Beyond The Pale- The Approach to the Anaemic Patient

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Disclosures:

Employee of IDEXX, UK

Employee of AniCura, France

Anaemia

+ Clinical sign NOT a diagnosis
+ Decreased PCV/Hb/RBC
+ Relative or absolute
+ Regenerative or non-regenerative

Regenerative vs Non-regenerative

+ The approach to the anaemic patient hinges on whether the anaemia is regenerative or non-regenerative

+ Good blood film essential!

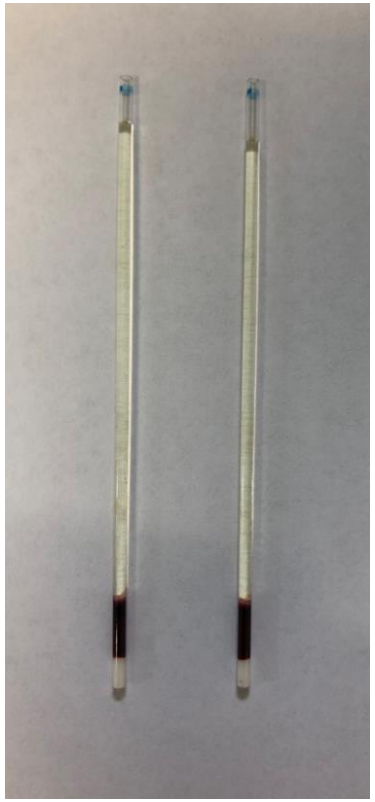
Avoid Sample Collection Errors

- Use largest vein possible
- Atraumatic sampling
- Avoid clots
- EDTA for haematology
  - Preserves red cell morphology
  - Fill tubes adequately
  - Analyse quickly

Haematocrit vs PCV

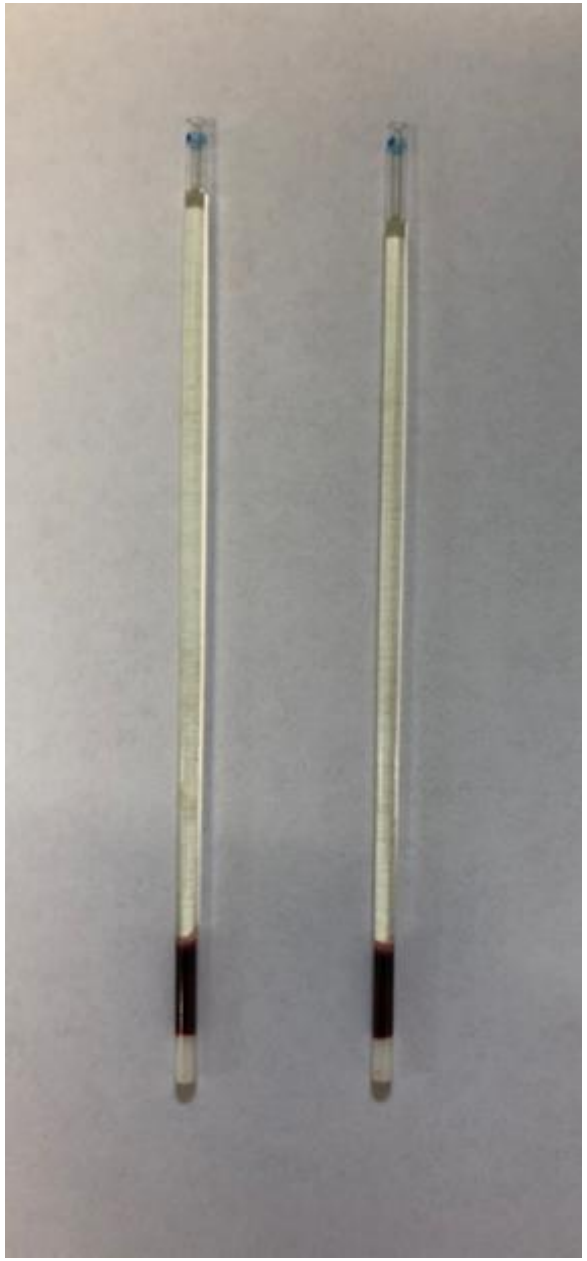
+ Ht is calculated
  + RBC x MCV x 0.1

+ PCV is measured
  + Normally 1-2% higher than Ht



Discordance Between Ht and PCV

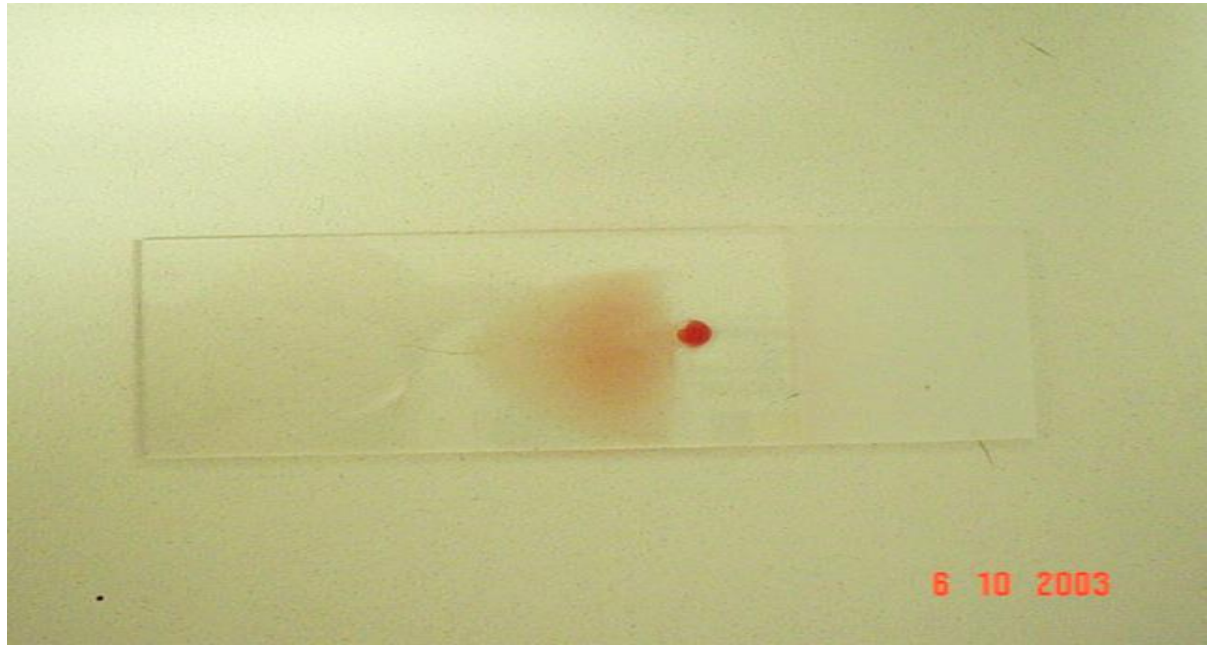
- Look at haemoglobin
- If Ht = 3 x Hb, then Ht is correct
- If Ht ≠ 3 x Hb then check PCV
  - Agglutination may affect results



Blood Film

- Good smear essential regardless of which analyser used!

- Prompt examination
  - Avoids age related changes
  - Make smear within 1hr



Preparing a Blood Film

+ Small drop of blood at one end

+ Draw spreader back into blood at 30º angle
+ Let blood spread along slide

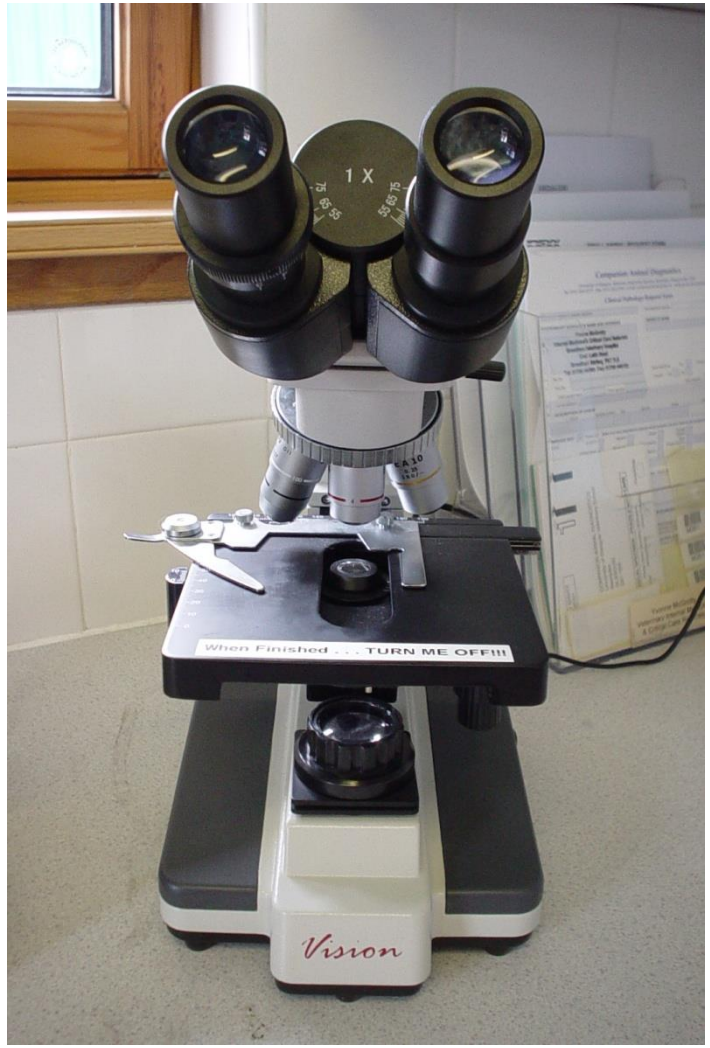
+ Push spreader along slide without lifting spreader
+ Air dry

Staining a Blood Film

- Modified Wright's Stain
  - Diff-Quick
  - Rapi-diff
- 3 solutions
  - Alcohol fixative
  - Eosinophilic stain
  - Dark blue stain
- Ideally separate stains for clean & contaminated samples

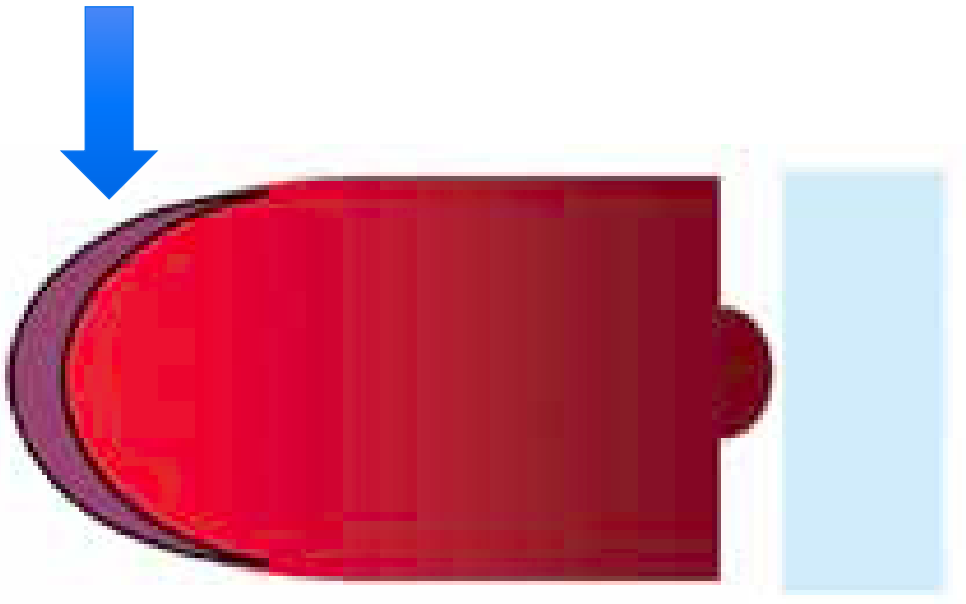
Film Examination

- Good microscope essential!
  - Binocular
  - 10x, 20x and 100x objectives
  - Variable rheostat
- Position sub-stage condenser close to stage
- Keep lenses clean
  - Remove oil after use



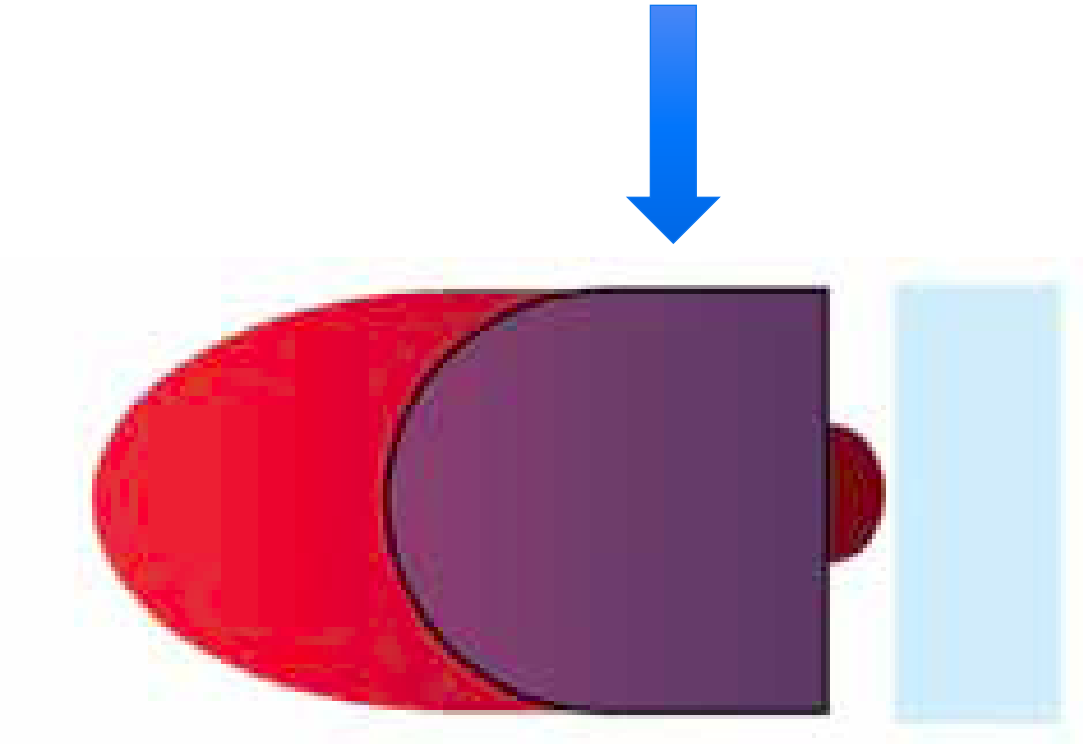
Feathered Edge of Film

+ Area furthest from blood spot
+ Platelet clumping
+ White cell clumping
+ Atypical cells
+ Microfilariae



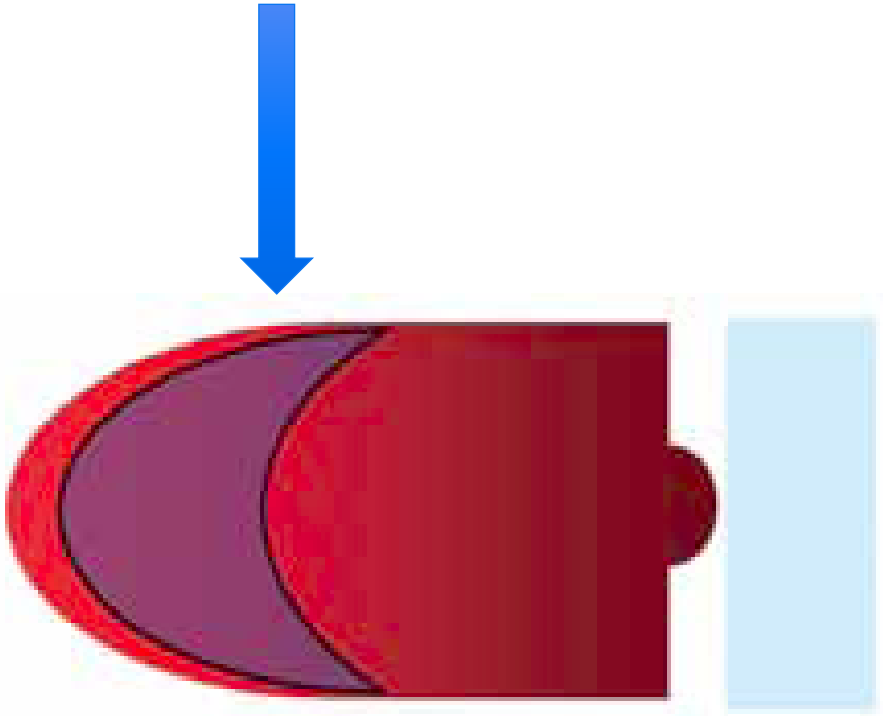
Body of the Film

+ Area closest to blood spot
+ Rouleaux
+ Agglutination



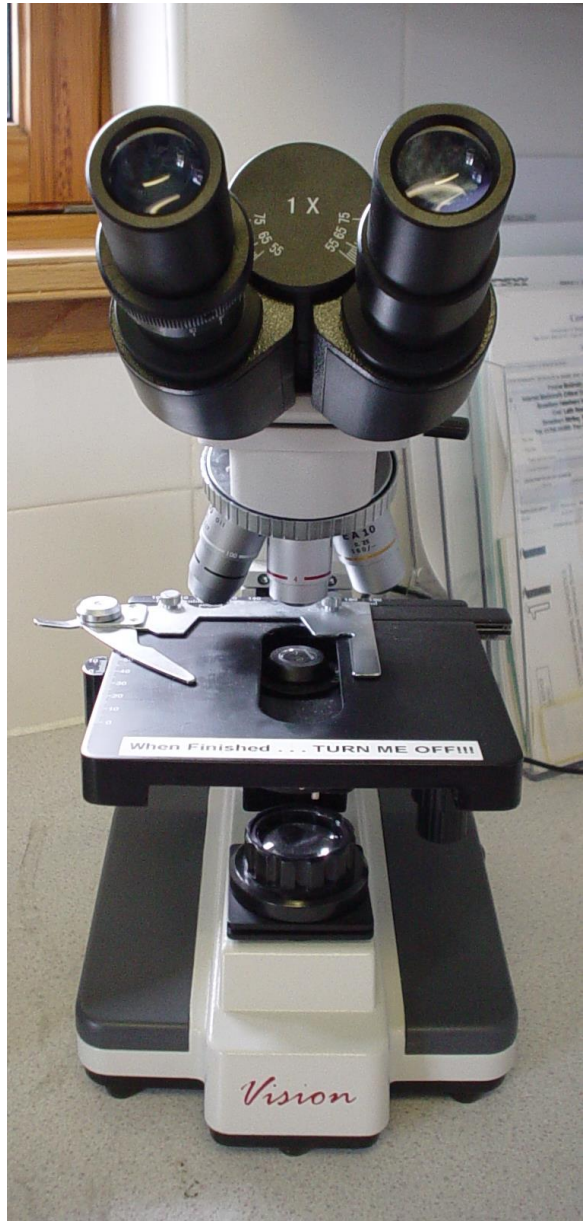
Monolayer

- Area between body and feathered edge
  - RBCs are touching without overlapping
- Estimate platelet count
- Estimate WCC
- Cell morphology



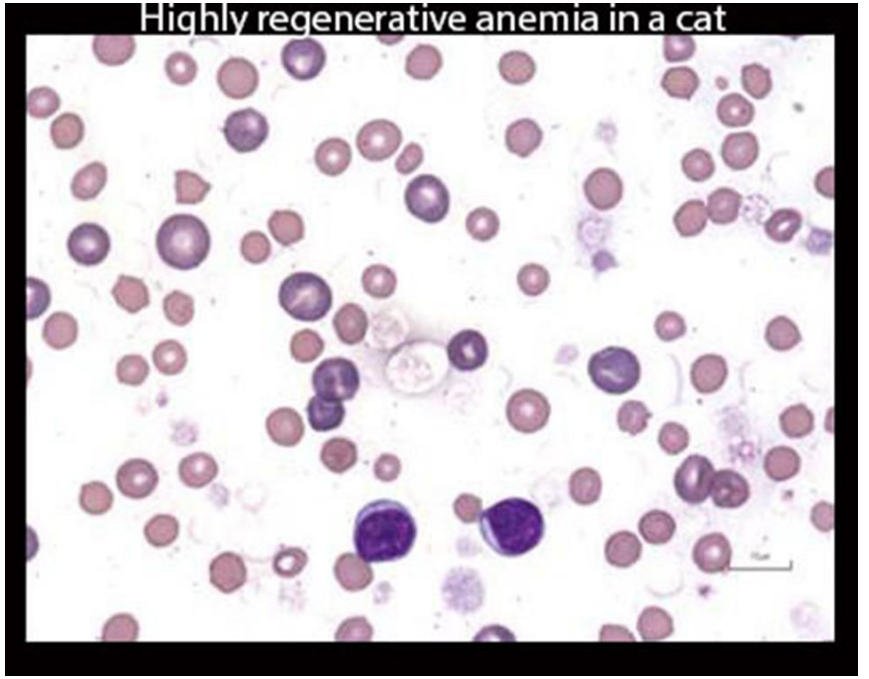
Film Examination

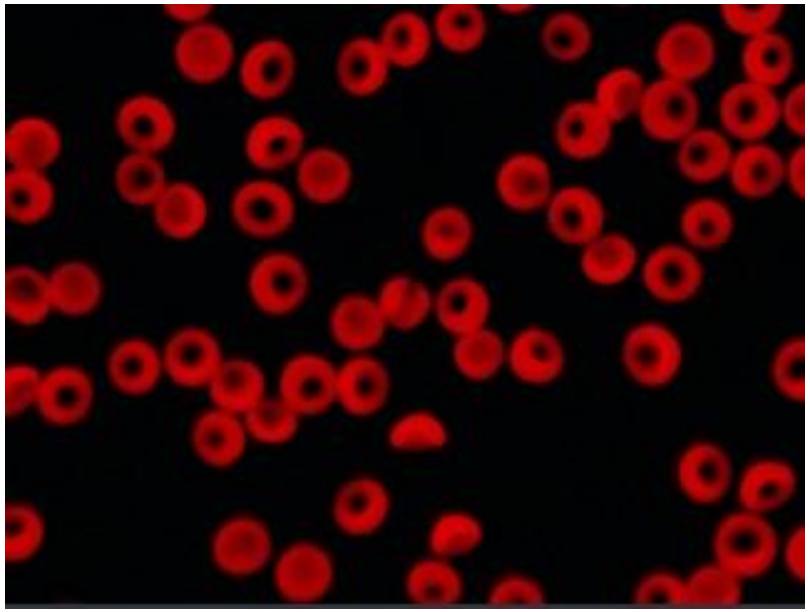
- Examine whole film at low magnification (x20)
  - Check film thickness
  - Look for platelet clumps & atypical cells
- Examine monolayer at low magnification (x20)
  - Estimate WBC count
- Examine monolayer with oil immersion (x100)
  - Examine cell morphology
  - RBC and WBC abnormalities



Blood Film Interpretation

- Regenerative response?
  - Anisocytosis
  - Polychromasia
  - NRBCs
    - Less numerous than polychromatophils
- Agglutination?
- Abnormal rbcs
  - Ghost cells
  - Spherocytes





IDEXX inVue Dx™ Cellular Analyzer's novel slide-free workflow gives time back to practices

Microscope

Often interrupted, multiple slides created

~10–20 minutes for prep

Collect sample → Affix sample → Spread cells → Air dry slide → Stain slides → Air dry again → View slide(s)

IDEXX inVue Dx™

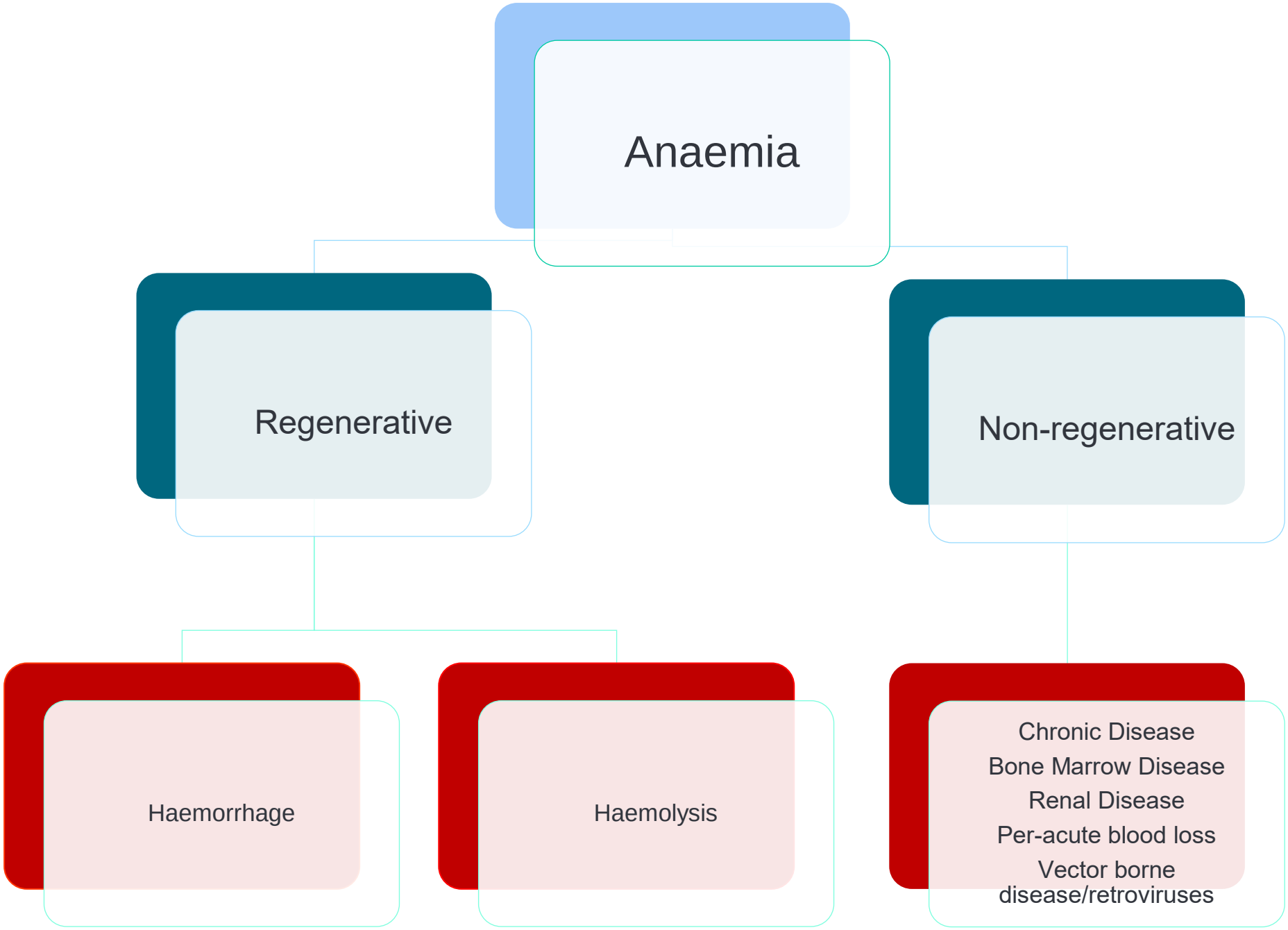
~1 minute

Collect sample → Load → Analysis

10 minutes

Hands-on time

Hands free



Regenerative Anaemias

Haemorrhage vs Haemolysis

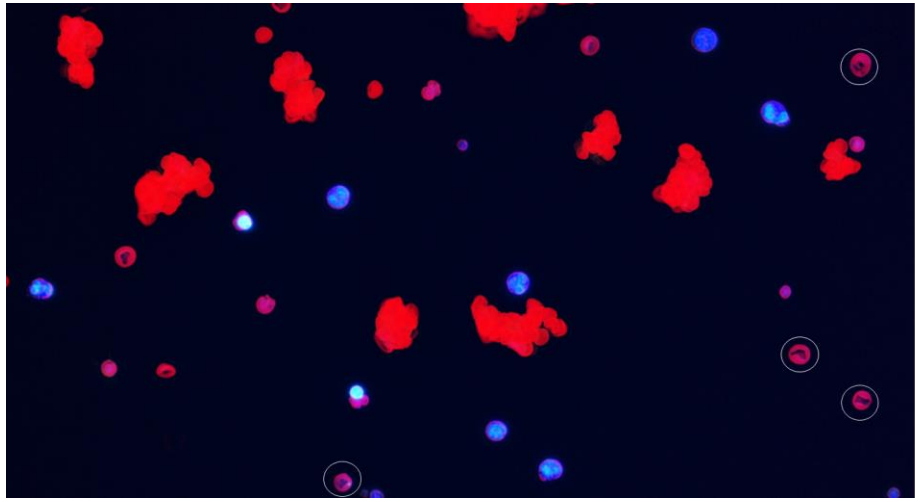
Regenerative Anaemia

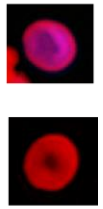
+ Can take 3-5 days for bone marrow to respond
+ Haemorrhage
+ Haemolysis
+ Polychromasia and reticulocytes assess degree of regeneration
+ Lasercyte & Procyte produce a reticulocyte count
+ InVue Dx provides RBC morphology assessment

Haematology

20/02/2025
17:47

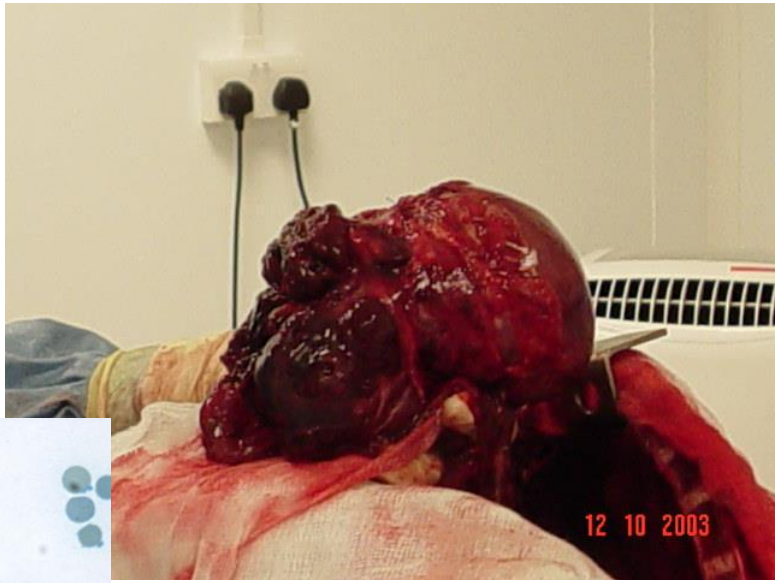
RBC	3.85	5.65 - 8.87 x10^12/L
Haematocrit	0.237	0.373 - 0.617 L/L
Haemoglobin	82	131 - 205 g/L
MCV	61.6	61.6 - 73.5 fL
MCH	21.4	21.2 - 25.9 pg
MCHC	347	320 - 379 g/L
RDW	14.1	13.6 - 21.7 %
% Reticulocytes	8.4	%
Reticulocytes	321.6	10.0 - 110.0 K/µL

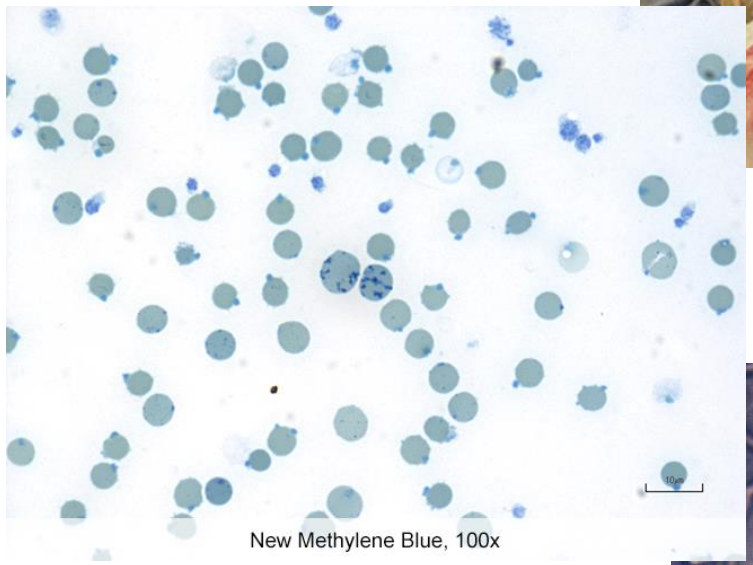




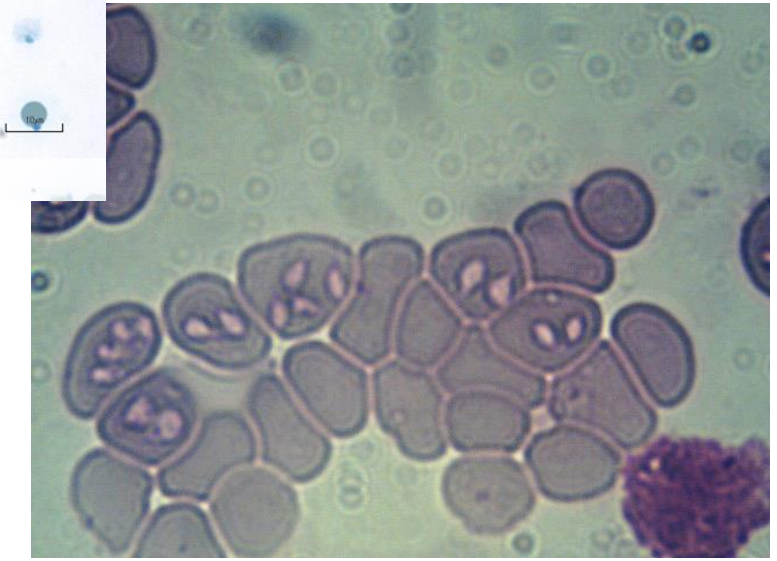
Haemolytic Anaemias

- Immune mediated
  - Associative or non-associative
- Mechanical Injury
  - Vascular neoplasms
  - Heartworm
- Oxidative Injury
  - Heinz bodies
- Red Cell Parasites
  - Haemomycoplasmas
  - *Babesia spp*



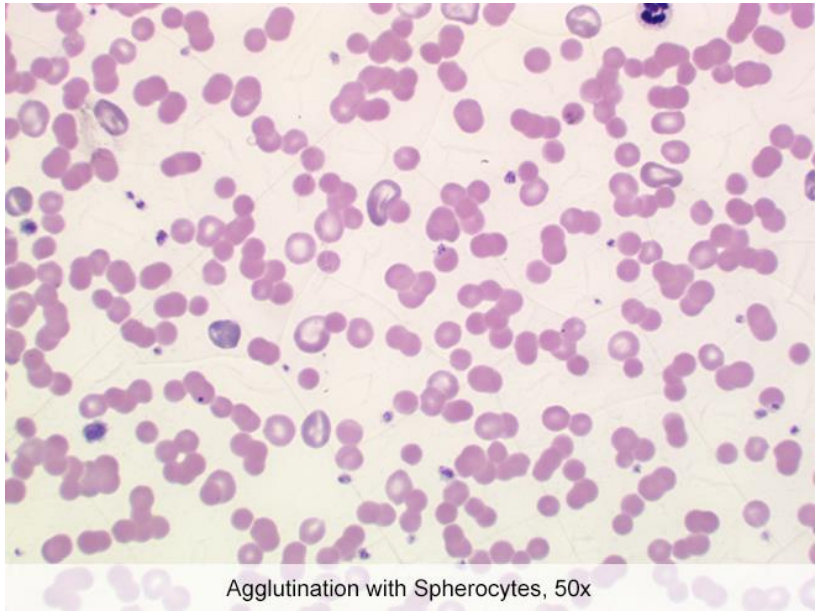


New Methylene Blue, 100x

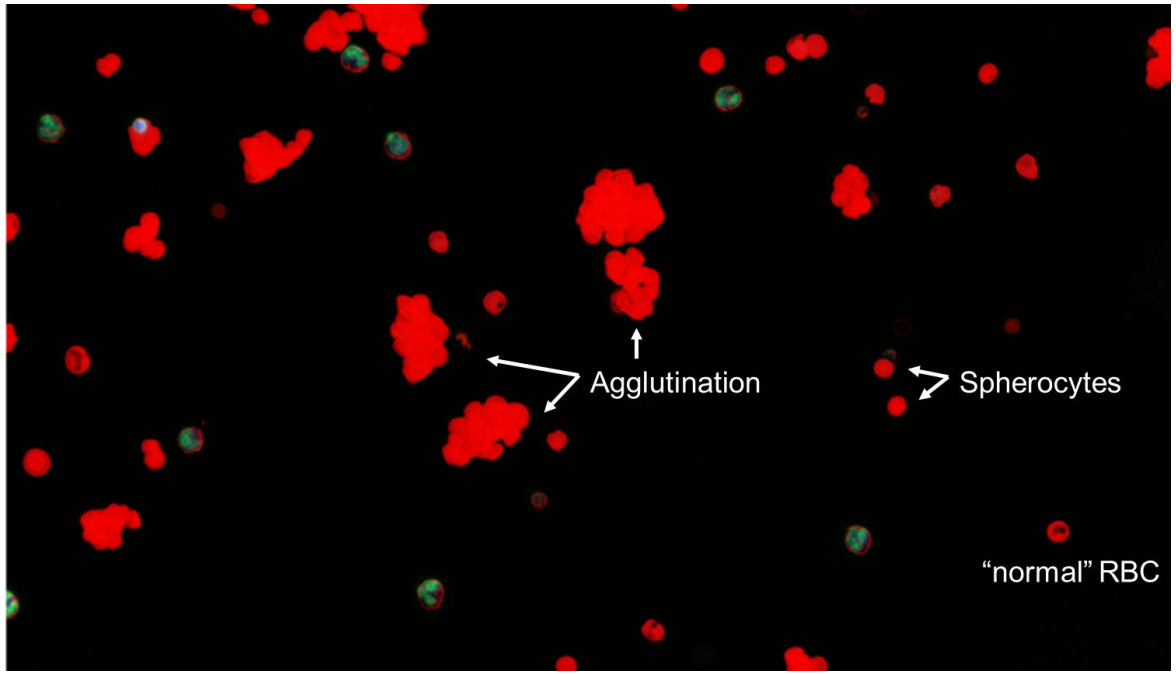


Haemolysis

- Regenerative response
  - Polychromasia
  - Anisocytosis
  - Reticulocytes
- Neutrophilia
- Spherocytes
  - Immune-mediated
- Autoagglutination?

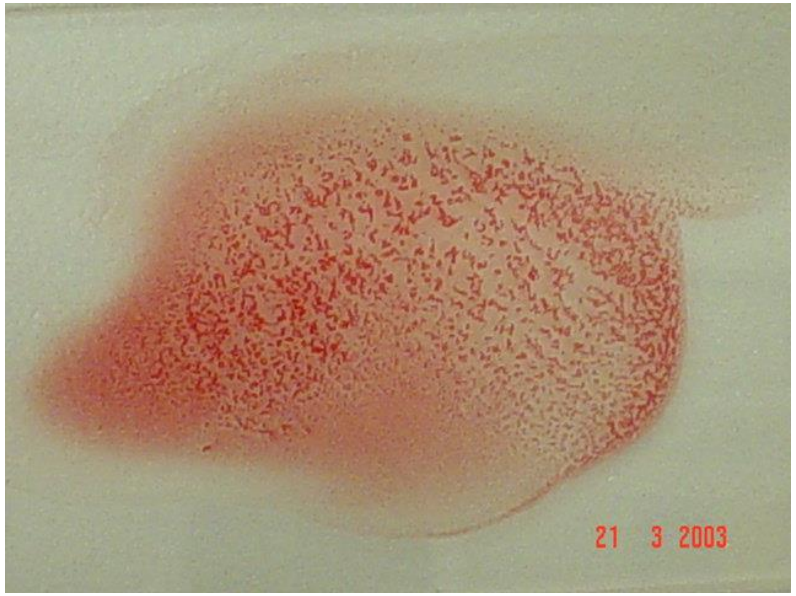


Agglutination with Spherocytes, 50x



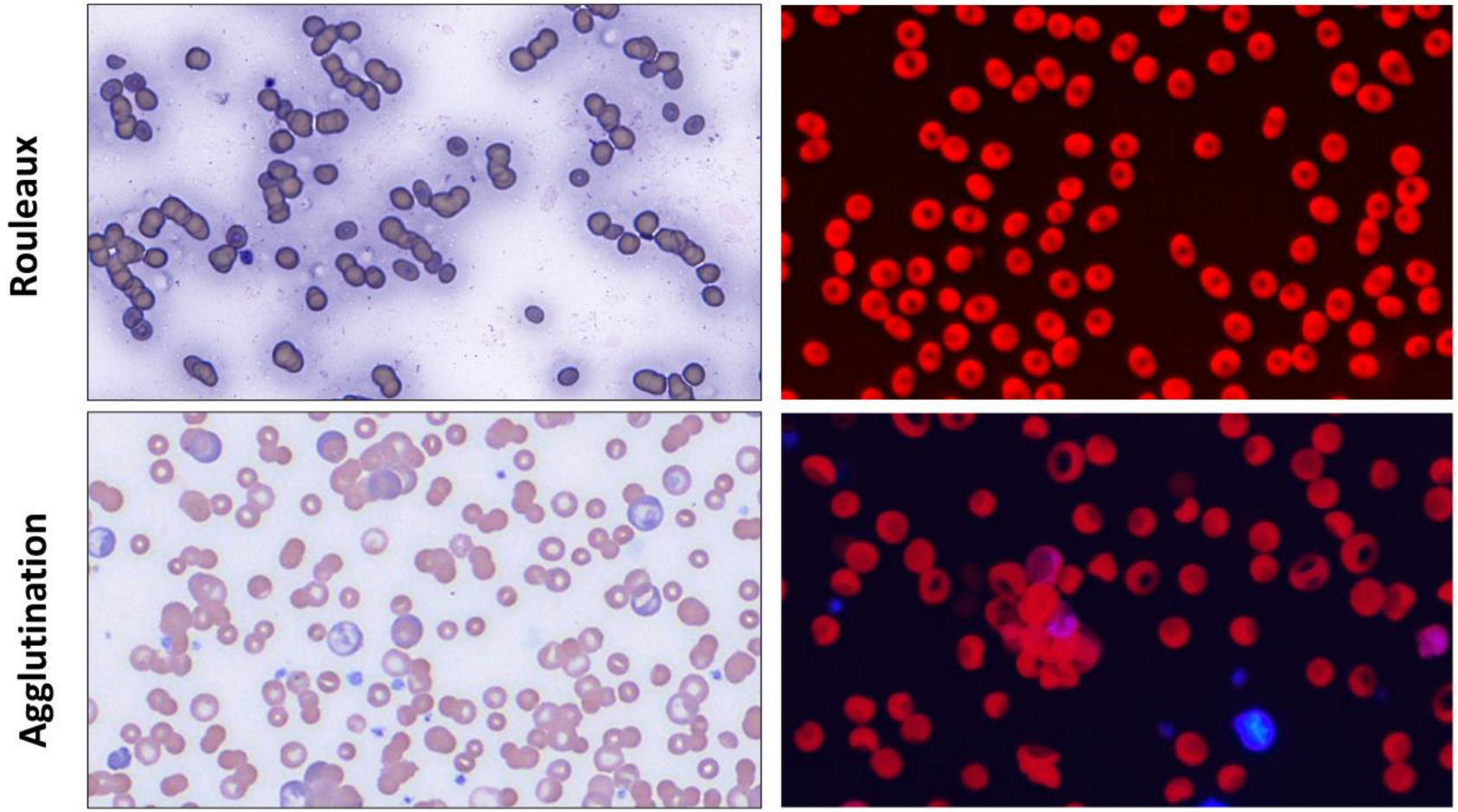
In Saline Agglutination Test

+ Place 1 drop of EDTA blood on glass slide
+ Add at least 4 drops of saline
+ Gently rock to mix blood and saline
+ Distinguishes rouleaux from agglutination



Agglutination vs Rouleaux

inVue Dx™ solves clinical confusion of rouleaux and agglutination



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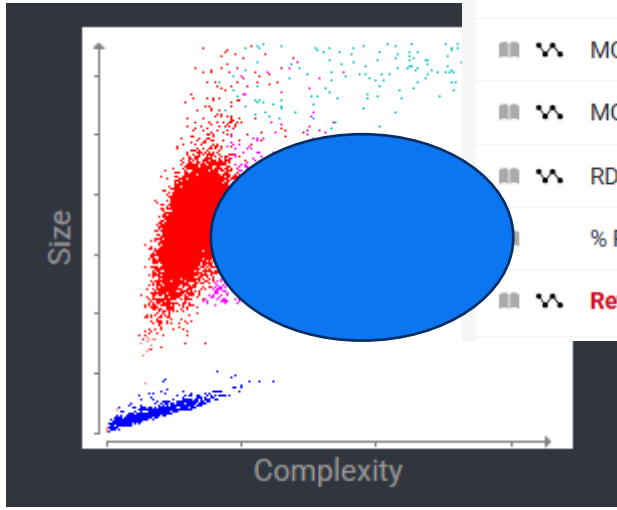
Reticulocytes

+ Evaluates response to anaemia
+ Immature RBCs
+ Increased mRNA
+ Identified with
  + Flow cytometry
  + New methylene blue stain
  + InVue Dx



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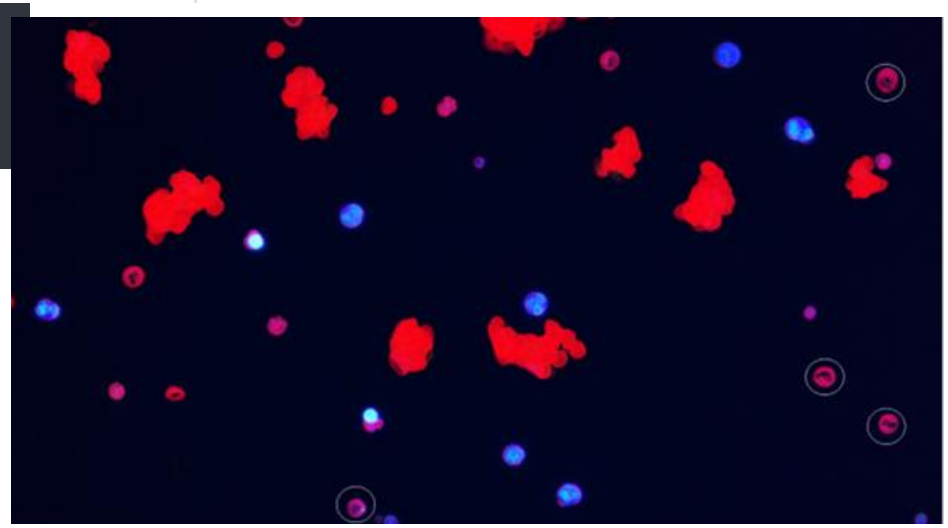
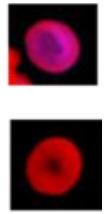
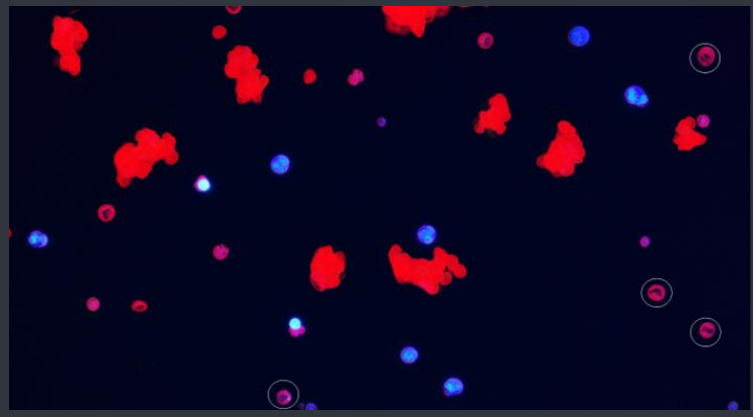


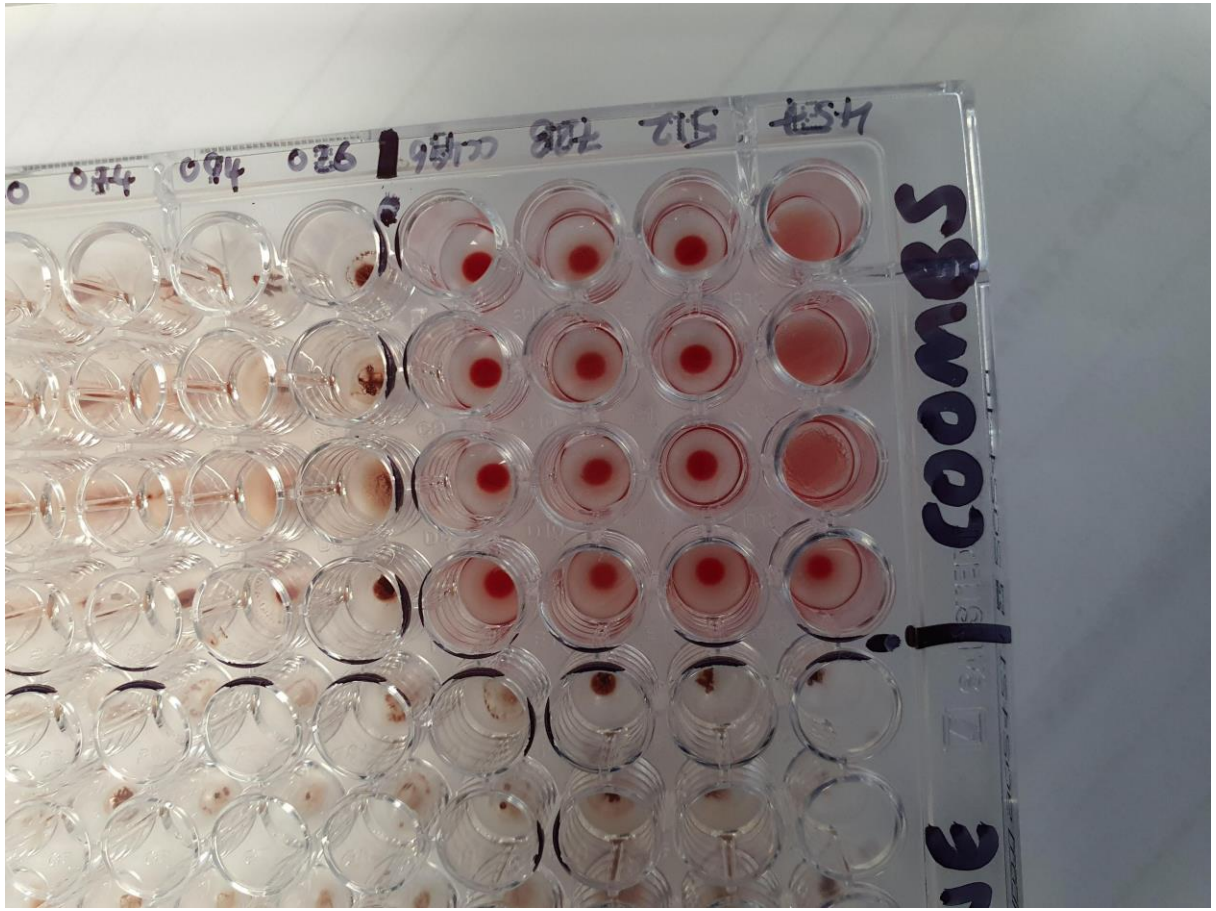


All polychromatophilic red blood cells are reticulocytes, *but* not all reticulocytes are polychromatophilic

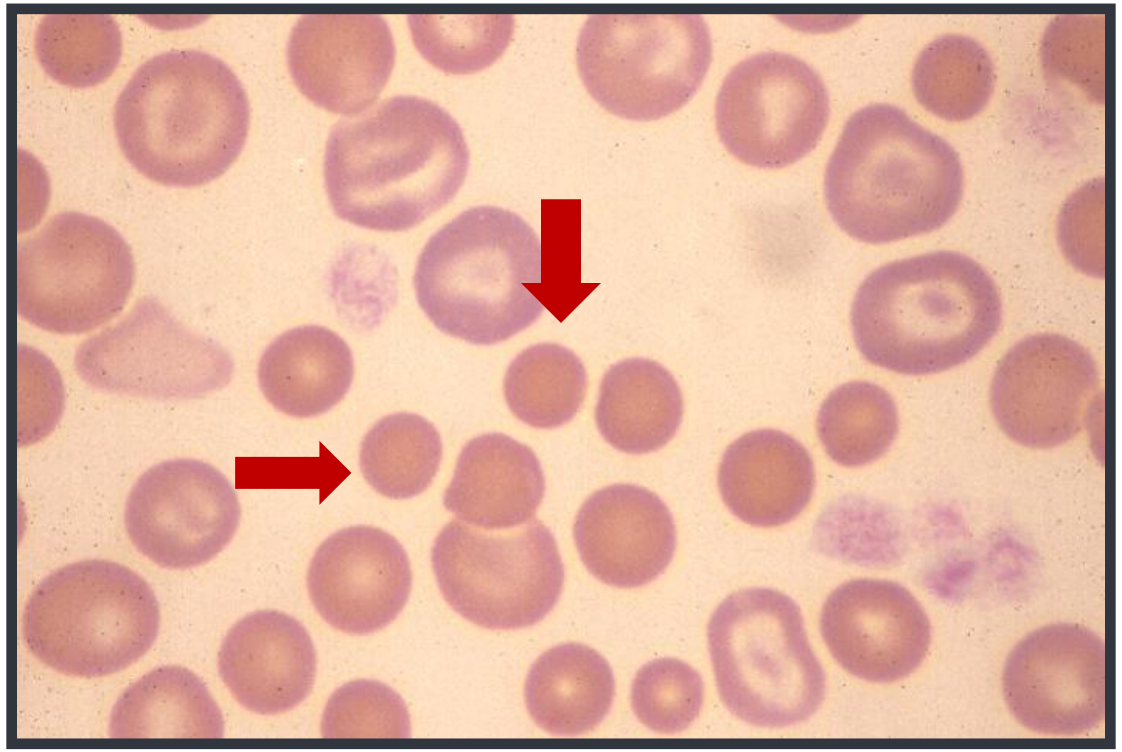


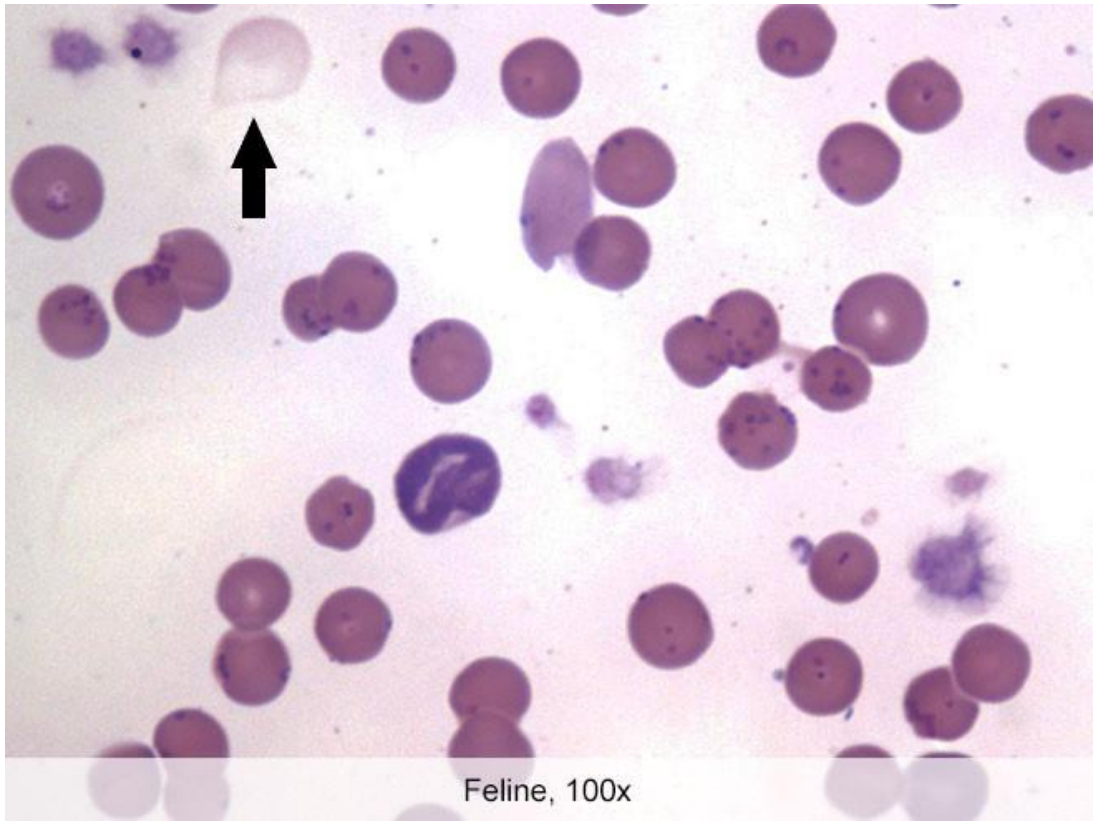
Coombs Test

+ Not required if agglutination positive
+ Detects Ab or complement on surface of rbc
+ Positive result supports diagnosis of IMHA
+ False +ves can occur



Spherocytes & Ghost Cells





IMHA in Dogs

- Associative or non-associative

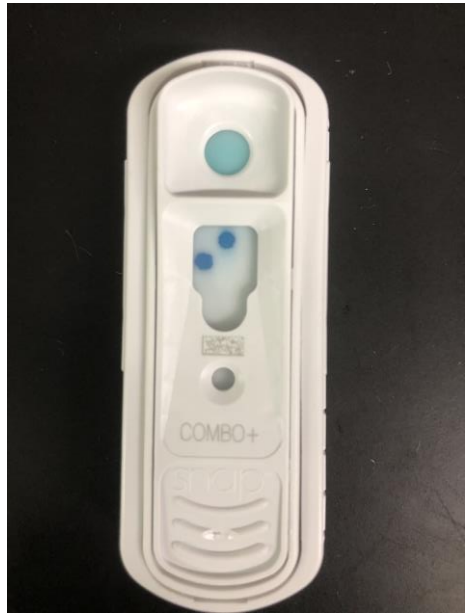
- Non-associative
  - Underlying cause not identified
  - Young adults
  - Cocker Sp, Springer Sp, OES

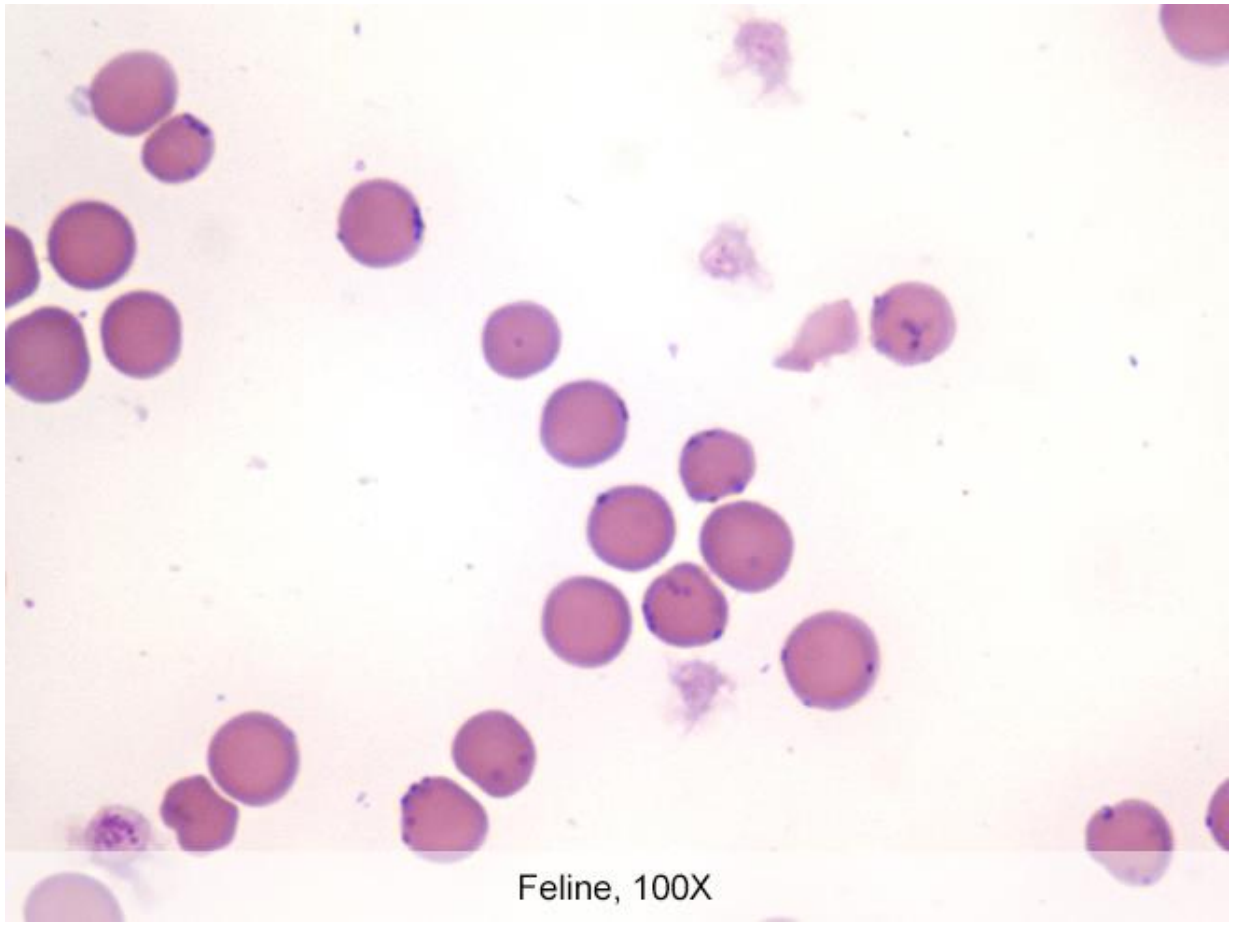
- Associative
  - Secondary to infectious/inflammatory/neoplastic cause
  - Drug reactions
  - Parasites

IMHA in cats

+ Associative or non-associative

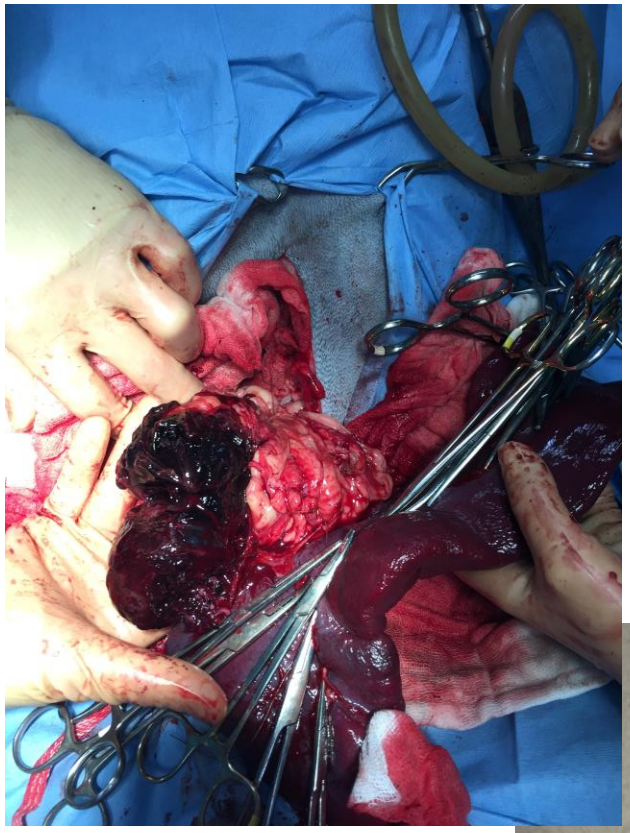
+ Associative
  + *Mycoplasma haemofelis sp*
  + FeLV




Feline, 100X

Haemorrhage

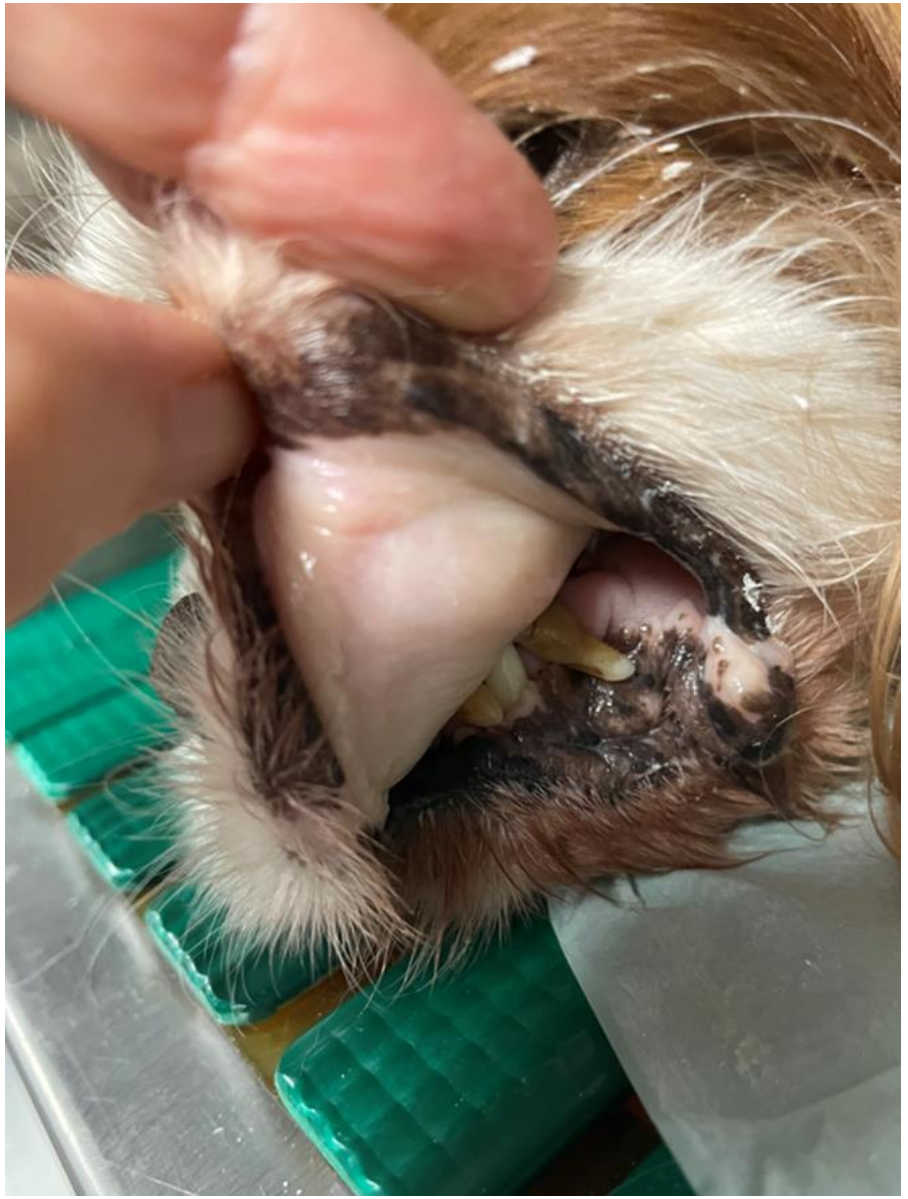
- Trauma
- Neoplasia
- Acquired coagulopathy
  - Rat bait ingestion
  - DIC
  - Liver dysfunction
- Congenital coagulopathy
  - Haemophilia



Non-Regenerative Anaemia

Non-regenerative Anaemia

- Peracute haemorrhage
  - Pre-regenerative
- Inflammatory disease
  - Mild non-regen anaemia
- Chronic kidney disease
  - Can be severe anaemia
- Chronic GI blood loss
- Bone marrow disease
- Vector borne disease
  - *Ehrlichia/Anaplasma/Babesia*



First steps for non-regenerative anaemia

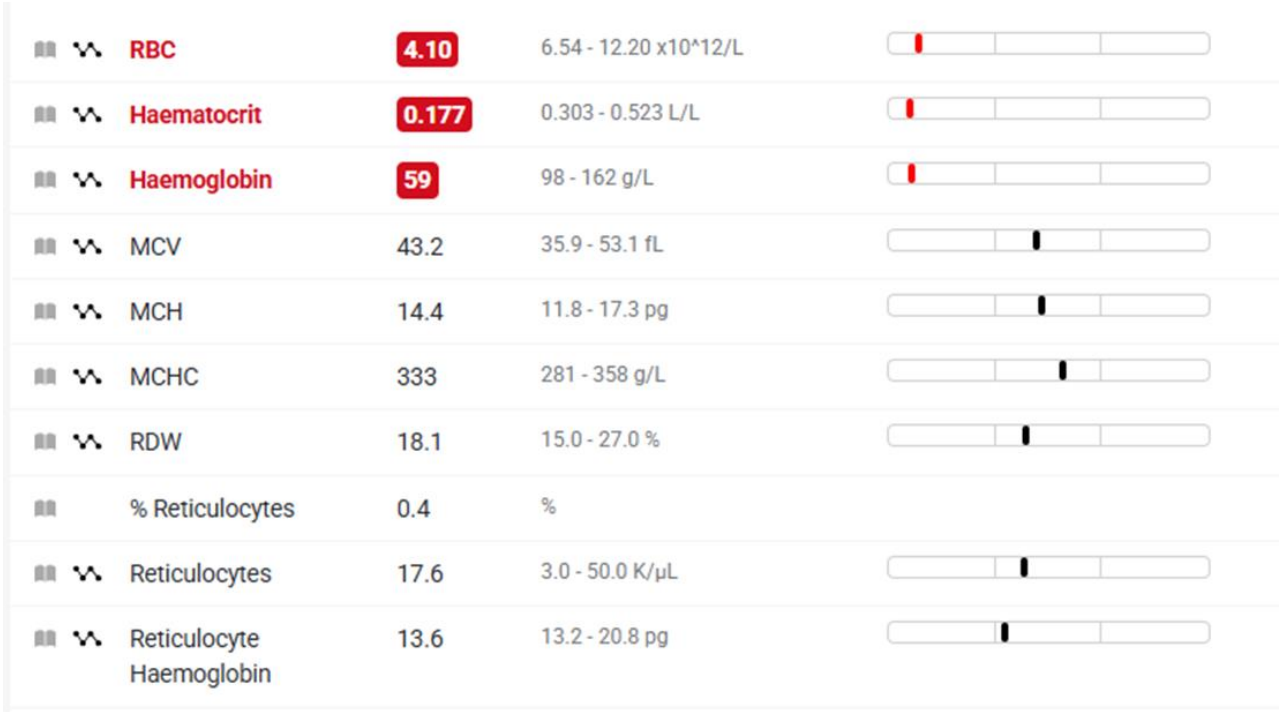
+ Consider if acute onset
  + Pre regenerative

+ Full CBC with film exam
  + Concurrent cytopenias?
  + Abnormal cells?
  + Reticulocyte haemoglobin

+ Check biochemistry
  + Azotaemia?

+ FeLV and FIV testing in cats

+ Vector borne disease testing in dogs
  + *Ehrlichia/Anaplasma/Babesia*



RBC	4.10	6.54 - 12.20 x10^12/L
Haematocrit	0.177	0.303 - 0.523 L/L
Haemoglobin	59	98 - 162 g/L
MCV	43.2	35.9 - 53.1 fL
MCH	14.4	11.8 - 17.3 pg
MCHC	333	281 - 358 g/L
RDW	18.1	15.0 - 27.0 %
% Reticulocytes	0.4	%
Reticulocytes	17.6	3.0 - 50.0 K/µL
Reticulocyte Haemoglobin	13.6	13.2 - 20.8 pg

Reticulocyte Haemoglobin

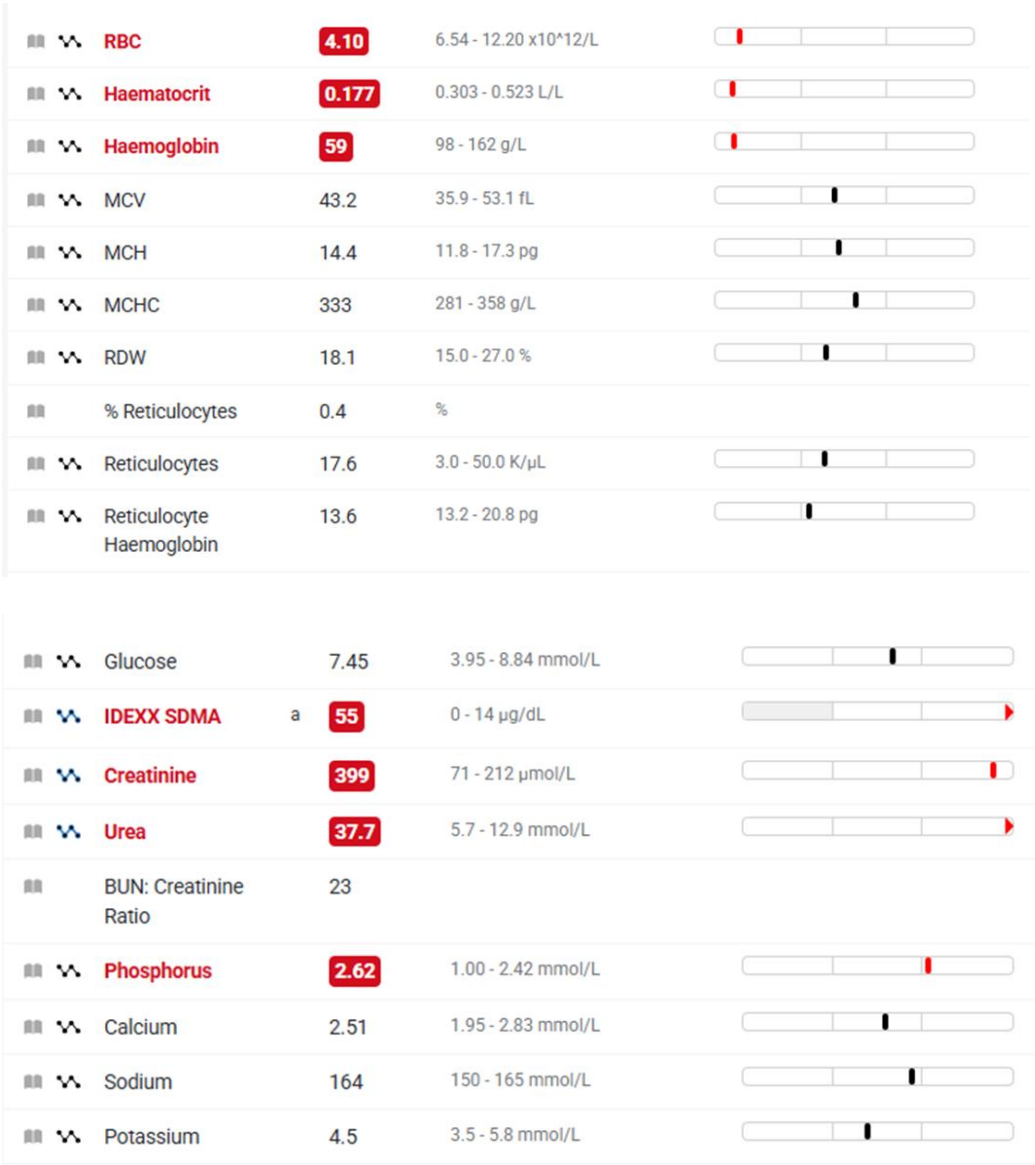
RBC		4.32	5.39 - 8.70 x10^12/L
Haematocrit		0.289	0.383 - 0.565 L/L
Haemoglobin		101	134 - 207 g/L
MCV		66.9	59.0 - 76.0 fL
MCH		23.4	21.9 - 26.1 pg
MCHC		349	326 - 392 g/L
Reticulocytes	a	60.0	<= 110.0 K/µL
Reticulocyte Haemoglobin		20.1	24.5 - 31.8 pg

+ Sensitive indicator of decreased iron availability

+ DDx
  + Blood loss
    + Haemorrhage
    + Parasitism
  + Chronic Inflammation
    + (iron sequestration)

Anaemia with CKD

+ Can result in severe non regenerative anaemia
+ Lack of EPO production
+ Treatment
  + Transfusions?
  + Recombinant EPO?
  + Varenzin



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% Reticulocytes	0.4	%	
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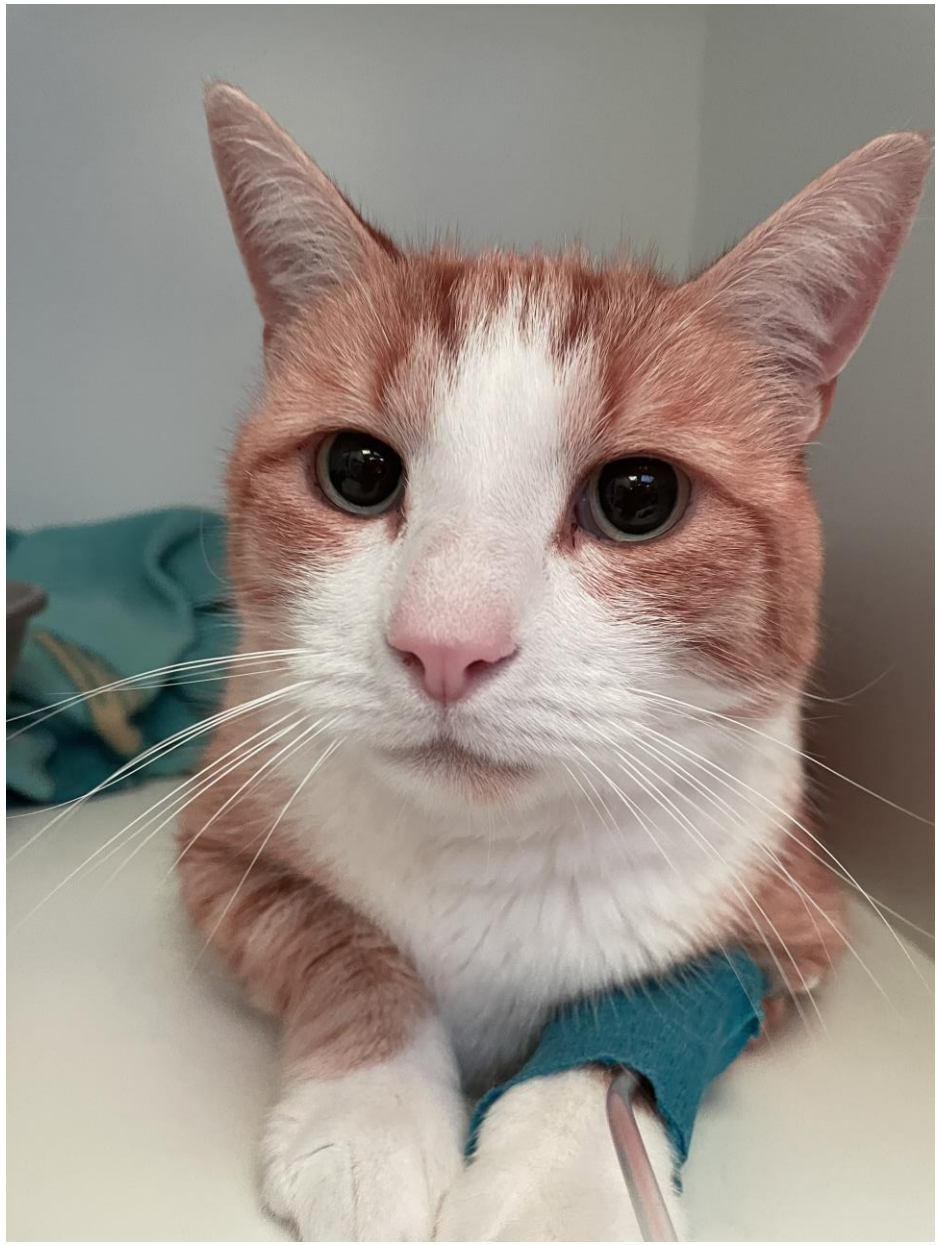
Reticulocyte Haemoglobin	13.6	13.2 - 20.8 pg	
Glucose	7.45	3.95 - 8.84 mmol/L	
IDEXX SDMA a	55	0 - 14 µg/dL	
Creatinine	399	71 - 212 µmol/L	
Urea	37.7	5.7 - 12.9 mmol/L	
BUN: Creatinine Ratio	23		
Phosphorus	2.62	1.00 - 2.42 mmol/L	
Calcium	2.51	1.95 - 2.83 mmol/L	
Sodium	164	150 - 165 mmol/L	
Potassium	4.5	3.5 - 5.8 mmol/L	

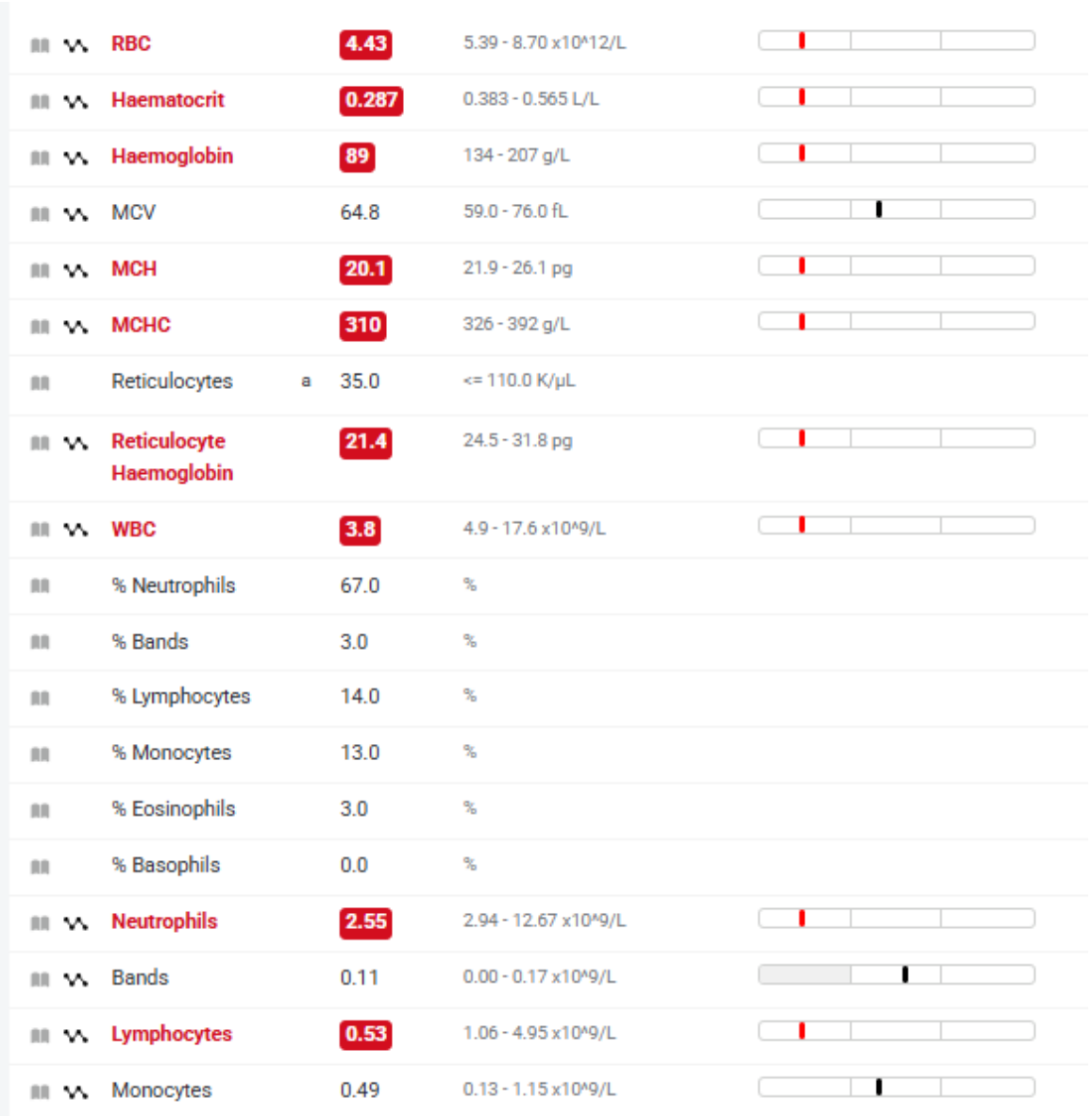
Future Horizons

- Varenzin- CA1
  - Molidustat
  - Increases EPO production by kidneys
- Control of non regenerative anaemia with CKD
  - Orally SID for 28 days
  - Can be repeated after 7 days
- Side effects
  - Vomiting
  - Hypertension
  - Thromboembolism



Bone Marrow Biopsy Indications

+ Unexplained non regenerative anaemia
  + Having excluded other causes
+ Bi or pancytopenia
+ Suspicion of non regenerative IMHA



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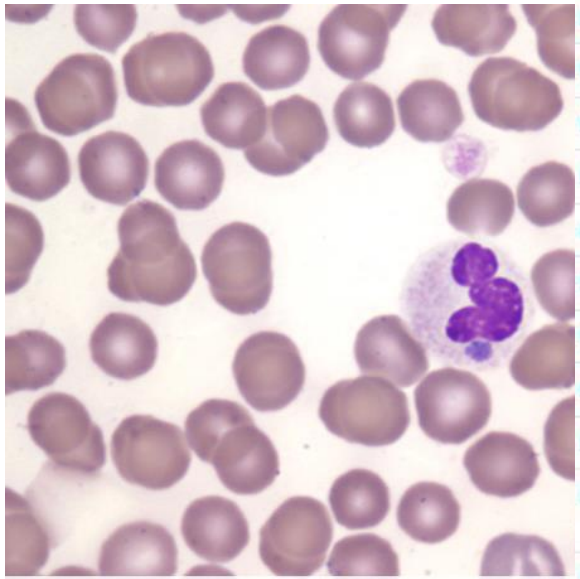
Haemoglobin		89	134 - 207 g/L	
MCV		64.8	59.0 - 76.0 fL	
MCH		20.1	21.9 - 26.1 pg	
MCHC		310	326 - 392 g/L	
Reticulocytes	a	35.0	<= 110.0 K/µL	
Reticulocyte Haemoglobin		21.4	24.5 - 31.8 pg	
WBC		3.8	4.9 - 17.6 x10^9/L	
% Neutrophils		67.0	%	
% Bands		3.0	%	
% Lymphocytes		14.0	%	
% Monocytes		13.0	%	
% Eosinophils		3.0	%	
% Basophils		0.0	%	
Neutrophils		2.55	2.94 - 12.67 x10^9/L	
Bands		0.11	0.00 - 0.17 x10^9/L	
Lymphocytes		0.53	1.06 - 4.95 x10^9/L	
Monocytes		0.49	0.13 - 1.15 x10^9/L	

Vector Borne Disease

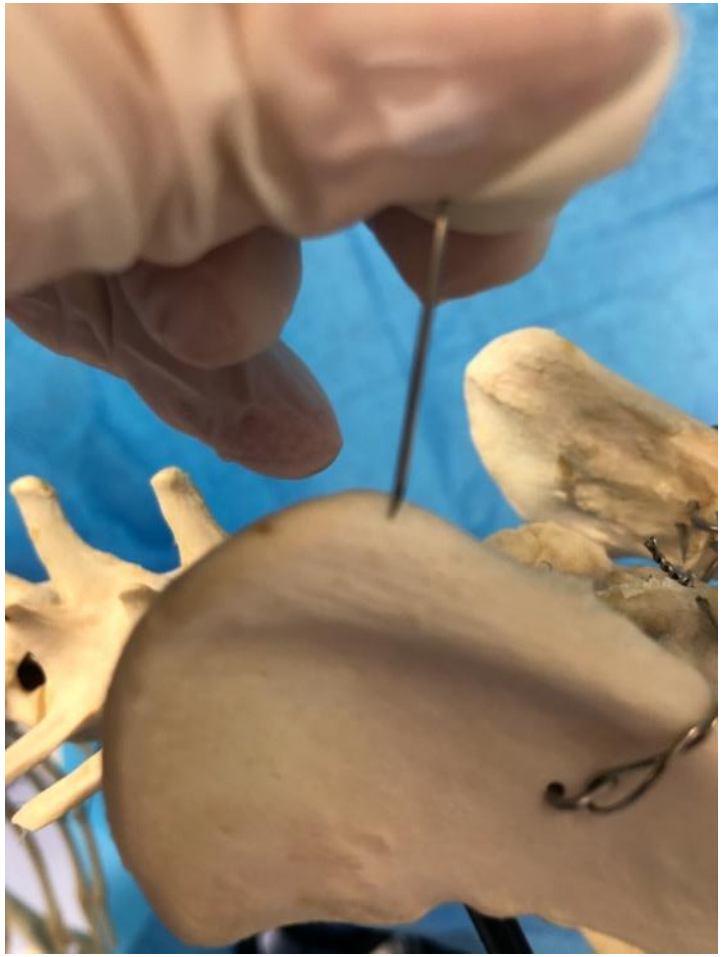
- *Ehlichia sp*
- *Anaplasma sp*
- *Babesia sp*



Canine, 100x

RBC	5.42	5.65 - 8.87 x10^12/L	
Haematocrit	0.379	0.373 - 0.617 L/L	
Haemoglobin	129	131 - 205 g/L	
MCV	69.9	61.6 - 73.5 fL	
MCH	23.8	21.2 - 25.9 pg	
MCHC	340	320 - 379 g/L	
RDW	17.8	13.6 - 21.7 %	
% Reticulocyte	0.5	%	
Reticulocytes	26.0	10.0 - 110.0 K/µL	
Reticulocyte Haemoglobin	25.2	22.3 - 29.6 pg	
WBC	3.01	5.05 - 16.76 x10^9/L	
% Neutrophils	39.9	%	
% Lymphocytes	56.1	%	
% Monocytes	3.0	%	
% Eosinophils	1.0	%	
% Basophils	0.0	%	
Neutrophils	1.20	2.95 - 11.64 x10^9/L	
Lymphocytes	1.69	1.05 - 5.10 x10^9/L	
Monocytes	0.09	0.16 - 1.12 x10^9/L	
Eosinophils	0.03	0.06 - 1.23 x10^9/L	
Basophils	0.00	0.00 - 0.10 x10^9/L	
Platelets	*7	148 - 484 x10^9/L	

Iliac Crest Site- Dogs



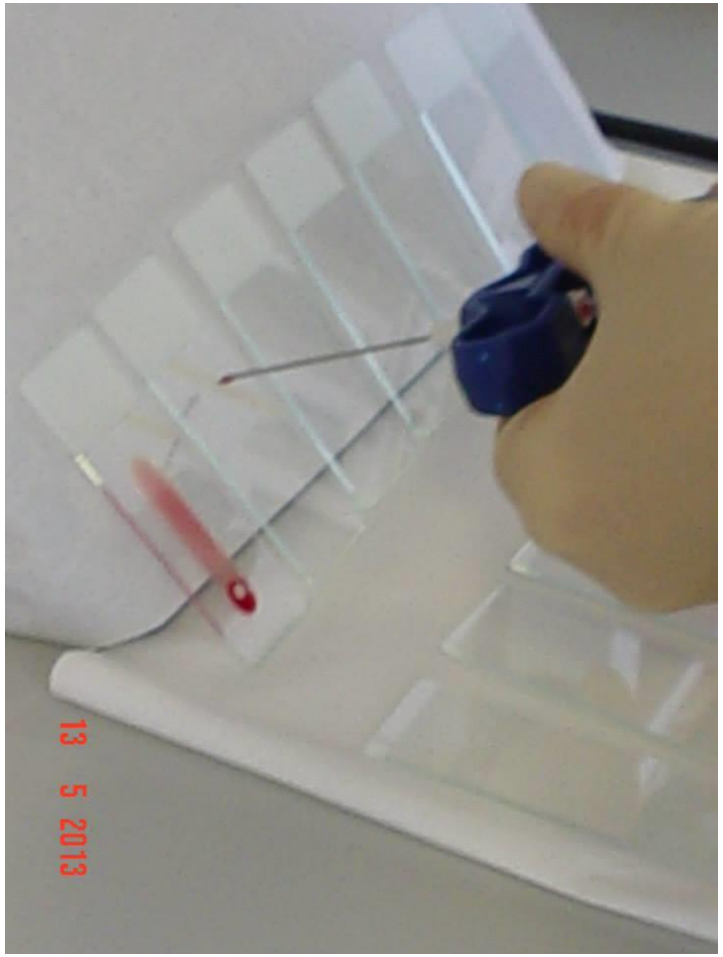
- Identify widest point on dorsal aspect of wing of ileum
- Make a small incision in the skin
- Hold the needle firmly
- Lodge the handle in the palm of the hand
- Position needle perpendicular to crest

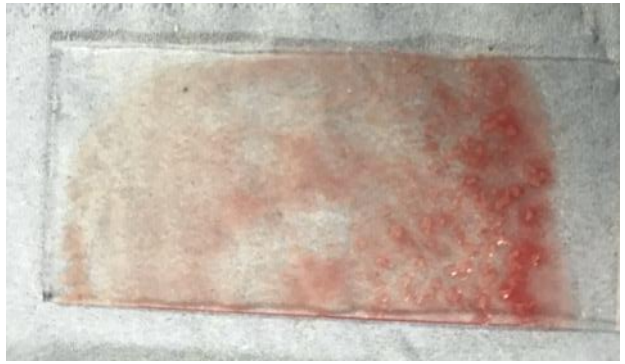
Humeral Bone Marrow Aspirate- Cats

+ Place animal in left lateral recumbency
+ Aseptically prepare over point of shoulder
+ Externally rotate humerus
+ Locate flat surface on proximal humerus

Bone Marrow Samples

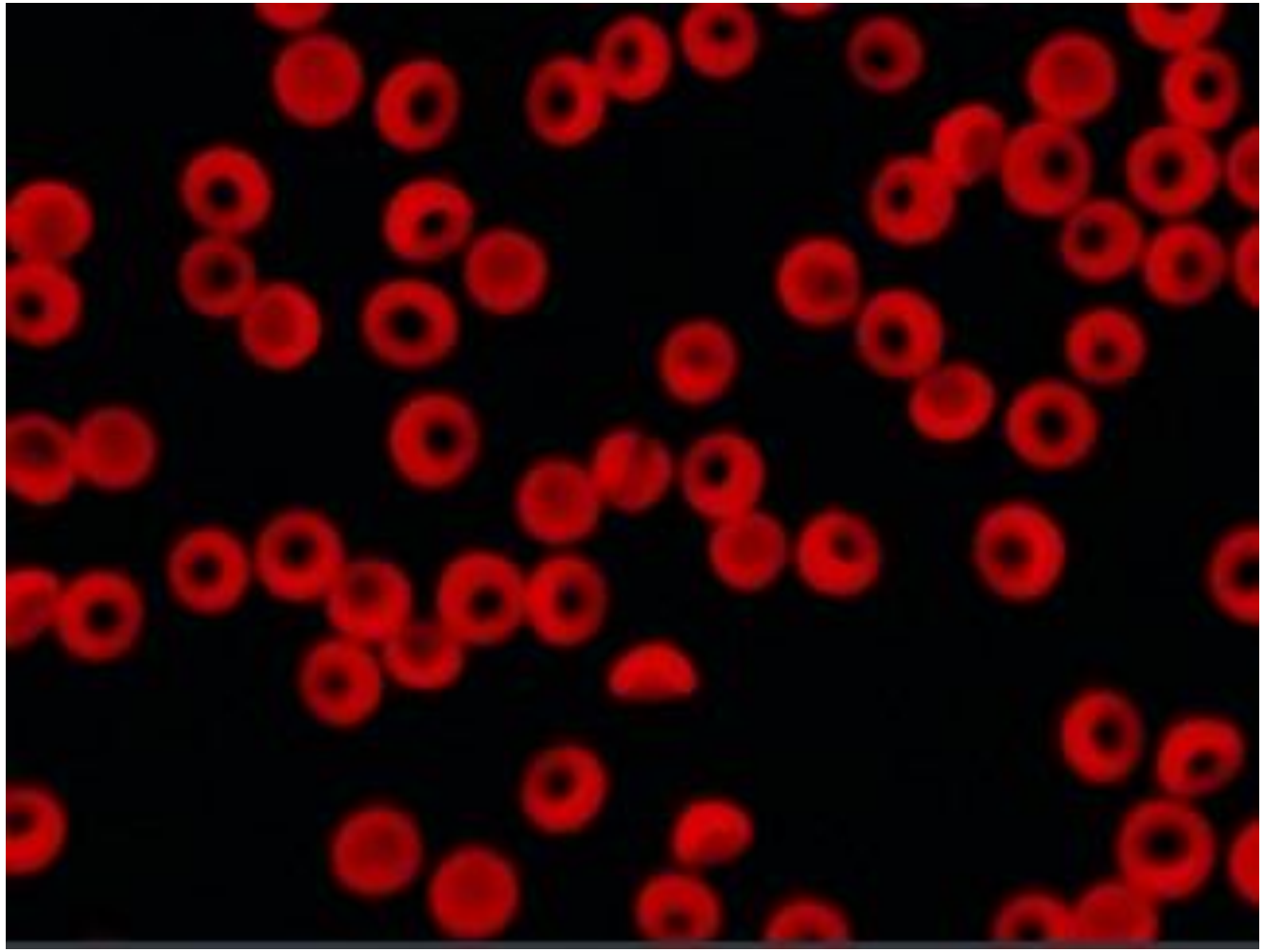
- Remove needle quickly
- Apply sample to tilted slides
  - Allows blood to run off
- Use squash technique to prepare slide
- Dry rapidly
- Send to lab with contemporaneous CBC





Summary

- Anaemia is a symptom NOT a diagnosis
- Logical work up depends on determining whether anaemia is regenerative or non regenerative
- Don’t forget a film examination!!!



Any Questions?