This is a composite image of a patients' blood morphology from the IDEXX InVue Dx™ Analyzer





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# The "Art" of Interpreting Blood Morphology:

## Let's paint cells!

Candice Chu, DVM, PhD, DACVP Casey Etter, LVT











- Blood smear introduction, platelet clumps, and others
- PAINT
- White cell abnormalities, red cell abnormalities
- PAINT
- Case study
- PAINT and prizes awarded





Veterinary Medicine & Biomedical Sciences

# Vet Clip Path Professor

#### Candice Chu, DVM, PhD, DACVP

#### Experience

2008-2013 DVM, National Taiwan University 2014-2018 PhD, Texas A&M University 2018-2021 Clinical Pathology Residency, Texas A&M 2021-2023 Assistant Professor, University of Pennsylvania

#### **Current position**

2024- Assistant Professor, Texas A&M University



#### **Conflict of Interest Disclosure**

I have financial interest, arrangement or affiliation with:

Name of Organization EveryCat Health Foundation IDEXX <u>Relationship</u> Grant/Research Support Speaker & Honorarium



# 

### **Thank You**



### **Blood smear review**







#### Blood smear

What are the two main areas to evaluate?





#### Blood smear

What are the two main areas to evaluate?





#### Blood smear

What are the two main areas to evaluate?





#### Comprehensive blood smear review

- 1. Feathered edge (10, 20x objectives)
  - 1. Platelet clumps
  - 2. Mast cells
  - 3. Fungal organisms
- 2. Monolayer (40, 50, 60, 100x objectives)
  - 1. WBC differential count
  - 2. WBC morphology
  - 3. RBC morphology
  - 4. Platelet estimate





#### Comprehensive blood smear review

- 1. Feathered edge (10, 20x objectives)
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  - 1. WBC differential count
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# Platelet clumps



#### Can the analyzer help?

Test	Results	Reference Interval		LOW	NORMAL	HIGH
ProCyte Dx	(September 30,	2016 9:20 AM)	)			
RBC	3.12 M/µL	5.65 - 8.87	LOW			
HCT	23.3 %	37.3 - 61.7	LOW			
HGB	7.8 g/dL	13.1 - 20.5	LOW			
MCV	74.7 fL	61.6 - 73.5	HIGH	2.		
MCH	25.0 pg	21.2 - 25.9		20		
MCHC	33.5 g/dL	32.0 - 37.9		S.		
RDW	18.0 %	13.6 - 21.7		30		8
%RETIC	10.9 %			-		- 100
RETIC	338.8 K/µL	10.0 - 110.0	HIGH	82		
WBC	* 11.56 K/µL	5.05 - 16.76		20		
%NEU	* 72.7 %					
%LYM	* 20.3 %					
%MONO	* 5.7 %					
%EOS	* 1.2 %					
%BASO	* 0.1 %					
NEU	* 8.40 K/µL	2.95 - 11.64		51		
LYM	* 2.35 K/µL	1.05 - 5.10		3		
MONO	* 0.66 K/µL	0.16 - 1.12				
EOS	* 0.14 K/µL	0.06 - 1.23		225		2
BASO	* 0.01 K/µL	0.00 - 0.10		2		
nRBC	* Suspected					
PLT	238 K/µL	148 - 484		2		
MP\/	12.1 fL	8.7 - 13.2		24		
	00.0.0	9.1 - 19.4	HIGH	20		
PDW	22.0 TL					





#### How ProCyte Dx<sup>TM</sup> works

1. Laminar Flow Impedance





#### How ProCyte Dx<sup>TM</sup> works

#### 2. Laser Flow Cytometry





#### ProCyte Dx<sup>TM</sup>: RBC dot plot





#### ProCyte Dx<sup>TM</sup>: RBC dot plot





#### ProCyte Dx<sup>TM</sup>: RBC dot plot





#### ProCyte Dx<sup>TM</sup>: WBC dot plot





#### Is it truly thrombocytopenic?



edge



#### Is it truly thrombocytopenic?





#### Platelet estimate

- 1. 100x oil objective
- 2. Count the number of platelets in 5 **monolayer** fields
- 3. Get an average number of platelets x 15,000-20,000
- For example:
- I saw 1, 2, 2, 1, 4 in 5 fields
- (1+2+2+1+4)/5 = 10/5 = 2
- 2\*15,000 = 30,000
- 2\*20,000 = 40,000
- Platelet estimate: 30,000-40,000

Normal platelet count: At least 200,000 = Roughly 13 per 100x field



#### Finding the monolayer





#### Finding the monolayer





#### Finding the monolayer





#### Platelet estimate in monolayer





# Mast cells



#### Mast cells



- Dog:
  - Acute inflammatory disease (parvoviral infections), inflammatory skin disease, regenerative anemias, neoplasia (MCT or others), and trauma
  - Nonspecific: No longer routinely performed for MCT staging
- Cat:
  - No mast cells seen in healthy or non-MCT ill cats (n=80)
  - 43% of cats with MCT had positive buffy coats, and most of these cats have splenic/visceral MCT
  - If circulating mast cells are seen, do a cancer hunt!


## Something unexpected...



#### 7 y/o MC Blue Point Siamese

	Result	Flag	Reference	Unit	
WBC	4.1	L	5.5 - 19.5	x10^	
Red Blood Cell Count	7.76		5.00 - 10.00	x10^	
Hemoglobin	12.2		8.0 - 15.0	g/dl	
Hematocrit (automated)	36.5		24.0 - 45.0	%	
Packed Cell Volume (Spun)	37			%	
Mean Corpuscular Volume	47.0		39.0 - 55.0	fl.	
Mean Corpuscular Hemoglobin Concentration	33.4		31.0 - 35.0	g/dl	
Plasma Protein	8.6	Н	6 - 8	TS-g/	
Fibrinogen (heat precipitation)	2			mg/dl	
Platelet Count (Automated)	126000	L	300000 - 800000	/ul	
Segmented Neutrophils	59		35 - 75	%	
Absolute Neutrophil	2419	L	2500 - 12500		
Band	2		0 - 3	%	
Absolute Bands	82		0 - 300		
Metamyelocyte	2			%	
Absolute Metamyelocyte					
Lymphocytes	30		20 - 55	%	
Absolute Lymphocyte	1230	L	1500 - 7000		
Monocytes	7	H	1 - 4	%	
Absolute Monocyte	287		0 - 850		
Eosinophil	2		2 - 12	%	
Absolute Eosinophil	82		0 - 1500		

#### 7 y/o MC Blue Point Siamese - Histoplasma





#### Comprehensive blood smear review

- 1. Feathered edge (10, 20x objectives)
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#### IDEXX inVue Dx<sup>™</sup> Cellular Analyzer



















#### IDEXX inVue Dx<sup>™</sup> Cellular Analyzer



# inVue Dx Provides: automated quantification, classification, and interpretation of blood morphology





Delivering reference lab-quality results in a real-time environment

• (*	Canine   Br	ARK 203AB ussels Griffon   F	Patient Management ¥ Female   8y						Order Nev	v Diagno
20	24 Jan 27 Jan	n 27								
Result	Details 🛩				Add to Order	•	~	¢		0
💧 He	ematology	1/27/24 9:43 AM			1/27/ 9:43	24 AM				
H 55	RBC	a. 1.09	5.65 - 8.87 M/µL	4	1.09					
H 55	Hematorcrit	b. <mark>9.8</mark>	37.3 - 61.7 %	۲	9.8					
88	Spherocytes	60% (Mark	ed)							
88	Agglutination	Present								
88	% Reticulocyte	17.0	56		17.0					
H 55	Reticulocytes	184.8	10.0 - 110.0 K/µL		184.	3				
H 55	WBC	c. 43.20	5.05 - 16.76 K/µL		43.2					
88	% Neutrophils	69.5	%		*69.3	2				
**	% Immature Neutrophils	18.5	%							
88	% Lymphocytes	1.9	%		*21.0	5				
88	% Monocytes	9.7	%		*8.9					
88	% Eosinophils	0.2	%		0.2					
88	% Basophils	0.1	%		0.1					
H 55	Neutrophils	30.02	2.95 - 11.64 K/µL		*29.	19				
88	Immature Neutrophils	7.99	K/pL							
H 55	Lymphocytes	0.84	1.05 - 5.10 K/µL		*9.3	3				
H 55	Monocytes	4.20	0.16 - 1.12 K/µL		*3.8	3				
m 55	Eosinophils	0.09	0.06 - 1.23 K/µL		0.09					
88 Y.	Basophils	0.03	0.00 - 0.10 K/µL		0.03					
88	Platelet Estimate	50-100 K/μ	L (Moderately decreased)					_		
	Diagnostic Considerations	The presen strongly sup features ind dysfunction as infection drugs/vacci	ce of regenerative anemia, spher ggestive of immune-mediated he Jude icterus, hyperbilirubinemia/ I), or hemoglobinemia/uria. Inves , neoplasia, concurrent inflamma nes.	ocytosis, and RBC agglutii molytic anemia. Other clir /bilirubinuria (in the abser tigate for underlying caus itory conditions, or history	ation are ical ice of liver es such e of recent					
		This platele platelets wi platelet cor from the bo unexpected thrombocy	t estimate incorporates enumera thin clumps. Moderately decreas isumption, immune-mediated de one marrow, and sequestration in f, please redraw a new sample to topenia (e.g., clot in the blood tub	tion of individual platelet: ed platelets may be seen struction, decreased prod n the spleen. If this finding rule out artifactual pe).	and with uction is					
	Images									

## Let's paint!



#### Tips and Tricks

- Create your "donuts" using your red and orange paint, dilute paint with water if you want your donuts too be semitransparent.
- Work on painting your reds and oranges and remember you can always add black paint back in to make your "donut holes"!
- You can paint the image in its entirety or only a part of the image – totally up to you!



## White blood cells



#### • Neutrophil

- Most numerous
- Colorless cytoplasm
- Segmented nucleus

#### Monocyte

- Bigger than neutrophils
- Gray-blue cytoplasm
- Pleomorphic nucleus



- Lymphocyte
- Single, round nucleus
- Minimal cytoplasm
- Neoplastic lymphocyte
- Larger
- Basophilic cytoplasm
- More numerous
- Nucleolar ring



- Eosinophil
- Eosinophilic granules
- Lobulated nucleus





- Basophil
- Basophilic granules
- Lobulated nucleus





#### ProCyte Dx<sup>TM</sup>: WBC dot plot





## White blood abnormalities



#### 10-year-old, female spayed, DSH cat

- Ongoing issue D+, V+ Occasionally, lethargic, hair loss, weight loss.
- Primary thinks cancer was waiting on u/s but worsened tonight - not eating, hardly moving, isolating



#### 10-year-old, female spayed, DSH cat

- Severe nonregenerative anemia (HCT 10.4%, Retic 0.4%)
- Bands suspected
- Severe monocytosis (5.71 K/uL)
- Eosinopenia (0.06 K/uL)
- Thrombocytopenia (PLT 34 K/uL)



#### 10-year-old, female spayed, DSH cat























#### IDEXX inVue Dx<sup>™</sup> Cellular Analyzer





# IDEXX inVue Dx<sup>™</sup> Cellular Analyzer Different light channels

- Large immature mononuclear cell
- Bottom left: see nuclear chromatin features
- Bottom right: arrow points to nucleolus



















## **Red blood cell abnormalities**



- Presents for lethargy and poor appetite
- Exam findings:
  - Quiet, alert, and responsive
  - Pale and icteric
  - Tachycardic



- Severe regenerative anemia (HCT 9.8%, Retic 17% = 184.8 K/uL)
- Marked leukocytosis (43.2 K/uL)
  - Neutrophilia (30 K/uL)
  - Suspected Band
  - Lymphocytosis (9.34 K/uL)
  - Monocytosis (3.85 K/uL)
- Thrombocytopenia (PLT 60 K/uL)









#### Immune-mediated hemolytic anemia (IMHA)



#### IDEXX inVue Dx<sup>™</sup> Cellular Analyzer








#### Spherocytes



Spherocyte



Normal canine RBC





#### Spherocytes



Spherocyte



Normal canine RBC

microscope

78



#### Reticulocytes



Reticulocyte



Normal canine RBC





#### Reticulocytes



#### Reticulocyte



Normal canine RBC

# IDEXX inVue Dx - Reticulocyte

#### Florescent – RNA



#### Composite







#### nRBC



nRBC



Normal canine RBC





#### nRBC



nRBC

#### Normal canine RBC

#### microscope



#### IDEXX inVue Dx - Reticulocyte





#### Red Blood Cells

CREATING CLARITY

Reference (Blood Smear)	Bright 1	Bright 2	Fluorescent 1	Fluorescent 2	Composite
	0	0			
		Ø		$\odot$	
	980				

# Let's paint!



# **Case study**



# Becky: 4 y/o FS Australian Shepherd dog

- Presents for vomiting, diarrhea, lethargy following eating something on a walk
- Previous hx of anaplasma positive
- Exam findings: T 104.8F, mildly icteric mmbr, markedly injected sclera and mild hyphema OU, port-colored urine, melena





## Initial diagnostic tests

- A/T/CFAST: negative
- Chem/SDMA/Lytes largely unremarkable
  - but T.bili13.3 mg/dl, K+ 3.3 meq/L, and hemoconcentration
- Markedly hemolyzed serum
- UA: USG 1.048, Black colored, produced a clot, Marked RBCs
- IOPs 14, 13 mm hg (WNL)
- Saline agglutination: negative
- PT/PTT: 19/106 seconds (mildly prolonged)
- Tick PCR sent out



#### **CBC** Results

Becky

#### Normal

• No anemia

Scattered plot:

- Challenged separation between platelets and RBCs
- Small pathologic RBCs
- Ghost RBC?







#### **CBC** Results

- No anemia
- Severely leukopenia
  - Severe neutropenia
  - Bands suspected
  - Moderate lymphopenia
  - Eosinopenia





Normal





## IDEXX inVue Dx<sup>™</sup> Cellular Analyzer





0

(ma)

63

SC

-

6

12

# Ghost cells

1

6

C

2

U

0

a

# Ghost cells

# Ghost cells

# **IDEXX InVue Dx Results**

- EccentrocytesGhost cells

## Eccentrocytes – oxidative damage



- Ingestion of onion and garlic (may contain in certain flavors of baby food)
- Acetaminophen toxicity
- Use of propofol
- Hyperthyroidism
- Lymphoma
- Diabetic mellitus



### Becky Case Summary

- Suspected toxin ingestion resulting in acute vasculitis and oxidative injury leading to regenerative anemia
- Resolved with time, antiinflammatory medication, and supportive care





### Conclusion – Blood morphology is important!



#### PLT/WBC

- Platelet clumps
- Mast cells
- Histoplasma
- Neoplastic cells

#### RBC

- Autoagglutination
- Reticulocytes
- Spherocytes
- nRBC
- Eccentrocytes



#### IDEXX inVue Dx

- Florescent images
- Integrated report
- Ready in 10 mins



#### QUESTIONS?



- Candice P. Chu, DVM, PhD, DACVP
- Assistant Professor, Texas A&M University
- cchu@cvm.tamu.edu





# Let's finish the painting!





# hie frank god, with all our hearts

#### Please rate your speaker and session in the app!



# **Additional slides**



# **Red blood cell abnormalities**



## 8-year-old, male neutered French bull dog

- Presents for vomiting and collapsing
- Exam findings:
  - Muffled heart sounds
  - CFAST mild pericardial effusion and heart based mass



#### Phil

- 8-year-old, male neutered French bull dog
- Presents for vomiting and collapsing
- Exam findings:
  - Muffled heart sounds
  - CFAST mild pericardial effusion and heart based mass





#### CBC Results – Phil RBC Findings







Test	Result	Reference Interval
RBC	5.18 M/uL	5.65 - 8.87 Low
нст	31.9 %	37.3 - 61.7 Low
HGB	11.3 g/dL	13.1 - 20.5 Low
MCV	61.6 fL	61.6 - 73.5
мсн	21.8 pg	21.2 - 25.9
мснс	35.4 g/dL	32 - 37.9
%RETIC	3.40 %	
RETIC	176.1 K/uL	10 - 110 High
RET-He	21.9 pg	

Mild regenerative anemia Small pathologic RBCs


#### CBC Results - Rico WBC Findings





WBC	8.66 K/uL	5.05 - 16.76
%NEU	83.1 %	
%LYM	10.6 %	
%MONO	6.0 %	
%EOS	0.1 %	
%BASO	0.2 %	
%BAND		
NEU	7.19 K/uL	2.95 - 11.64
LYM	0.92 K/uL	1.05 - 5.1 Low
ΜΟΝΟ	0.52 K/uL	0.16 - 1.12
EOS	0.01 K/uL	0.06 - 1.23 Low
BASO	0.02 K/uL	0 - 0.1

#### Mild lymphopenia

- Mild eosinopenia
- nRBCs suspected



#### CBC Results - Rico Platelet Findings





PLT	78 K/uL	148 - 484 Low
MPV	15.5 fL	8.7 - 13.2 High
PDW	fL	9.1 - 19.4
DOT	0 12 %	0.14 - 0.46 Low

#### Mild thrombocytopenia



#### Blood film results

Polychromasia

Acanthocytes

Keratocytes

nRBC



#### Blood film results

Polychromasia

Acanthocytes

Schistocytes

Blister cell



IDEXX inVue Dx

Schistocyte Acanthocyte



#### IDEXX inVue Dx results

Acanthocytes





#### IDEXX inVue Dx results

Schistocytes





## IDEXX inVue Dx Reticulocyte

- Florescent RNA
- Composite





### IDEXX inVue Dx nRBC

- Bright
- Florescent DNA
- Composite





#### Phil Case Summary

• 8-year-old, male neutered French bull dog

CFAST – mild pericardial effusion and heartbased mass IDEXX inVue Dx results reveal fragmentation injury to RBCs





Elliptocyte

Echinocyte

Eccentrocyte

**Blister Cells** 

Acanthocytes









Bright 1















# IDEXX inVue Dx- Innovating Point of Care Cytology





# hie frank god, with all our hearts

#### Please rate your speaker and session in the app!

