

Veterinærdagene 2024

13.-15. mars, Bergen



Seksjonen er sponset av



Torsdag 14. mars

Program for Smådyr

The anaemic patient – Diagnostic approach

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Anaemia

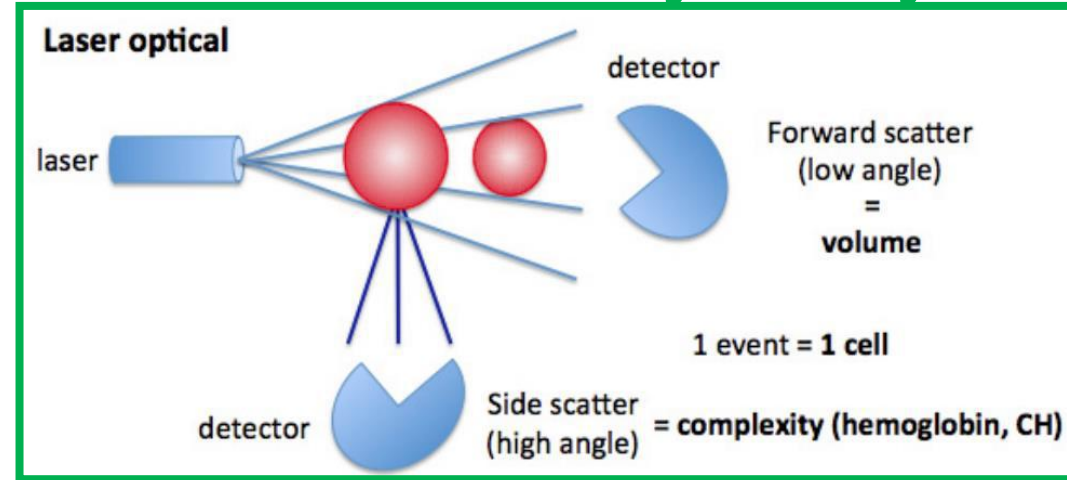
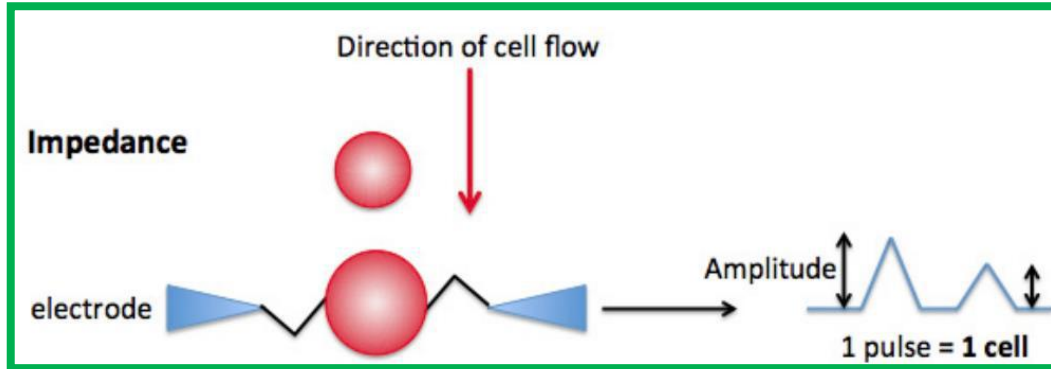
- **“Anaemia”**
 - Clinical syndrome
 - Not a diagnosis
 - Variety of clinical signs
 - Hypoxia
- **Decreased**
 - Concentration of haemoglobin (Hgb)
 - Haematocrit (HCT)
 - Red Blood Cell (RBC) count



In-clinic haematology analysers – *How do they work?*

Laser-flow cytometry

Impedance (electronic resistance)



- COULTER® A^c.T diff™
- Heska CBC-diff
- Vet abc™
- Scil Vet ABC
- VetScan® HMT
- ABACUS (Diatron®)
- Medonic CA620-VET
- CA530-VET
- Hemavet 950
- MS45
- Celltac Alpha
- Mythic 18



Laser analysers



LaserCyte™
(Idexx)



ProCyte DX™
(Idexx)



BC-5000Vet
(Mindray)

Results - Impedance and Laser flow cytometry analysers

Haemoglobin (Hb) (use a biochemical method)

Haematocrit (HCT)

calculated (HCT = MCV x RBC)

Red blood cell (RBC) count

RBC parameters (calculated)

Mean cell volume (MCV); average size of RBCs

Mean corpuscular Hgb concentration (MCHC) [MCHC=Hgb/HCT]

Mean cell Hgb (MCH)

[MCH=Hgb/RBC] average Hgb concentration in RBCs

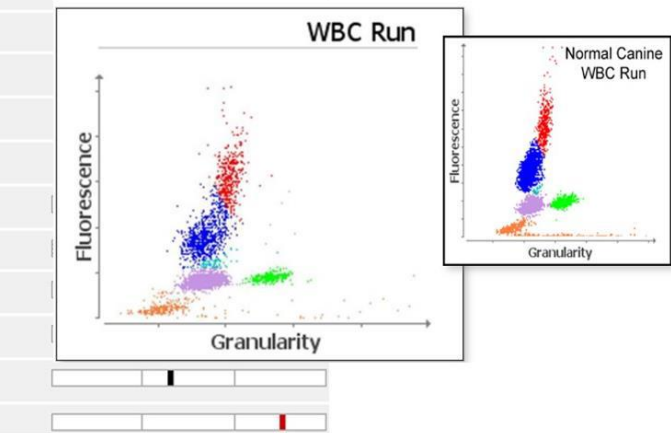
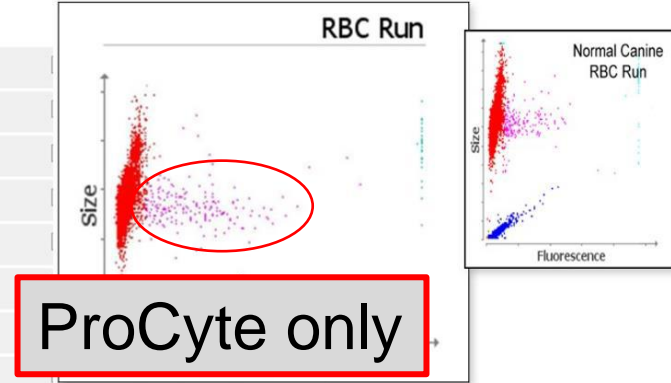
Platelet count

Total white blood cell (WBC) count

WBC differential counts

Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils

▶ RBC	5.9	5.39 - 8.7 x10 ¹² /L
▶ Haematocrit	0.334	0.383 - 0.565 L/L
▶ Haemoglobin	91	134 - 207 g/L
▶ MCV	56.6	59.0 - 76.0 fL
▶ MCH	15.4	21.9 - 26.1 pg
▶ MCHC	272	326 - 392 g/L
▶ Reticulocyte	^a 290.9	<= 110 K/ μ L
▶ WBC	10.9	4.9 - 17.6 x10 ⁹ /L
▶ % Neutrophil	69	%
▶ % Band	1	%
▶ % Lymphocyte	14	%
▶ % Monocyte	12	%
▶ % Eosinophil	4	%
▶ Neutrophil	7.52	2.94 - 12.67 x10 ⁹ /L
▶ Band	0.11	0 - 0.17 x10 ⁹ /L
▶ Lymphocyte	1.53	1.06 - 4.95 x10 ⁹ /L
▶ Monocyte	1.31	0.13 - 1.15 x10 ⁹ /L
▶ Eosinophil	0.44	0.07 - 1.49 x10 ⁹ /L
▶ Platelet	702	143 - 448 x10 ⁹ /L



Anaemia based on **MCV**

- **Normocytic** (MCV within normal limits): normal size RBC
 - Non-regenerative
- **Microcytic** (MCV decreased):
 - Iron deficiency
- **Macrocytic** (MCV increased):
 - Regenerative
 - Presence of immature RBCs (larger than mature RBCs)
 - In some bone marrow disorders

Anaemia and **MCH/MCHC**

- **Normochromic**: MCHC/MCH are within normal limits
 - Non-regenerative
- **Hypochromic** (MCHC/MCH decreased):
 - Iron deficiency
 - Regenerative
 - Presence of immature RBCs (are not fully haemoglobinised)
- **Hyperchromic** (MCHC/MCH increased):
 - Not physiologically possible
 - Always artefact (i.e. haemolysis)

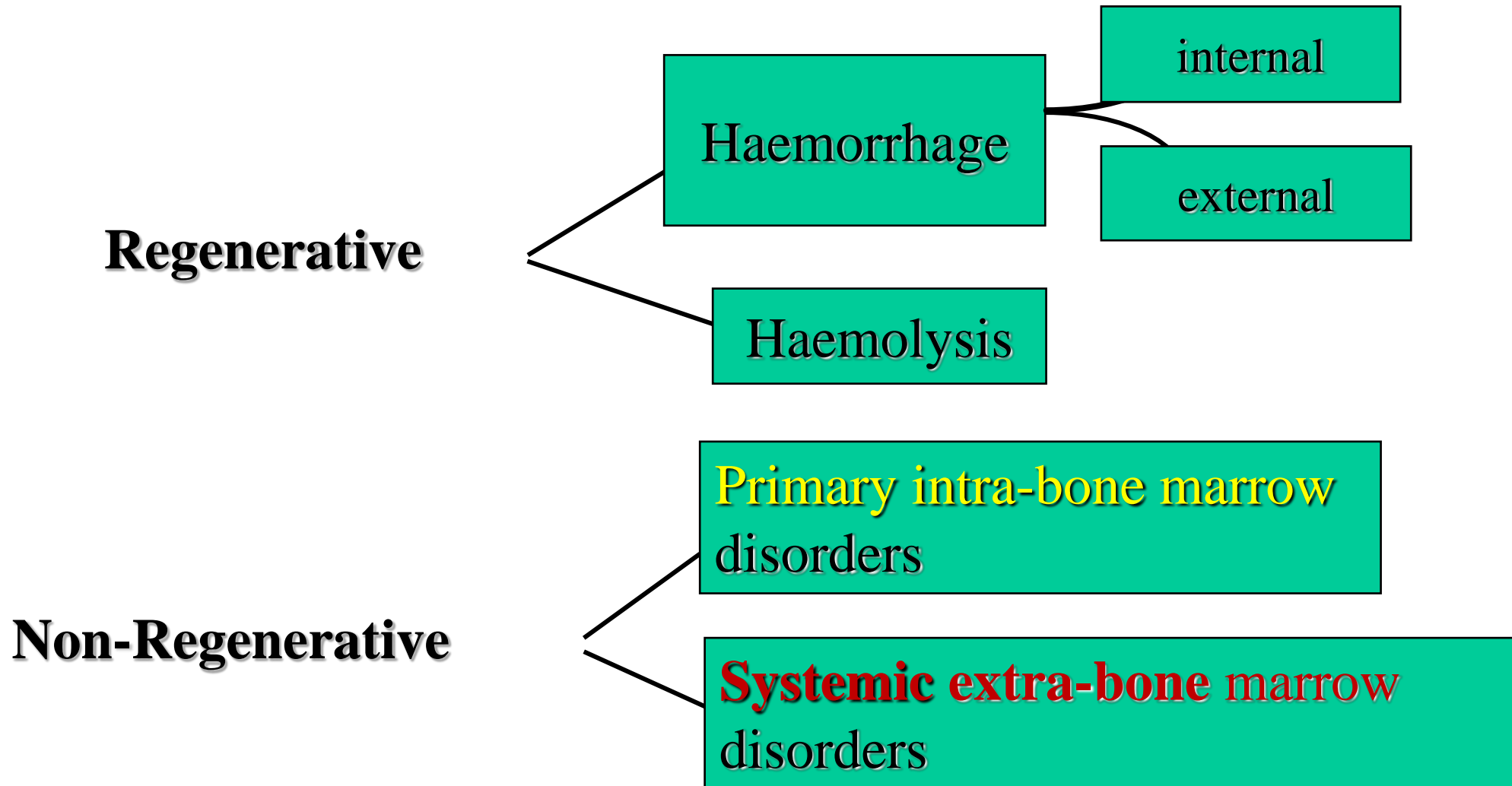
Regenerative / Non- Regenerative

- **Regeneration is the body's response to a fall in oxygenation**
- **The kidneys respond by releasing erythropoietin (EPO)**
- **This stimulates the bone marrow to increase RBC production**

- **Takes 2-3 days, and younger RBCs (polychromatophils/ reticulocytes) increase in circulation**

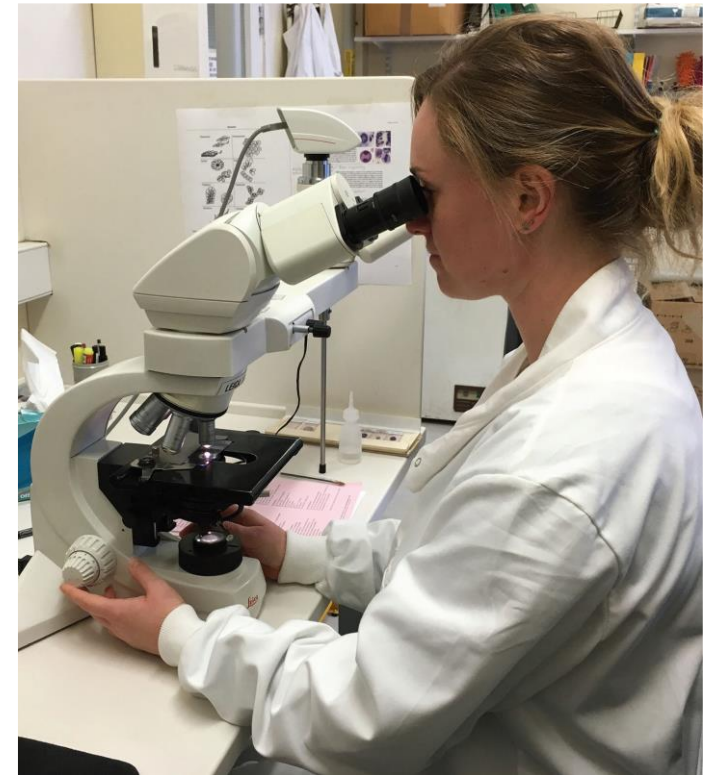
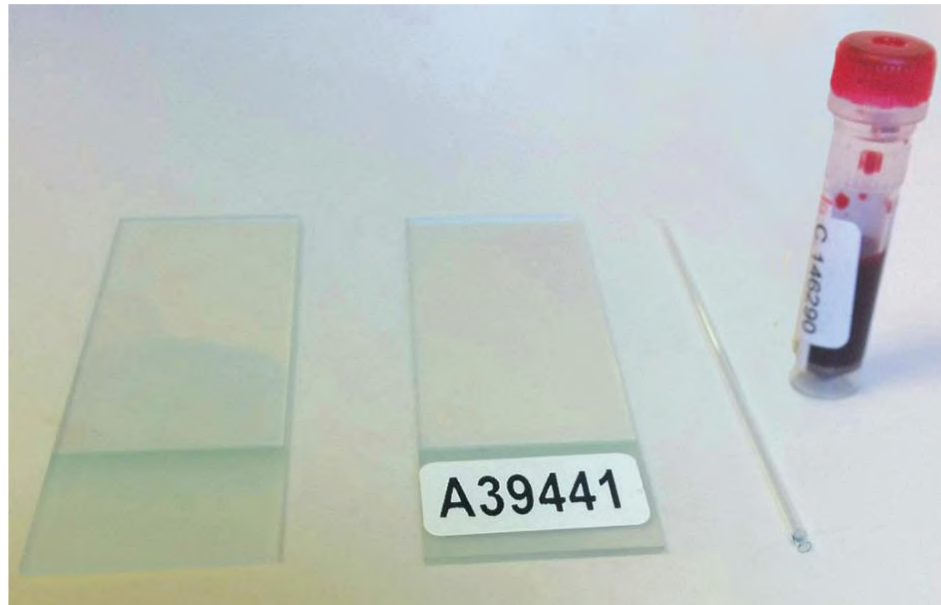


Anaemia: DECISION MAKING



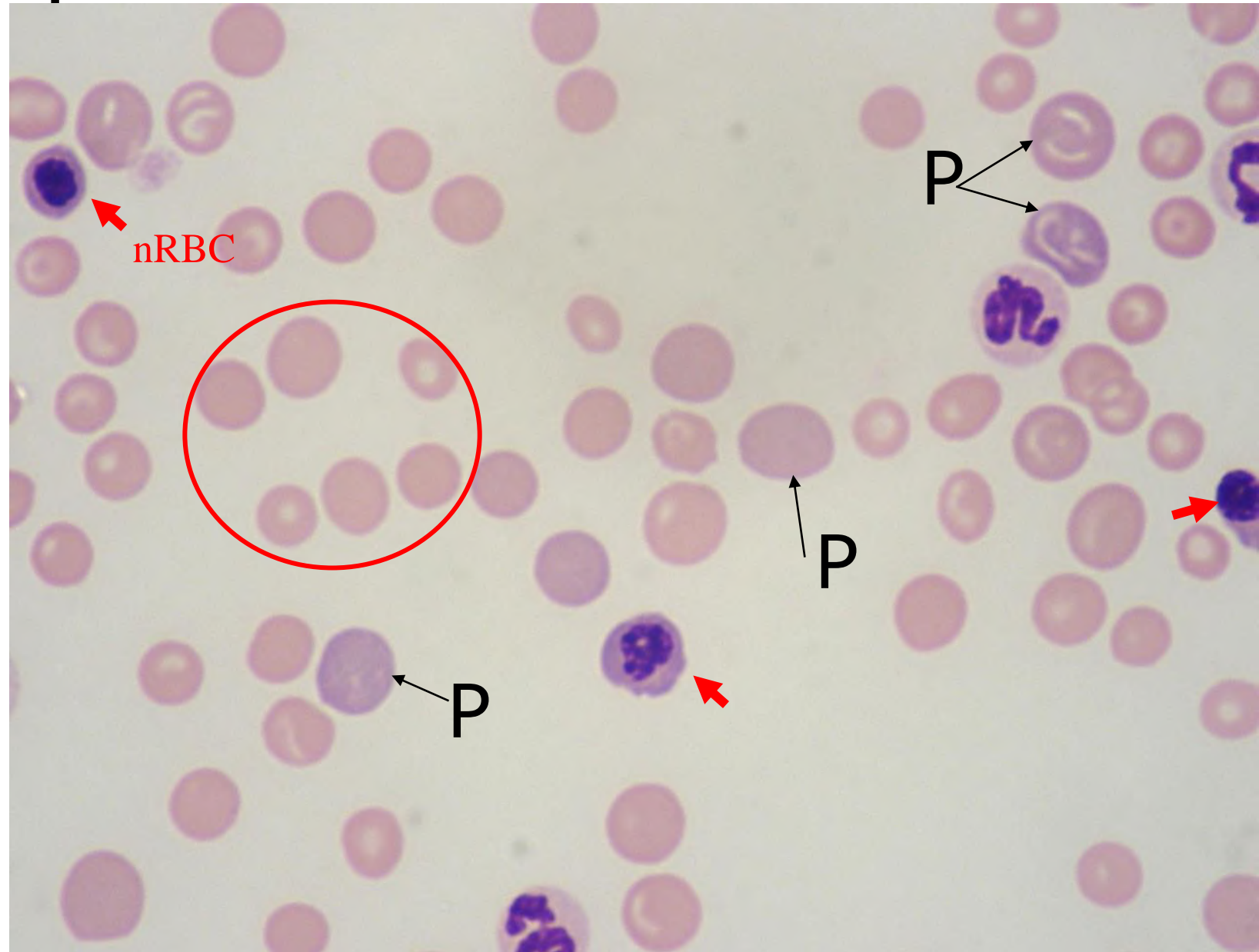
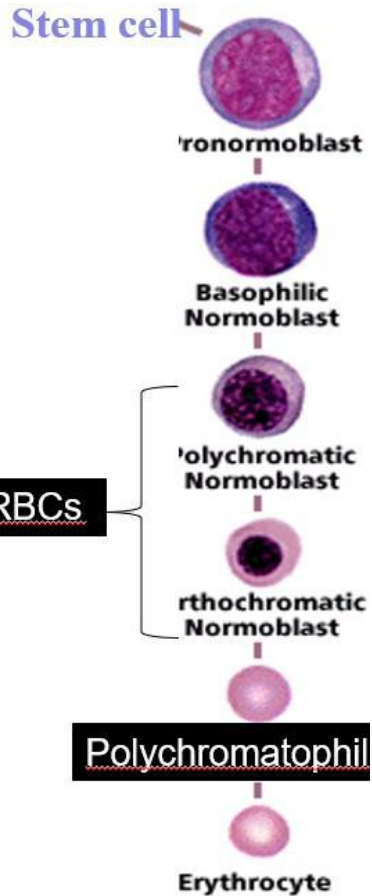
If a Reticulocyte count is NOT provided by the Haematology analyser

- **HOW CAN WE DETERMINE IF THE ANAEMIA IS REGENERATIVE OR NOT?**
- **MCV may or may not be Increased**
 - **Blood smear examination**

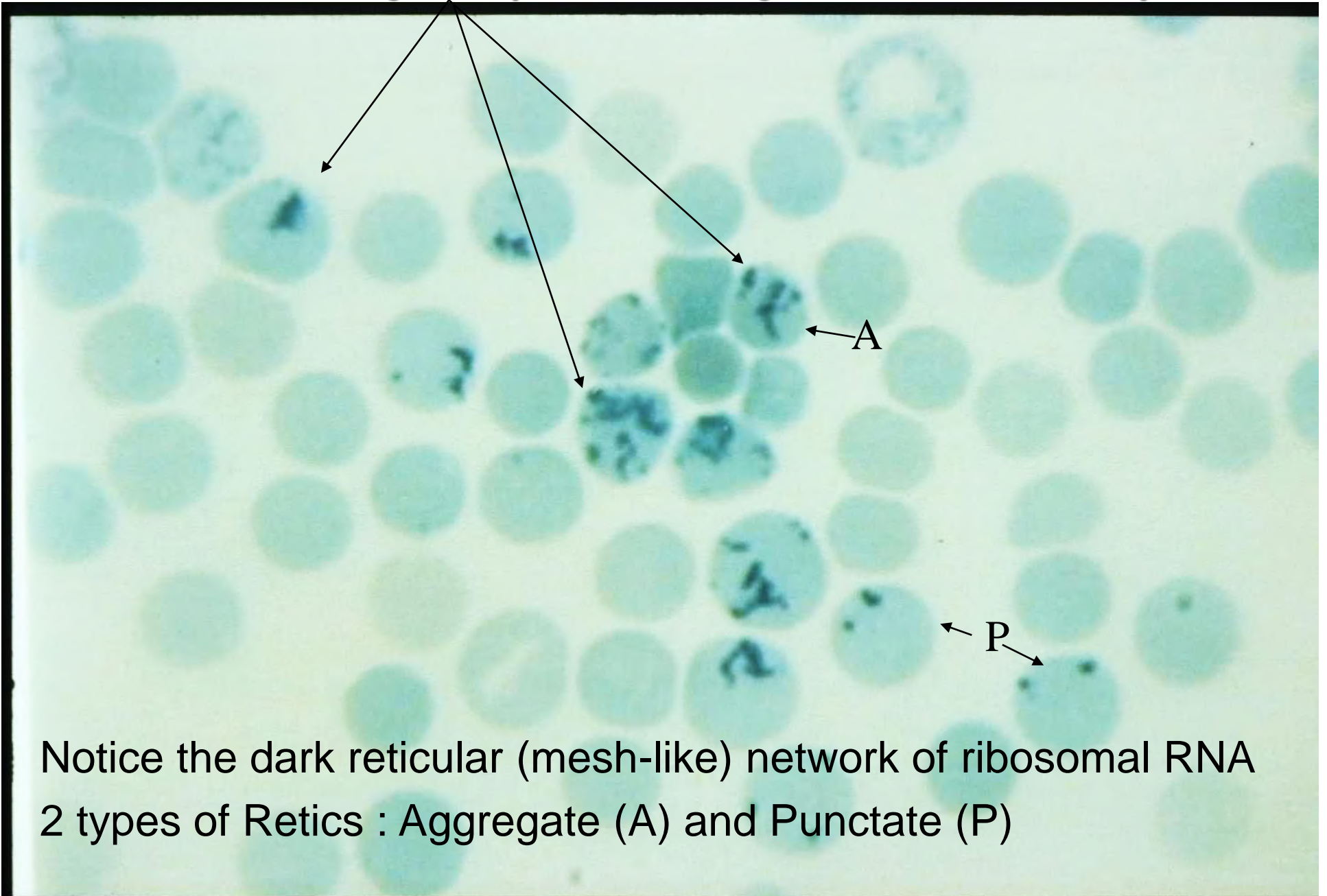


- Anisocytosis
- Polychromatophils
- Nucleated RBC (nRBCs)
- Reticulocytes

Evidence of regeneration (Diff-Quik stain)

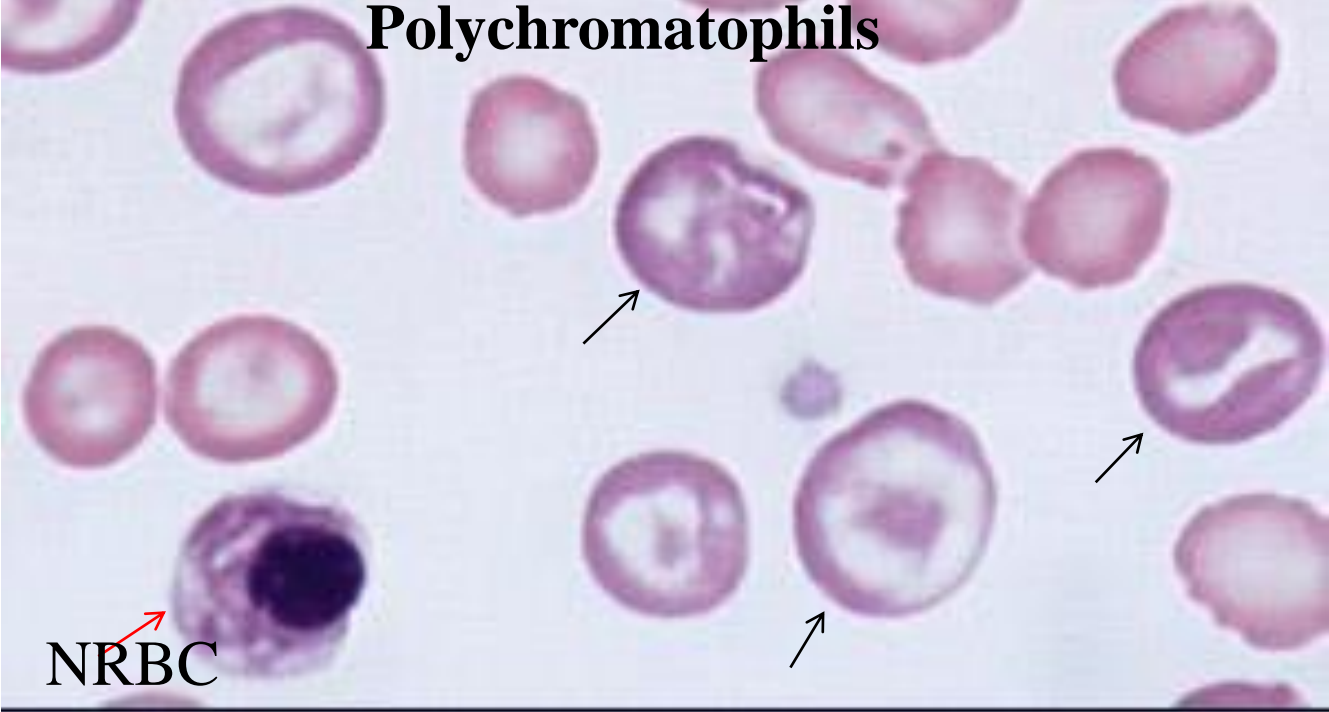


Reticulocytes (new methylene blue stain)



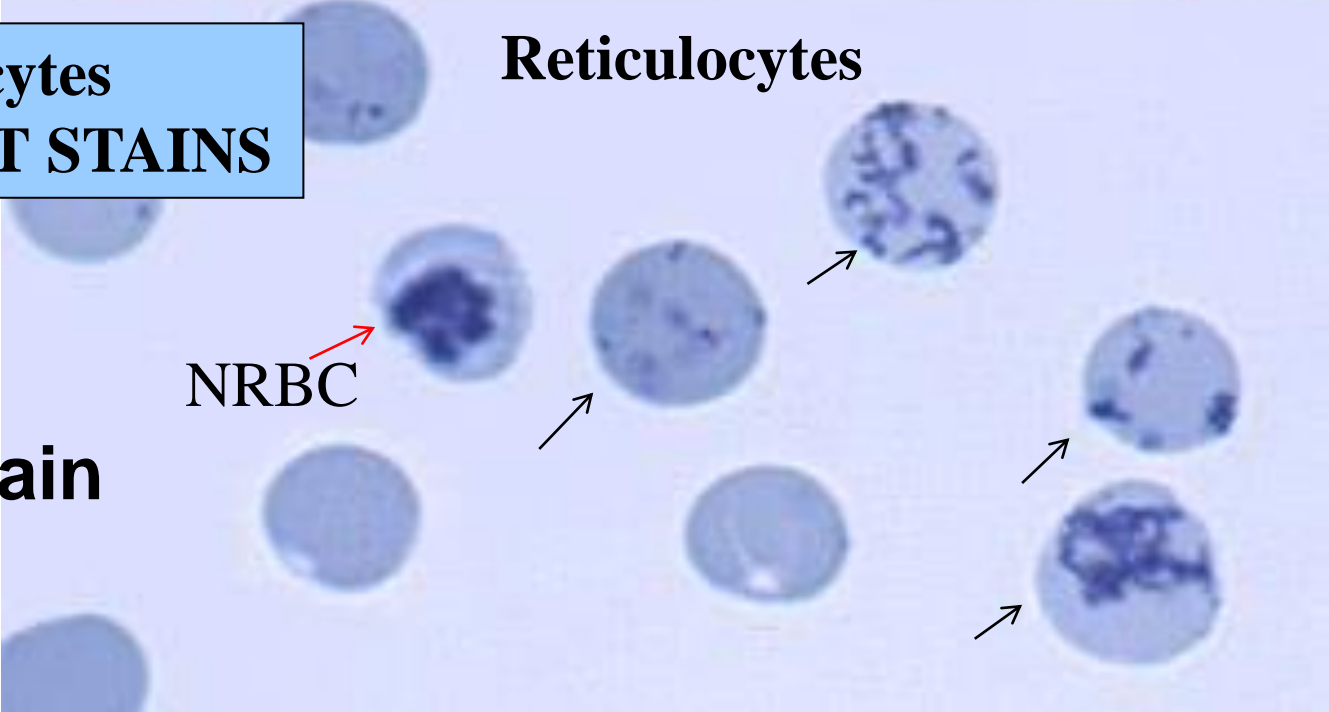
Notice the dark reticular (mesh-like) network of ribosomal RNA
2 types of Retics : Aggregate (A) and Punctate (P)

Diff-Quik stain

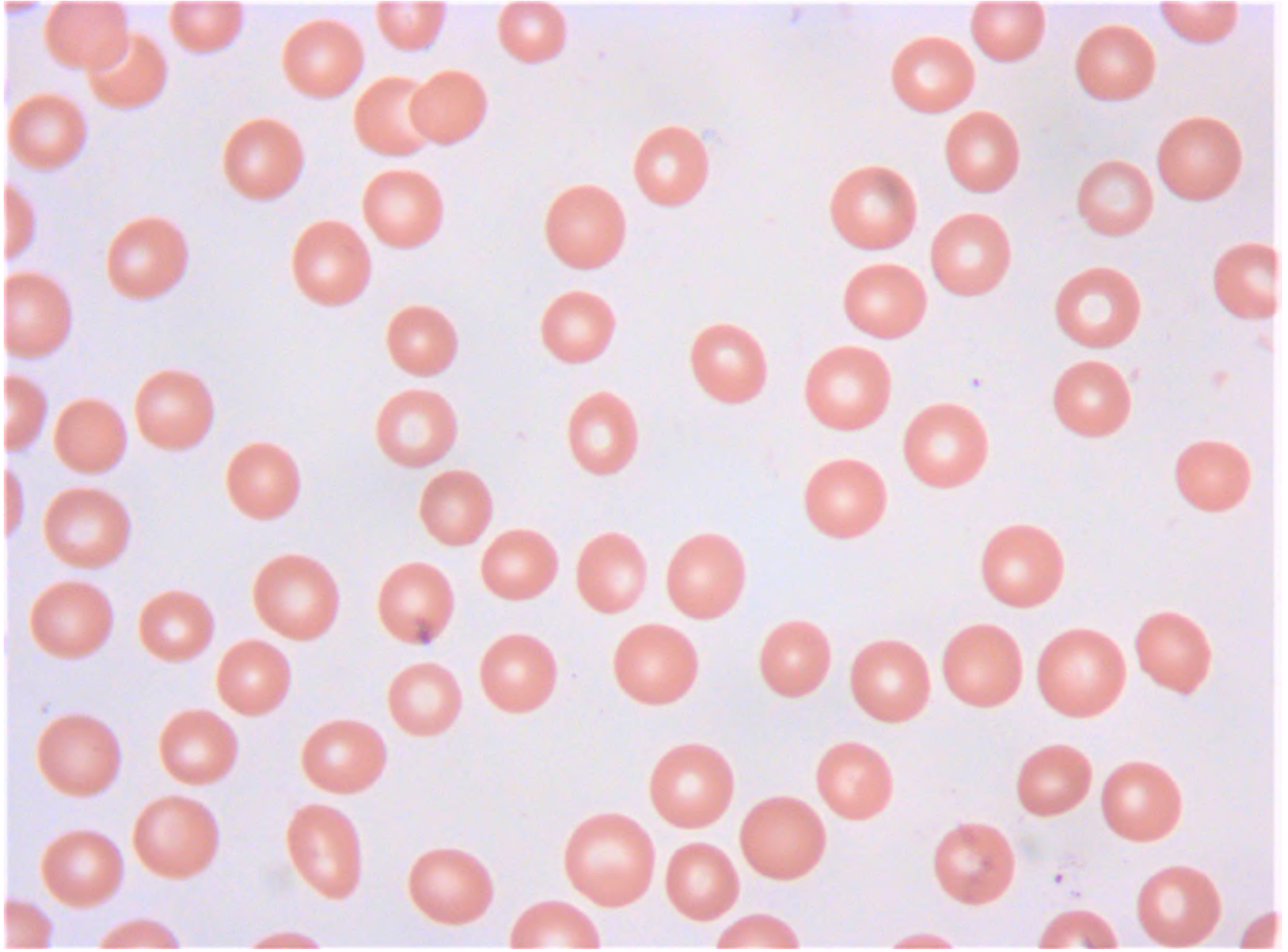


**Polychromatophils –Reticulocytes
SAME CELLS – DIFFERENT STAINS**

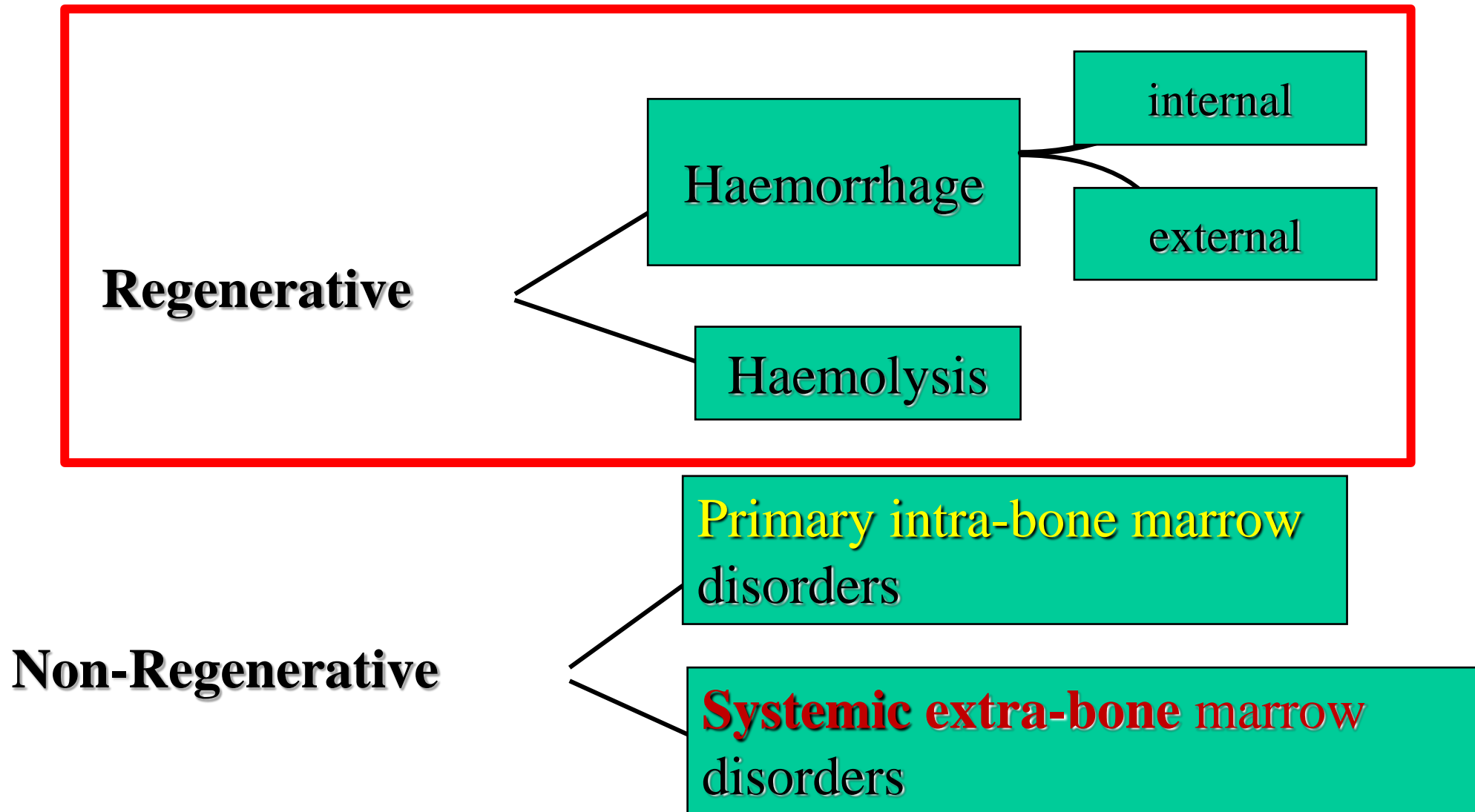
New methylene blue stain



Non-regenerative anaemia (= absence of Anisocytosis, Polychromasia/Reticulocytosis, nRBCs = normocytic, normochromic)



Anaemia: DECISION MAKING



HAEMORRHAGIC ANAEMIA

- **EXTERNAL**
 - First 2-4 days: Non-regenerative
 - Next few days: Regenerative
 - After weeks or months:
 - Chronic haemorrhage, Iron deficiency
 - Microcytic, hypochromic
- **INTERNAL**
 - First 2-4 days: Non-regenerative
 - Next few days: Regenerative
 - No iron-deficiency
 - Iron from the breakdown of RBCs (e.g. haemoglobin/iron) are available for recycling/use
- **Common causes**
 - Trauma
 - Parasites
 - Surgery
 - Neoplasia
 - Coagulopathies



HAEMOLYTIC ANAEMIA (HA)- Causes

• 1. IMHA

- Primary (autoimmune)
- Secondary to
 - Infections
 - Neoplasia
 - Lymphoma, Leukaemia
 - Drugs
 - Antibiotics
 - NSAID

2. HA due to mechanical destruction – microvascular

- Some diseases of the liver, spleen, myocardium
- Haemangiosarcoma
- Dirofilariasis
- DIC

3. HA due to oxidation

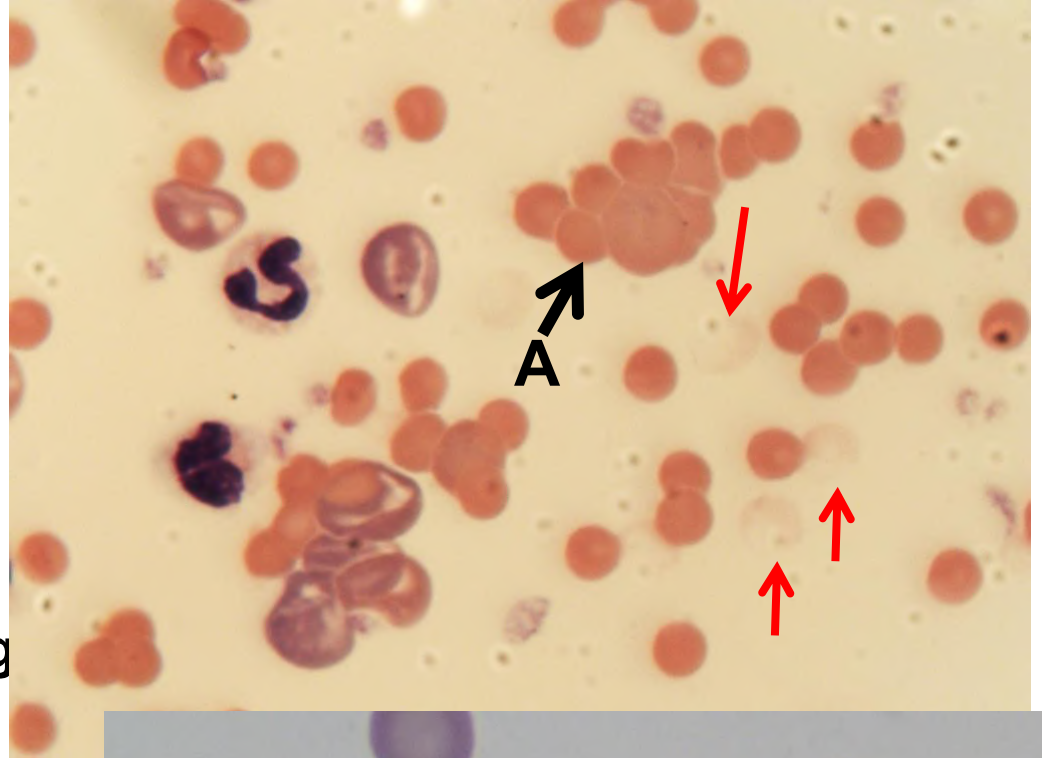
- Drugs, garlic, onion
- Diabetes m., HyperT4, Lymphoma

4. HA due to RBC metabolic defects

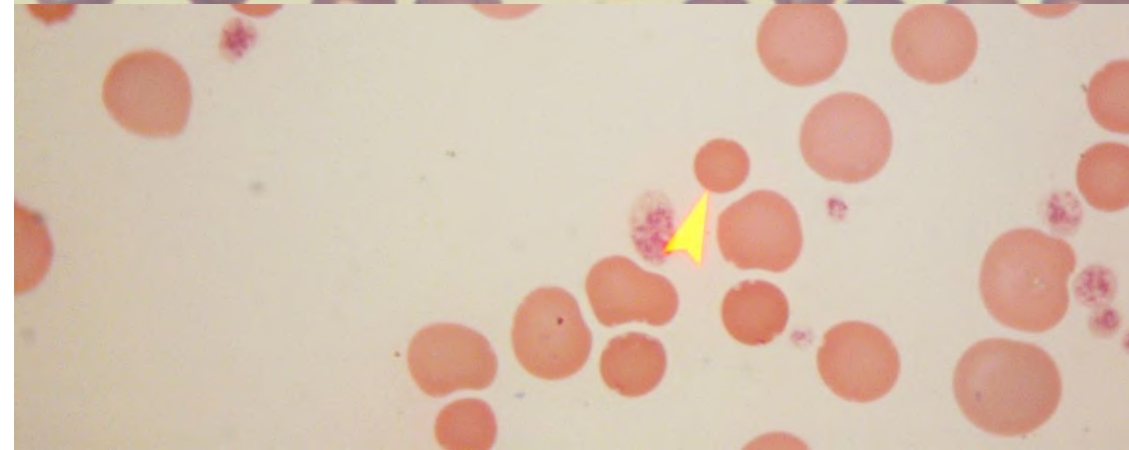
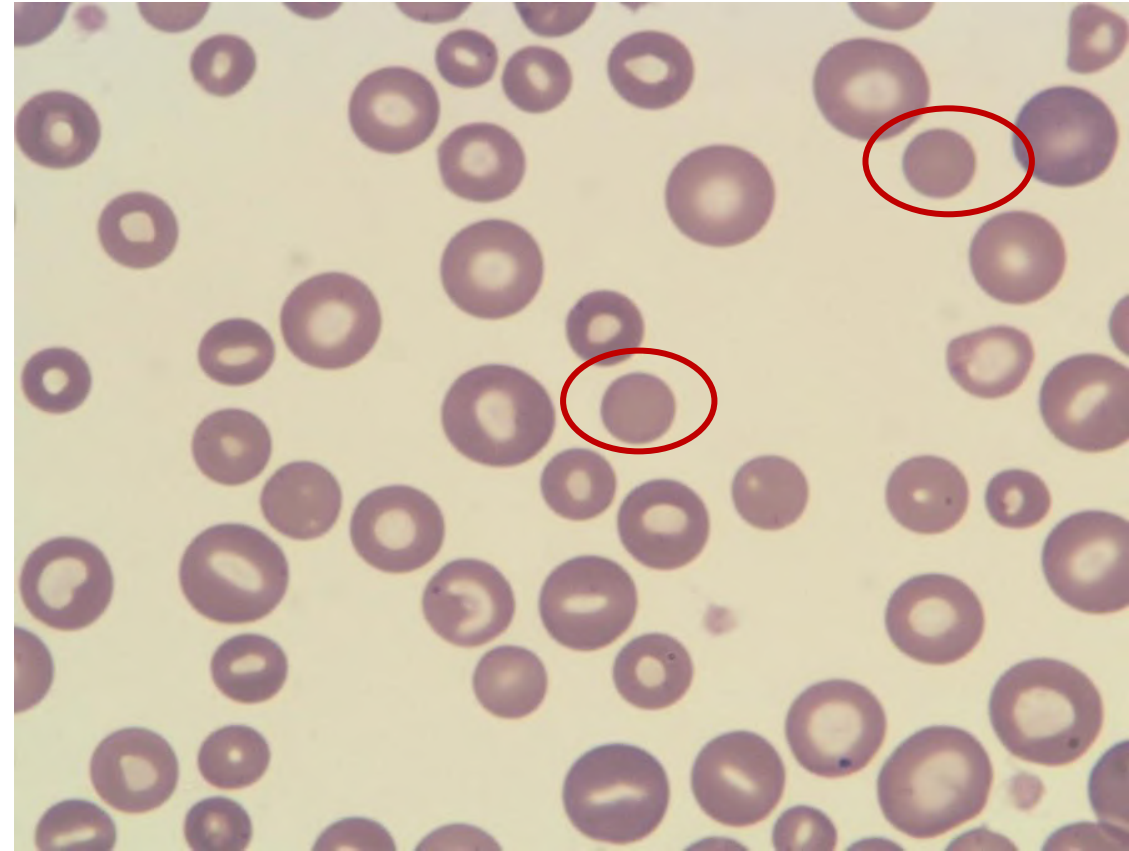
- Membrane, Enzymes

Primary IMHA

- Most common
- More common in Dogs vs Cats
- **Haematology**
 - Most frequently regenerative anaemia
 - Agglutination (A) (crosslinking of Ab on the surface of RBCs)
 - Presence of spherocytes (S)
 - Presence of **ghost cells**
 - remnant cell membranes (empty cells); created after intravascular IMH
- **Positive saline agglutination test**
- **Positive Coombs' test**
 - Test detects the presence of antibodies on the RBC



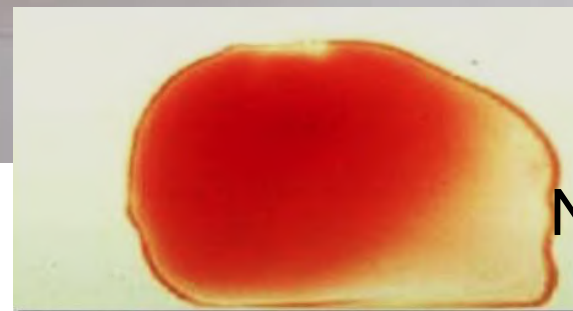
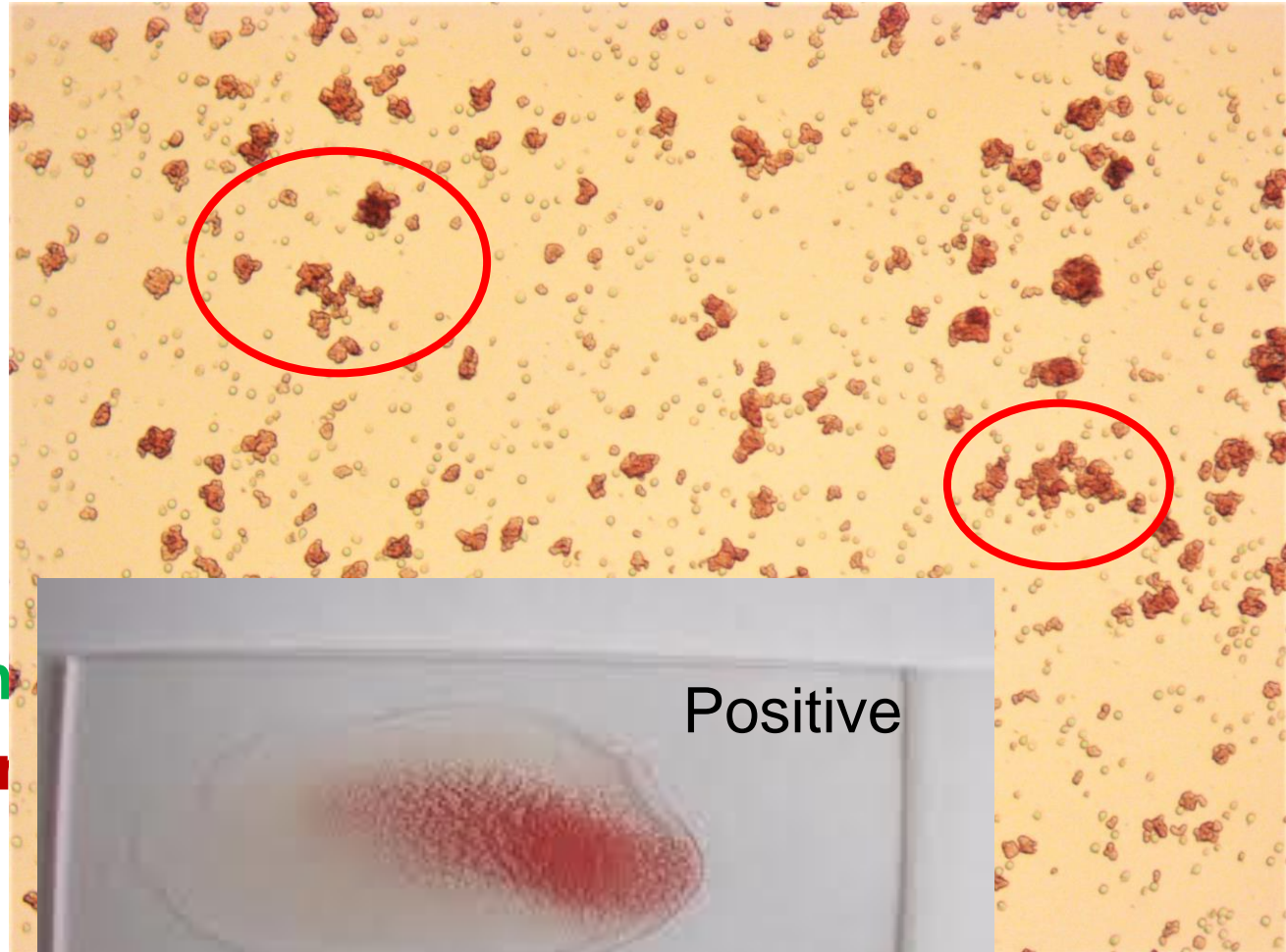
Spherocytes



- **Formed**
 - when the splenic macrophages remove portions of the membrane with bound antibody
 - the RBC reseals with a smaller volume
- **Diagnosis of IMHA**
 - ≥ 5 spherocytes/x100 oil field
- **Consistent with IMHA**
 - 3-4 spherocytes/x100 oil field
 - no other cause of non-immune-mediated spherocytosis identified
 - snake envenomation, bee sting
 - zinc toxicity
 - histiocytic sarcoma
- **Non-specific finding**
 - < 3 spherocytes/x100 oil field
- **Spherocytes cannot be identified reliably in cats**

IMHA-Diagnosis


- **Saline agglutination test**
- To confirm microscopic agglutination
- Mix on a slide
 - 1 drop of EDTA blood
 - 4 drops of saline
- Microscopic examination
 - If RBC agglutination persists
 - **POSITIVE**



Negative

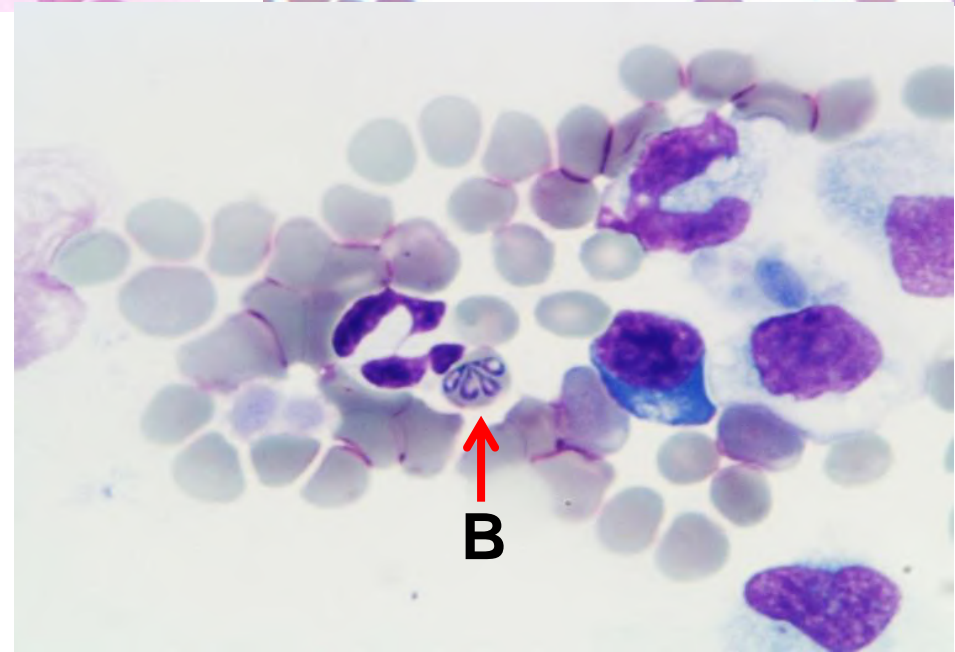
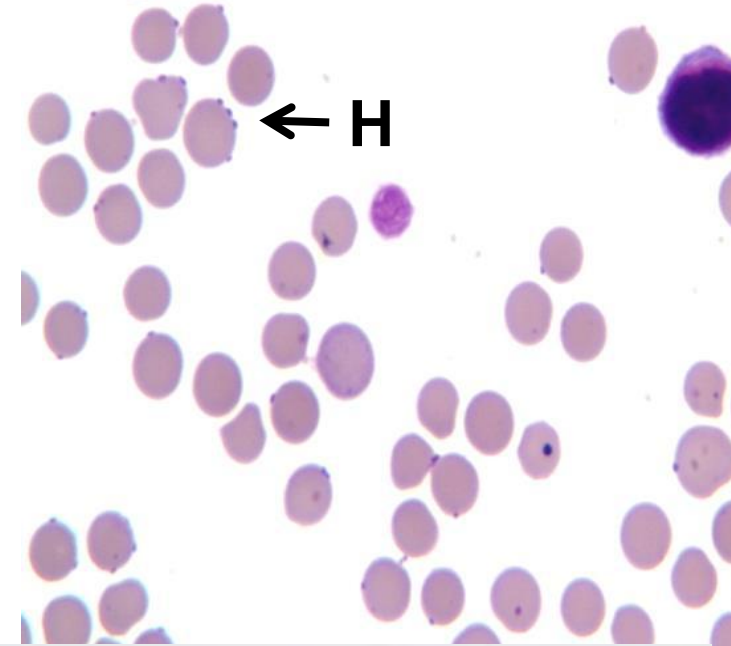
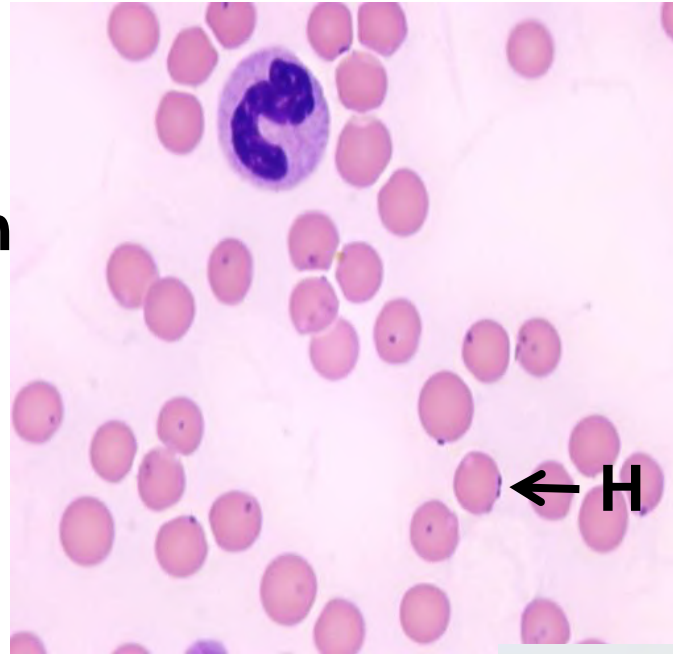
Coombs' test

- A test for in-clinic use is available
 - Clinically useful

 <p>Caviezel et al <i>JVIM</i> 2014;28:583-591</p>	Reference method Positive (n=20)	Reference method Negative (n=26)
Positive	19	1 (weak positive)
Negative	1	25

2. Haemolytic anaemia – Secondary to Infection

- Direct RBC destruction
- Indirect destruction
 - Harmful products
 - Presence of antibodies
- Parasites
 - Haemoplasmas (H)
 - Babesia (B)
- *Bacteria*
 - *Leptospira*
 - *Clostridium*
- *Ehrlichia, Leishmania, Dirofilaria*



HAEMOLYTIC ANAEMIA- *Causes*

1. IMHA

- Primary (autoimmune)
- Secondary to
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 - Neoplasia
 - Lymphoma, Leukaemia
 - Drugs
 - Antibiotics
 - NSAID

2. HA due to mechanical destruction – microvascular destruction

- **Some diseases of the liver, spleen, myocardium**
- **Haemangiosarcoma**
- **Dirofilariasis**
- **DIC**

3. HA due to oxidation

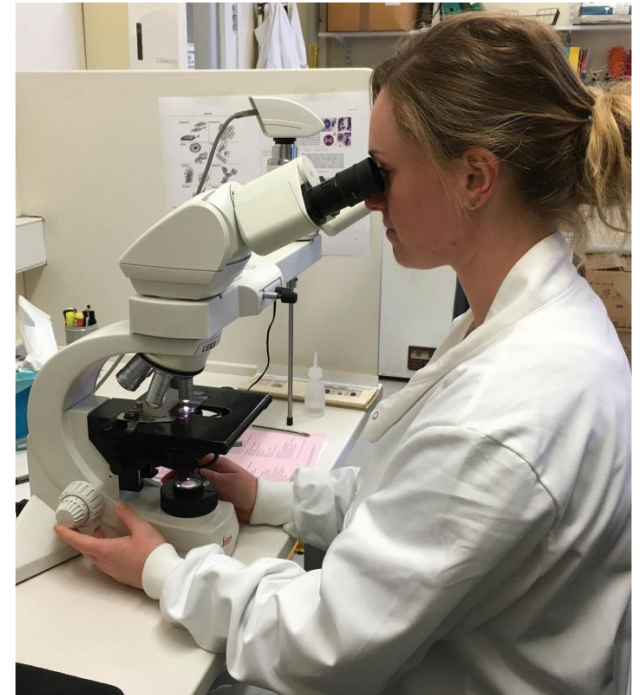
- **Drugs, garlic, onion**
- **Diabetes m., HyperT4, Lymphoma**

4. *HA due to RBC metabolic defects*

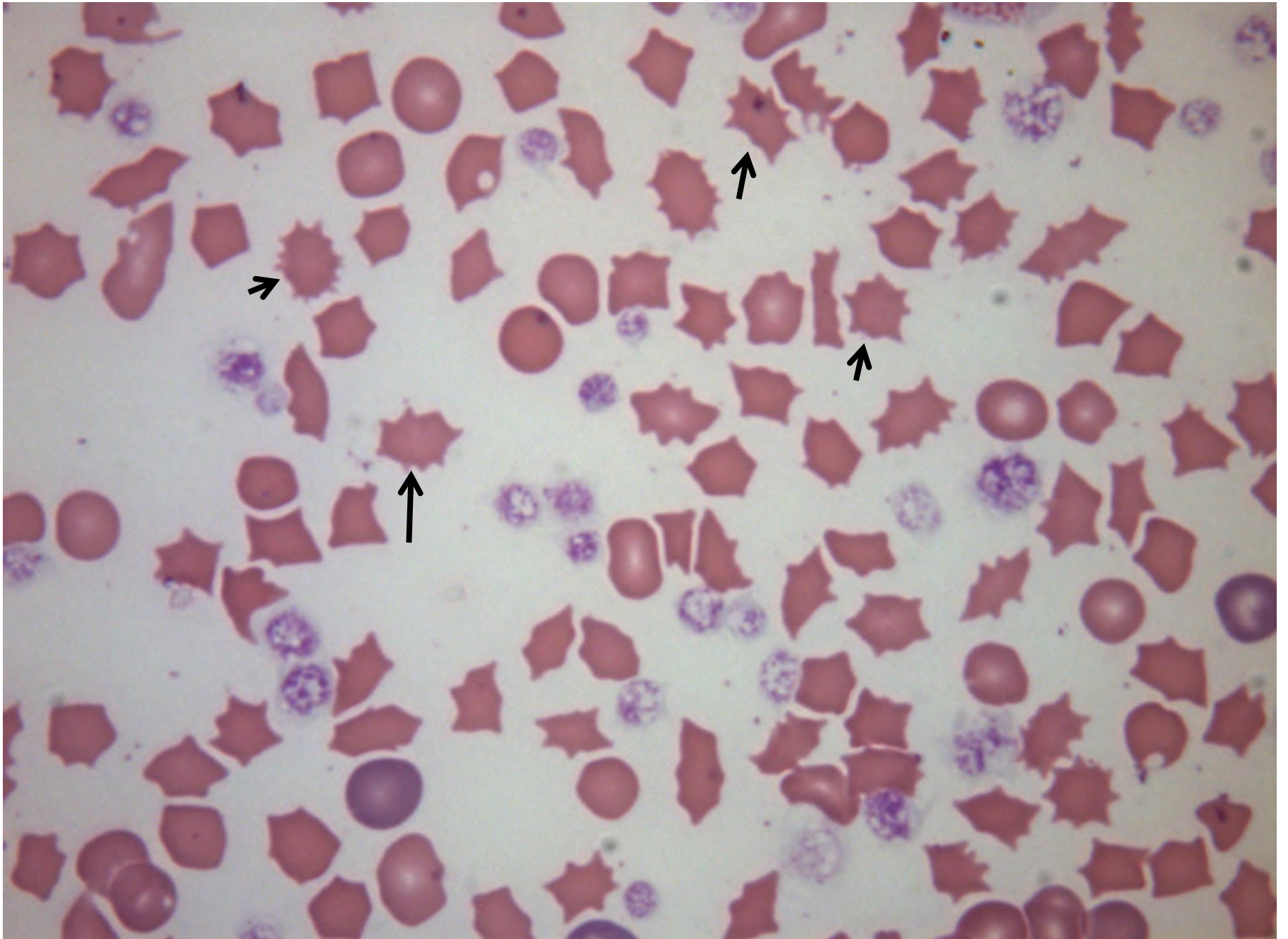
- **Membrane, Enzymes**

Blood smear examination

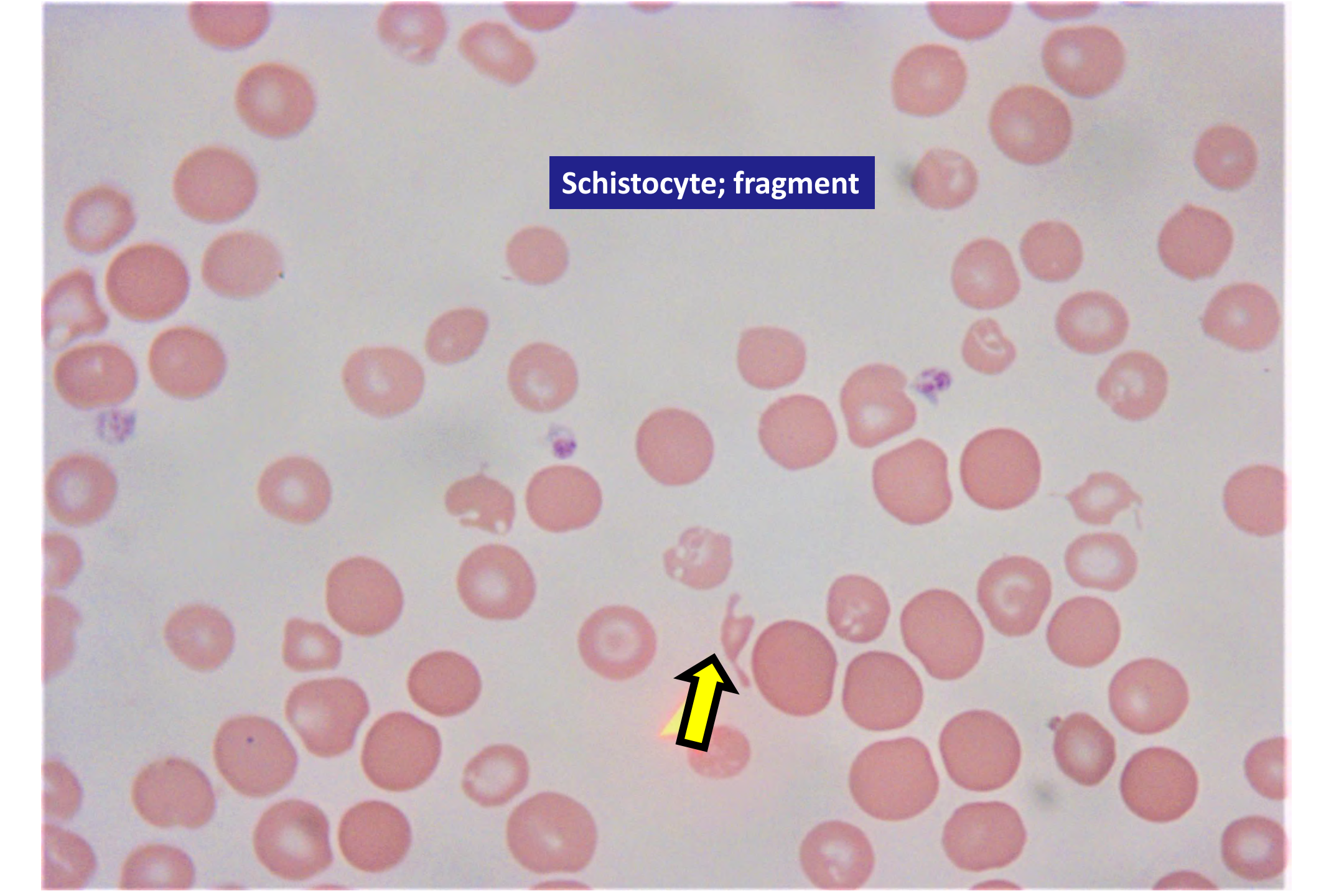
- **Morphologically abnormal RBCs**
 - due to mechanical/shear injury –microvascular destruction
 - **Acanthocytes**
 - **Schistocytes**



Acanthocytes



Schistocyte; fragment



HAEMOLYTIC ANAEMIA- *Causes*

1. IMHA

- Primary (autoimmune)
- Secondary to
 - Infections
 - Neoplasia
 - Lymphoma,
 - Leukaemia
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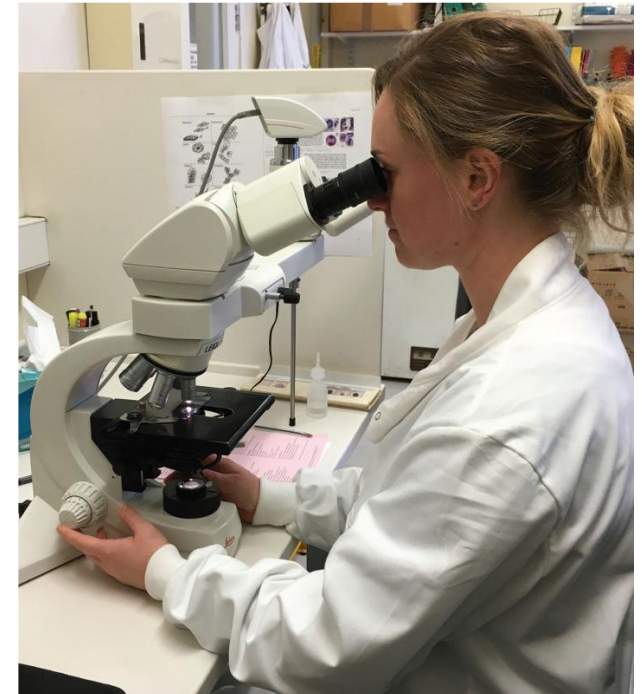
- Drugs, garlic, onion, Paracetamol
- Diabetes m., HyperT4, Lymphoma

4. *HA due to RBC metabolic defects*

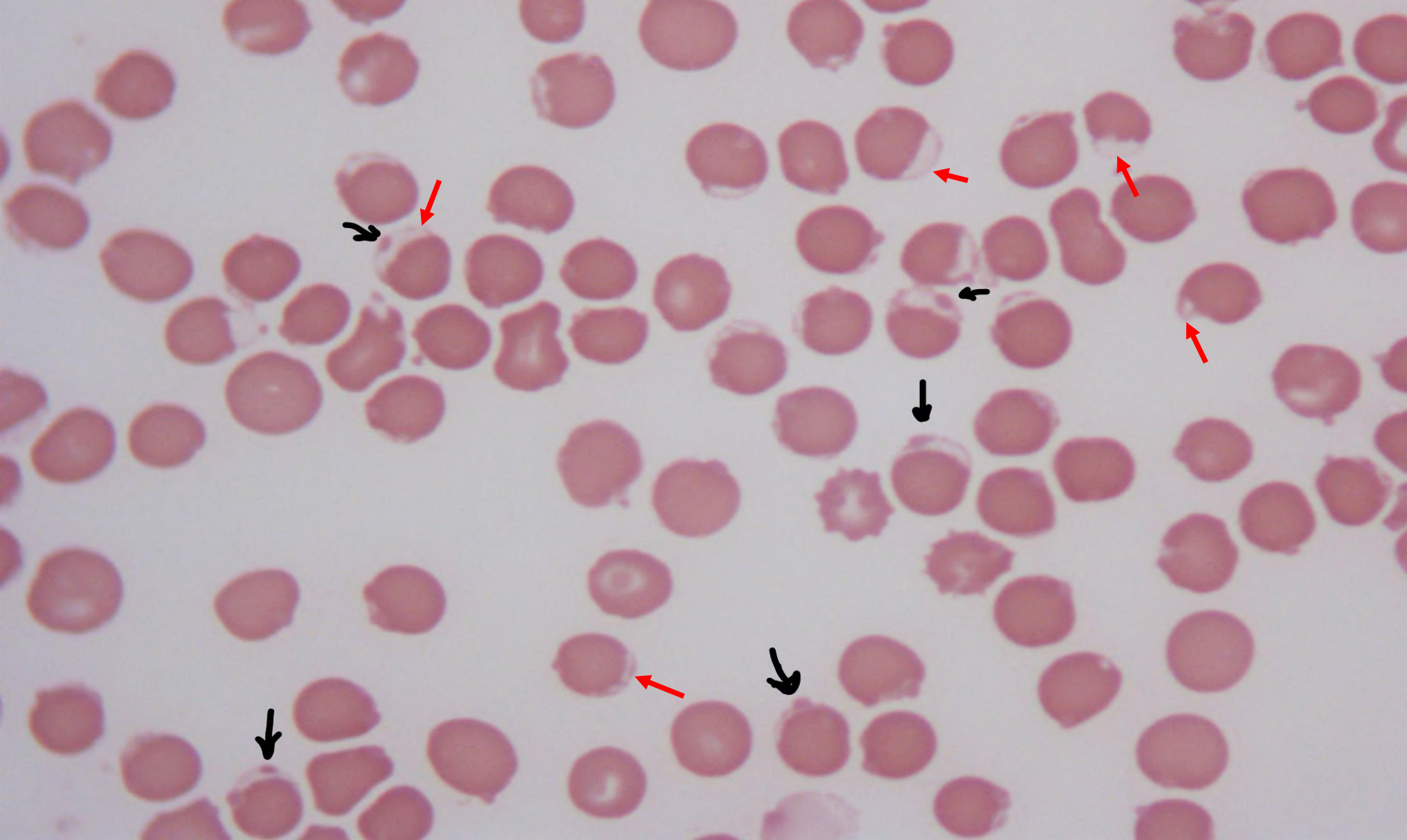
- Membrane, Enzymes

Blood smear examination

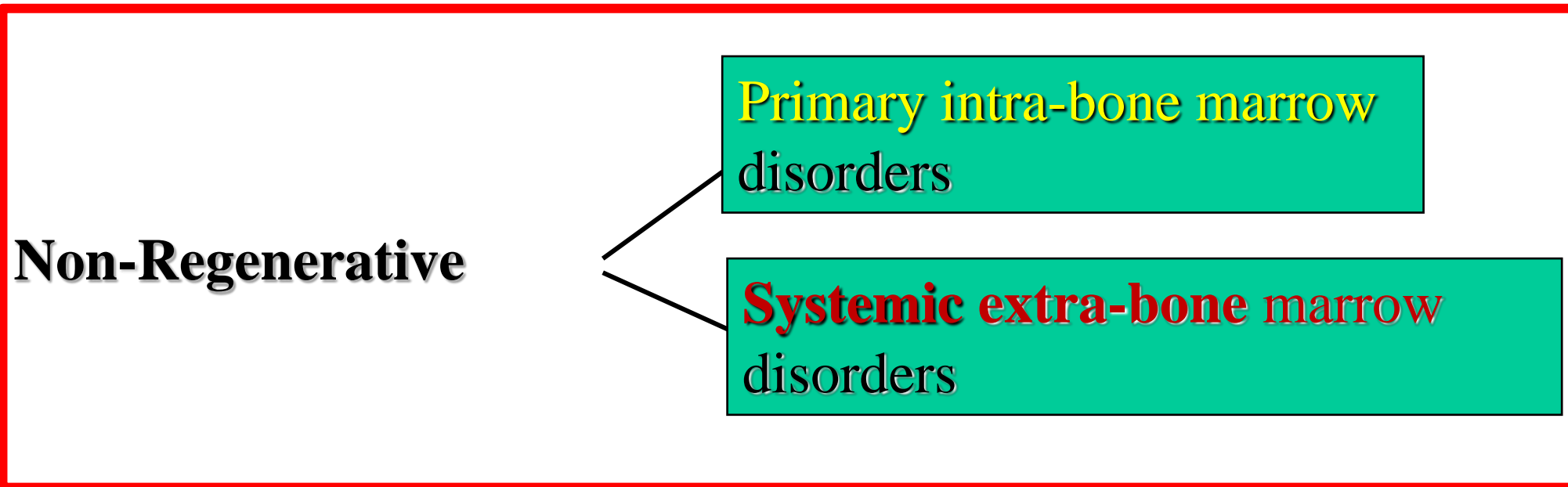
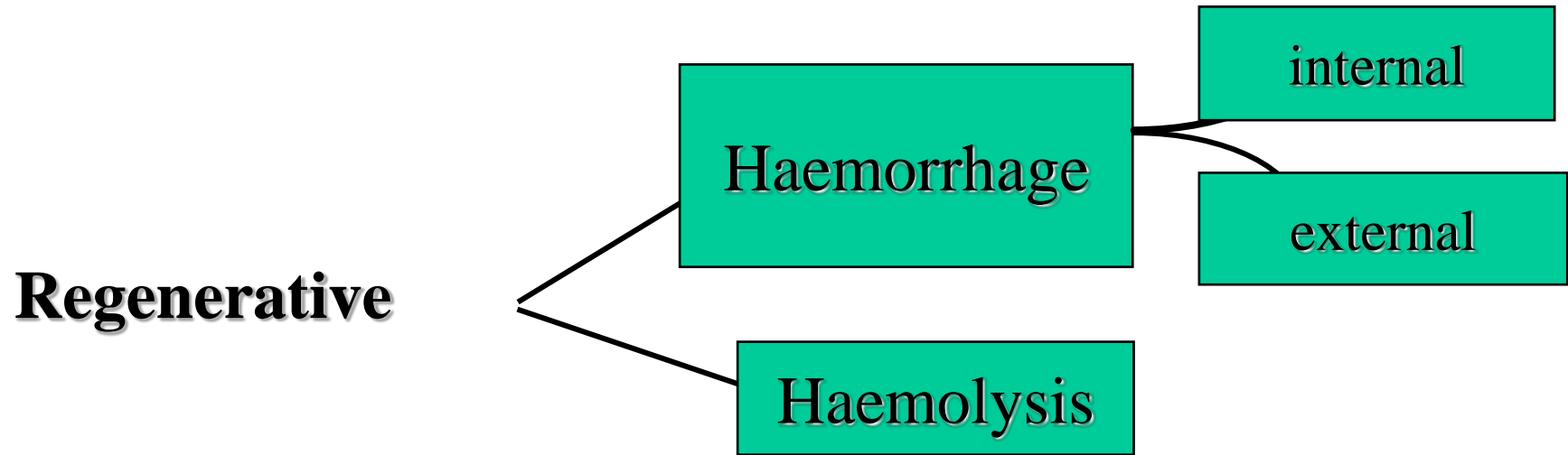
- **RBC morphological abnormalities**
 - **due to oxidation**
 - **Eccentrocytes**
 - **Heinz bodies**



Eccentrocytes (clear area; damaged cell membrane)
& Heinz bodies (dark body; oxidised haemoglobin)

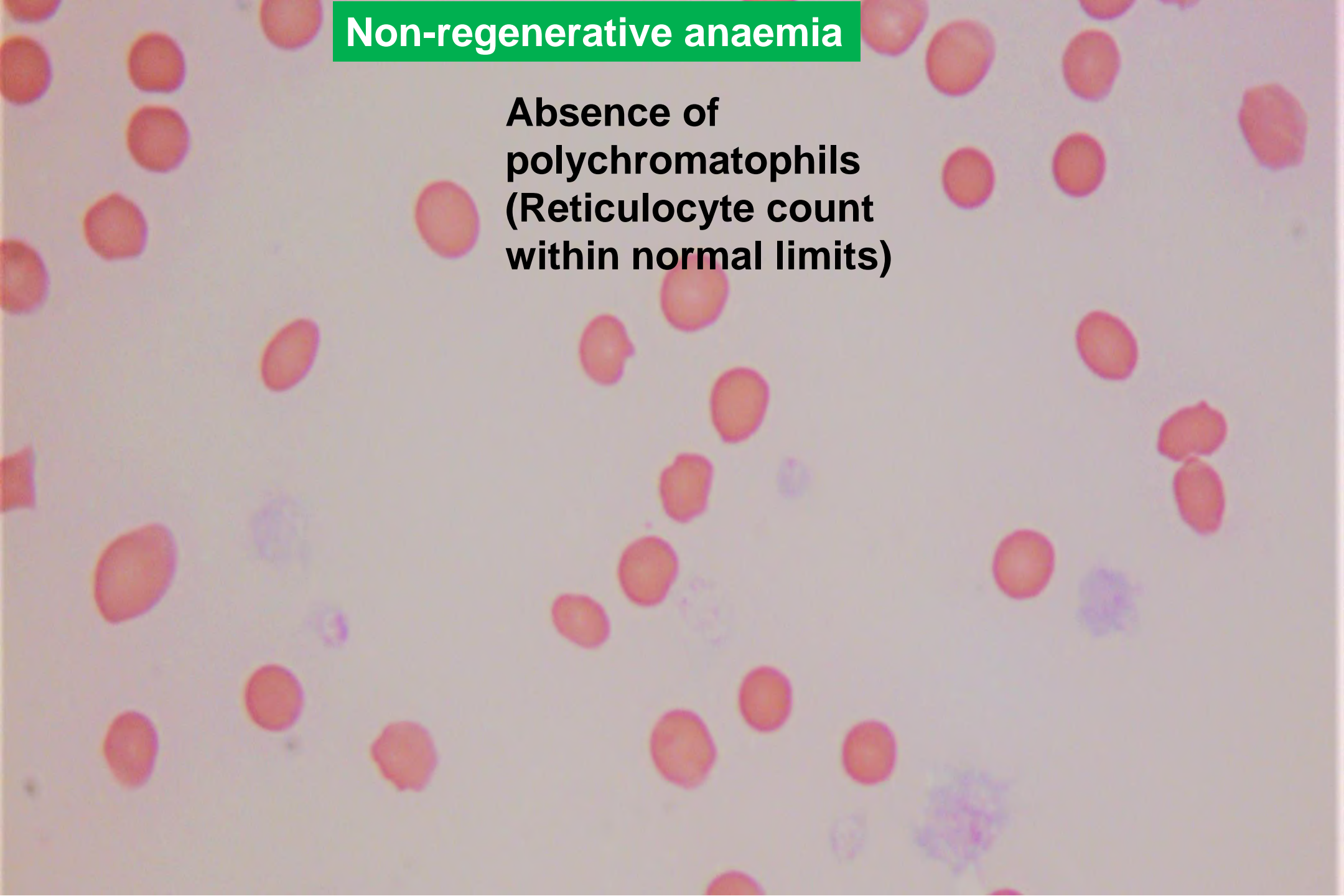


Anaemia: DECISION MAKING



Non-regenerative anaemia

**Absence of
polychromatophils
(Reticulocyte count
within normal limits)**



Non-regenerative anaemia

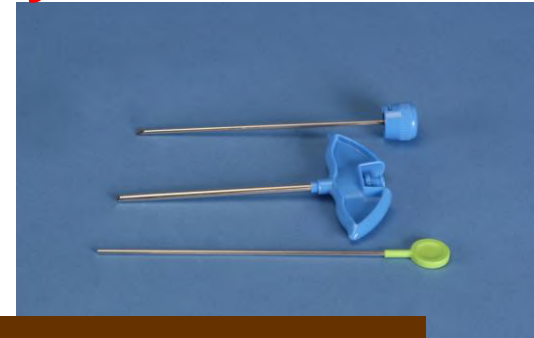
Extra-marrow disorders

- Most common
- Frequently moderate anaemia
- **CHRONIC DISEASES**
 - Inflammatory, Degenerative, Neoplastic
- **CHRONIC KIDNEY DISEASE**
- **ENDOCRINOPATHIES**
 - Hypothyroidism, Hypoadrenocorticism
- **VITAMIN DEFICIENCY**
 - Folate, B₁₂
 - Anaemia may be macrocytic-normochromic
- **CHRONIC IRON DEFICIENCY**

Intra-marrow disorders

Frequently the anaemia is severe. Commonly there is bi- or pancytopenia.

Aplastic anaemia,
Pure red cell aplasia,
Non-Regenerative IMHA
Neoplasia-Leukaemia
Myelodysplastic syndromes



Final diagnosis:
Bone marrow examination
(Cytology and Histopathology)

Tusen takk!

Har du noen spørsmål?

Seksjonen er sponset av

 TRIOLAB

 **MSD**
Animal Health

Torsdag 14. mars

Program for Smådyr