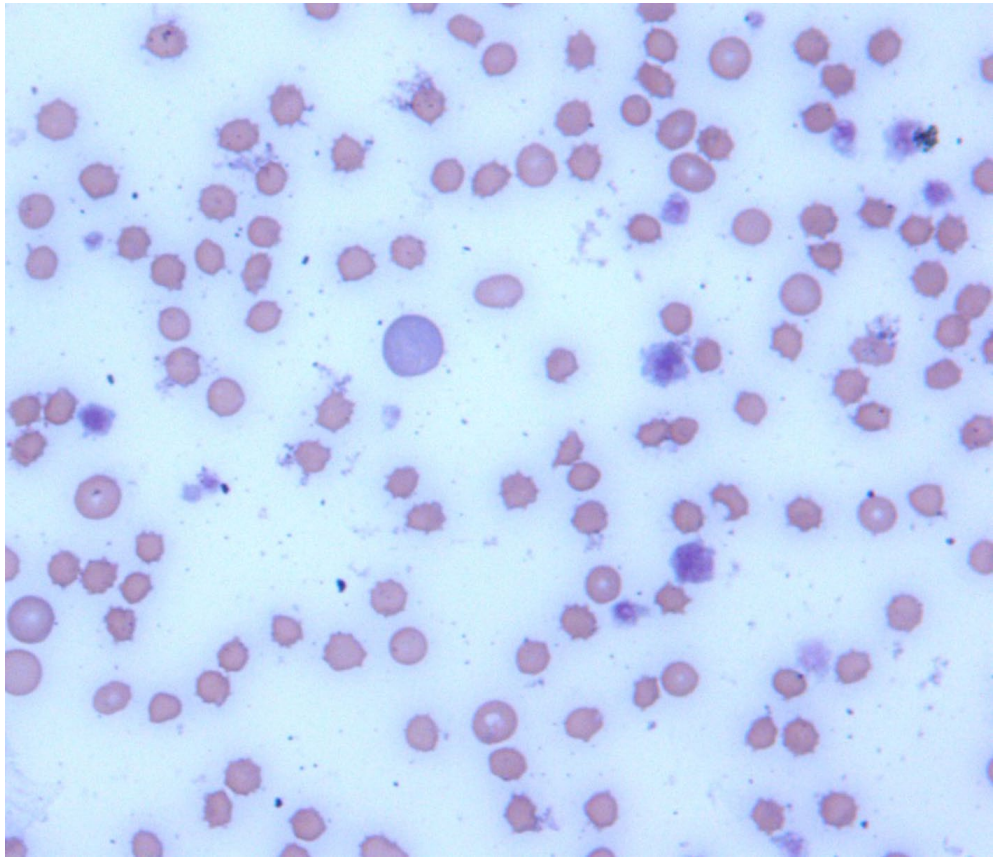
+ Clots
+ Platelet clumps
+ Macroplatelets
+ RBC agglutination
+ nRBC
+ Heinz bodies
+ Lipemia
+ Leukocyte agglutination
+ Delay in sample handling (increase in VCM, Haemolysis, etc)

For Platelets

Platelet clumps

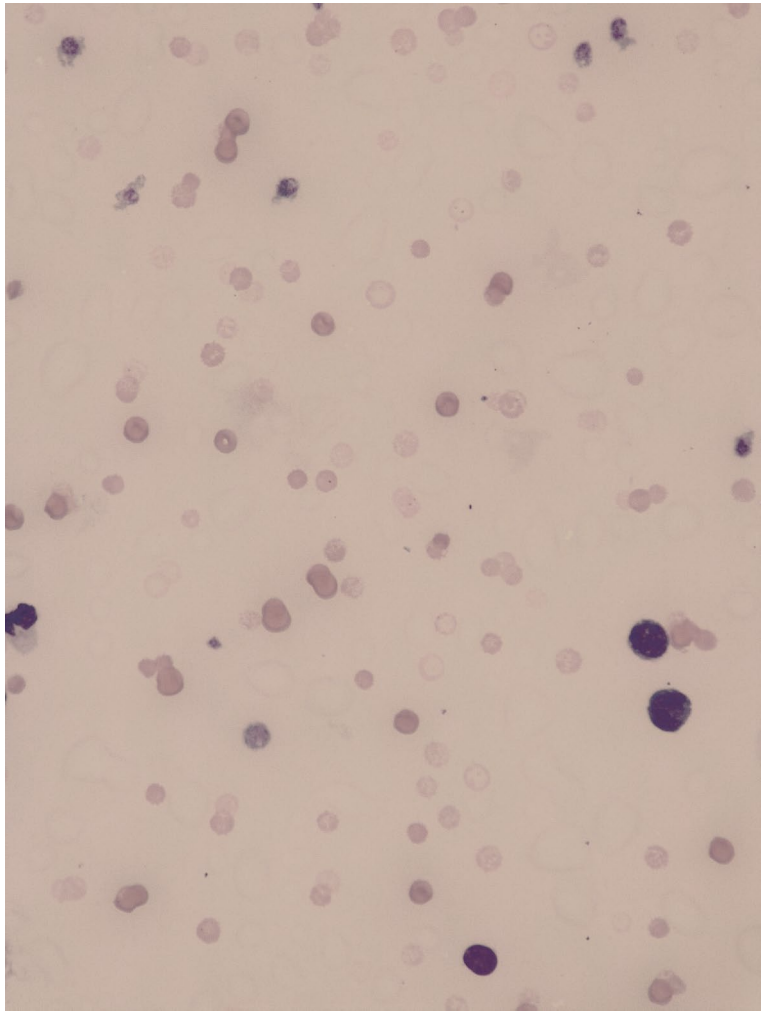


Macroplatelets



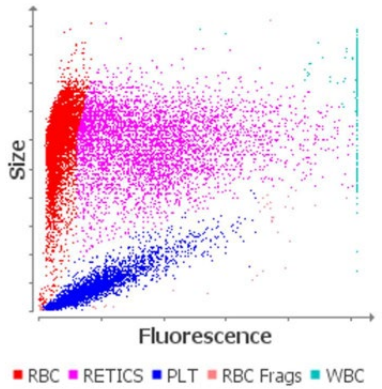
For the RBC we are evaluating 3 main things…

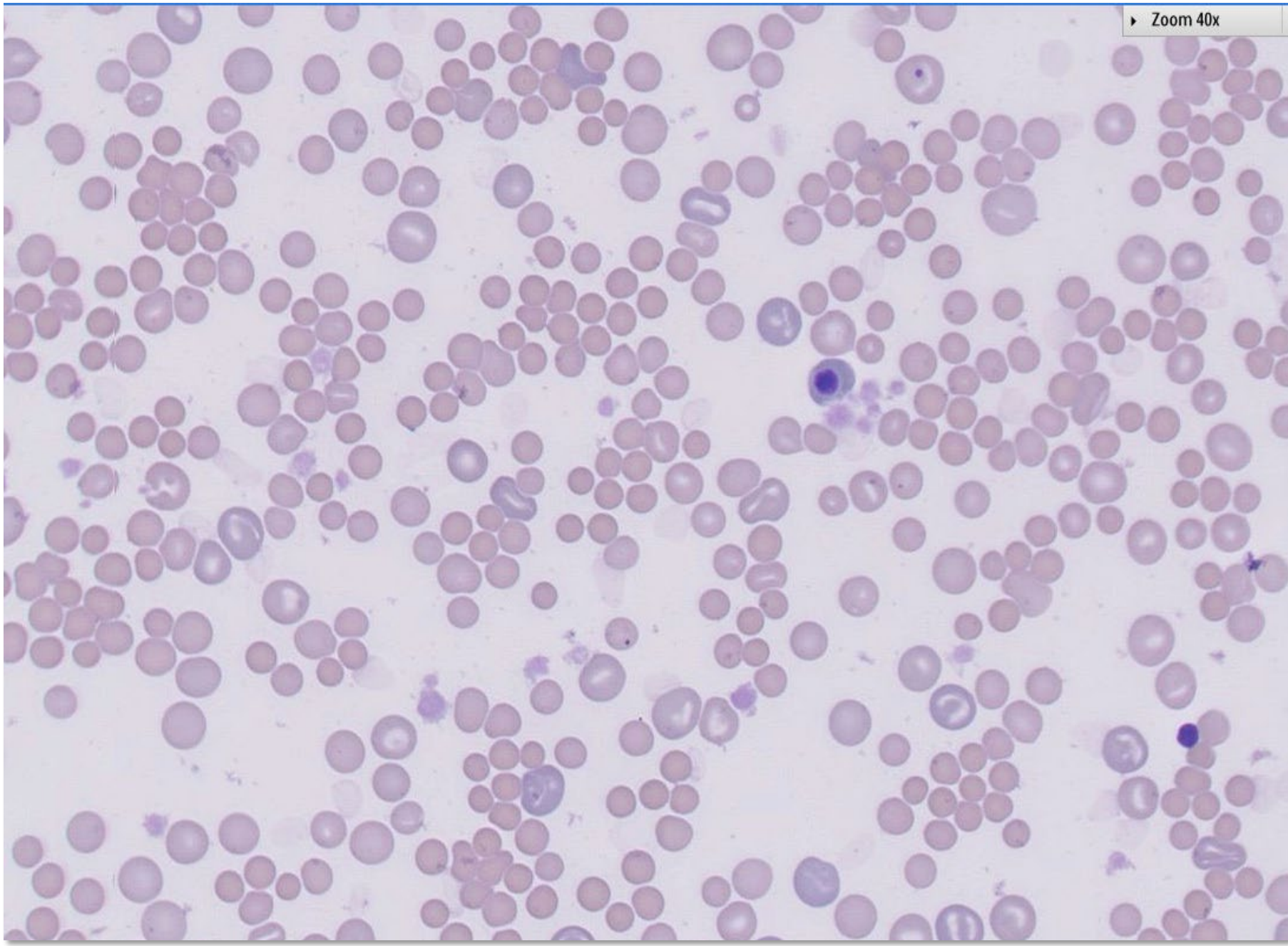
- Red blood cell density and spread – does it look anaemic? Is it agglutinating or rouleaux?
- Red blood cell regeneration – is the marrow trying to regenerate? Are there polychromatophils?
- Red blood cell morphology – are there any clues to the cause of an anaemia? (E.g., spherocytes, organisms, etc) Are there any changes that can lead to the cause of disease?



Polychromasia

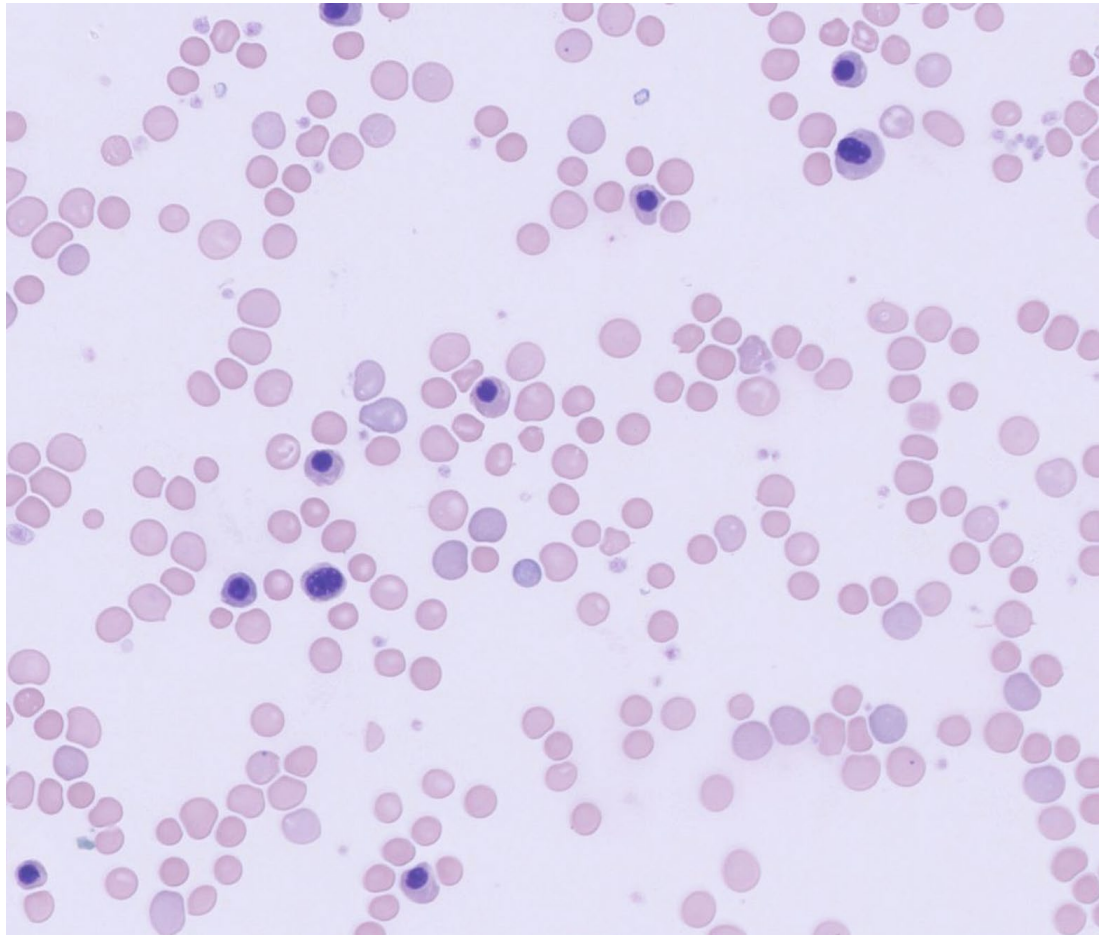
Polychromasia is indicative of regeneration
Polychromatophils are “younger RBC”
All polychromatophils are reticulocytes
More noticeable in some species e.g. dog



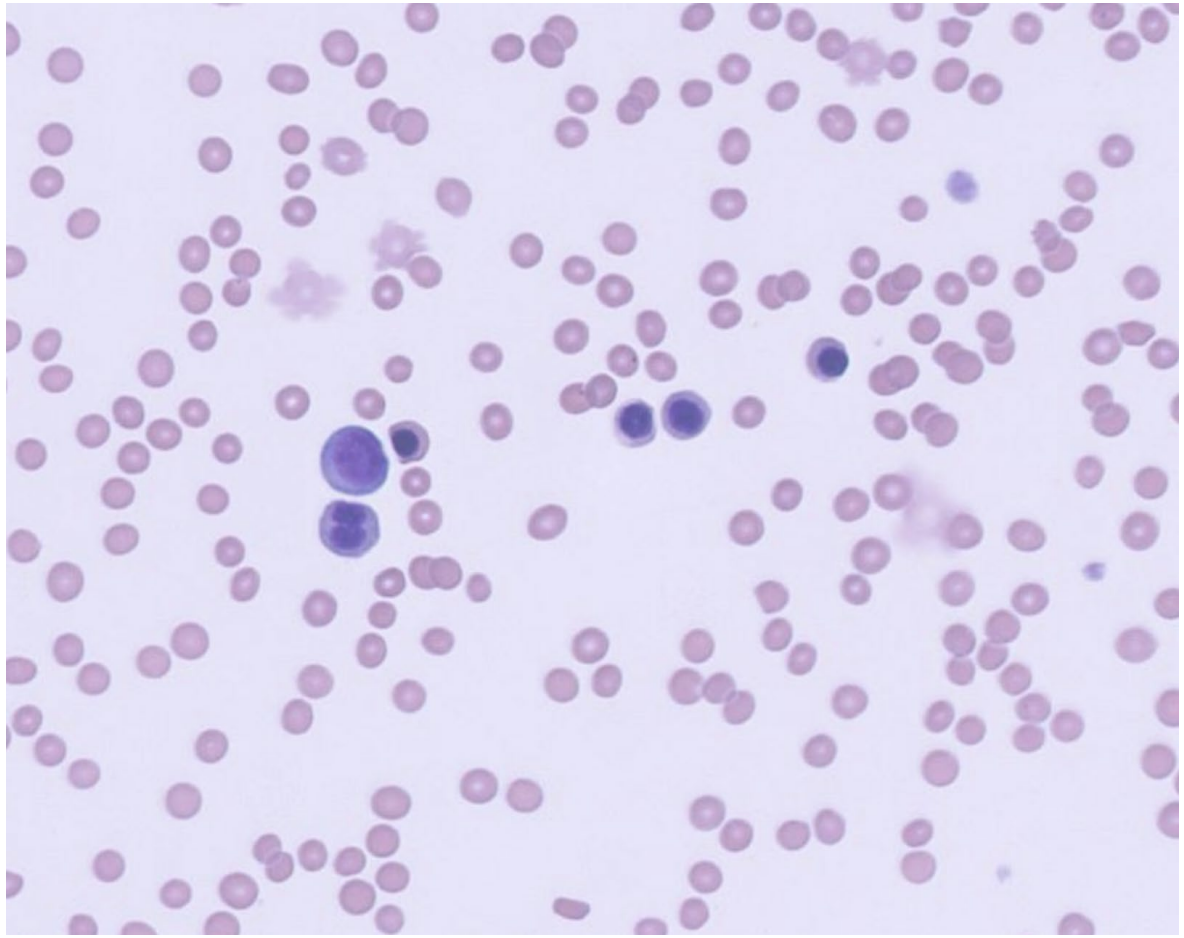


Nucleated RBC

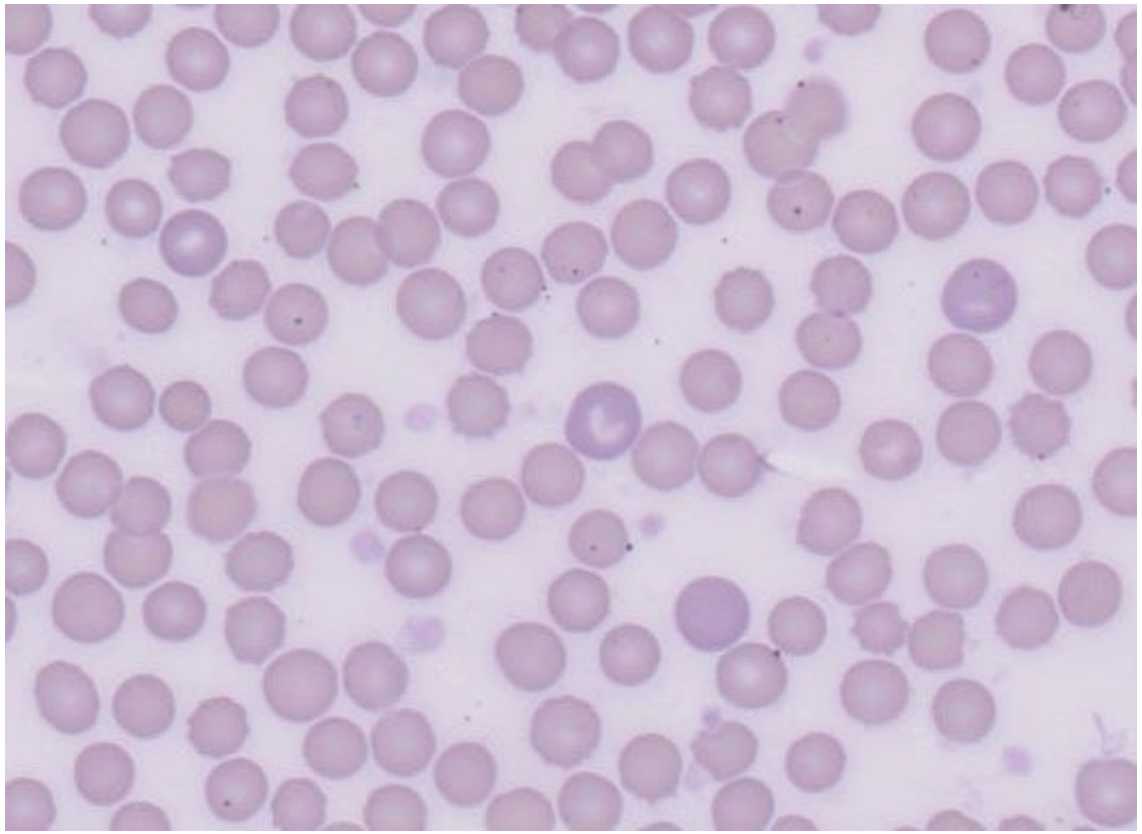
As part of regeneration



Inappropriate rubricytosis

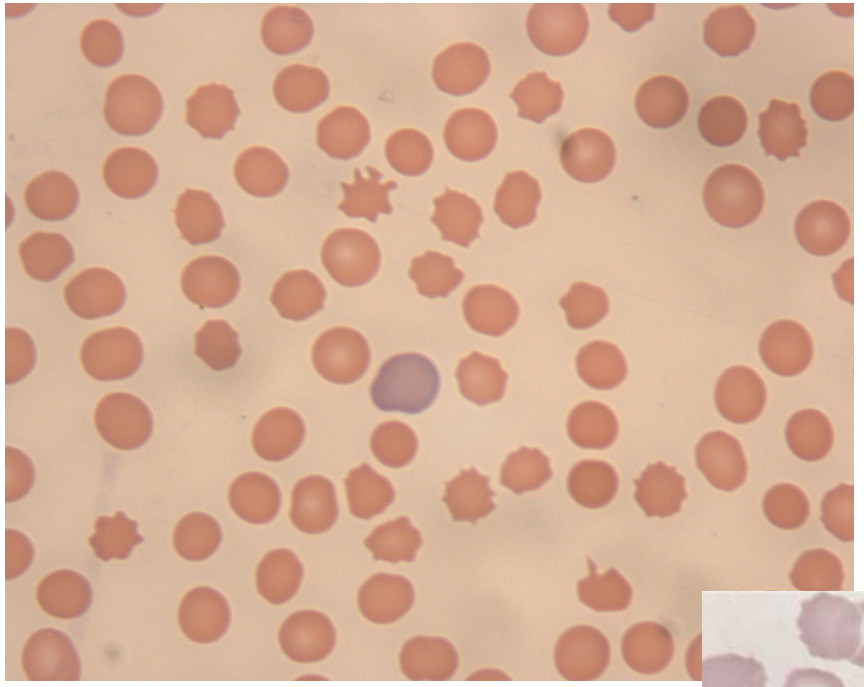


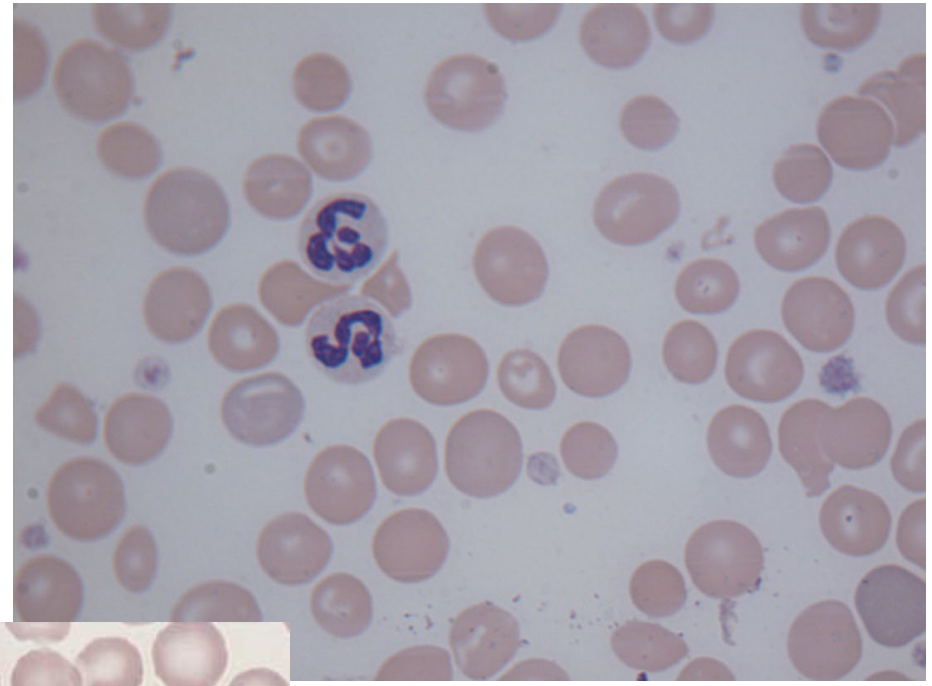
Regeneration without anaemia?

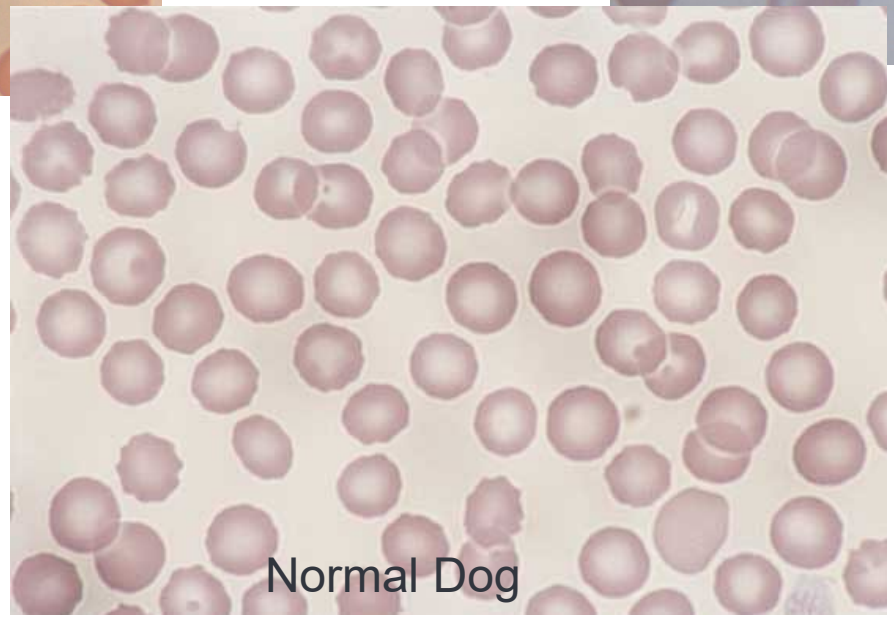


- Compensation
- Splenic contraction
- Chronic hypoxia
- Anaemia obscured by dehydration
- Breed appropriate Hct?

Are there abnormal shapes ???







Normal Dog

RBCs – Morphological changes

Most common/specific

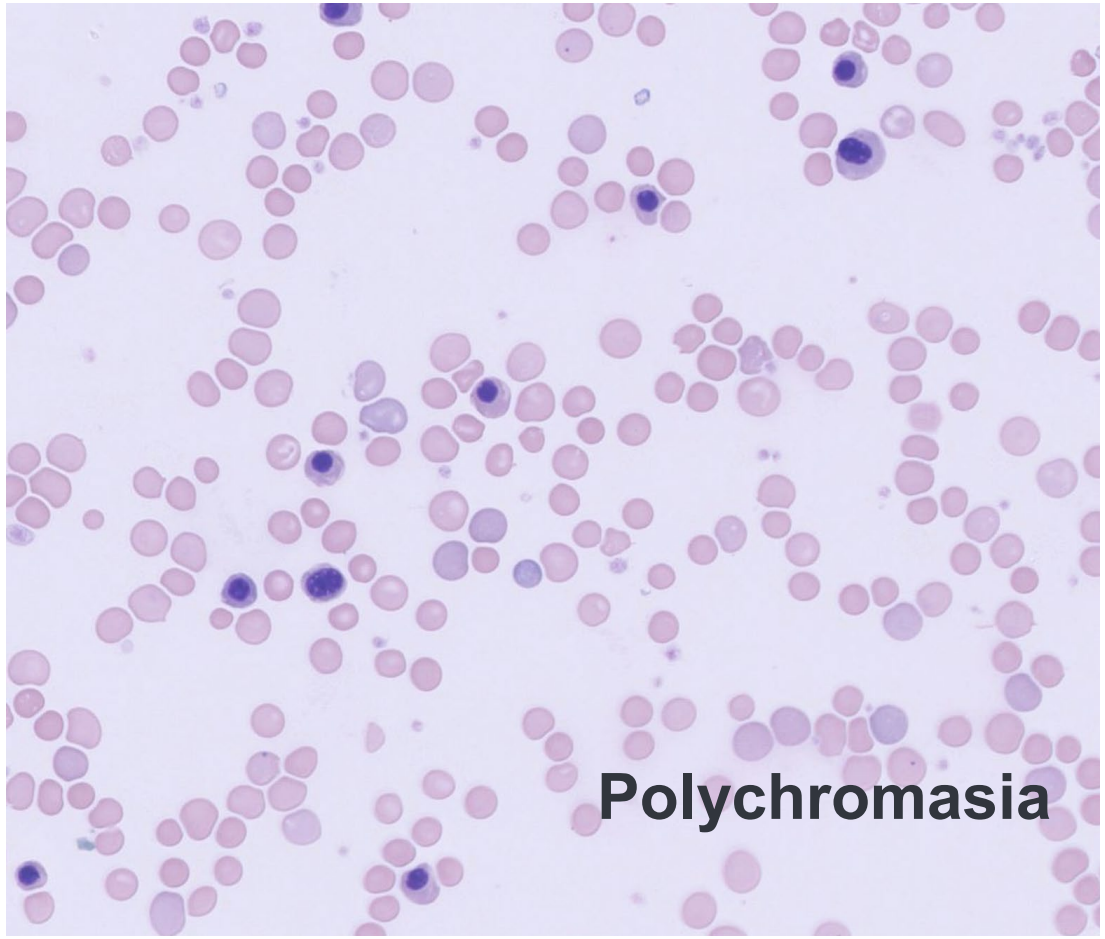
- Anisocytosis
- Polychromasia
- Macro- or microcytosis
- Hypochromasia
- Spherocytes/ghost cells
- Acanthocytes
- Keratocytes and Blister cells
- Schistocytes
- Heinz bodies

Less common/helpful/specific

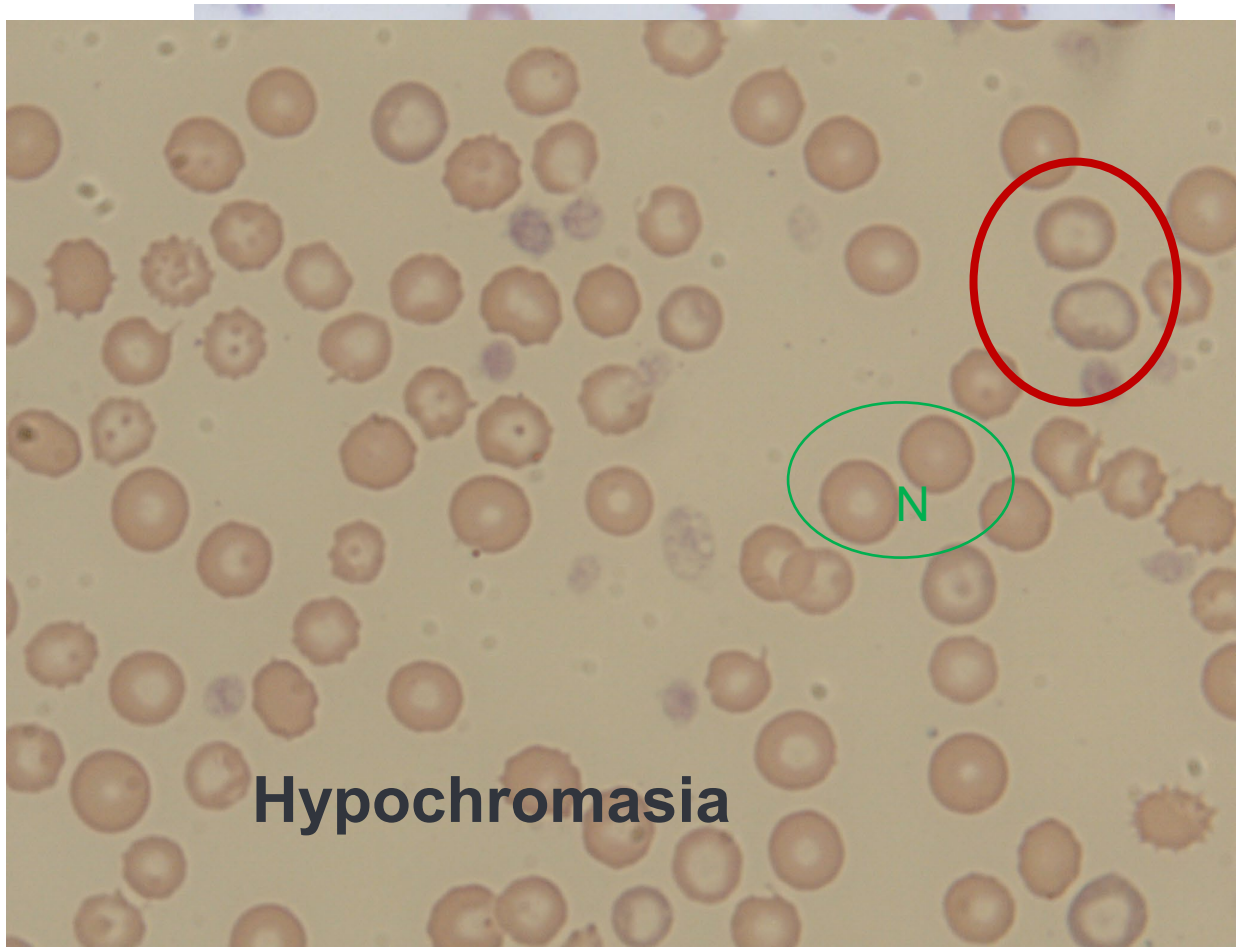
- Poikilocytes (common)
- Echinocytes (crenated RBC) (common)
- Torocytes
- Codocytes or Target cells (common)
- Eccentrocytes
- Ovalocytes
- Dacrocytes
- Knizocytes
- Leptocytes
- Organisms and inclusion bodies

Anisocytosis

Macrocytosis



Microcytosis



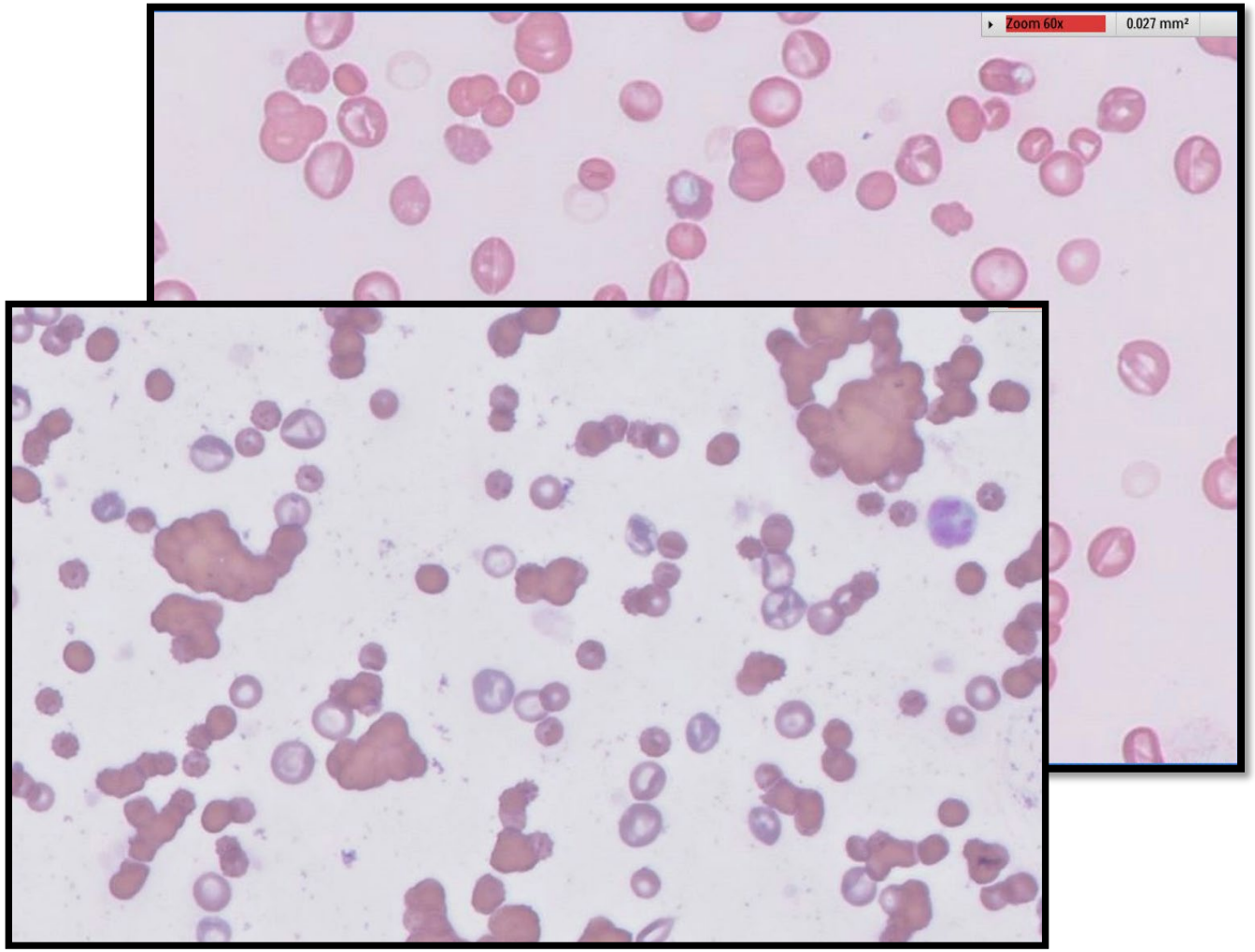
RBC morphology: Features of IMHA

- Spherocytosis (Dogs)

- Ghost cells
- Ideally on a fresh smear

- Questionable results?
  - In-saline agglutination
  - +/- Coomb's test

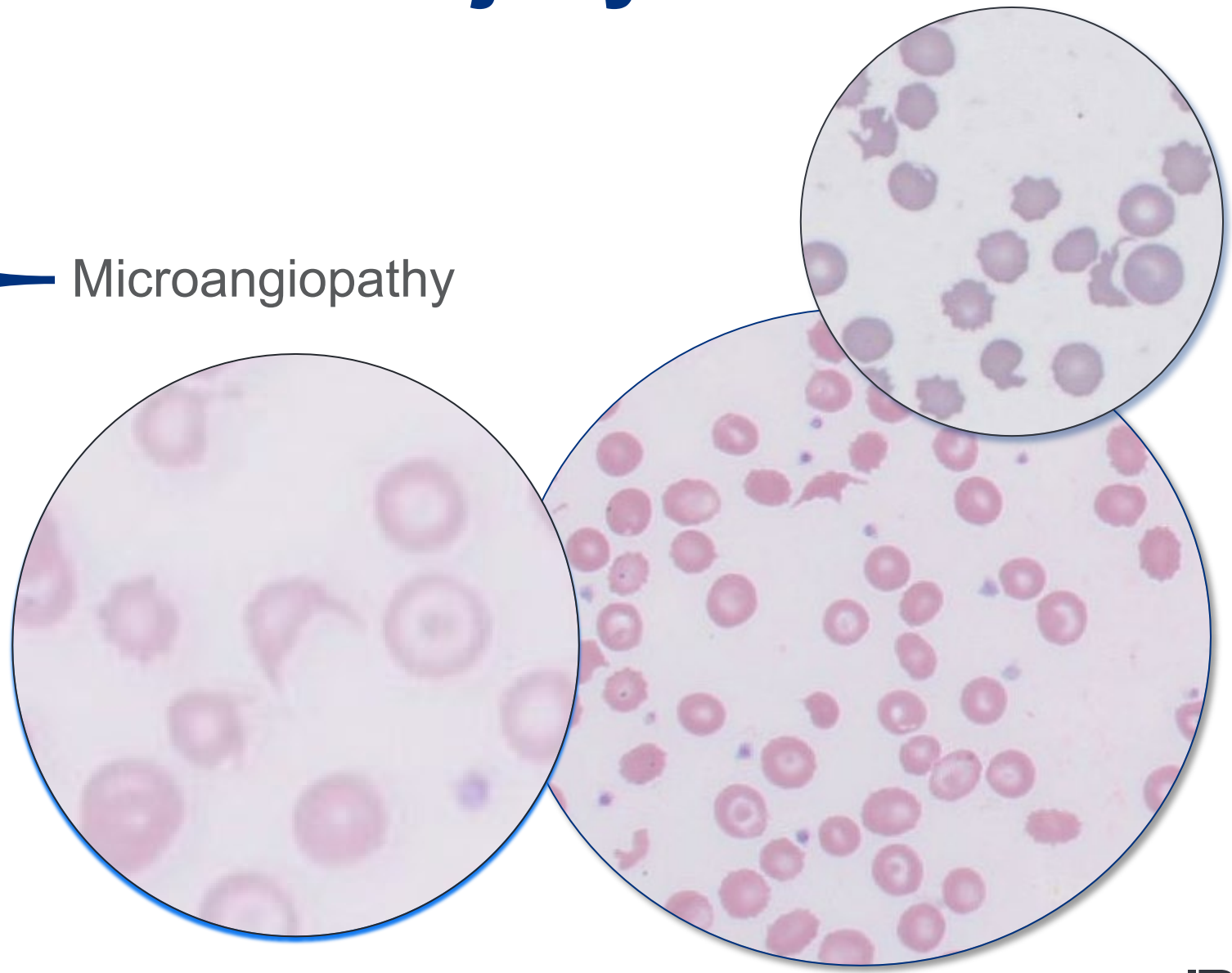
- Regenerative vs Non-regenerative



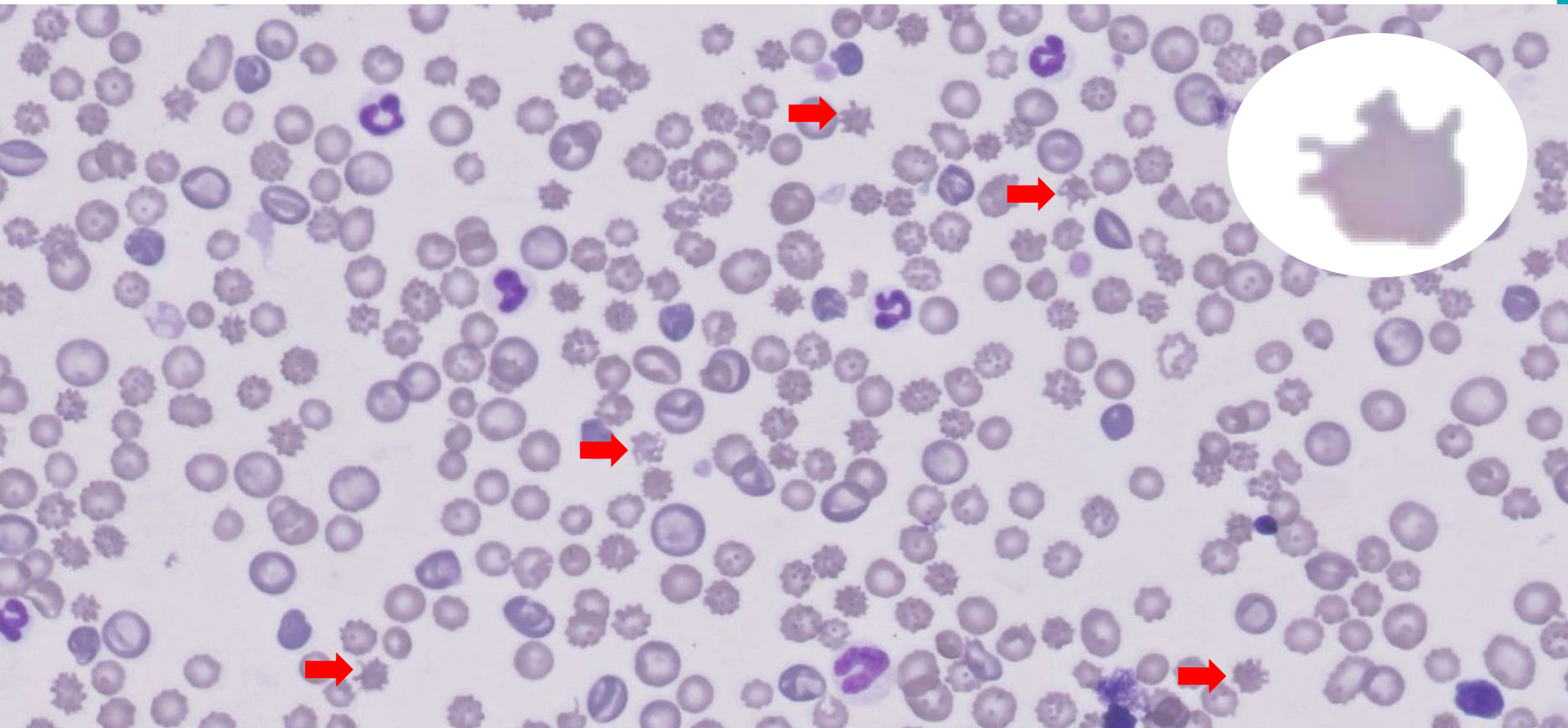
Poikilocytes: Shear injury

- Schistocytes
- Keratocytes
- Acanthocytes

Microangiopathy



Acanthocytes or echinocytes?



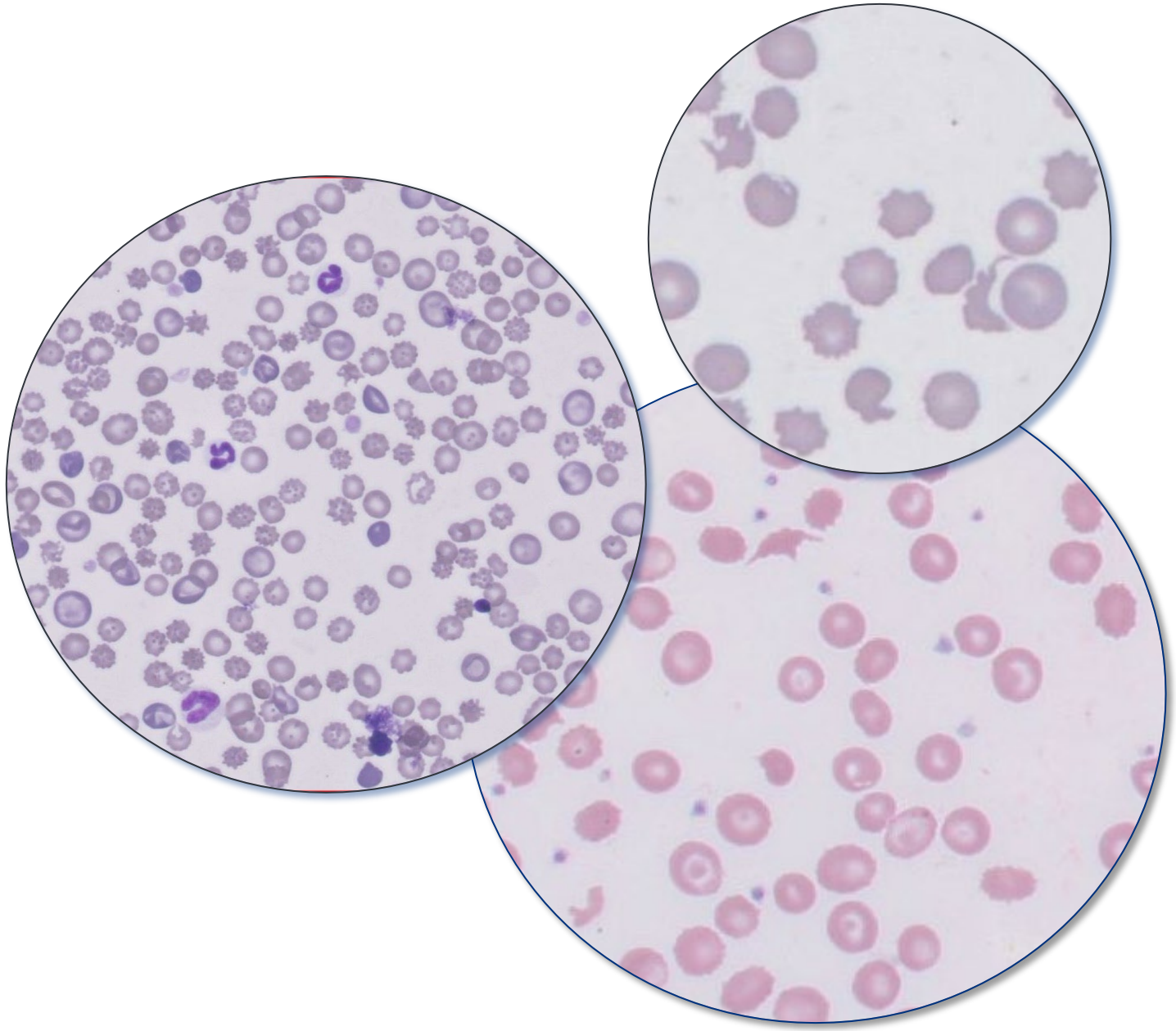
Poikilocytes

Significance?

- >1/2 per 100X field?
- Associated anaemia?
- Polychromasia?
- Keratocytes in cats?

Common differentials:

- Vascular tumour
- Hepatic/splenic disease
- DIC
- +++

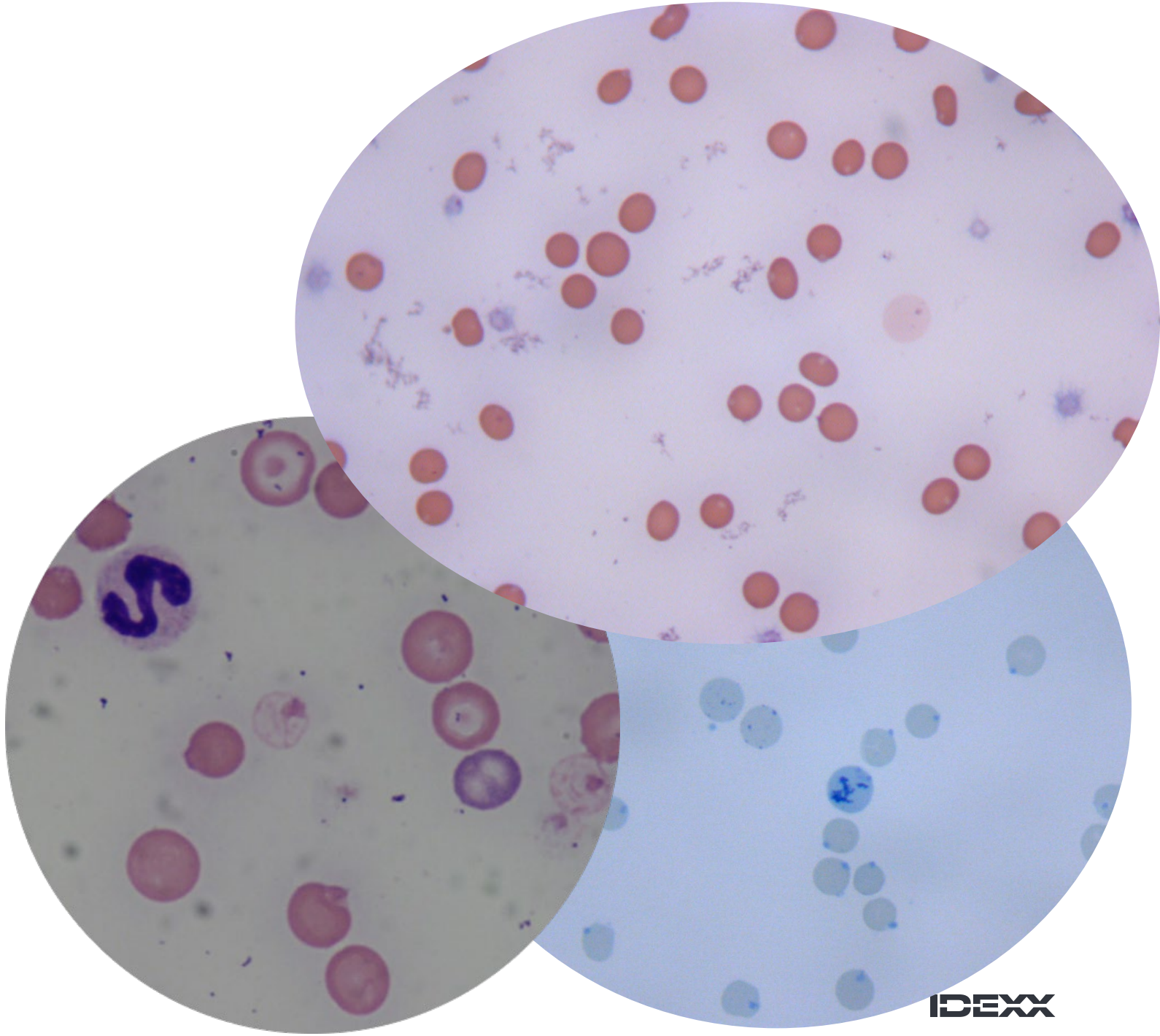


IDEXX

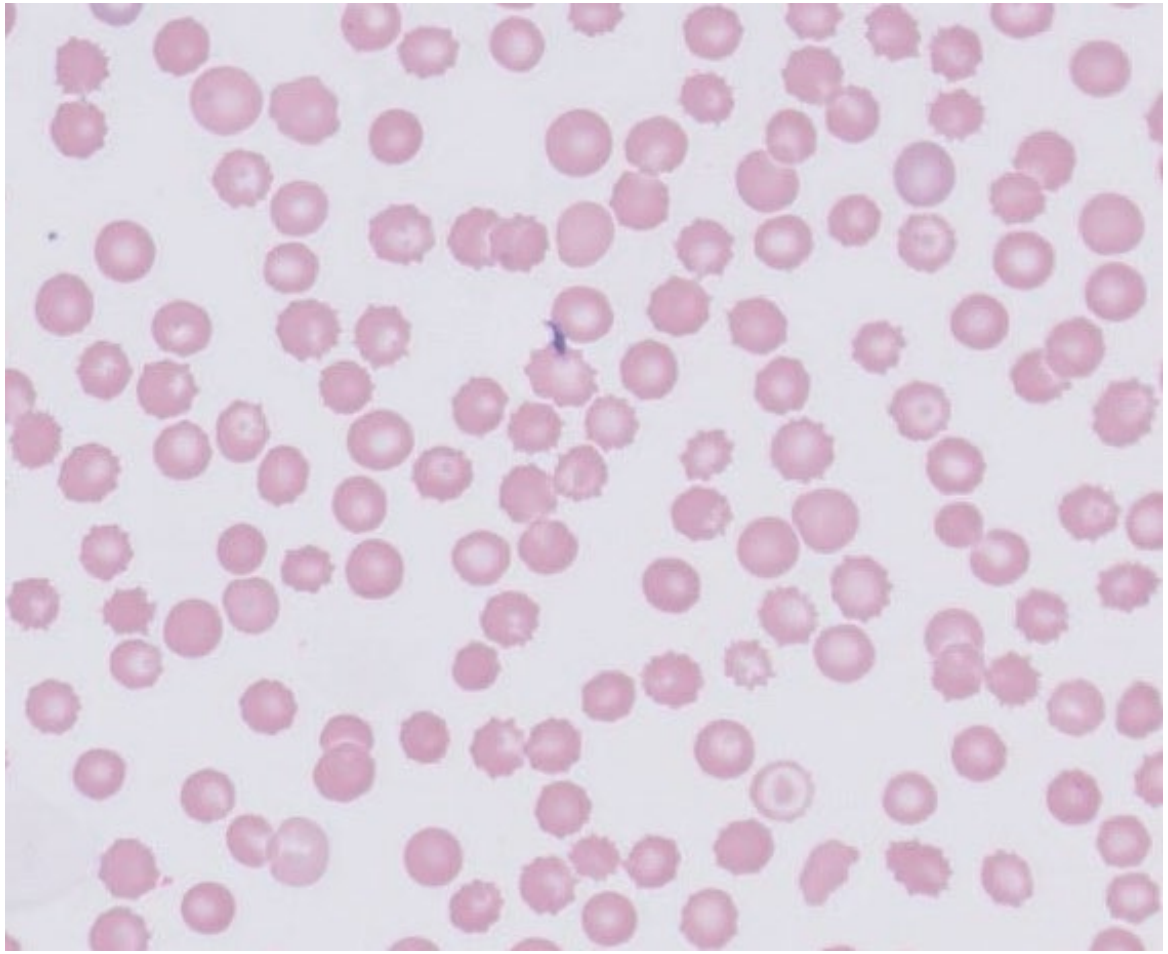
Oxidative insult

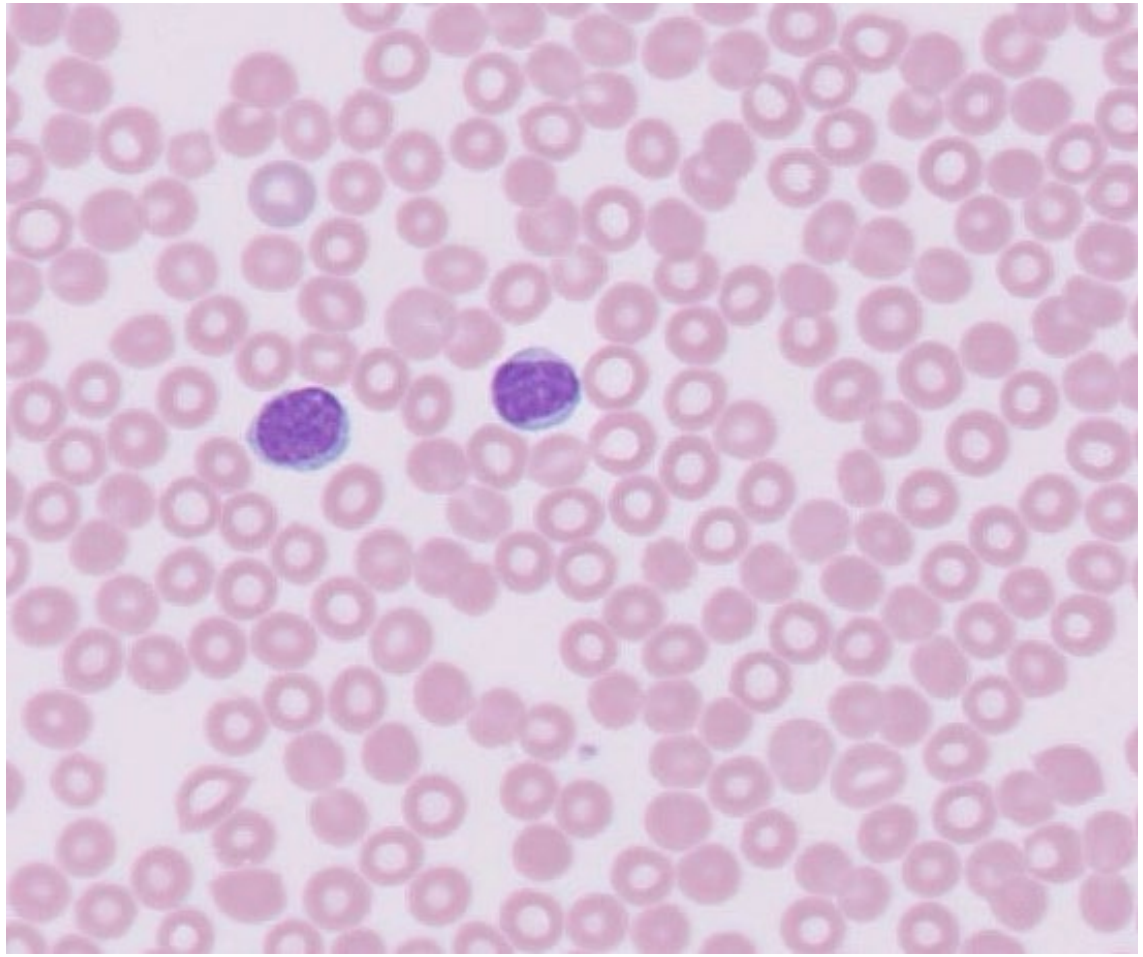
Heinz Bodies

- Often incidental/endogenous in cats
- More likely associated with toxin ingestion and haemolysis in dogs
- More easily identified with NMB or other vital stains



Storage and Drying artefacts: echinocytes, codocytes (target cells) and torocytes

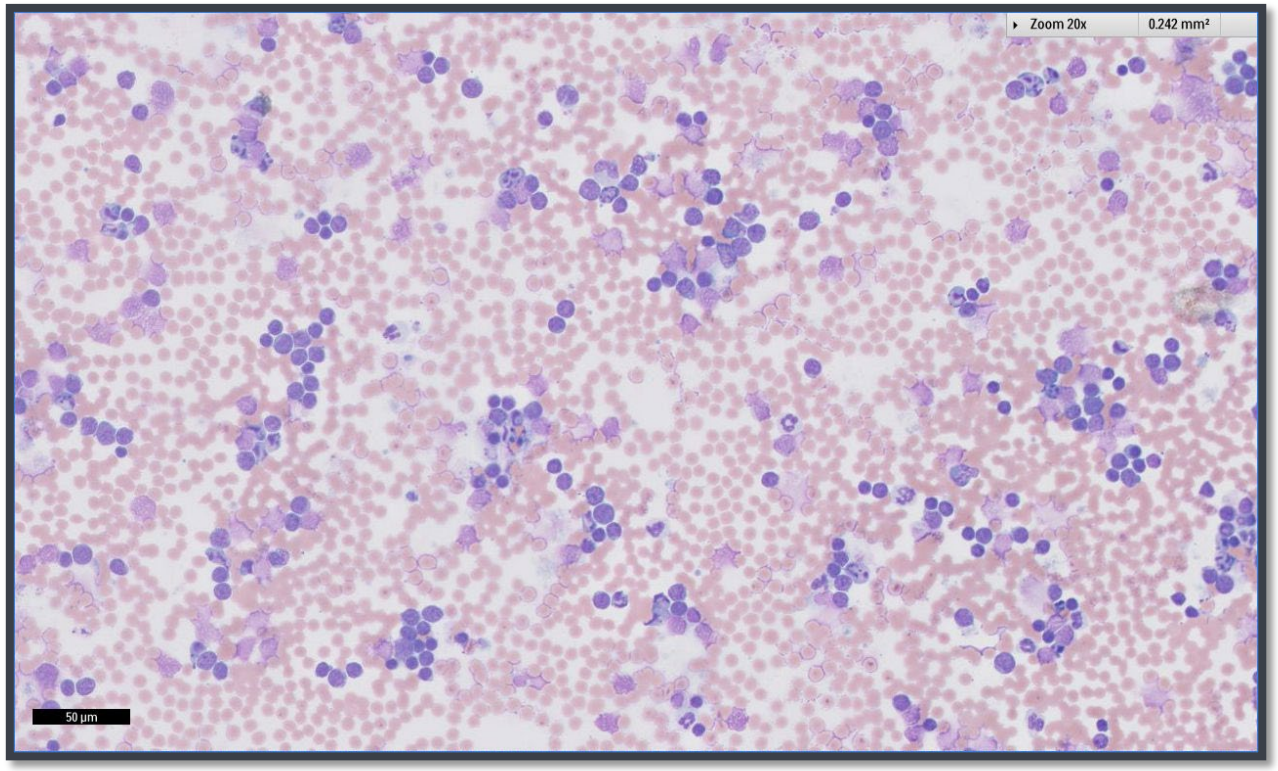


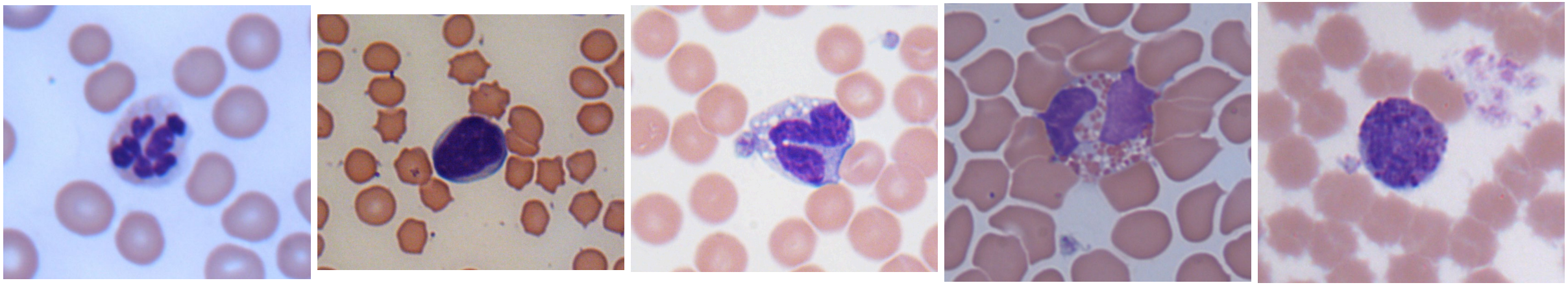


Nucleated cells

- Low power:
- Do numbers agree with [WBC]?
- Is distribution even?

- High power:
- Does the spread agree with WBC differential counts?
- Are there morphological abnormalities?





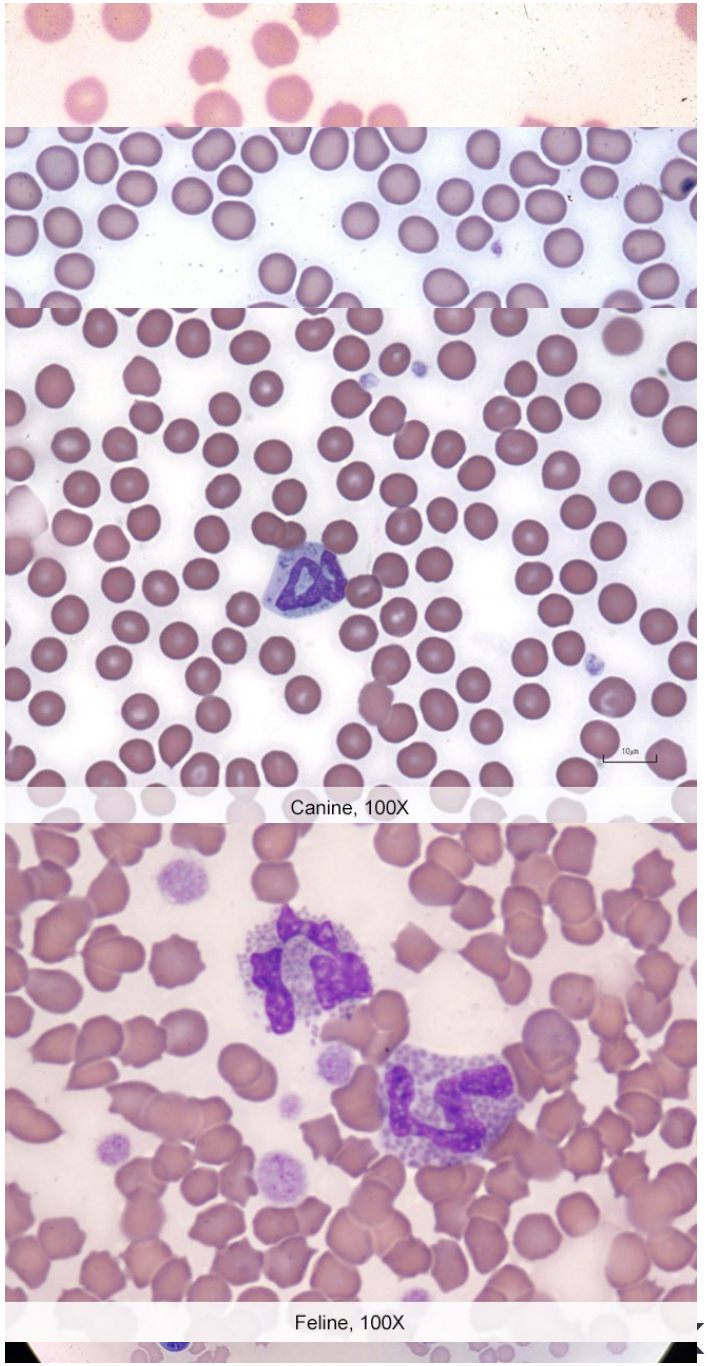
WBC Differential - leucogram

+ Count in monolayer or along edges, not in feathered edge
+ Always do one if analyser suggests confirmation
+ Percentages MUST be converted to absolute numbers using WBC before interpreted
+ Ideal opportunity to assess leucocyte morphology

IDEXX

Where you might get into trouble

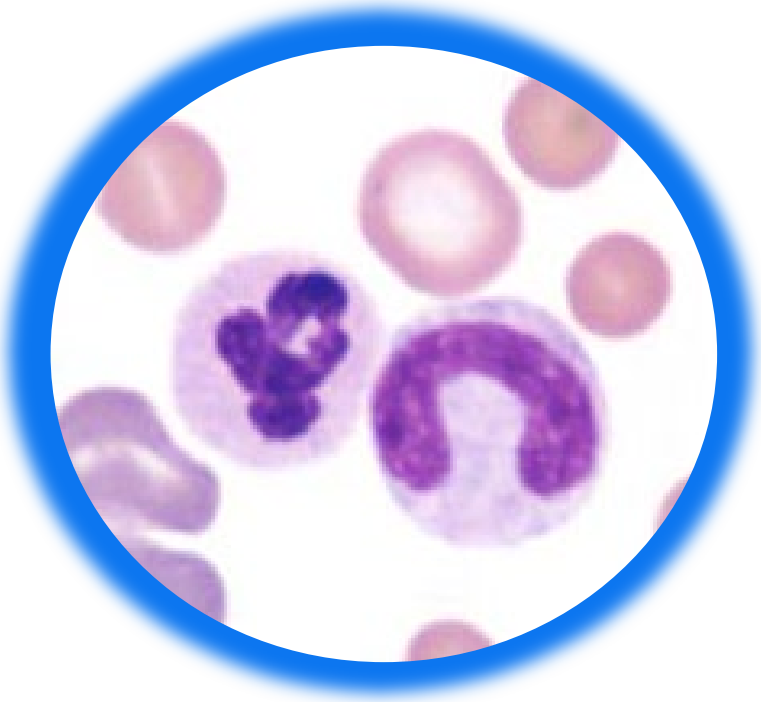
- Band neutrophil *vs* mature segmented neutrophil
- Toxic changes vs old samples
- Band *vs* metamyelocyte / monocyte
- Lymphocyte *vs* nRBC
- Reactive lymphocytes vs atypical cells
- Degranulated eosinophil/ Monocyte
- Basophils/ PMN



Neutrophil morphology and left-shift

- Neutrophils:
  - Segmented nuclei
  - Poorly staining granules
  - Coarse chromatin
- Bands
  - Hypo segmented
  - Fine chromatin

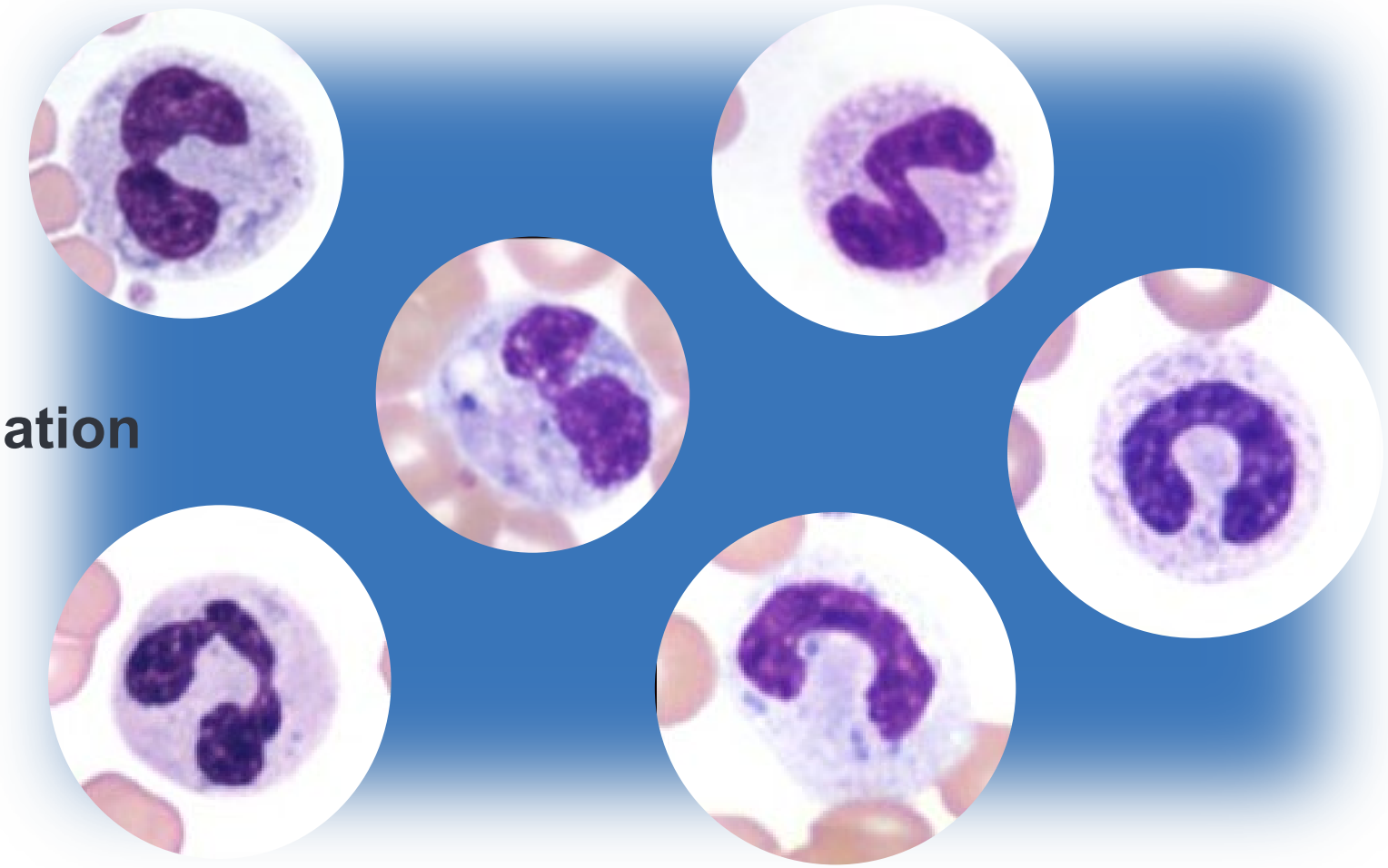
Left-shift = active inflammation



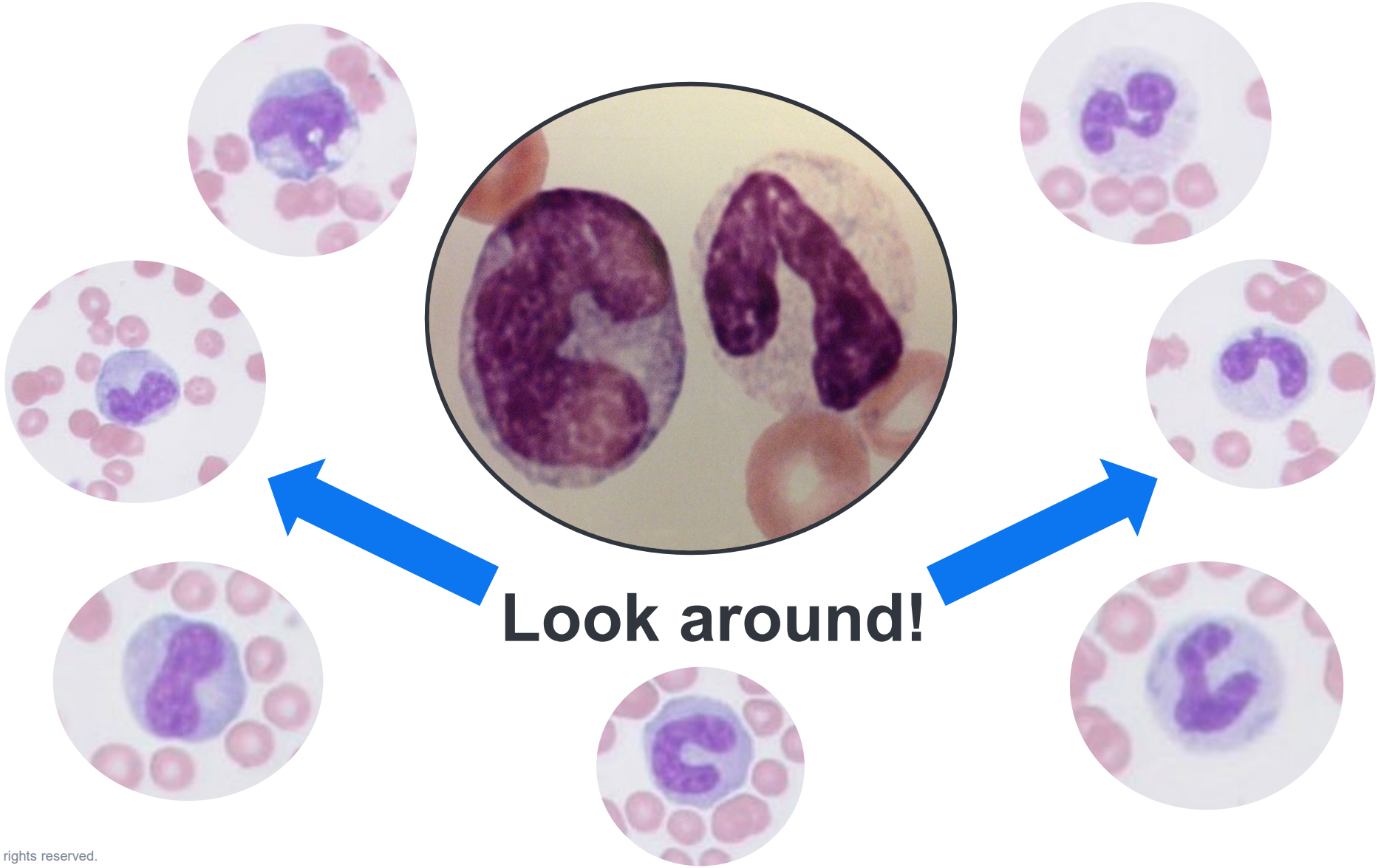
Neutrophil morphology: toxic change

- Basophilia
- Döhle bodies
- Foamy cytoplasm

Toxicity = active inflammation

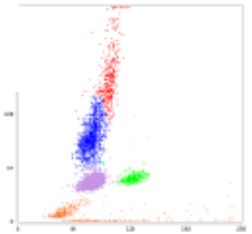


Differentiating the monocyte from the toxic band

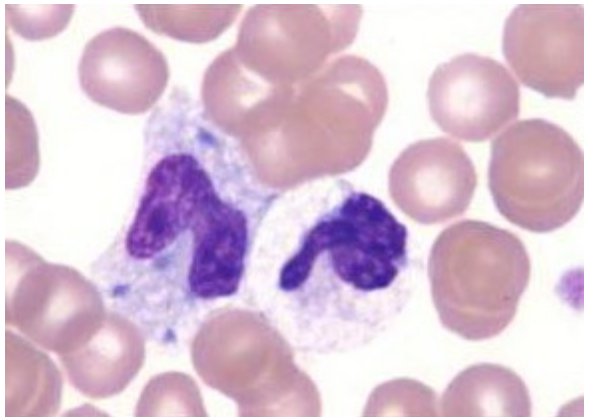


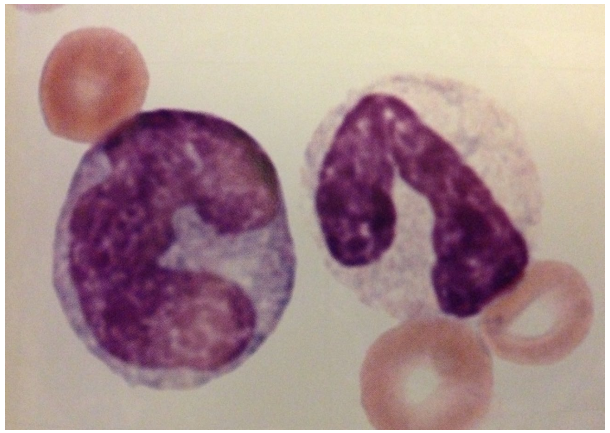
Automated scatterplots

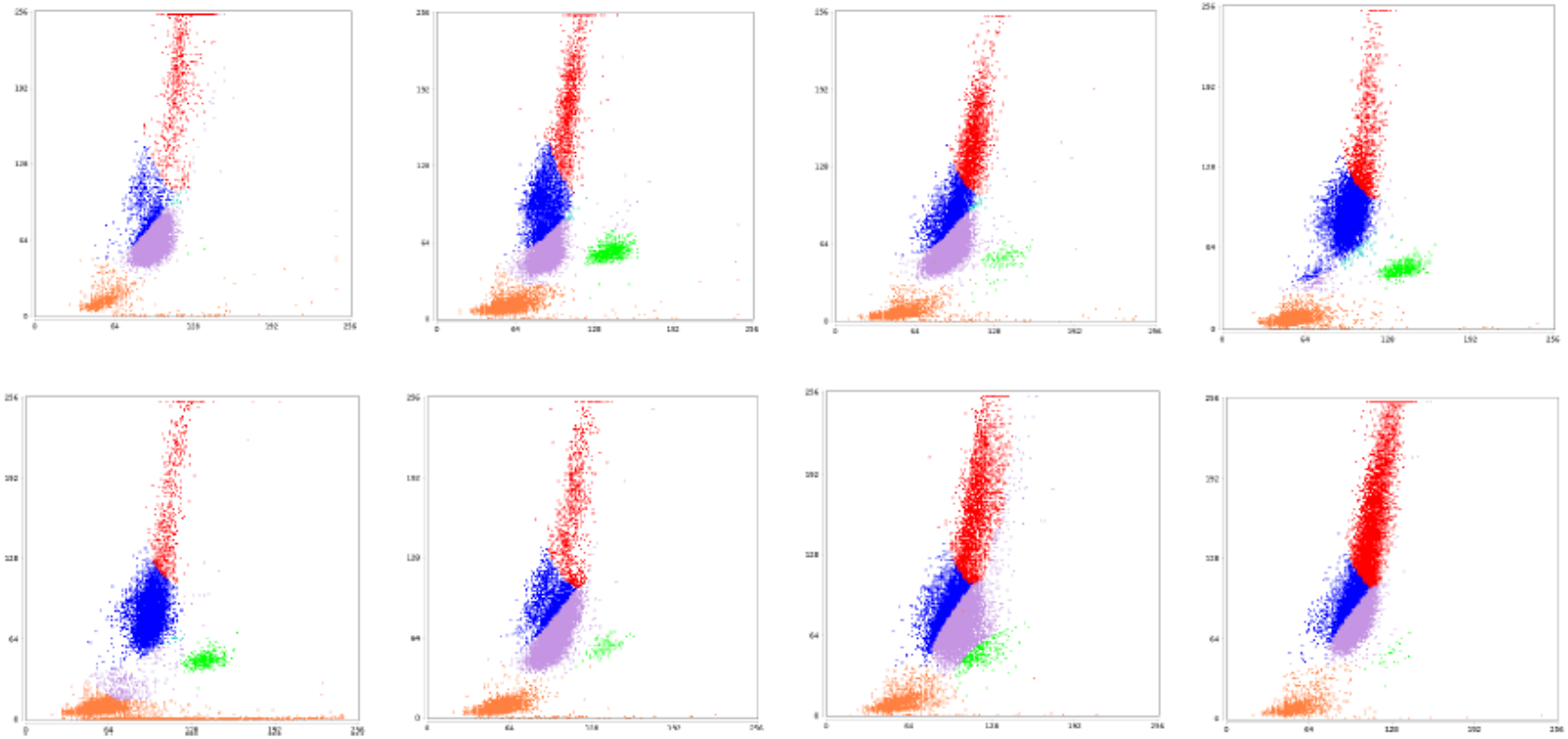
ProCyte Dx – Examples left shift



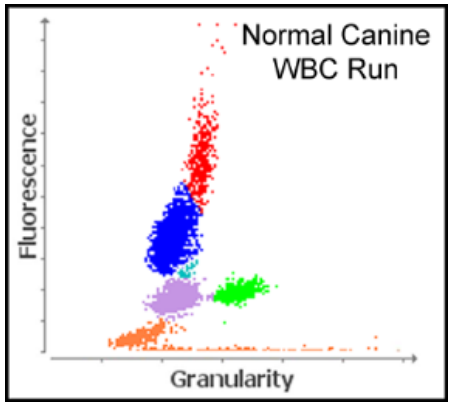
Normal



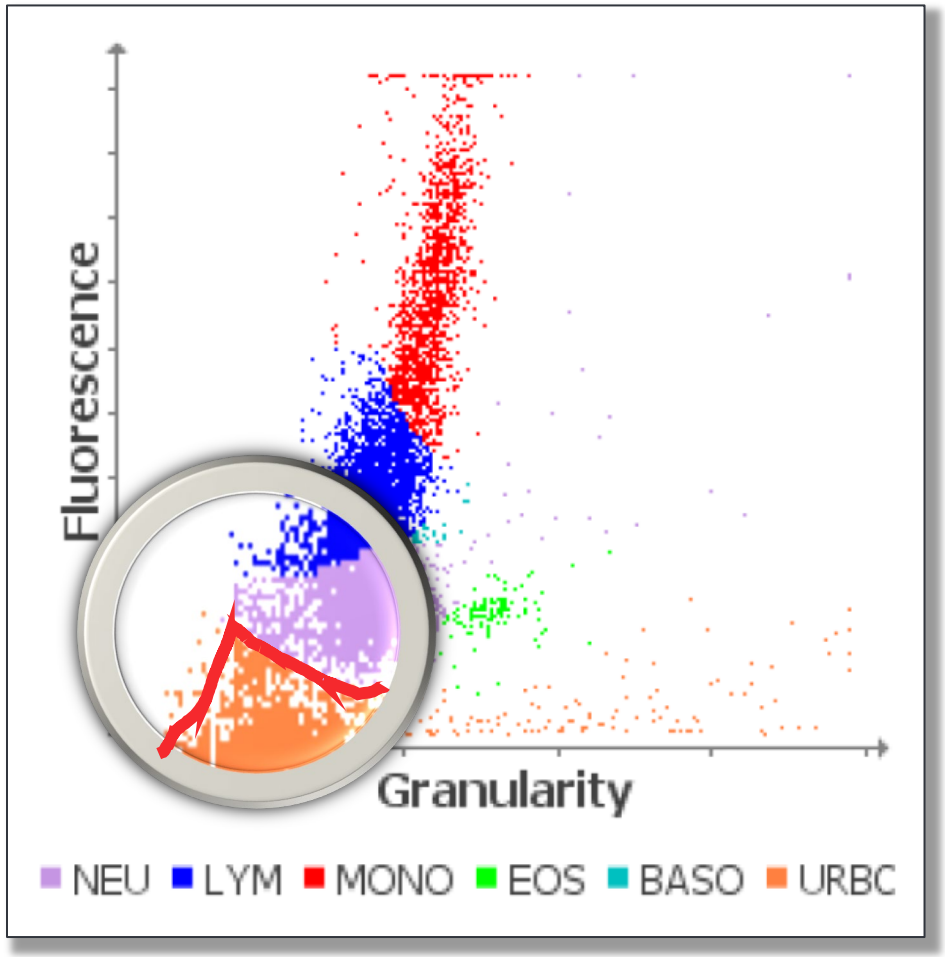


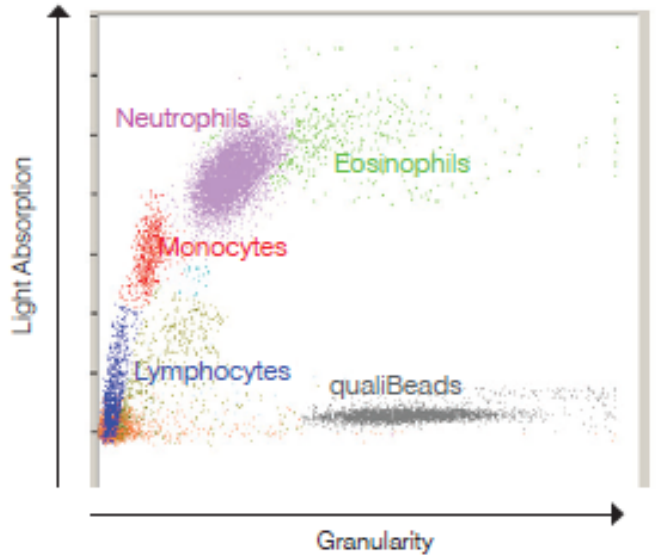


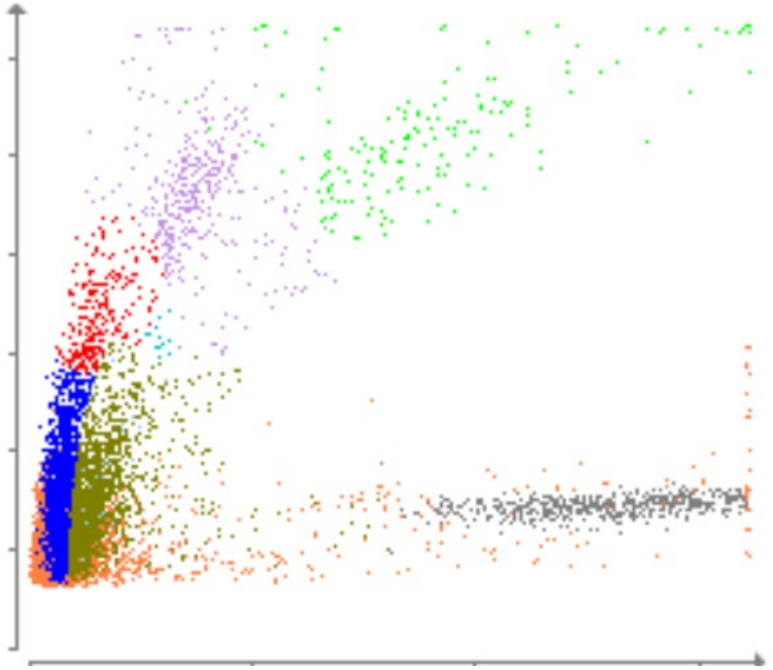
nRBC on the dot plot



Test	Results	Reference Interval	
ProCyte Dx (October 7, 2013 8:27 PM)			
WBC	* 34.01 K/µL	5.05 - 16.76	HIGH
NEU	* 19.77 K/µL	2.95 - 11.64	HIGH
BAND	* Suspect presence		
LYM	* 12.14 K/µL	1.05 - 5.10	HIGH
MONO	* 1.94 K/µL	0.16 - 1.12	HIGH
EOS	* 0.13 K/µL	0.06 - 1.23	
BASO	* 0.03 K/µL	0.00 - 0.10	
nRBC	* Suspect presence		



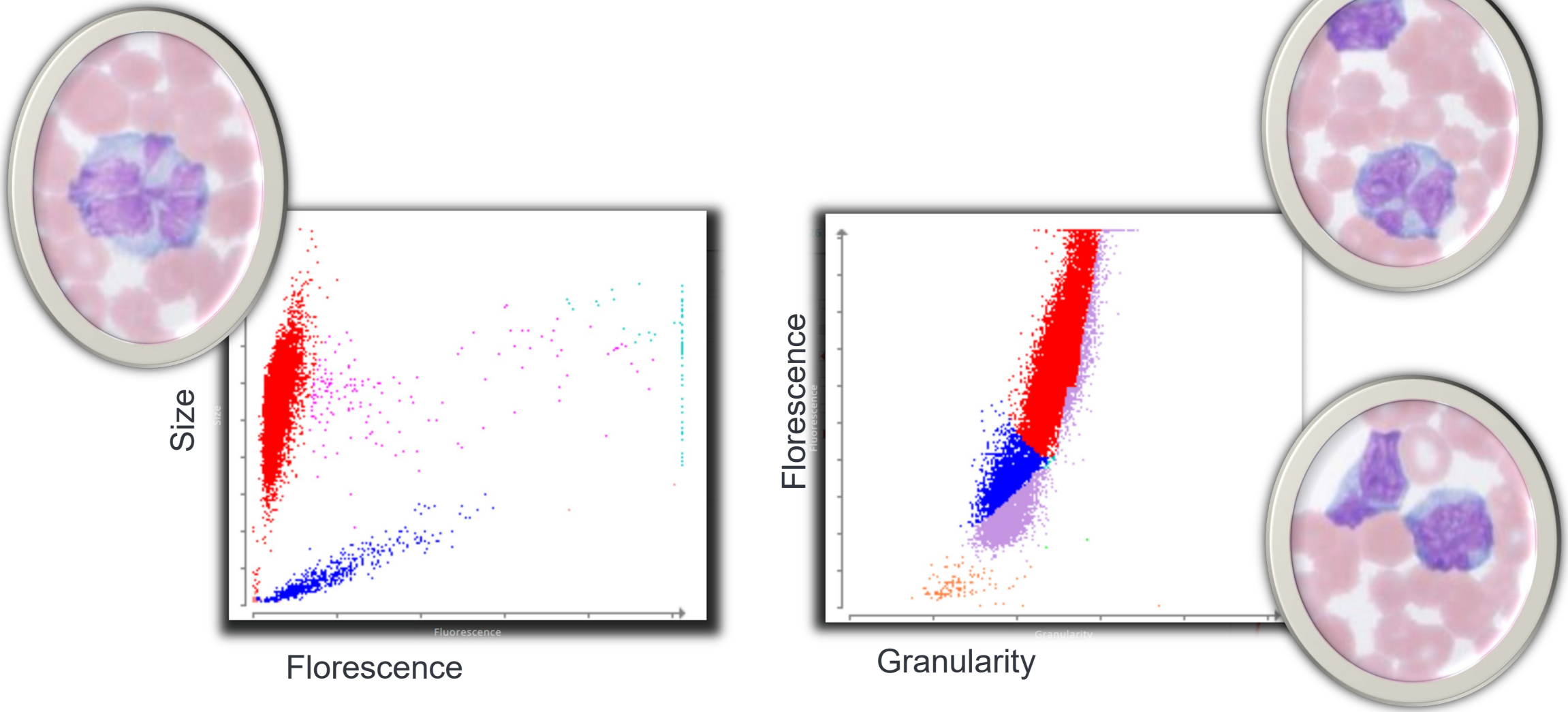




nRBC * Suspect presence

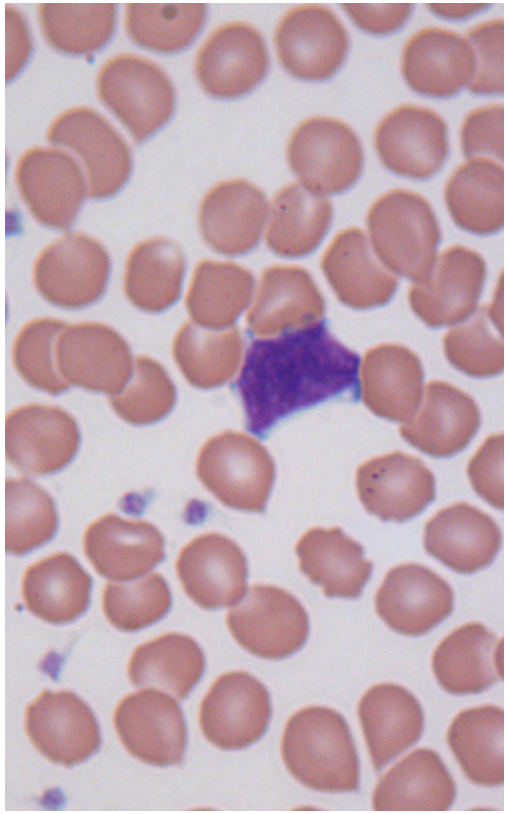
IDEXX

Atypical cells in the dot plot

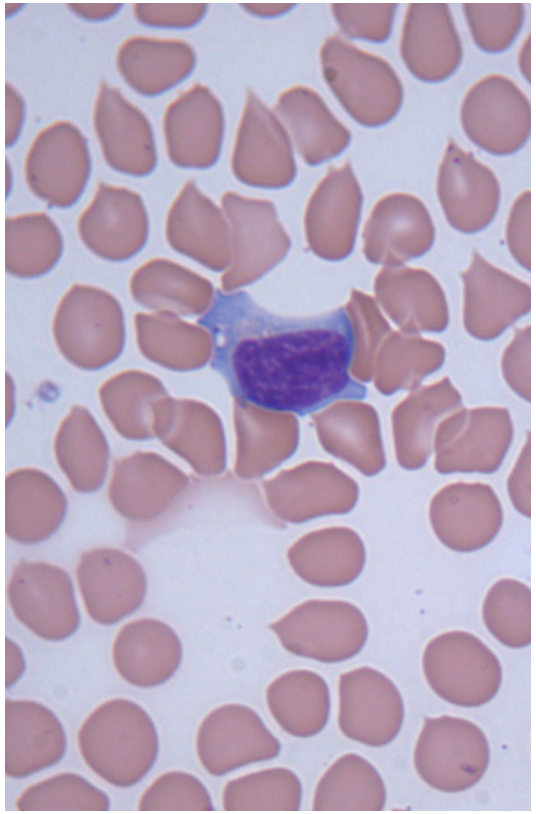


Lymphocytes morphological assessment

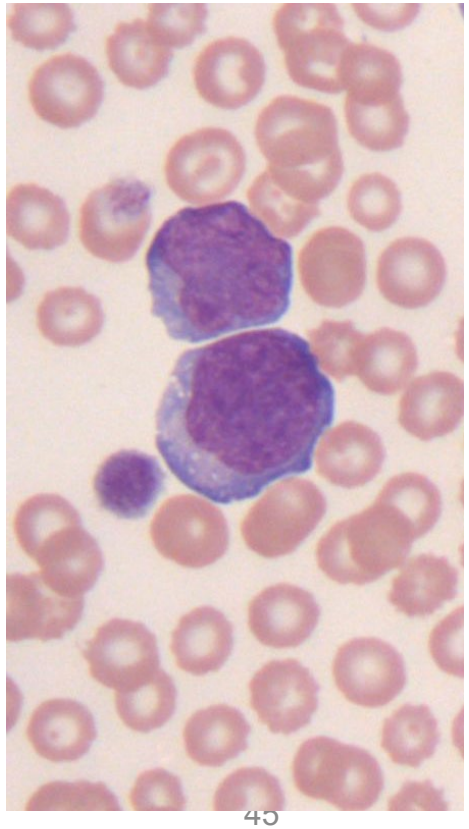
Normal Lymphocyte



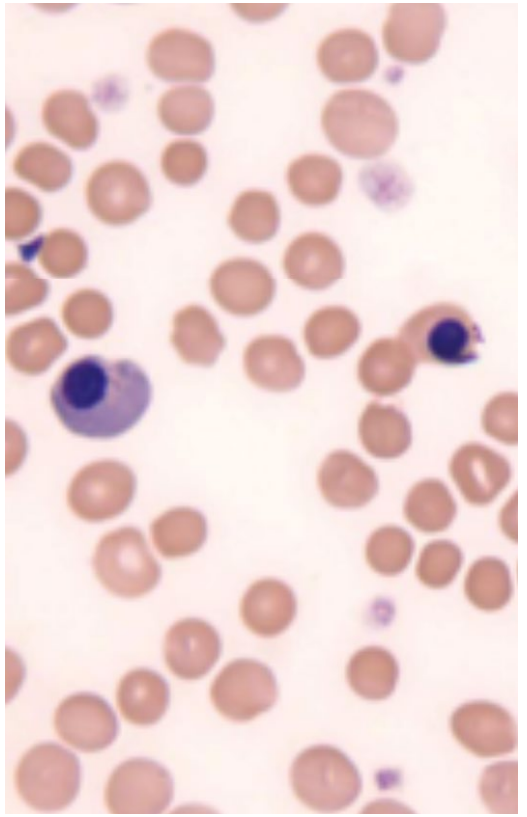
Reactive Lymphocyte



Lymphoblast



nRBC



Atypical cells and blasts

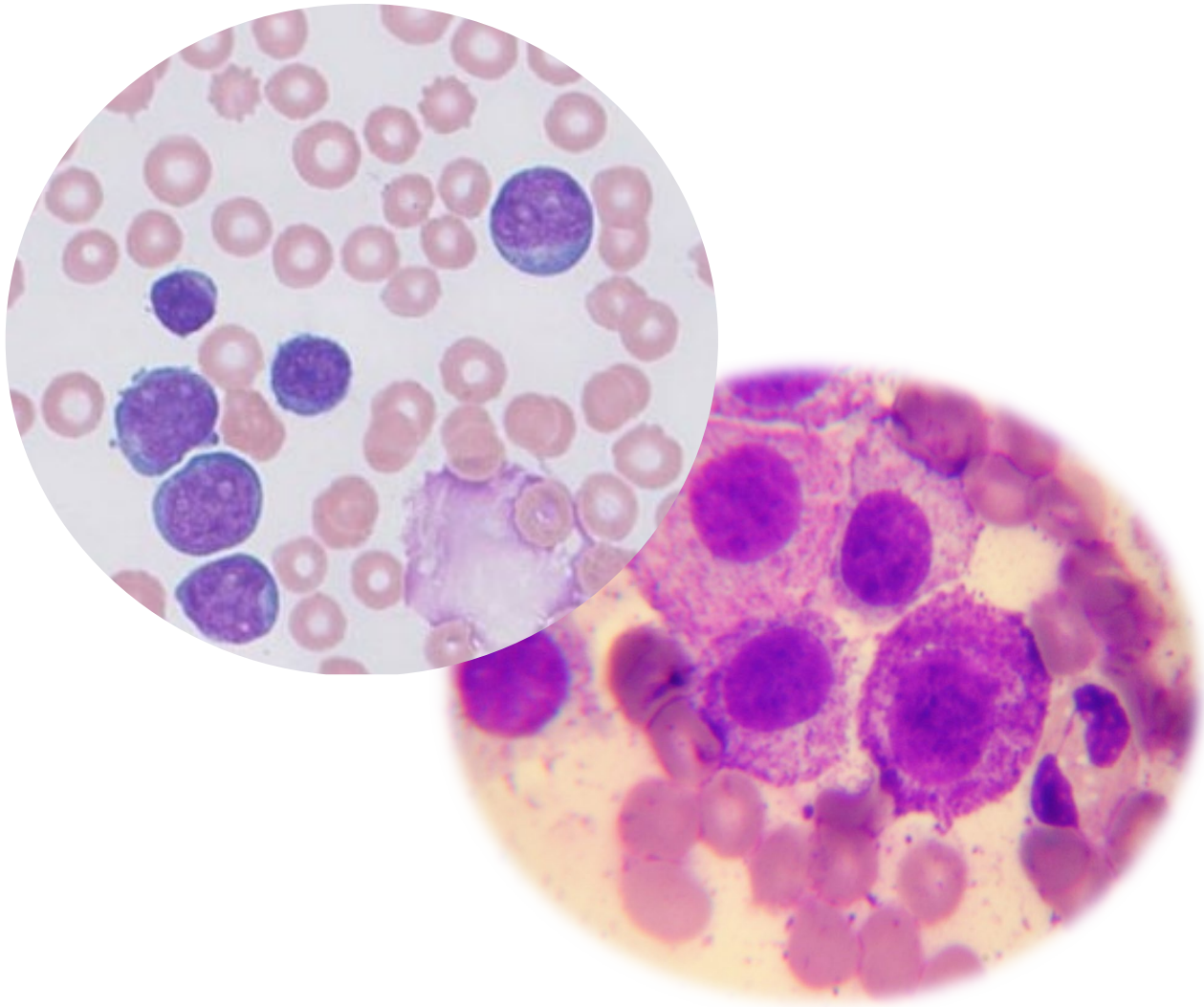
- Morphological features
- [atypical cells]
- [Total WBC]
- Clinical picture

Monitoring

Further investigation

- Imaging
- cytology

Phenotyping



PLANNING REFERRAL

Blood smear evaluation

+ Ideally all smears should be evaluated

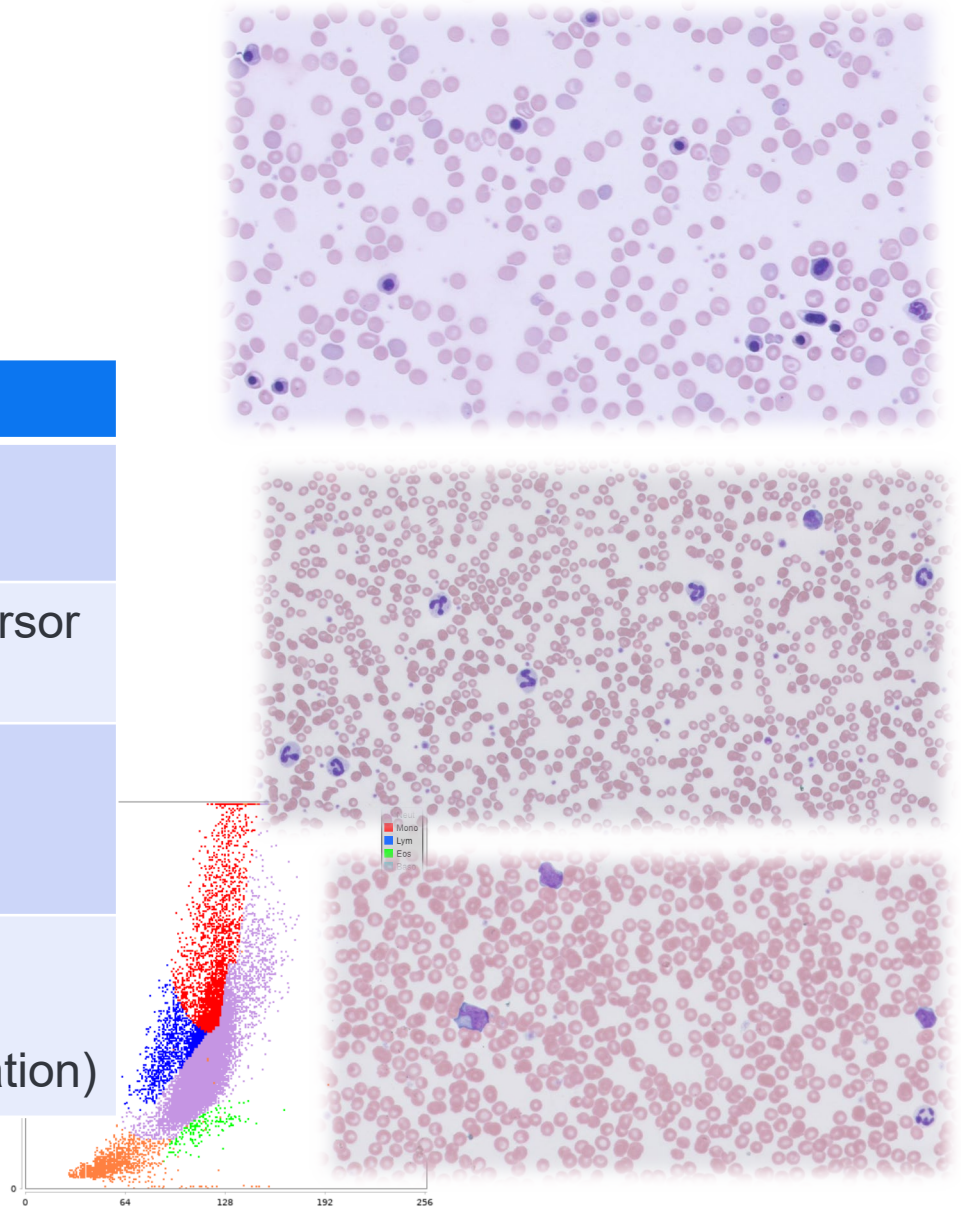
+ Minimum recommended
  + Review all critically ill patients
  + CBCs with unusual or suspicious results
+ If no time and expertise available, any cases which require a blood smear exam should be referred

+ Some cases it is recommended that further evaluation is done

ASVCP recommendations

In-Clinic Manual WBC Differential Count by Trained Personnel

Presence of	Suggested Cut-Off value
Nucleated RBCs	More than rare
Neutrophil left shift	>1 band or earlier myeloid precursor
Unclassified (unidentified) cells	Any
Automated WBC differential count may not be accurate	N/A (e.g., flags, unclear cloud separation)

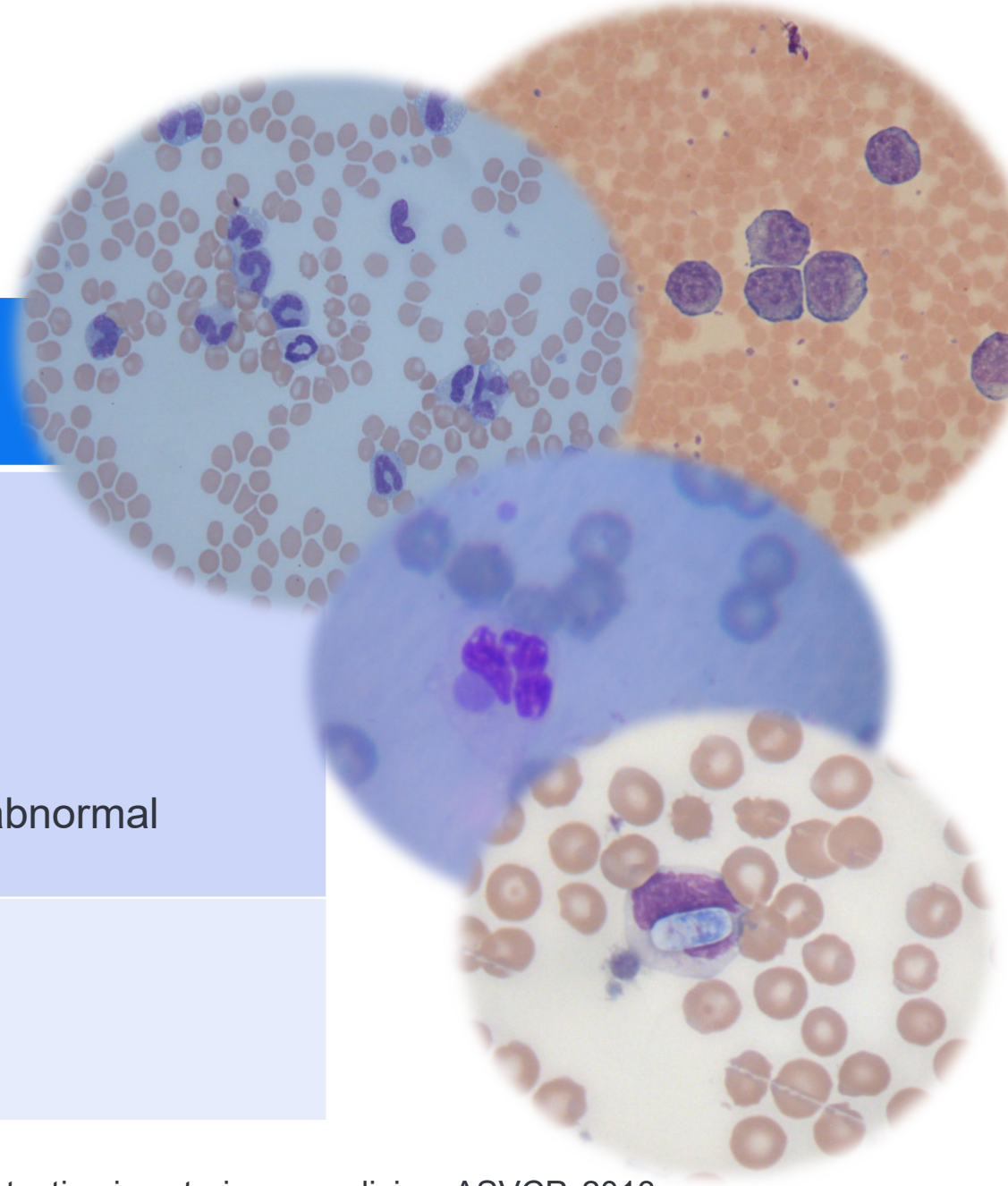


ASVCP guidelines: quality assurance for point-of-care testing in veterinary medicine. ASVCP, 2013. Available at www.asvcp.org. Accessed September 2023.

ASVCP recommendations

Medical Review of Blood Smears and Concurrent CBC Data

Blood Smear	Criteria Triggering a Review
WBC	Left shift (if marked or degenerative) **Marked leukopenia** < 3,000 WBC/µL **Marked leukocytosis** > 30,000 WBC/µL **Lymphocytosis > 10,000 cells/µL** Any **unclassified** cells Any **organisms or suspected organisms** Presence of vacuoles in non-monocytes and abnormal granulation in any leukocyte
Platelets	Platelet count > 900,000 cells/µL Thrombocytopenia < 100,000 cells/µL Abnormal MPV (if reported by instrument) Suspected inclusions or abnormal granulation



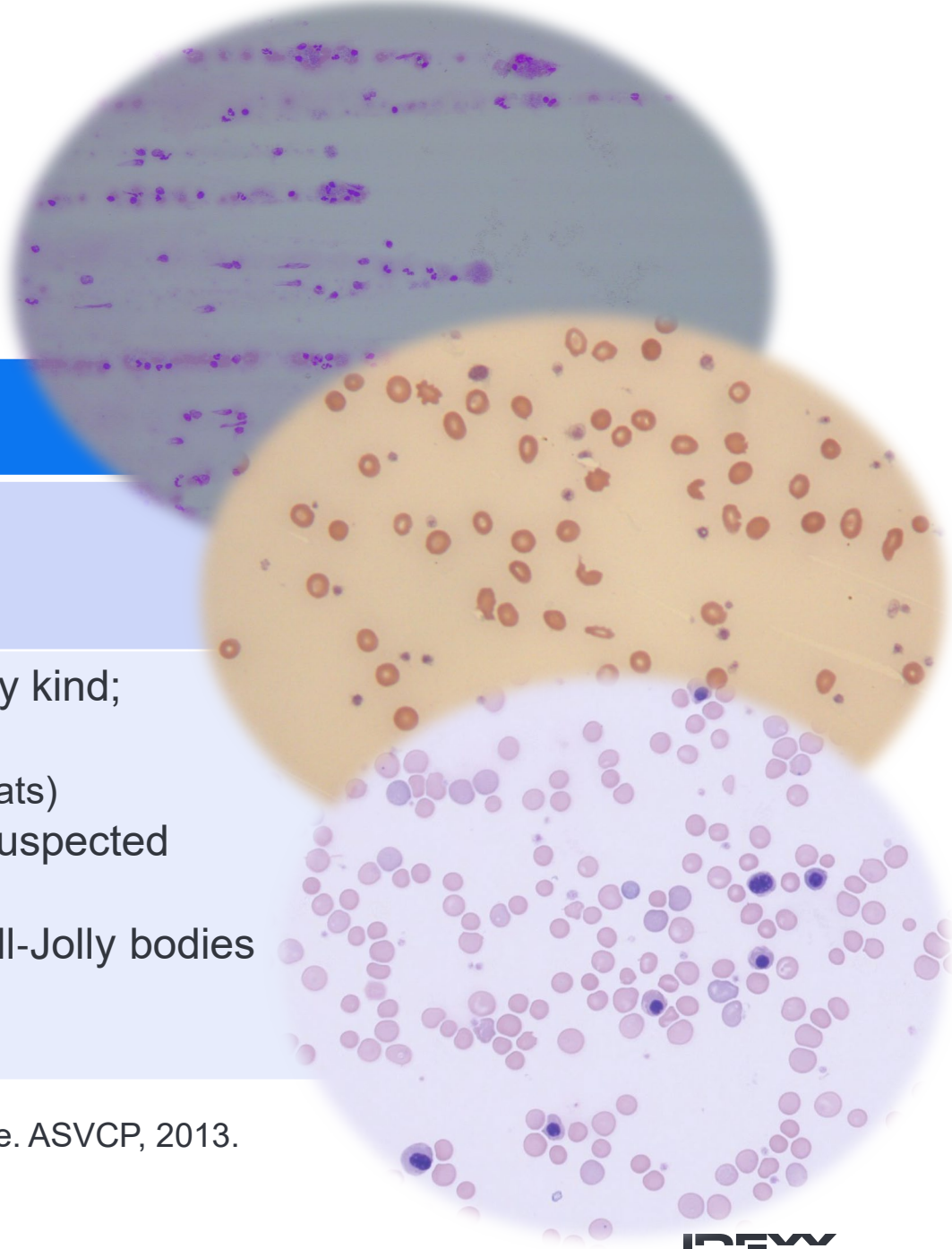
ASVCP guidelines: quality assurance for point-of-care testing in veterinary medicine. ASVCP, 2013. Available at www.asvcp.org. Accessed September 2023.

IDEXX

ASVCP recommendations

Medical Review of Blood Smears and Concurrent CBC Data

Blood Smear	Criteria Triggering a Review
Background	Unusual background matrix Unusual background color **Organisms or suspected organisms**
Red Blood Cells	Moderate to marked **poikilocytosis** of any kind; Moderate to **severe anaemias** Any **Heinz bodies** (> 10% Heinz bodies in cats) Any **inclusions** (including organisms or suspected organisms) Basophilic stippling, siderocytes, or Howell-Jolly bodies **> 5 nRBC/100 WBC** Abnormal MCV



ASVCP guidelines: quality assurance for point-of-care testing in veterinary medicine. ASVCP, 2013. Available at www.asvcp.org. Accessed September 2023.

IDEXX

For submission

- Blood smears and tubes are labeled with 1-2 unique patient IDs and date of collection.
- Good quality smears.
- Unstained smears are preferred.
- Stored at room temperature and sent in slide carriers
- Accompanied by available instrument derived data and relevant clinical history.

Take home MSG

- Blood smear exam allows you to get more out of your CBC results
- Start with a good quality smear
- Have a systematic approach
- Cross check findings with analyzer and history
- If in doubt… refer…get us to help out!

Happy to take any QUESTIONS …

Questions?

References

- American Society for Veterinary Clinical Pathology (ASVCP), 2013. ASVCP guidelines: quality assurance for point-of-care testing in veterinary medicine. ASVCP, 2013. Available at www.asvcp.org. Accessed September 2023.
- Flatland B, Freeman KP, Vap LM, Harr KE. ASVCP guidelines: quality assurance for pointof-care testing in veterinary medicine. Veterinary Clinical Pathology 2013; 42(4):405- 423.
- Schalm's Veterinary Hematology , 7th Ed, 2023. (eds M.B. Brooks, K.E. Harr, D.M. Seelig, K.J. Wardrop and D.J. Weiss).