Breaking down the haematology - what do all these parameters really mean?

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IDEXX

Disclosure:

I am an employee of IDEXX Laboratories Ltd.

Disclaimer:

The information contained herein is intended to provide general guidance only. As with any diagnosis or treatment, you should use clinical discretion with each patient based on a complete evaluation of the patient, including history, physical presentation, and complete laboratory data. With respect to any drug therapy or monitoring program, you should refer to product inserts for a complete description of dosages, indications, interactions, and cautions. Diagnosis and treatment decisions are the ultimate responsibility of the primary care veterinarian.

Presentation overview:

+ Familiarise with the basics of in-house analysers technology to critically assess results

+ Understand the significance of erythrocyte and leukocyte parameters provided by automated analysers

+ Recognise and interpret pattern of changes

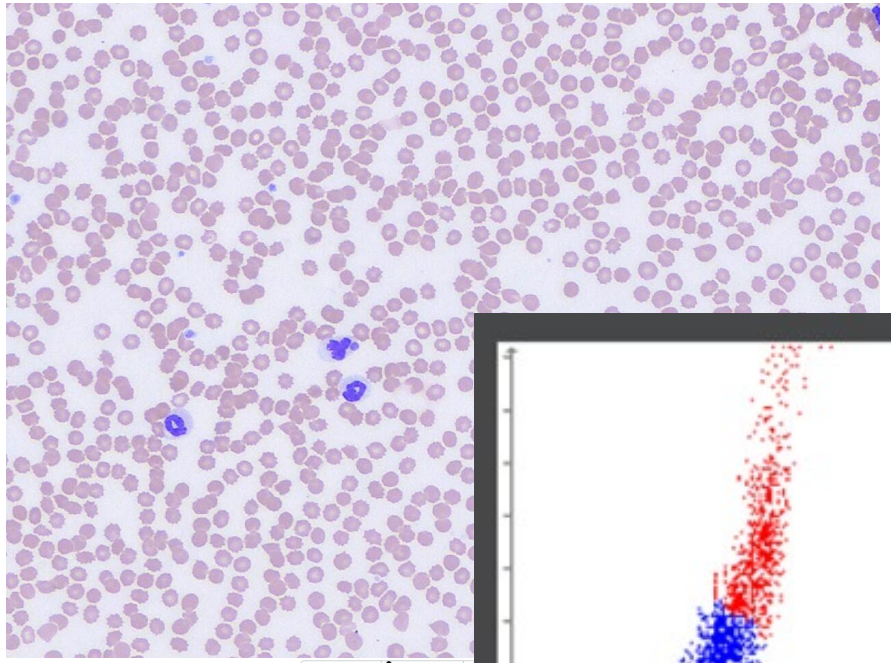
+ Integrate information provided by dot plots to interpret results and guide blood smear review

Haematology is an important diagnostic tool

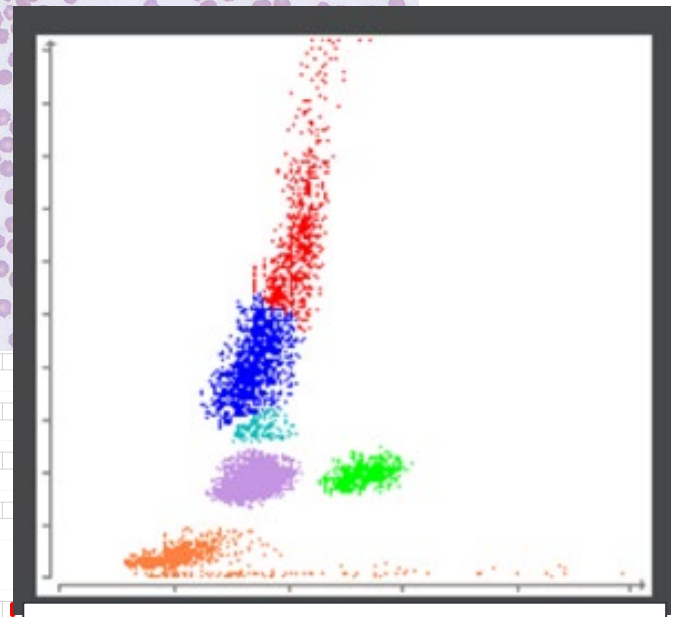
+ Part of minimal data base
+ Investigation of every sick patient

Components of haematology results

+ Automated analysis
  + Numerical values
  + Dot plot analysis
+ Blood film evaluation
+ Advanced ancillary tests



Neutrophils	*19.68	
Lymphocytes	*1.29	1.05 - 5.10 x10^9/L
Monocytes	*0.70	0.16 - 1.12 x10^9/L
Eosinophils	*0.78	0.06 - 1.23 x10^9/L
Basophils	*0.08	0.00 - 0.10 x10^9/L
Nucleated RBC	*Suspected	
Platelets	*533	148 - 484 x10^9/L
PDW	--.--	9.1 - 19.4 fL
MPV	18.7	8.7 - 13.2 fL
Plateletcrit	1.00	0.14 - 0.46 %

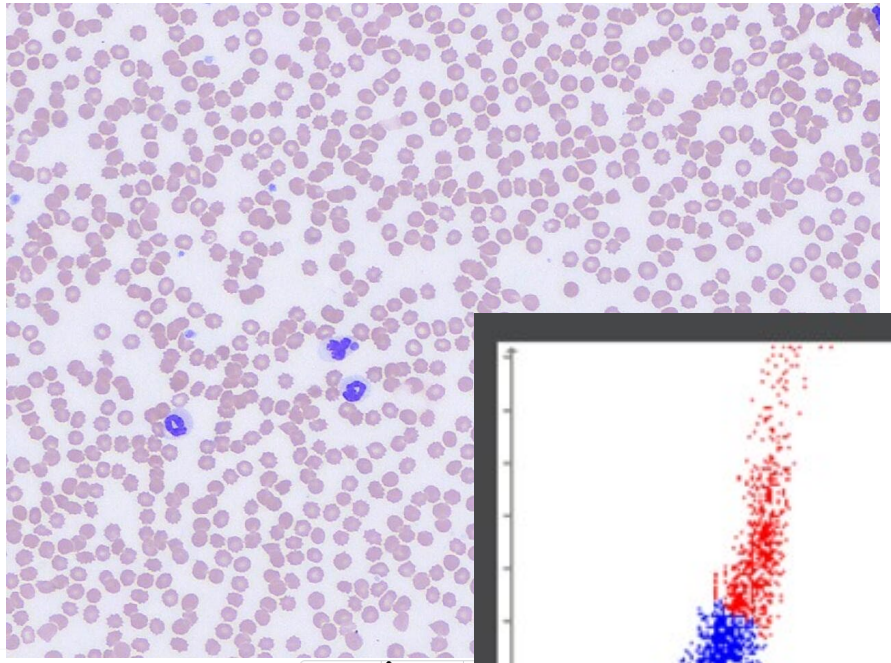


ProCyte Dx - Canine
Normal WBC Dot Plot

Haematology is an important diagnostic tool

Each strong but together provide more complete picture

+ Make use of all available data
+ Correlate with clinical picture
+ Monitor to assess trends



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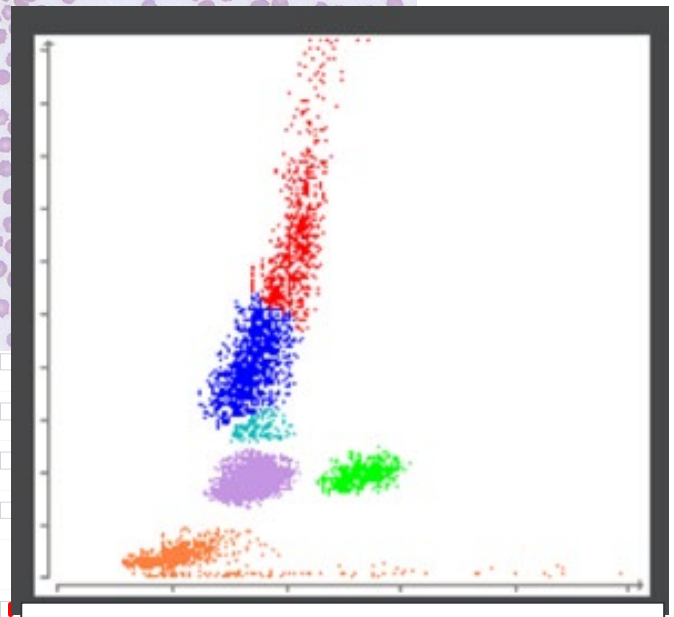


ProCyte Dx - Canine
Normal WBC Dot Plot

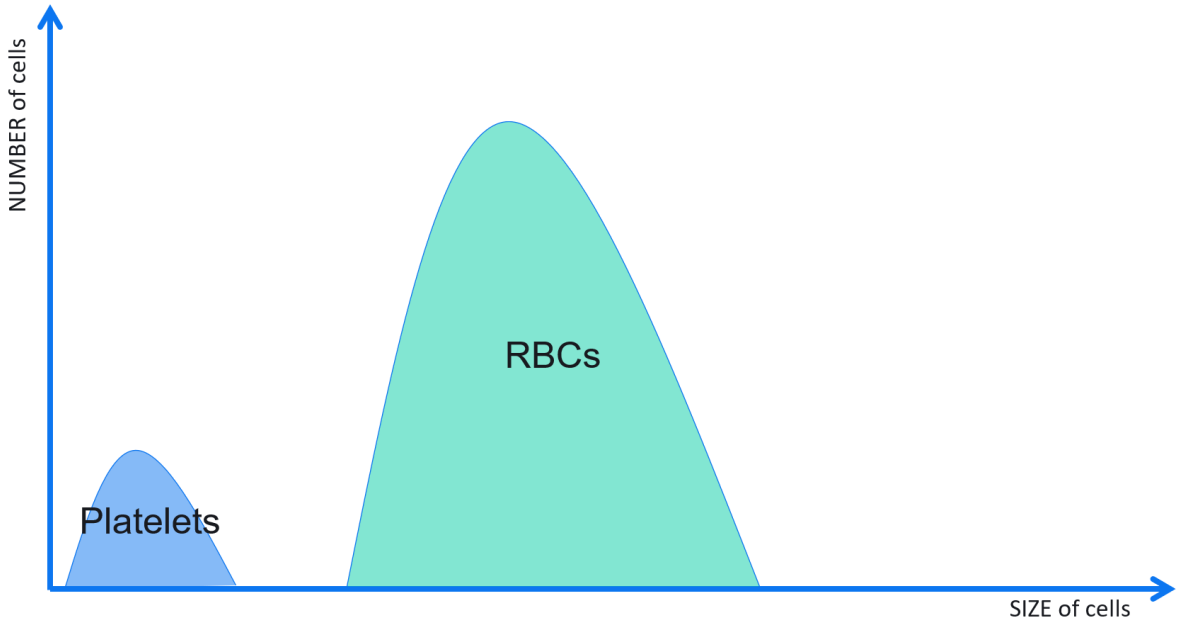
Numerical haematology analysis: evolution over time

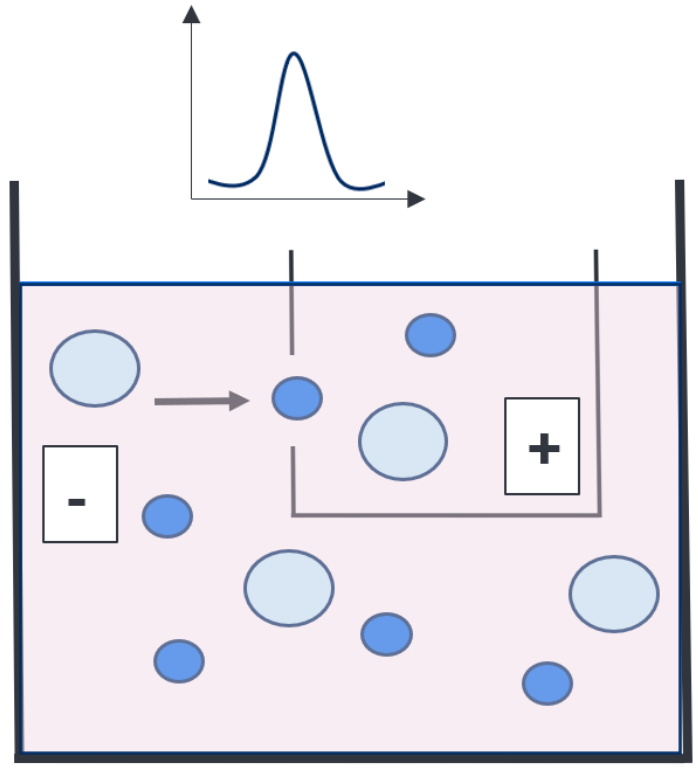
Packed cell volume

+ Manual technique
+ Red cells read as a % of column = PCV
+ Provides info on macroscopic appearance of plasma and total protein

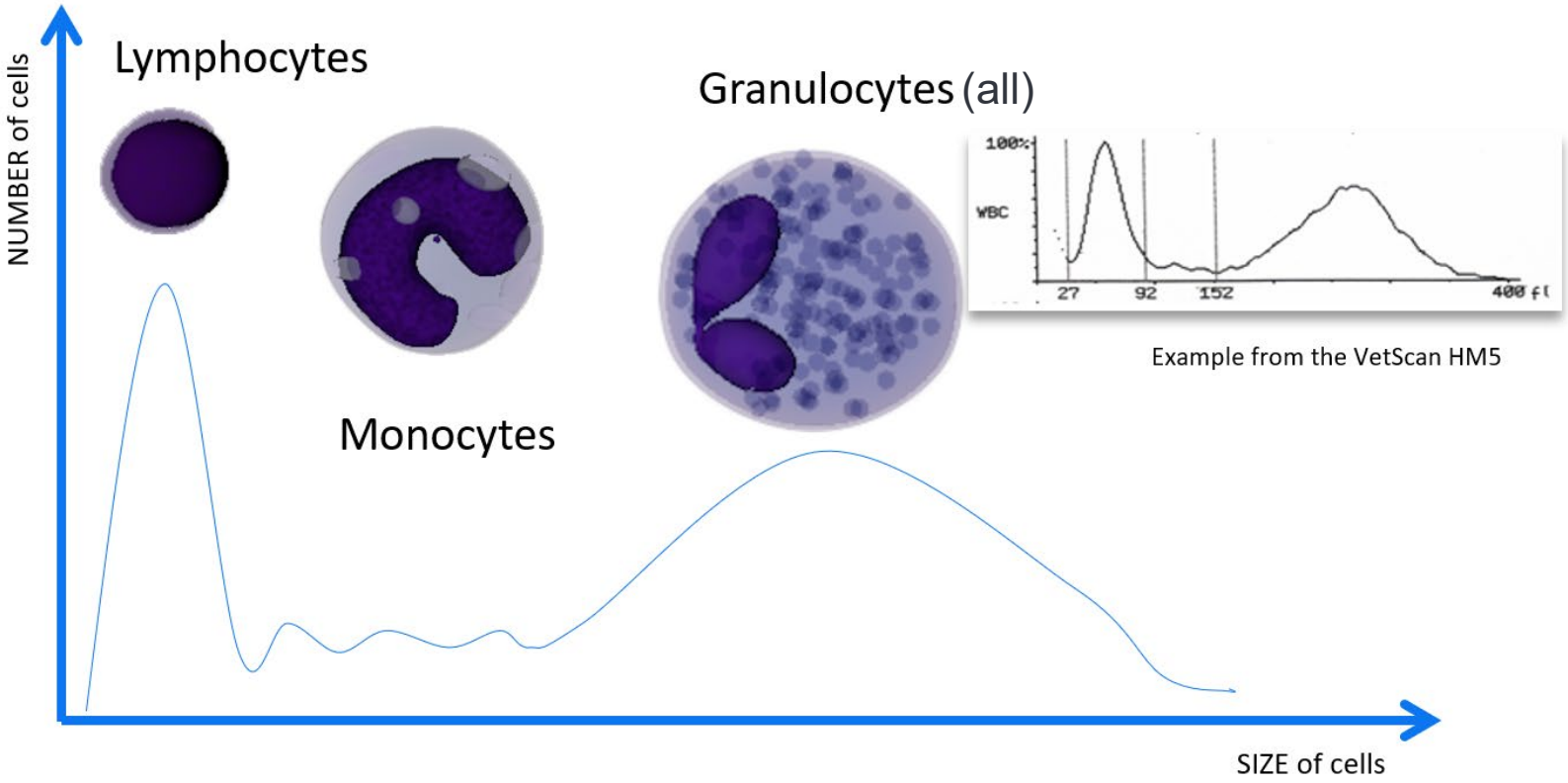
Impedance haematology analysers

+ Cells are counted and divided by size based on the measurement of changes in electrical impedance



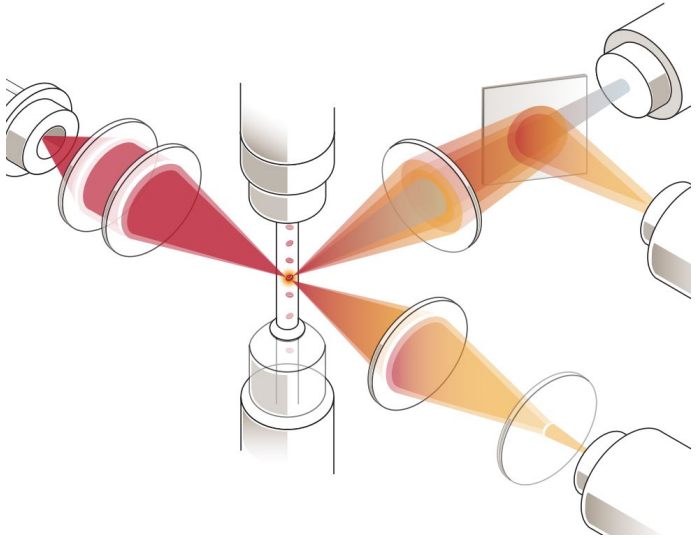


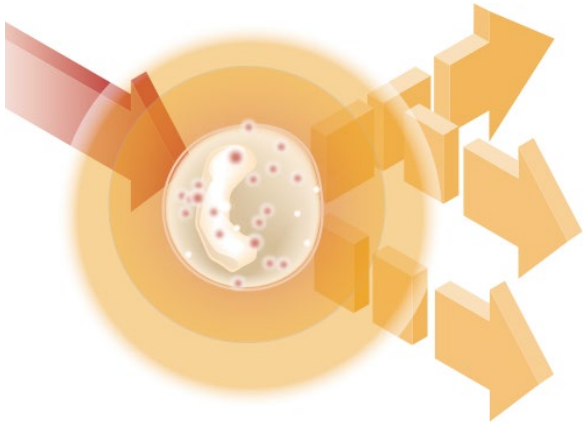
Impedance haematology analysers

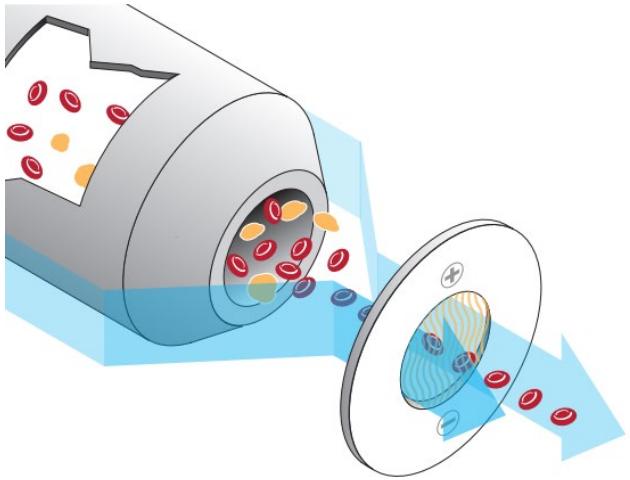


Example from the VetScan HM5

Technologies in laser haematology analysers







Laser flow Cytometry

Optical Fluorescence

Laminar flow Impedance

- **Lasercyte Dx®**
- **ProCyte One®**
- **ProCyte Dx®**

IDEXX

…so much data!

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Haematocrit	0.265	0.373 - 0.617 L/L
Haemoglobin	94	131 - 205 g/L
MCV	70.7	61.6 - 73.5 fL
MCH	25.1	21.2 - 25.9 pg
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% Reticulocyte	3.1	%
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WBC	54.21	5.05 - 16.76 x10^9/L
Neutrophils	*0.82	2.95 - 11.64 x10^9/L
Bands	*Suspected	
Lymphocytes	*10.93	1.05 - 5.10 x10^9/L
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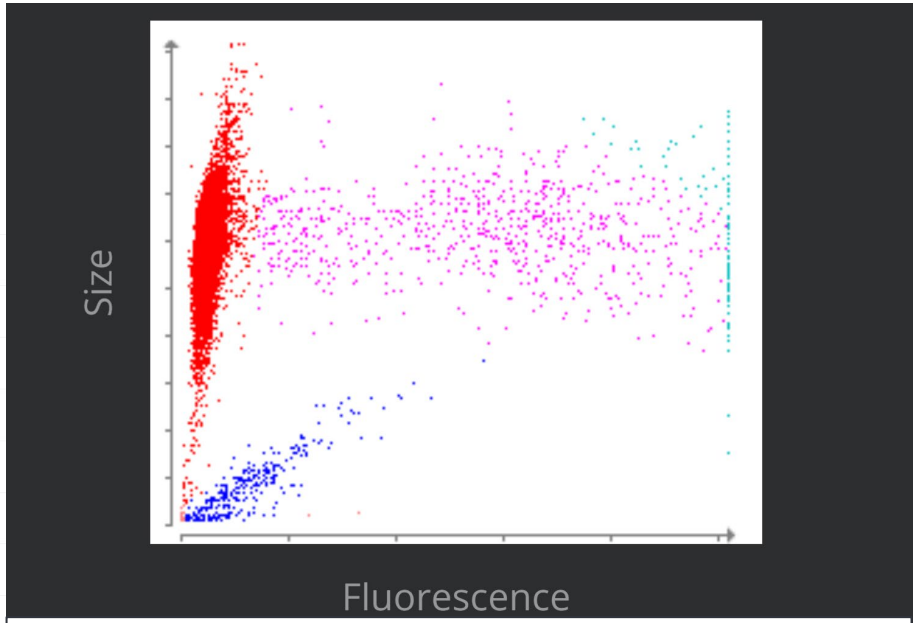
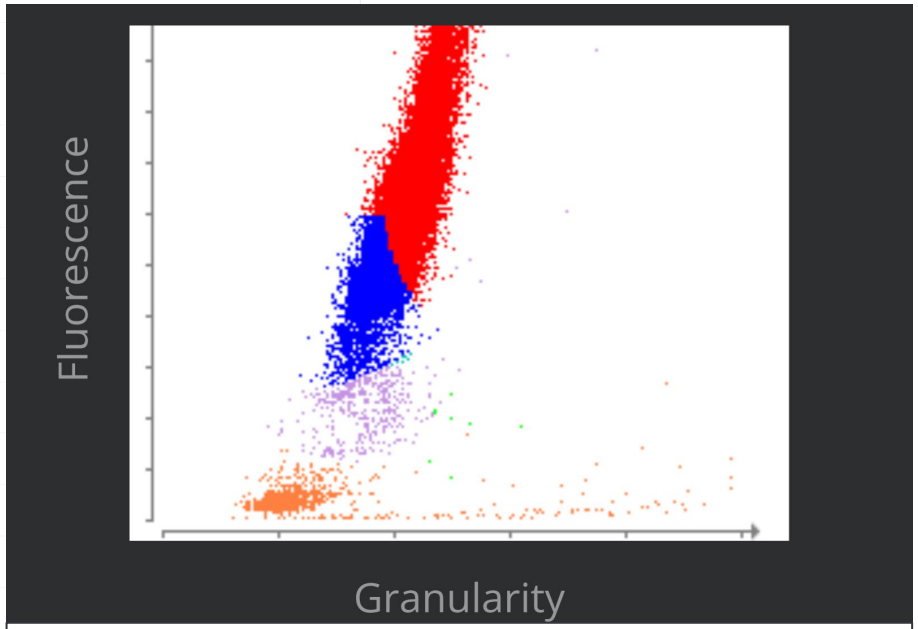
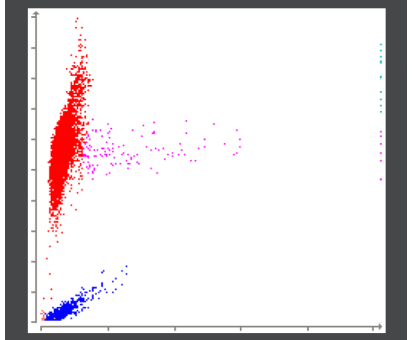


ProCyte Dx – Canine Patient RBC Dot Plot



ProCyte Dx – Canine Patient WBC Dot Plot

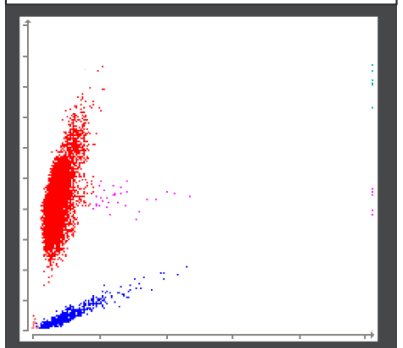
ProCyte Dx - Canine Normal RBC Dot Plot



Dot Plot Legend

RBC_FRAG RETICS
WBC RBC
PLT

ProCyte Dx - Feline Normal RBC Dot Plot



Dot Plot Legend

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RETICS

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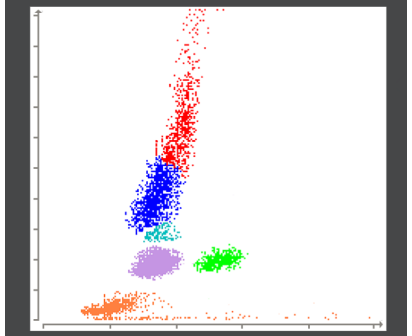
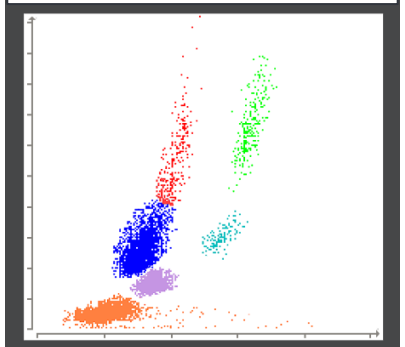


Dot Plot Legend

BASO EOS
NEU URBC
MONO LYM

ProCyte Dx - Feline Normal WBC Dot Plot



Dot Plot Legend

LYM URBC
MONO NEU
BASO EOS

Haematology…so much data!

Numerical data

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Red blood cells

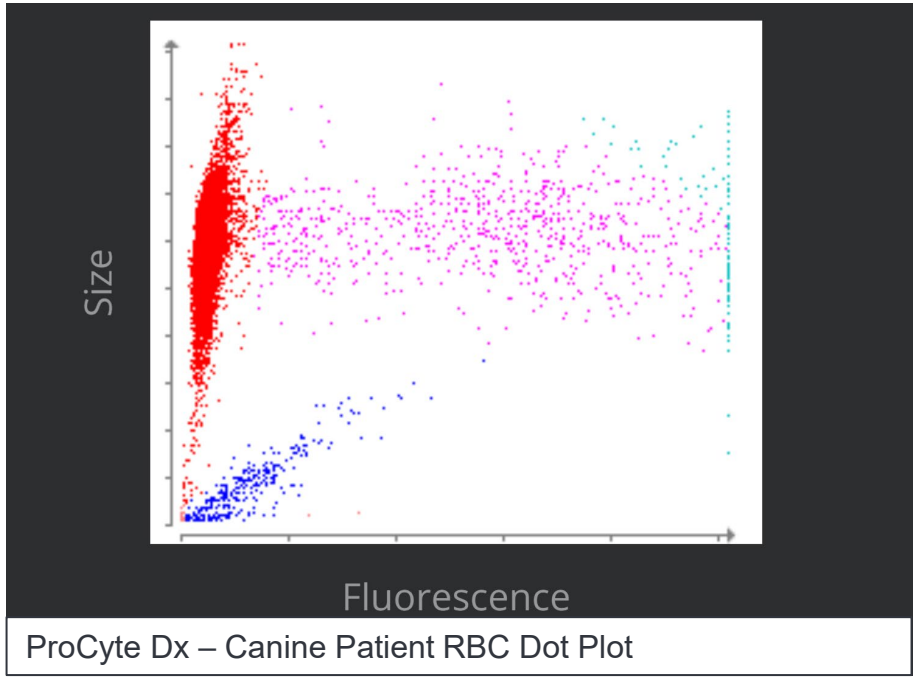
White blood cells

Platelets

Dot plots

Red blood cell run

White blood cell run



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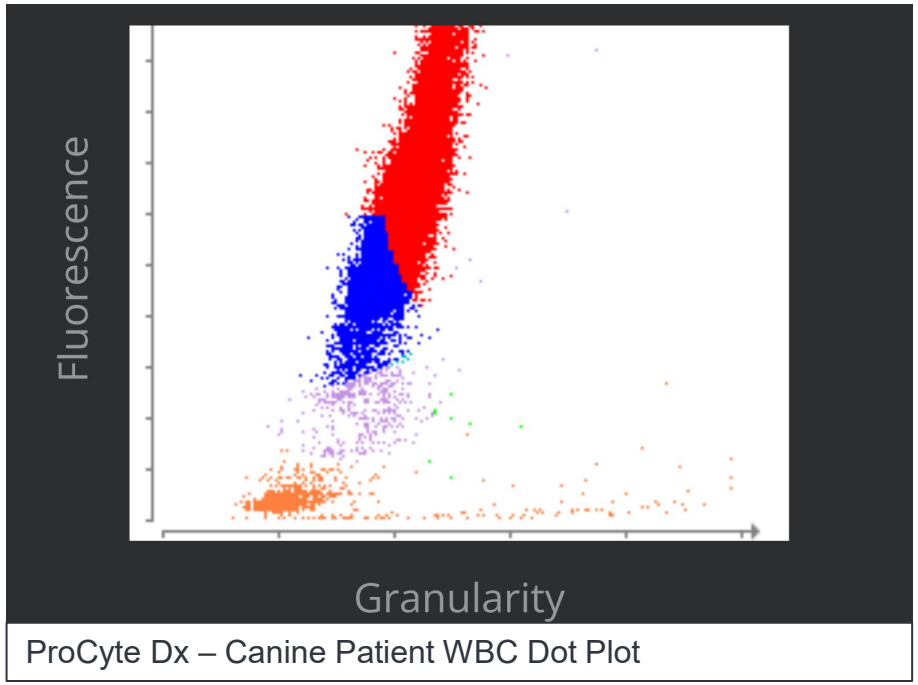

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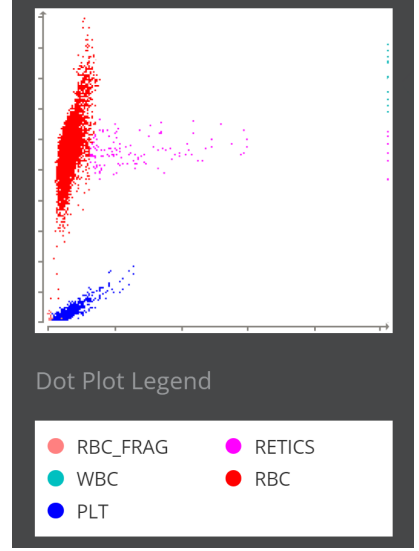

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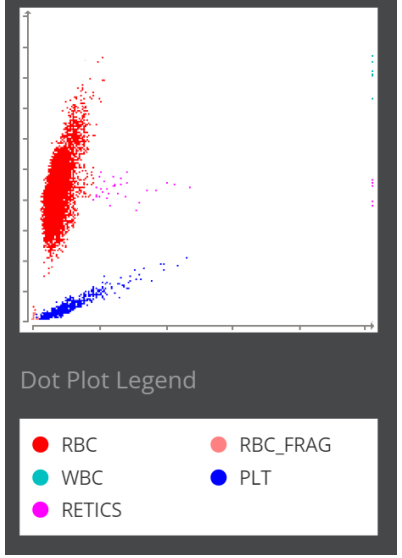

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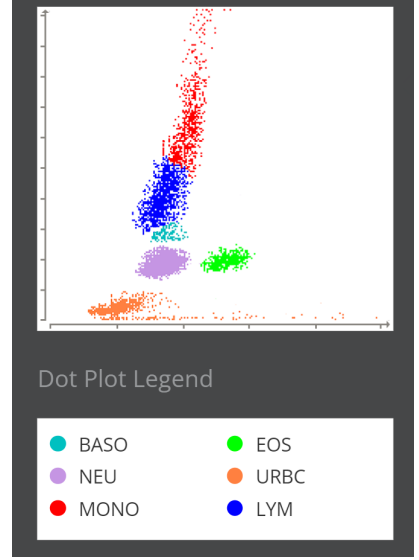

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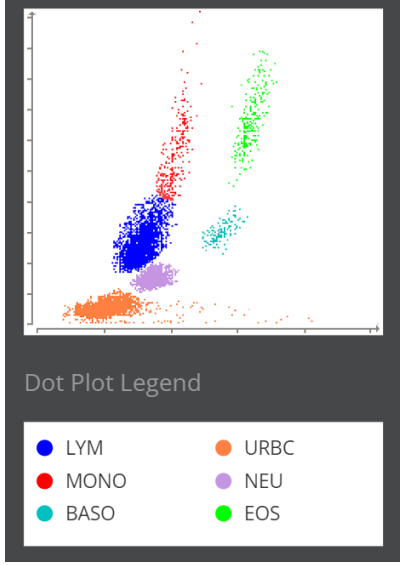

We have all these numerical data…
Why should we bother with the dot plots?

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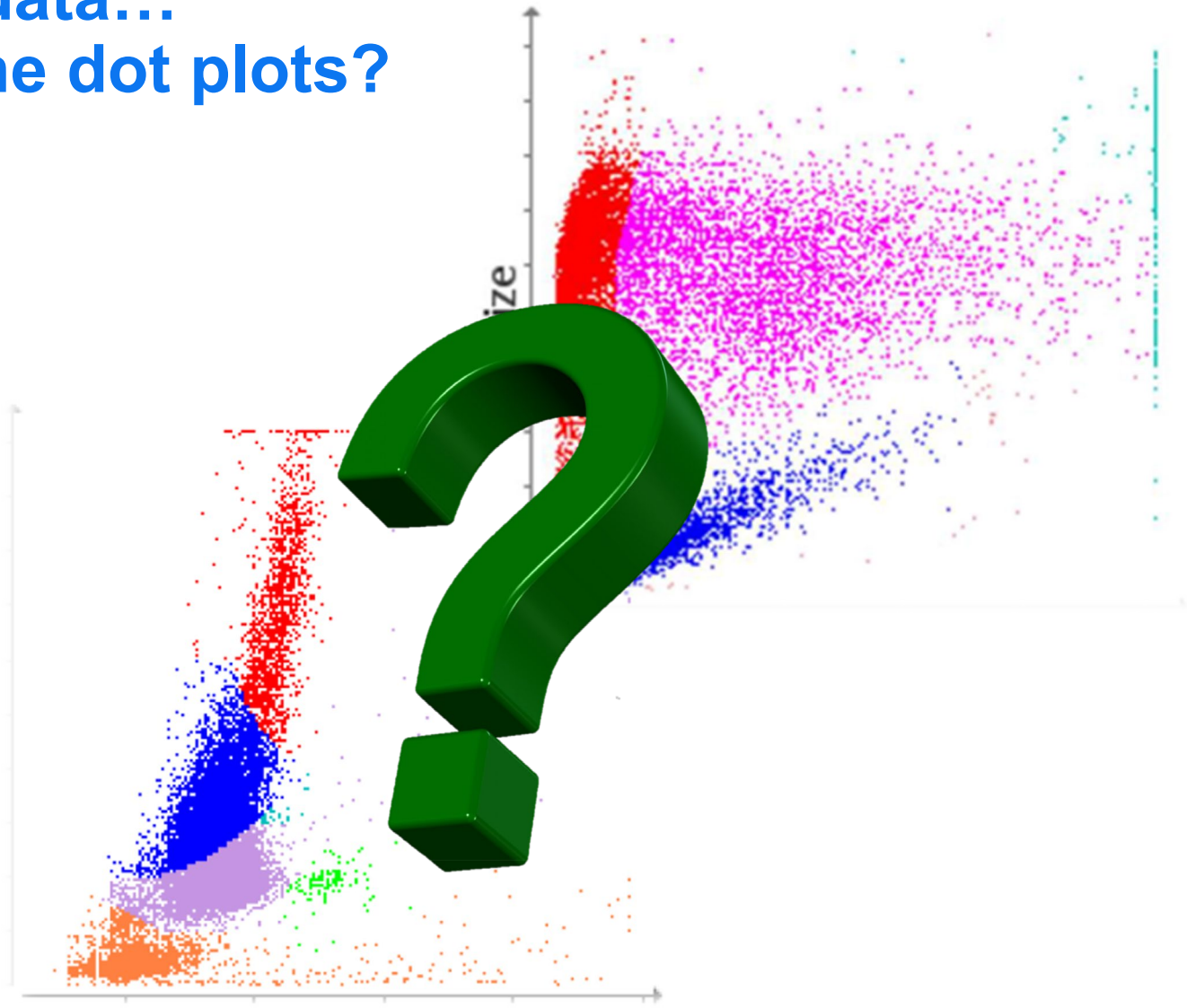
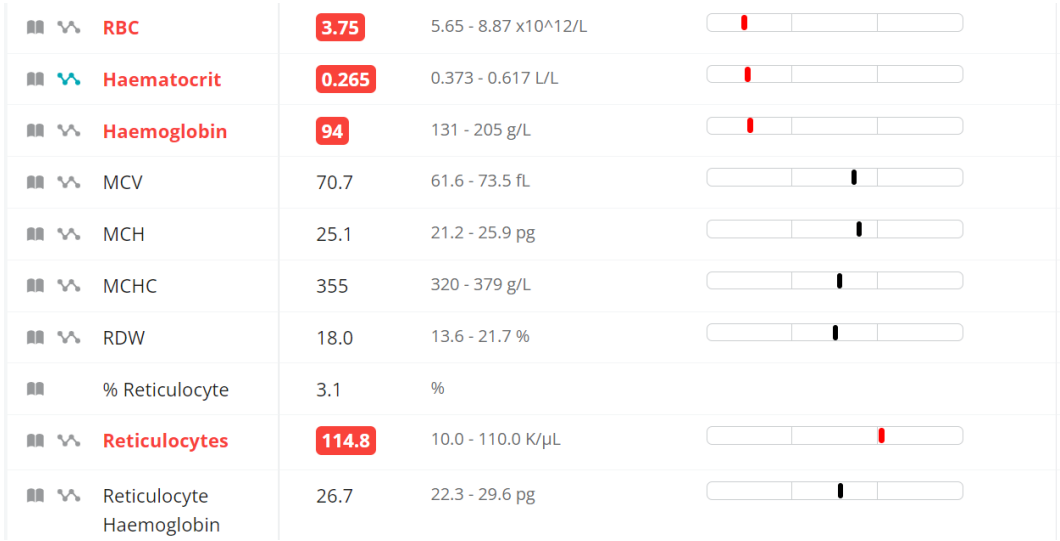


Starting with Red Blood Cells



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Anaemia, erythrocytosis

Erythrocyte indices

Reticulocytes

Classification of anaemia:

- Degree of severity (mild – moderate – severe)
- Regenerative vs non-regenerative
- By erythrocyte indices

	DOG Hct (%)	CAT Hct (%)
Mild	30 - 37	20 - 26
Moderate	20 - 29	14 - 19
Severe	< 20	<13

Modified from: Douglas J. Weiss, K. Jane Wardrop; Schalm's Veterinary Hematology, 6th ed, Wiley, 2010

Erythrocyte indices

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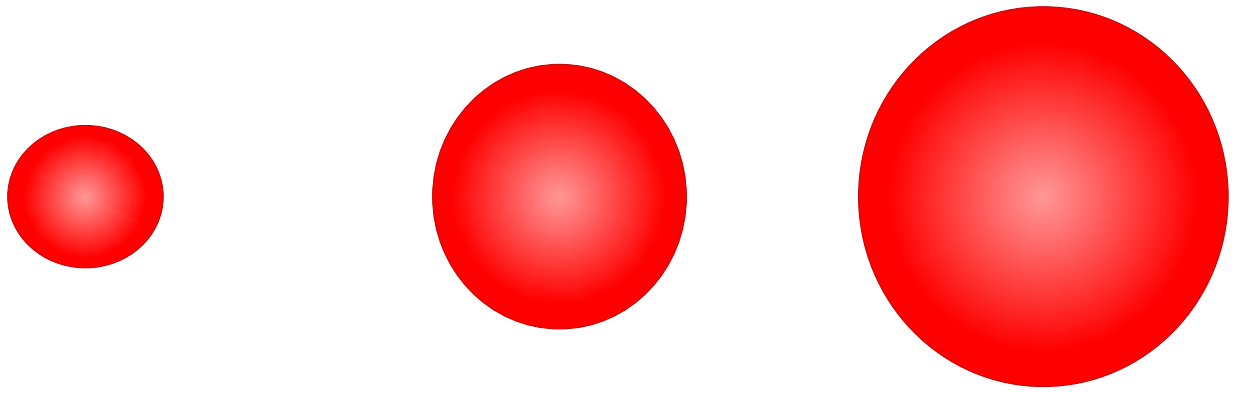
Reticulocyte Haemoglobin		

Mean corpuscular volume (MCV): average **volume** of erythrocytes

- iron deficiency
- portosystemic shunt
- age (young animals)
- breed (e.g., Akita, Shiba Inu, Chow Chow, Abyssinian)



MICROCYTIC **NORMOCYTIC** **MACROCYTIC**

- regeneration
- breed (e.g., Poodles)
- FeLV
- BM disorders
- artefacts

Erythrocyte indices

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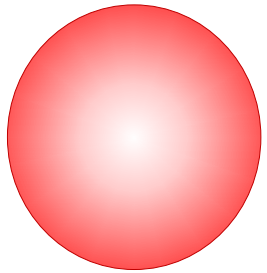
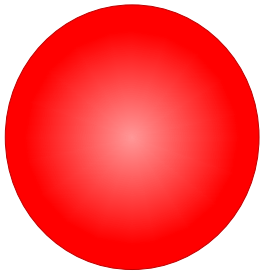
Mean corpuscular haemoglobin concentration (MCHC):

average **concentration of Hb** in the erythrocytes

- regeneration
- iron deficiency
- artefacts



HYPOCHROMIC



NORMOCHROMIC

Increased MCHC is generally artefactual

Erythrocyte indices

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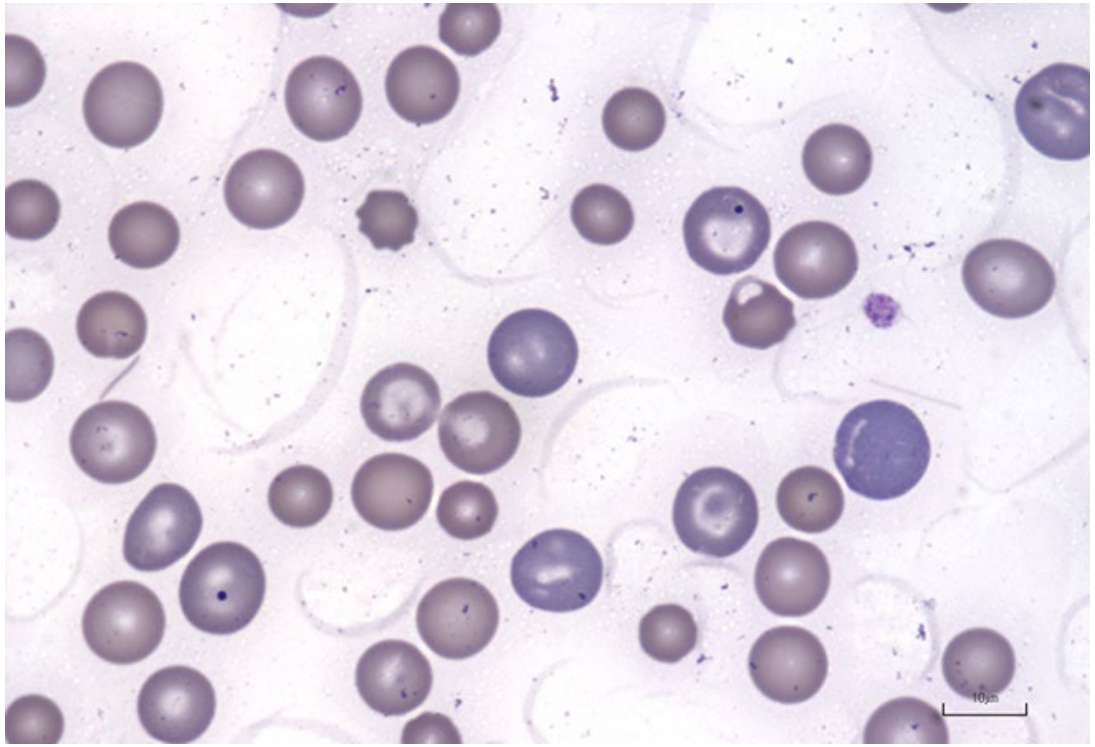


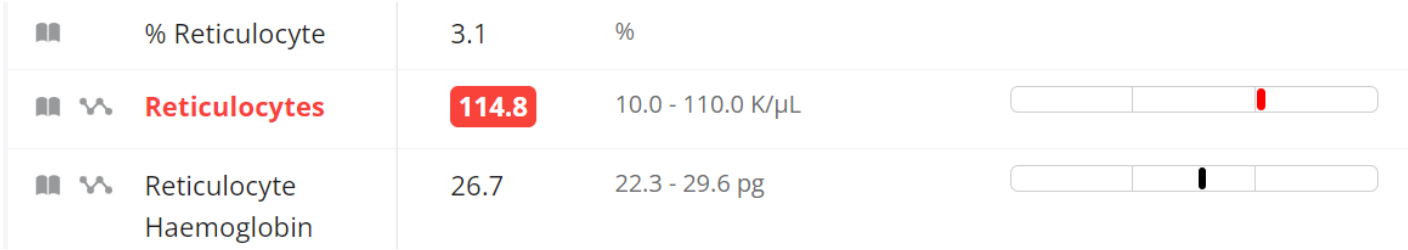
And what is RDW?

Red blood cell Distribution Width (RDW):

Reflects variation in size of RBCS (marker of **ANISOCYTOSIS**)

Increases when the cell size is more variable (i.e., increased number of larger or smaller RBCs) e.g., regeneration, iron deficiency

Reticulocyte parameters



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Absolute reticulocyte count:

+ Regenerative vs non-regenerative anaemia

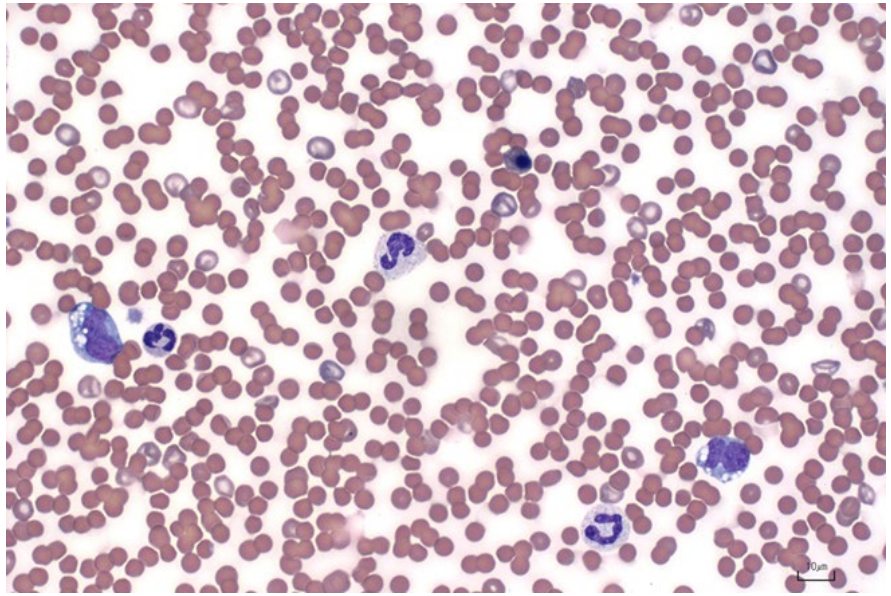
+ Degree of regeneration

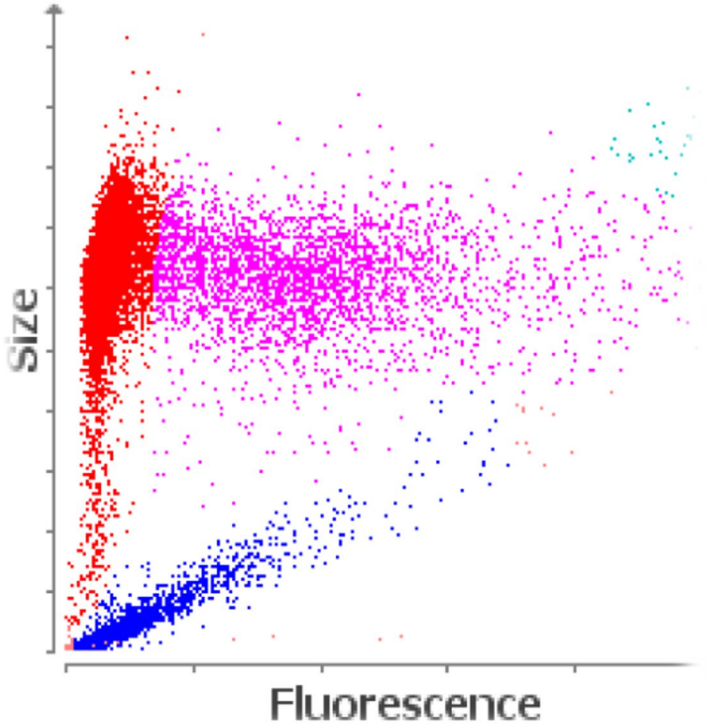
	DOG Retics (K/uL)	CAT Retics (K/uL)
Mild	110 - 200	50 - 100
Moderate	200 - 300	100 - 200
Marked	> 300	> 200

Modified from: https://eclinpath.com/hematology/tests/absolute-reticulocyte-count/

Increased reticulocytes in absence of anaemia?

+ Catecholamine-mediated splenic contraction (e.g., excitement, fear)
+ Mild compensated RBC lysis or blood loss
+ Chronic hypoxia (e.g., cardio-respiratory disease)
+ Recovery from previous anaemia
+ Anaemia obscured by dehydration





ProCyte Dx – Canine Patient RBC Dot Plot

Reticulocyte haemoglobin

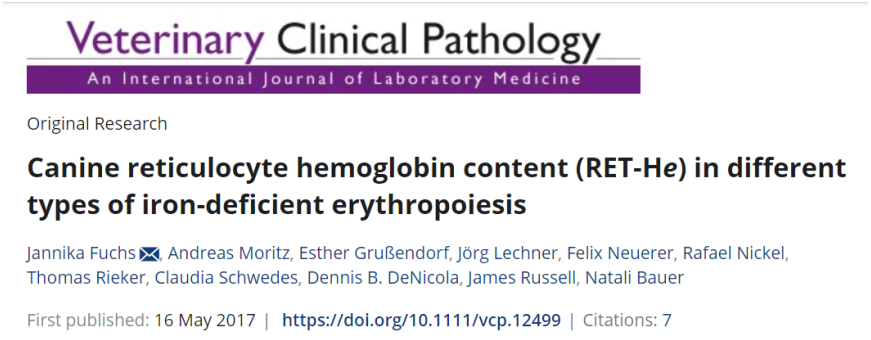
+ Marker of iron availability
+ Reflects the availability of iron for erythropoiesis
+ Reflects functional iron available for erythropoiesis over the past 2-4 days; reticulocytes circulate 1-2 days before maturation, mature RBC circulate for a long time (120d dog, 70d cat)
+ Earlier detection of decreased iron availability compared to erythrocyte indices (MCV and MCHC)

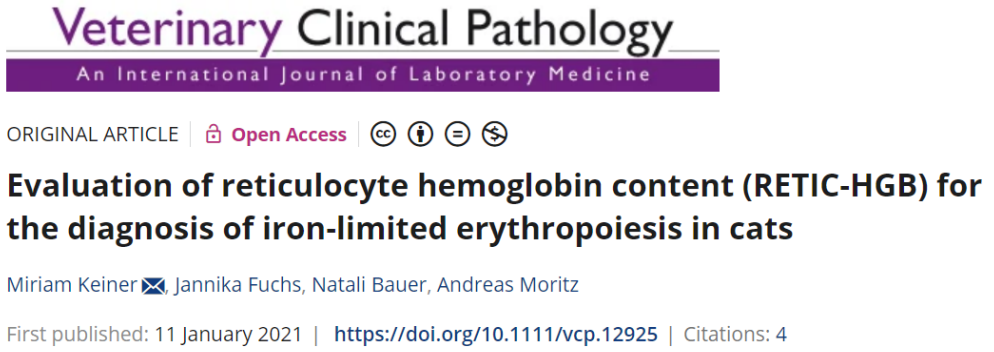
- Fuchs, J., et al. (2017). Canine reticulocyte hemoglobin content (RET-He) in different types of iron-deficient erythropoiesis. *Veterinary Clinical Pathology,* 46(3), 422-429.
- Keiner, M., Fuchs, J., Bauer, N., & Moritz, A. (2020). Evaluation of reticulocyte hemoglobin content (RETIC-HGB) for the diagnosis of iron-limited erythropoiesis in cats. *Veterinary Clinical Pathology*, *49*(4), 557-566.

Reticulocyte haemoglobin

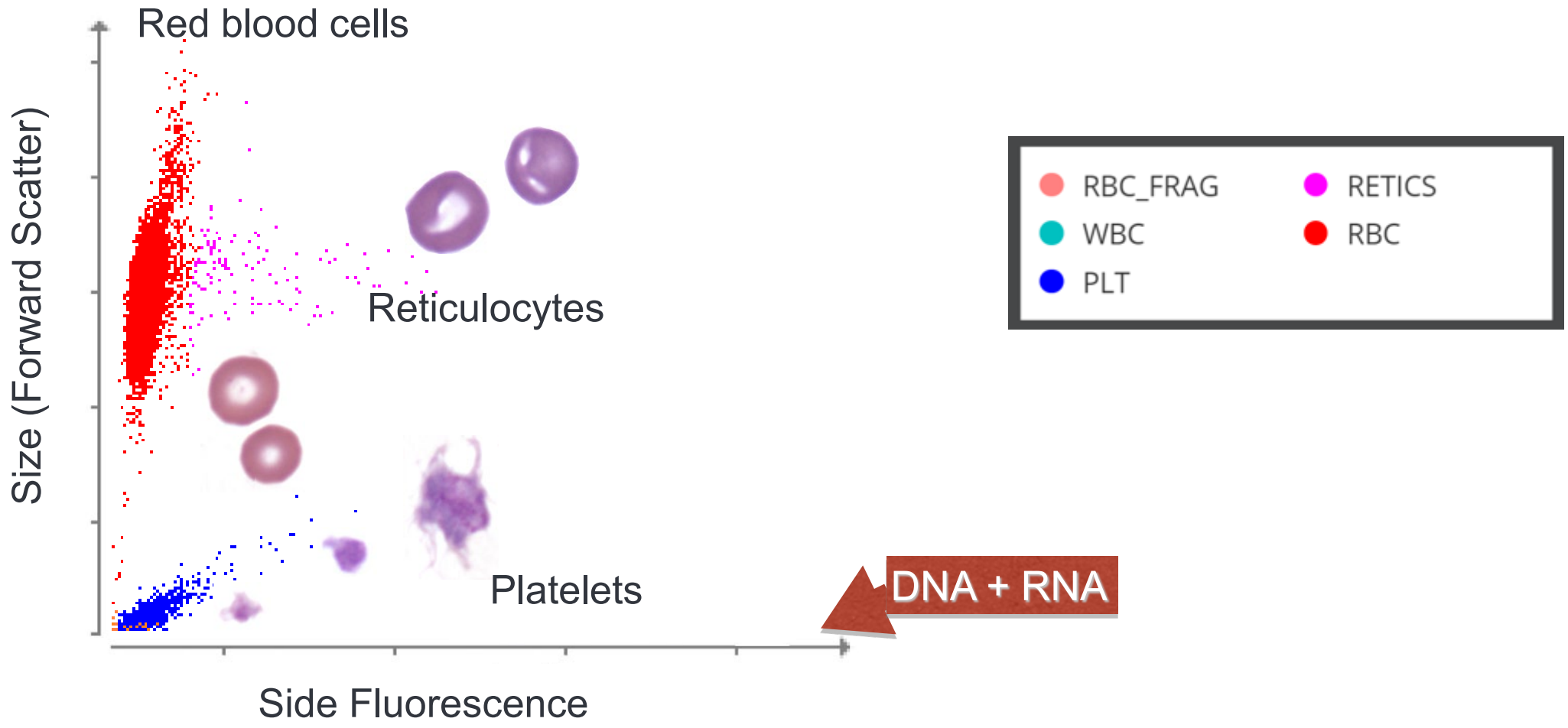
Causes of decreased reticulocyte haemoglobin:

+ Inflammatory conditions (functional iron deficiency)
+ Absolute iron deficiency
+ Portosystemic shunt
+ Breed-related microcytosis (Akita, Shiba, Shar Pei)



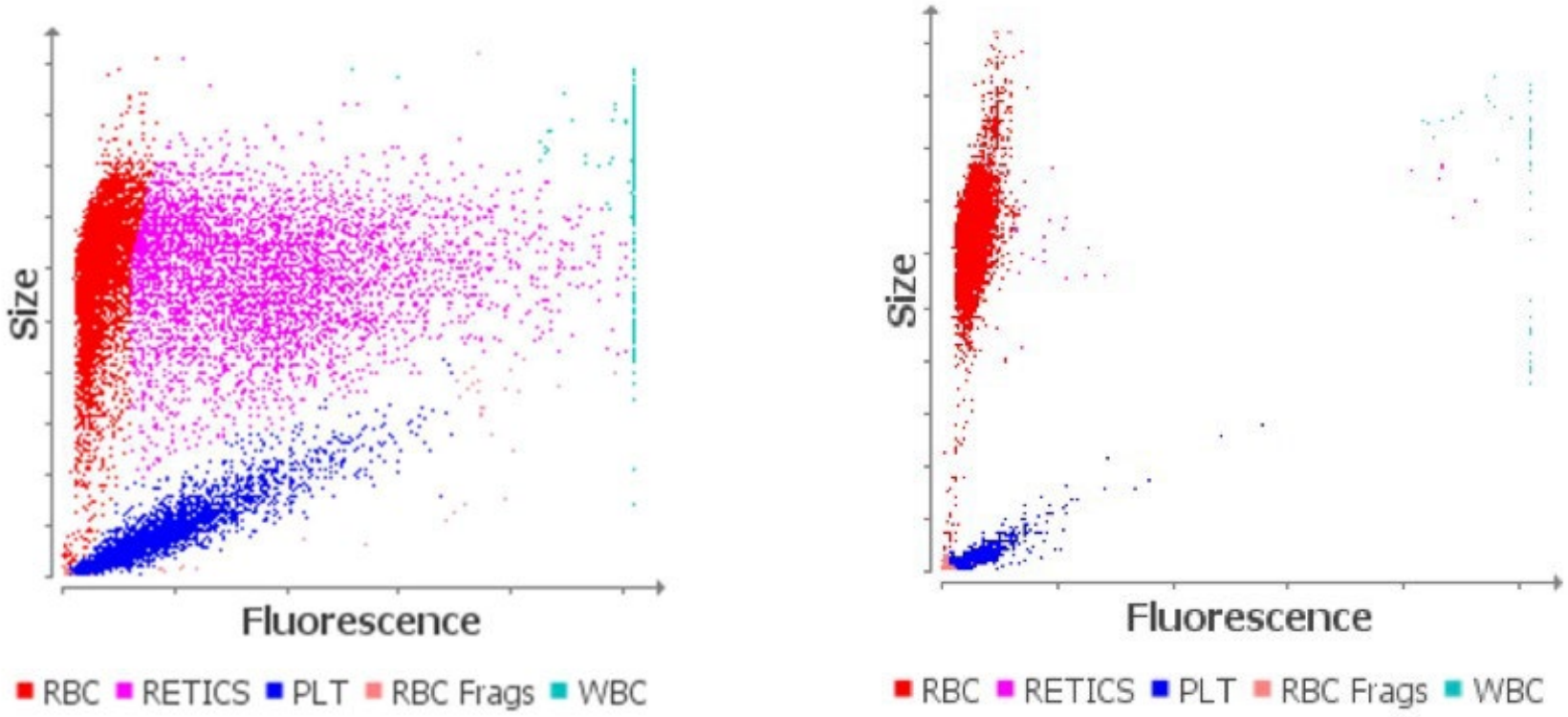


Dot plot for RBC run



ProCyte Dx – Canine Normal RBC Dot Plot

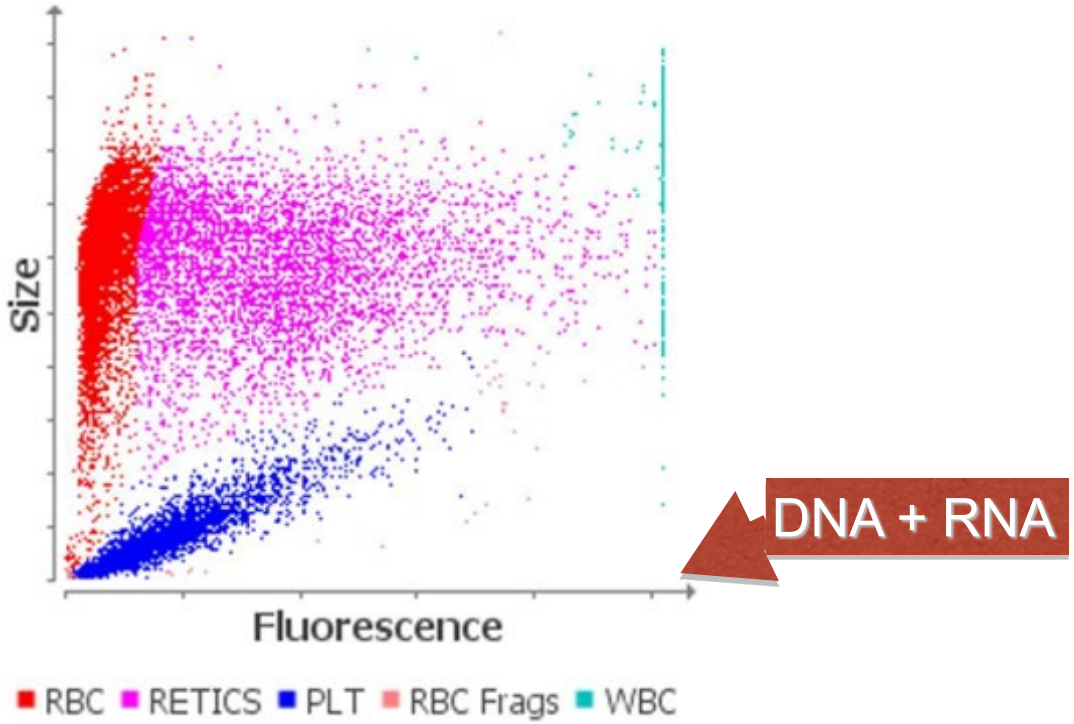
Dot plot for RBC run



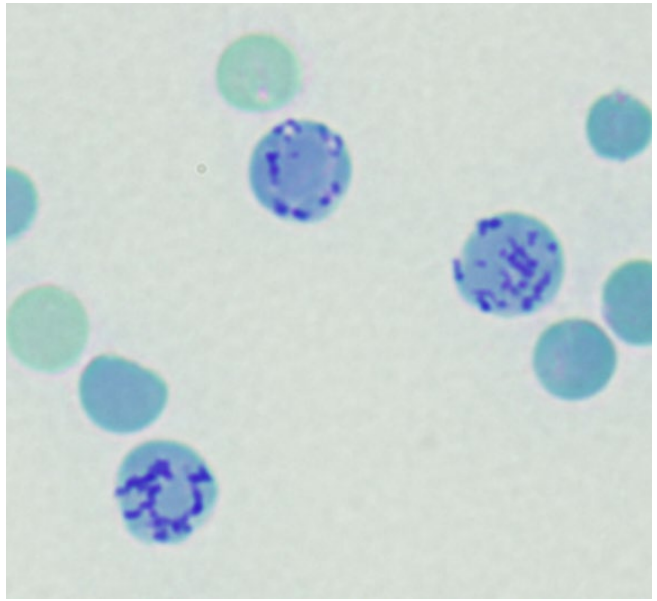
ProCyte Dx – Canine patient RBC Dot Plots

Which one is regenerative?

Dot plot for RBC run



ProCyte Dx – Canine patient RBC Dot Plot



Canine - New methylene blue (NMB) stain

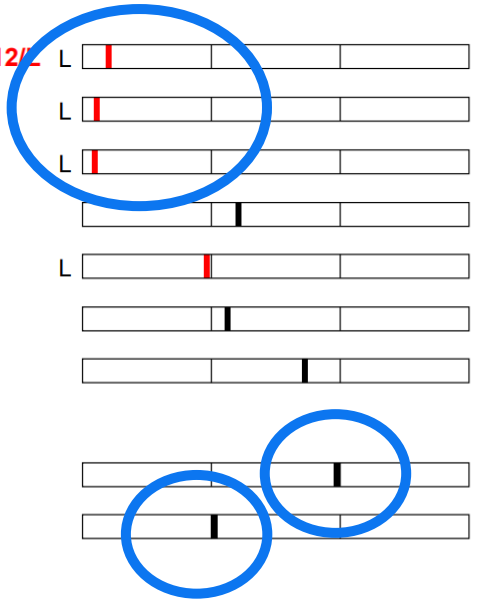
Regenerative

Observing the patterns of change

Haematology

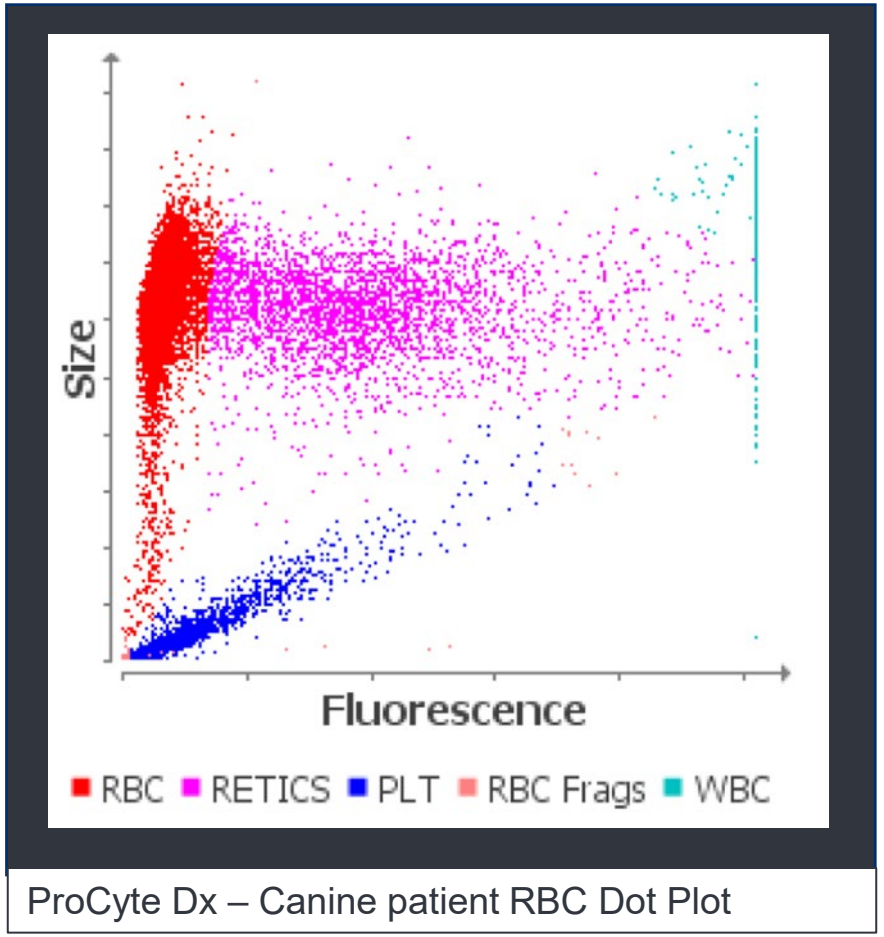
1/4/2022 **10:55 AM**

TEST	RESULT	REFERENCE VALUE	
RBC	**2.69**	**5.65 - 8.87 x10^12/L**	L
Haematocrit	**0.172**	**0.373 - 0.617 L/L**	L
Haemoglobin	**56**	**131 - 205 g/L**	L
MCV	63.9	61.6 - 73.5 fL	
MCH	**20.8**	**21.2 - 25.9 pg**	L
MCHC	326	320 - 379 g/L	
RDW	19.3	13.6 - 21.7 %	
% Reticulocyte	3.9	%	
Reticulocytes	105.7	10.0 - 110.0 K/µL	
Reticulocyte Haemoglobin	22.3	22.3 - 29.6 pg	



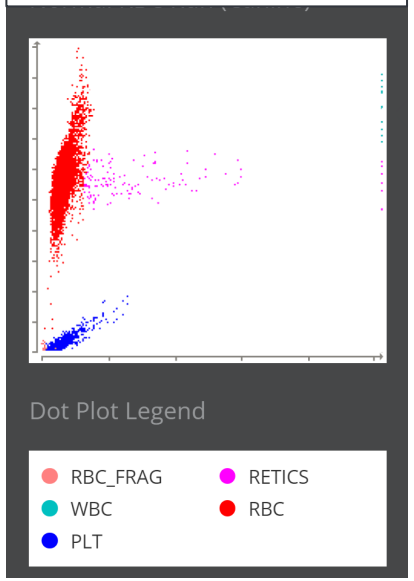
Recognising pattern of changes

RBC	2.44	5.65 - 8.87 x10^12/L
Haematocrit	0.196	0.373 - 0.617 L/L
Haemoglobin	62	131 - 205 g/L
MCV	80.3	61.6 - 73.5 fL
MCH	25.4	21.2 - 25.9 pg
MCHC	316	320 - 379 g/L
RDW	19.5	13.6 - 21.7 %
% Reticulocyte	7.0	%
Reticulocytes	170.6	10.0 - 110.0 K/µL



ProCyte Dx – Canine patient RBC Dot Plot

ProCyte Dx - Canine
Normal RBC Dot Plot

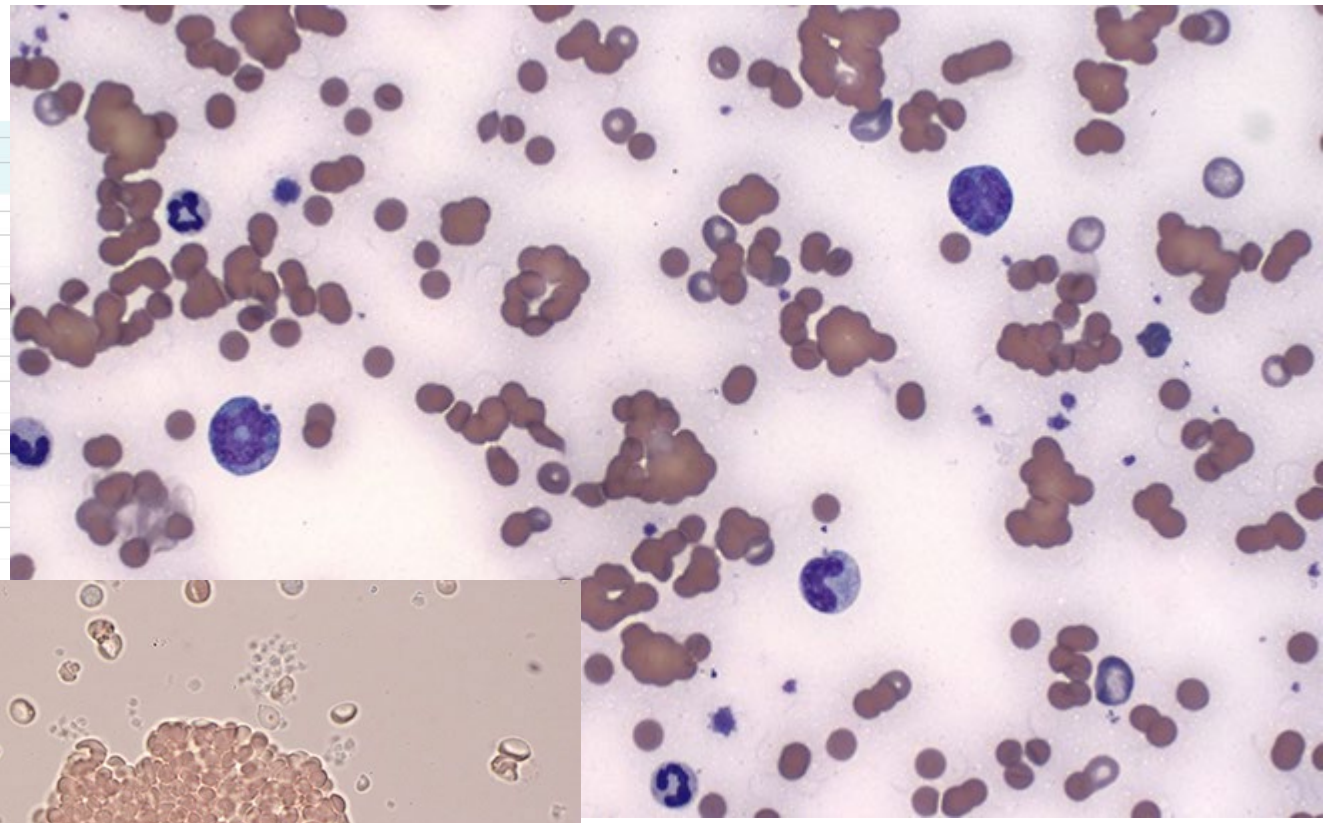


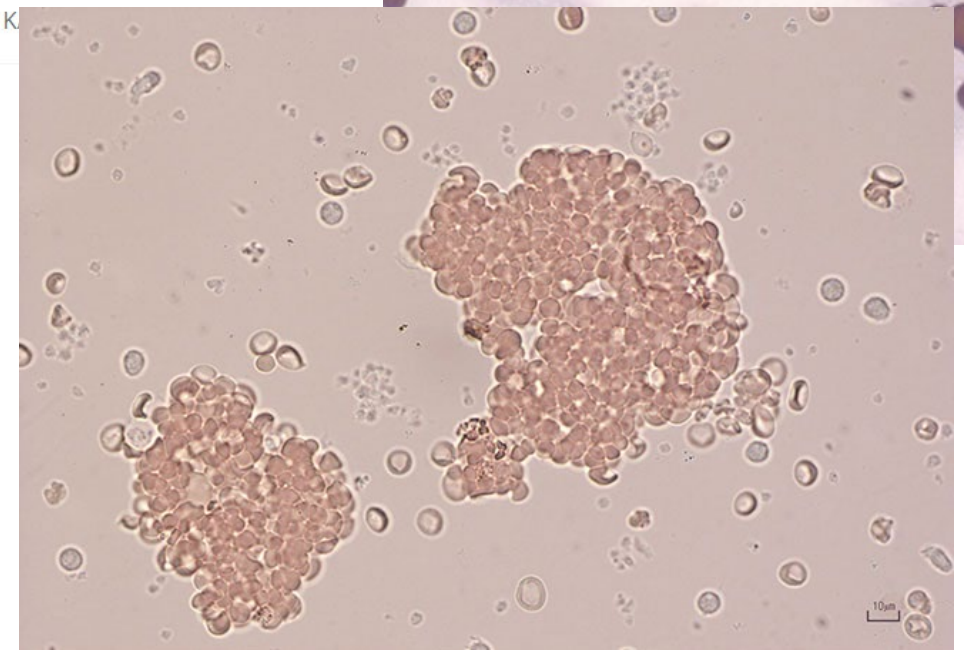
- Macrocytic
- Hypochromic
- Mildly increased reticulocytes

→ Regenerative anaemia

Are the numbers always accurate?

RBC	1.85	5.39 - 8.70 x10^12/L	
Haematocrit	0.192	0.383 - 0.565 L/L	
Haemoglobin	52	134 - 207 g/L	
MCV	103.8	59.0 - 76.0 fL	
MCH	28.1	21.9 - 26.1 pg	
MCHC	271	326 - 392 g/L	
Reticulocytes	b. 361.9	<= 110.0 K	





AGGLUTINATION

Other common causes of artefactual changes:

Increased MCV and decreased MCHC in absence of regeneration (in-vitro swelling of erythrocytes)

- Prolonged sample storage
- Persistent hypernatremia
- Excess EDTA may also be a cause

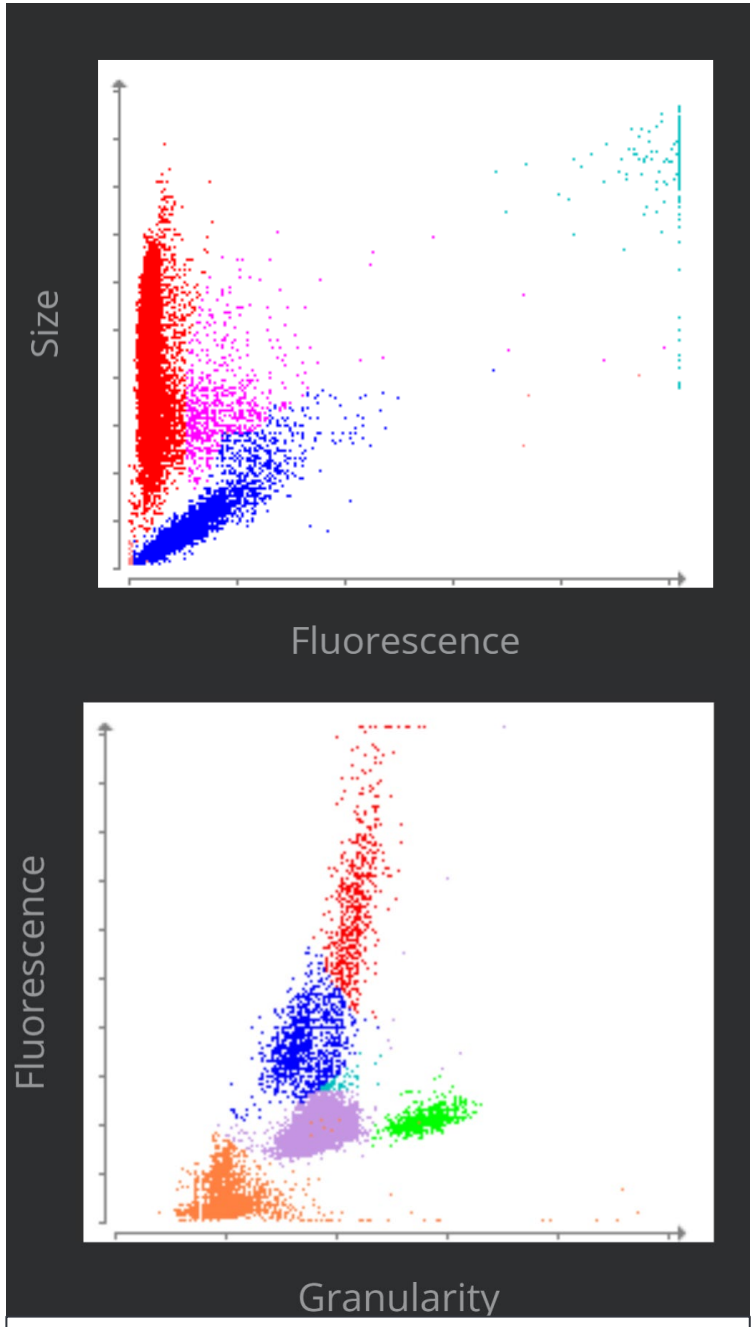
Artefactually increased MCHC

- Haemolysis
- Lipaemia
- Heinz bodies

Sadie 9 years old NF Boxer dog

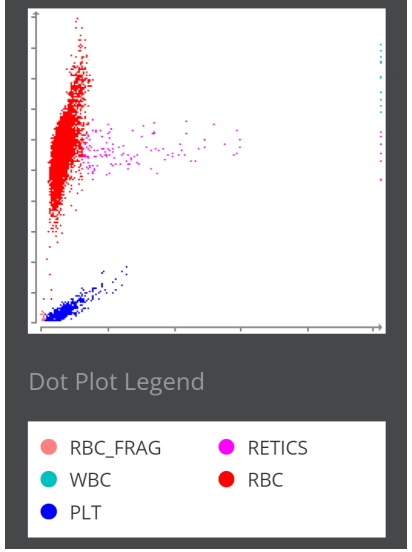
- Vomiting
- Inappetence
- Occasional diarrhoea

RBC	3.27	5.65 - 8.87 x10^12/L	
Haematocrit	0.153	0.373 - 0.617 L/L	
Haemoglobin	49	131 - 205 g/L	
MCV	46.8	61.6 - 73.5 fL	
MCH	15.0	21.2 - 25.9 pg	
MCHC	320	320 - 379 g/L	
RDW	31.5	13.6 - 21.7 %	
% Reticulocyte	4.1	%	
Reticulocytes	135.1	10.0 - 110.0 K/µL	
Reticulocyte Haemoglobin	13.6	22.3 - 29.6 pg	
WBC	*22.53	5.05 - 16.76 x10^9/L	
Neutrophils	*19.68	2.95 - 11.64 x10^9/L	
Lymphocytes	*1.29	1.05 - 5.10 x10^9/L	
Monocytes	*0.70	0.16 - 1.12 x10^9/L	
Eosinophils	*0.78	0.06 - 1.23 x10^9/L	
Basophils	*0.08	0.00 - 0.10 x10^9/L	
Nucleated RBC	*Suspected		
Platelets	*533	148 - 484 x10^9/L	

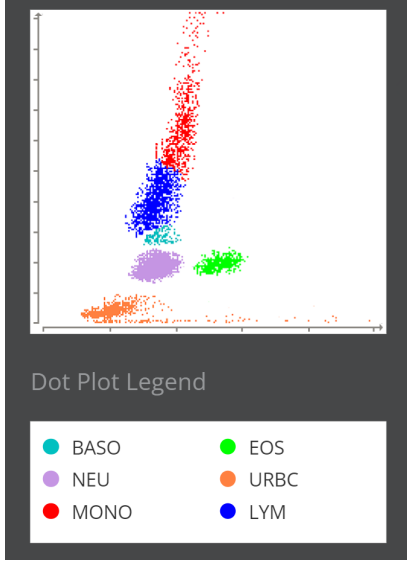


ProCyte Dx – Canine patient RBC&WBC Dot Plot

ProCyte Dx - Canine Normal RBC Dot Plot



ProCyte Dx - Canine Normal WBC Dot Plot



Sadie 9 years old NF Boxer dog

- Vomiting
- Inappetence
- Occasional diarrhoea

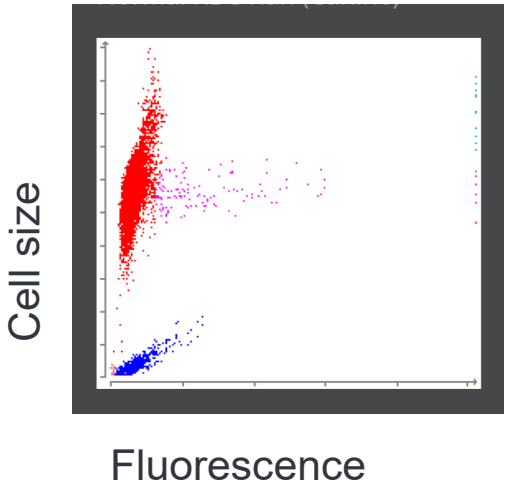
RBC	3.27	5.65 - 8.87 x10^12/L
Haematocrit	0.153	0.373 - 0.617 L/L
Haemoglobin	49	131 - 205 g/L
MCV	46.8	61.6 - 73.5 fL
MCH	15.0	21.2 - 25.9 pg
MCHC	320	320 - 379 g/L
RDW	31.5	13.6 - 21.7 %
% Reticulocyte	4.1	%
Reticulocytes	135.1	10.0 - 110.0 K/µL
Reticulocyte Haemoglobin	13.6	22.3 - 29.6 pg
WBC	*22.53	5.05 - 16.76 x10^9/L
Neutrophils	*19.68	2.95 - 11.64 x10^9/L
Lymphocytes	*1.29	1.05 - 5.10 x10^9/L
Monocytes	*0.70	0.16 - 1.12 x10^9/L
Eosinophils	*0.78	0.06 - 1.23 x10^9/L
Basophils	*0.08	0.00 - 0.10 x10^9/L
Nucleated RBC	*Suspected	
Platelets	*533	148 - 484 x10^9/L

+ Severe anaemia
  + Microcytic
  + Hypochromic
  + Mildly regenerative
+ Markedly reduced reticulocyte haemoglobin

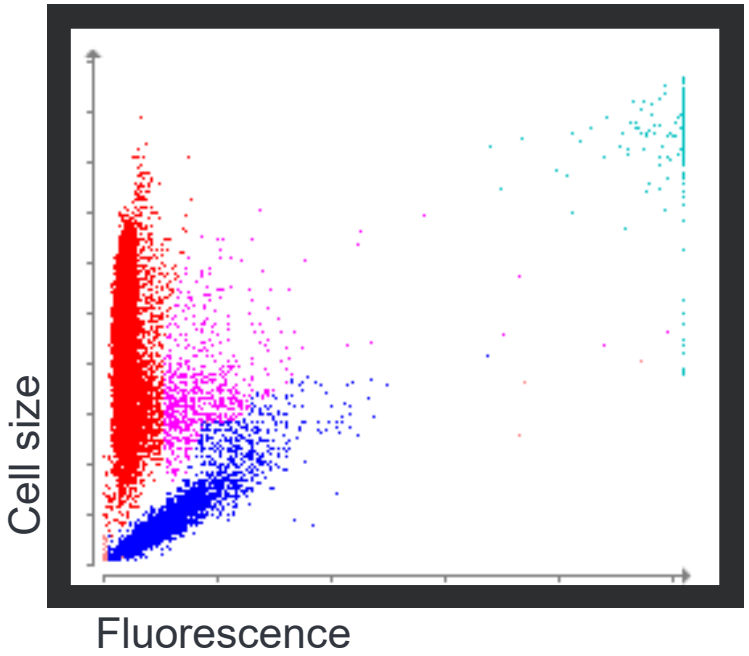
Dot plot for RBC run

ProCyte Dx - Canine
RBC Dot Plots

Normal



Patient results

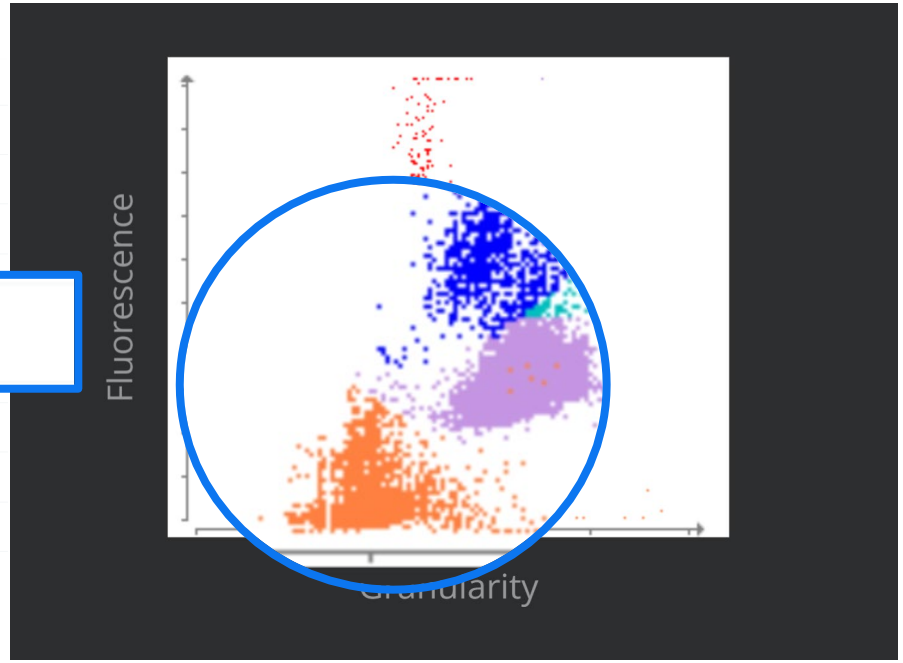


Iron deficiency anaemia

+ Most often chronic blood loss
+ Commonly GI tract

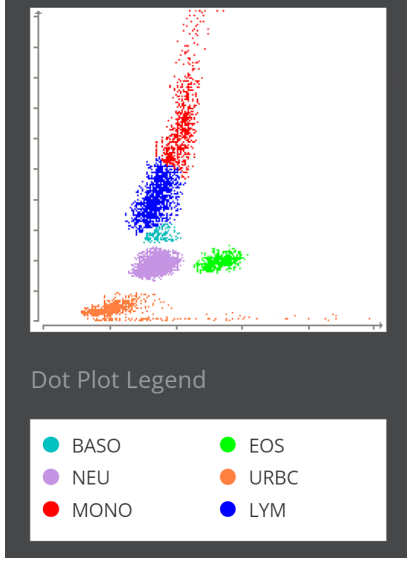
Any additional info on RBCs that can be gathered by flags on the leukocyte run?

WBC	*22.53	5.05 - 16.76 x10^9/L
Neutrophils	*19.68	2.95 - 11.64 x10^9/L
Lymphocytes	*1.29	1.05 - 5.10 x10^9/L
Monocytes	*0.70	0.16 - 1.12 x10^9/L
Eosinophils	*0.78	0.06 - 1.23 x10^9/L
Nucleated RBC	*Suspected	
PDW	--.--	9.1 - 19.4 fL
MPV	18.7	8.7 - 13.2 fL
Plateletcrit	1.00	0.14 - 0.46 %

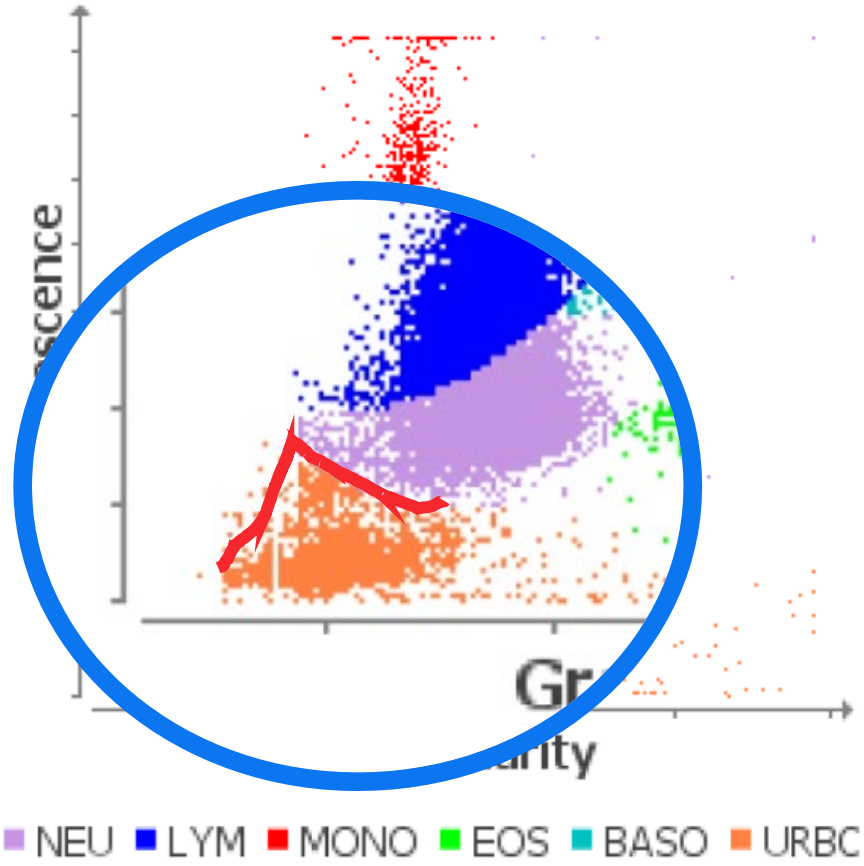


ProCyte Dx – Canine patient WBC Dot Plot

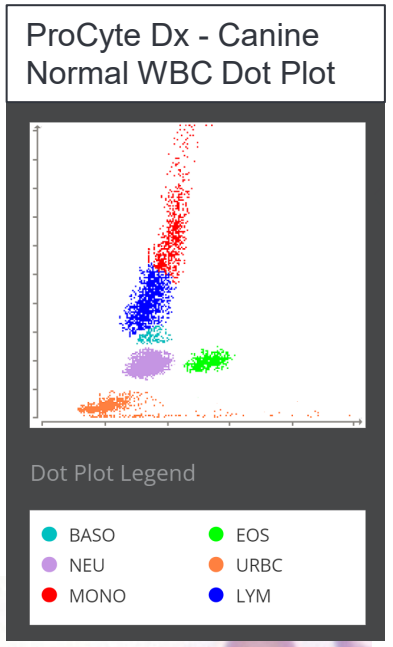
ProCyte Dx - Canine Normal WBC Dot Plot

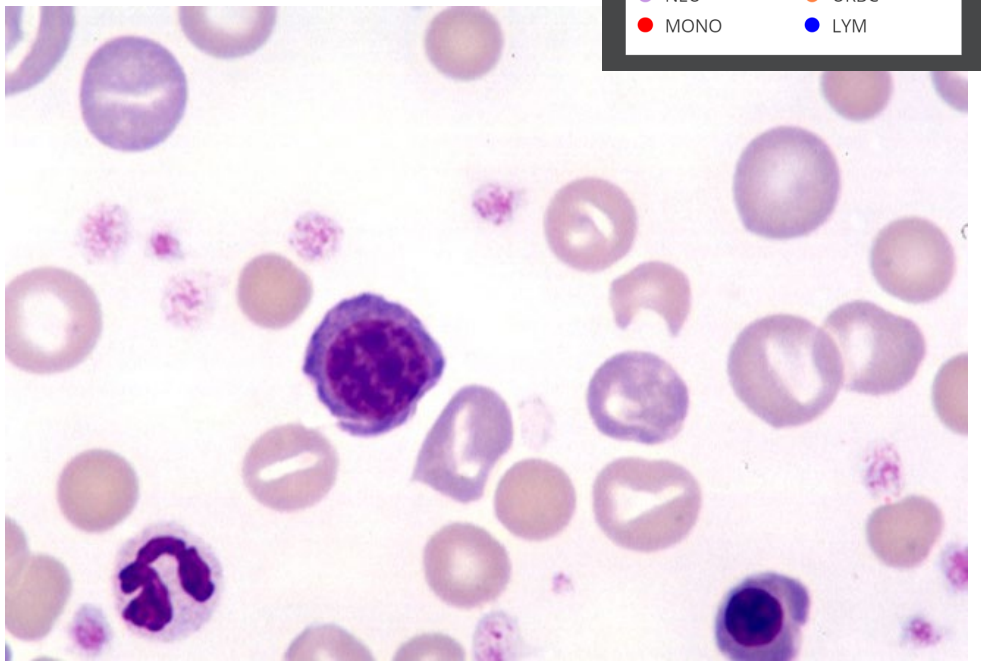


nRBC on the scatterplot



ProCyte Dx – Canine patient WBC Dot Plot





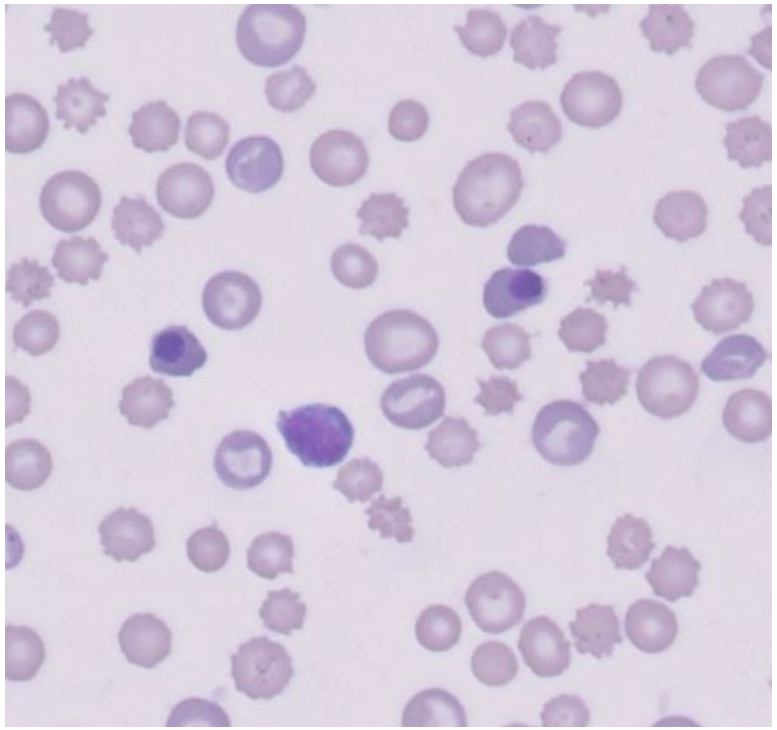
Nucleated RBCs and regeneration

+ Nucleated RBCs are part of the regenerative response, and their number should be proportionate to level of regeneration

+ In absence of proportionate regeneration:

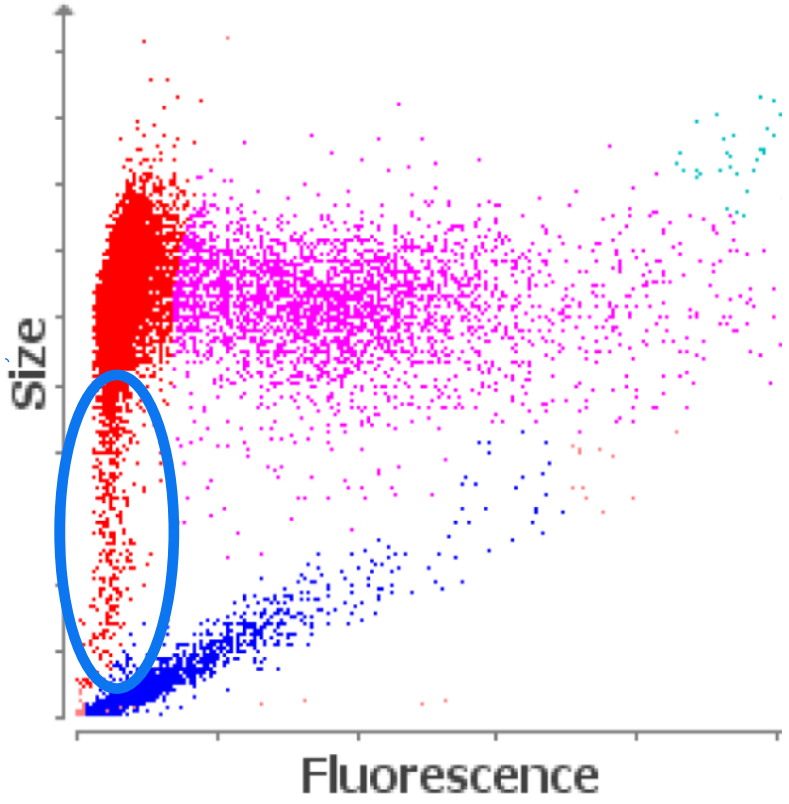
INAPPROPRIATE RUBRICYTOSIS, e.g.

- Bone marrow damage
- Lead poisoning
- Heat stroke
- Dyserythropoiesis
- Splenic disorder



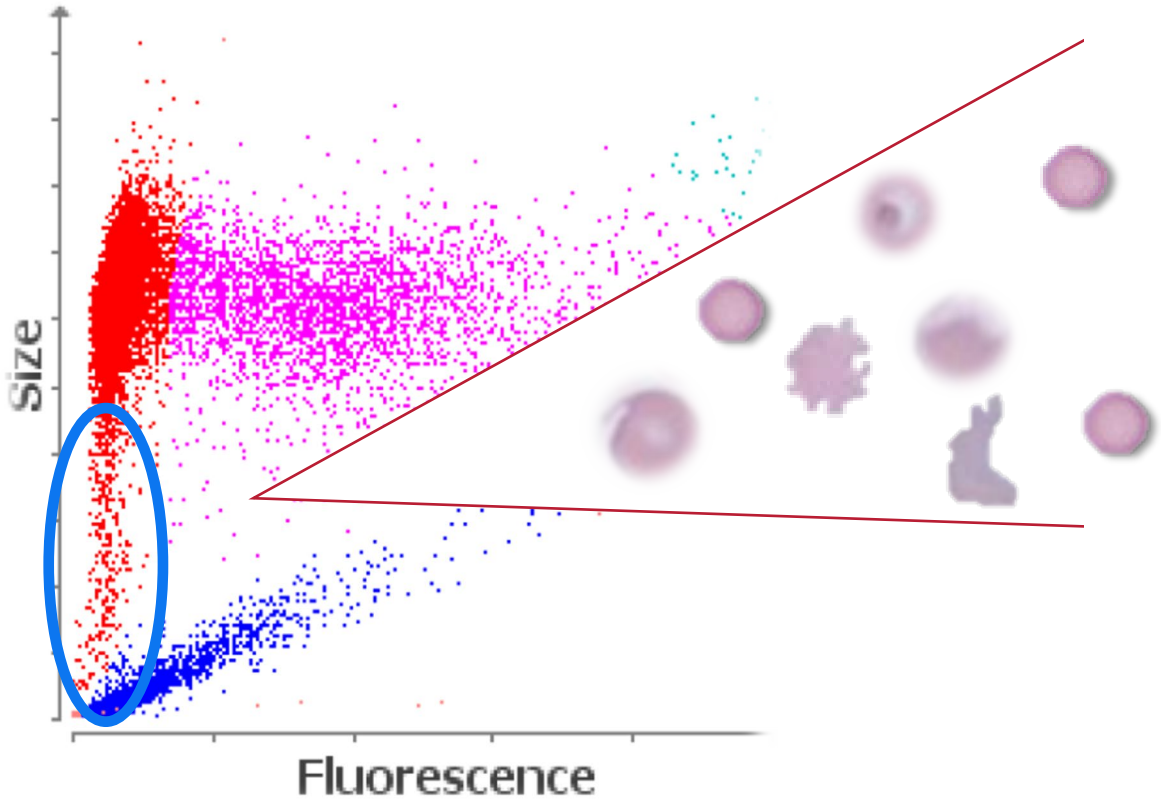
Can there sometimes be additional clues in the red cell run?

‘tail’ highlighted



ProCyte Dx – Canine patient RBC Dot Plot

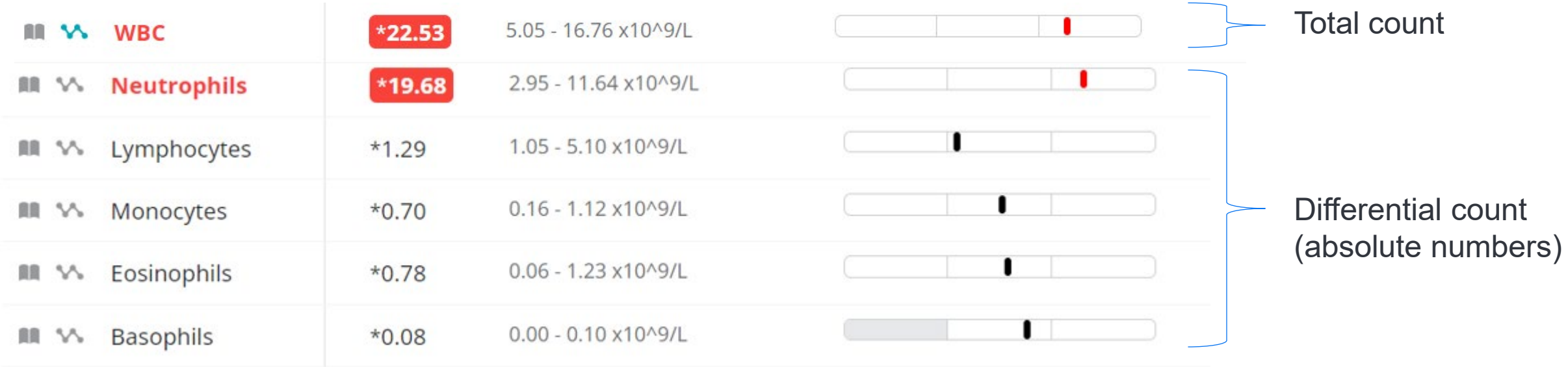
Poikilocytes on the red cell run



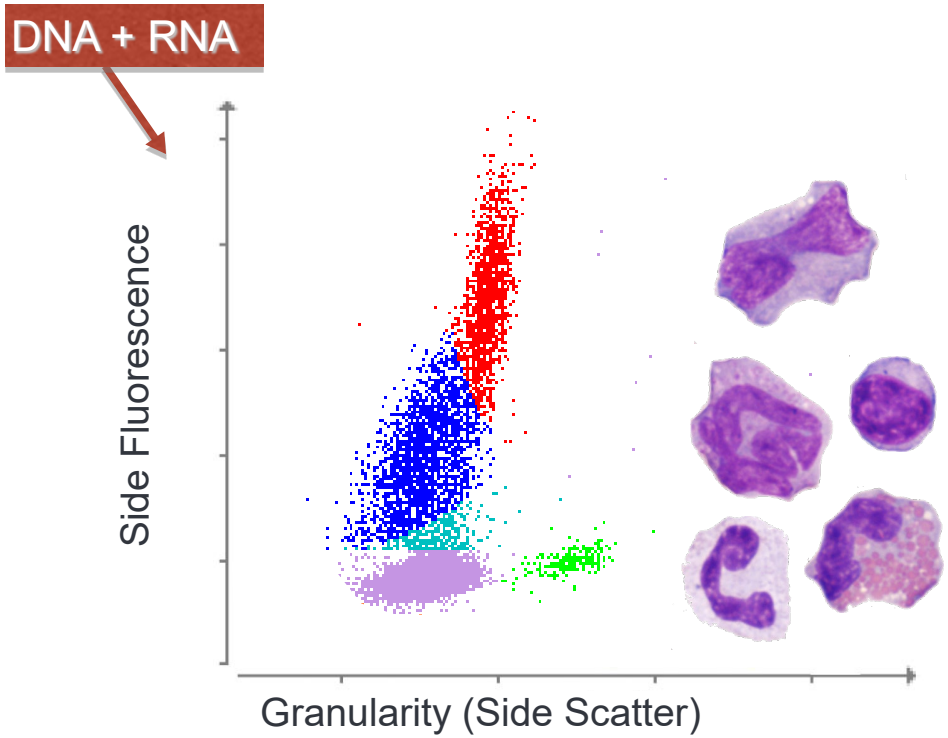
ProCyte Dx – Canine patient RBC Dot Plot

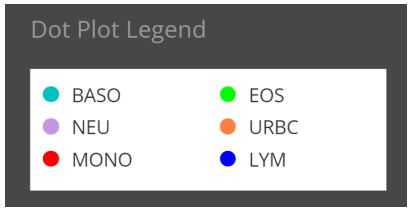
Moving onto the leukocytes

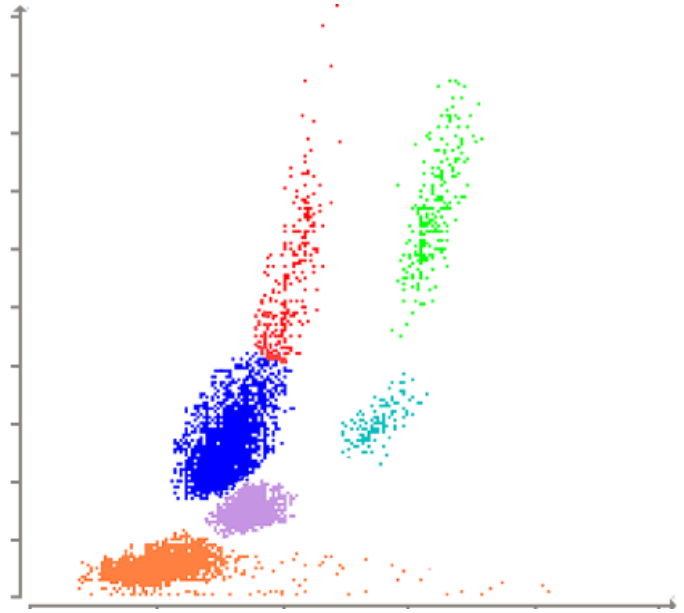
Numerical data:



Dot plots: leukocyte run - normal







ProCyte Dx – Normal WBC Dot Plot

Pattern of changes in numerical data

Is there a decrease/increase in total and individual WBC numbers?

Recognisable patterns, e.g.

- Corticosteroid-mediated stress leukogram
- Catecholamine-mediated leukogram
- Inflammatory leukogram

Degree of change is relevant in interpretation, e.g.

- Corticosteroid-mediated: Neutrophilia up to 2x upper reference range in dogs, 3x in cats
- Reactive vs neoplastic lymphocytosis

- Stockham, Steven L., and Michael A. Scott. Fundamentals of veterinary clinical pathology. 2nd ed, Blackwell, 2008

But do the numbers always tell us everything?

Trixie Yorkshire terrier, 8 year old FN

- Dog not doing well
- Anorexia
- Lethargy

On examination:

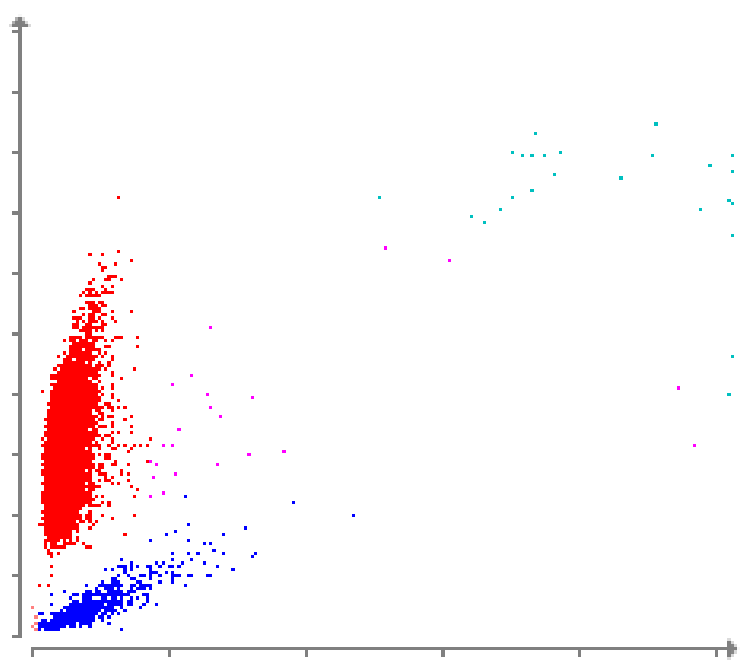
- Painful abdomen
- Limited palpation
- HR 160
- Panting
- T 39.8 C

Automated haematology results

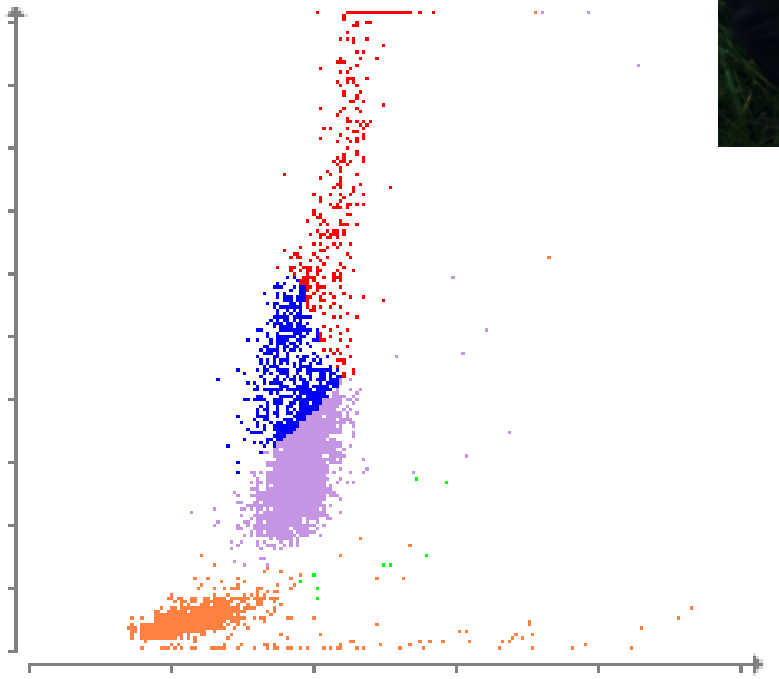
RBC	7.18	6.54 - 12.20 x10^12/L
Haematocrit	**0.266**	0.303 - 0.523 L/L
Haemoglobin	**91**	98 - 162 g/L
MCV	37.0	35.9 - 53.1 fL
MCH	12.7	11.8 - 17.3 pg
MCHC	342	281 - 358 g/L
RDW	25.9	15.0 - 27.0 %
% Reticulocyte	0.1	%
Reticulocytes	7.9	3.0 - 50.0 K/µL
Reticulocyte Haemoglobin	13.2	13.2 - 20.8 pg
WBC	* 6.26	2.87 - 17.02 x10^9/L
Neutrophils	* 4.05	2.30 - 10.29 x10^9/L
Lymphocytes	* 1.42	0.92 - 6.88 x10^9/L
Monocytes	* 0.24	0.05 - 0.67 x10^9/L
Eosinophils	* 0.46	0.17 - 1.57 x10^9/L
Basophils	* 0.09	0.01 - 0.26 x10^9/L
Platelets	202	151 - 600 x10^9/L
MPV	14.9	11.4 - 21.6 fL
Plateletcrit	0.30	0.17 - 0.86 %

IDEXX

Dot plots



ProCyte Dx – Canine patient RBC Dot Plot

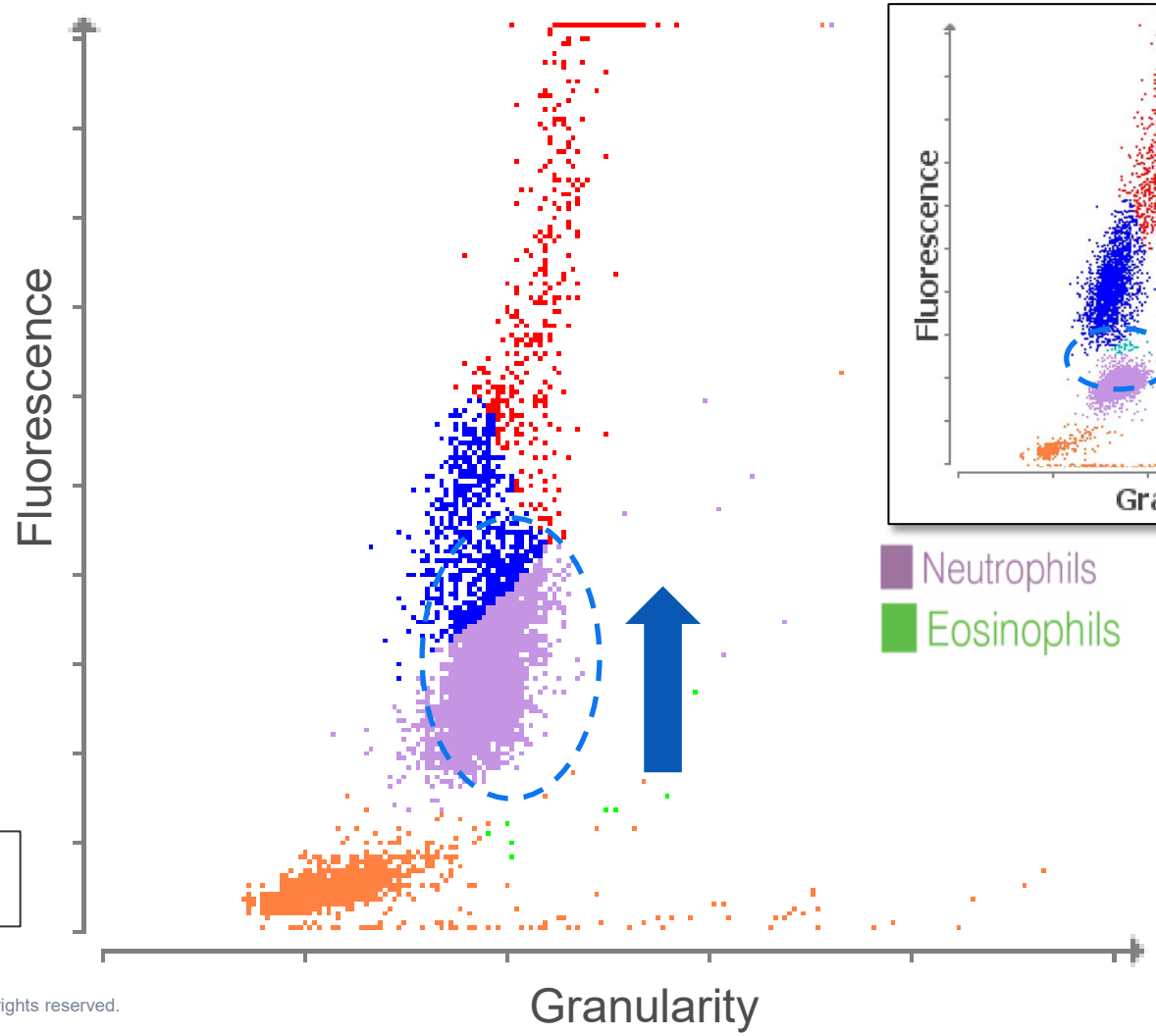


ProCyte Dx – Canine patient WBC Dot Plot

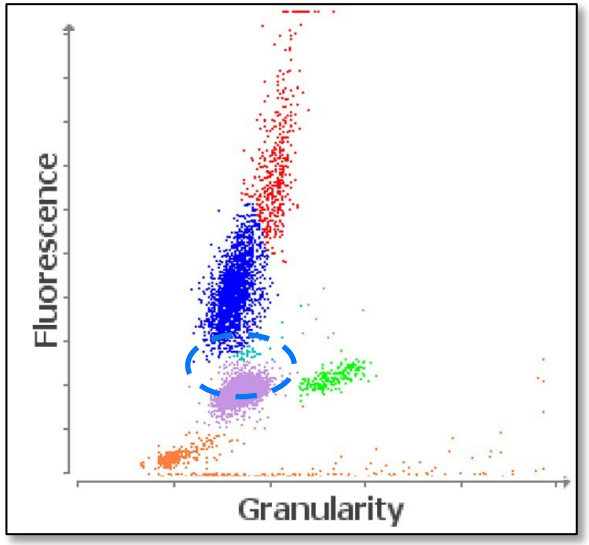
- Expectations from counts ?
- Suspected possible additional features?

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

Compare the dot plots to normal examples



ProCyte Dx – Canine
Patient WBC Dot Plot



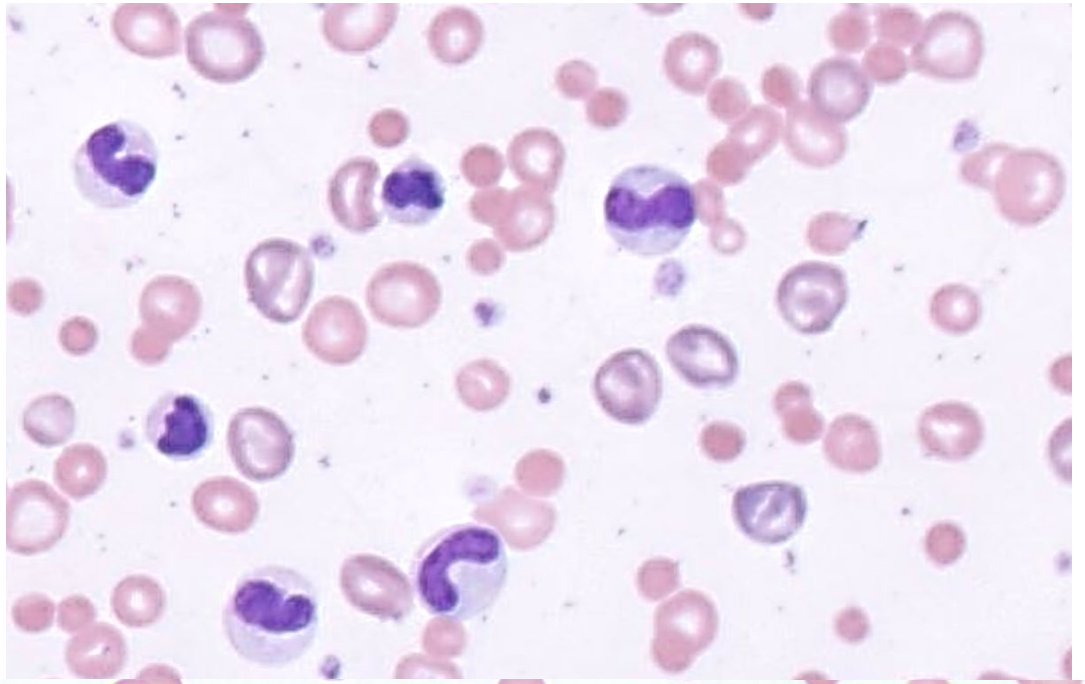
ProCyte Dx – Canine
Normal WBC Dot Plot

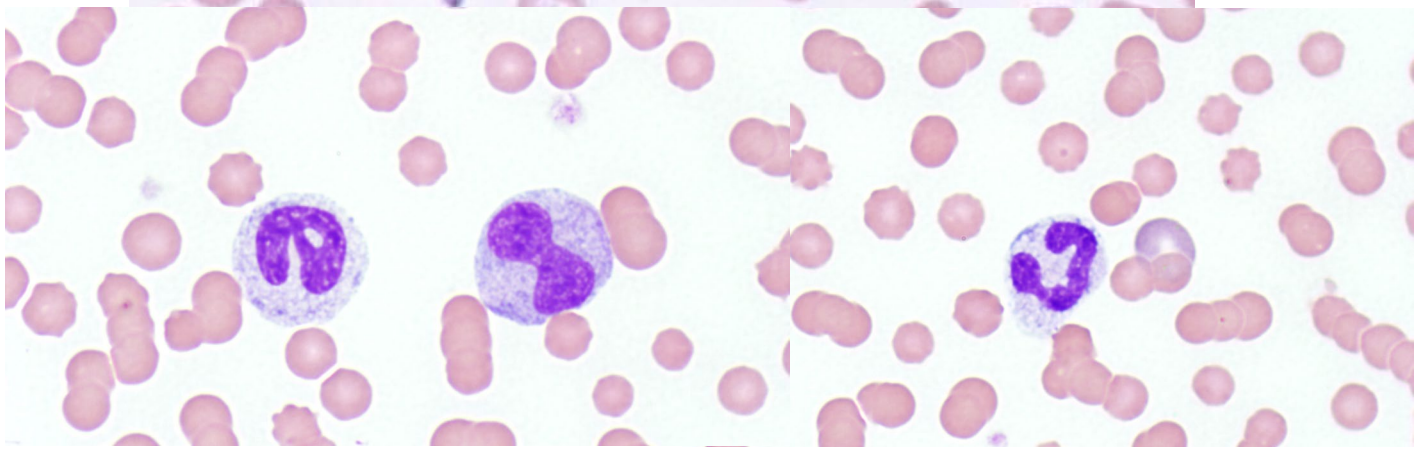
Neutrophils
Monocytes
Lymphocytes
Eosinophils
Basophils
RBC Ghosts

IDEXX

Blood smear findings

% Neutrophils	76.0	%
% Bands	12.0	%
% Lymphocytes	4.0	%
% Monocytes	7.0	%
% Eosinophils	1.0	%
% Basophils	0.0	%

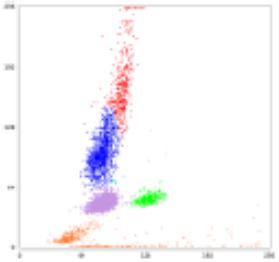




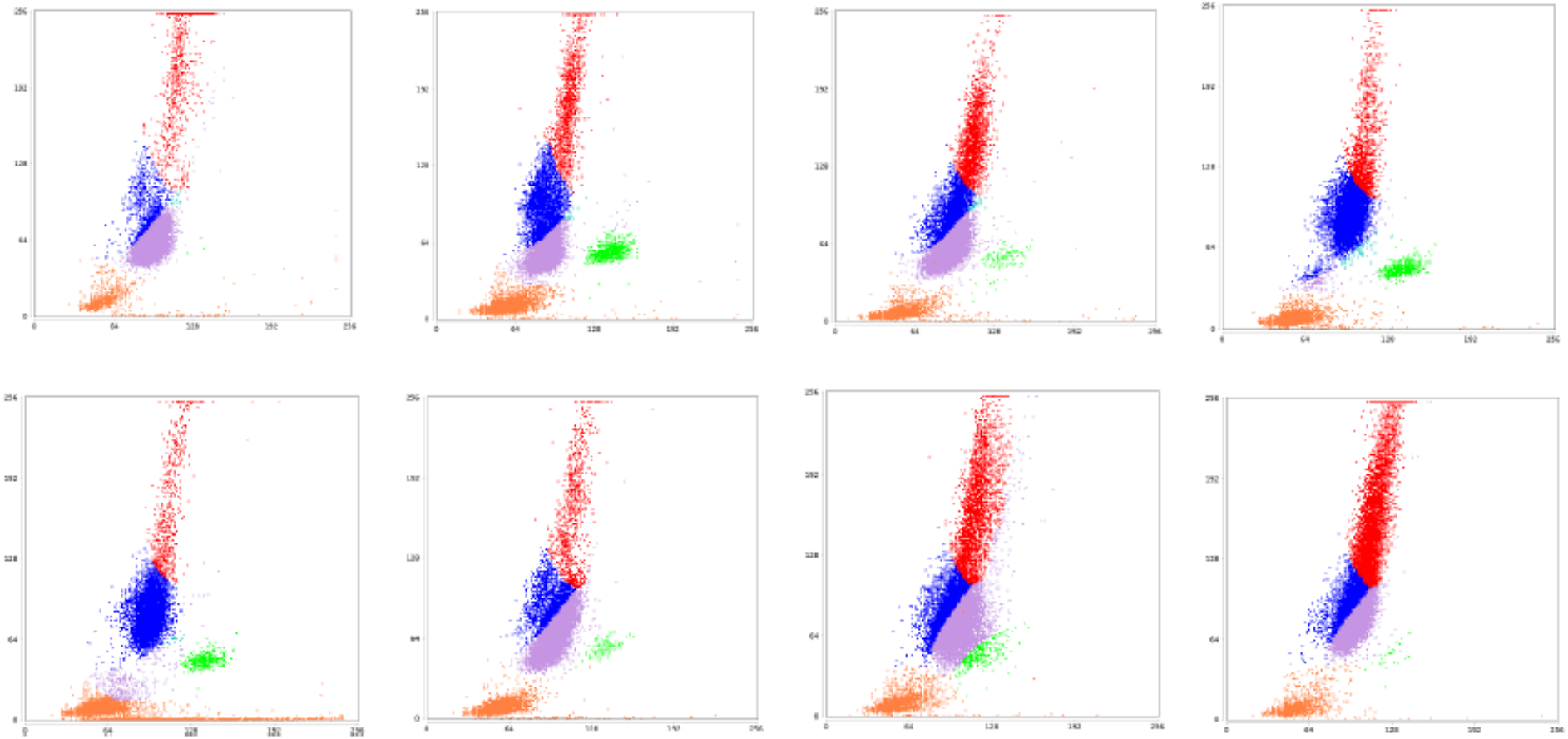
The following day…..

WBC	*** 20.02**	**5.05 - 16.76 K/µL**	
% Neutrophil	* 81.4	%	
% Lymphocyte	* 9.7	%	
% Monocyte	* 6.7	%	
% Eosinophil	* 2.2	%	
% Basophil	* 0.0	%	
Neutrophil	*** 16.28**	**2.95 - 11.64 K/µL**	
Band	* Suspected		
Lymphocyte	* 1.95	1.05 - 5.1 K/µL	
Monocyte	*** 1.34**	**0.16 - 1.12 K/µL**	
Eosinophil	* 0.45	0.06 - 1.23 K/µL	
Basophil	* 0	0 - 0.1 K/µL	
Platelet	* 395	148 - 484 K/µL	

ProCyte DX – examples of left shift



Normal



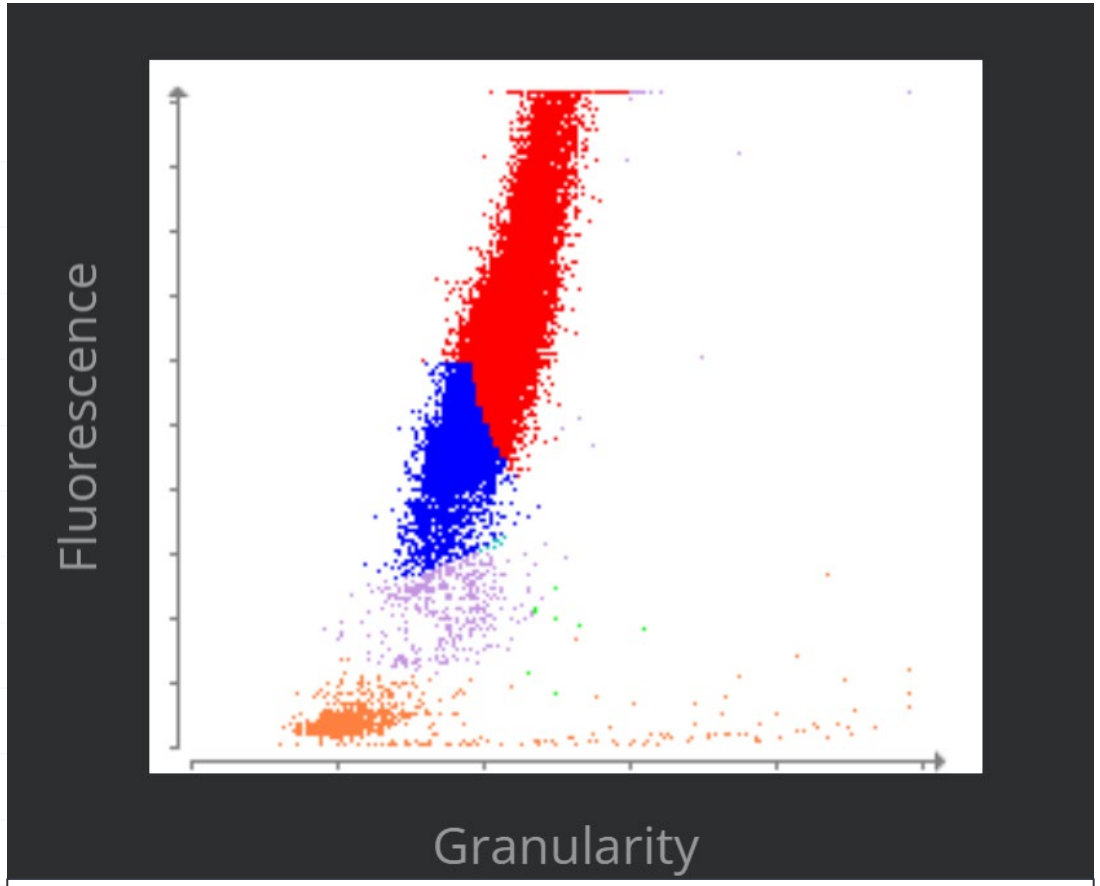
Poppy Boxer 9y Female neutered

Off food, Lethargy

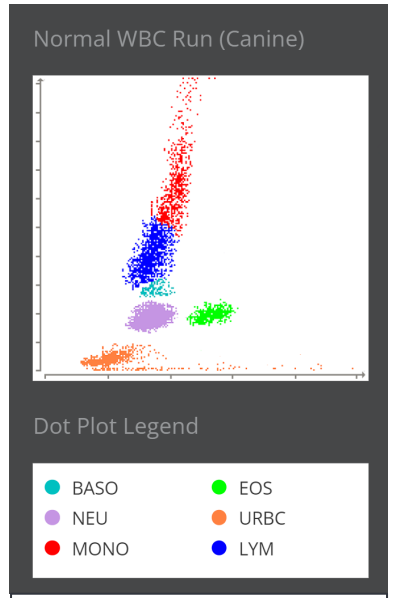
On examination:

- Temperature 39.5C
- Pale mucous membranes

WBC	**54.21**	5.05 - 16.76 x10^9/L
% Neutrophils	*1.5	%
% Lymphocytes	*20.2	%
% Monocytes	*78.3	%
% Eosinophils	0.0	%
% Basophils	0.0	%
Neutrophils	***0.82**	2.95 - 11.64 x10^9/L
Bands	*Suspected	
Lymphocytes	***10.93**	1.05 - 5.10 x10^9/L
Monocytes	***42.44**	0.16 - 1.12 x10^9/L
Eosinophils	**0.01**	0.06 - 1.23 x10^9/L
Basophils	0.01	0.00 - 0.10 x10^9/L

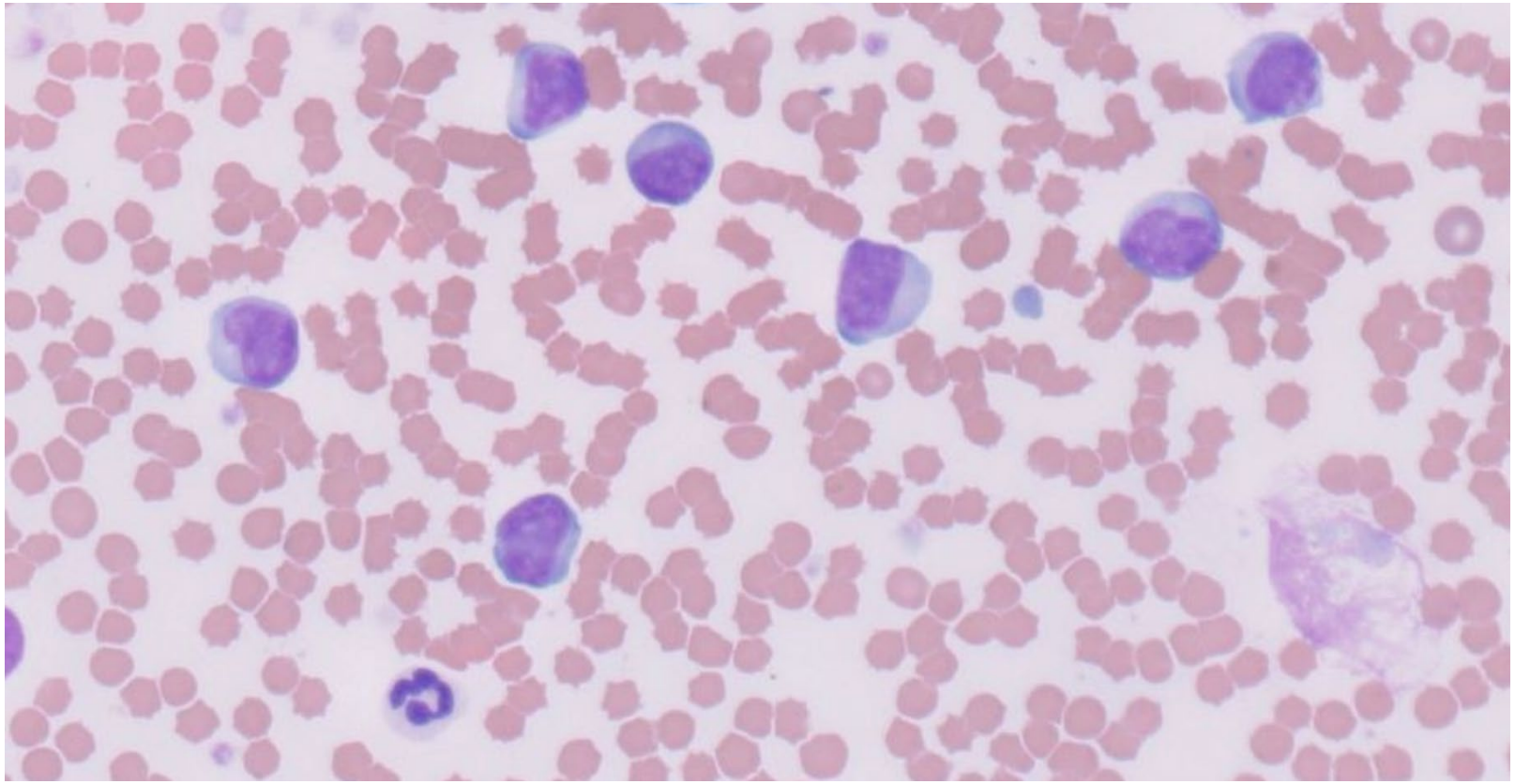


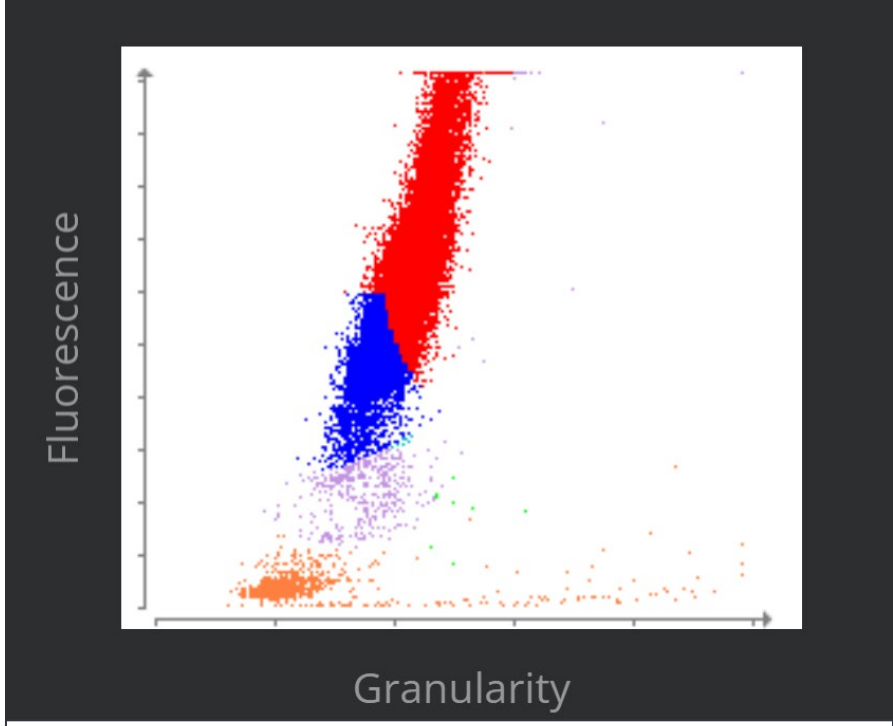
ProCyte Dx – Canine Patient WBC Dot Plot



ProCyte Dx – Canine Normal WBC Dot Plot

Blood smear findings:





ProCyte Dx – Canine Patient WBC Dot Plot

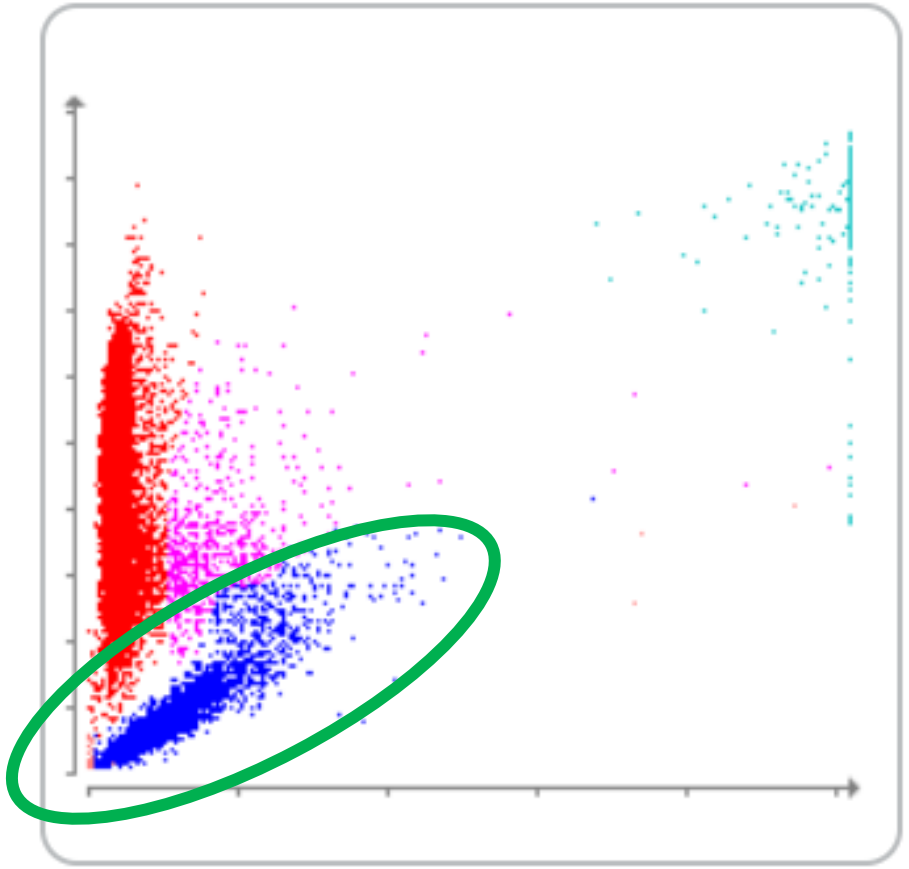
Flow cytometry:
Acute B cell leukemia

And what about platelets?

THROMBOCYTOSIS

Generally, a very non-specific finding

+ Inflammation
+ Redistribution (e.g. splenic contraction due to excitement)
+ Rebound after previous thrombocytopenia
+ Iron deficiency



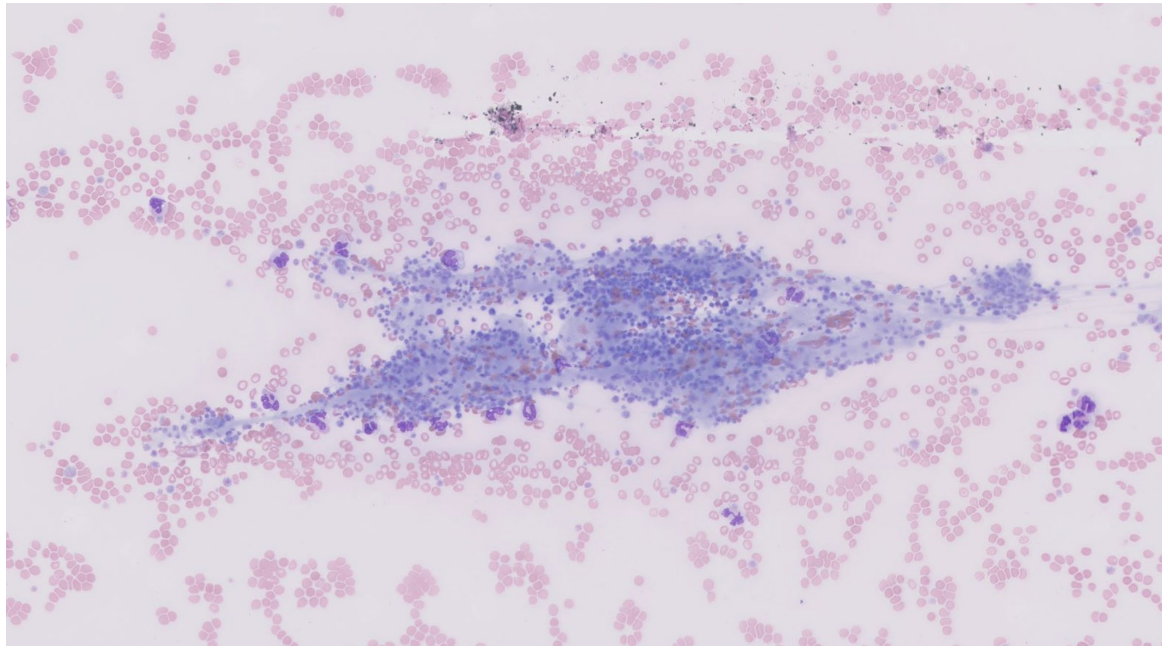
ProCyte Dx – Canine Patient RBC Dot Plot

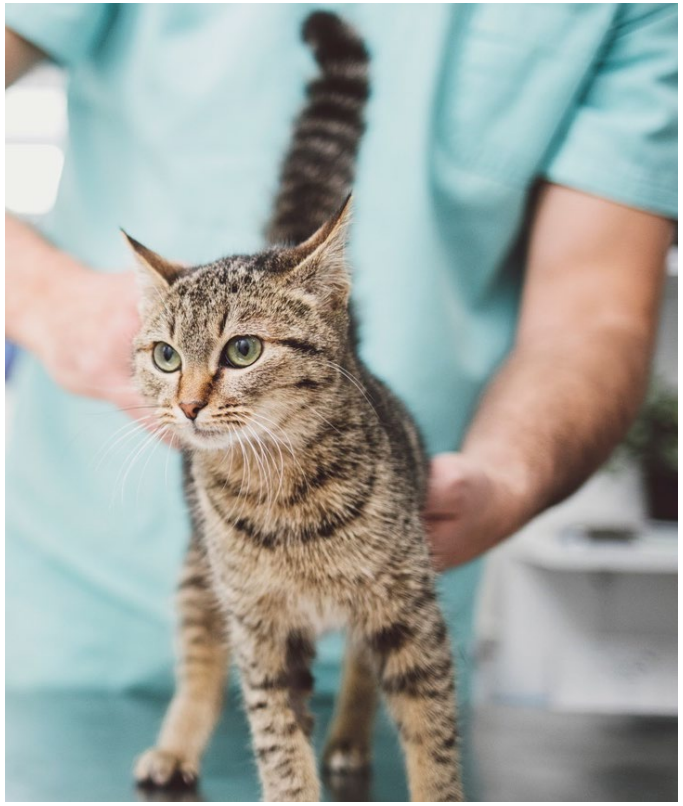
And what about platelets?

THROMBOCYTOPENIA

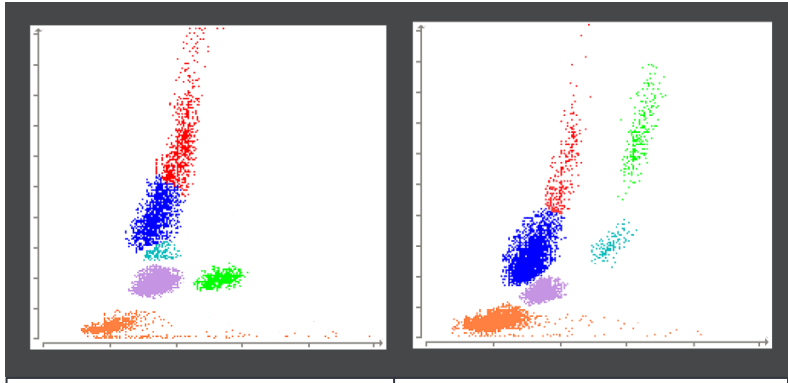
Platelets	*32	148 - 484 x10^9/L	

+ First question to ask: is it genuine?



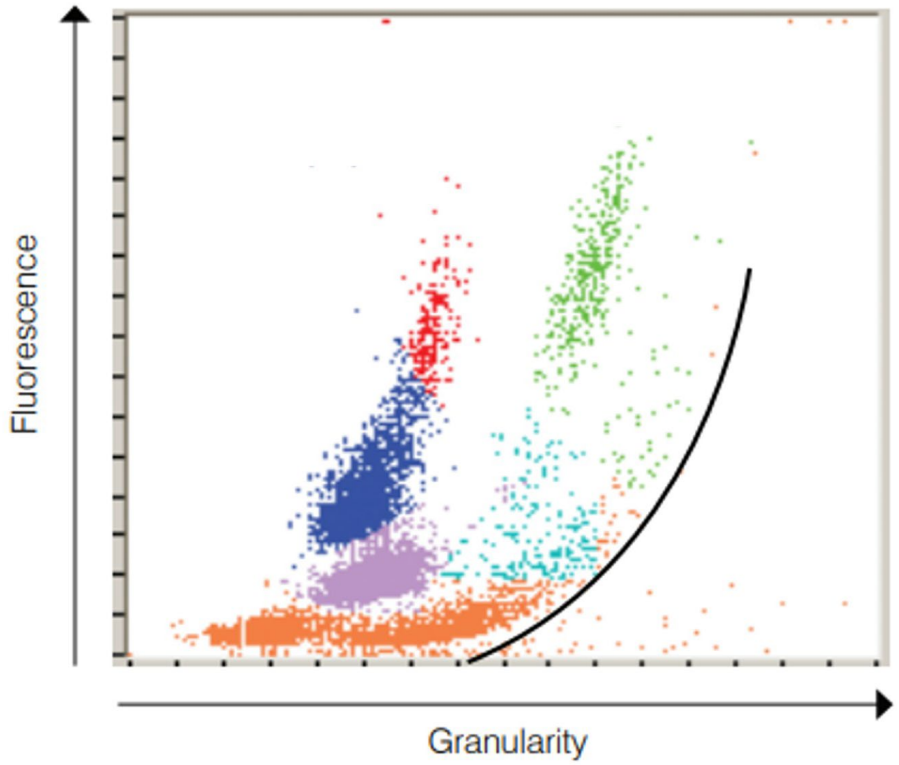


Platelet clumps in dot plots

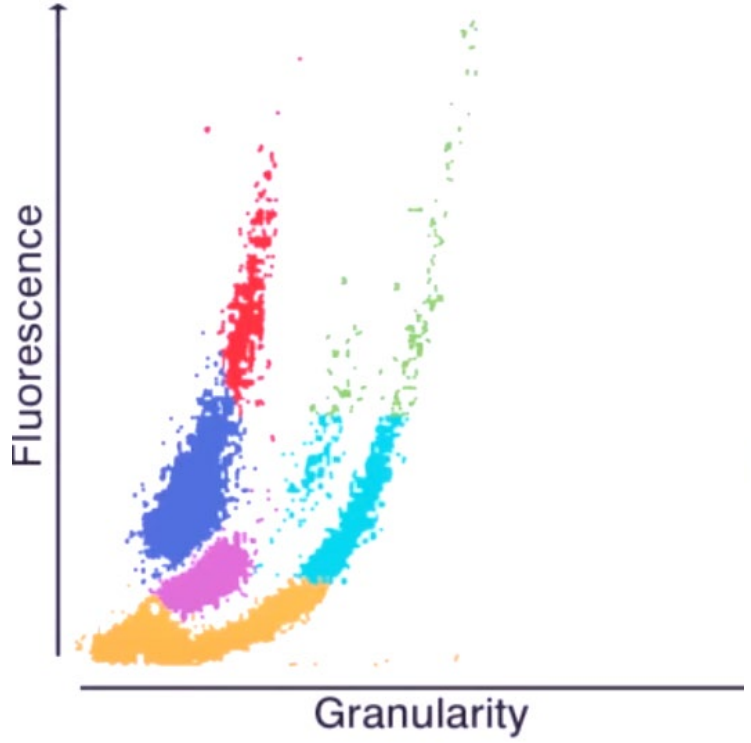


ProCyte Dx – Canine Normal WBC Dot Plot

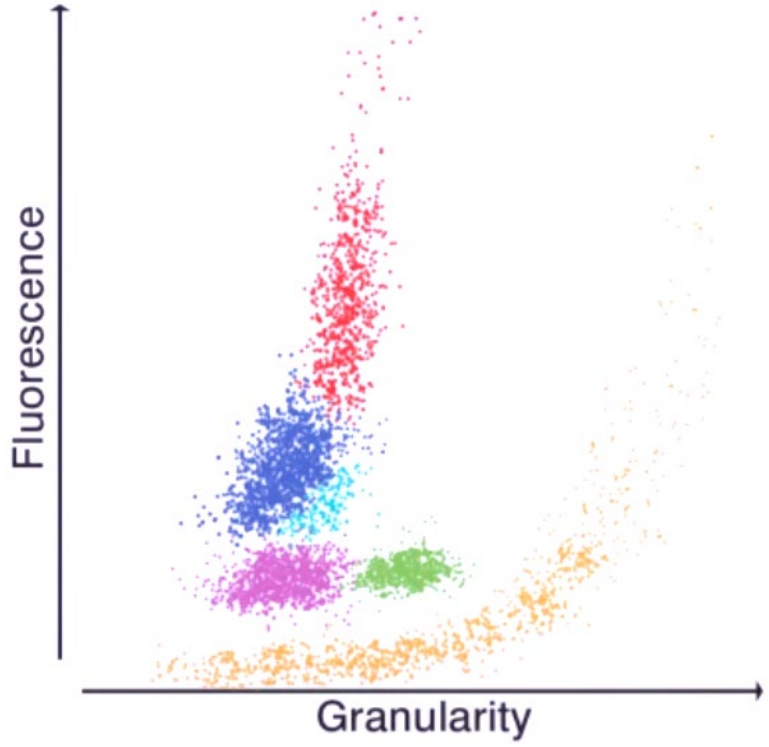
ProCyte Dx – Feline Normal WBC Dot Plot



ProCyte Dx – Feline Patient WBC Dot Plot

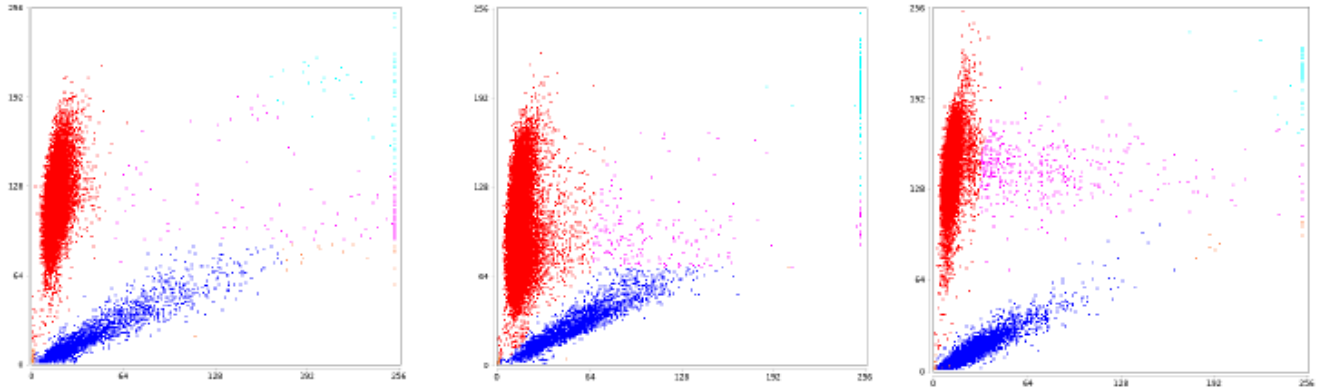


ProCyte Dx – Feline Patient WBC Dot Plot

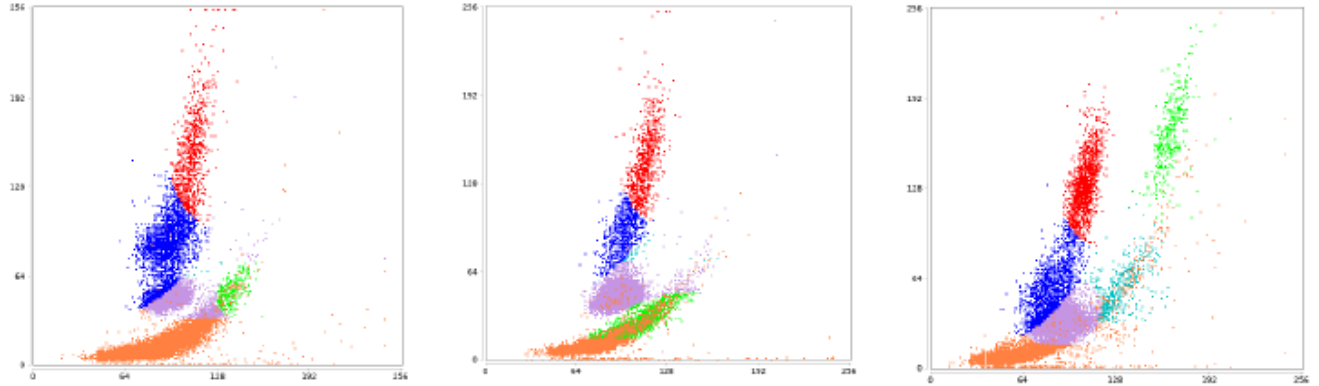


ProCyte Dx – Canine Patient WBC Dot Plot

Some other platelet clumps examples



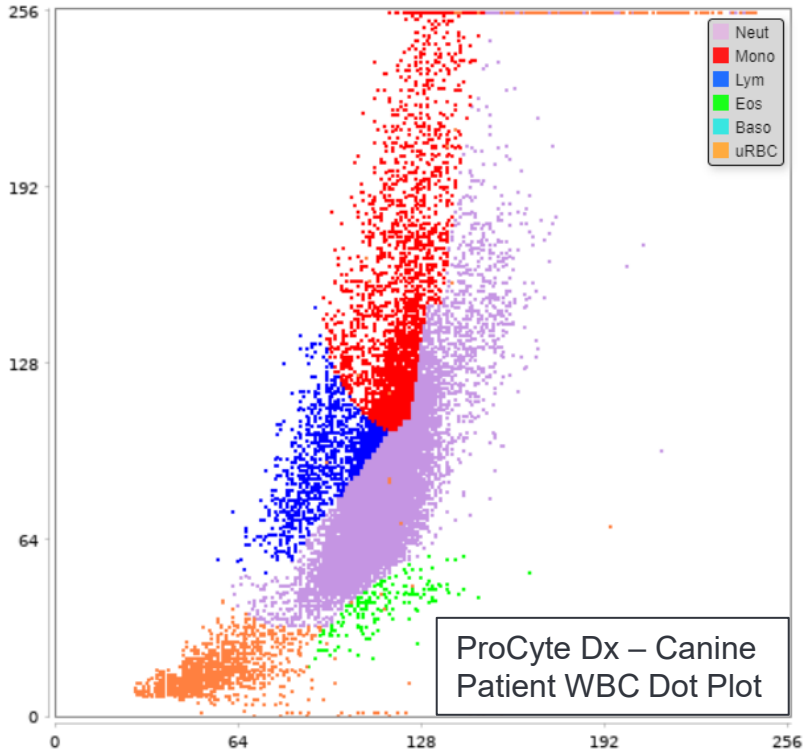
ProCyte Dx – Feline
Patient RBC Dot Plots



ProCyte Dx – Feline
Patient WBC Dot Plots

When should you prepare and review a blood film

+ Anaemic patients
+ High WBC counts
+ Low WBC/Plt counts
+ Flags in dot plots/indistinct separation of clusters
+ Unexpected or suspicious instrument results
+ Investigation of critically ill patient



Bands	*Suspected

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

Immature and/or toxic neutrophils likely present - consider inflammation
```

ASVCP guidelines

Some suggested numerical guidelines for medical review of blood smear and CBC data

	CRITERIA
WBC	Leukopenia < 3 x10e9/L
	Leukocytosis > 30 x10e9/L
	Lymphocytosis > 10 x10e9/L
Platelets	Thrombocytopenia < 100 x10e9/L
	Thrombocytosis > 900 x10e9/L

Modified from ASVCP Guidelines: Quality Assurance for Point-of-Care Testing in Veterinary Medicine Version 1.0 (May 2013)

IDEXX

Points to remember

+ Automated haematology results provide crucial information, and all components need to be evaluated to obtain a complete picture
+ Numerical data may not always be accurate or tell the whole story
+ Assessment of dot plots allows us to obtain valuable additional information
+ Always correlate findings with clinical picture
+ Effective blood smear review is guided by integration of numerical data and dot plots as well as clinical considerations

References:

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IDEXX

Any questions?