
United States of America
State Boundary
Road
River
National Capital
State Capital
City or Town
0 300 KM
0 300 Miles
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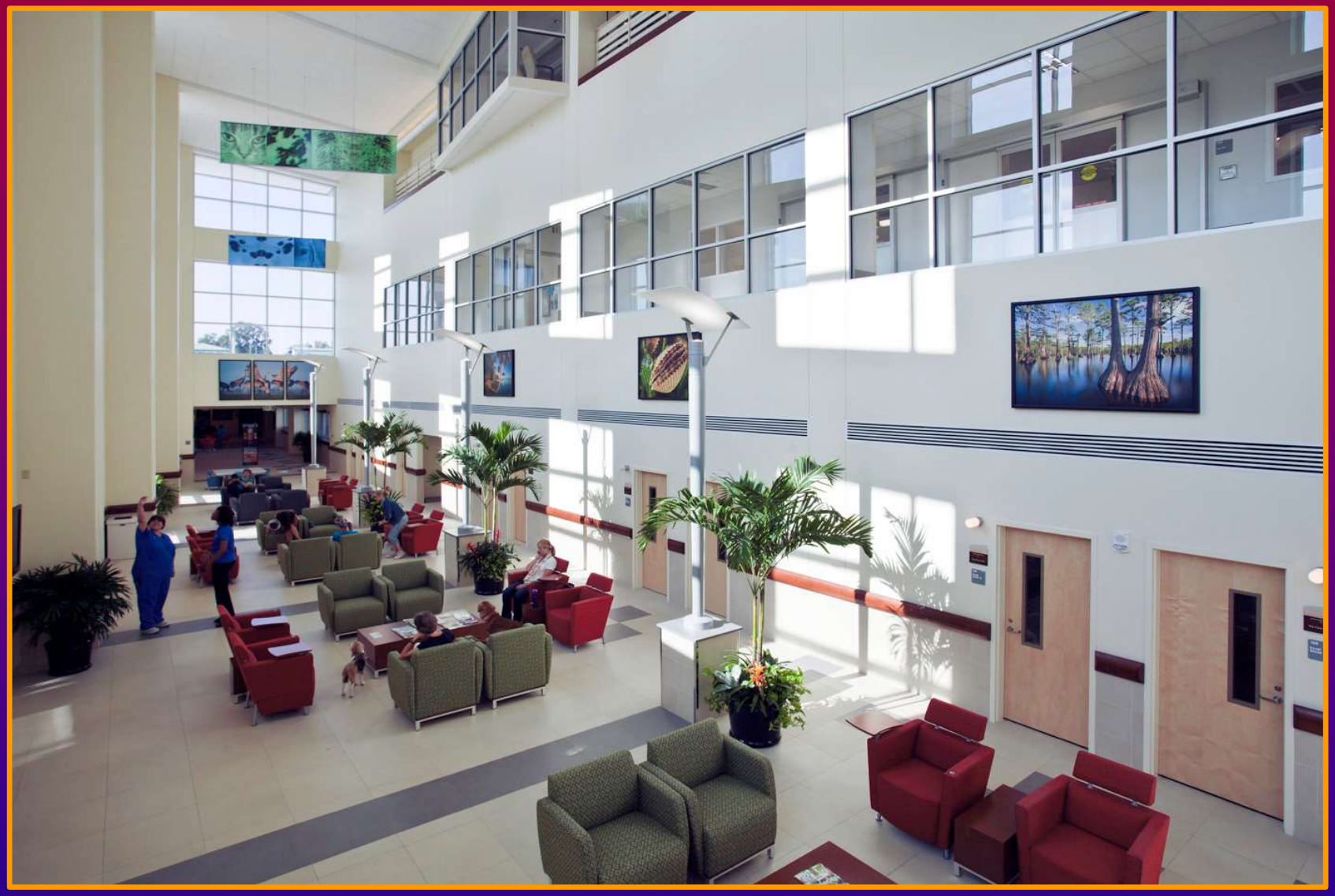
CANADA
MEXICO
U.S.A.
Pacific Ocean
Atlantic Ocean
Gulf of Mexico
Washington, D.C.
Ottawa
Mexicali
Hermosillo
Chihuahua
Saltillo
Monterrey
Laredo
Corpus Christi
Nassau
THE BAHAMAS
RUSSIA
ALASKA
HAWAII
Anchorage
Juneau
Honolulu
0 500 KM
0 500 Miles
0 100 KM
0 100 Miles

University of Florida
College of Veterinary Medicine

Small Animal Hospital

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SALT CREEK
GULF OF MEXICO

Unique Feline Hematology and the Impact of FeLV and FIV Infections

John W. Harvey, DVM, PhD, DACVP
College of Veterinary Medicine
University of Florida

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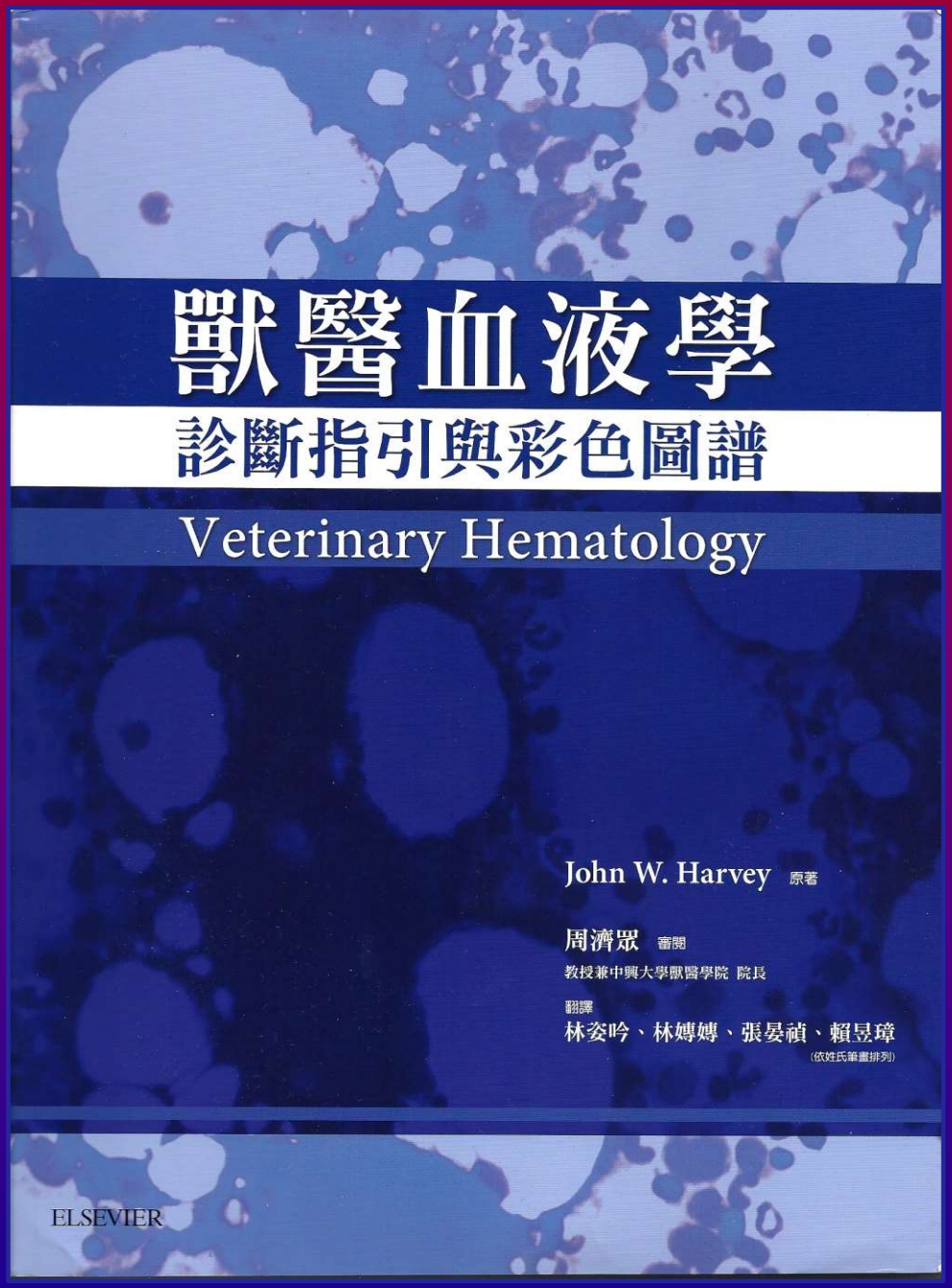
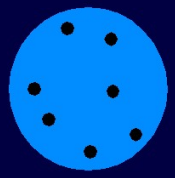
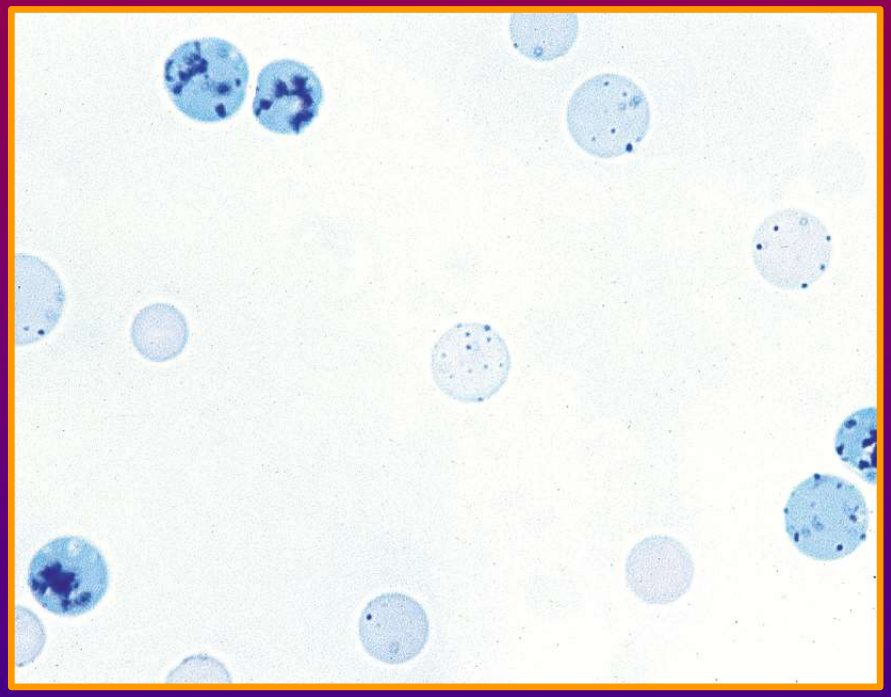

獸醫血液學
診斷指引與彩色圖譜
Veterinary Hematology
John W. Harvey 原著
周濟眾 審閱
教授兼中興大學獸醫學院 院長
翻譯
林姿吟、林嫥嫥、張晏禎、賴昱璋
(依姓氏筆畫排列)
ELSEVIER

Feline Reticulocytosis

Aggregate Reticulocytes

Punctate Reticulocytes



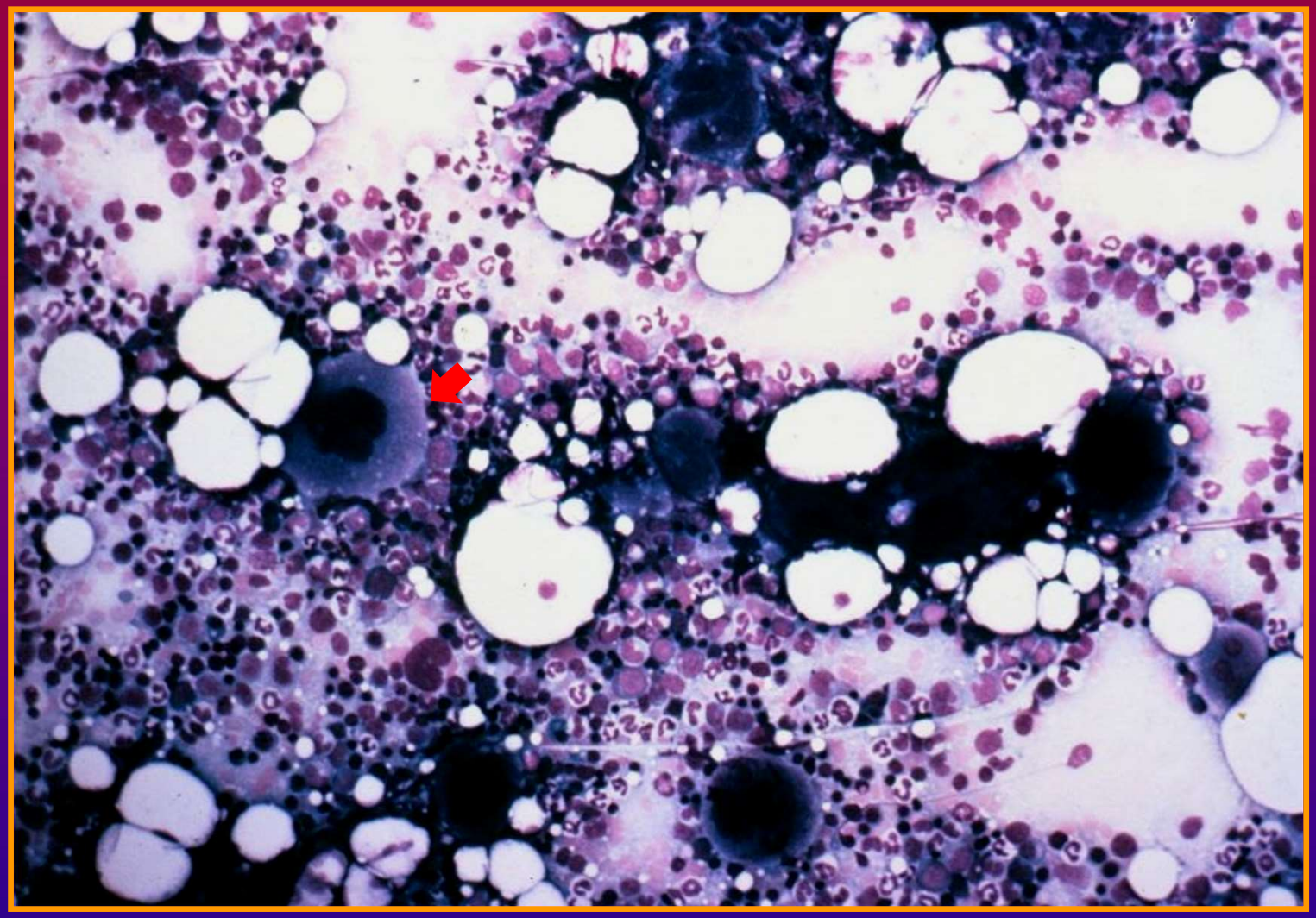


New Methylene Blue Reticulocyte Stain

Reticulocyte Counts

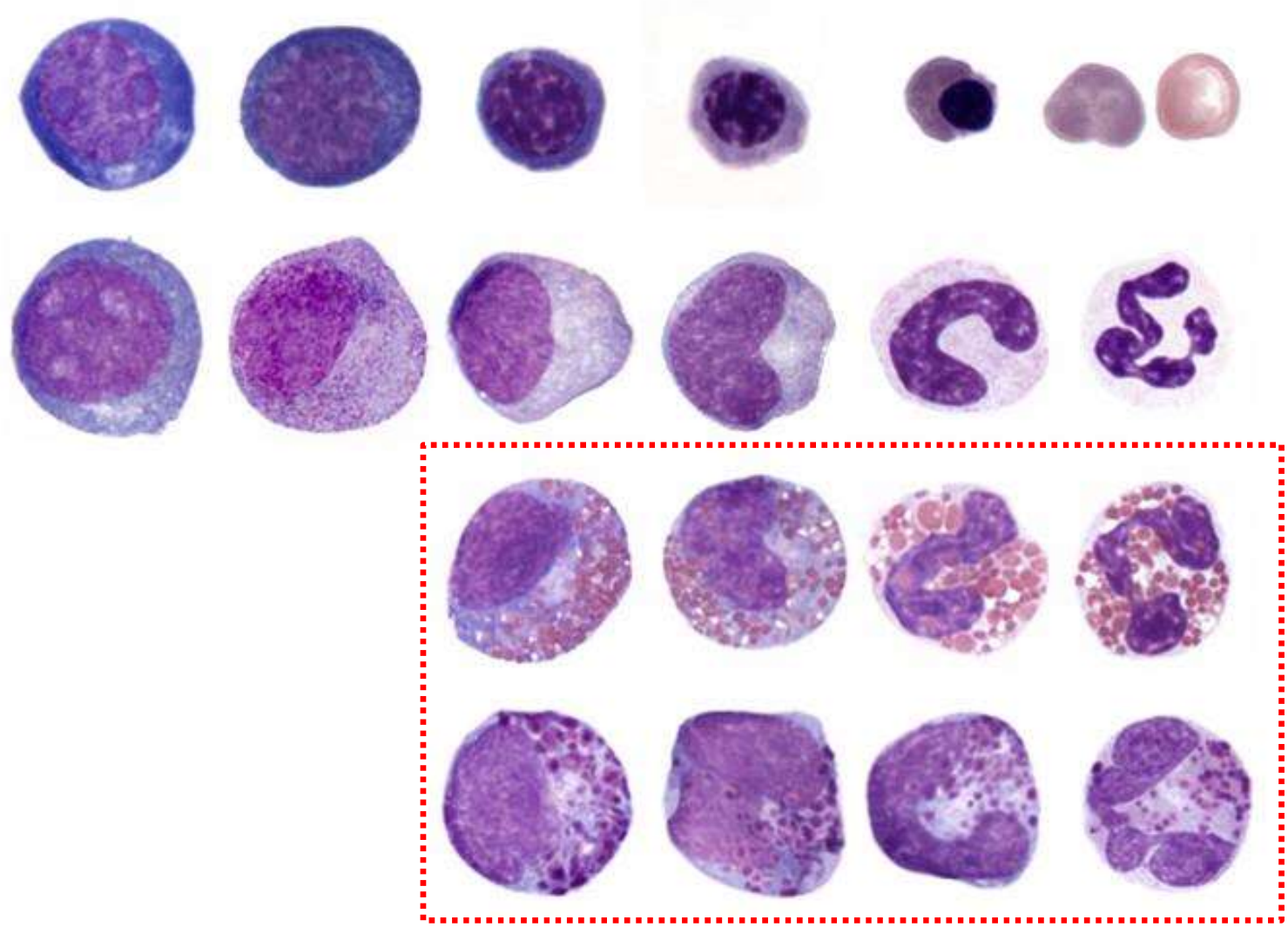
- Counting the numbers of reticulocytes in blood is critical for assessing anemias.
- Immature reticulocytes are produced in the bone marrow and generally undergo final maturation to mature erythrocyte in the blood and/or the spleen.
- Increased numbers of reticulocytes are produced in response to erythrocyte destruction or hemorrhage when bone marrow is healthy.

Normal Marrow Aspirate



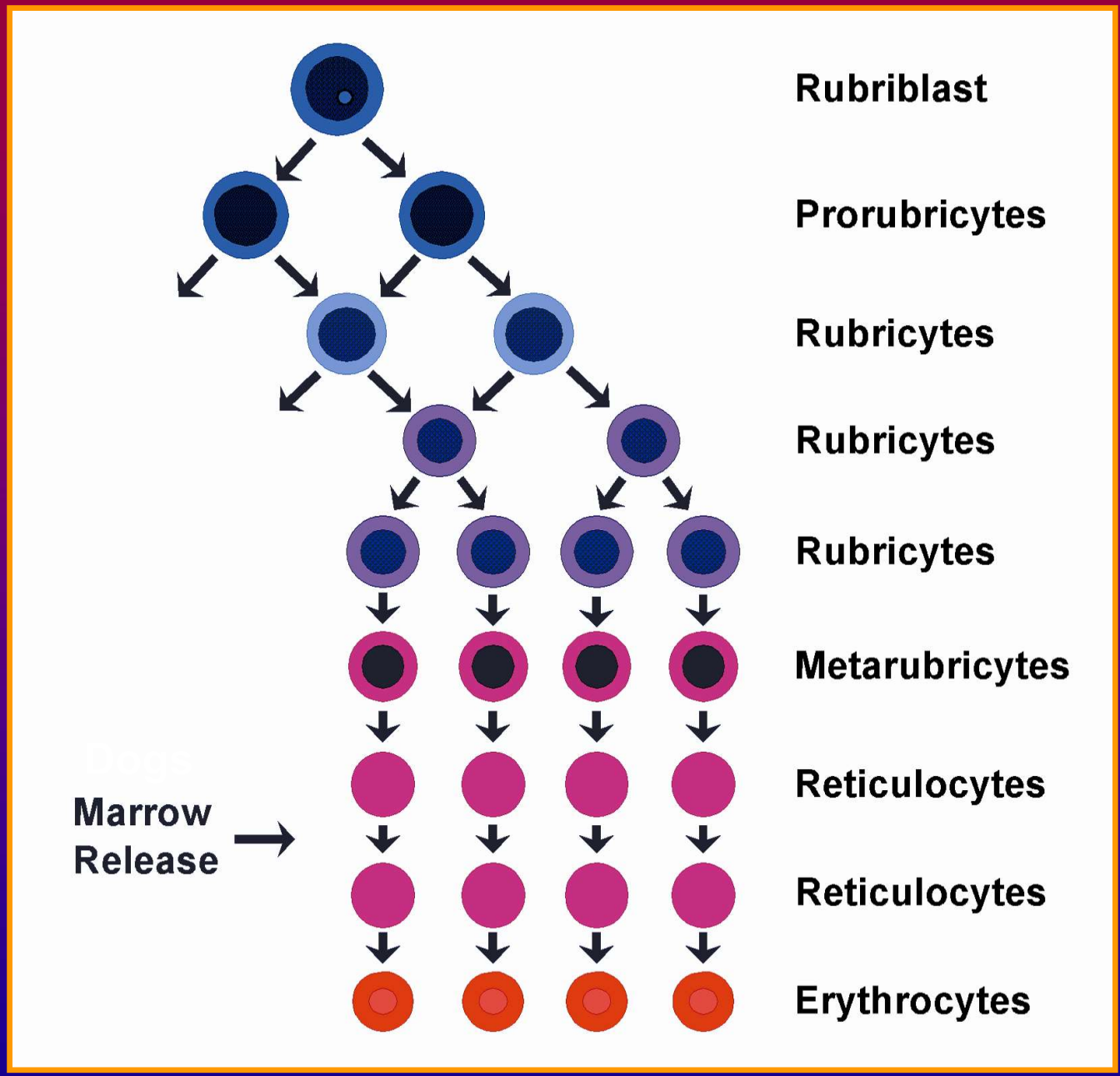
Wright-Giemsa Stain

Canine Precursor Cells

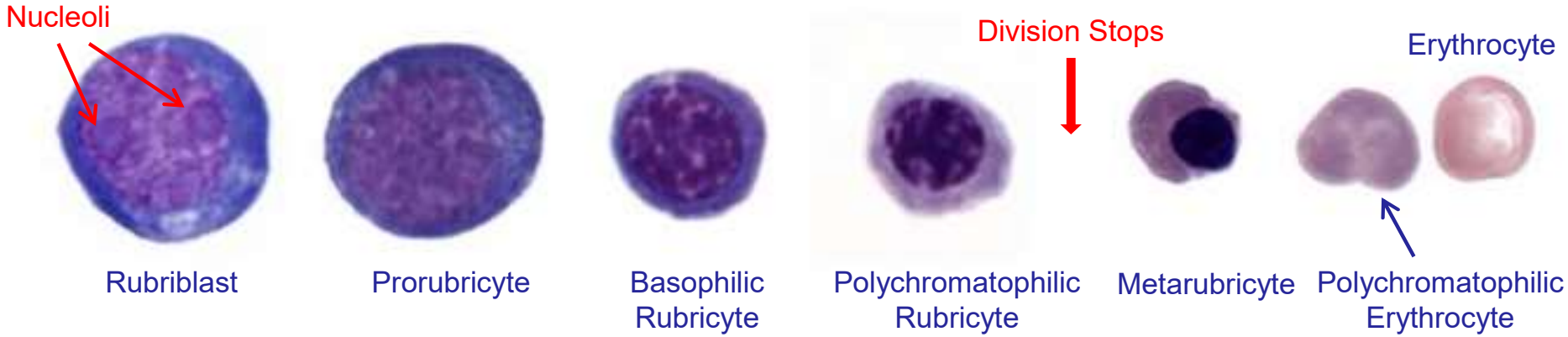


Harvey JW: Veterinary Hematology: A Diagnostic Guide and Color Atlas, 2012

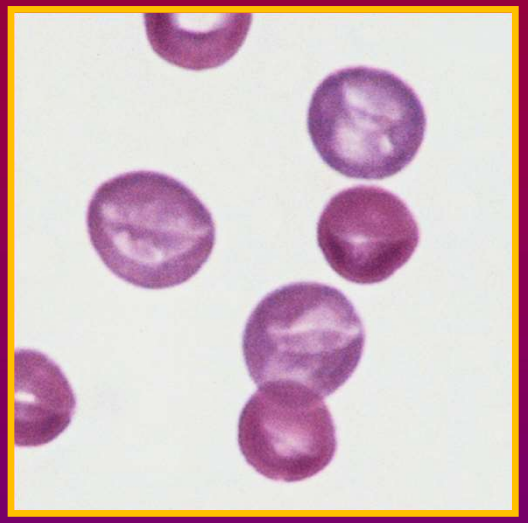
Maturational Divisions



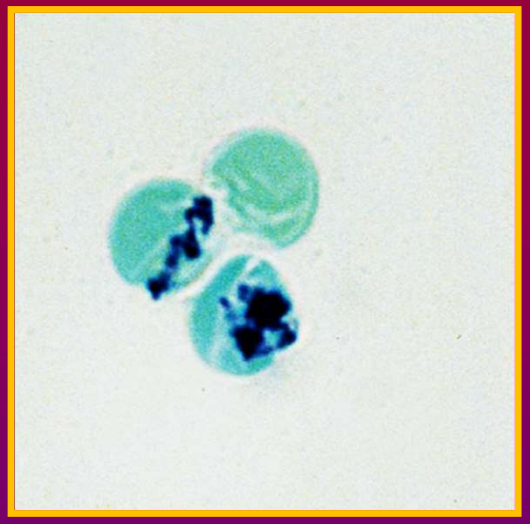
Erythropoiesis



Immature Reticulocytes

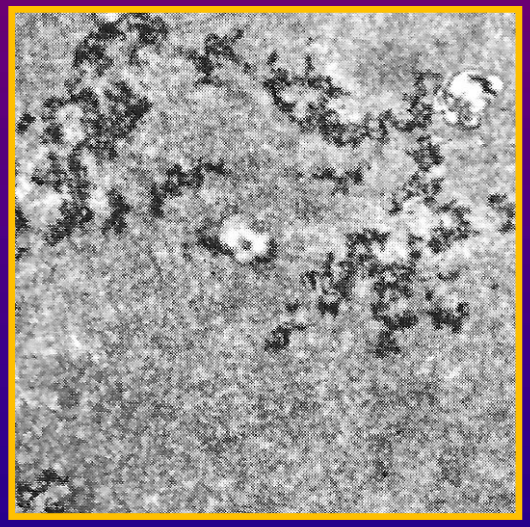


Wright-Giemsa Stain



Reticulocyte Stain

Electron Microscopy



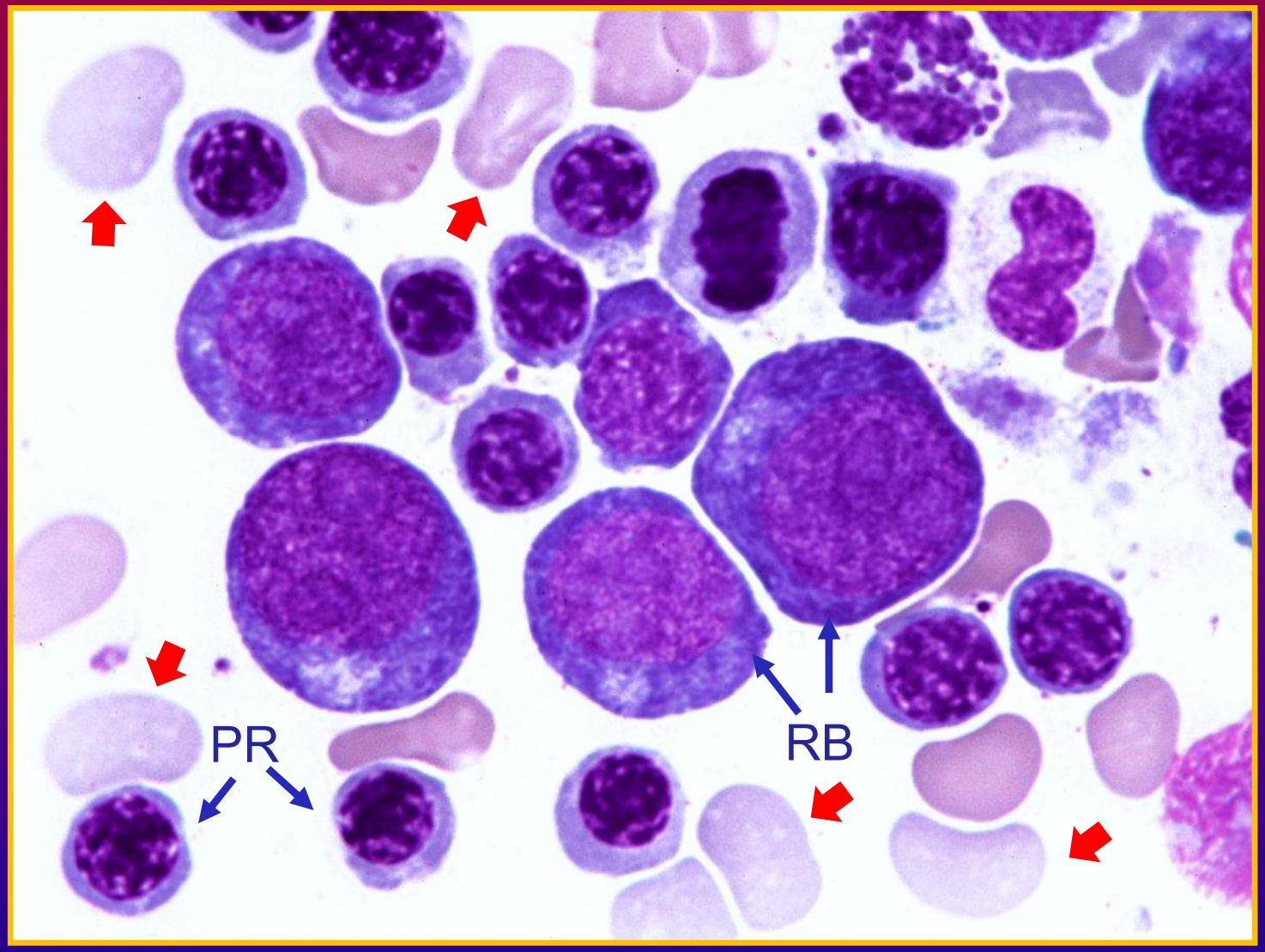
Electron Microscopy

Reticulocyte Formation
Nuclear Extrusion

Metarubricyte

Polychromatophilic Erythrocyte is an Immature Aggregate Reticulocyte

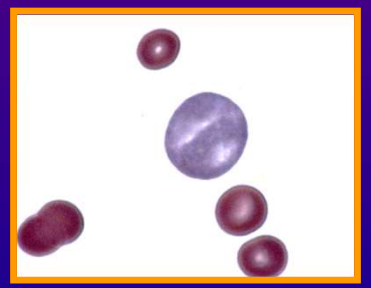
Bone Marrow Erythroid Hyperplasia Response to Anemia



PR – polychromatophilic rubricyte, RB = rubriblast

Reticulocytosis

- Regenerative anemia - Anemic animal with increased numbers of reticulocytes/µL blood.
- The presence of a reticulocytosis indicates that the anemia was caused by either:
  - Hemorrhage or
  - Increased erythrocyte destruction
- Slight, rapid increase in blood reticulocytes (stress reticulocytes) may occur in response to severe anemia, due to premature reticulocyte release from marrow; however, marrow stores are small.



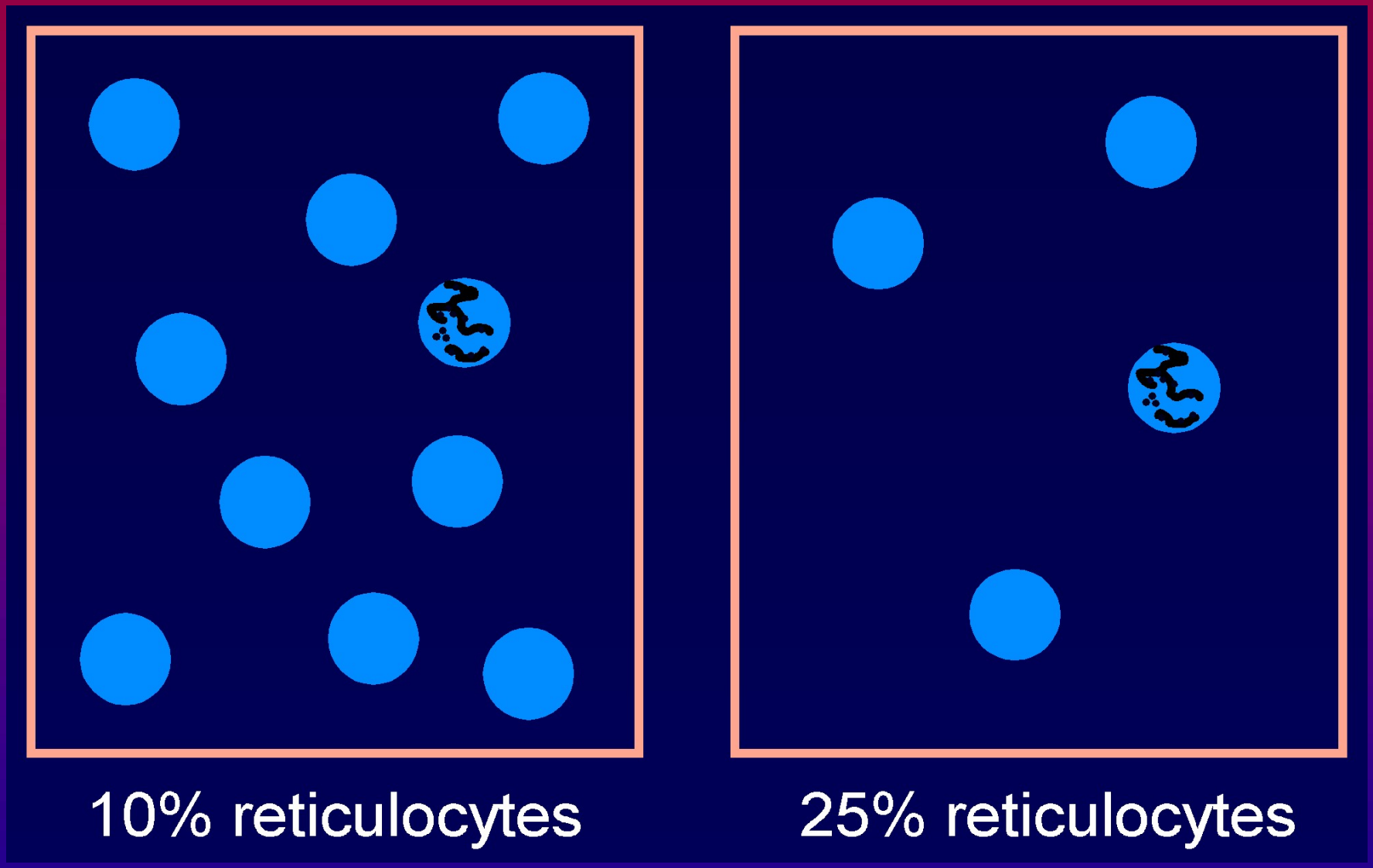
Reticulocytosis (continued)

- About 3 to 4 days are required for increased synthesis and release of reticulocytes from bone marrow.
- Nonregenerative anemia - Lack of reticulocytosis after 3-4 days of anemia indicates there is a disorder with decreased erythrocyte production.

Percentage of reticulocytes can be misleading

Percentage depends on the number of mature erythrocytes present, as well as the number of reticulocytes present

Percentage Reticulocytes

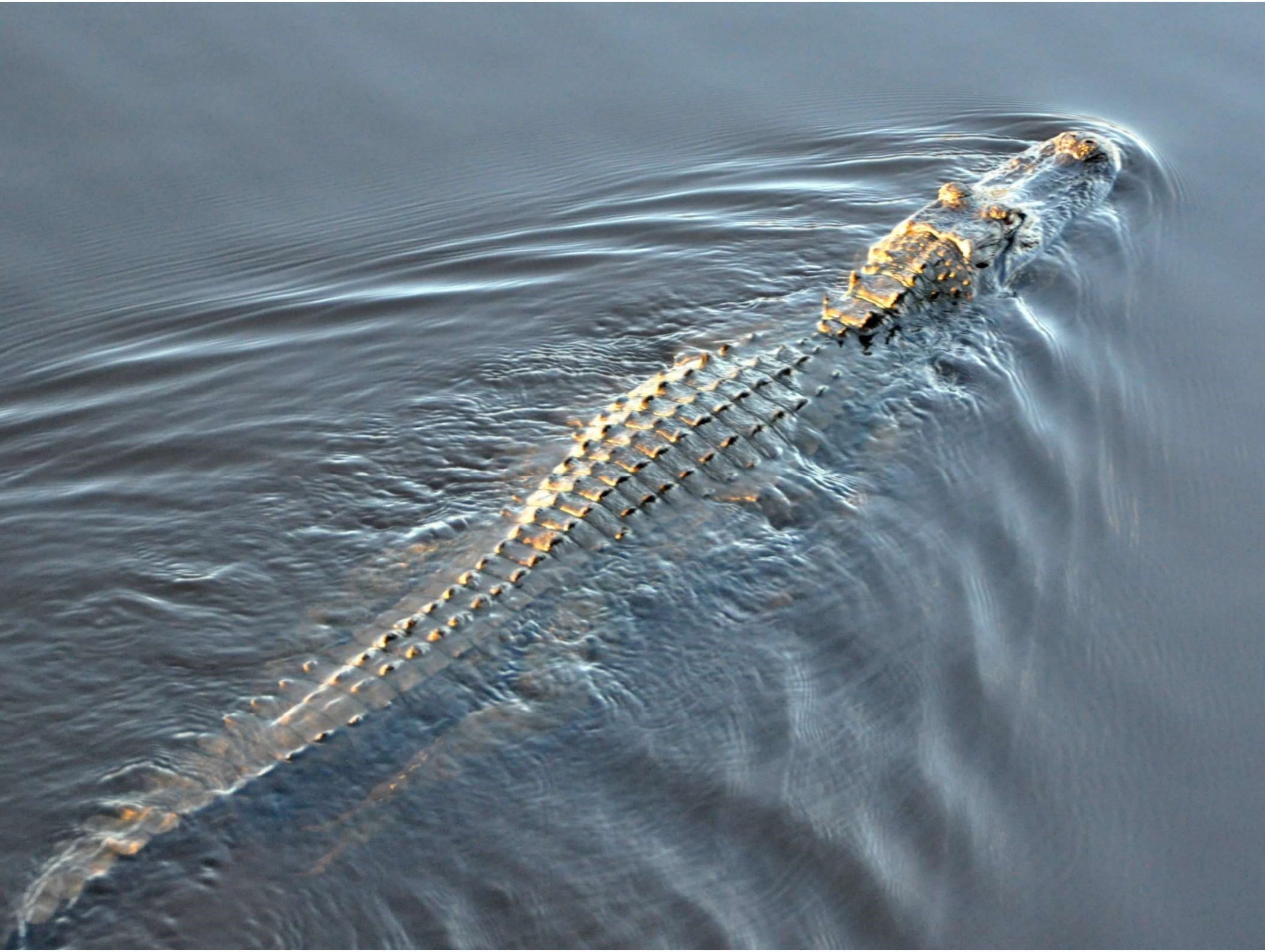


Reticulocytosis Classification

Reticulocytes/μL blood

NOT

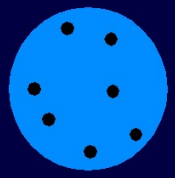
Reticulocyte %

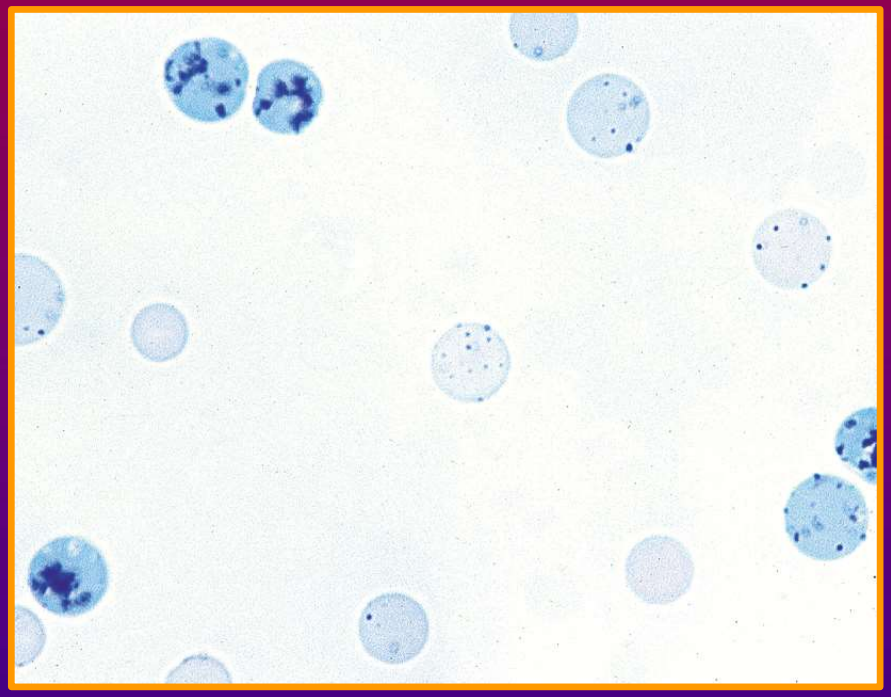


Feline Reticulocytosis

Aggregate Reticulocytes

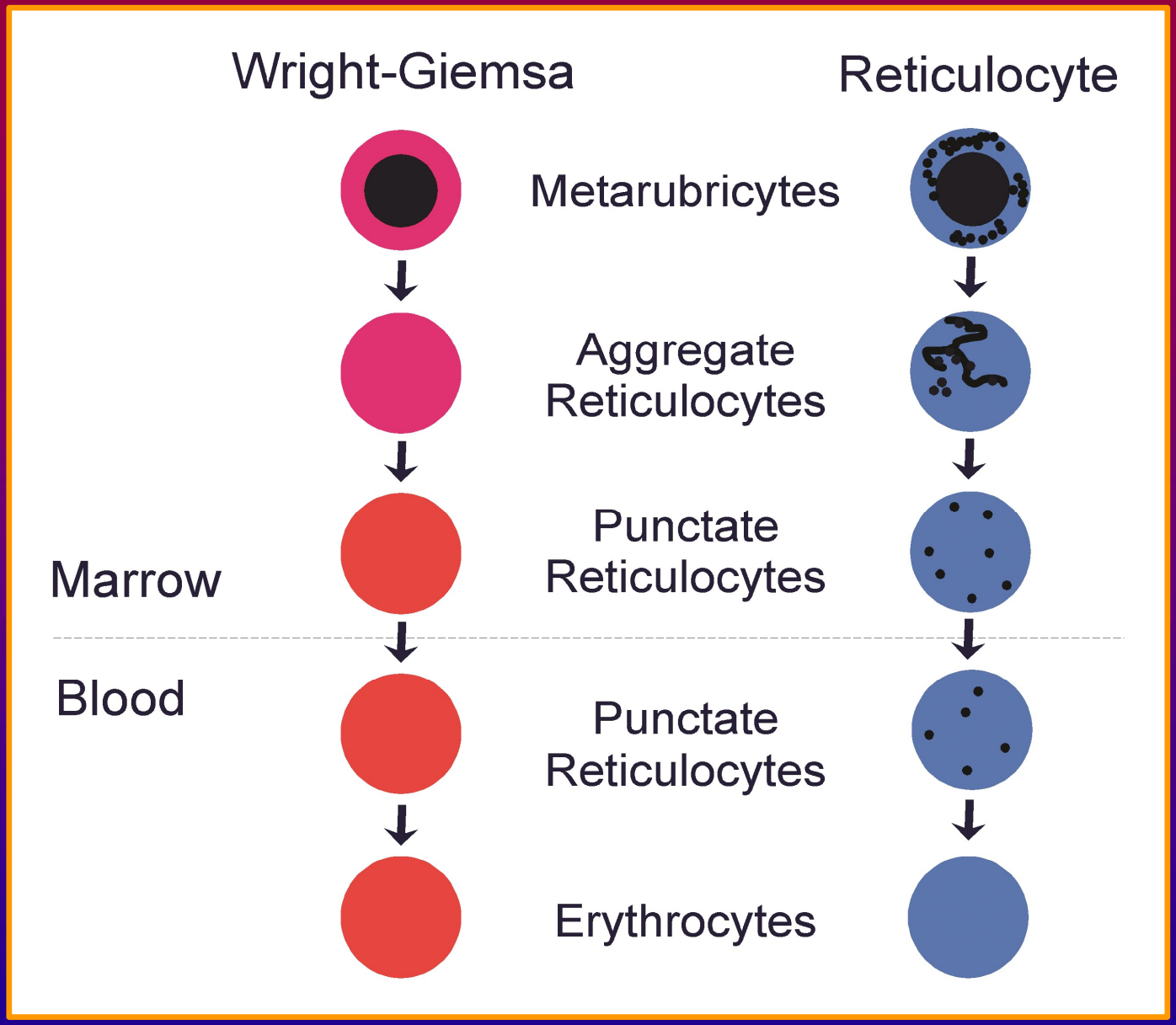
Punctate Reticulocytes





New Methylene Blue Reticulocyte Stain

Feline Reticulocytes



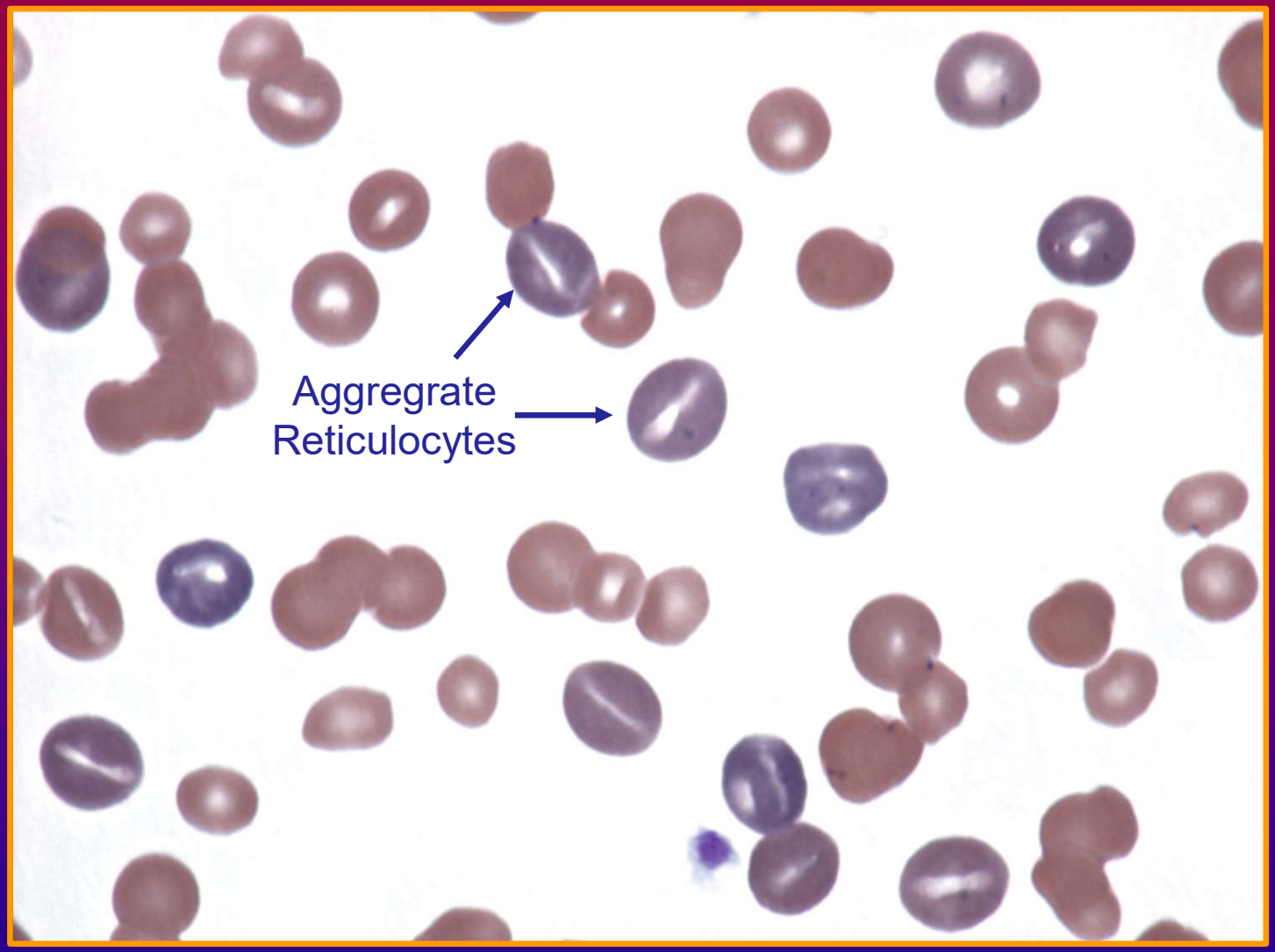
Reticulocyte Counts in Healthy Mammals

Species	Aggregate	Punctate	Total
Dogs (n=10)	43 ± 50	14 ± 23	58 ± 56
Cats (n=7)	41 ± 61	142 ± 60	183 ± 108
Cattle (n=11)	0	0	0
Humans (n=10)	74 ± 21	24 ± 20	98 ± 36

Values (x $10^3/\mu L$) are mean ± SD

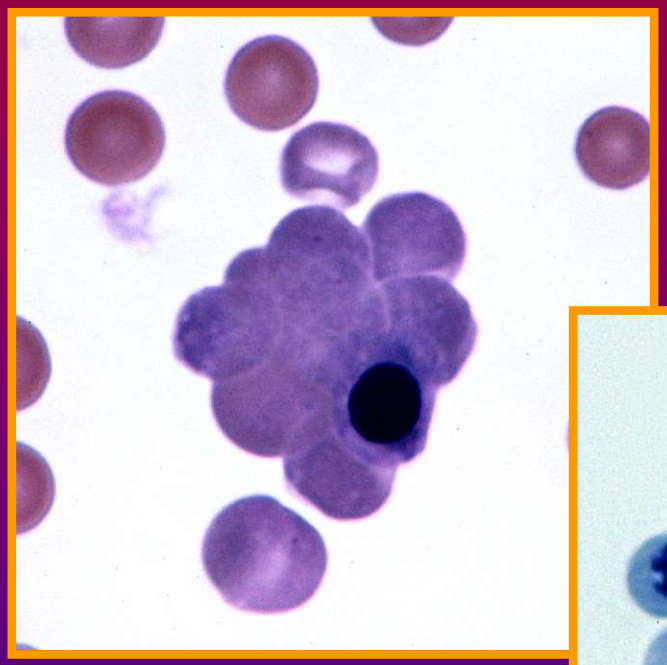
Hossain MA, et al. J Vet Med Sci 65(2):193-197, 2003

M. haemofelis infection



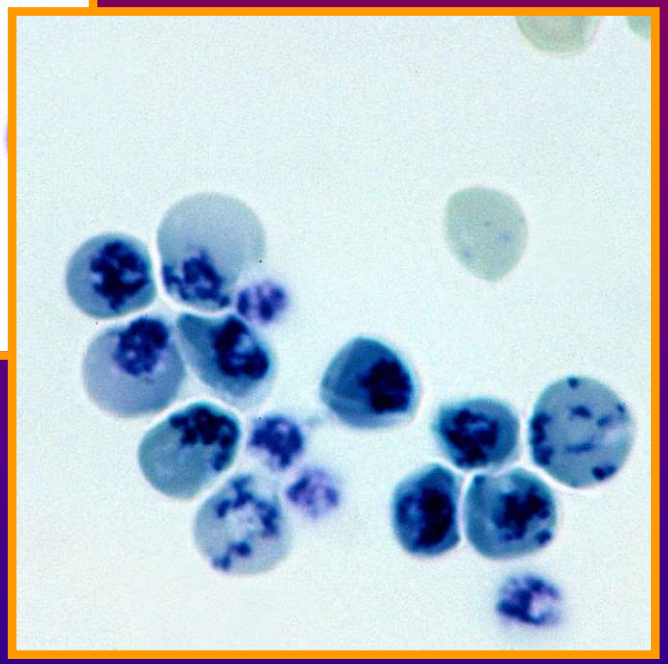
Wright-Giemsa Stain

Immune-Mediate Hemolytic Anemia in a Cat



Wright-Giemsa Stain

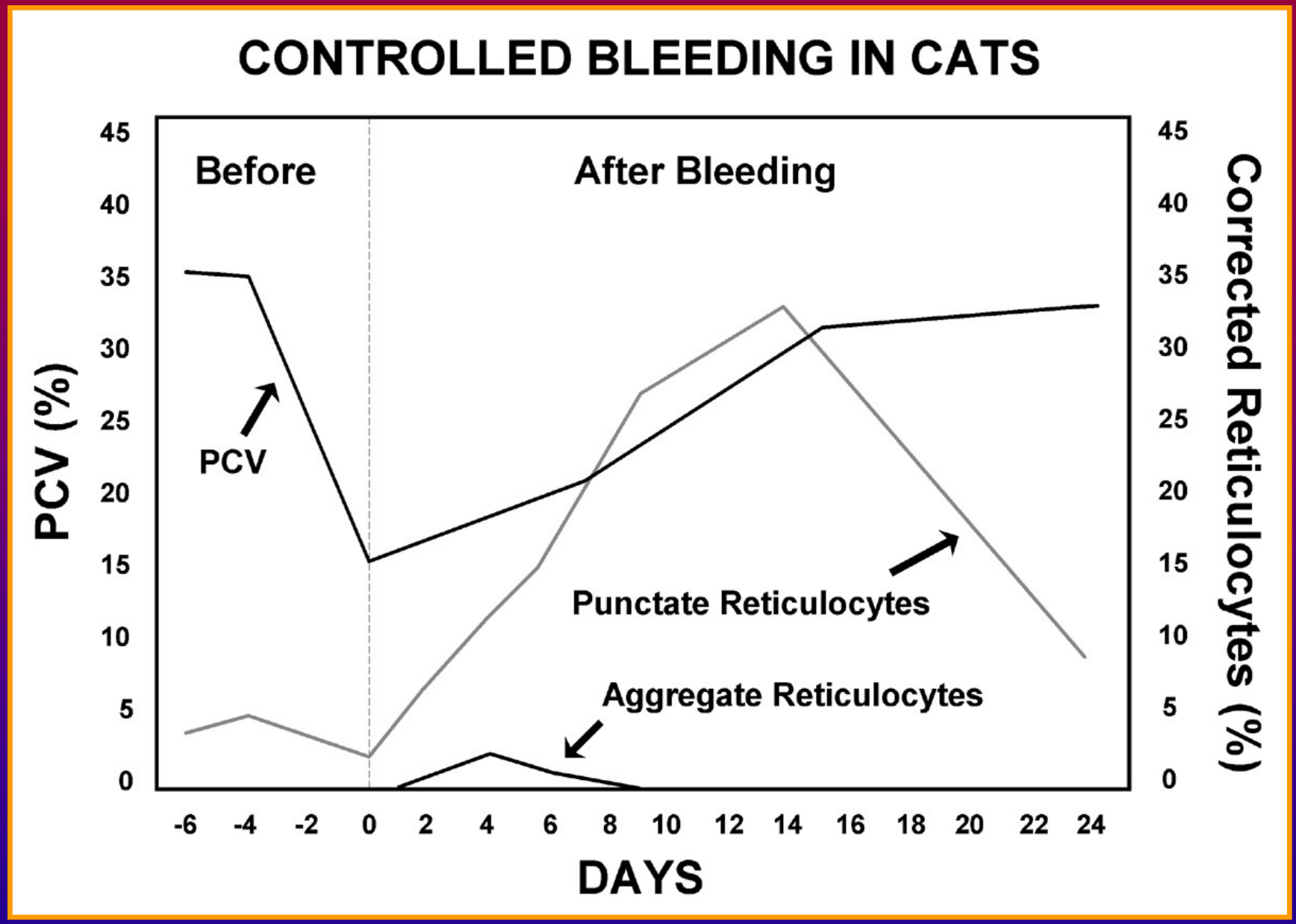
Agglutination of Aggregate Reticulocytes



Reticulocyte Stain

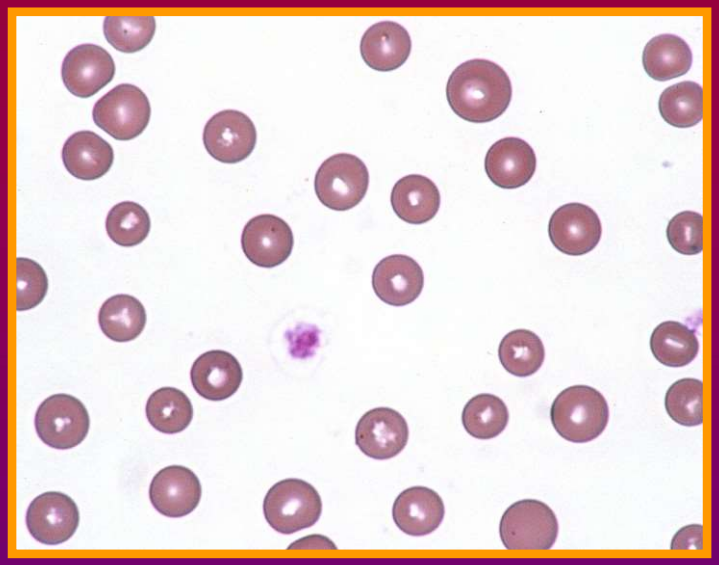
Blood Reticulocyte Maturation

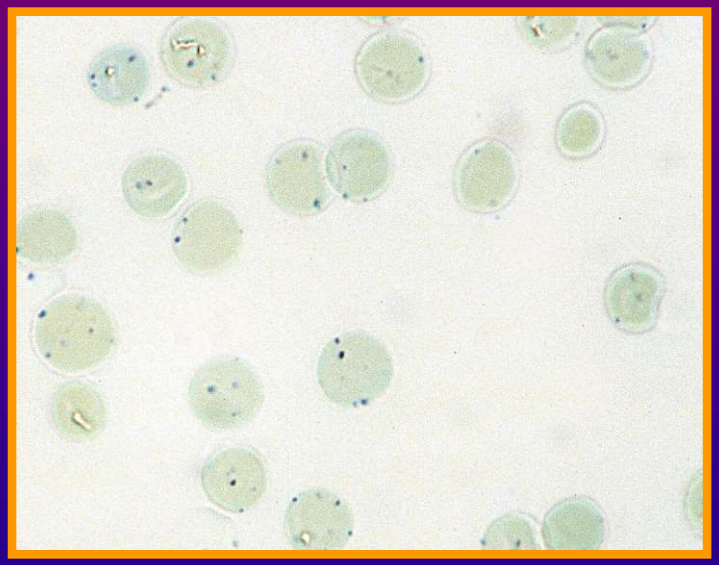
- Aggregate reticulocytes in the blood of cats mature to punctate reticulocytes in a day or less.
- A week or more is required for maturation (total disappearance of ribosomes) of punctate reticulocytes in cat blood.


CONTROLLED BLEEDING IN CATS
Before
After Bleeding
PCV
Punctate Reticulocytes
Aggregate Reticulocytes
PCV (%)
Corrected Reticulocytes (%)
DAYS
45
40
35
30
25
20
15
10
5
0
-6
-4
-2
0
2
4
6
8
10
12
14
16
18
20
22
24

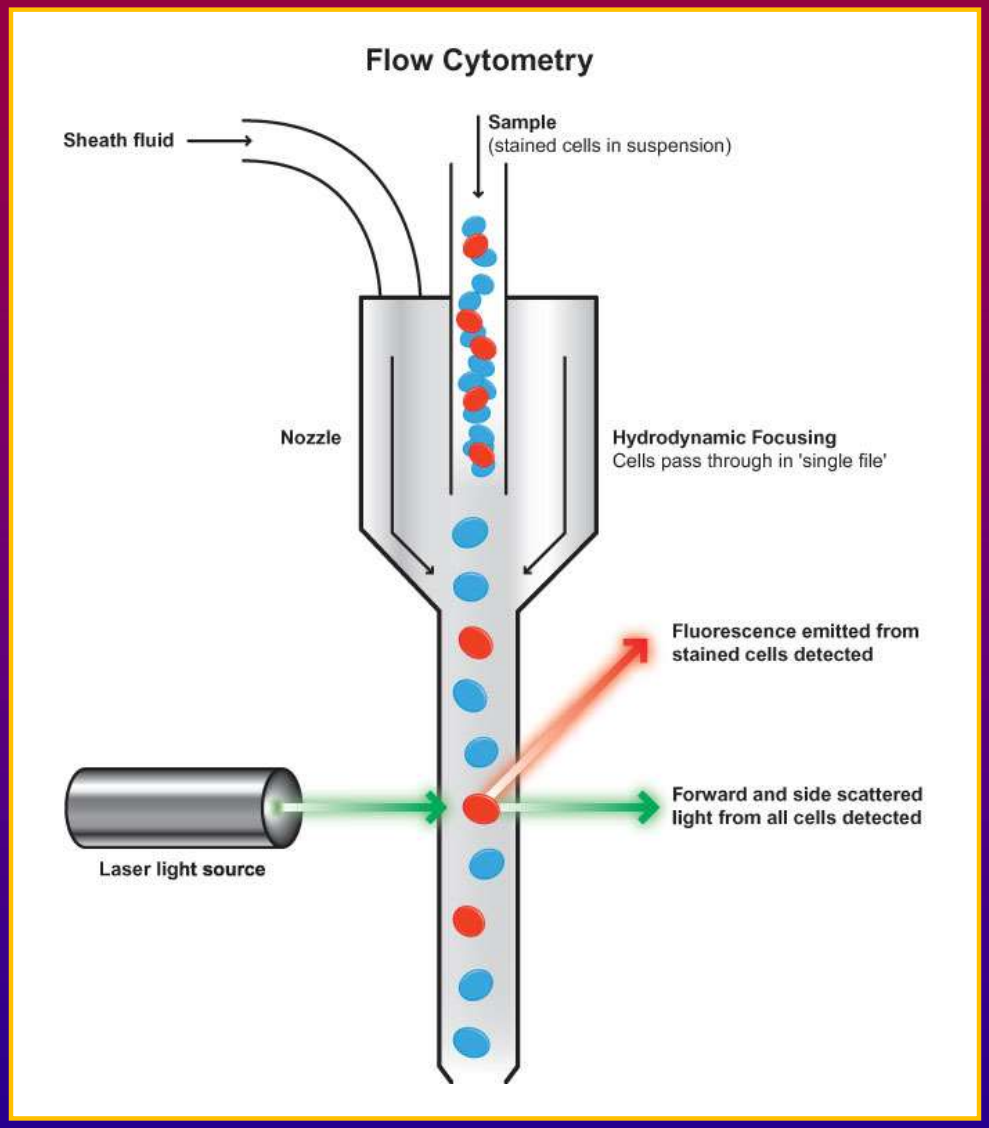
Cat Punctate Reticulocytes

- HCT 23%
- MCV 70 fL
- MCHC 32 g/dL
- A-retic 0.2%
- P-retic 83%



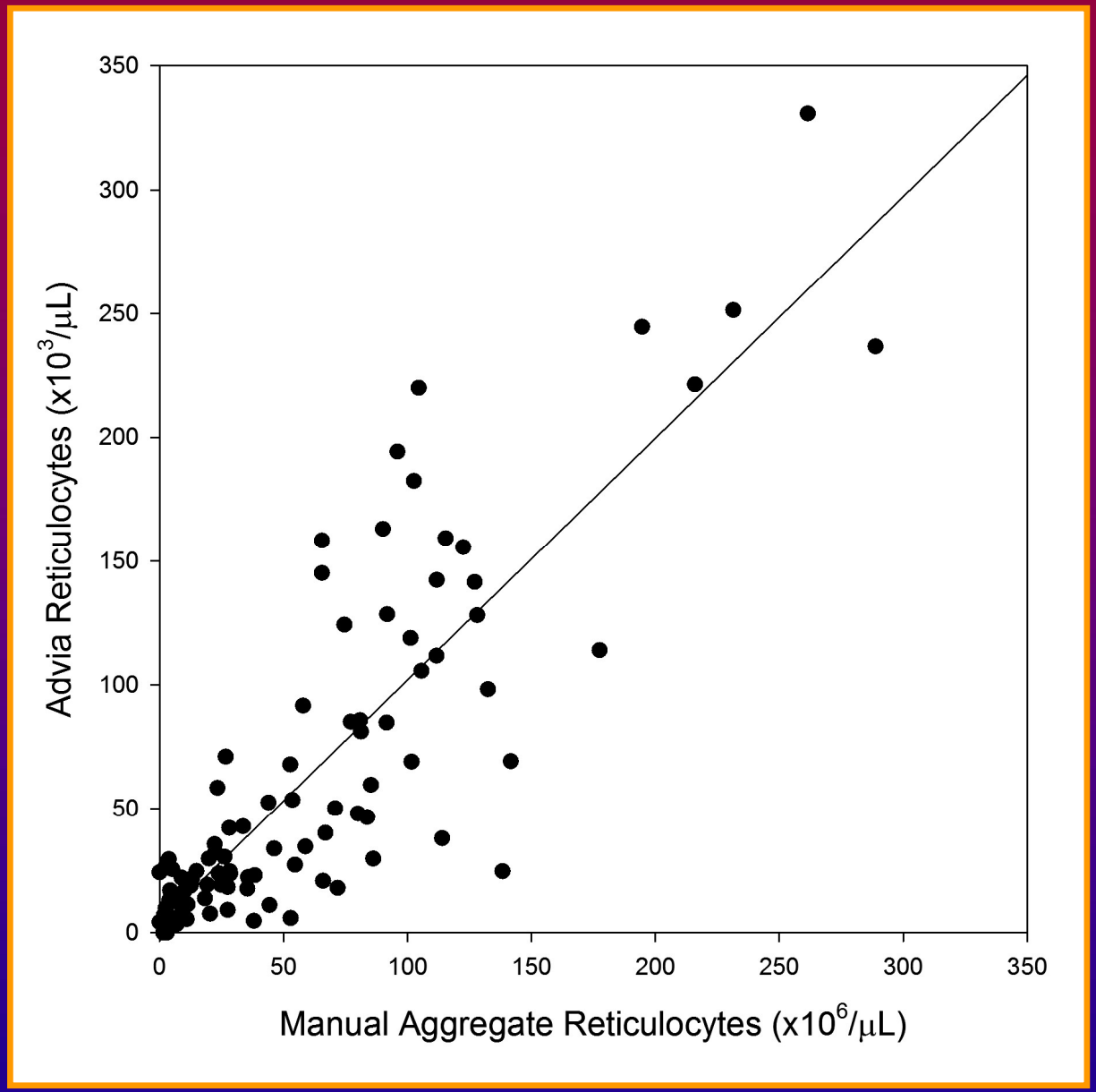


Laser Flow Cytometry

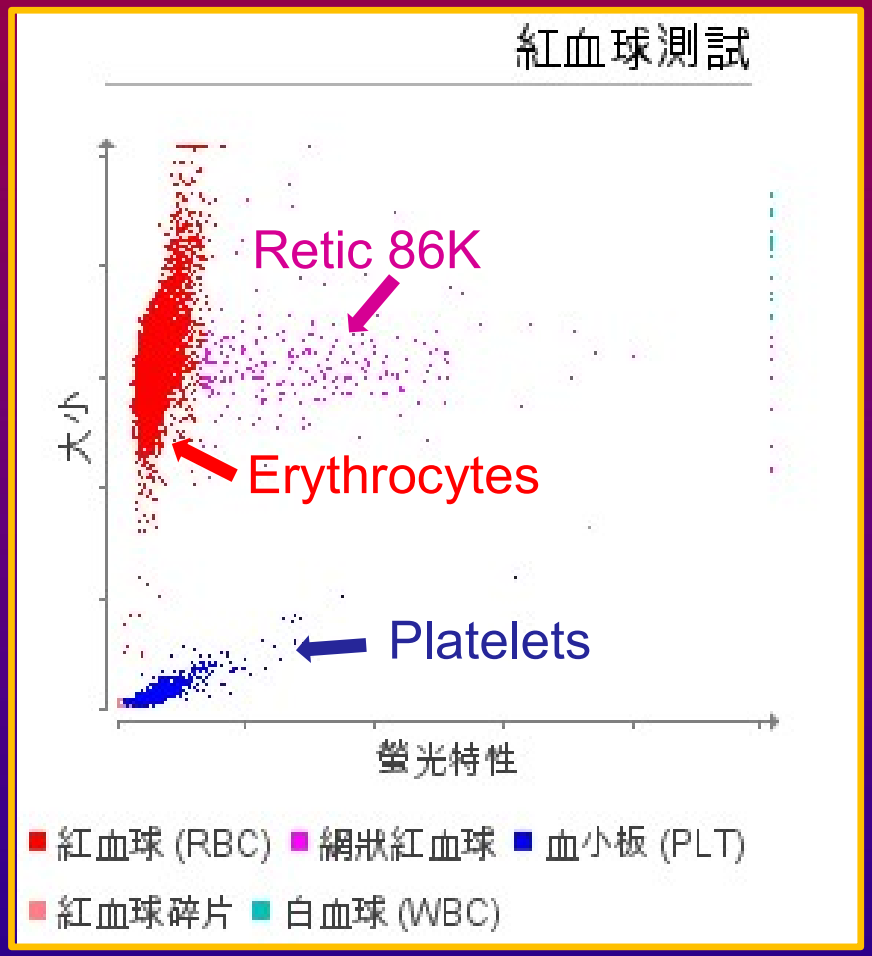


Fluorescent labels can be used to detect reticulocytes containing RNA

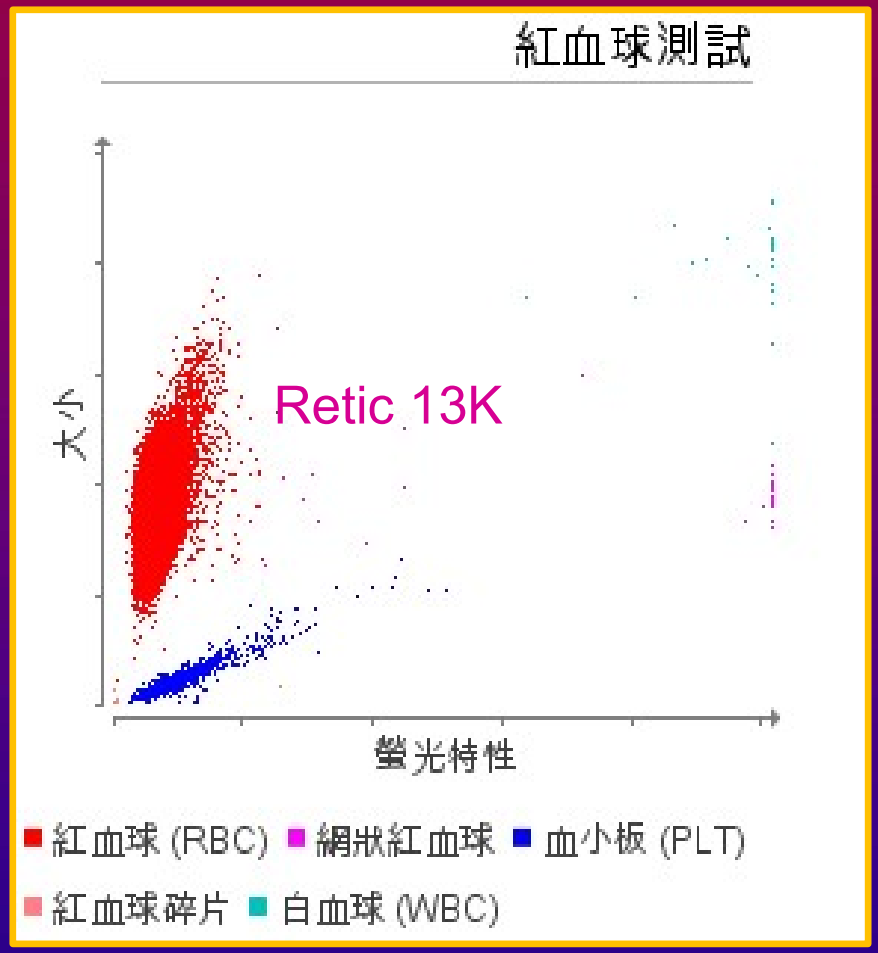
Advia vs Aggregate Reticulocytes



ProCyte Dx
RBC-PLT Dot Plots (NCHU Taiwan)

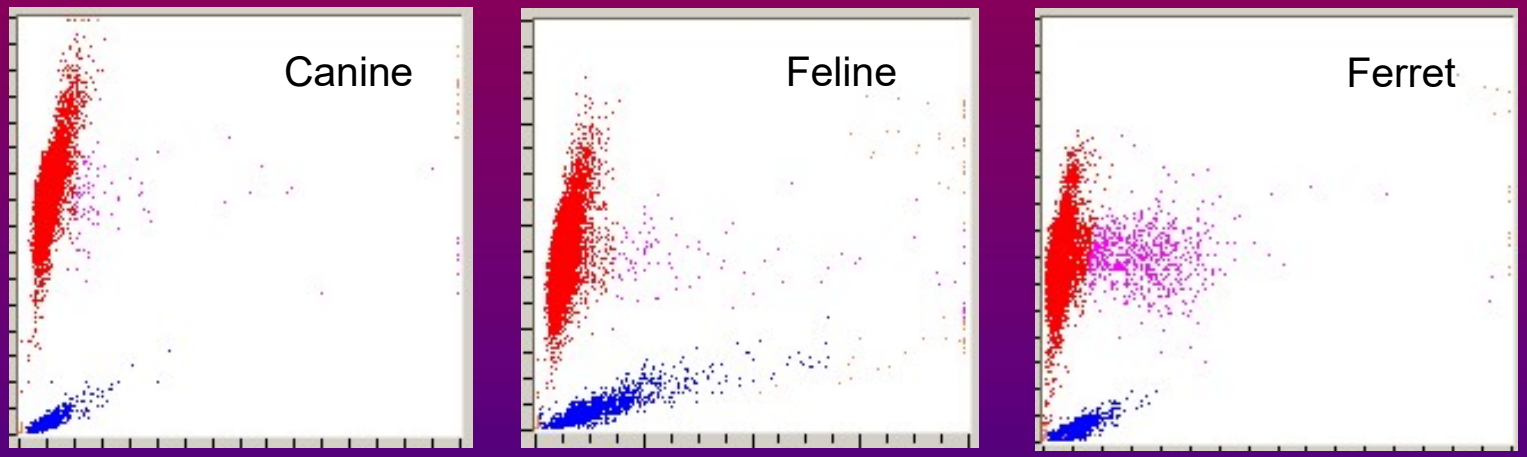


Dog



Cat

ProCyte Dx
CBC: RBC-PLT Dot Plot

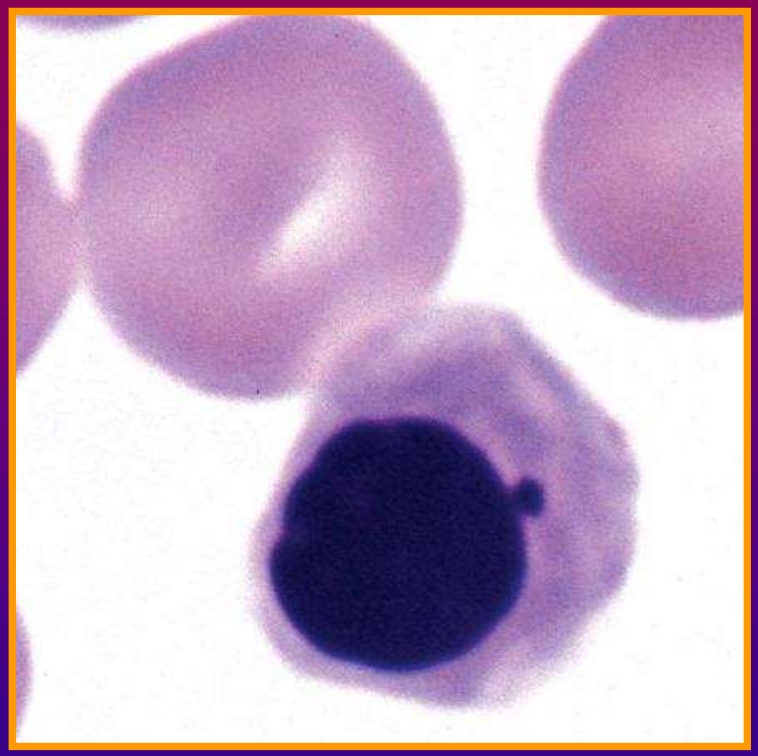


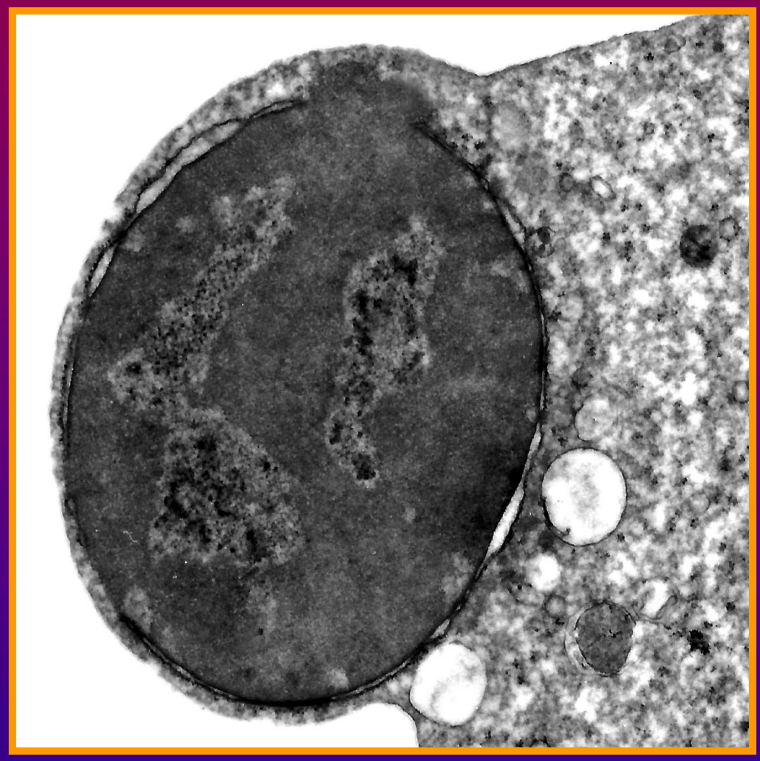
Red Blood Cells Platelets Reticulocytes

Howell-Jolly Bodies

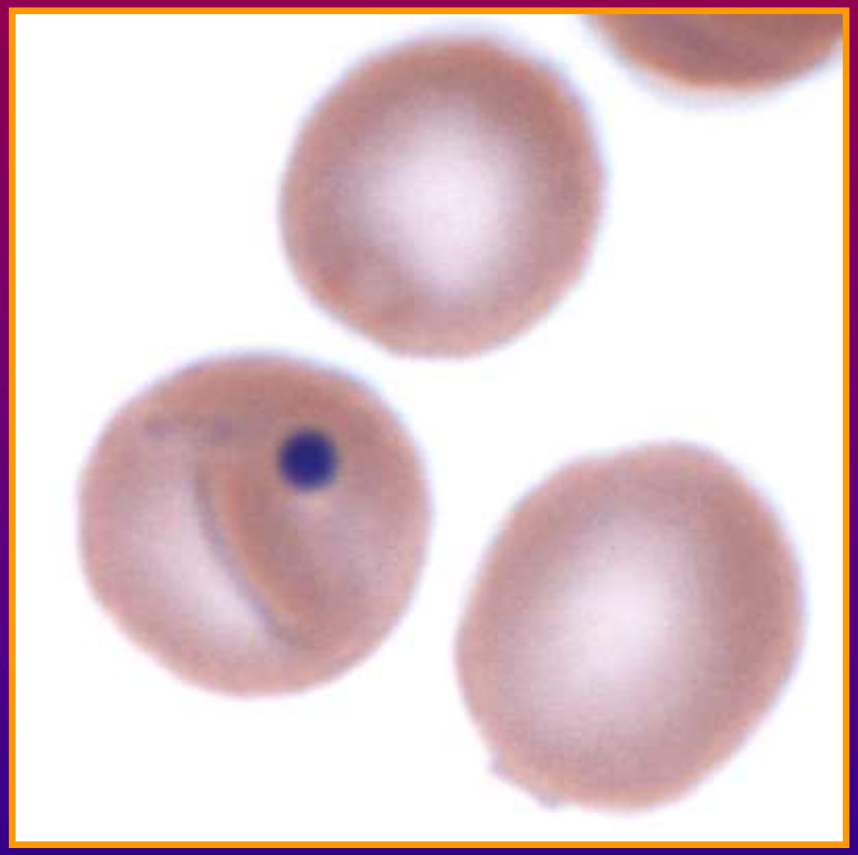
Wright-Giemsa Stain

Howell-Jolly Body Formation





Howell-Jolly vs Heinz Bodies



Wright-Giemsa Stain

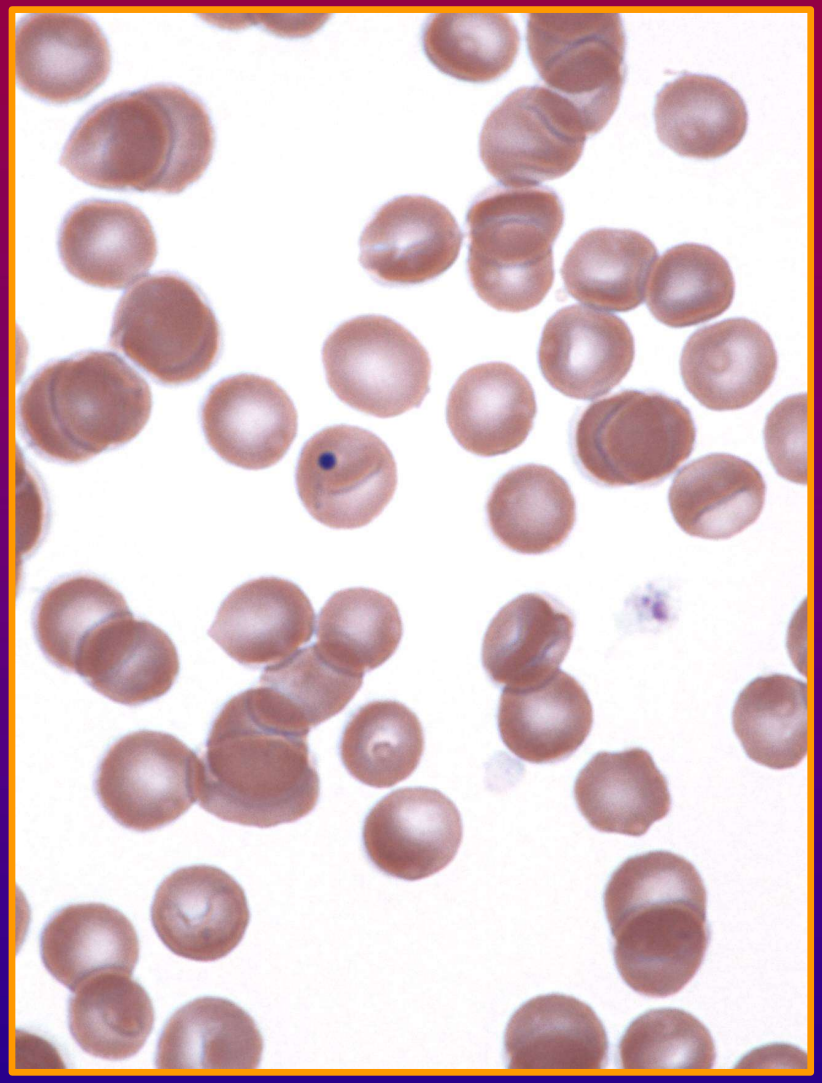
Reticulocyte Stain

Feline Spleen
Poor Pitting Function

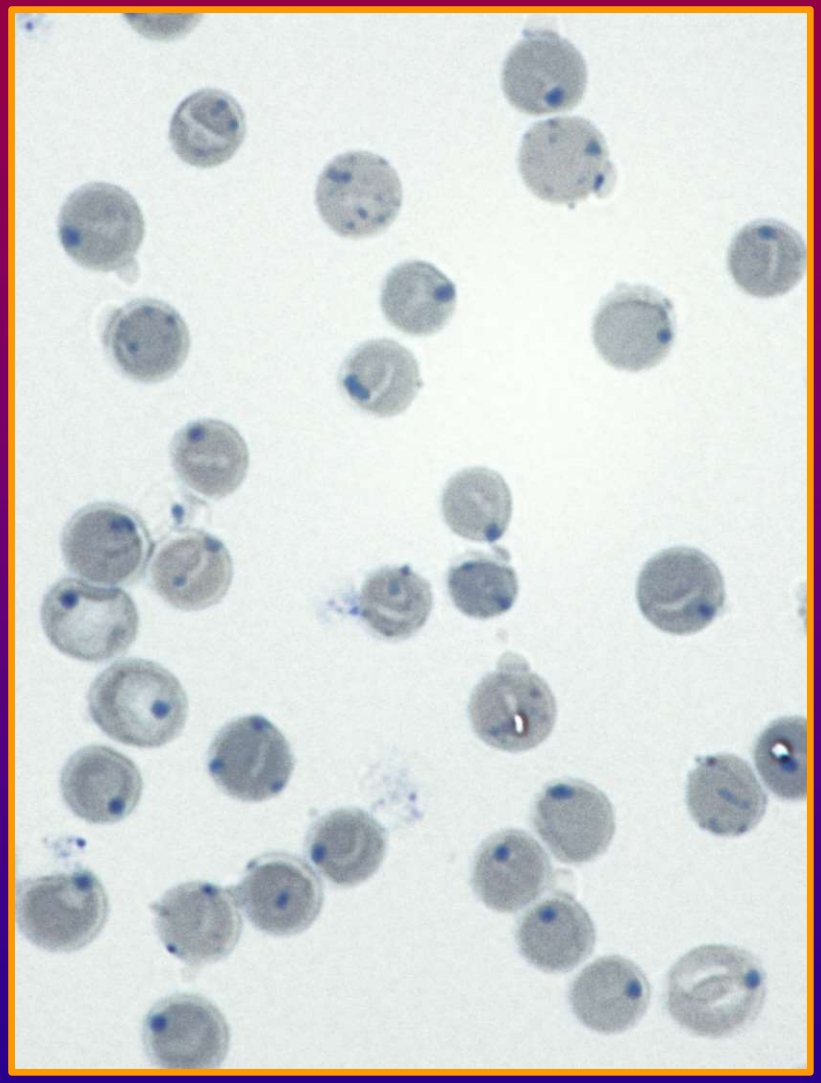
Heinz Bodies in Cats

- Up to 5% in normal cats
- Increased with oxidant damage
- Increased with some diseases (diabetes, lymphoma, hyperthyroidism)
- Onion and garlic ingestion
- Propylene glycol in soft-moist food
- Acetaminophen, methylene blue, methionine, phenazopyridine,
- Prolonged propofol anesthesia

Heinz Bodies in Cat Erythrocytes

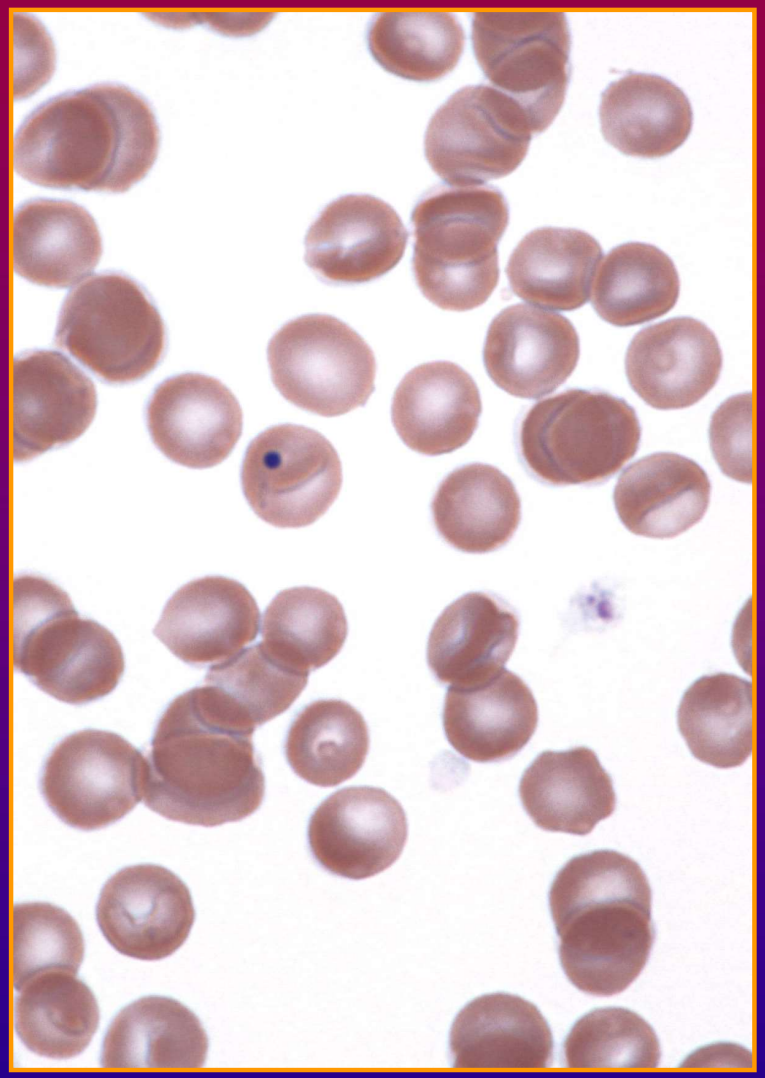


Wright-Giemsa Stain

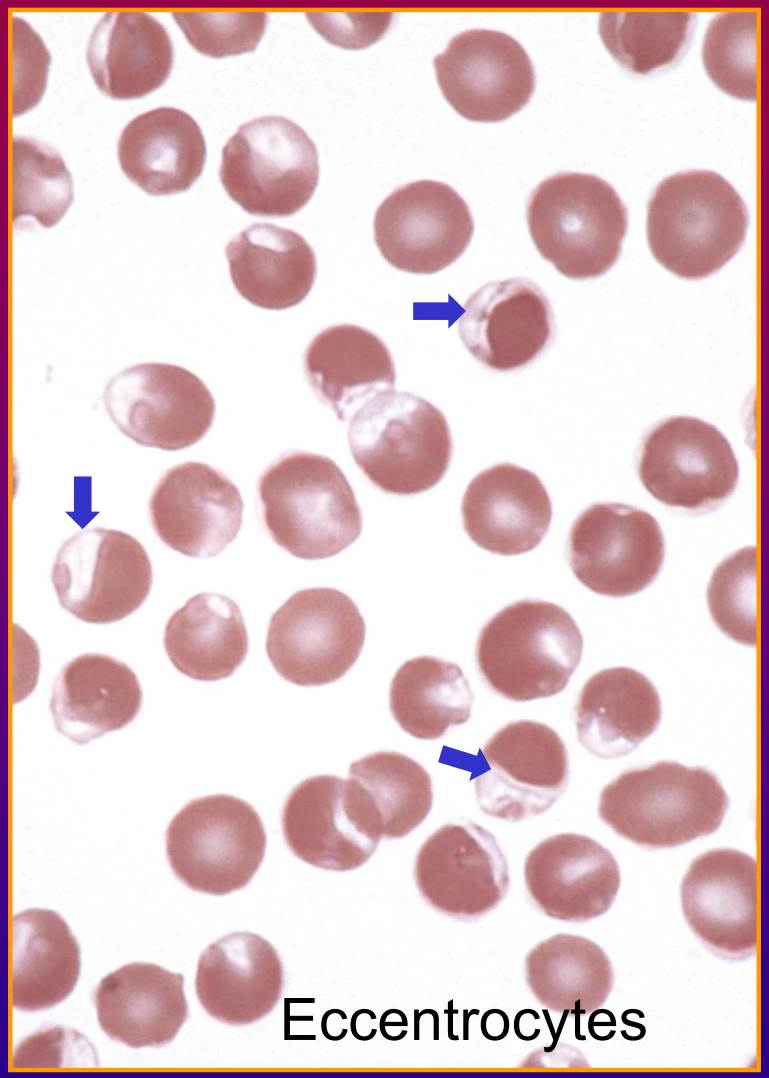


NMB Reticulocyte Stain

Feline Acetaminophen Toxicity

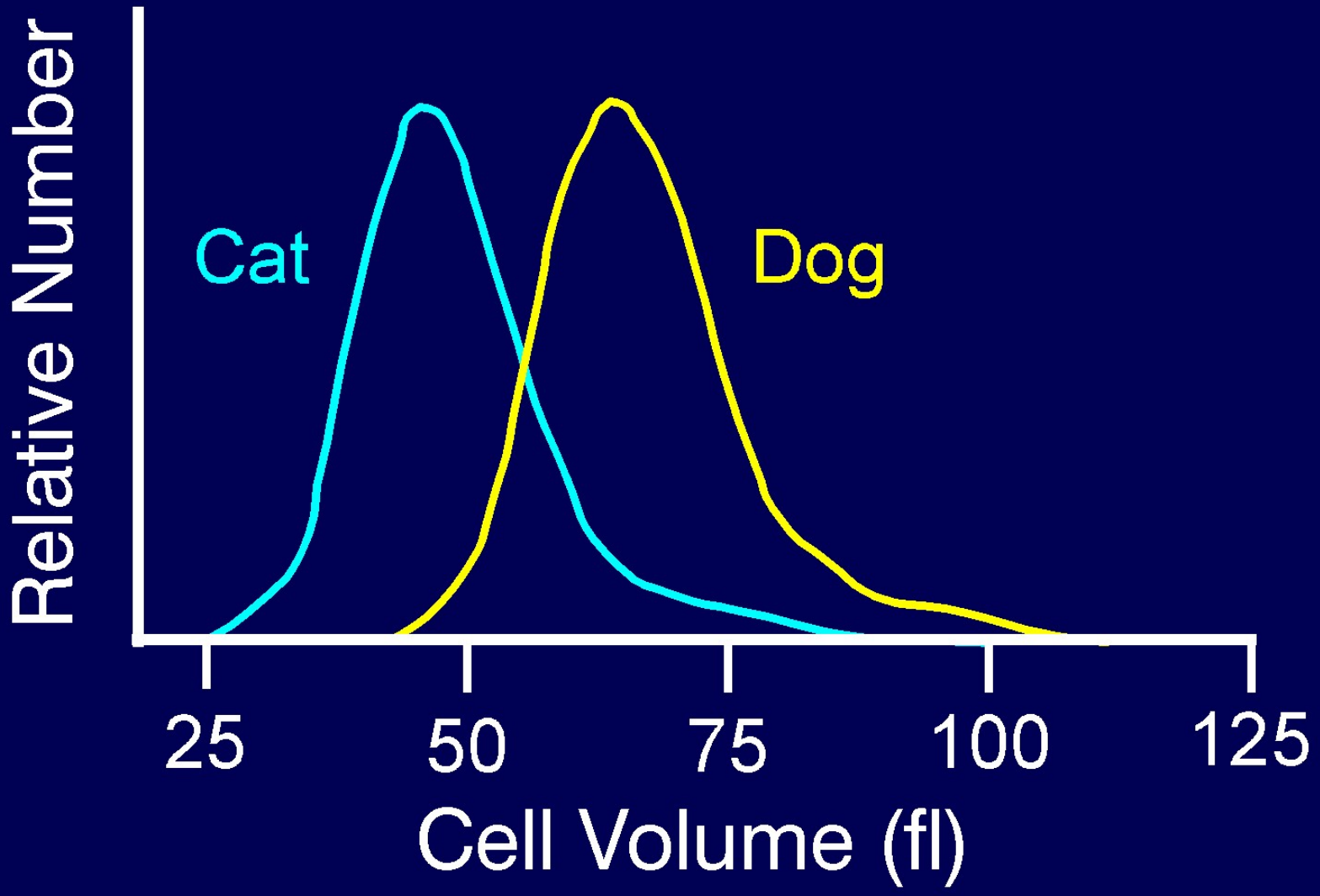


At Admission



2 Days Later

Erythrocyte Histograms

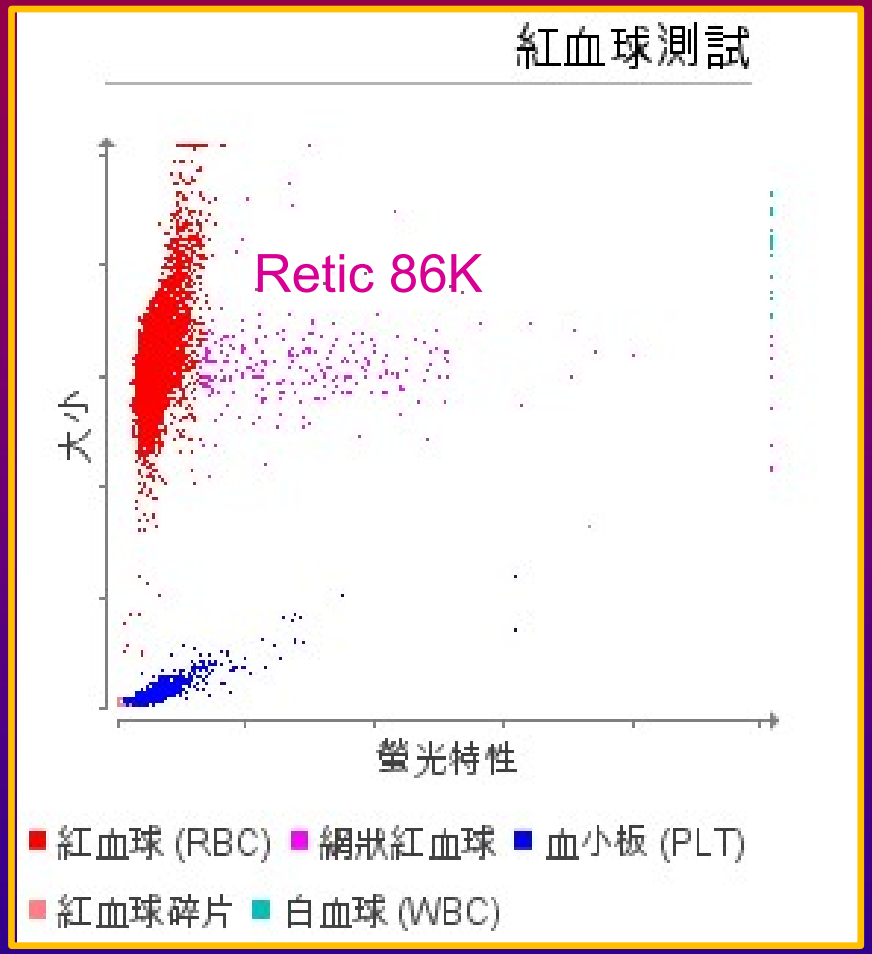


Average MCV

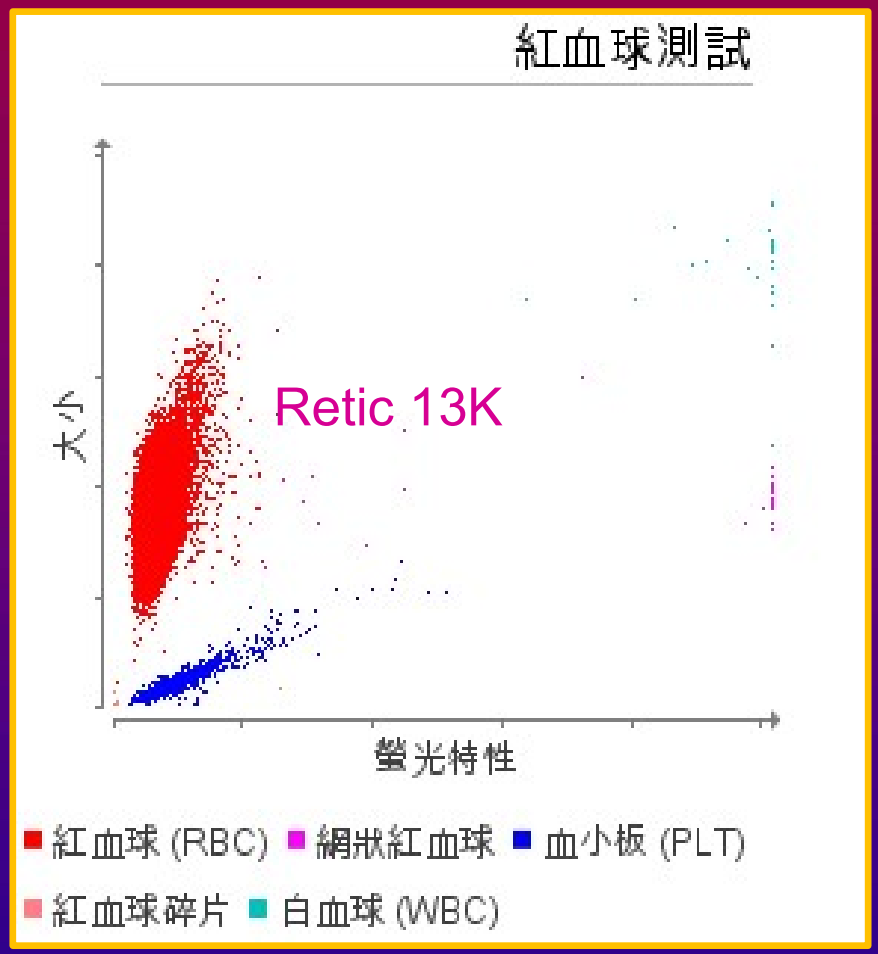
Species	MCV (fL)
Dog	70
Cat	45

MCV = mean volume of a single erythrocyte

ProCyte Dx
RBC-PLT Dot Plots (NCHU Taiwan)



Dog



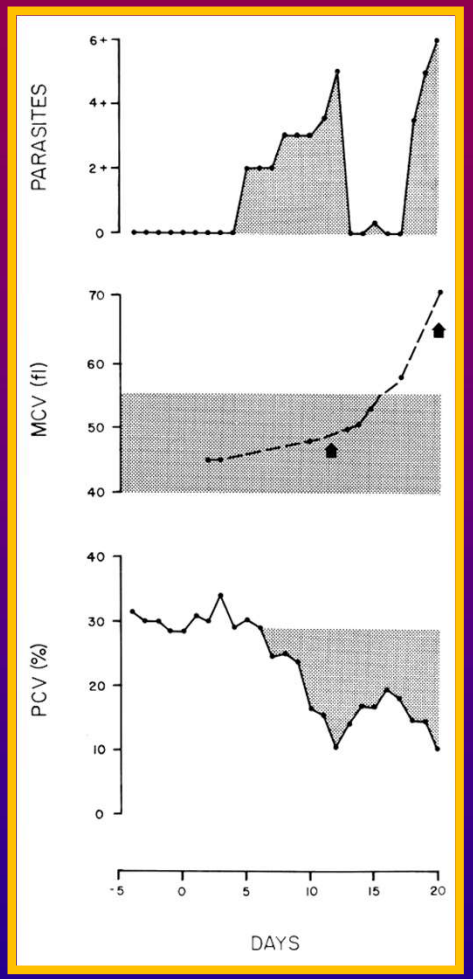
Cat

Macrocytosis (High MCV) in Cats

- Some regenerative anemias, especially hemolytic anemias
- Some FeLV-positive cats (usually nonregenerative)
- Myeloid neoplasms including MDS (nonregenerative) – often FeLV positive
- Abyssinian and Somali cats with increased erythrocyte osmotic fragility (overhydrated, swollen erythrocytes)
- Plant-based “vegan” pet food with multi-nutrient (especially folate) deficiency

Macrocytosis in a Regenerative Anemia

- MCV increases slower than reticulocyte counts in response to hemolytic anemia
- MCV may not increase outside the reference range, especially in response to hemorrhage



M. haemofelis
Infection

Microcytosis in Cats

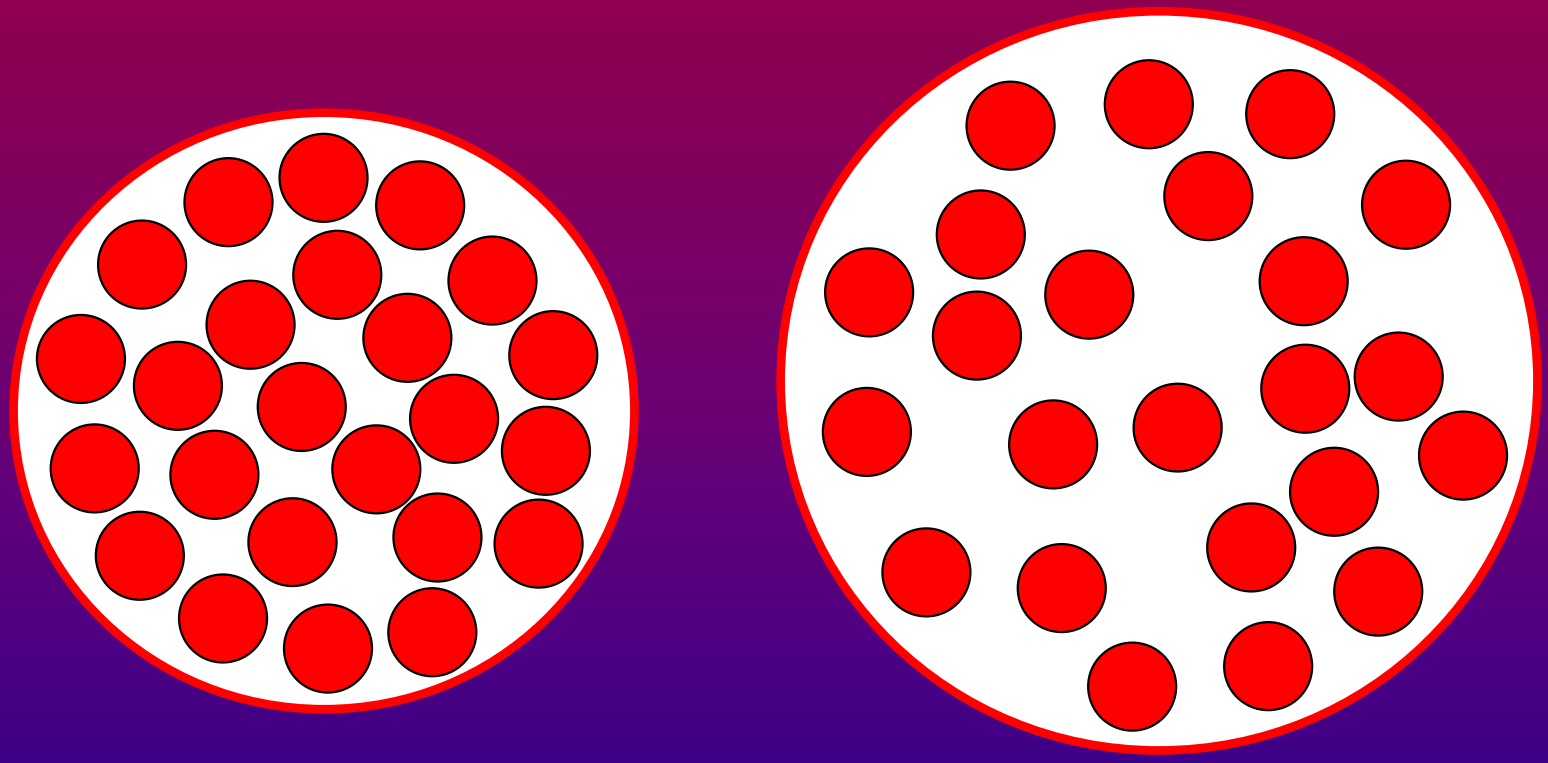
- Chronic iron deficiency anemia (uncommon in adult cats)
- Some cats with hepatic lipidosis
- Anemia of inflammatory disease (minimal effect)

Mean Cell Hemoglobin Concentration (MCHC)

$$\text{MCHC (g/dL)} = \frac{\text{Hb}}{\text{HCT}} \times 100$$

Classification – MCV and MCHC

Marked Reticulocytosis



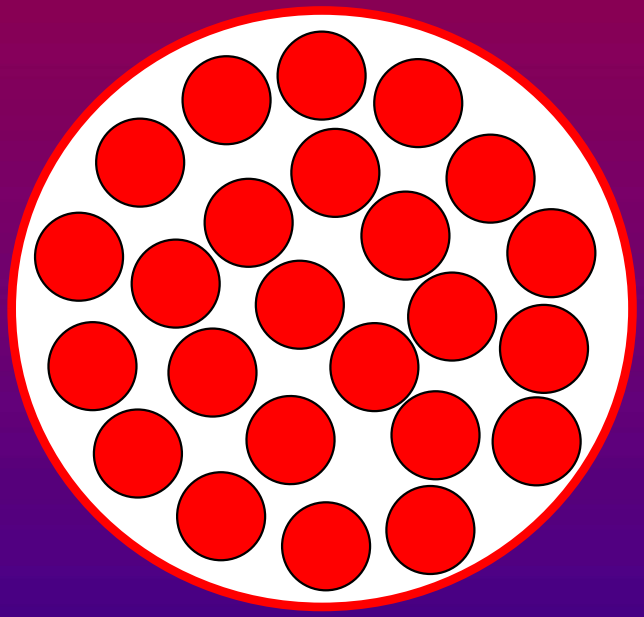
Normocytic/Normochromic

Macrocytic/Hypochromic

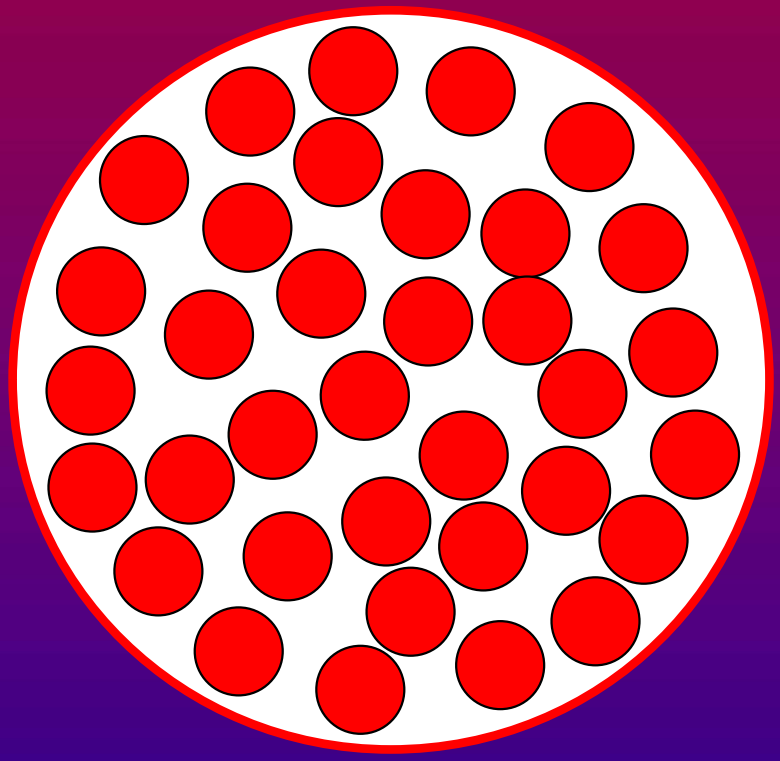
Represents an amount of hemoglobin

Classification – MCV and MCHC

Some FeLV-Positive Cats



Normocytic/Normochromic



Macrocytic/Normochromic

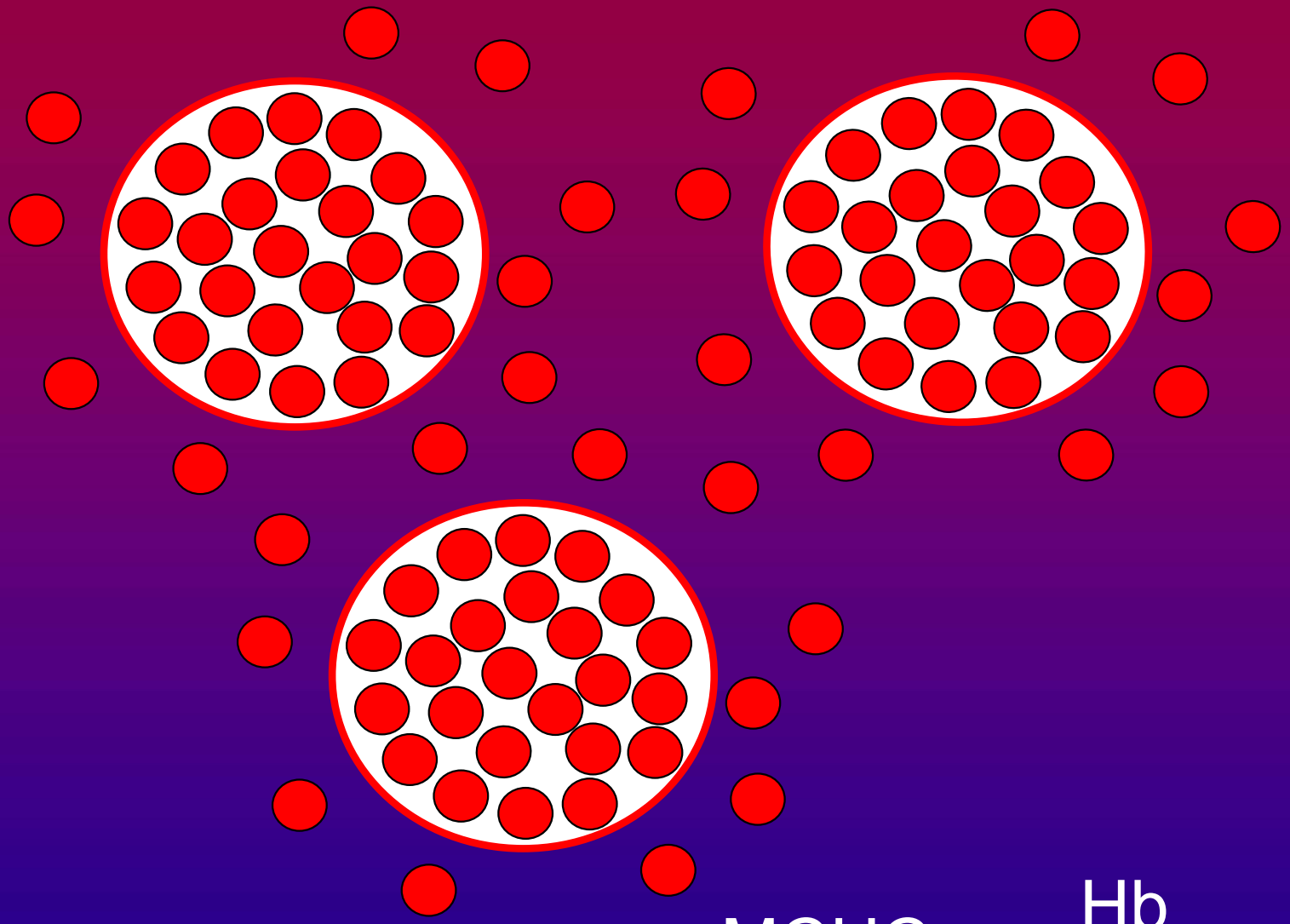
Represents an amount of hemoglobin

High MCHC Values (Artifacts)

- Intravascular hemolysis
- *In vitro* hemolysis
- Heinz bodies within erythrocytes
- Lipemia
- Erythrocyte agglutination in electronic cell counters

$$MCHC = \frac{Hb}{HCT\downarrow} \times 100$$

High MCHC Hemolysis Artifact



$$MCHC = \frac{Hb}{HCT\downarrow} \times 100$$

High MCHC Values (Artifacts)

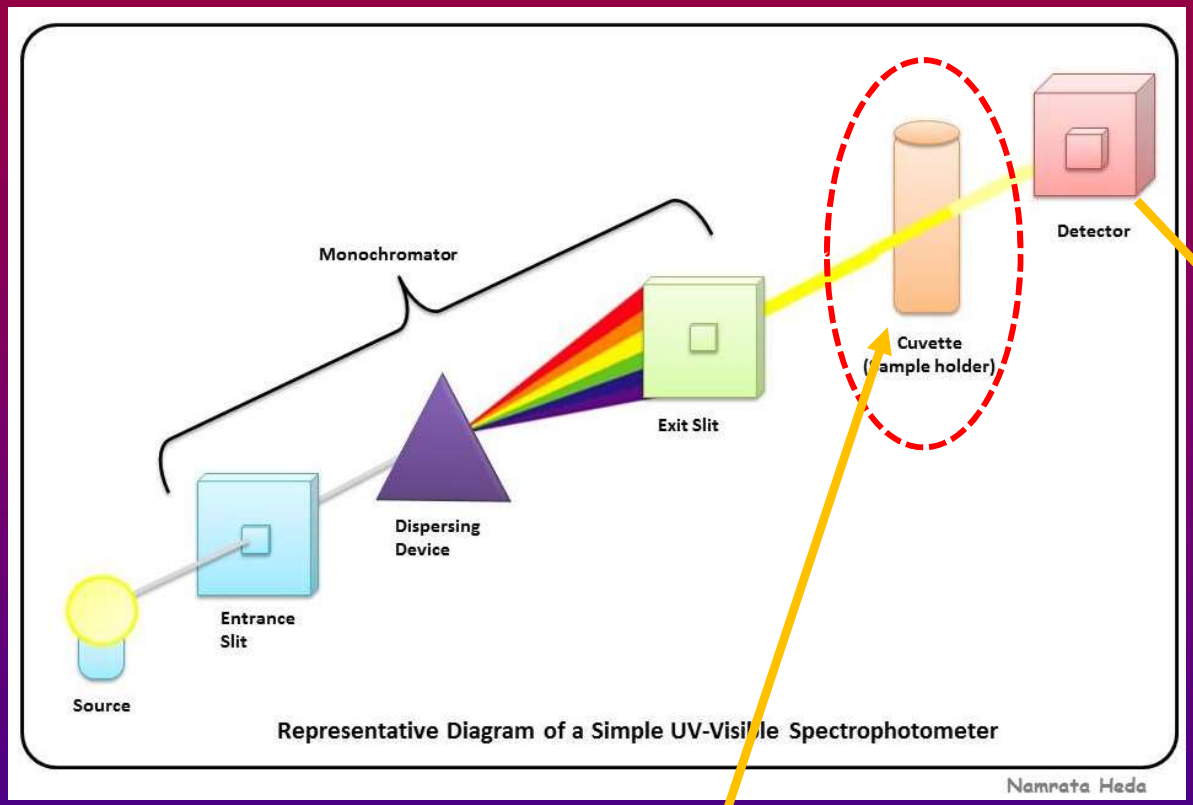
- Intravascular hemolysis
- In vitro hemolysis
- Heinz bodies within erythrocytes
- Lipemia
- Erythrocyte agglutination in electronic cell counters

$$MCHC = \frac{Hb\uparrow}{HCT} \times 100$$

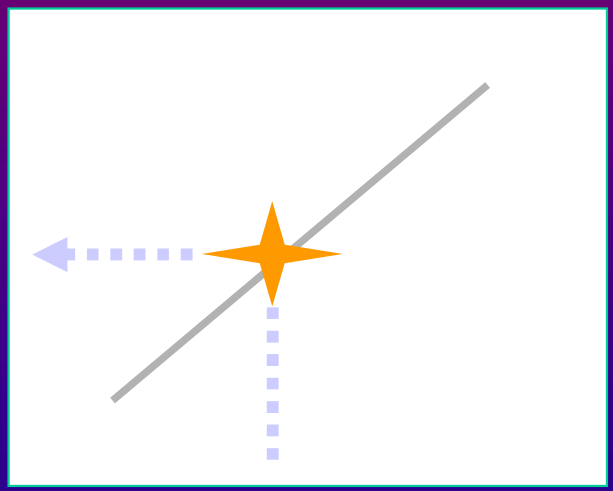
Whole Blood Hemoglobin Assay
Blood is diluted & RBC lysed

Hb is converted to stable form for spectrophotometry

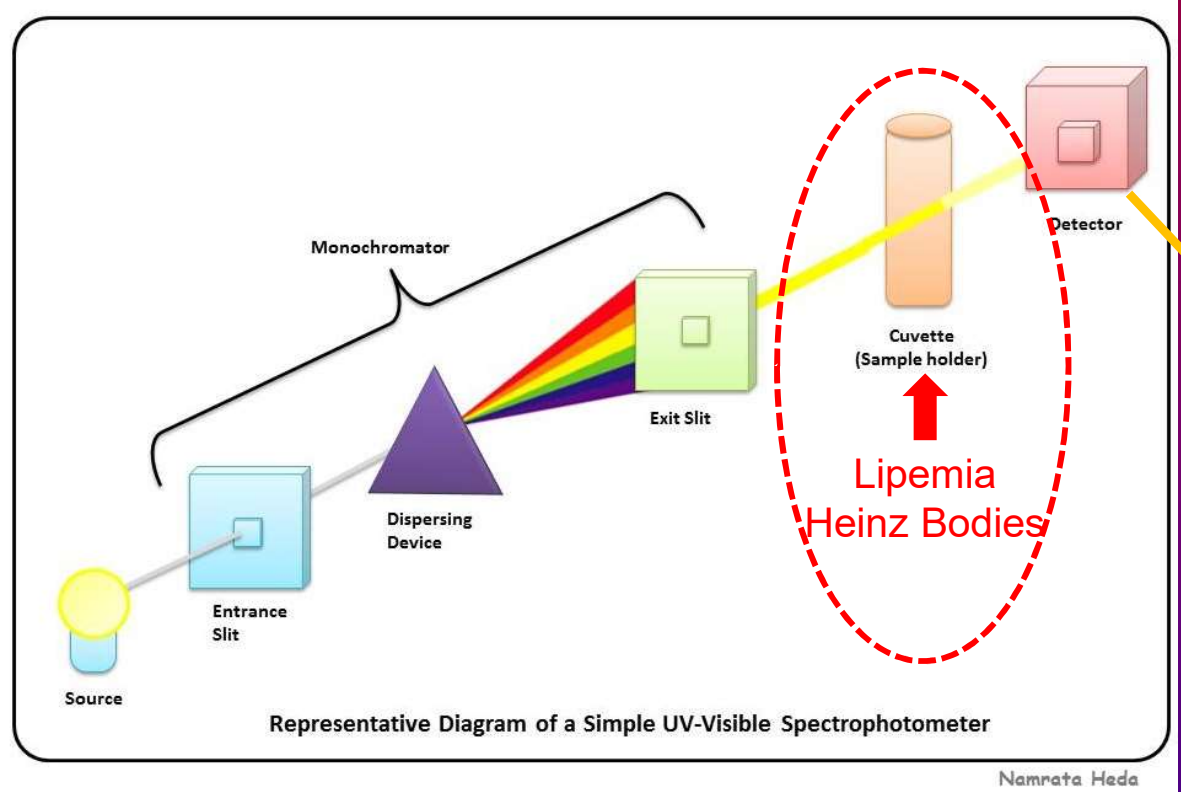
Spectrophotometry



Detector measures light absorbance



Spectrophotometry



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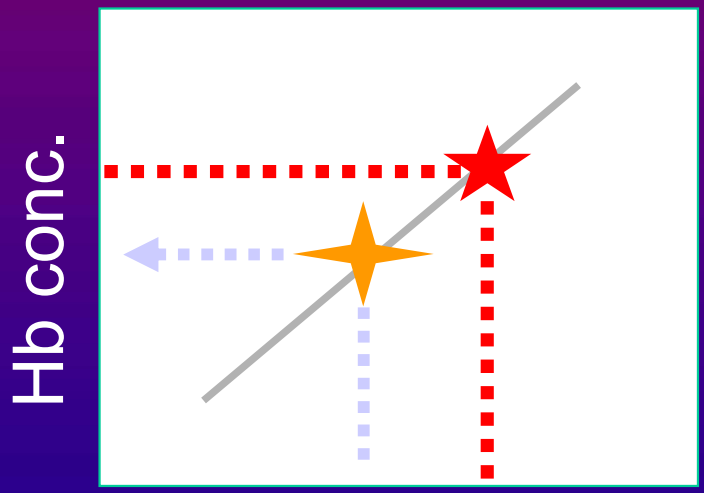

High MCHC Values (Artifacts)

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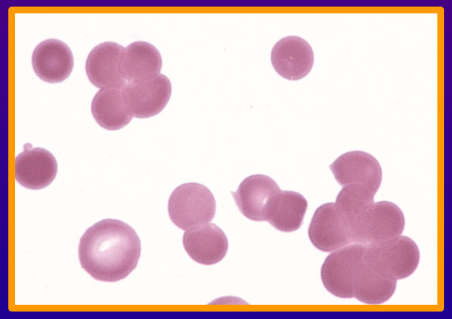


$$HCT = RBC \times MCV$$

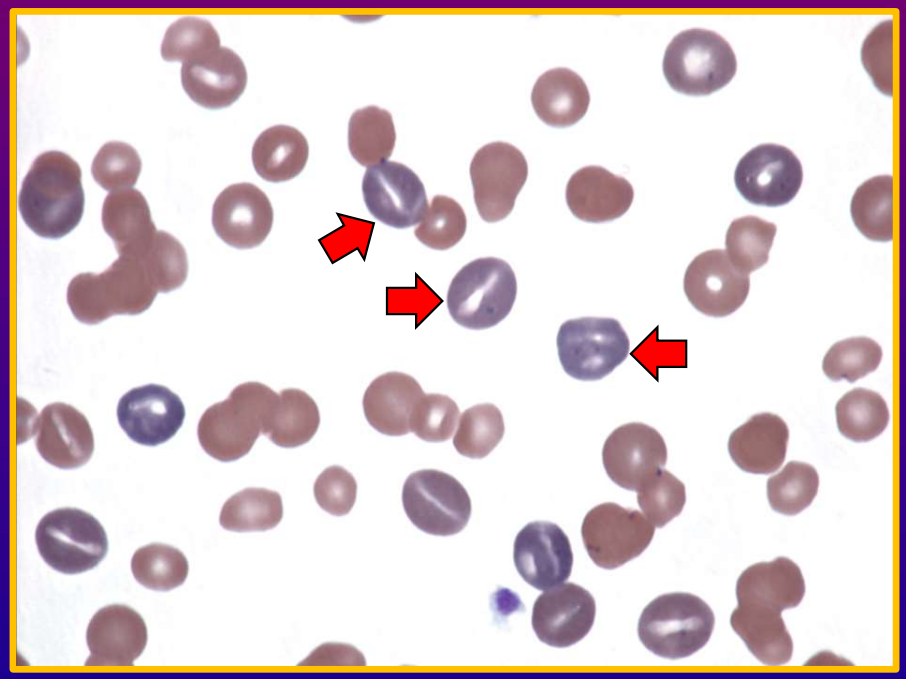
$$MCHC = \frac{Hb}{HCT\downarrow} \times 100$$

Low MCHC Values

- Some regenerative anemias, especially when high numbers of aggregate reticulocytes are present
- Abyssinian and Somali cats with erythrocyte osmotic fragility and swollen erythrocytes
- Chronic iron deficiency anemia (rare in cats)

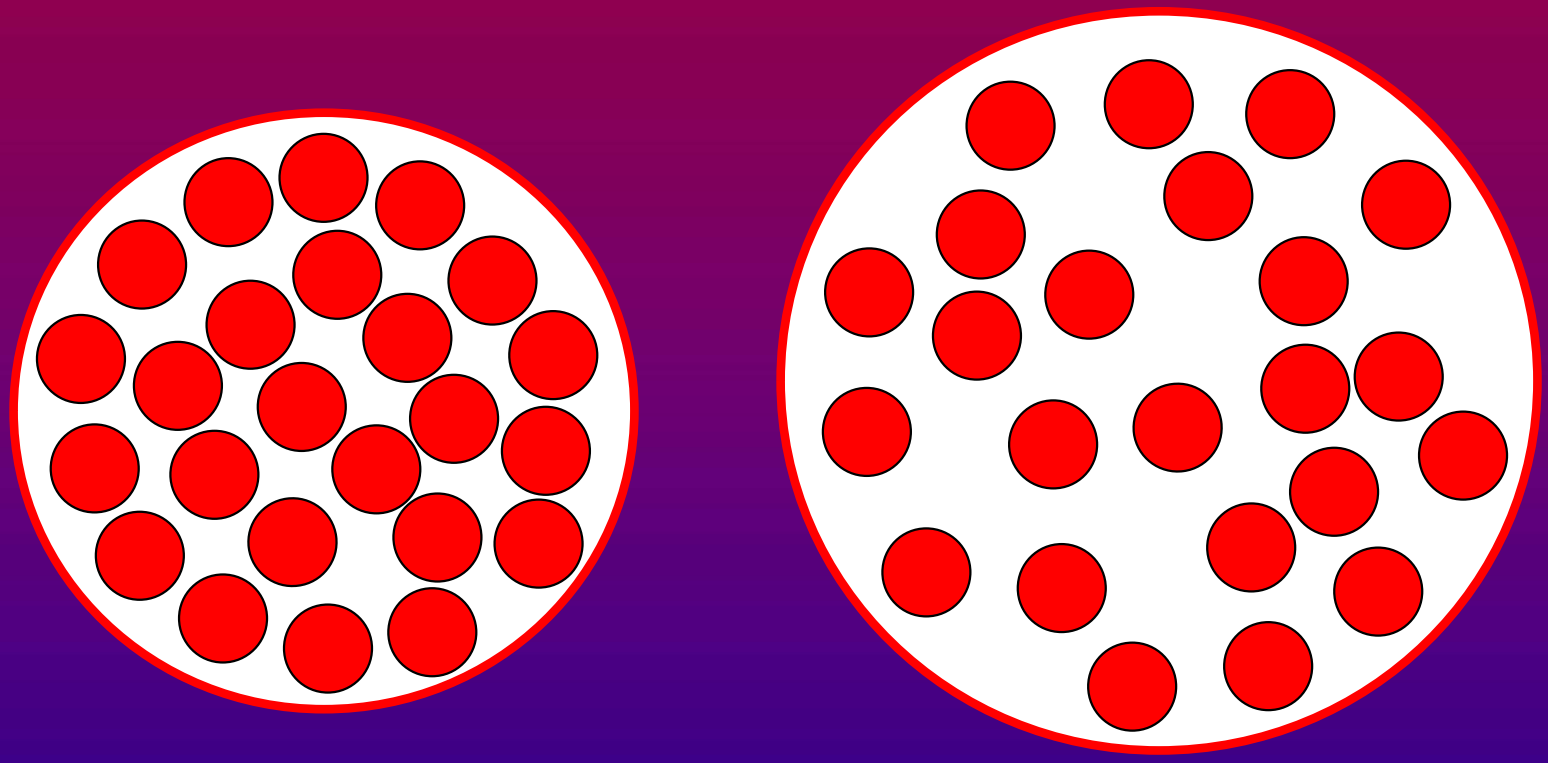
MCHC in Regenerative Anemia

- Within the reference range early and with mild regenerative anemias
- Low with a high percentage of reticulocytes, especially “stress” reticulocytes are released in response to hemolytic anemias.



Classification – MCV and MCHC

Marked Reticulocytosis



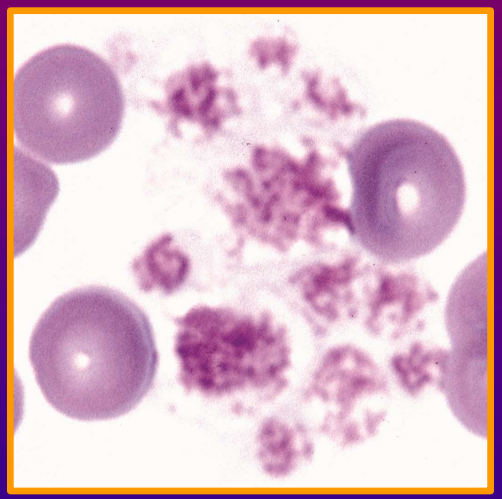
Normocytic/Normochromic

Macrocytic/Hypochromic

Represents an amount of hemoglobin

Cat Platelets

- Large with high mean platelet volume (MPV).
- Especially sensitive to activation during blood sample collection.

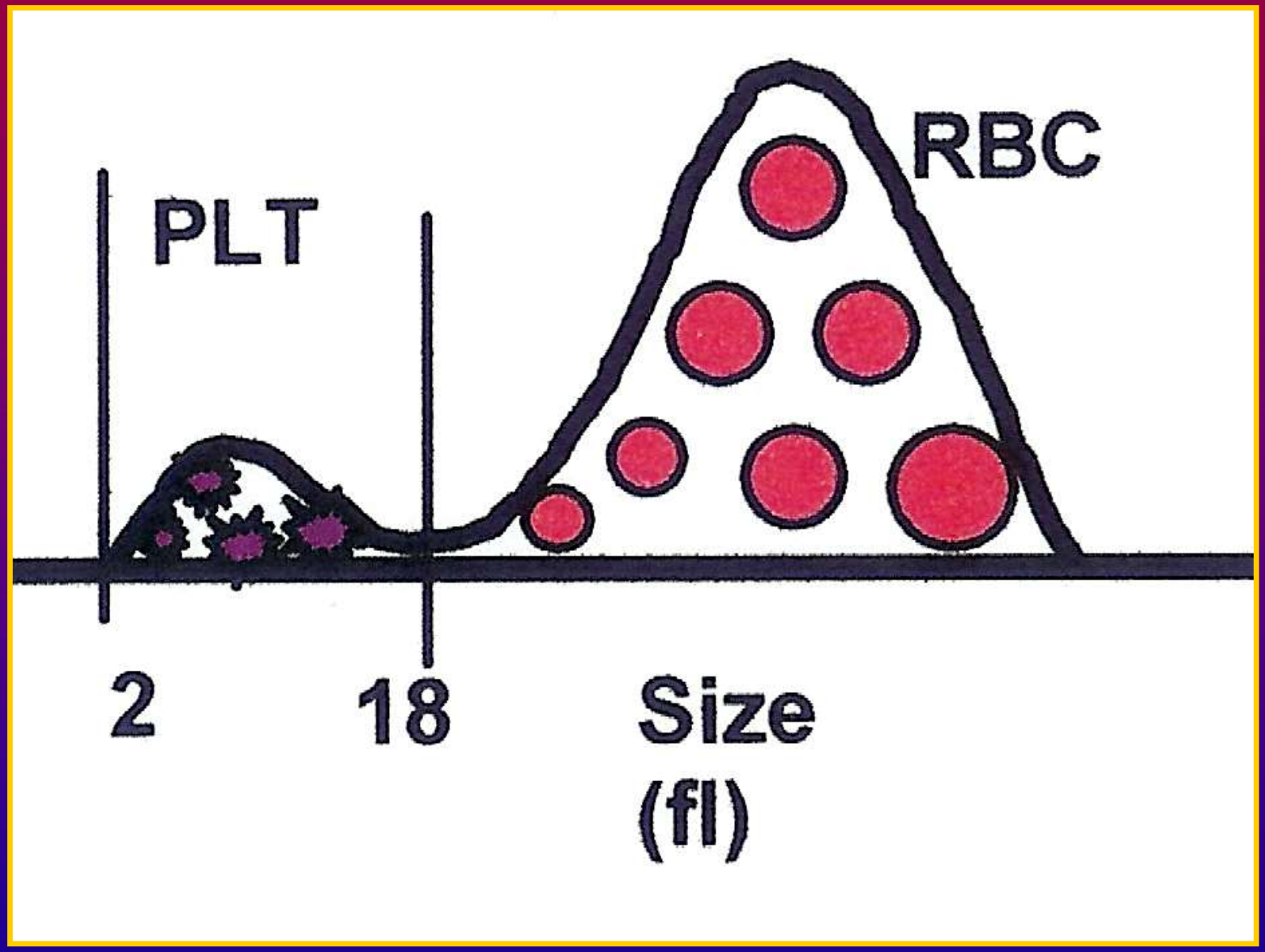


MPV in Domestic Animals

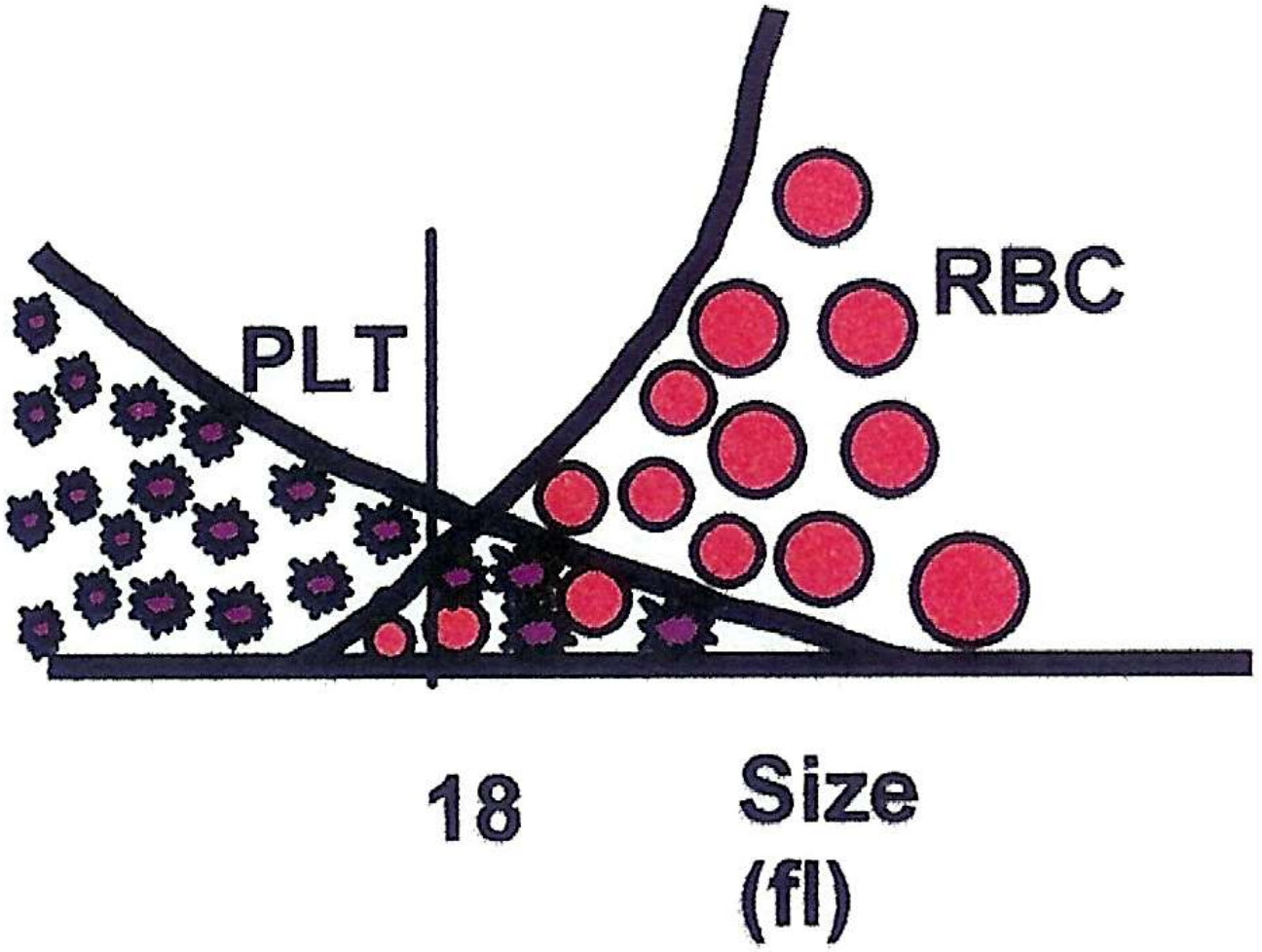
Species	Reference Interval
Cats	11.0 – 18.1
Dogs	6.7 – 11.1
Horses	4.6 – 7.3
Cattle	4.5 – 6.7

Values vary considerably by method used.

Platelet and RBC Volume Histograms Pattern in Dogs

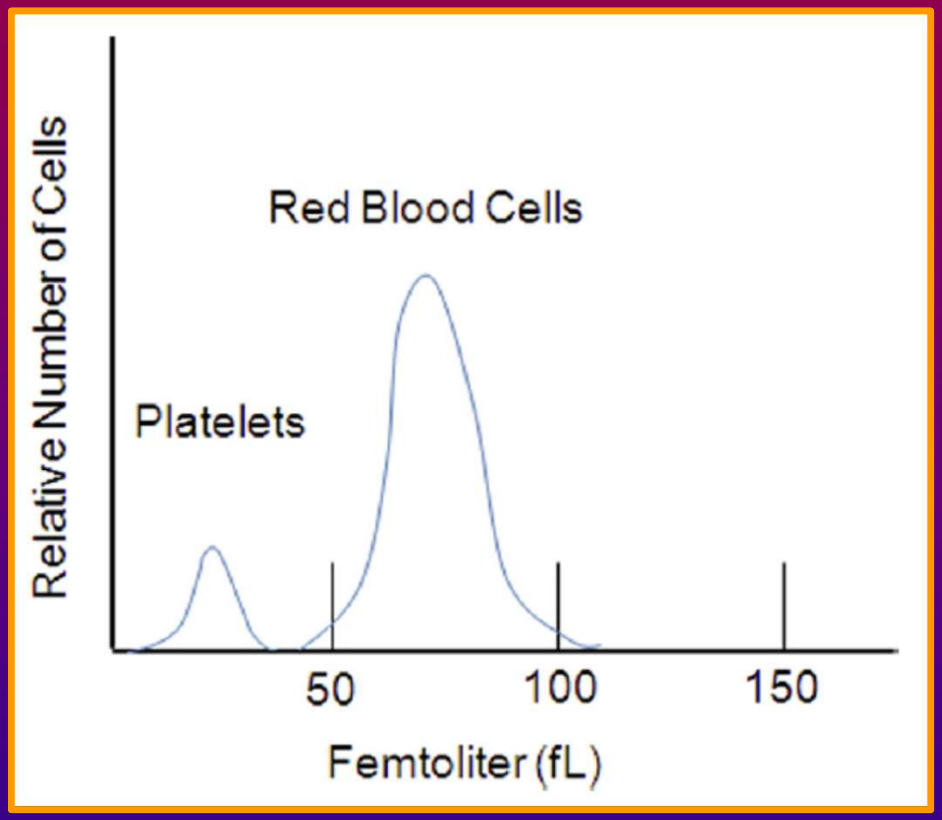


Platelet and RBC Volume Histograms

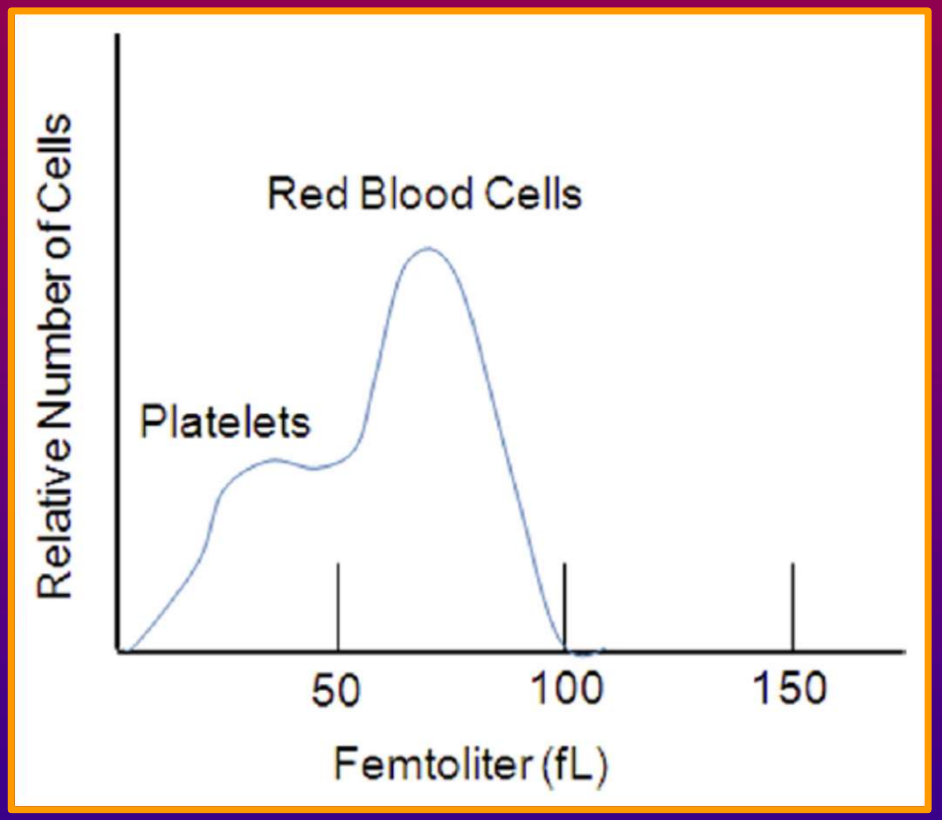


Erythrocyte versus Platelet Size

Dog



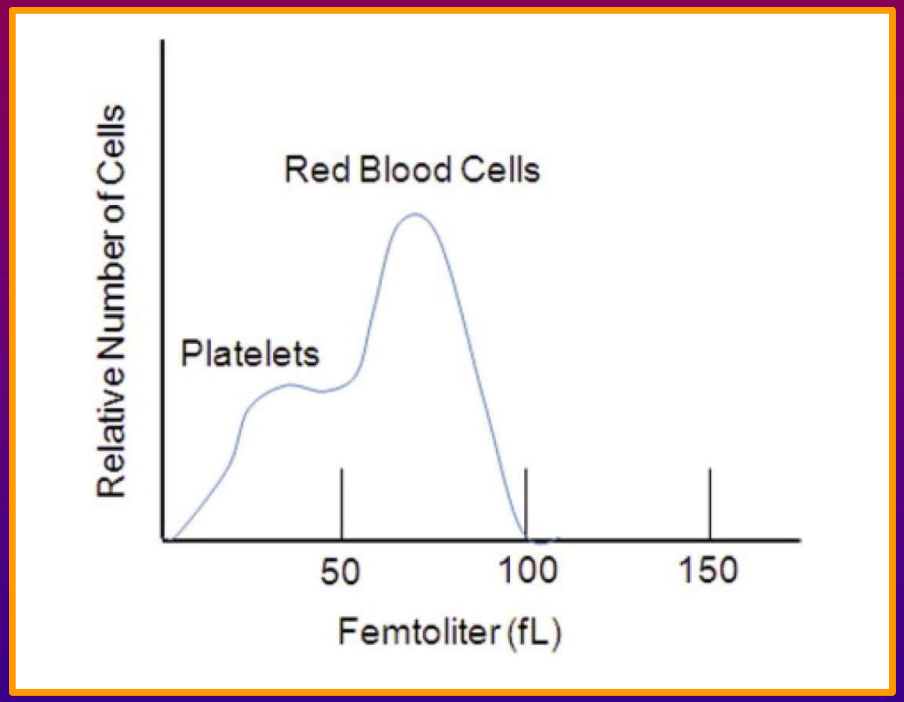
Cat



(DeNicola, 2011)

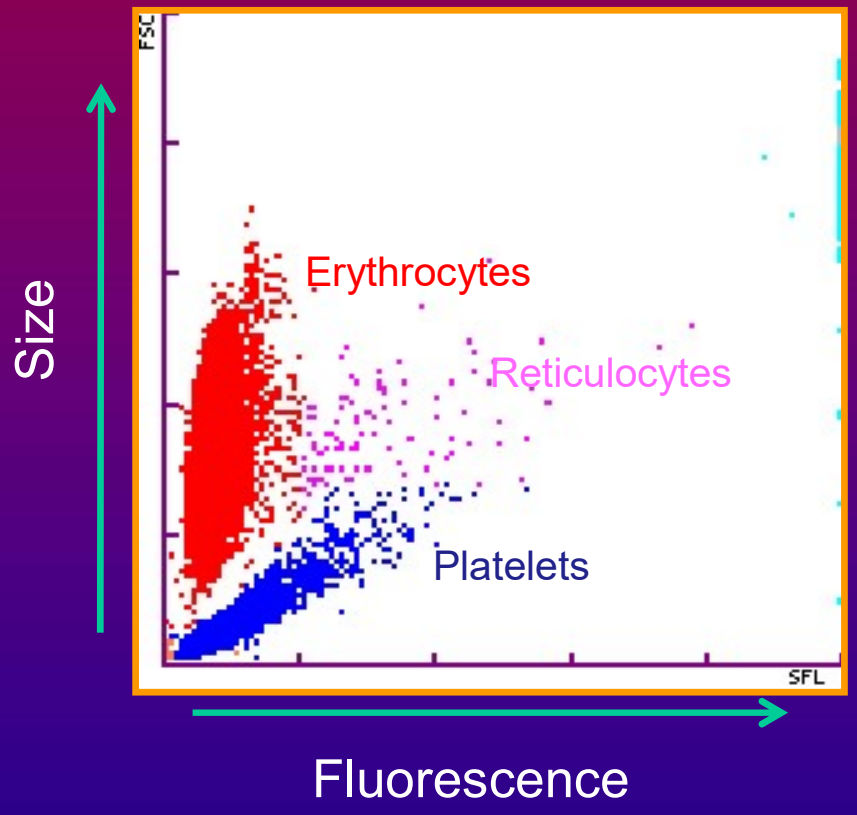
Platelet Counting in Cats

Impedance

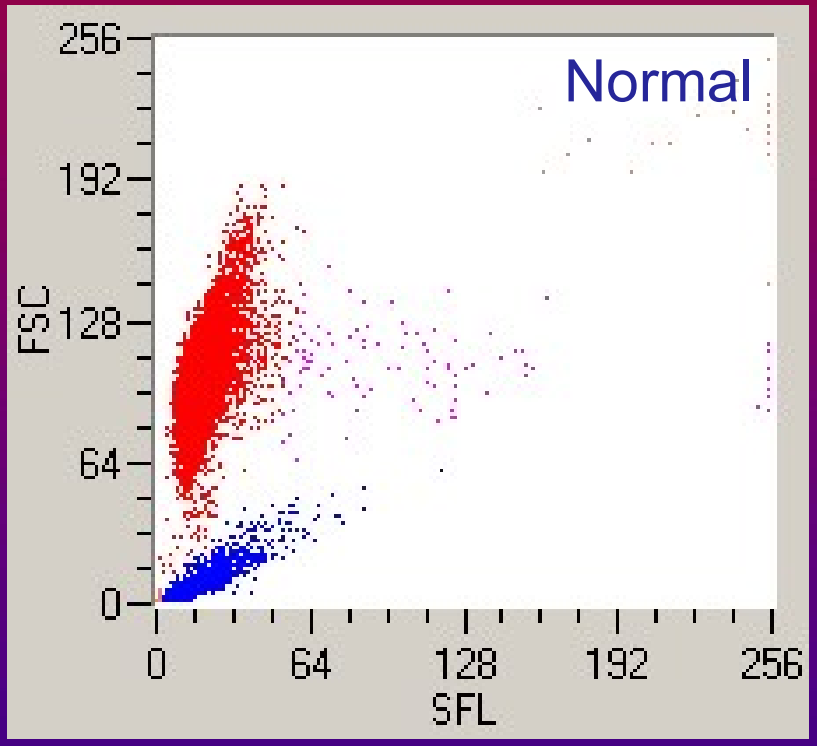


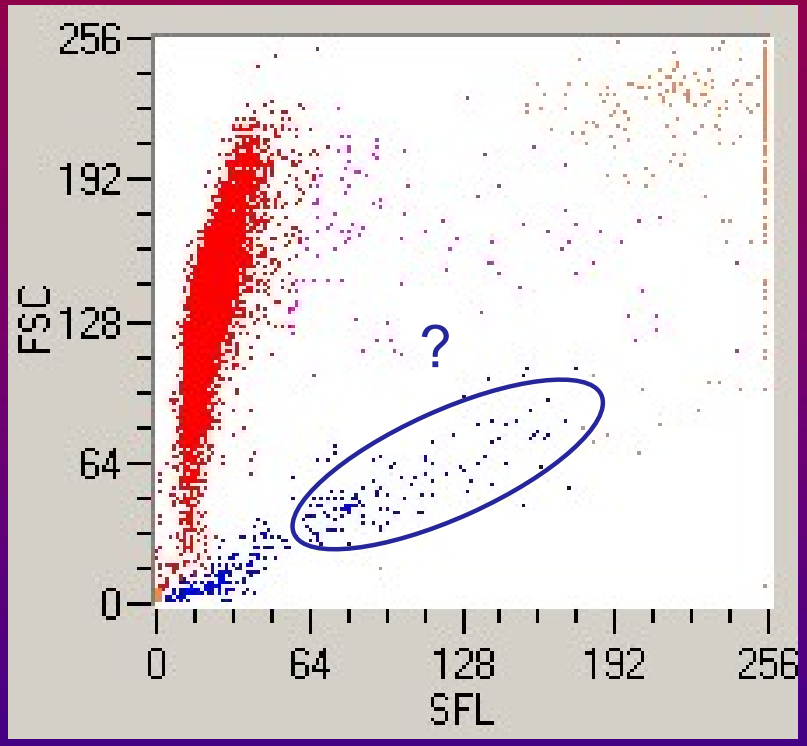
Inadequate Separation

Laser Flow



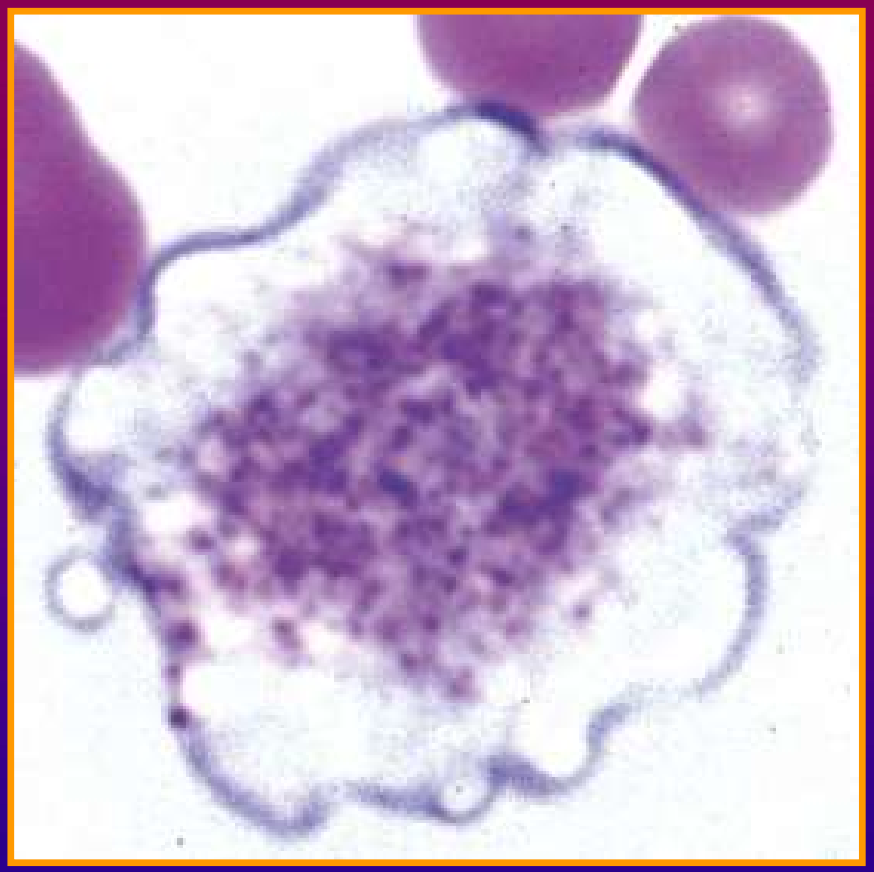
RBC-PLT Dot Plots



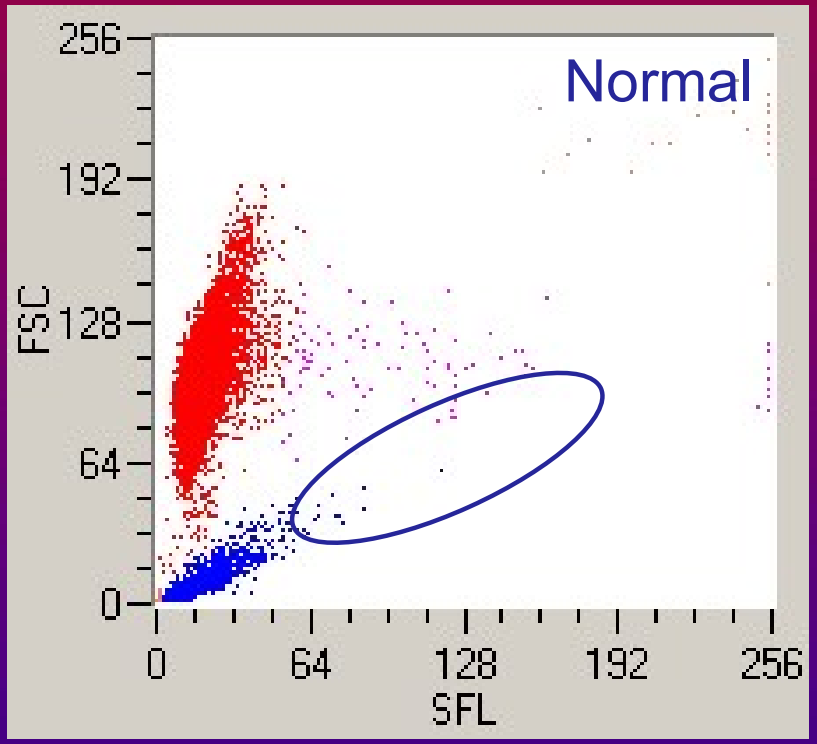


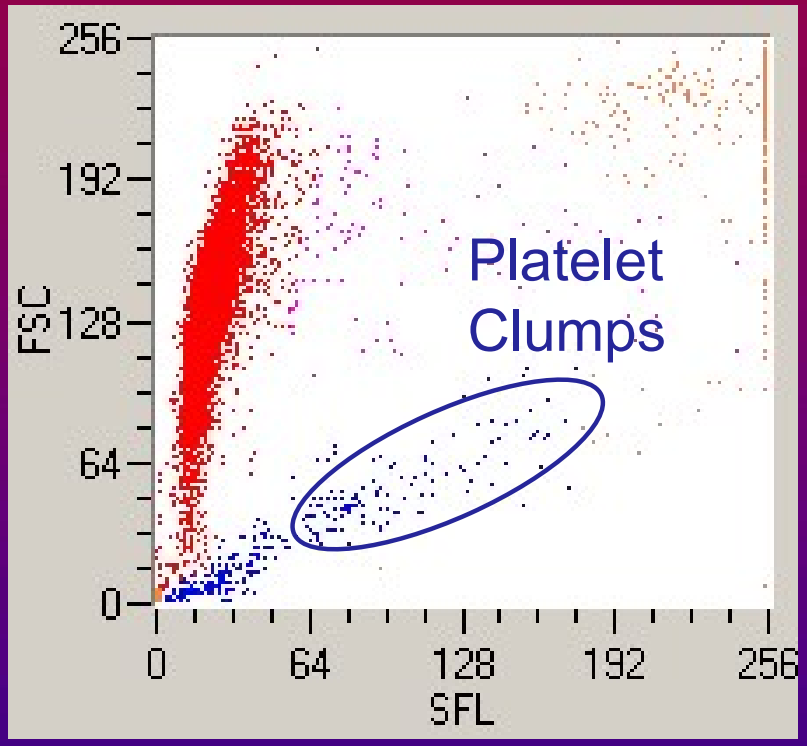
Red Blood Cells
RBC Fragments
Reticulocytes
Platelets

Cat Macroplatelet



RBC-PLT Dot Plots

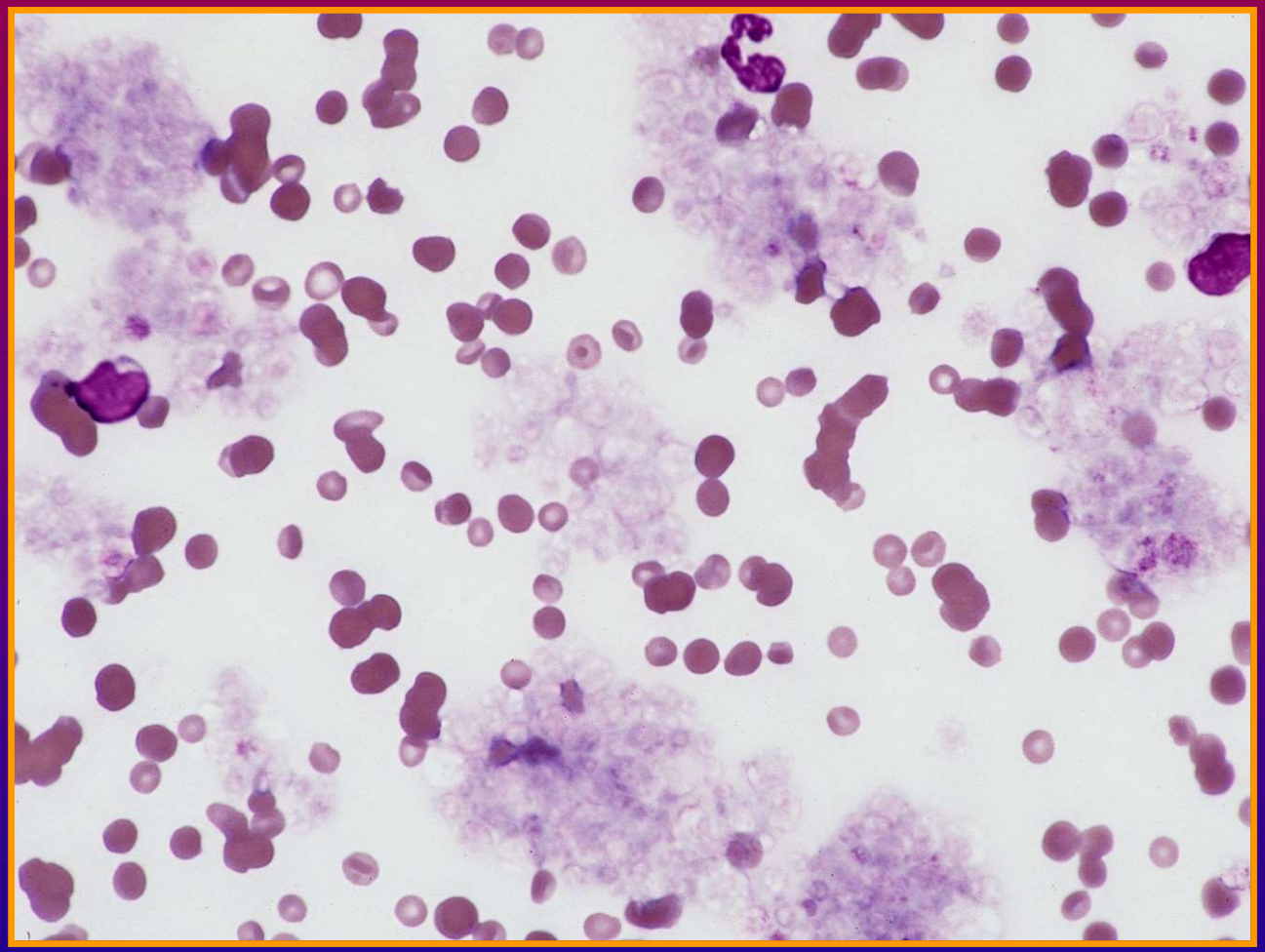




Red Blood Cells
RBC Fragments
Reticulocytes
Platelets

Low Platelet Count?

MUST confirm with blood film review.



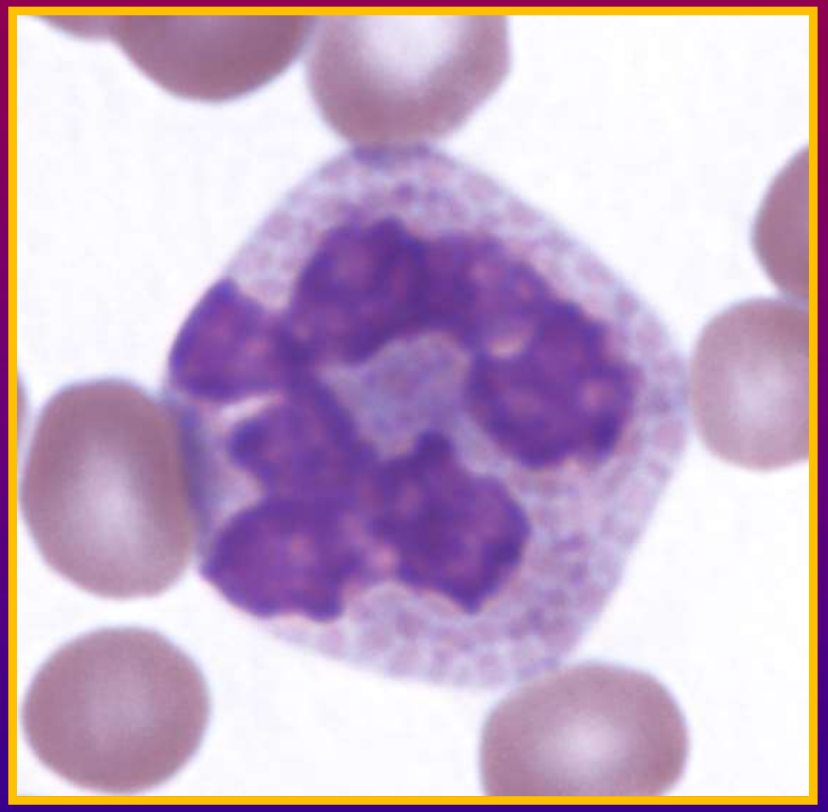
Eosinophils

Dog

Cat

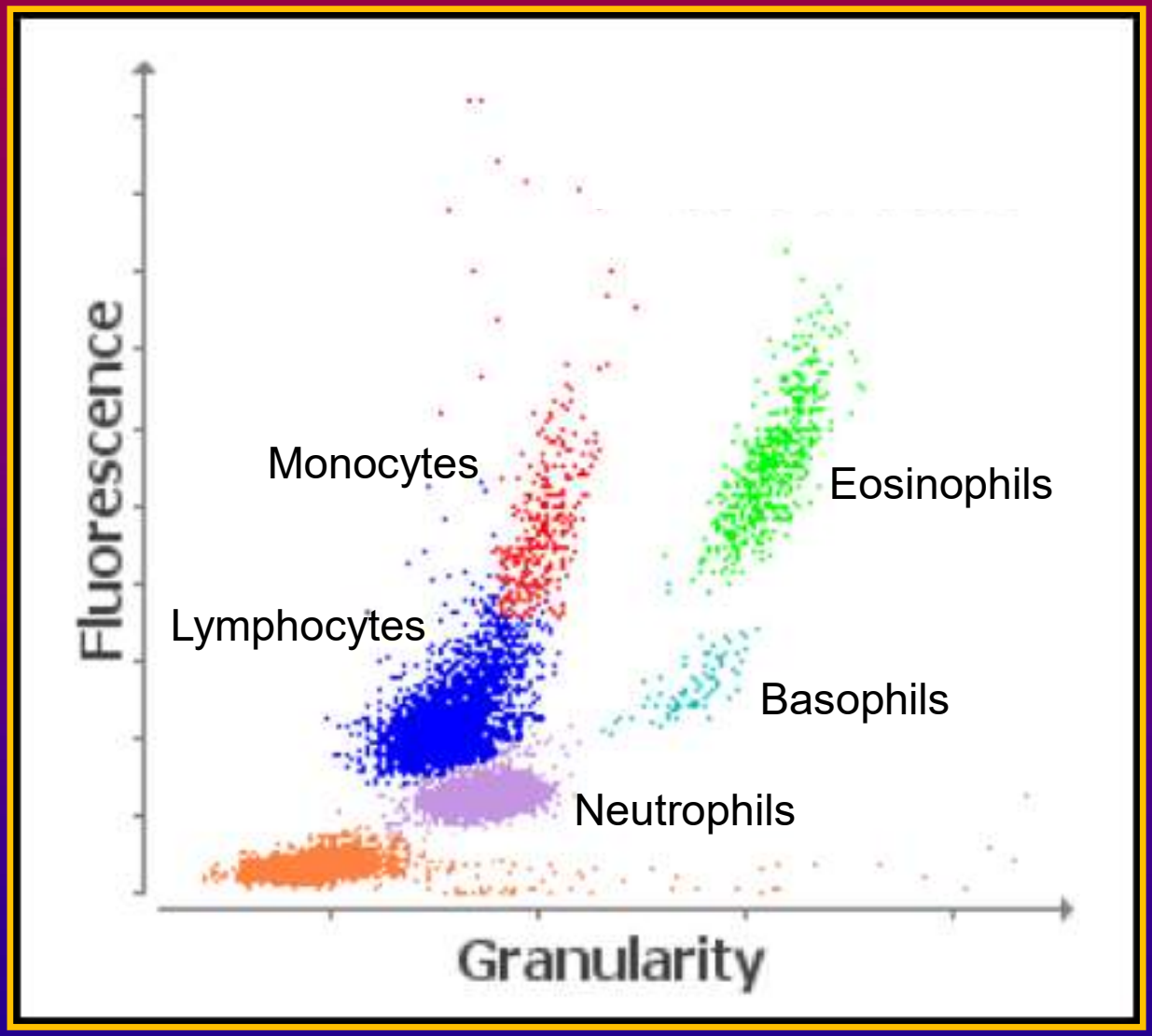
Basophils

Dog



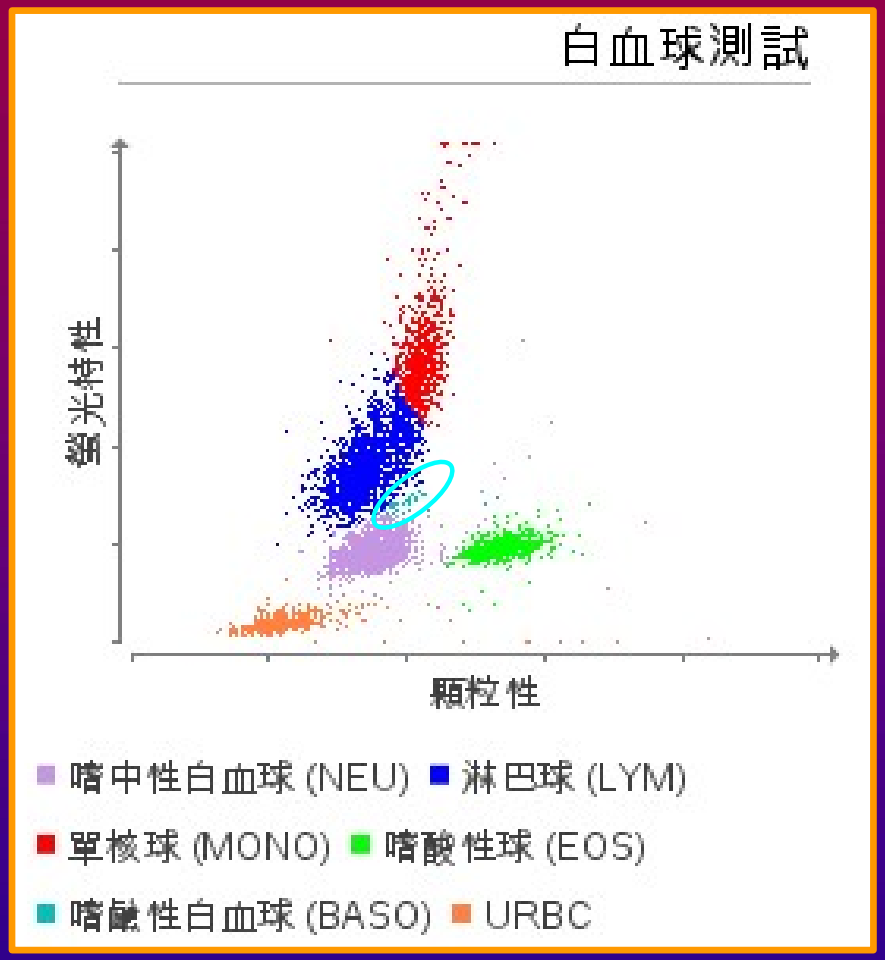
Cat

Cat Leukocyte Dot Plot

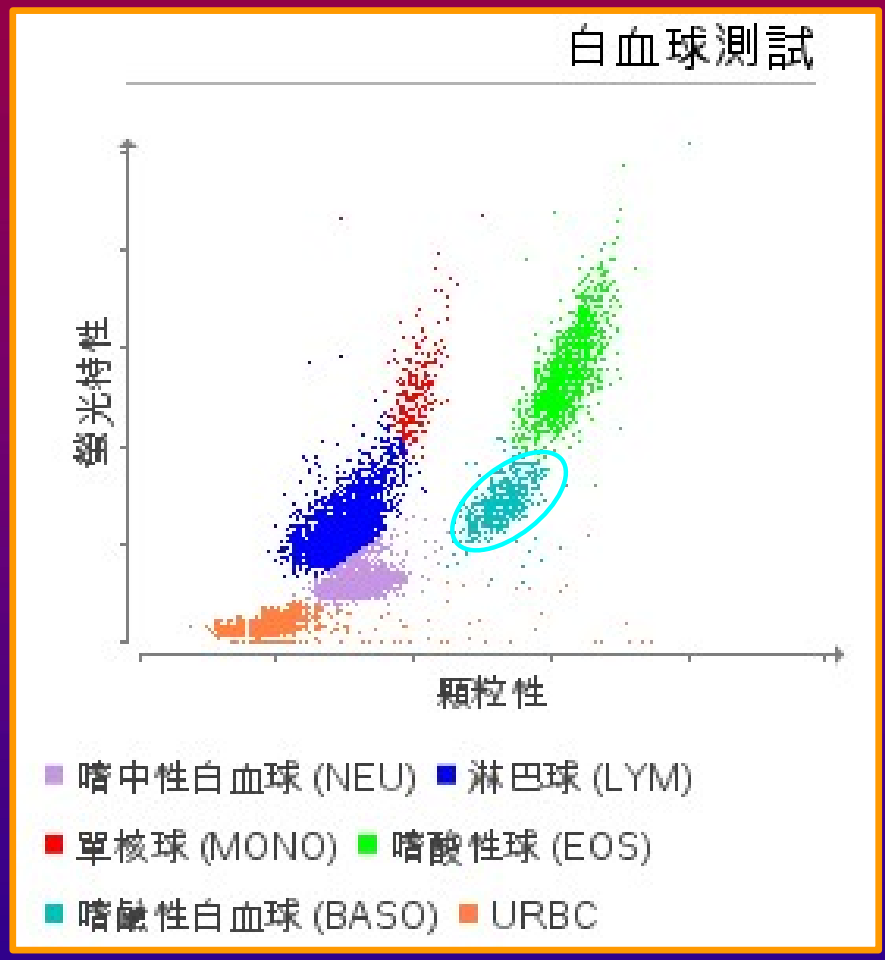


IDEXX ProCyte Dx

ProCyte Dx
Leukocyte Dot Plots (NCHU Taiwan)

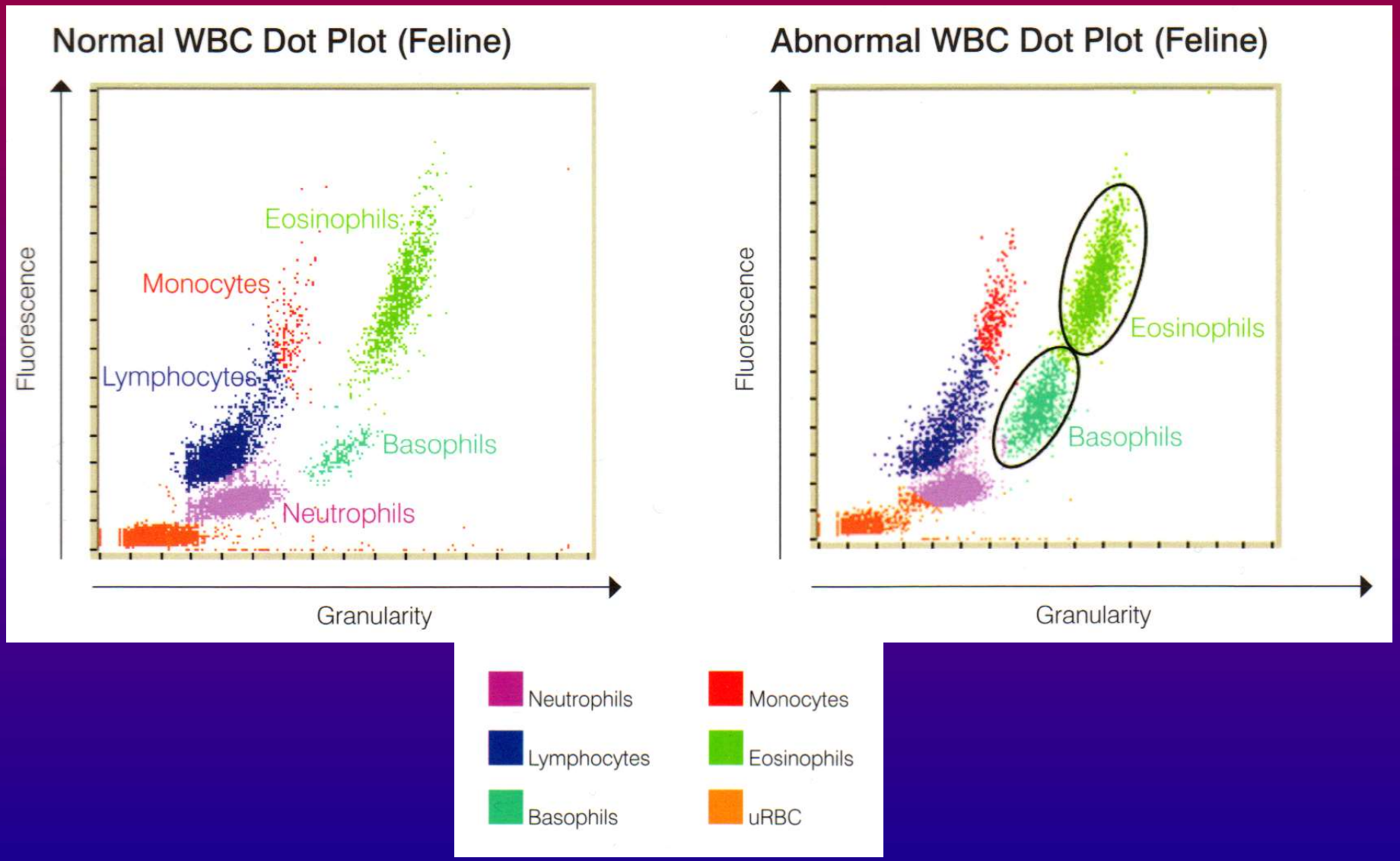


Dog



Cat

WBC Dot Plot Interpretation



Neutrophil Morphology

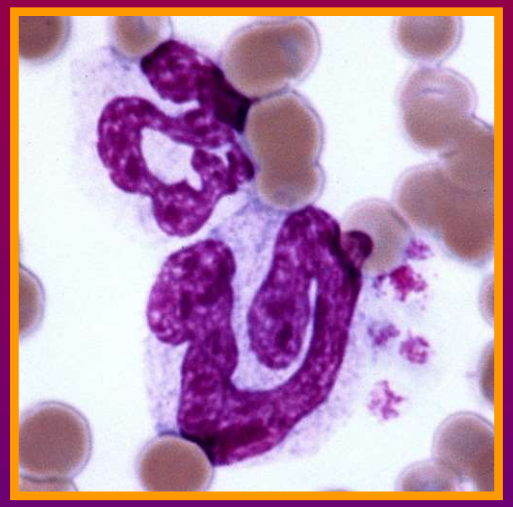
Neutrophil

Band Neutrophil

Cat Neutrophil Morphology

Döhle Body

Giant Neutrophil



Toxic Cytoplasm

Impact of FeLV and FIV Infections on Feline Hematology

Clinical Syndrome	FeLV	FIV
Neoplasms	62-fold increase direct role Mainly T-cell lymphoma	5-fold increase indirect role Mainly B-cell lymphoma
Marrow Suppression	common anemia, thrombocytopenia neutropenia, primary infection of marrow cells	rare mainly neutropenia, soluble factors inhibit marrow function
Immunodeficiency	common replication of virus in all marrow cell types, changes in cytokines	common Several mechanism, decreased CD4+ cells, changes in cytokines
Immune-mediated	Rare e.g., IMHA	Sometimes, hyperglobulinemia common with immune- complex deposition
Neurologic disorders	rare	rare
Stomatitis	common	very common

Hartmann K, Viruses (2012) 4:2684-2710

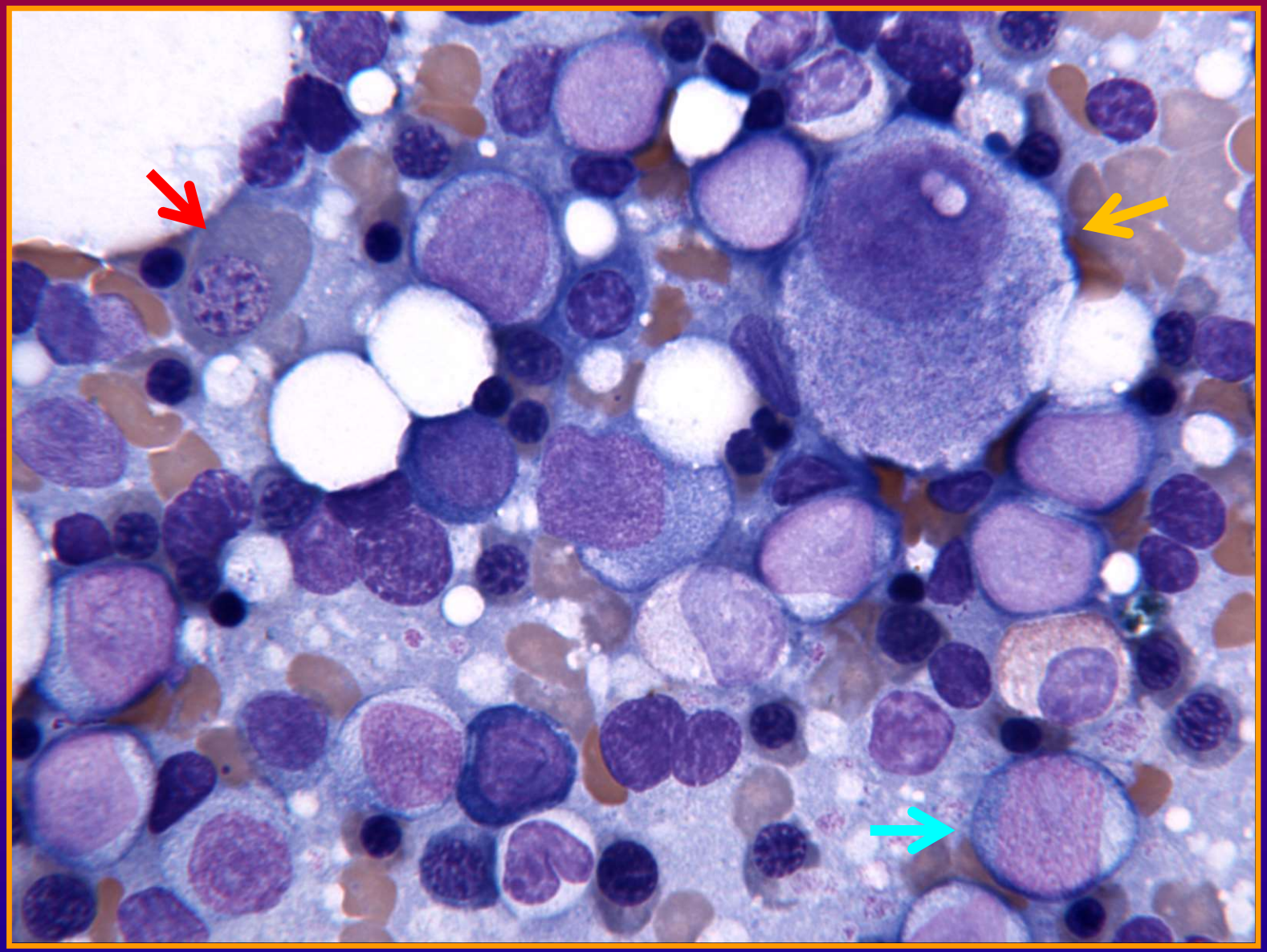
FeLV Subgroups

- FeLV-A is the primary strain transmitted between cats.
  - Minimally pathogenic
- FeLV-B – recombination of FeLV-A gene encoding for its envelope (Env) protein with endogenous retroviral sequences in cat genome
  - Anemia, lymphoma, leukemia
- FeLV-C – Mutation of the FeLV-A Env gene
  - Erythroid aplasia
- Additional subgroups D, E, T from multiple recombination events with feline endogenous retroviruses

Receptors for FeLV subgroups

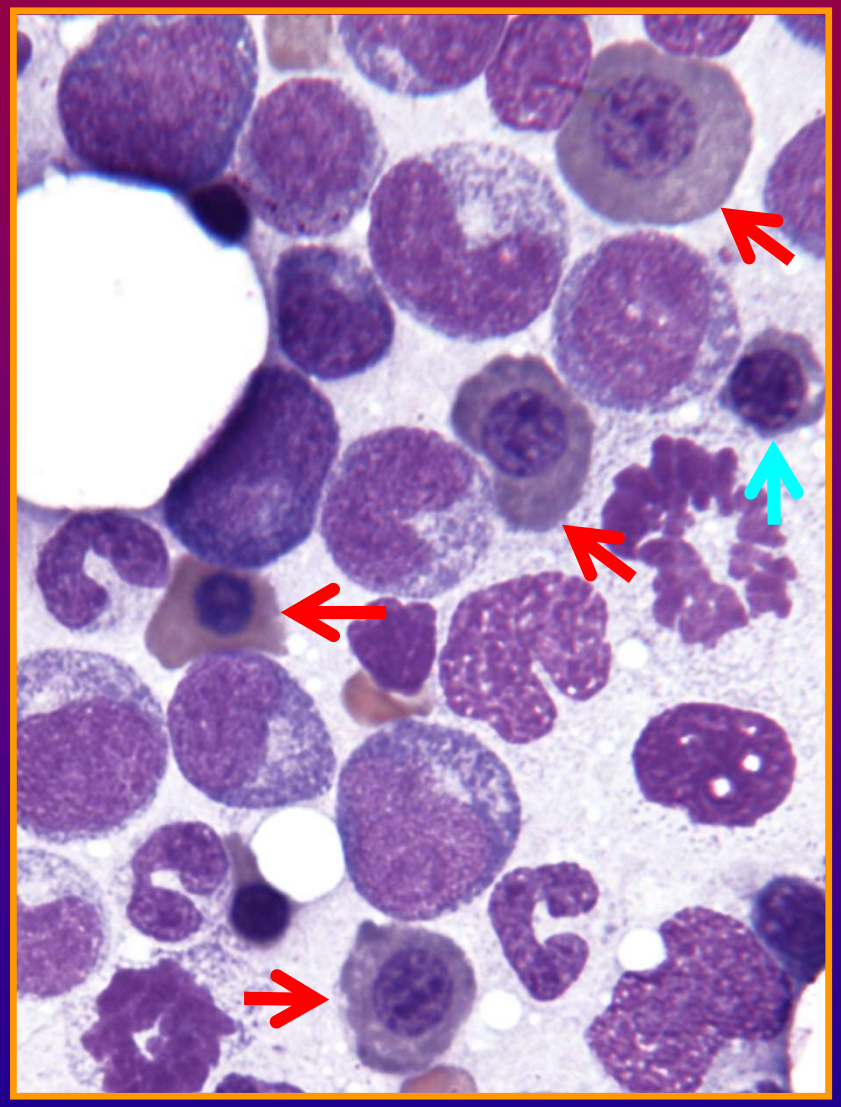
- Env gene recombinations and mutations determine host receptors used by different FeLV subgroup
- FeLV-A uses host receptor THTR1, a thiamine transporter
- FeLV-B uses host receptors Pit1 and Pit2, phosphate transporters
- FeLV-C uses host receptor FLVCR1, a heme exporter

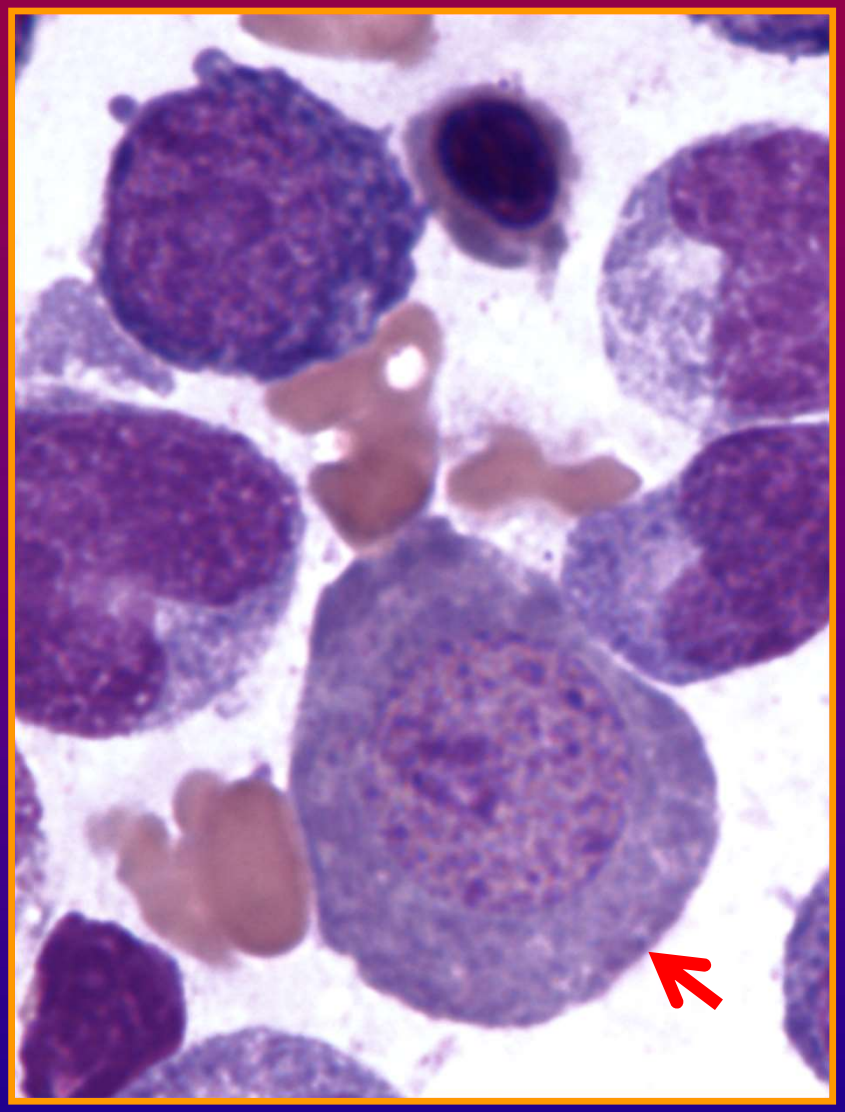
Pancytopenic Cat with MDS



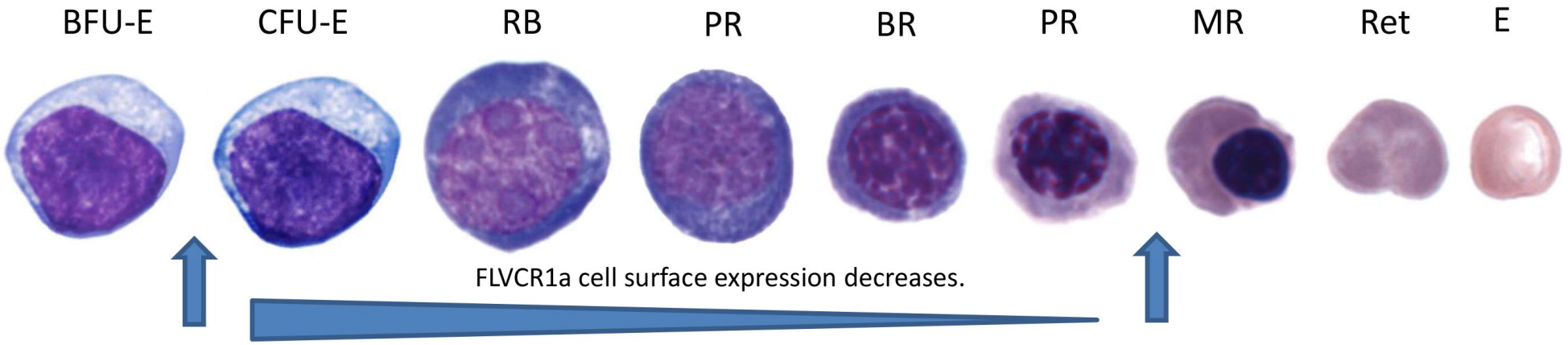
Dysplastic Erythroid Cells

Cat with MDS





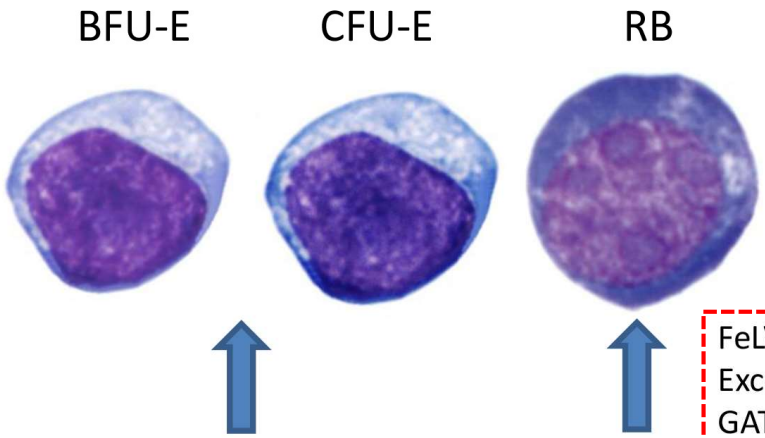
Normal Hematopoiesis



GATA1 upregulates ALAS2, and heme synthesis intensifies, which increases ribosome content and globin chain synthesis.

Excess heme accumulates and down-regulates GATA1 terminating erythropoiesis.

FLVCR1a inhibited by FeLV type C

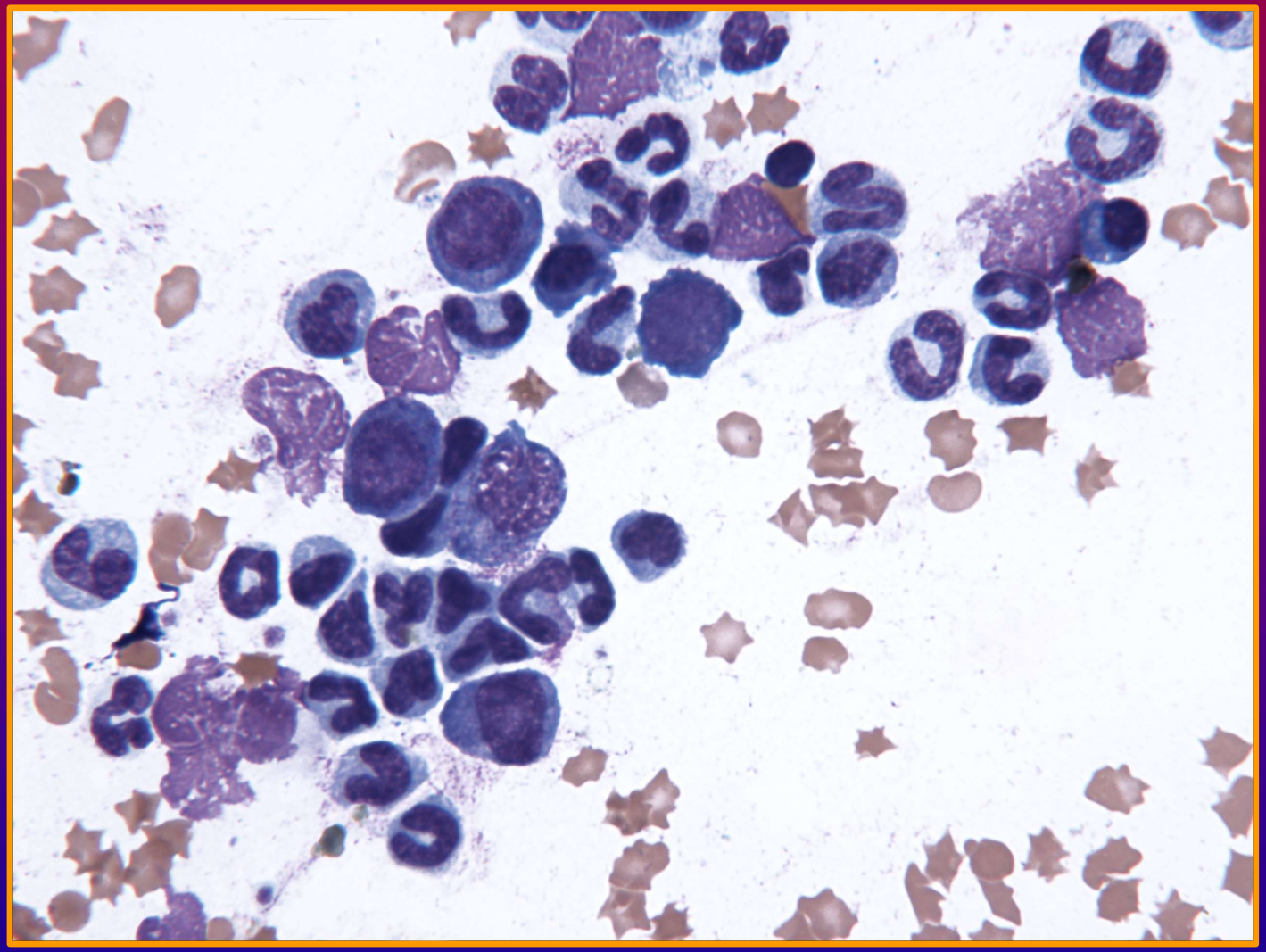


FeLV type C inhibits FLVCR1a.
Excess heme accumulates and down-regulates GATA1, prematurely terminating erythropoiesis.

GATA1 upregulates ALAS2, and heme synthesis intensifies, which increases ribosome content and globin chain synthesis.

Harvey JW. Clinical Biochemistry in Domestic Animals, in press

Hypocellular Marrow with Erythroid Aplasia



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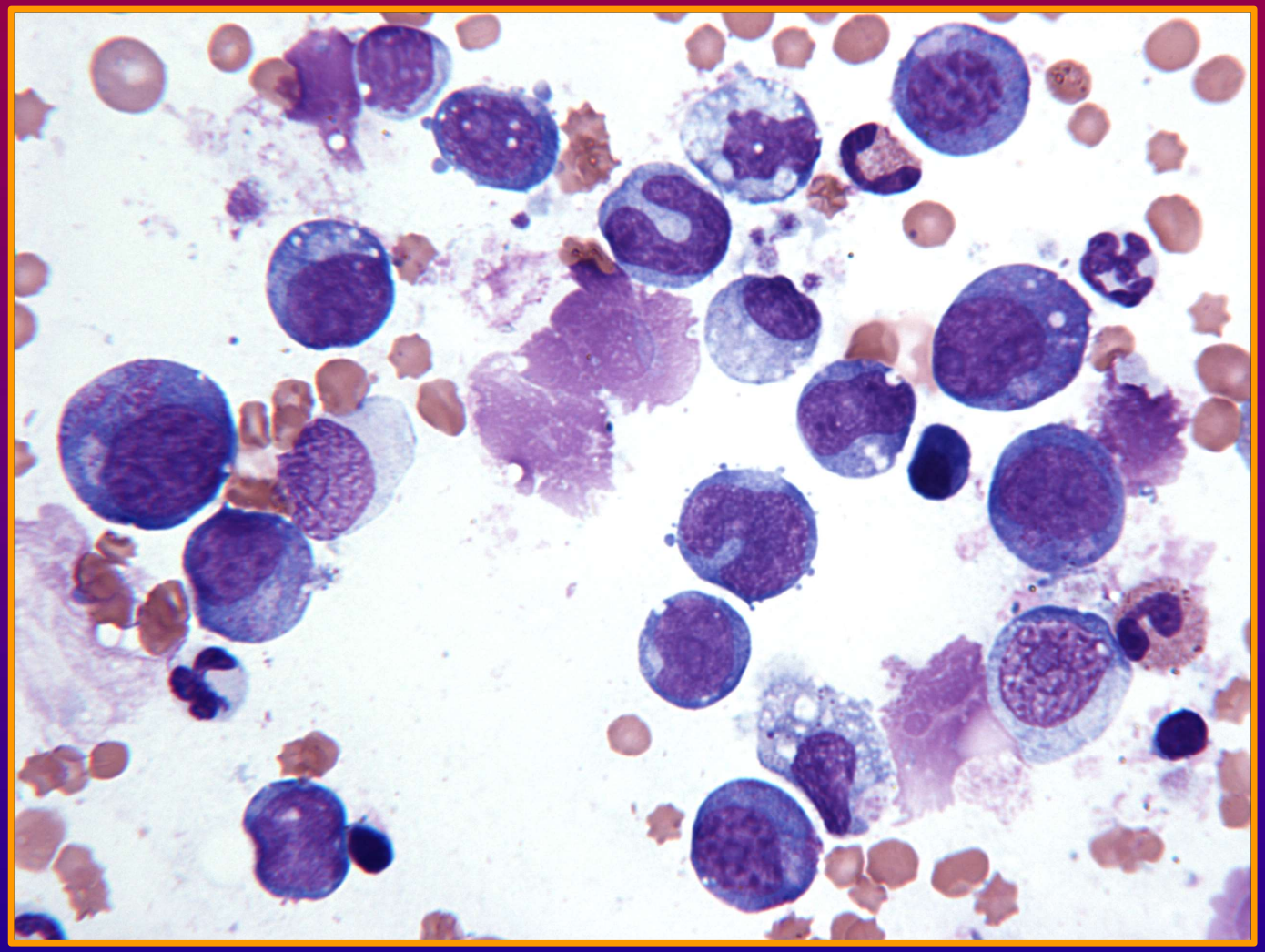
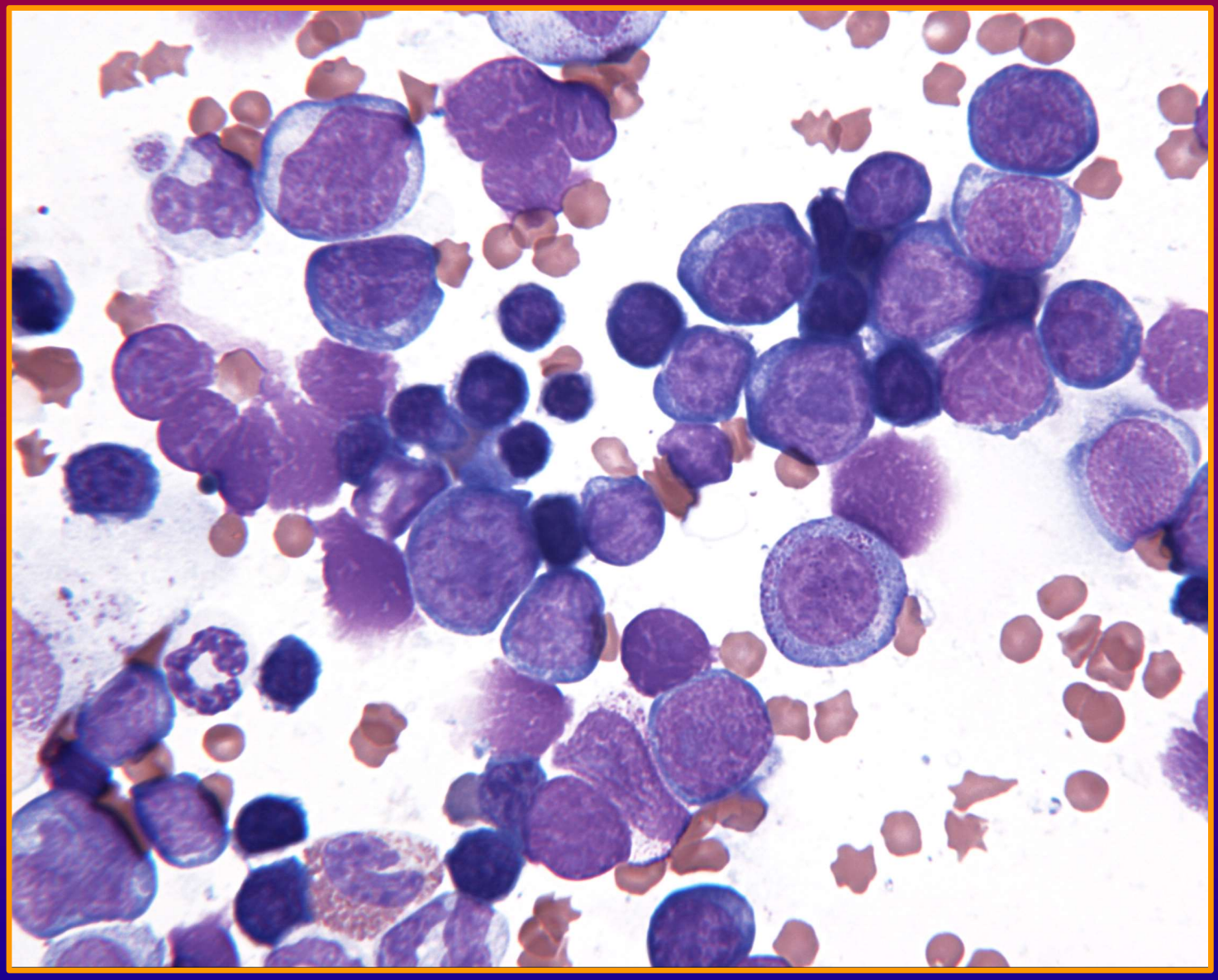
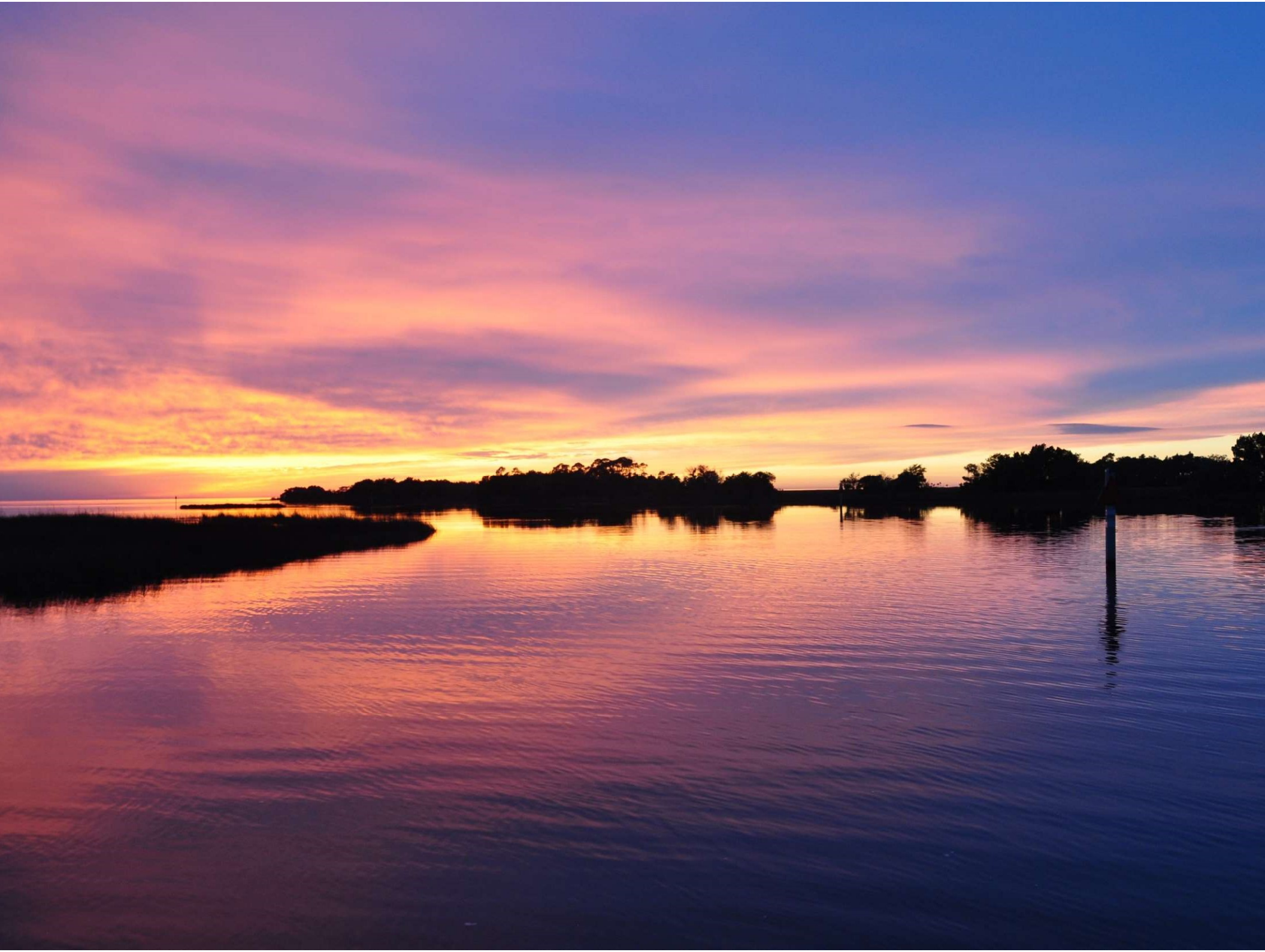


Erythroleukemia AML-M6





History and Clinical Findings

Adult mixed breed male cat

- Presented with a primary complaint of posterior paresis
- Abdominal enlargement present
- Unable to urinate
- Spinal reflexes present
- Sensation present in rear limbs
- Hepatomegaly or splenomegaly palpated
- Temperature was normal

Hematology Findings

Parameter	Patient	Reference
HCT (%)	21	30 - 47
MCV (fL)	59	41 - 51
MCHC (%)	33	31 - 35
Icterus Index (units)	<5	<5
Plasma Protein (g/dL)	8.5	6.2 - 8.0
Fibrinogen (mg/dL)	500	100 - 300
Platelets ($x10^3/\mu L$)	normal	300 - 800

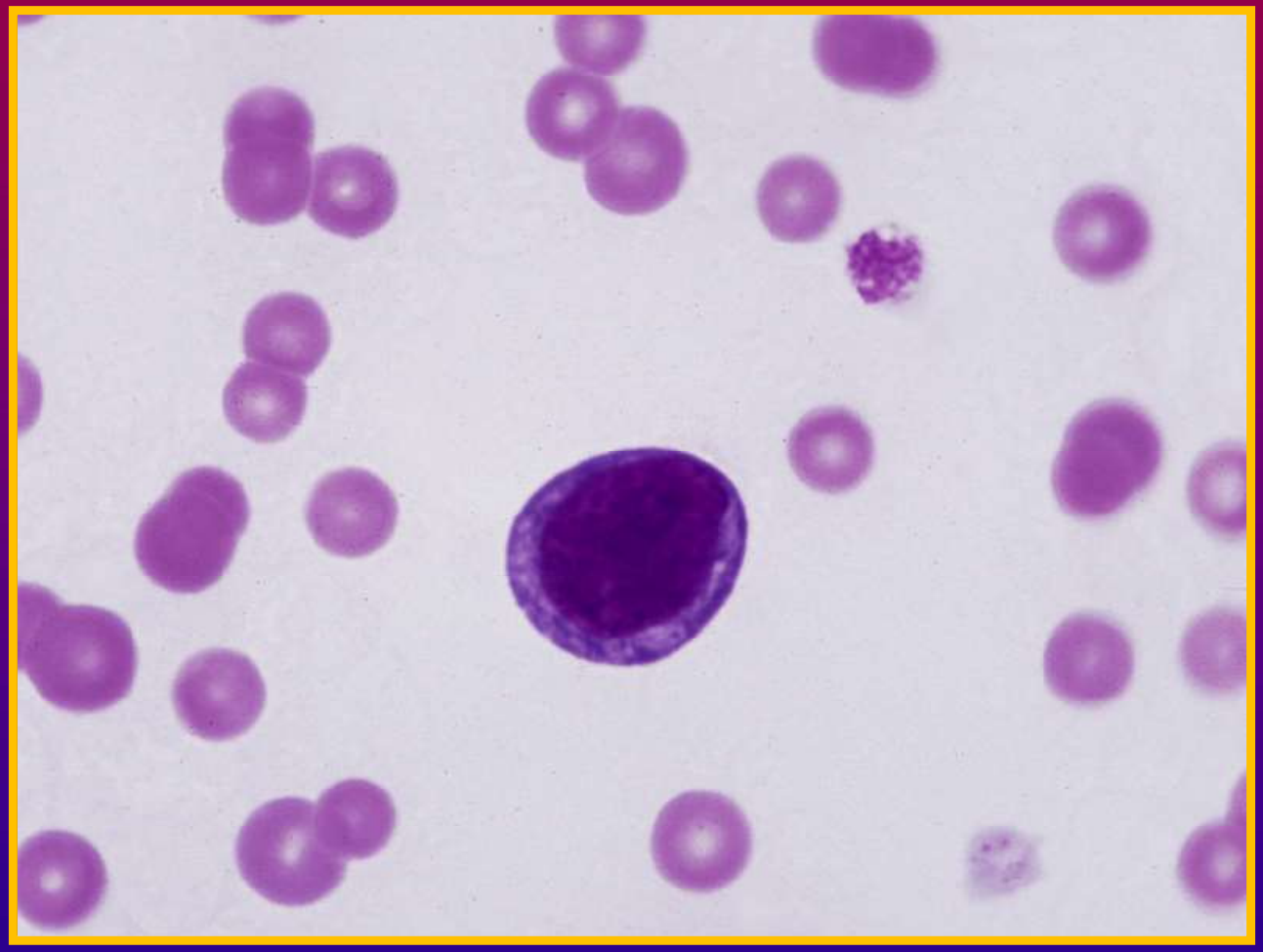
Erythrocyte Morphology: slight anisocytosis, no polychromasia

Leukogram Findings

Parameter	Patient	Reference
Total WBC ($x10^3/\mu L$)	4.0	5.1 – 15.4
Bands ($x10^3/\mu L$)	0.1	0 - 0.3
Neutrophils ($x10^3/\mu L$)	1.9	2.3 – 9.8
Lymphocytes ($x10^3/\mu L$)	1.9*	0.9 – 5.5
Monocytes ($x10^3/\mu L$)	0.1	0 - 0.8
Eosinophils ($x10^3/\mu L$)	0	0 - 1.8
Basophils ($x10^3/\mu L$)	0	0 – 0.2

*Some lymphocytes appear to be blasts

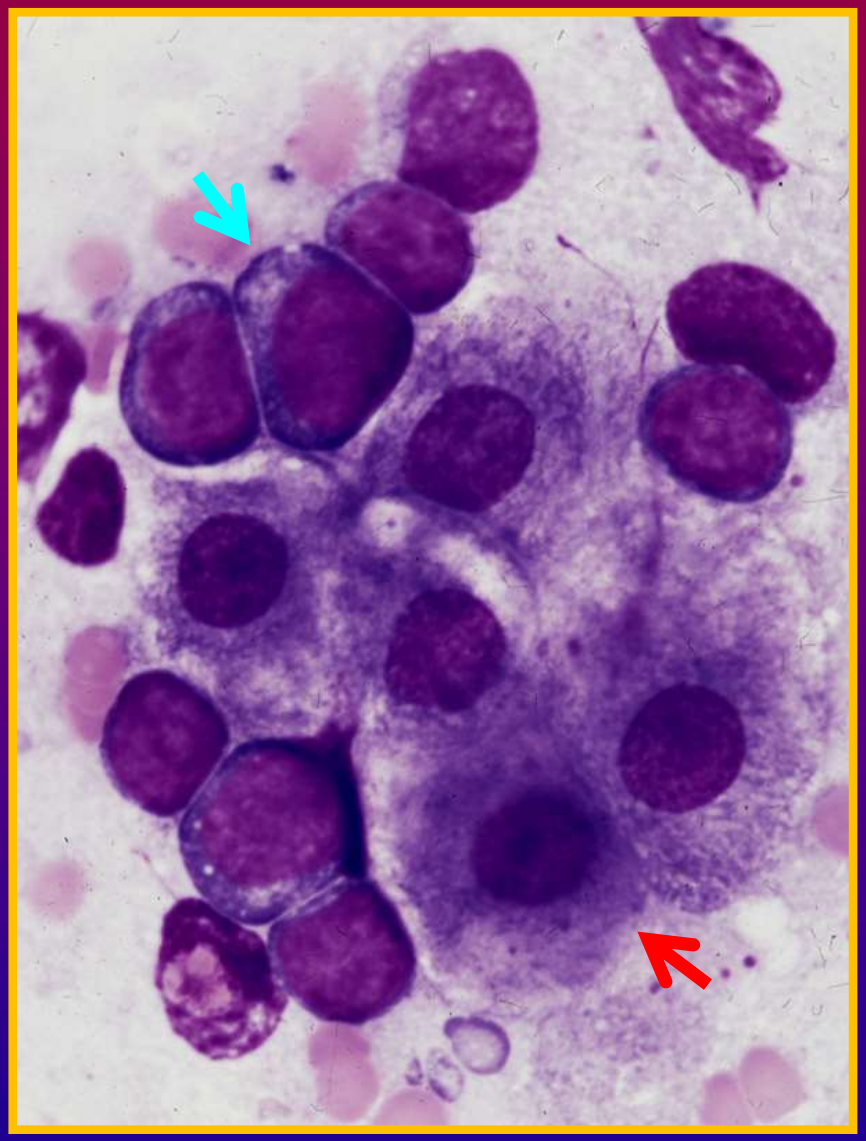
Stained Blood Film

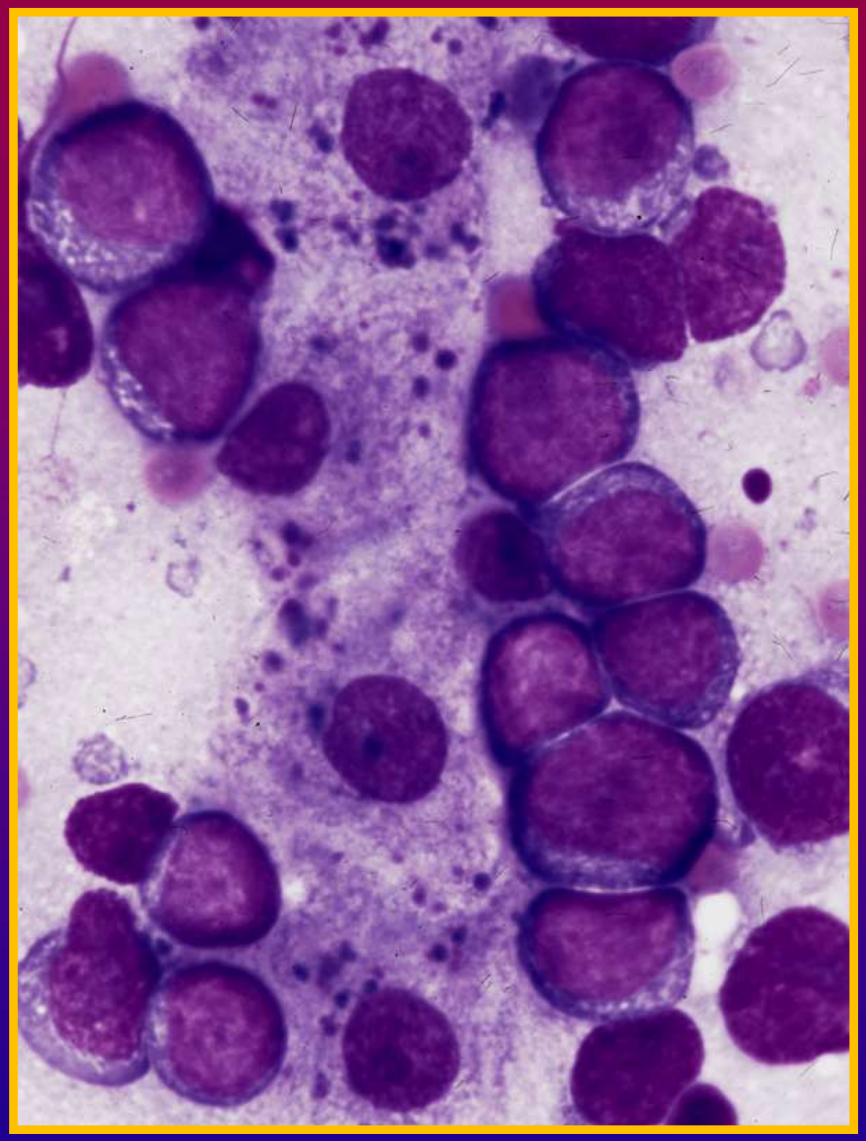


Additional Findings

- Radiology
  - Mild hepatic enlargement
  - No abdominal masses seen
- Antigen/serology tests
  - FeLV: positive
  - FIV: negative

Liver Aspirate Cytology





History and Examination

8-year-old spayed female cat

- History of progressive weight loss, with anorexia and lethargy recognized for one week
- Depressed, lethargic, cachexic and 10% dehydrated, dry hair coat
- Icteric mucous membranes
- Temperature of 104°F

FE76402

Hematology Findings

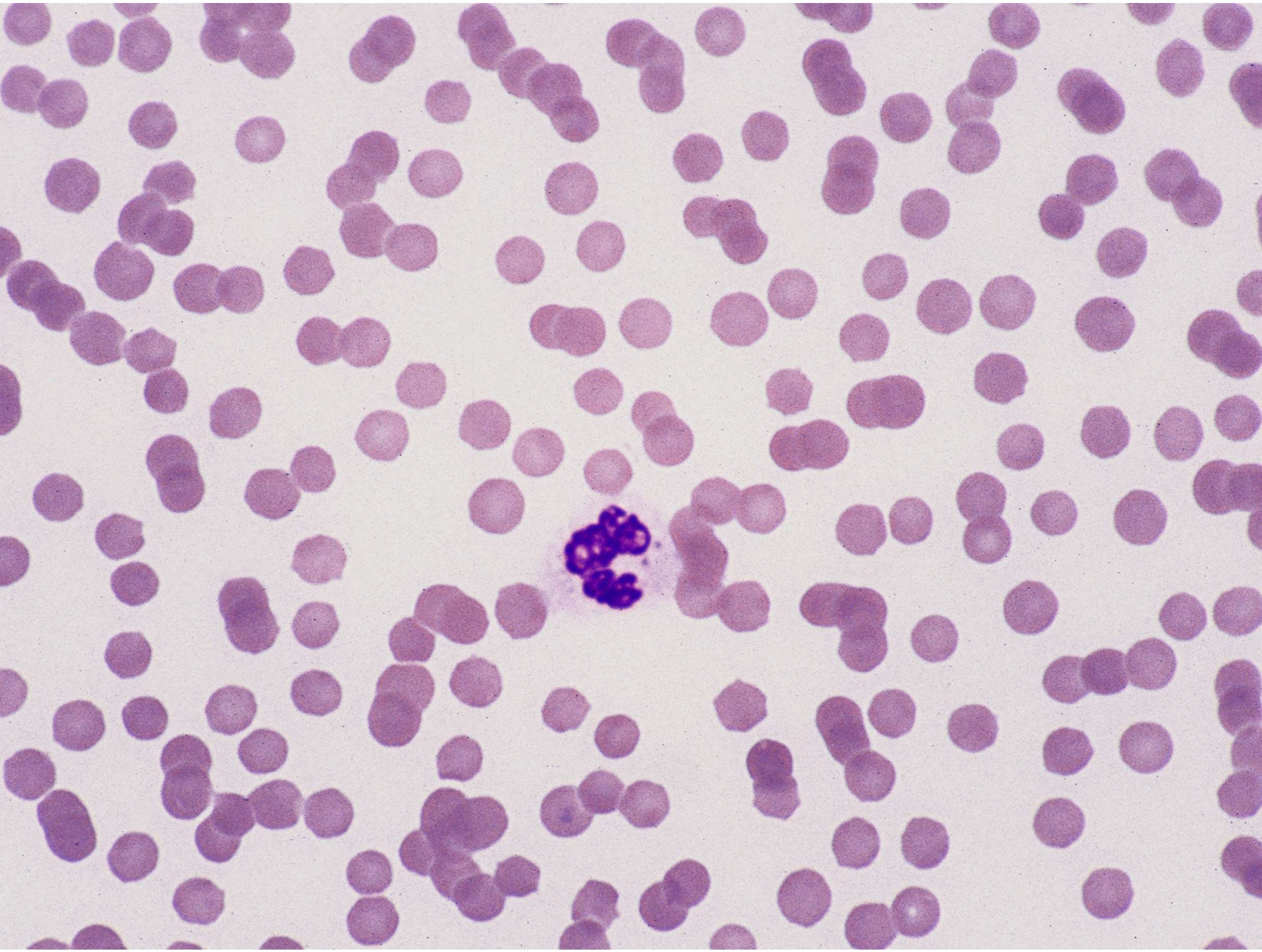
Parameter	Patient	Reference
HCT (%)	26	30 - 47
MCV (fL)	49	41 - 51
MCHC (%)	35	31 - 35
Retics (x10^3/µL)	0 A, 11P	<30 A, <500 P
Ict Ind (units)	25	<5
TPP (g/dL)	6.3	6.2 - 8.0
Fibrinogen (mg/dL)	200	100 - 300
Platelets (x10^3/µL)	16	300 - 800

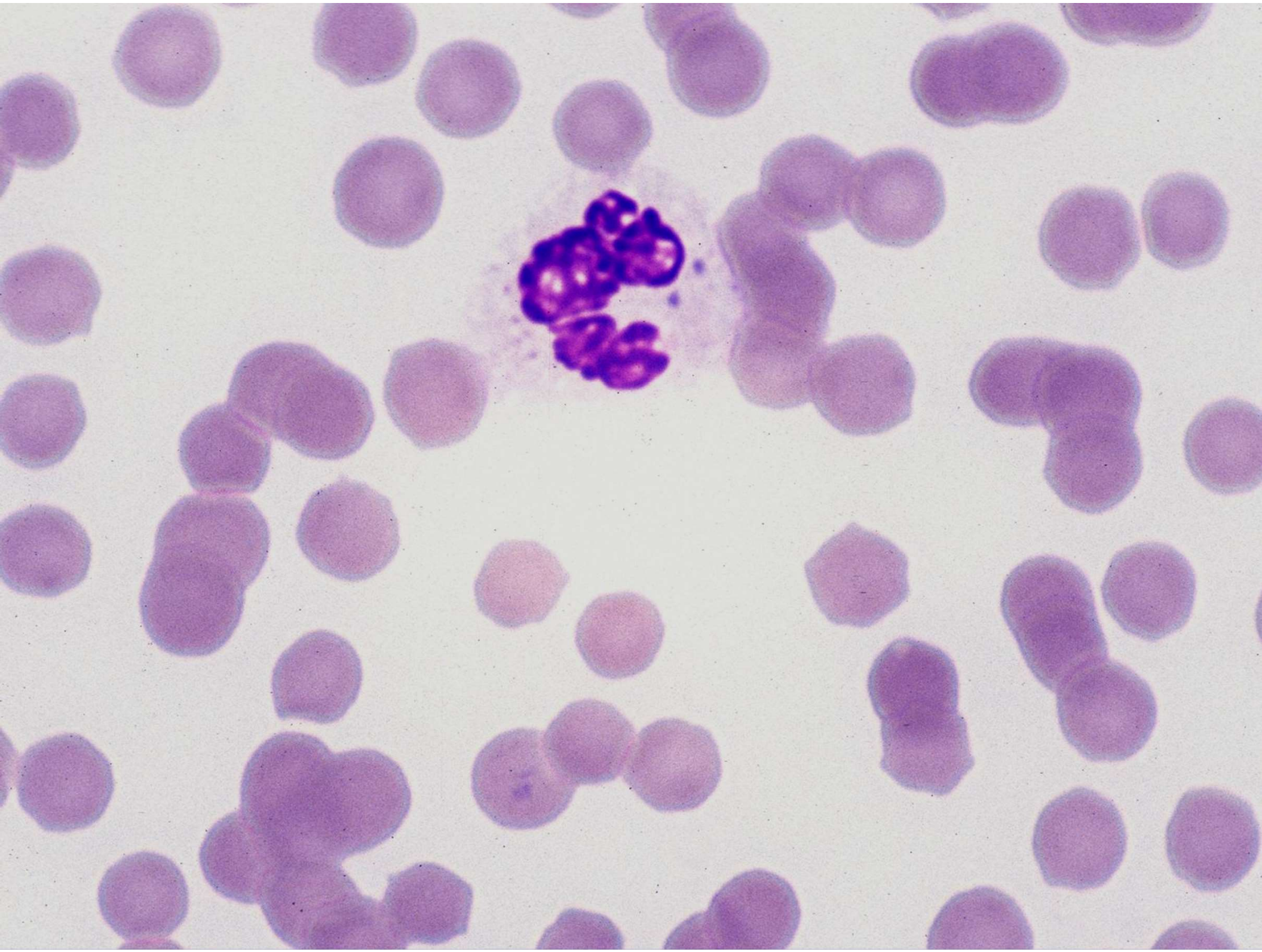
Erythrocyte Morphology: normal

Leukogram Findings

Parameter	Patient	Reference
Total WBC ($x10^3/\mu L$)	2.6	5.5 - 19.5
Bands ($x10^3/\mu L$)	0.1*	0 - 0.3
Neutrophils ($x10^3/\mu L$)	1.6*	2.5 - 12.5
Lymphocytes ($x10^3/\mu L$)	0.7	1.5 - 7.0
Monocytes ($x10^3/\mu L$)	0.2	0 - 0.8
Eosinophils ($x10^3/\mu L$)	<0.1	0 - 1.5
Basophils ($x10^3/\mu L$)	0	<0.1
NRBCs ($x10^3/\mu L$)	0	0

*Toxic cytoplasm

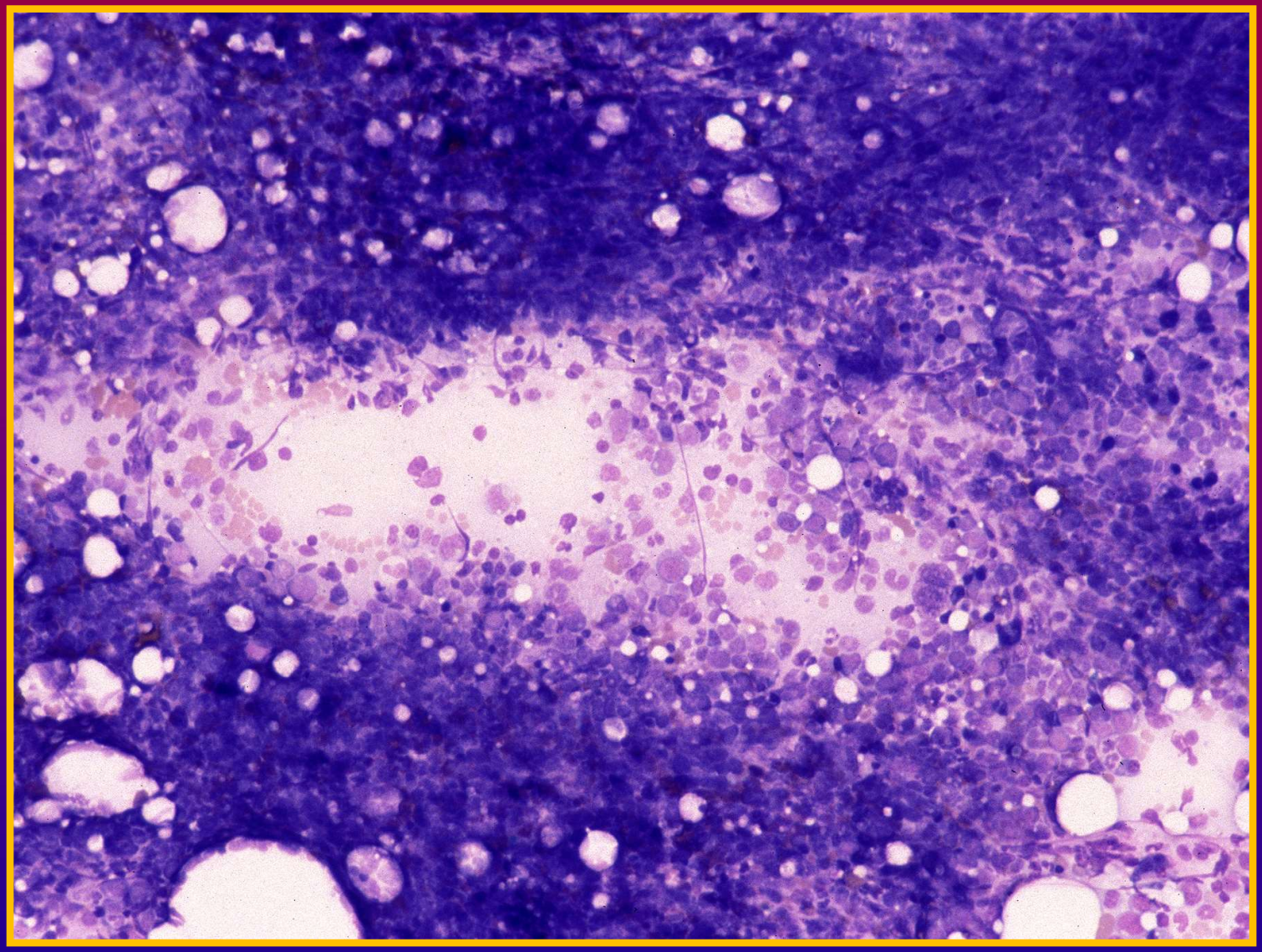




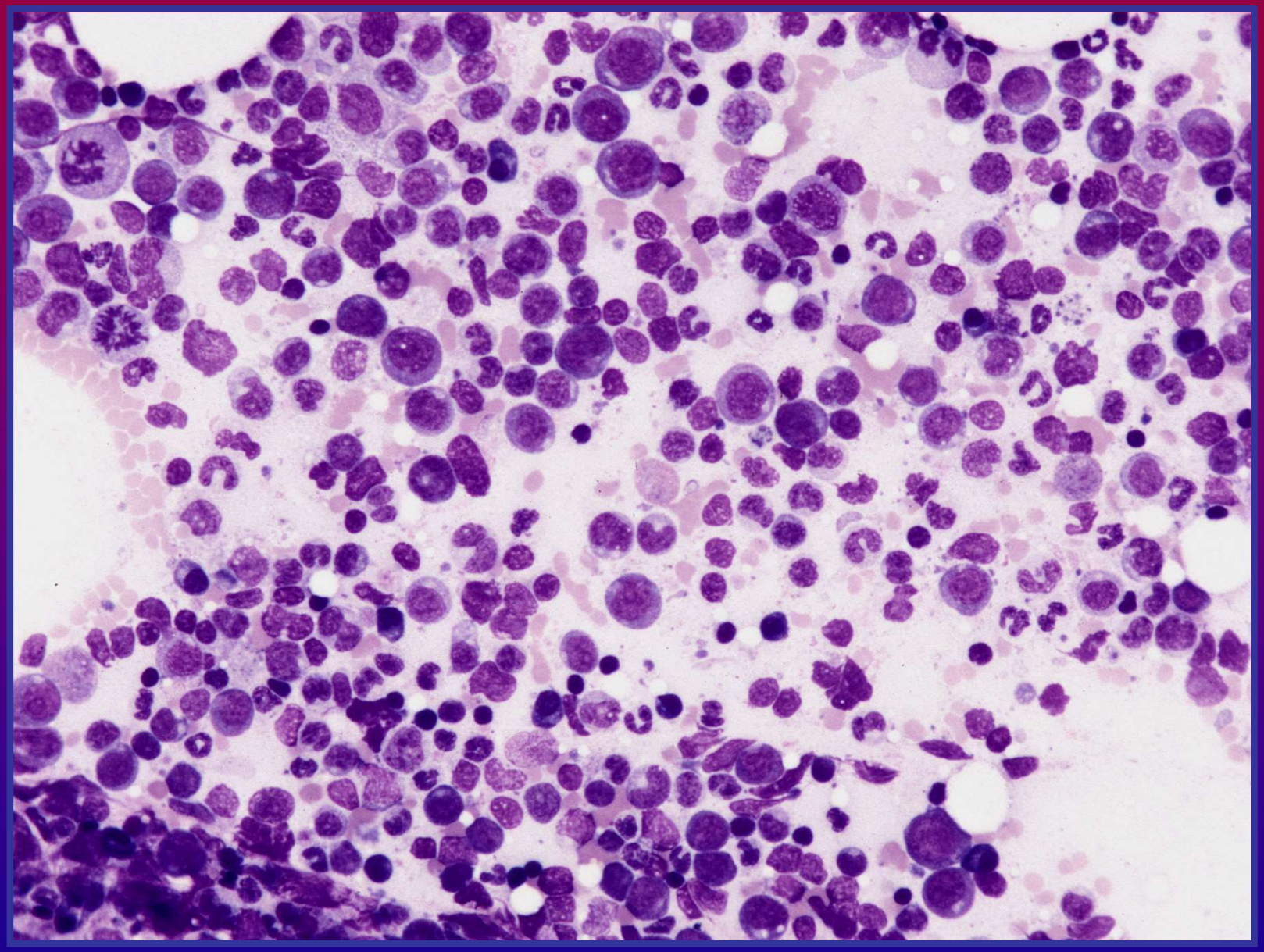
Additional Findings

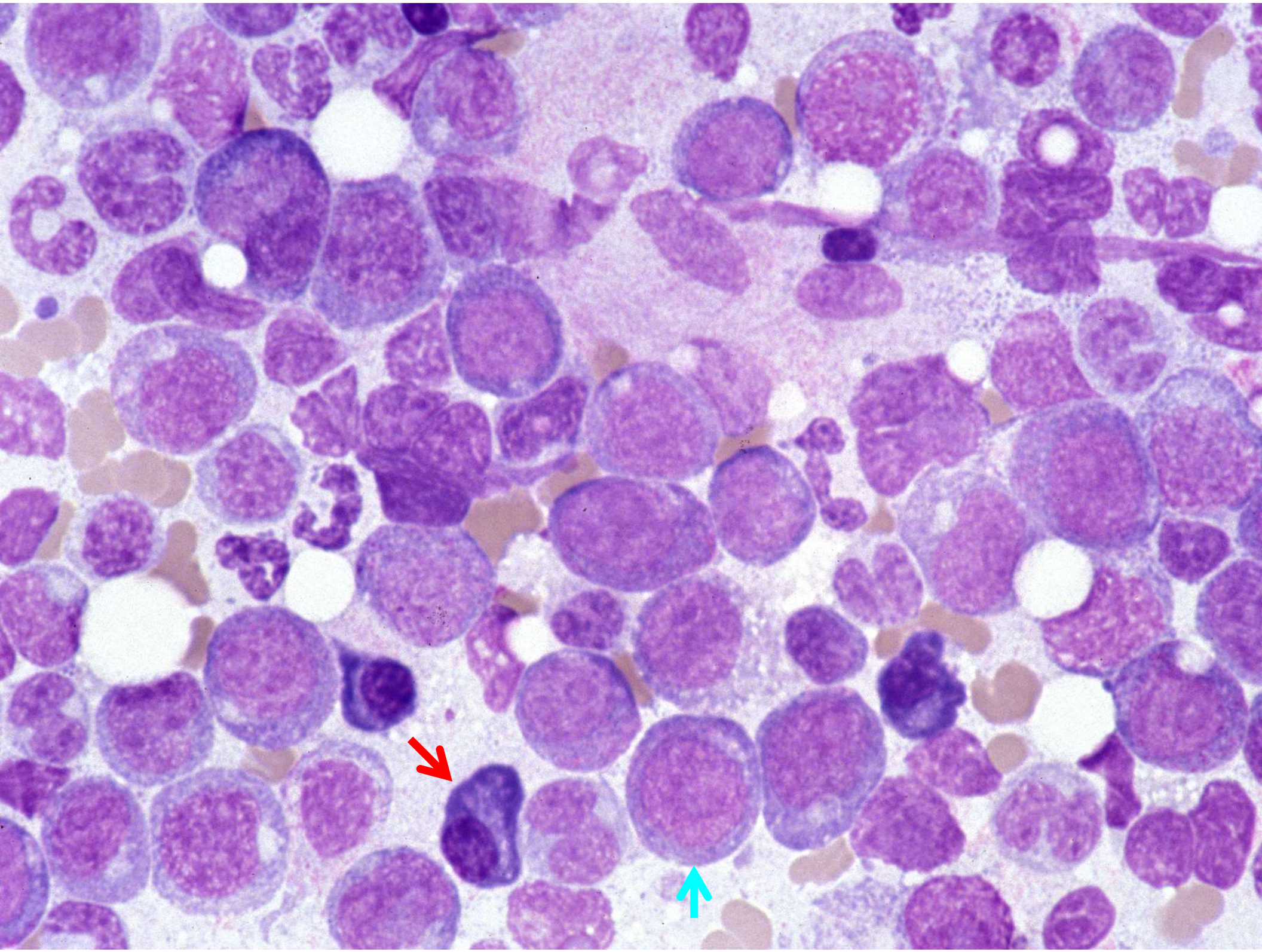
- Radiograph and sonogram: normal abdomen
- Clinical chemistry: bilirubin 4.1 mg/dL, albumin 1.9 g/dL
- PT normal, APTT slightly prolonged
- FeLV test: negative
- FIV test: positive

Marrow Aspirate



Marrow Aspirate





Marrow Cytology

- Hypercellular aspirate
- Some megakaryocytes are dwarf megakaryocytes
- Erythroid aplasia with no polychromasia
- Myeloid hyperplasia, left shift, with increased myeloblasts (11%)
- Moderately increased plasma cells
- Interpretation: Myelodysplastic Syndrome –Refractory Anemia with Excess Blasts (MDS-RAEB)

History and Clinical Findings

- 12-year-old male castrated cat
- Dilated left pupil for one month
- Unilateral lens luxation and glaucoma
- Thin body condition, gingivitis, afebrile
- Preoperative evaluation for ocular surgery

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Hematology Findings

Parameter	Patient	Reference
HCT (%)	25	30 - 47
MCV (fl)	58	41 - 51
MCHC (%)	35	31 - 35
Icterus Index (units)	<5	<5
Plasma Protein (g/dl)	7.8	6.2 - 8.0
Fibrinogen (mg/dl)	200	100 - 300
Platelets ($x10^3/\mu l$)	122	300 - 800
RBC Morphology	sl aniso	normal

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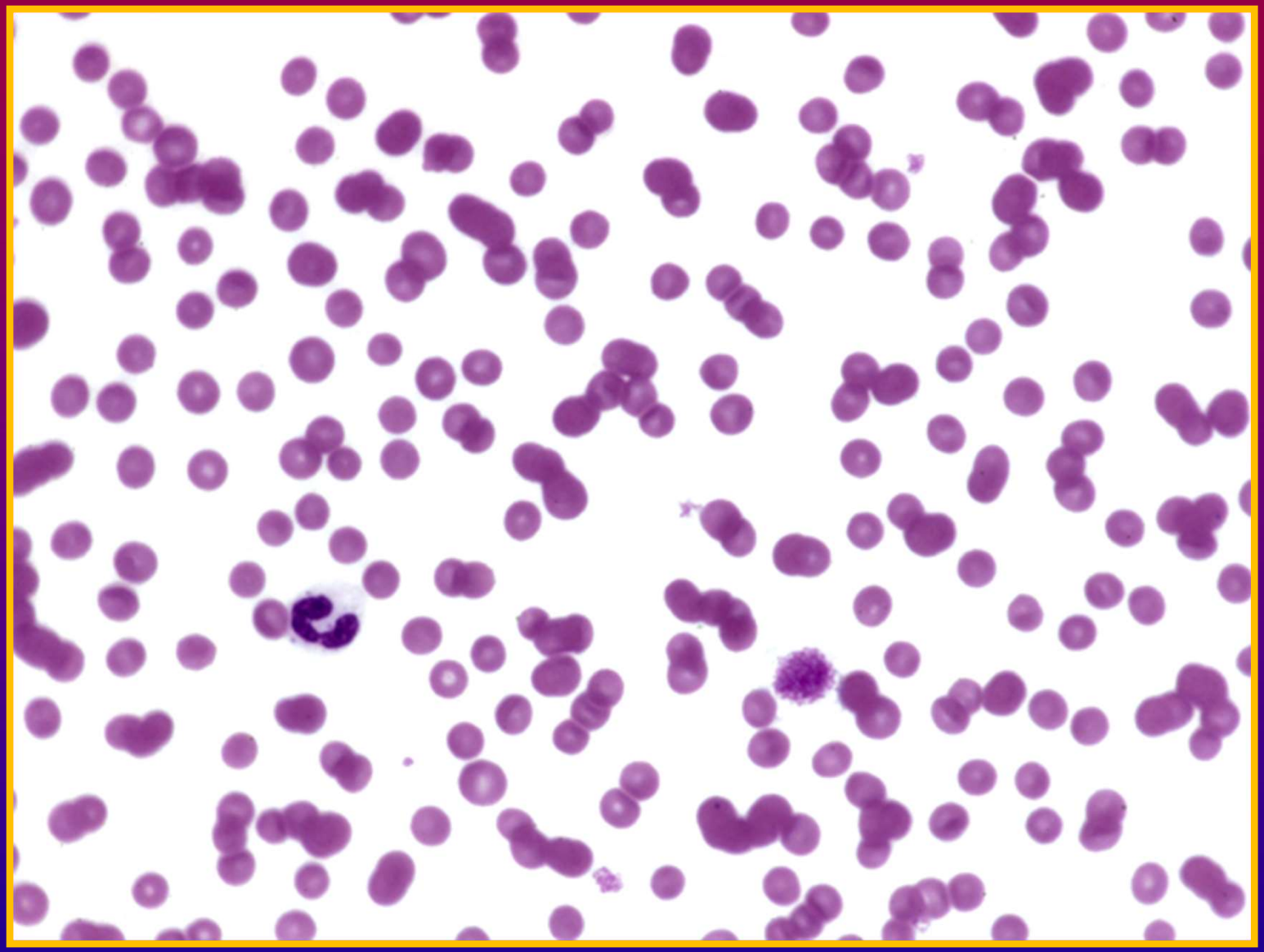
Cat Blood (Day 1)



Leukogram Findings

Parameter	Patient	Reference
Total WBC ($x10^3/\mu l$)	2.5	5.5 - 19.5
Bands ($x10^3/\mu l$)	0.1	0 - 0.3
Neutrophils ($x10^3/\mu l$)	1.7	2.5 - 12.5
Lymphocytes ($x10^3/\mu l$)	0.6	1.5 - 7.0
Monocytes ($x10^3/\mu l$)	0.1	0 - 0.8
Eosinophils ($x10^3/\mu l$)	0	0 - 1.5
Basophils ($x10^3/\mu l$)	0	<0.1

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Additional Findings

- FeLV test positive
- FIV test negative
- Clinical chemistry profile was within normal limits

Bone Marrow Aspirate

- Normal overall cellularity
- Frequent dwarf megakaryocytes
- Maturation abnormality in the neutrophilic line with increase numbers of myeloblasts (less than 20%) and promyelocytes
- Erythroid series was orderly but exhibited little polychromasia
- M:E ratio 2.0
- Interpretation: MDS-RAEB

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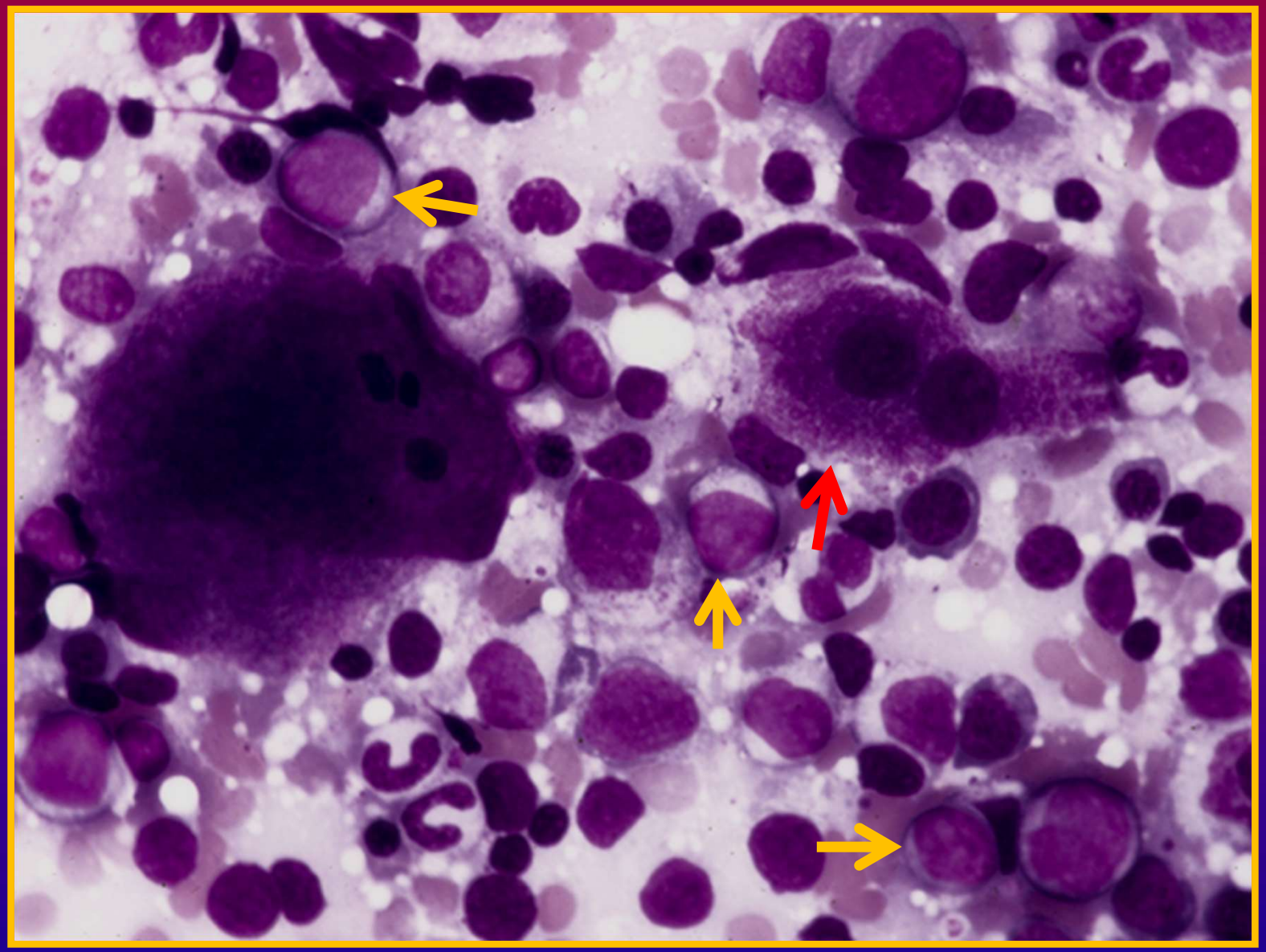
Marrow Aspirate (Day 1)



Hematology Findings

Parameter	Day 1	Month 2	Month 4	Reference
HCT (%)	25	26	13	30 - 47
MCV (fl)	58	62	62	41 - 51
MCHC (%)	35	34	35	31 - 35
PP (g/dl)	7.8	6.9	6.5	6.2 - 8.0
Platelets ($x10^3/\mu l$)	122	118	70	300 - 800
RBC Morphology	sl aniso	sl aniso	sl aniso	normal
Corr Retics (%)	ND	ND	0.1A/1.5 P	<0.4 A <10 P

ND = not done

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Leukogram Findings

Parameter	Day1	Month 2	Month 4	Reference
Total WBC ($x10^3/\mu l$)	2.5	1.8	4.9	5.5 - 19.5
Blasts ($x10^3/\mu l$)	0	0	1.7	0
Metas ($x10^3/\mu l$)	0	0	0.1	0
Bands ($x10^3/\mu l$)	0.1	0	0.3	0 - 0.3
Neutrophils ($x10^3/\mu l$)	1.7	1.3	0.9	2.5 - 12.5
Lymphocytes ($x10^3/\mu l$)	0.6	0.5	0.6	1.5 - 7.0
Monocytes ($x10^3/\mu l$)	0.1	0	0.1	0 - 0.8
Lysed cells ($x10^3/\mu l$)	0	0	1.2	0 - 1.5
NRBC ($x10^3/\mu l$)	0	0	0.8	<0.1

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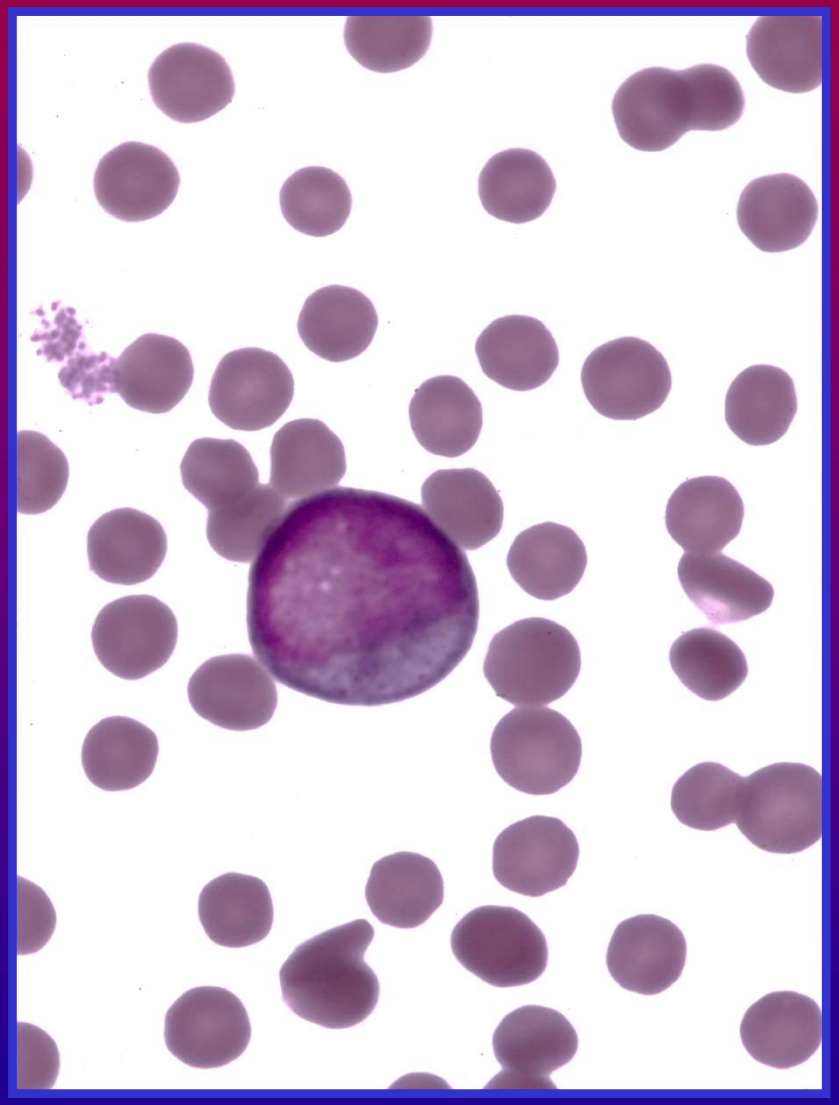
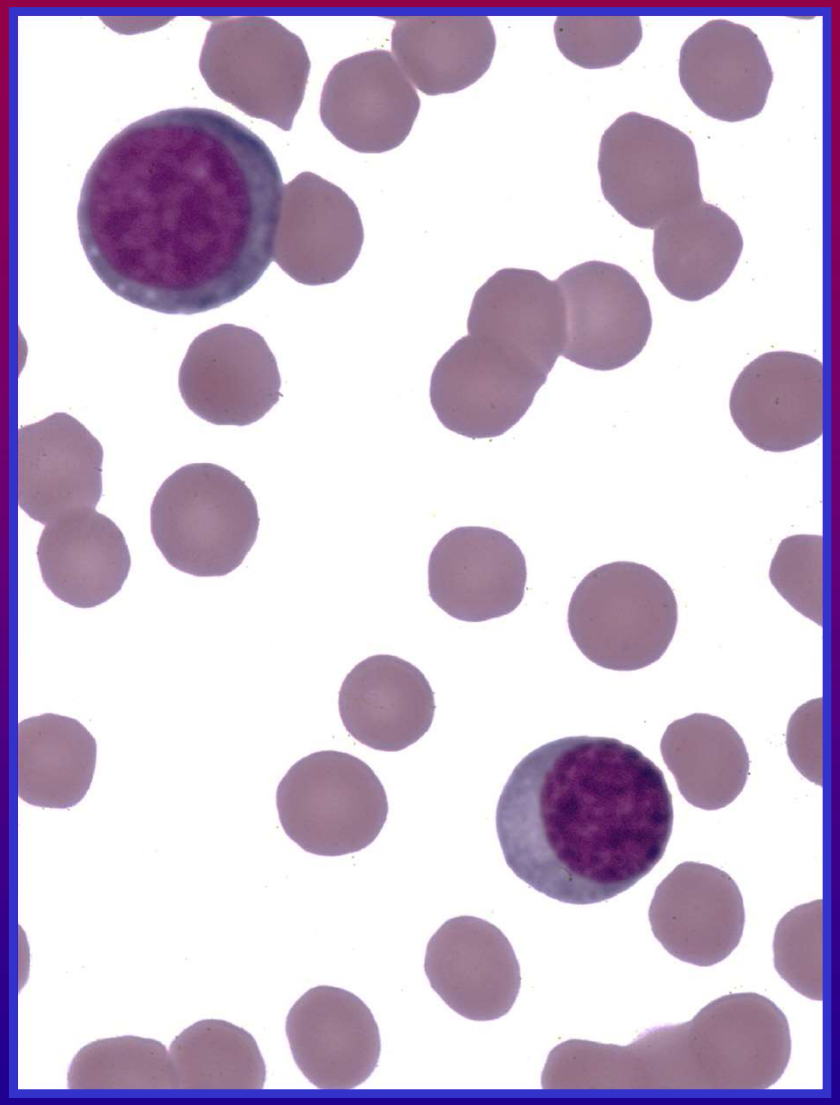
Blood Month 4





Bone Marrow Aspirate
4 months

- Hypercellular marrow
- Decreased megakaryocytes with some dwarf megakaryocytes
- Myeloblasts 60% of all nucleated cells (ANCs)
- Promyelocytes and myelocytes 20% of ANC
- Erythroid cells 20% of ANC, with more than half being rubriblasts
- M:E ratio was 4.0
- Interpretation: Acute myelogenous leukemia AML-M2

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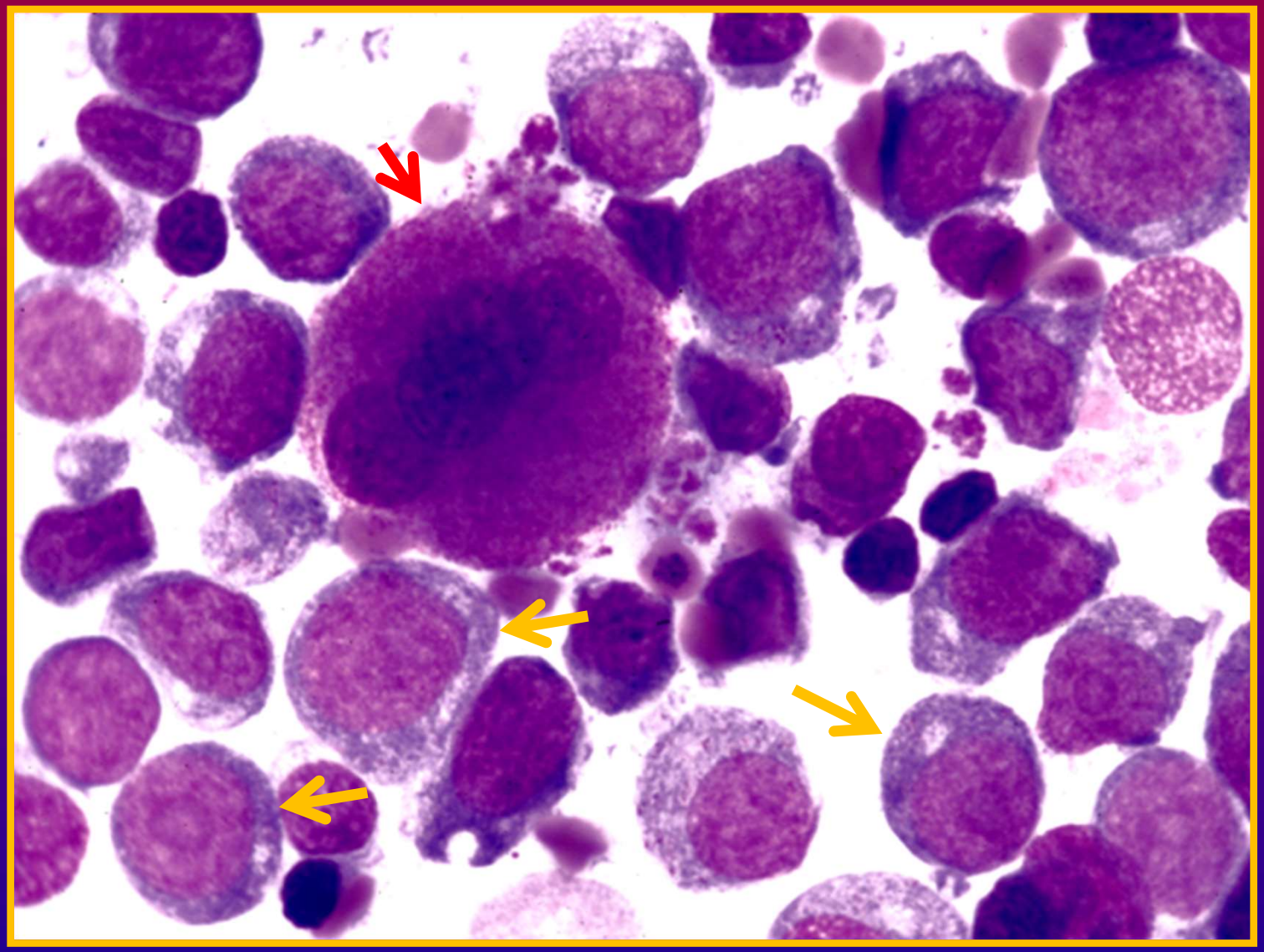
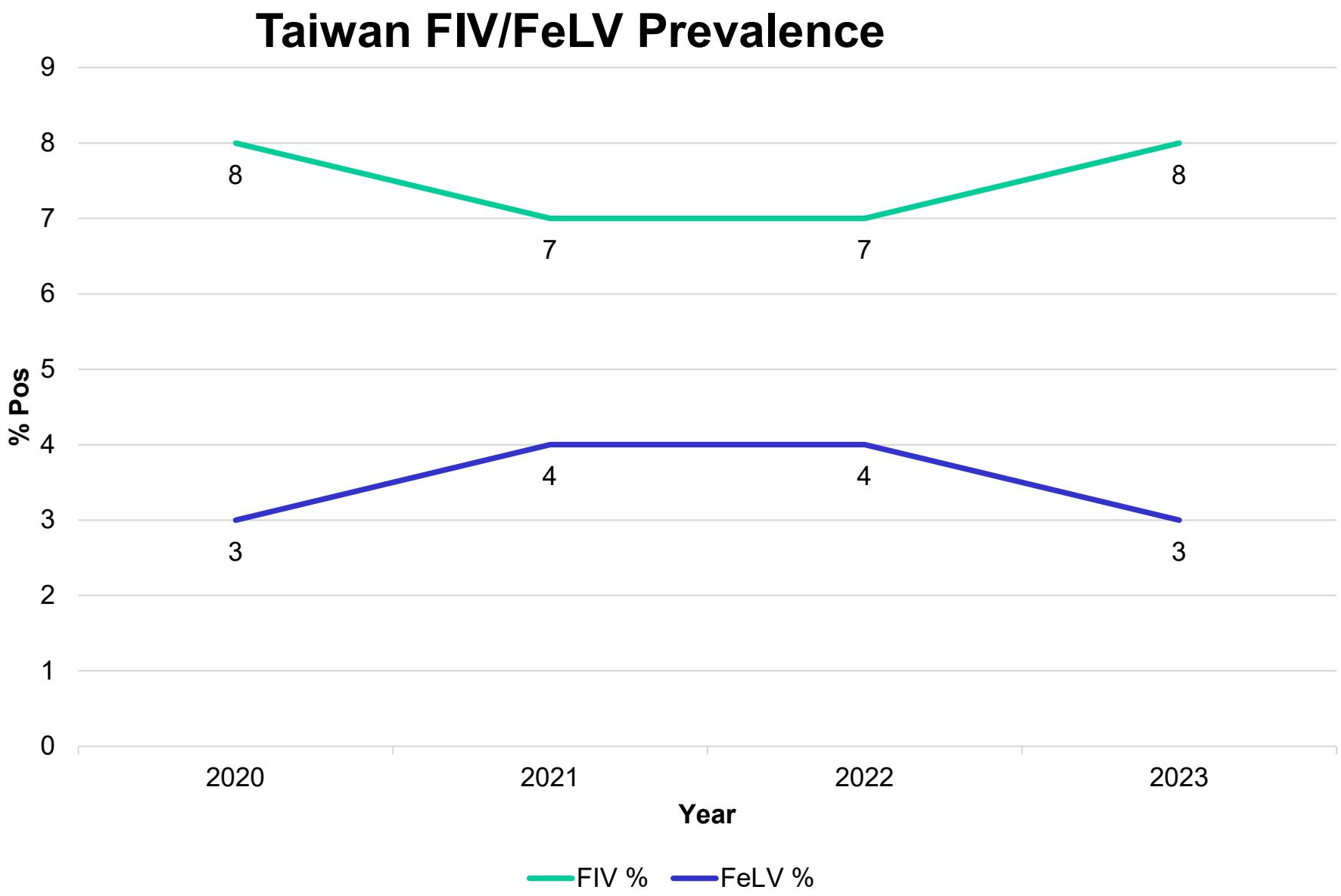
Marrow Month 4

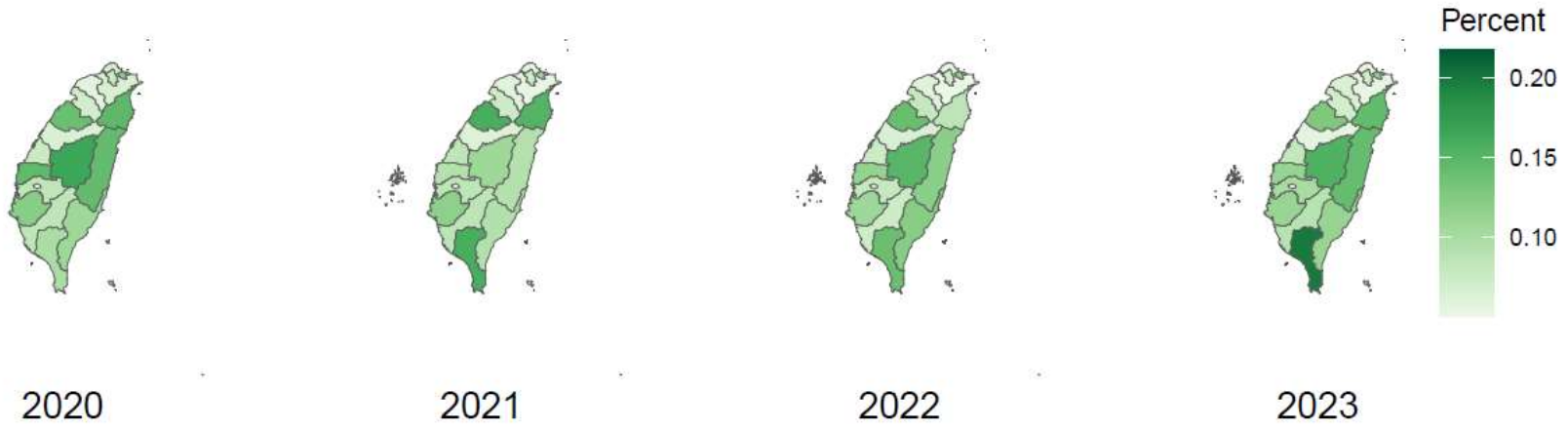


FeLV and FIV Testing

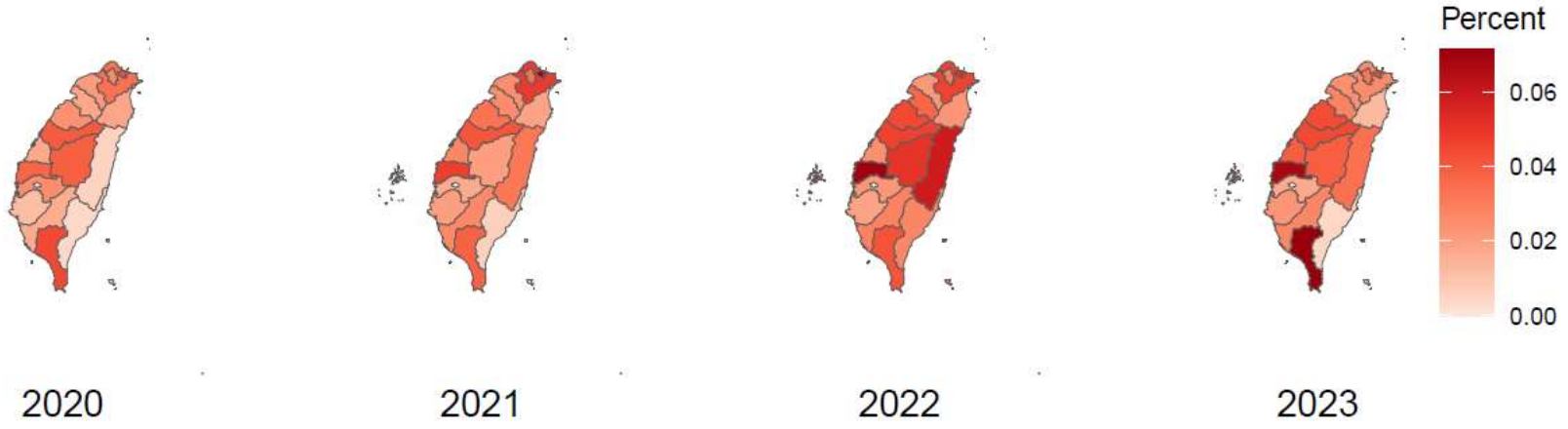
- Routine tests for chronically ill cats, especially when one or more cytopenias (nonregenerative anemia, leukopenia, thrombocytopenia) are discovered.
- Wellness examinations, especially when and cat is new to the owner.


Taiwan FIV/FeLV Prevalence
9
8
7
6
5
4
3
2
1
0
% Pos
8
7
7
8
4
4
3
3
2020
2021
2022
2023
Year
FIV %
FeLV %

FiV Positivity Rate by Region



FeLV Positivity Rate by Region



Summary

- FIV %
  - Consistently overall 8% prevalence in Taiwan (2020-2023)
  - In 2023, the prevalence on city-based data ranged from (5%-21%), with double digit prevalence in 11 cities.
- FeLV%
  - Consistently overall 3% prevalence in Taiwan (2020-2023)
  - In 2023, the prevalence on city-based data ranged from (1%-7%).

What do you say to the owner when the FeLV or FIV test is positive?

- It depends on which test is positive.
- It depends on whether the animal is ill or healthy