Blood Smear Reviews In Clinic: ways to optimise your in-clinic haematology results

# Session Sponsor

# Blood Smear Reviews In Clinic: ways to optimise your in-clinic haematology results

**Presenter:**

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June 15, 2024

**IDEXX**

# Outline

- Sample collection
- Blood smear preparation
- Setting up and using the microscope
- How to assess a blood smear
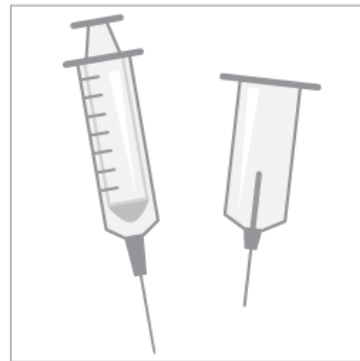- Common blood smear review findings
- Case Study

# Sample collection

- Results are only as good as the sample obtained

- Collection with larger gauge needles (pink 18g, green 21g, black 22g)

- Minimise trauma to the blood vessel
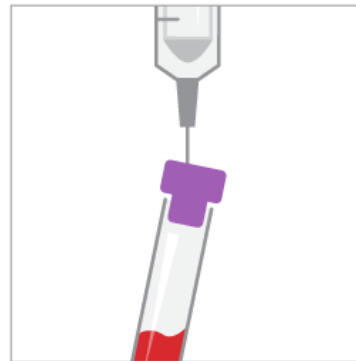
- Appropriate restraint
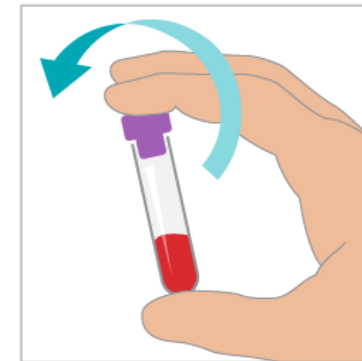
**Blood collection and sample preparation steps**



1. Use an EDTA tube that can be secured in the adapter (13 mm x 75 mm, 1.3 mL, 650 µL).
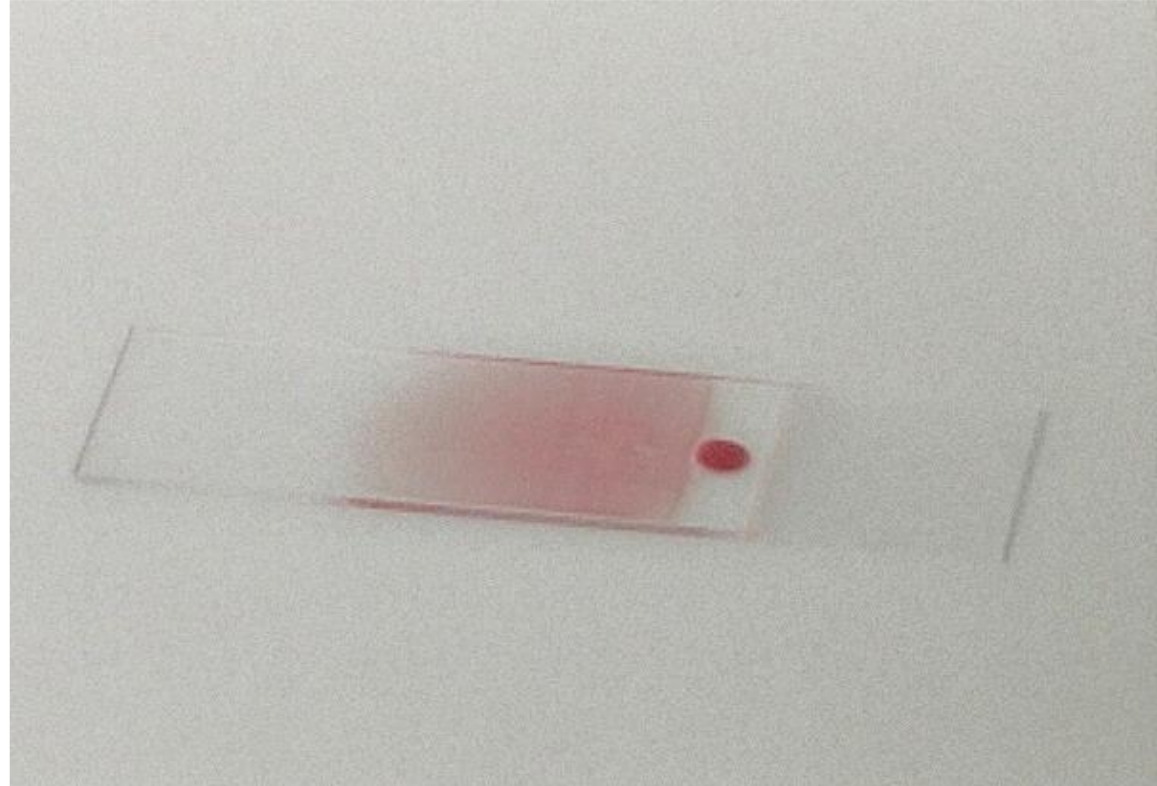
2. Use a syringe or vacuum collection system.
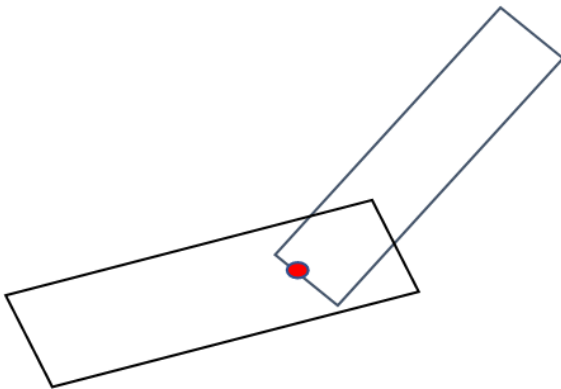
3. Draw the sample and transfer, if necessary.

4. Invert the tube to mix.
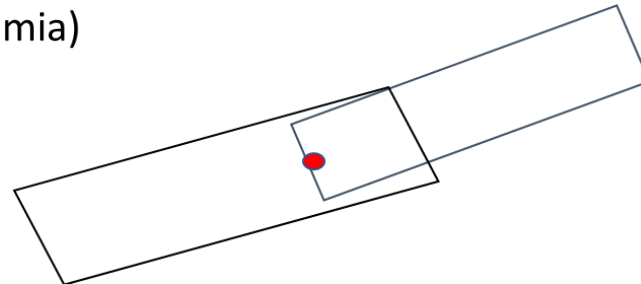
# Blood smear preparations



Tips:
- Smooth and purposeful
- Smear has symmetrical body, monolayer and feathered edge
- Practice makes progress
- Quickly dry the smears (air dry or fan on cool setting)

- Make 2 blood smears
    - 1 stained with diff quik
    - 1 air dried and unstained

Higher angle = less spread (anaemia)

Lower angle = more spread (erythrocytosis)

# Blood smear preparation



Complement your in-house haematology with blood morphology

Manual blood film preparation

**Instructions**

1. Use fresh, well-mixed, anti-coagulated blood to avoid sample deterioration.
2. Maintain an angle of approximately 30° between the spreader and sample slide throughout.
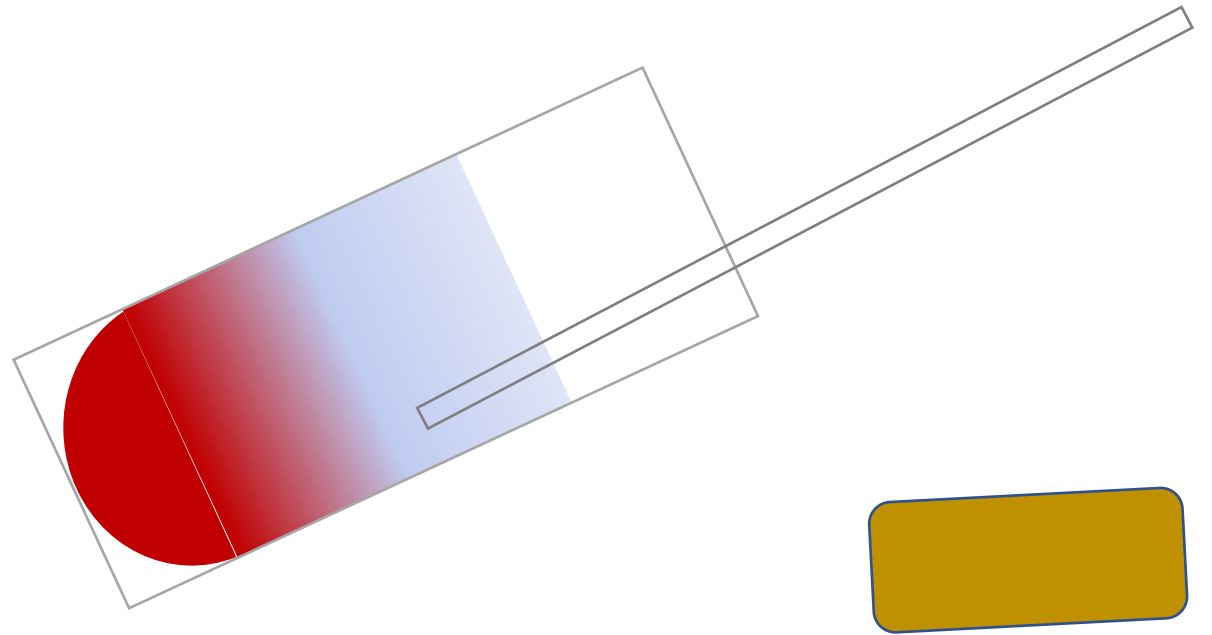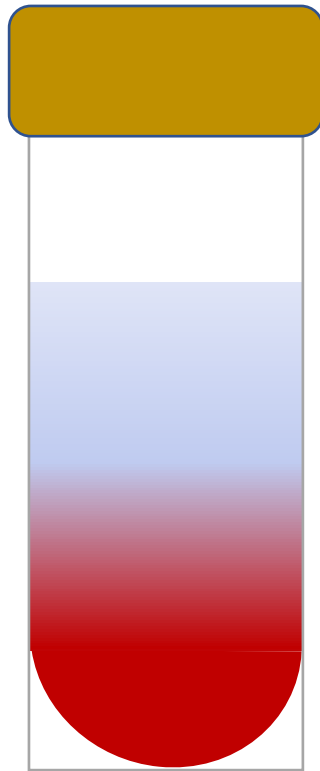3. Spread the sample in one smooth, steady motion.

**Tips**

- Include a blood film when results do not match clinical expectations.
- Make sure the film has a symmetrical body, a monolayer and a feathered edge.
- Avoid sample deterioration by limiting storage to 4 hours or less.
- Quickly dry slides (air dry or use a fan on the cool setting).

https://www.idexx.com.au/en-au/veterinary/analyzers/hematology/hematology-resources/
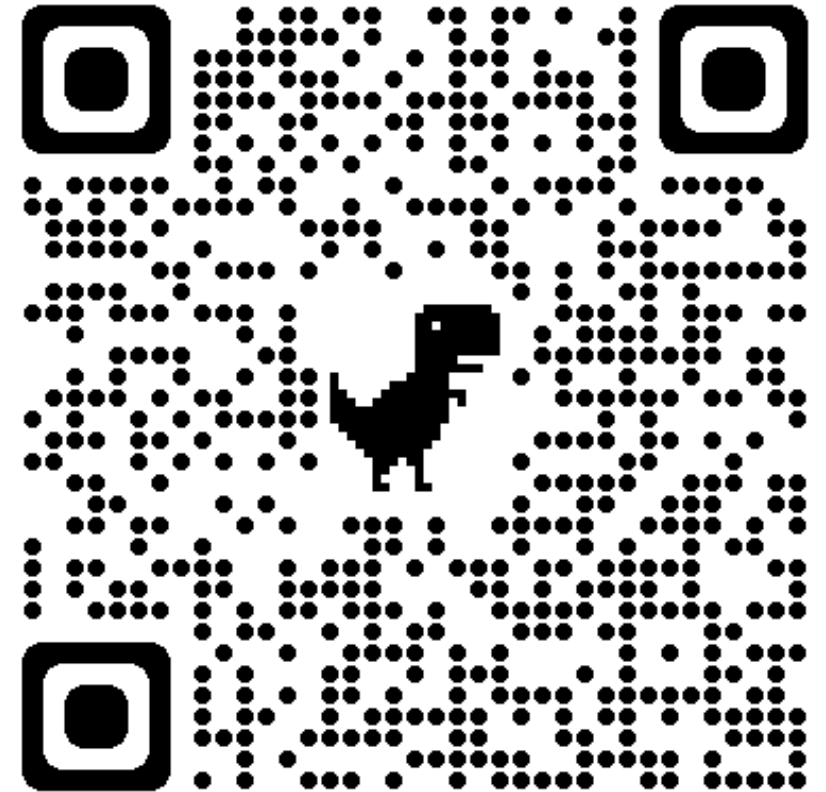
# Errors in PCV

- Not mixing tube

# Staining

- Follow manufacturers recommendations
- The Diff-Quick stain:
  - fixative agent (methanol, blue)
  - solution I (eosinophilic, orange)
  - solution II (basophilic, blue)
- Slides are dipped sequentially into each solution 6 times (or left for 10-15 seconds in each solution)
- Then water rinse (dip) and air dry
- Keep 2 stations – "clean" and "dirty" procedures



loudoun.nvcc.edu

# Anatomy of the microscope
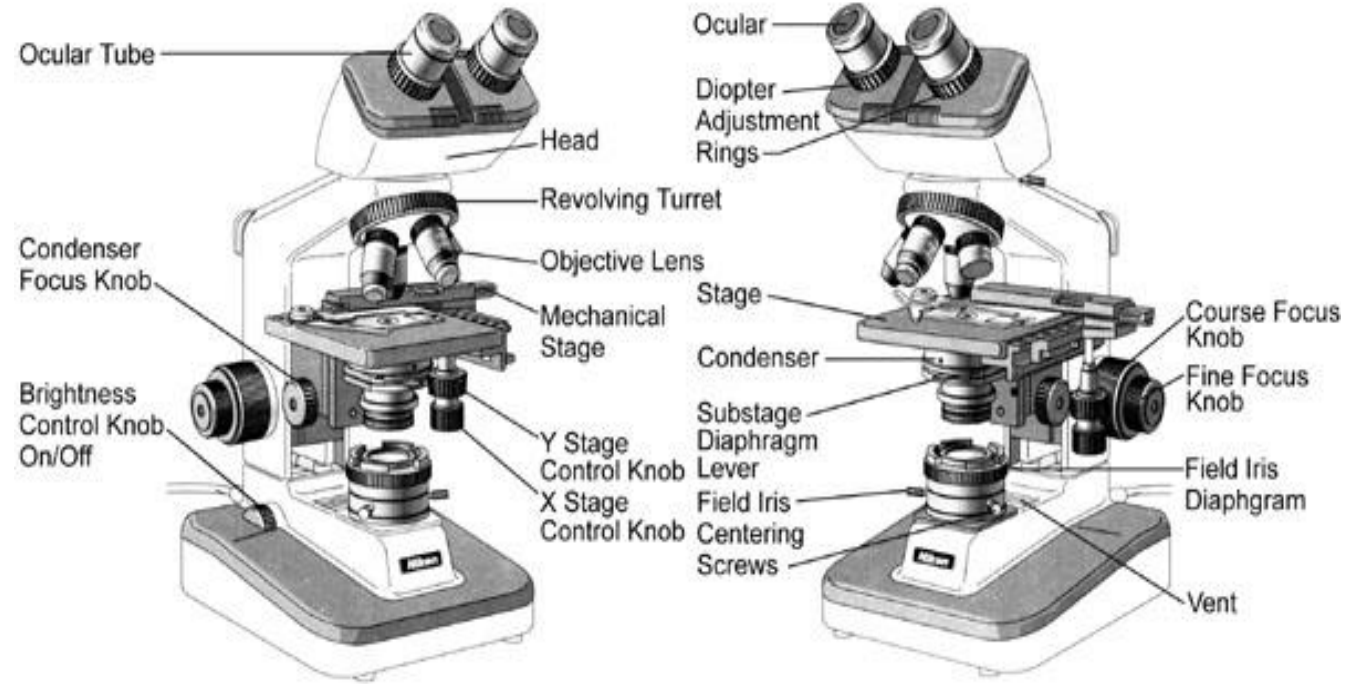
# Setting up the microscope

- Make sure the scope is clean!
- Kohler illumination
  - Optimal contrast and sharpness of the image
1. Adjust ocular interpupillary distance
2. When turning on light source – use mid brightness
3. Make sure the circle is in the centre at 4x mag – adjust accordingly
4. Condenser is adjusted all the way up close to the stage
5. Condenser opening setting – Olympus (0.6-0.9 or 60-90% open)
6. Field iris diaphragm is open – 90-100%

  - Blue light filter optional



IDEXX Newsletter – Under the Microscope
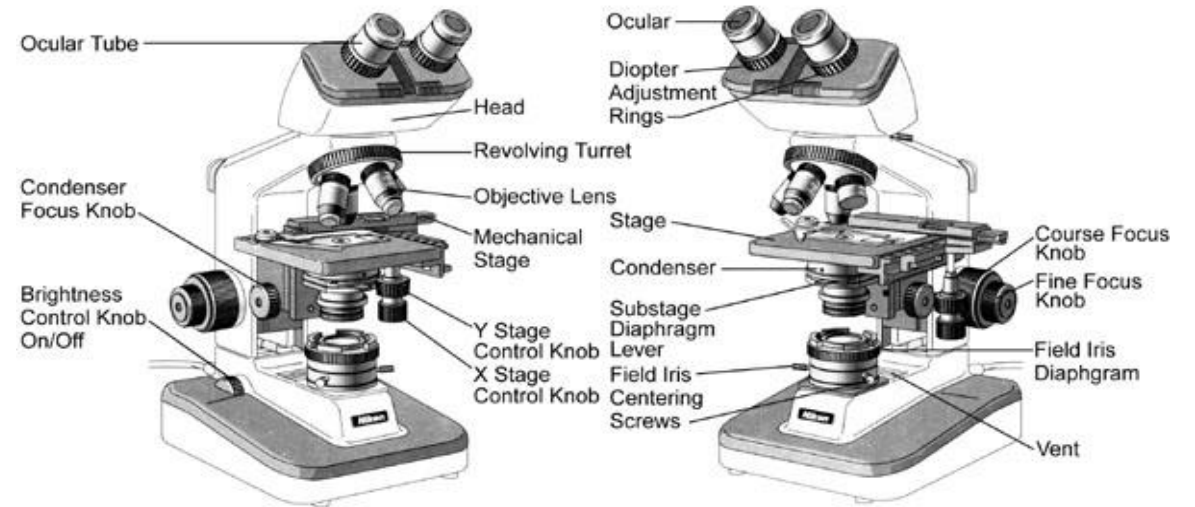
# Tips on using the microscope

- Don't go straight to 100x
    - Start at 4x

- Use both hands to "drive" and focus the slide on the stage

- Use appropriate immersion oil

- Use a cover slip on your glass slide when using objective lens 20x and 40x **(i.e. 1 drop of oil, place cover slip)**
    - Add another drop of oil on top of the cover slip for 100x immersion oil objective
    - Microscope light refractility index is optimal with use of a cover slip

# Value of a peripheral blood smear evaluation

- Thrombon (Platelets)
  - Validate numerical data – platelet count
  - Assessing clumping

- Leukon (WBCs)
  - Validate numerical data – WBC count and differential
  - Characterise an inflammatory process (left shift and toxic changes)
  - Presence of any abnormal cells

- Erythron (Erythrocytes or RBCs)
  - Validate numerical data – RBC density
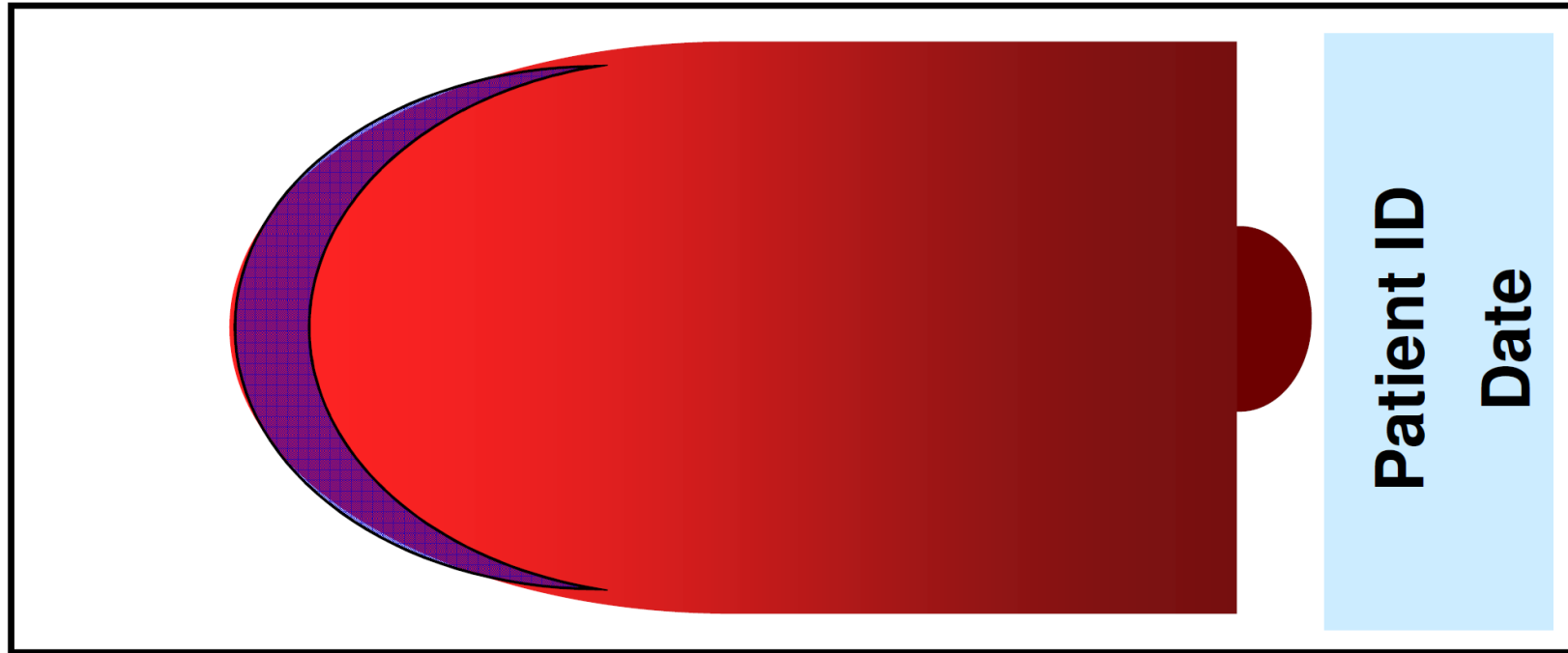  - May provide information on pathophysiology of an anaemia

## Know what is normal!

# How to effectively assess the blood smear
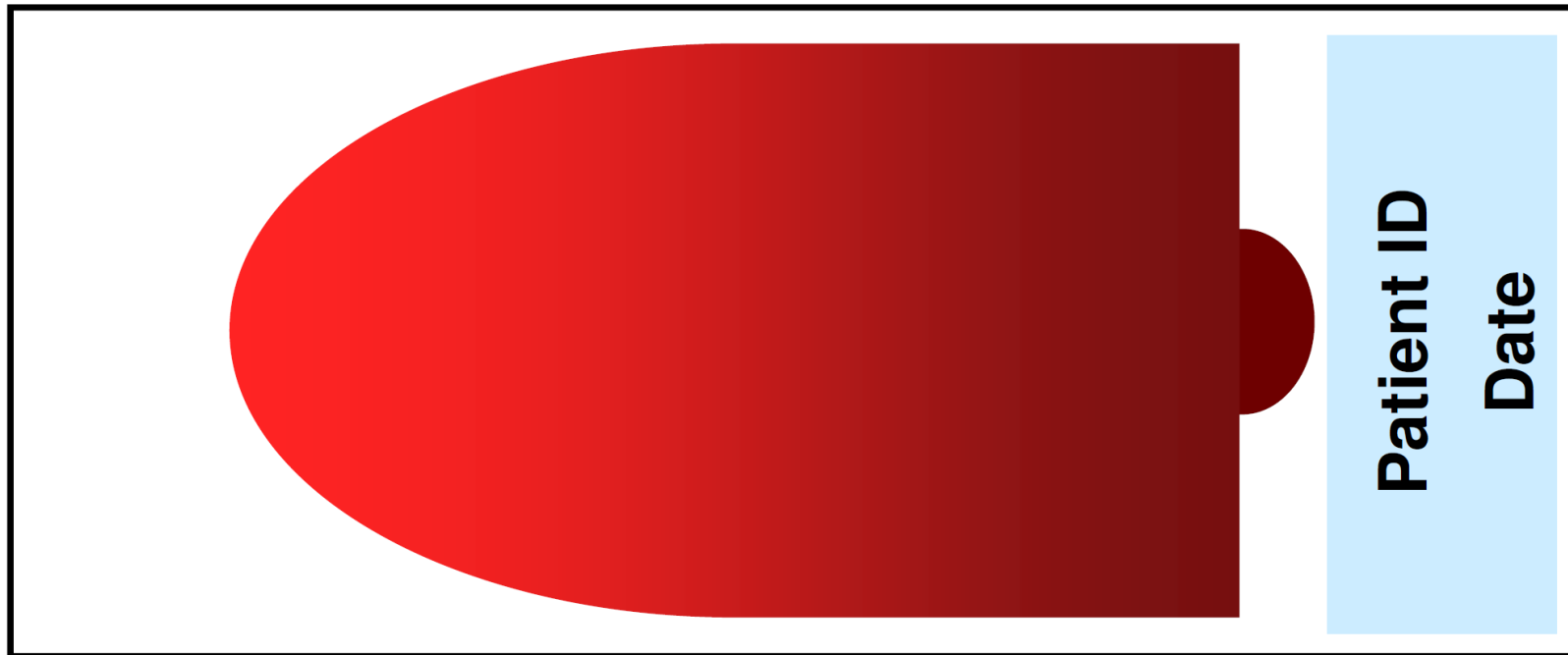
- Feathered edge
- Body
- Monolayer

Patient ID

Date

# How to effectively assess the blood smear



Feathered edge
- Platelet clumps
- Microfilaria
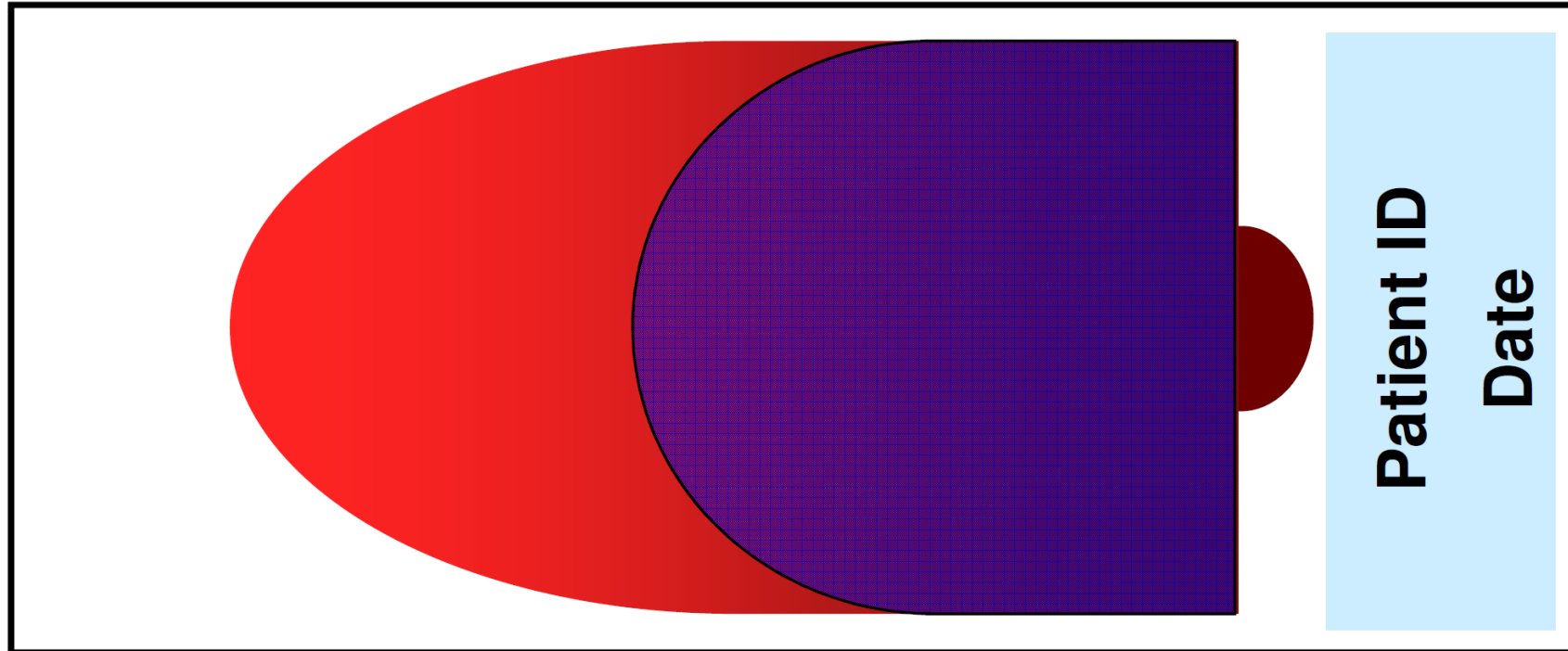- Large atypical cells

Patient ID
Date

# What magnification?

- Start at 4x or 5x

- Is your smear smooth or is there irregularity?

- Identify the feathered edge

- Check for platelet clumps (at 10x)

- Look for
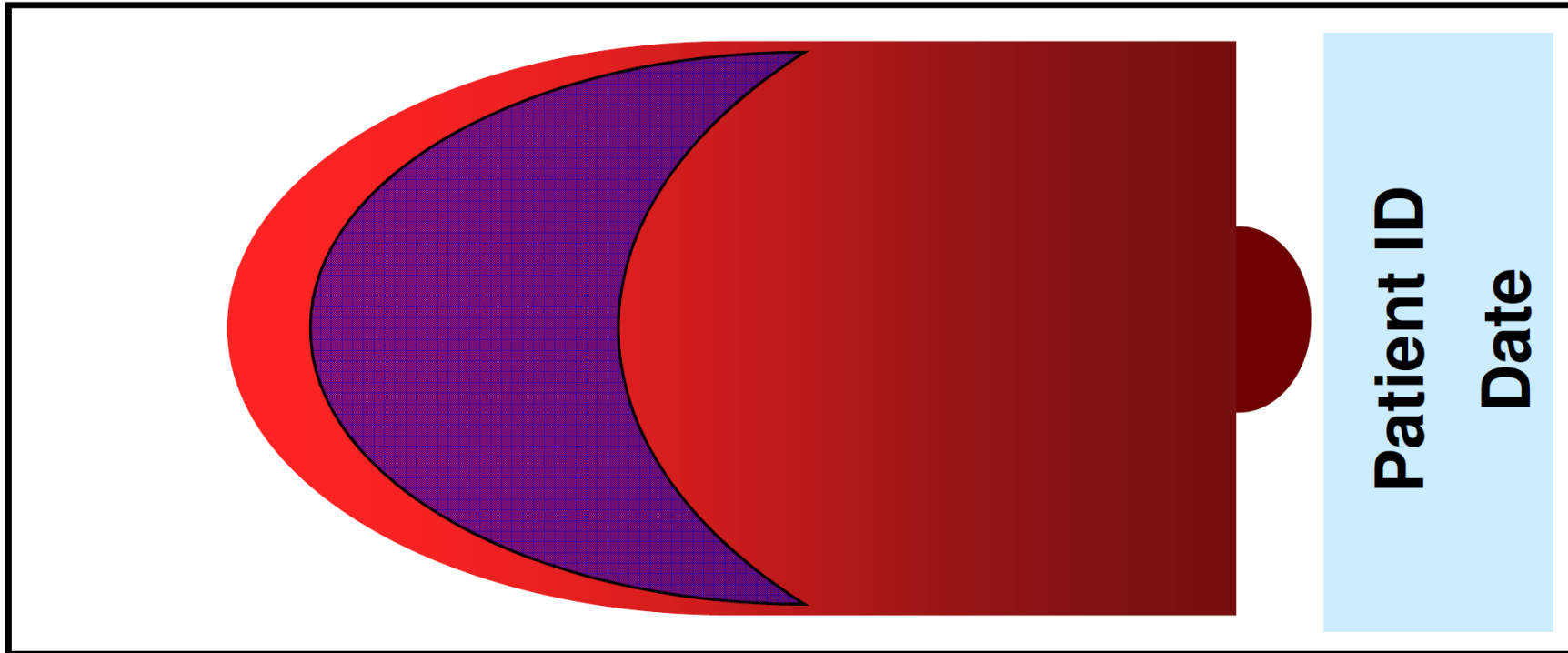    - Atypical cells – mast cells, neoplastic
    - Parasites – e.g. heartworm
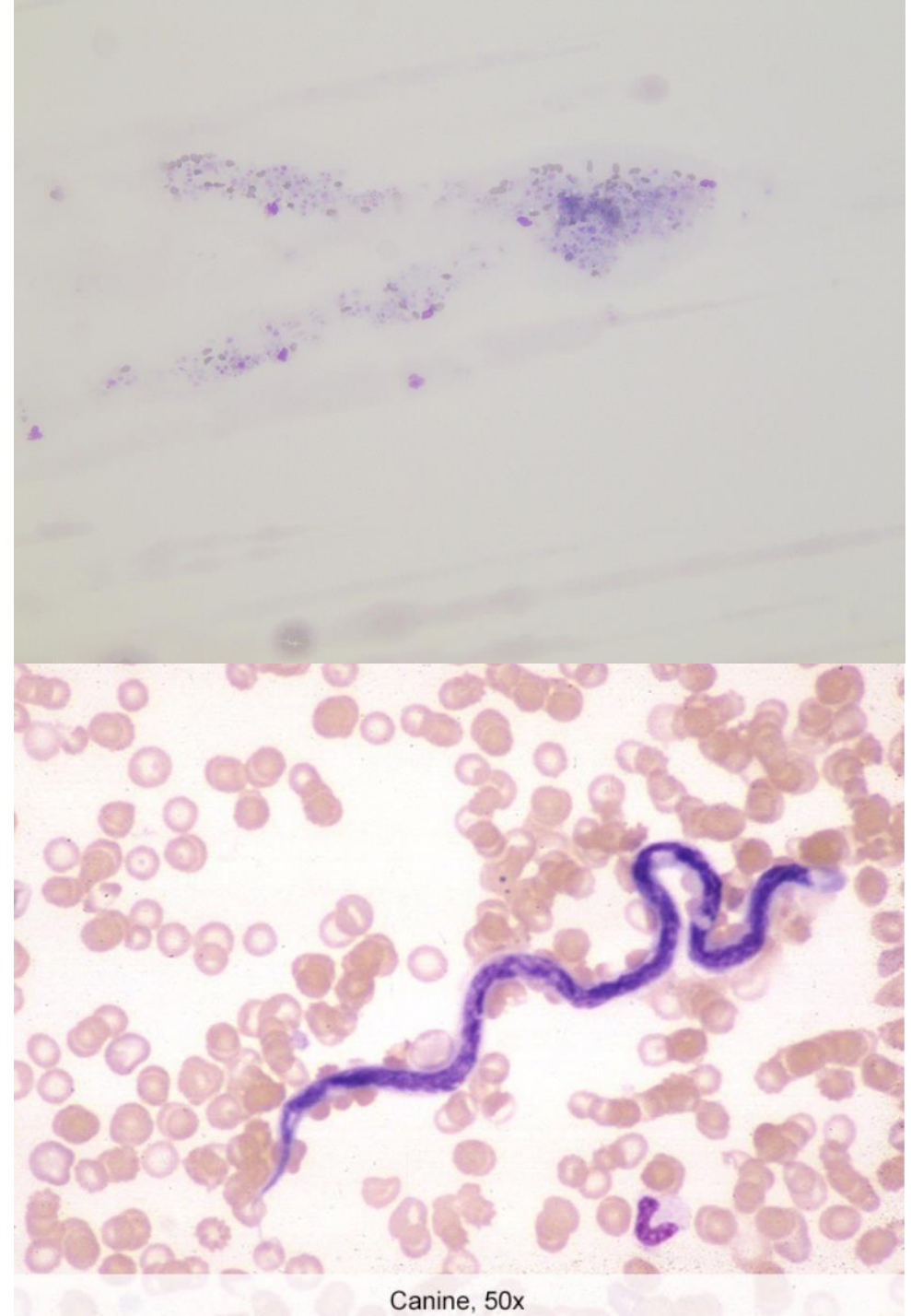
Canine, 50x

# How to effectively assess the blood smear



Body
- Rouleaux
- Agglutination

# How to effectively assess the blood smear



Monolayer
 - Platelet number estimation
 - Leucocyte number estimation
 - Manual differential
 - Morphologic evaluation of WBCs and RBCs
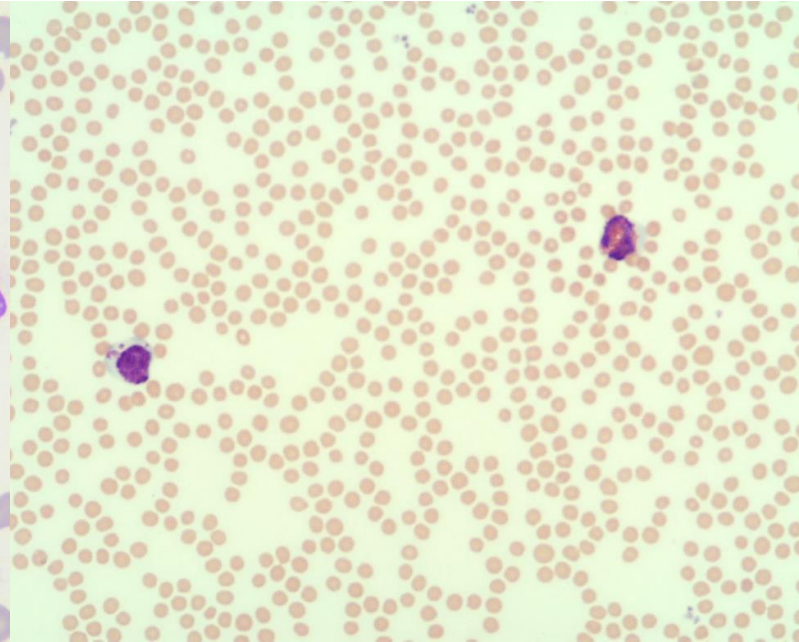
# How to look at a blood smear

- Find the monolayer - Move to 20x mag

- Look at the overall cellularity - get the broad picture
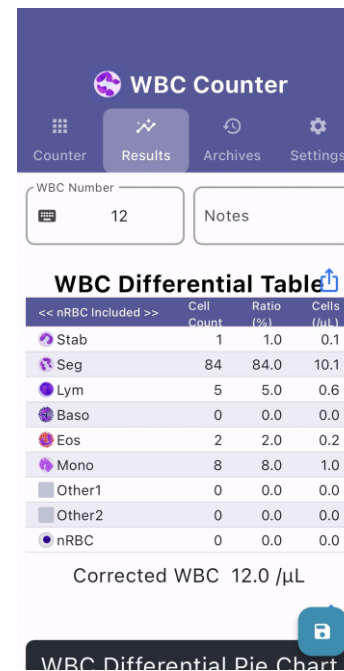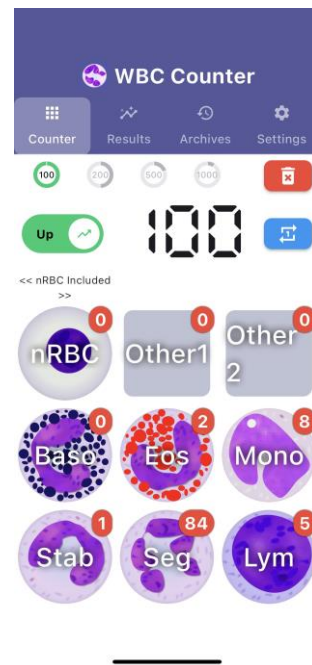
- Are leukocytes and platelets clumped?
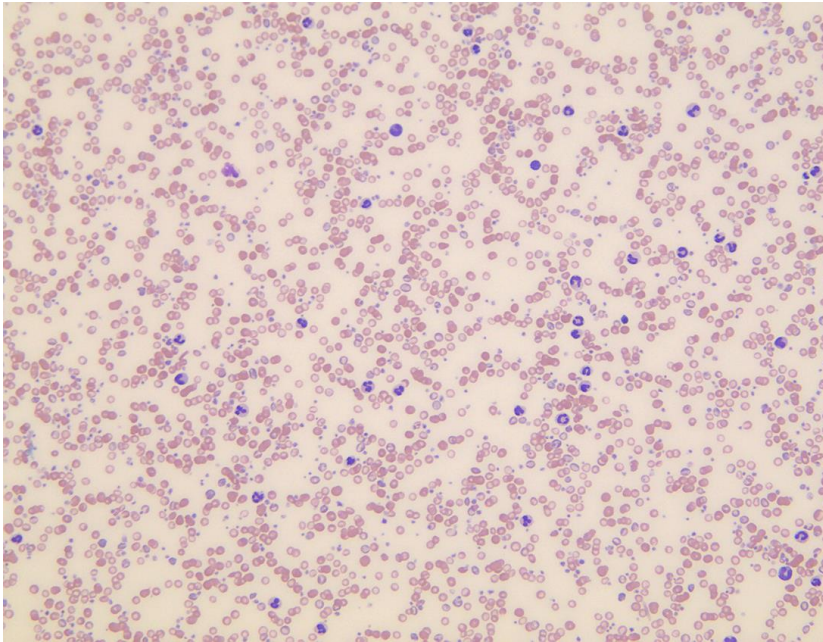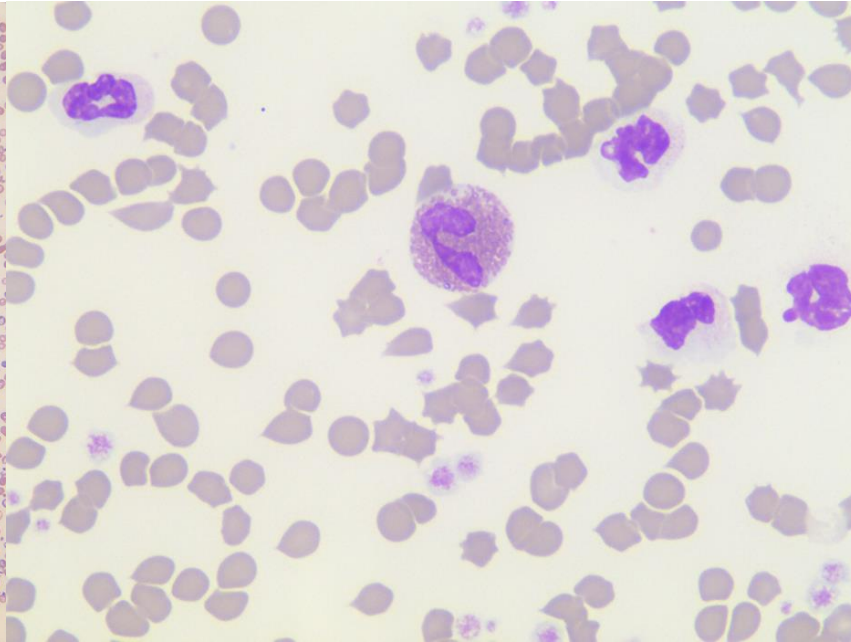


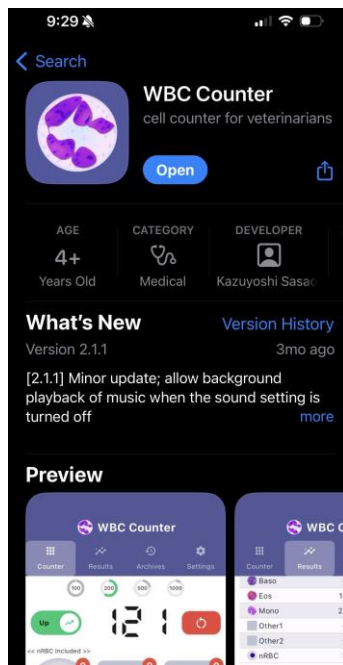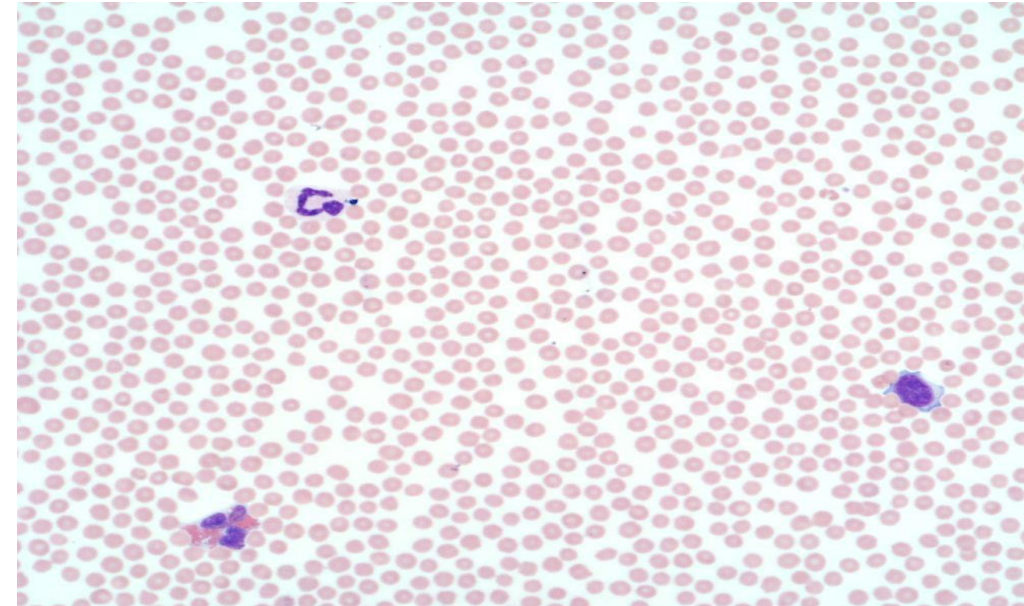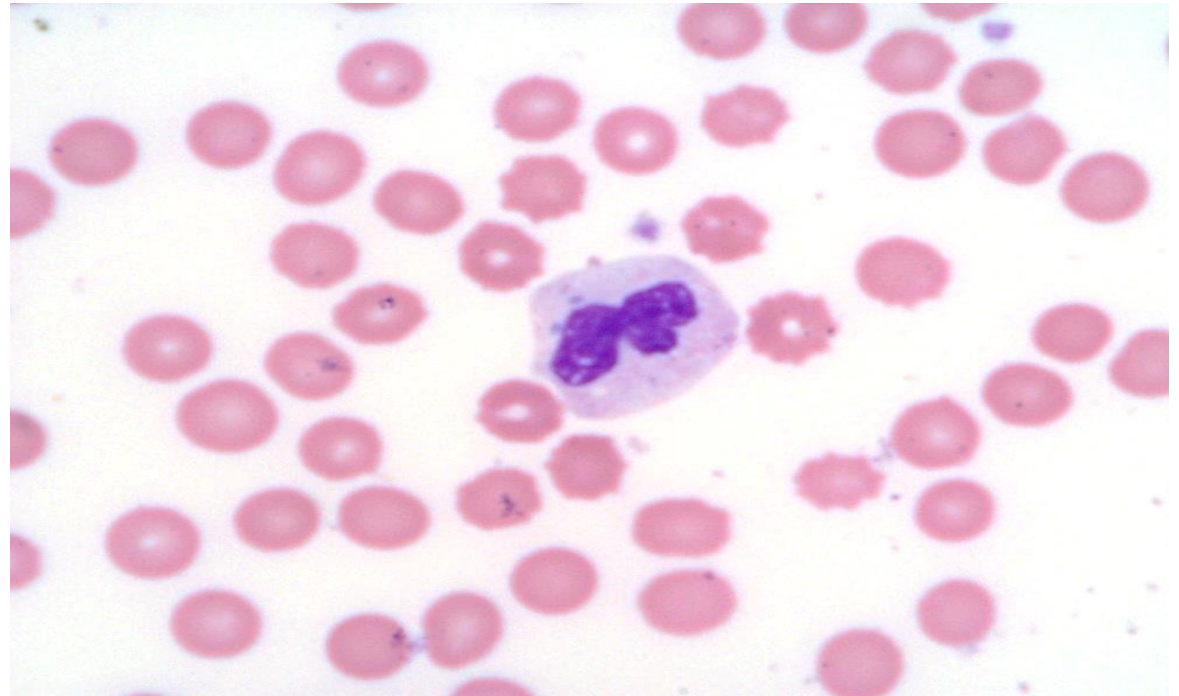A                                    B                                    C

# How to look at a blood smear

- Perform a 100-cell differential count at 40x

- Write it down or use apps
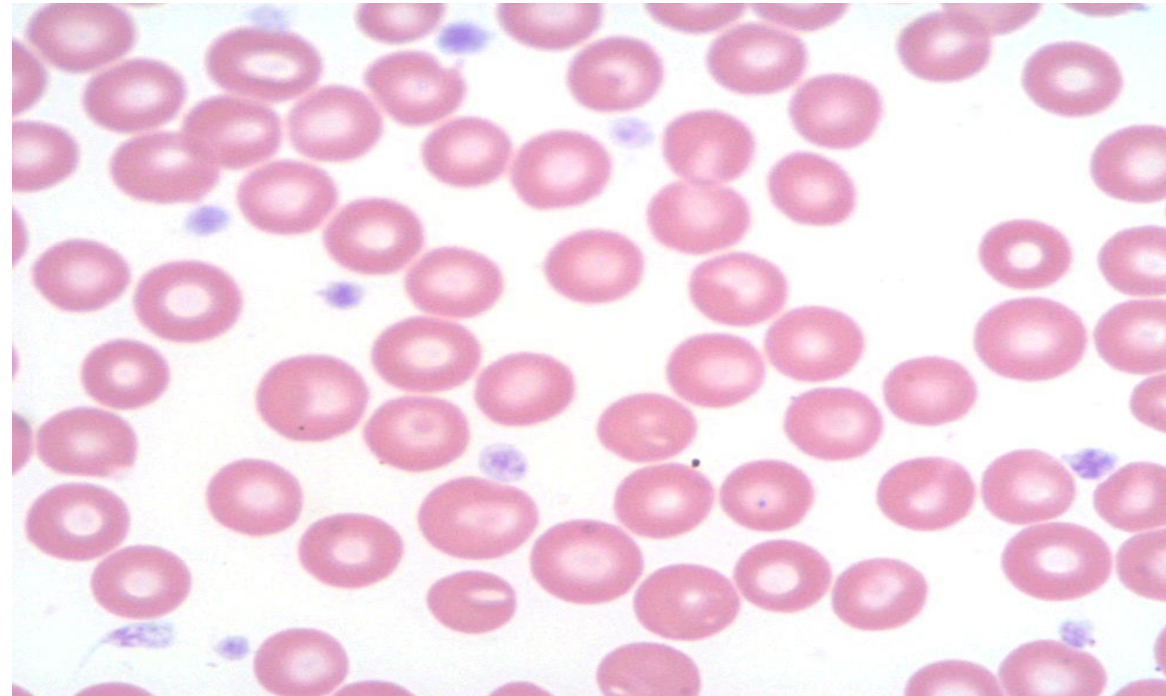
# How to look at a blood smear

- Move to 40x lens
- Look at morphology – platelets, erythrocytes and WBCs
- Look at the background!

# How to look at a blood smear

- Move to 100x lens

- Look again at the erythrocytes

- Look for uniformity, colour, shape, size and parasites
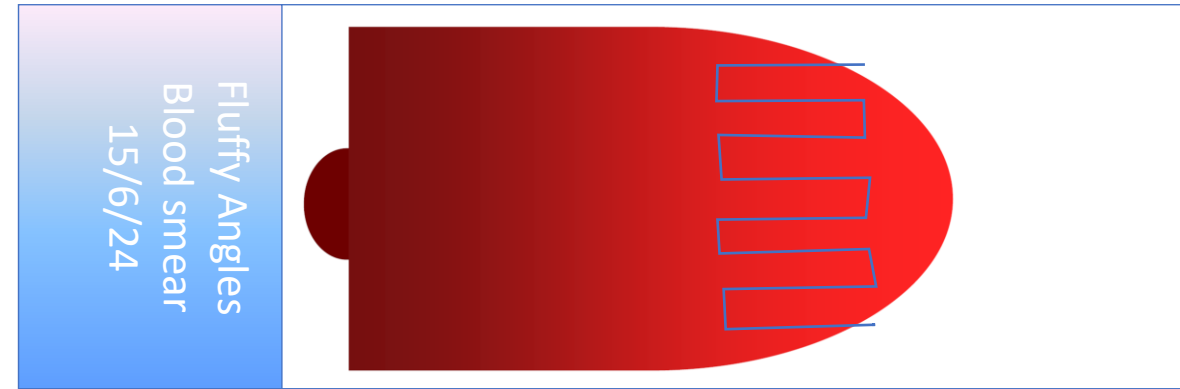
- Count the platelets per 100x field

*Average plt/100x field x 20 = est. plt count*

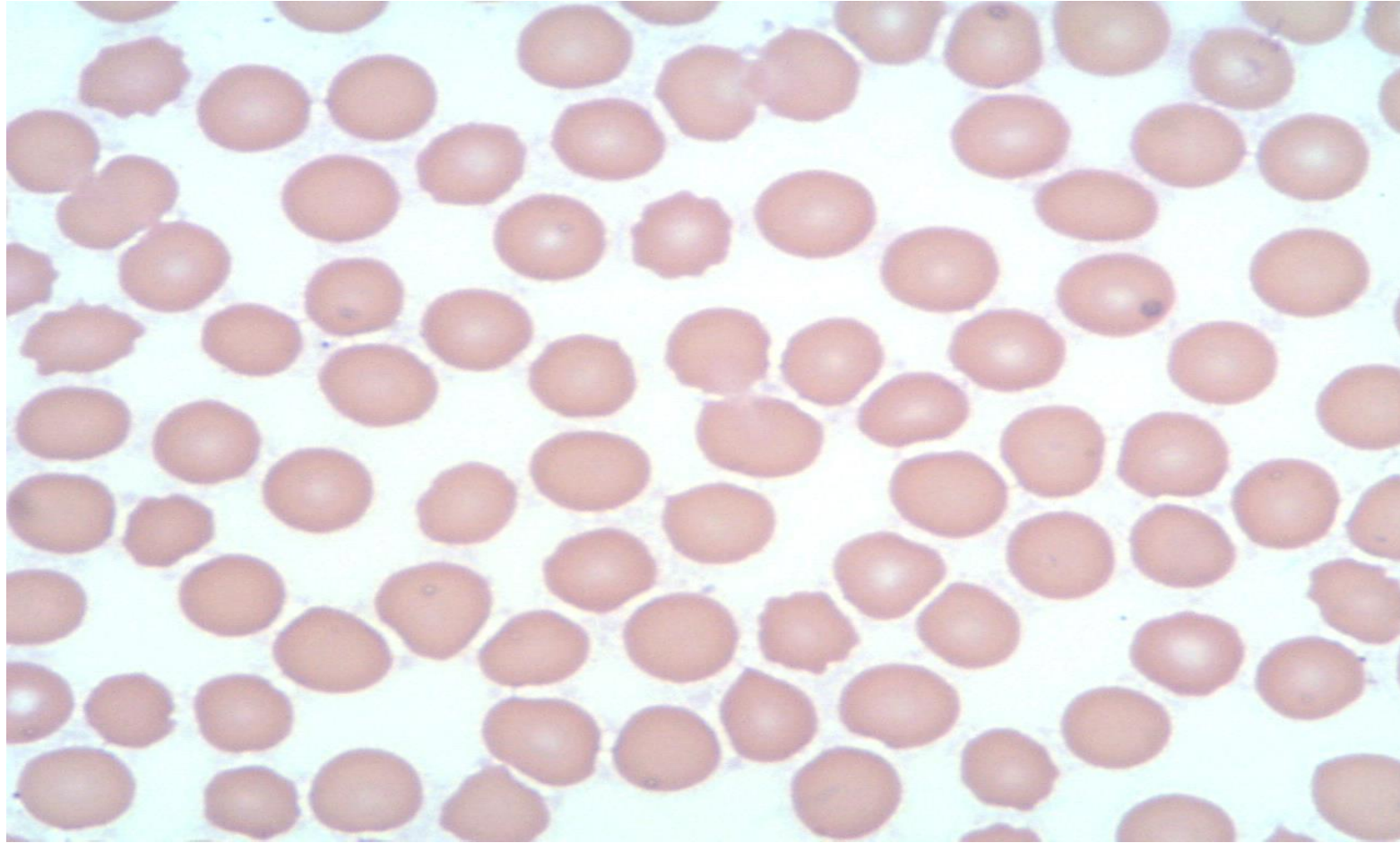# A Systematic Approach to a Slide



hutton-in-the-forest.co.uk

Castellation

Fluffy Angles
Blood smear
15/6/24
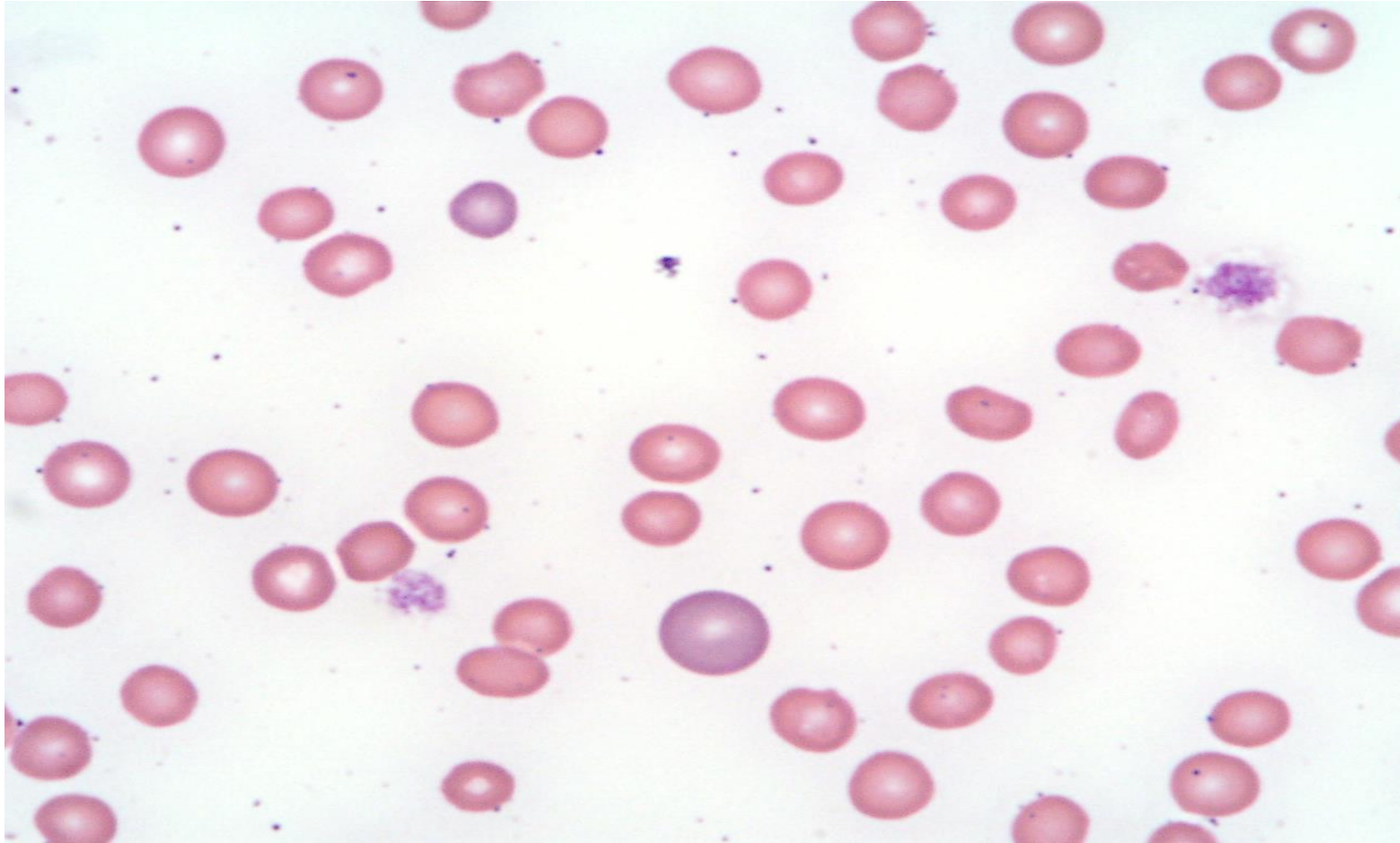
Be consistent

# Normal RBC density



Dog blood

x 1000

# Abnormal RBC density - anaemia



Dog blood - anaemia          x 1000

# Five common blood smear review findings (RBCs)

1. Anisocytosis

2. Polychromasia

3. Rouleaux vs Agglutination

4. Spherocytes

5. Echinocytes (vs acanthocytes)

# Anisocytosis

- Variation in RBC size between the smallest and largest cells

- Increased proportions of larger red blood cells than normal, smaller red blood cells than normal or a combination of both.

- Correlates with MCV and RDW



# Polychromasia

- Variation in colour among RBCs

- Bluish colour in RBCs = RNA

- Larger than mature RBCs

- Polychromatophils = reticulocytes
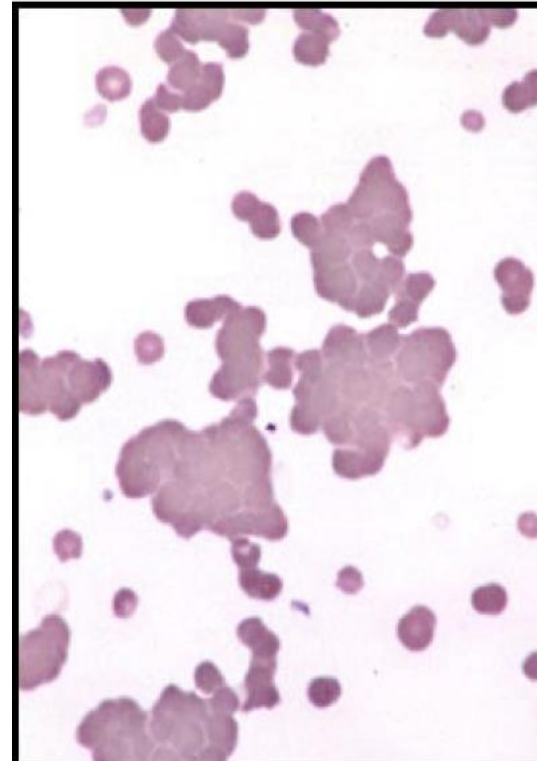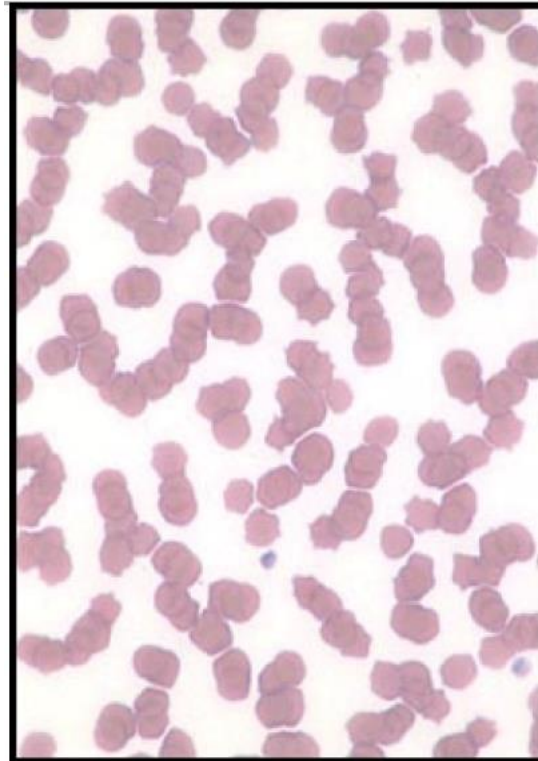
- Few are normal in dogs and cats
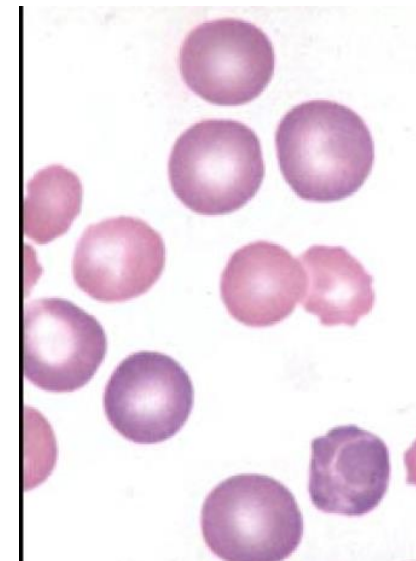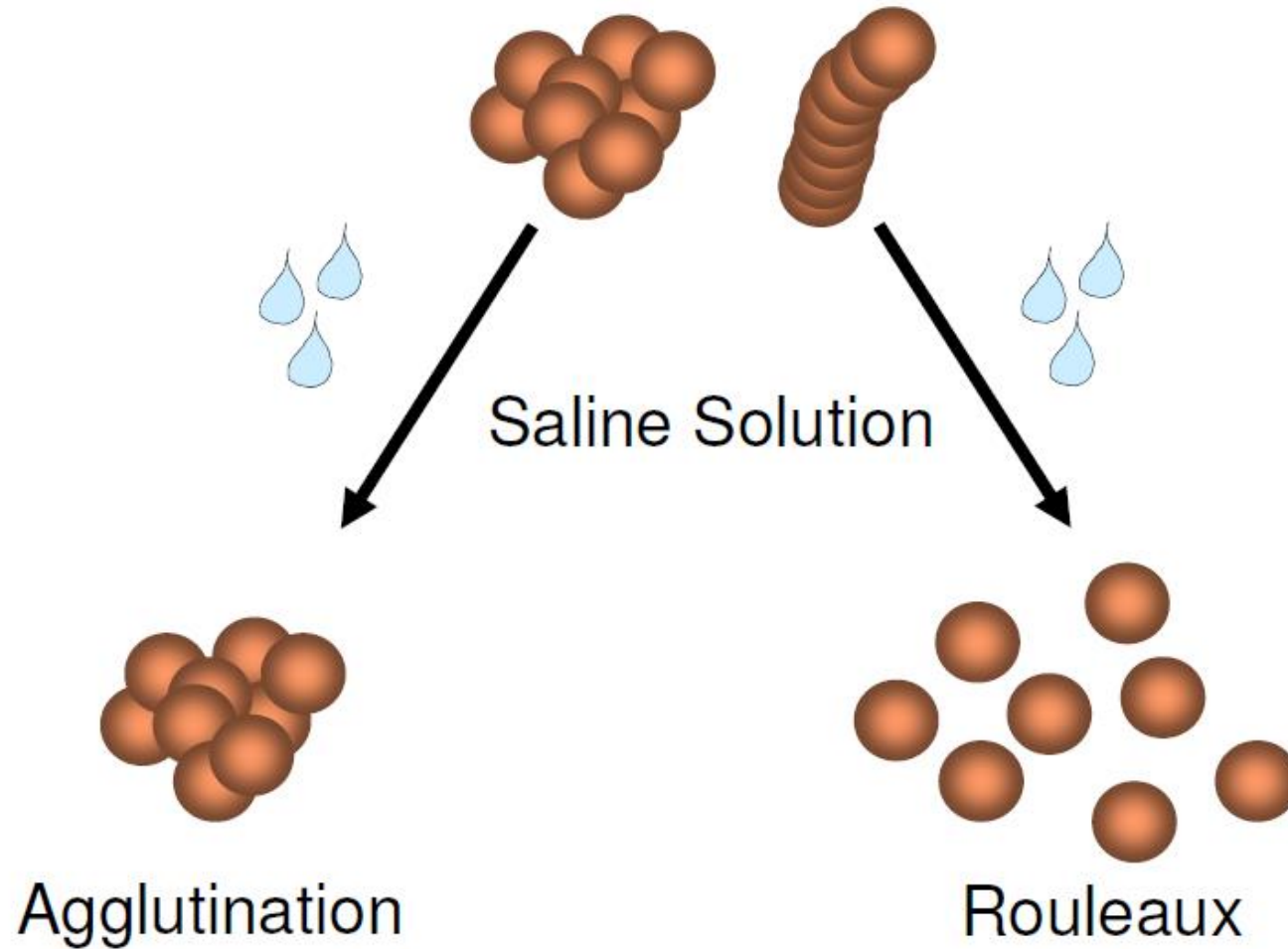
# Is it truly agglutinating?

**Rouleaux**                    **Agglutination**

- Lining up
- "Stack of coins"
- Low numbers in cats and horses
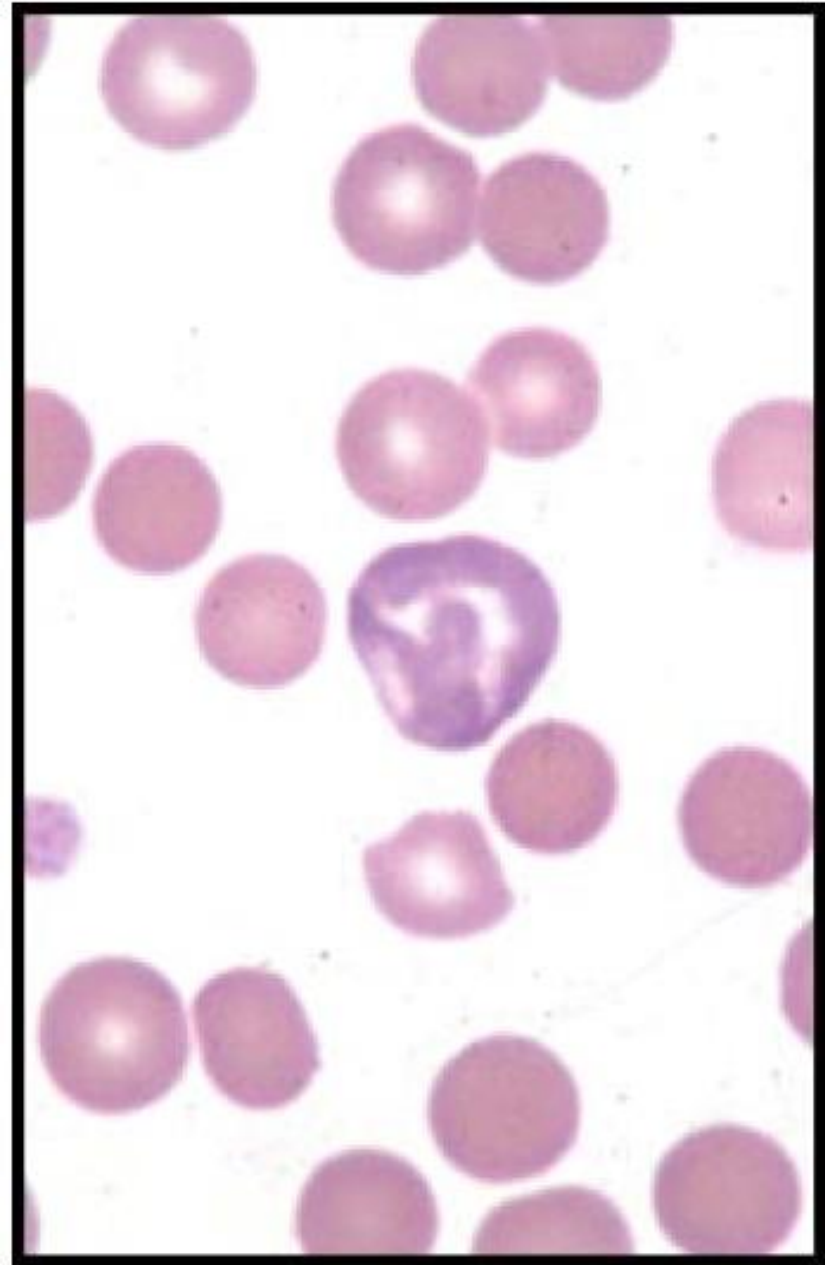- Can be seen with high protein levels



- 3D clumps of RBCs
- Disorganised
- Immune-mediated mechanism

# Saline agglutination test

# Spherocytes

- Smaller than normal mature RBC
- Dense staining
- No central pallor (in dogs)
- Very difficult to ID in cats
- Lose their normal biconcave shape
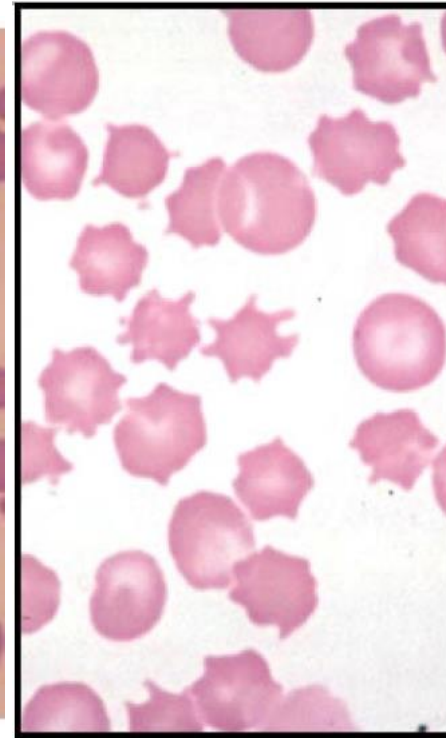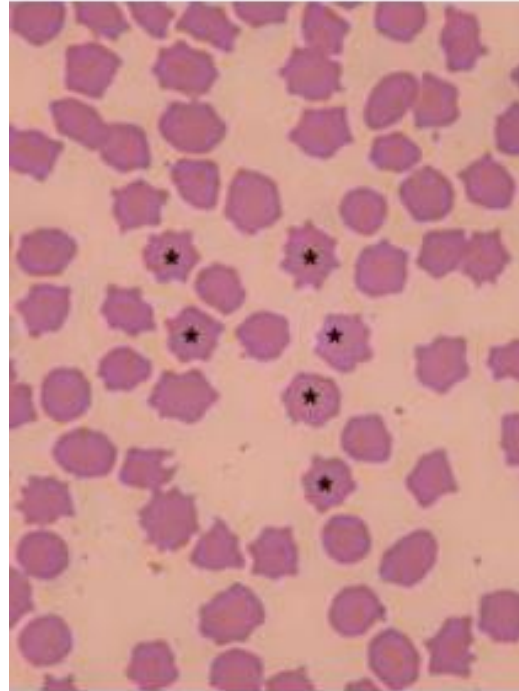- Immune mediated destruction (extravascular)
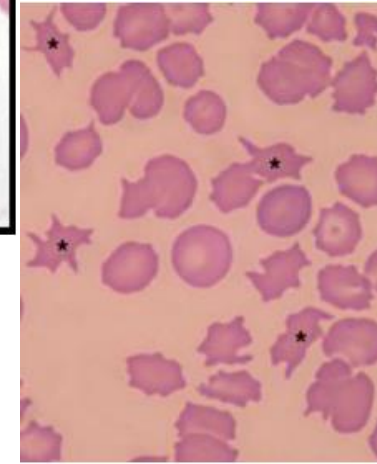
# They look spiky?!

**Echinocytes**

**Acanthocytes**

- Expansion of outer leaflet of RBC
- Small surface projections
  - Regular in size
  - Small spikes
- Artifact, electrolyte depletion, snake envenomation, some bacterial infections
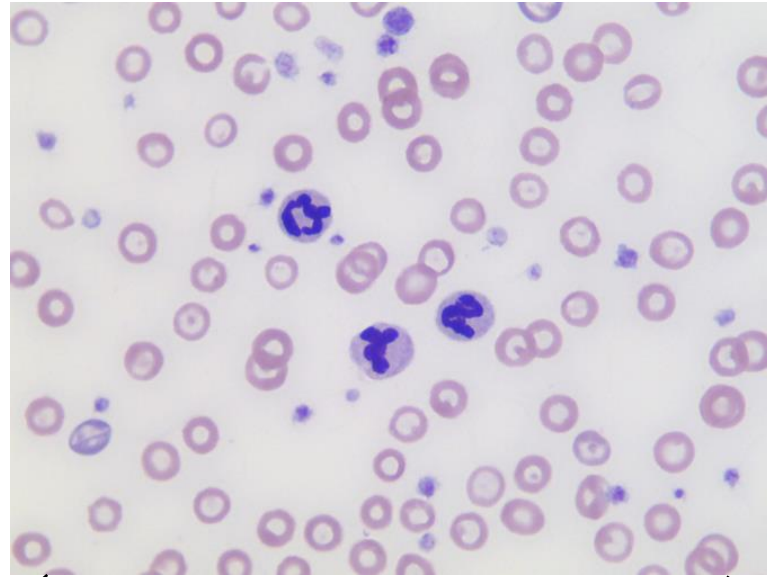




- Spherical with 2-10 surface projections
  - Variably sized
  - "Finger-like"
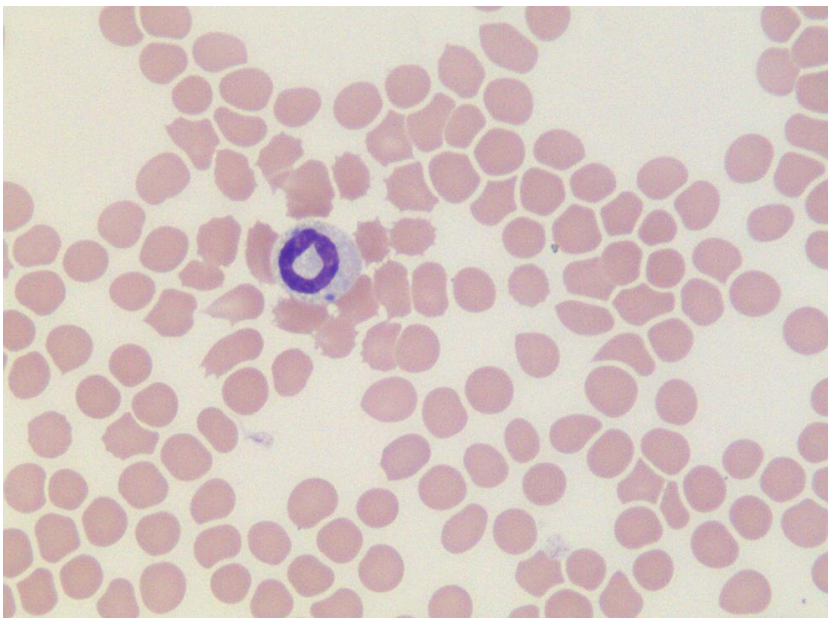- Supportive of potential liver, splenic or metabolic disorders

# Five blood smear review findings (WBCs)

1. Neutrophil identification

2. Band neutrophil ID
   - and toxic changes

3. Eosinophil ID
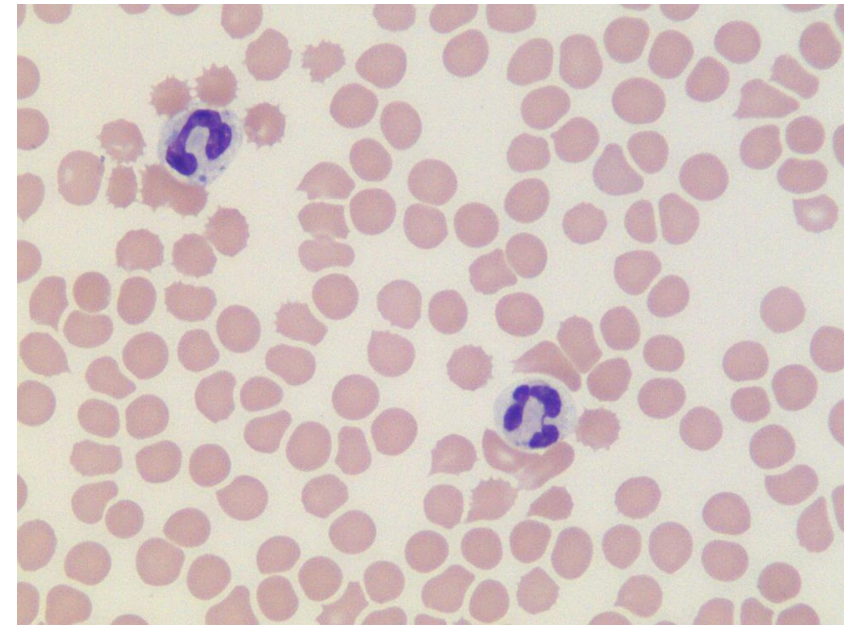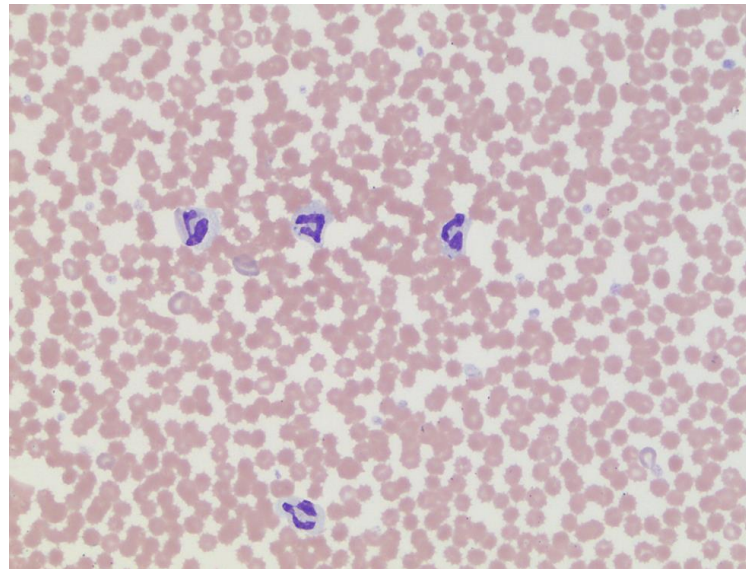
4. Lymphocyte ID

5. Monocyte ID

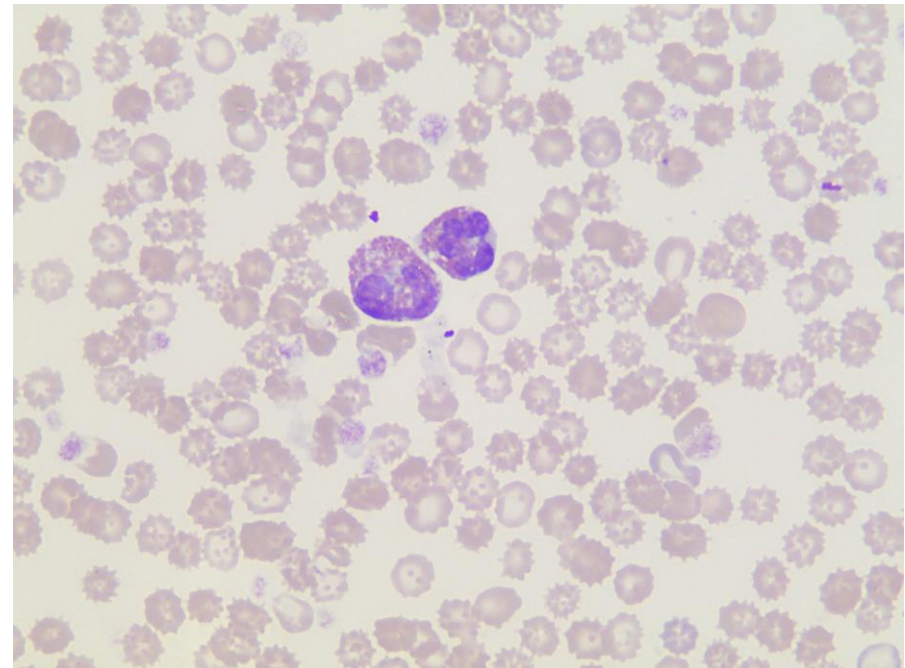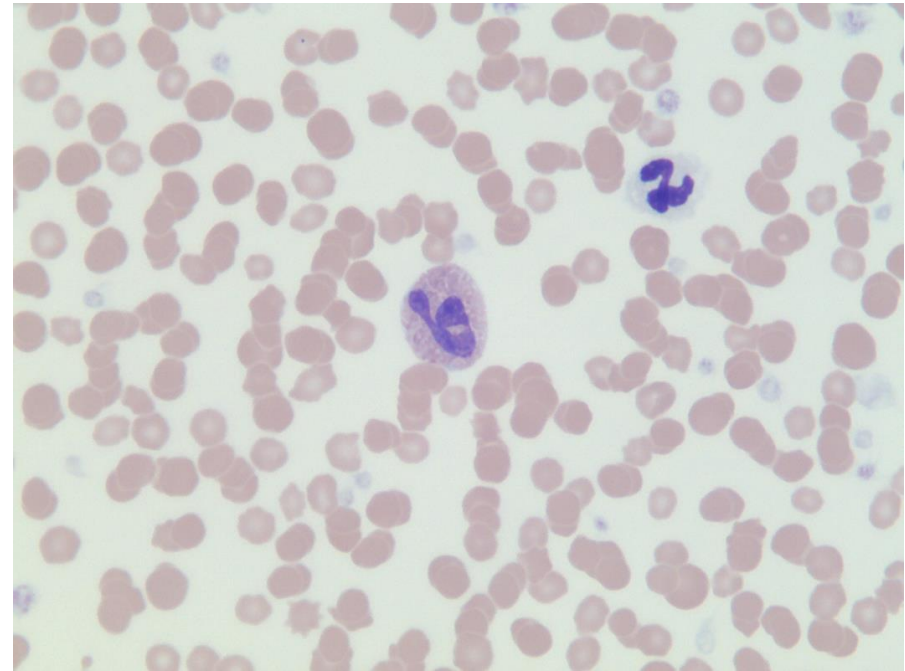# Neutrophils



## Band neutrophils



## Toxic changes

# Eosinophils

- Absent or present in low numbers
- Slightly larger than a neutrophil
- Nucleus is similar to a neutrophil
- Multiple reddish to red-orange granules

# Lymphocytes

- Second most common leukocytes
- 8-10 micrometres in diameter
- Round
- Round to oval nucleus with smooth and clumped nuclear chromatin
- Scant cytoplasm
- High NC ratio
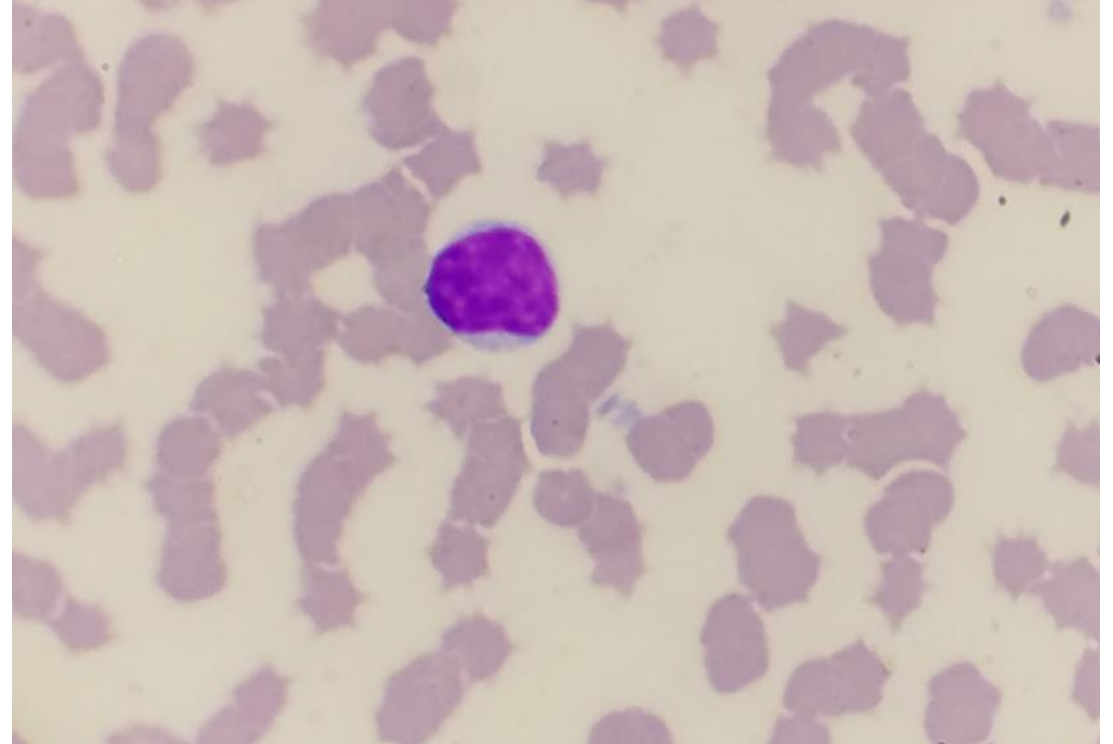
# Monocytes

- Largest leukocyte
  - 15-20 micrometres in diameter
- Round to angular
- Nucleus is variable shape
- Chromatin is lacey
- Cytoplasm is blue grey and can contain vacuoles
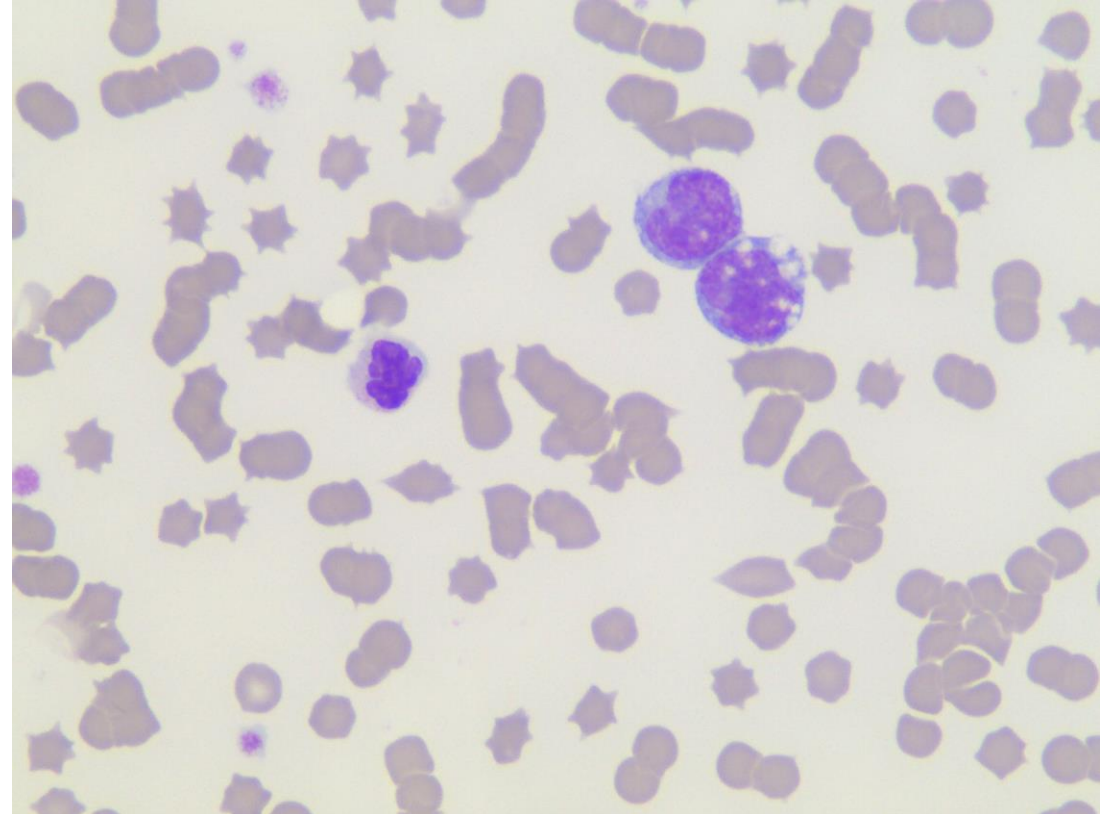
# Case

- Signalment: 12 yo MN Maltese Terrier

- History: Acute onset lethargy, anorexia, weakness.
  - PCV13/73
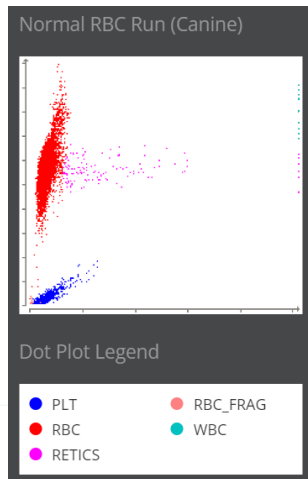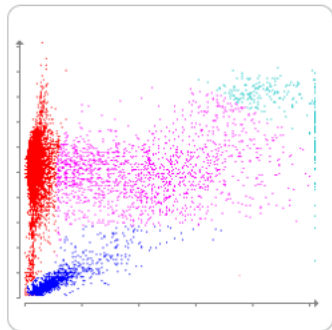  - Icteric serum
  - Suspect IMHA
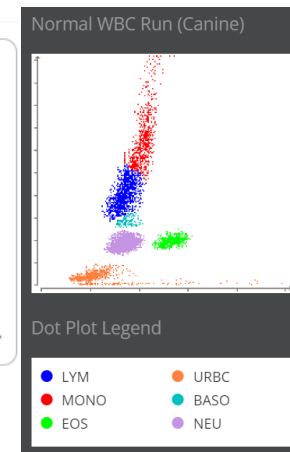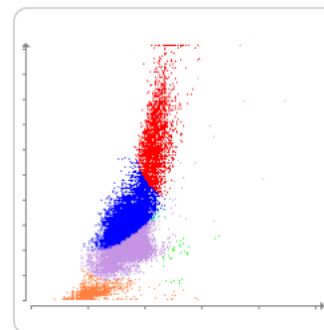
# Case

## Hematology
5/7/24 4:03 AM

| Test | Value | Reference Range | |
|------|-------|-----------------|--|
| RBC | 1.46 | 5.65 - 8.87 x10^12/L | ◄ |
| Hematocrit | 0.139 | 0.373 - 0.617 L/L | ◄ |
| Hemoglobin | 73 | 131 - 205 g/L | ▮ |
| MCV | 95.2 | 61.6 - 73.5 fL | ► |
| MCH | 50.0 | 21.2 - 25.9 pg | ► |
| MCHC | 525 | 320 - 379 g/L | ► |
| RDW | 26.7 | 13.6 - 21.7 % | ▮ |
| % Reticulocytes | 6.9 | % | |
| Reticulocytes | 100.3 | 10.0 - 110.0 K/µL | ▮ |
| Reticulocyte Hemoglobin | 19.1 | 22.3 - 29.6 pg | ▮ |

| Test | Value | Reference Range | |
|------|-------|-----------------|--|
| WBC | 33.27 | 5.05 - 16.76 x10^9/L | ▮ |
| % Neutrophils | *52.1 | % | |
| % Lymphocytes | *38.9 | % | |
| % Monocytes | *8.8 | % | |
| % Eosinophils | 0.1 | % | |
| % Basophils | 0.1 | % | |
| Neutrophils | *17.35 | 2.95 - 11.64 x10^9/L | ▮ |
| Bands | *Suspected | | |
| Lymphocytes | *12.94 | 1.05 - 5.10 x10^9/L | ► |
| Monocytes | *2.93 | 0.16 - 1.12 x10^9/L | ▮ |
| Eosinophils | 0.03 | 0.06 - 1.23 x10^9/L | ▮ |
| Basophils | 0.02 | 0.00 - 0.10 x10^9/L | ▮ |
| Platelets | 125 | 148 - 484 x10^9/L | ▮ |
| PDW | 26.0 | 9.1 - 19.4 fL | ▮ |
| MPV | 14.4 | 8.7 - 13.2 fL | ▮ |
| Plateletcrit | 0.18 | 0.14 - 0.46 % | ▮ |

## RBC Run



Normal RBC Run (Canine)

Dot Plot Legend
- PLT
- RBC
- RETICS
- RBC_FRAG
- WBC

## WBC Run



Normal WBC Run (Canine)

Dot Plot Legend
- LYM
- MONO
- EOS
- URBC
- BASO
- NEU

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

Immature and/or toxic neutrophils likely present - Consider inflammation.

Increased RDW - Anisocytosis present - review blood film.

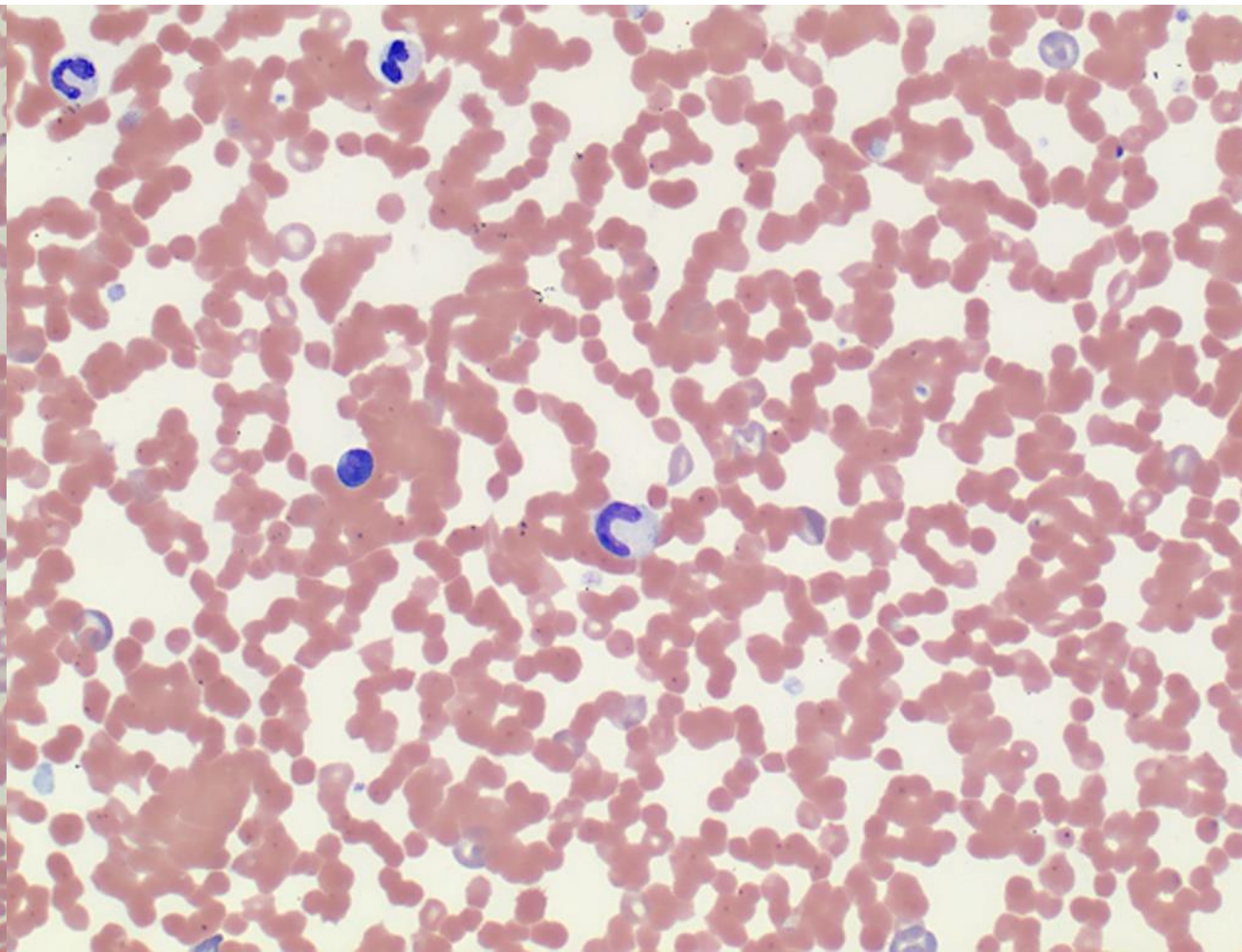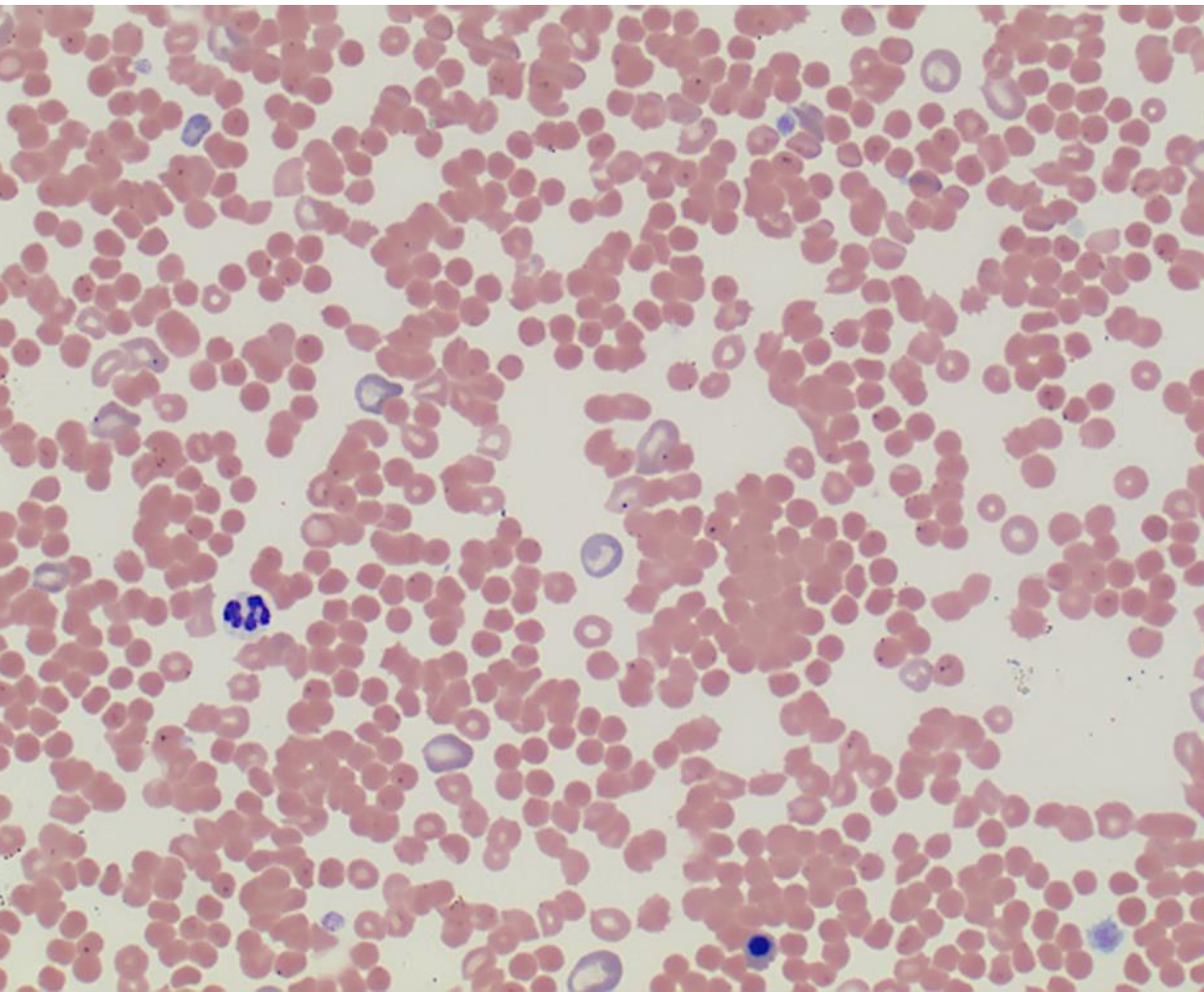Normal PCT - Likely adequate platelet mass.

Low RETIC-HGB - Decreased iron availability (consider inflammation, iron deficiency, PSS, breed-related microcytosis).

Increased MCHC or MCH - Consider hemolysis (including sample collection/handling), lipemia, and Heinz bodies.

# Case
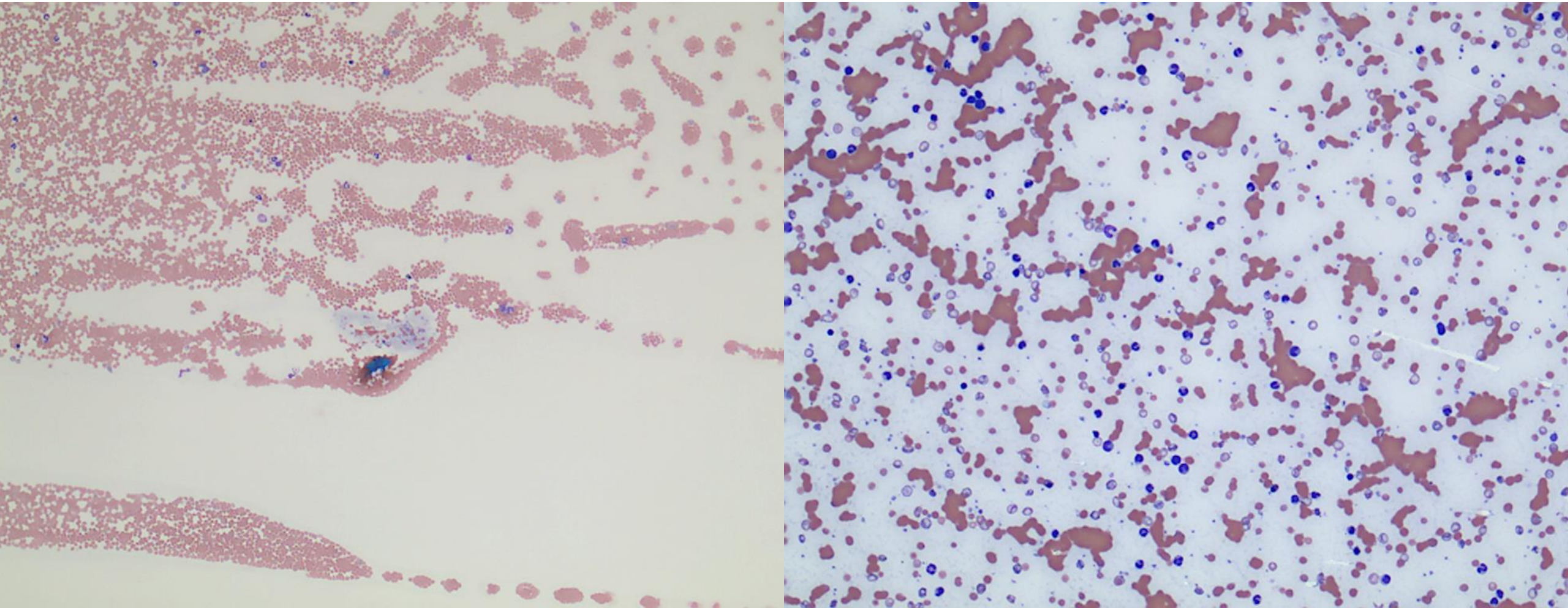
# Case

# Case

## Hematology — 5/7/24 7:45 AM / 5/7/24 4:03 AM

| | Test | Result (7:45 AM) | Reference | | Result (4:03 AM) |
|---|---|---|---|---|---|
| | RBC | 1.2 | 4.9 - 8.2 x10^12/L | | 1.46 |
| | Hematocrit | 0.10 | 0.35 - 0.58 L/L | | 0.139 |
| | Hemoglobin | 66 | 100 - 206 g/L | | 73 |
| | MCV | 83 | 64 - 76 fL | | 95.2 |
| | MCH | 55 | 21 - 26 pg | | 50.0 |
| | MCHC | 660 | 310 - 360 g/L | | 525 |
| | % Reticulocytes | 5.0 | 0.0 - 1.5 % | | 6.9 |
| | Reticulocytes | 60 | 10 - 110 K/µL | | 100.3 |
| | Reticulocyte Hemoglobin | 19.6 | 22.3 - 29.6 pg | | 19.1 |

| | Test | Result | Reference | | Result |
|---|---|---|---|---|---|
| | WBC | 21.5 | 4.5 - 17.0 x10^9/L | | 33.27 |
| | % Neutrophils | 74.0 | % | | *52.1 |
| | % Bands | 8.0 | % | | |
| | % Lymphocytes | 11.0 | % | | *38.9 |
| | % Monocytes | 7.0 | % | | *8.8 |
| | % Eosinophils | 0.0 | % | | 0.1 |
| | % Basophils | 0.0 | % | | 0.1 |
| | % Nucleated RBCs | 58.0 | 0.0 - 2.0 per 100wbc | | |
| | Neutrophils | 15.9 | 3.5 - 12.0 x10^9/L | | *17.35 |
| | Bands | 1.7 | 0.0 - 0.2 x10^9/L | | *Suspected |
| | Lymphocytes | 2.4 | 0.9 - 3.5 x10^9/L | | *12.94 |
| | Monocytes | 1.5 | 0.0 - 1.1 x10^9/L | | *2.93 |
| | Eosinophils | 0.0 | 0.0 - 1.4 x10^9/L | | 0.03 |
| | Basophils | 0.0 | 0.0 - 0.1 x10^9/L | | 0.02 |
| | Platelets | 208 | 200 - 500 x10^9/L | | 125 |

**Platelet Observations:** Clumped and adequate

**Blood Film Evaluation:**
Mild toxic changes with Dohle bodies.
Marked agglutination
Positive for agglutination 1:5 at room temperature and 1:10 at 37°C.
Mild anisocytosis
Mild polychromasia
Marked spherocytosis

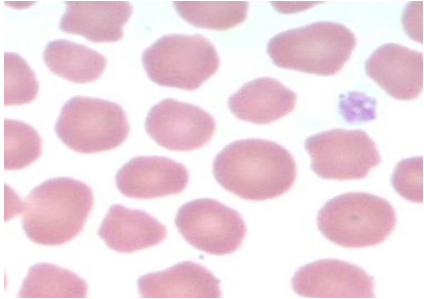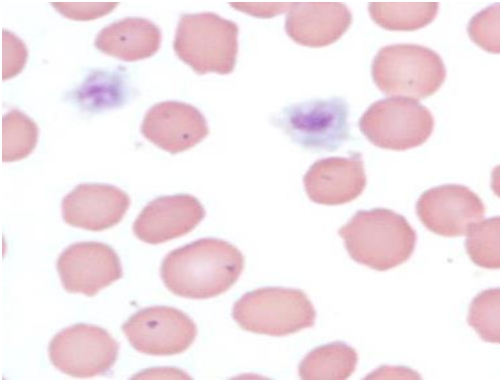Diagnosis – Immune mediated haemolytic anaemia (IMHA)
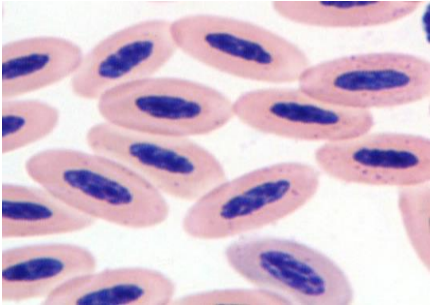
Questions?

# Normal RBC morphology - Quiz
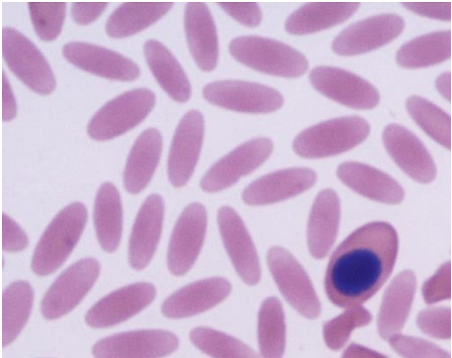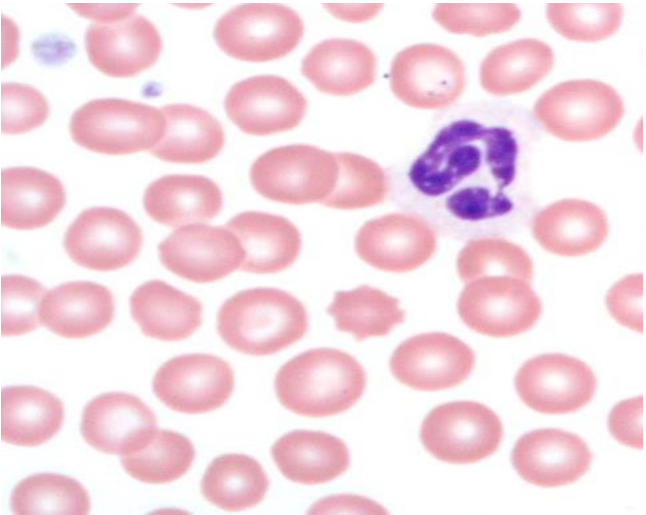


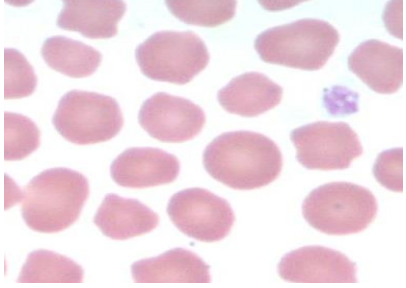Alpaca          Bird          Cat          Dog          Elephant          Horse          Sheep
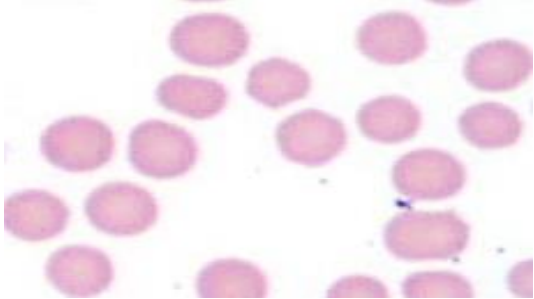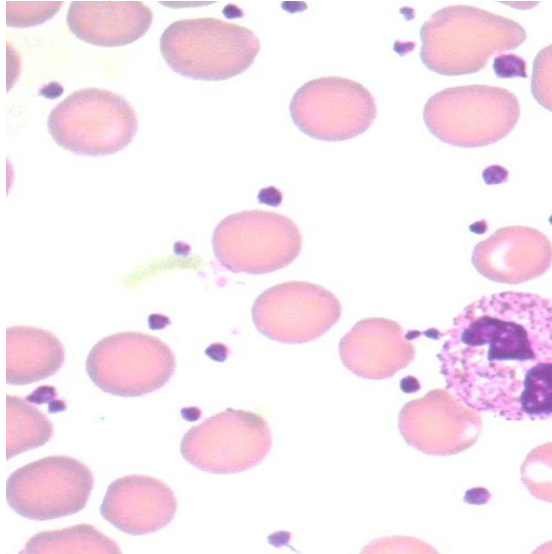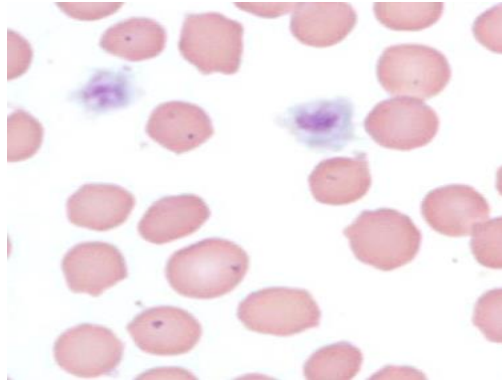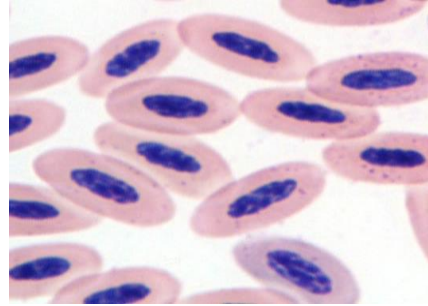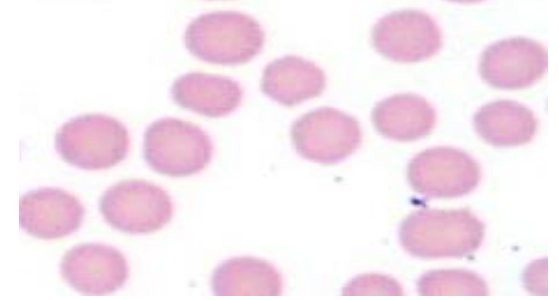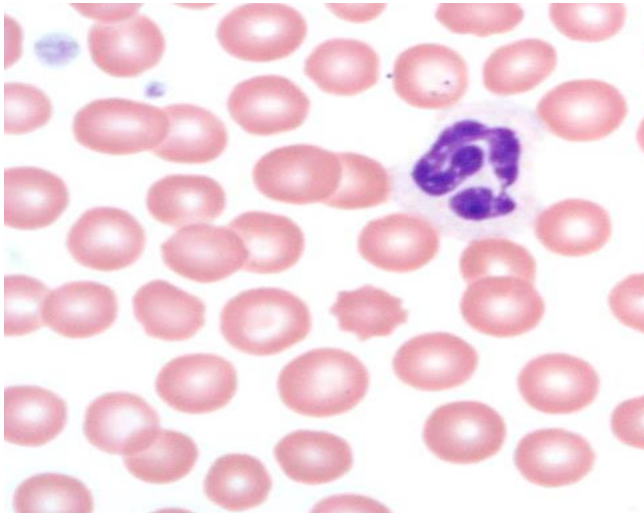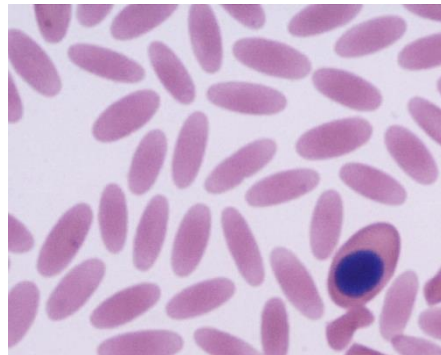
# Normal RBC morphology - Quiz


Cat


Sheep


Bird


Horse


Dog


Alpaca


Elephant