



Why Do I Need to Look at a Blood Film Regardless of What Type of Hematology Analyzer I Use?

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Hematology

- Hematology is only one part of the complete laboratory data profile



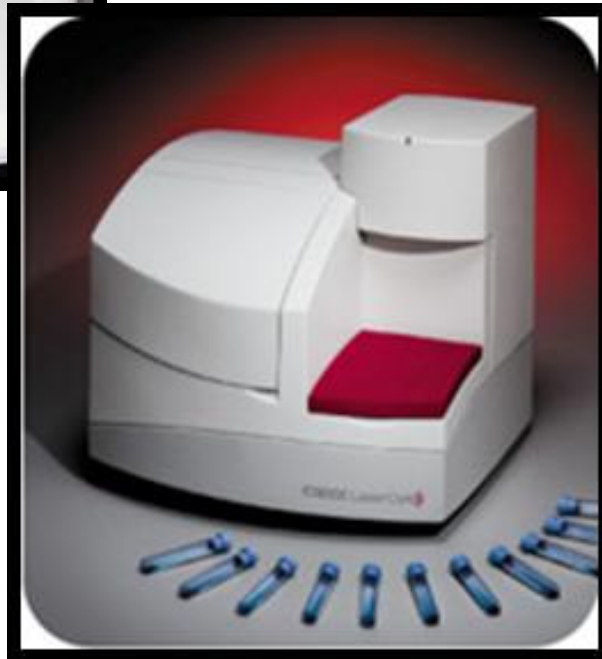
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IDEXX
LABORATORIES

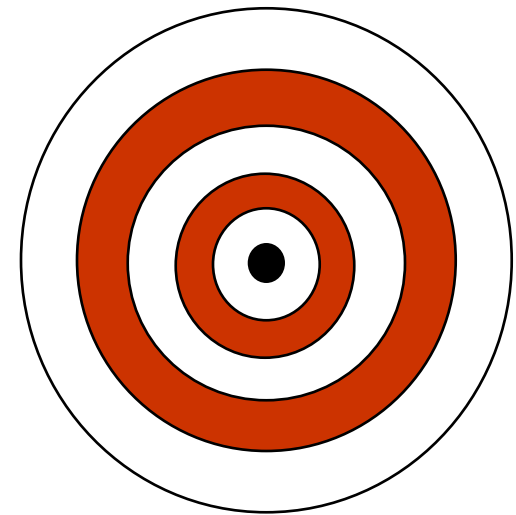
Advanced Automated Instrumentation



Impedance Analyzers



Flow Cytometry (laser)



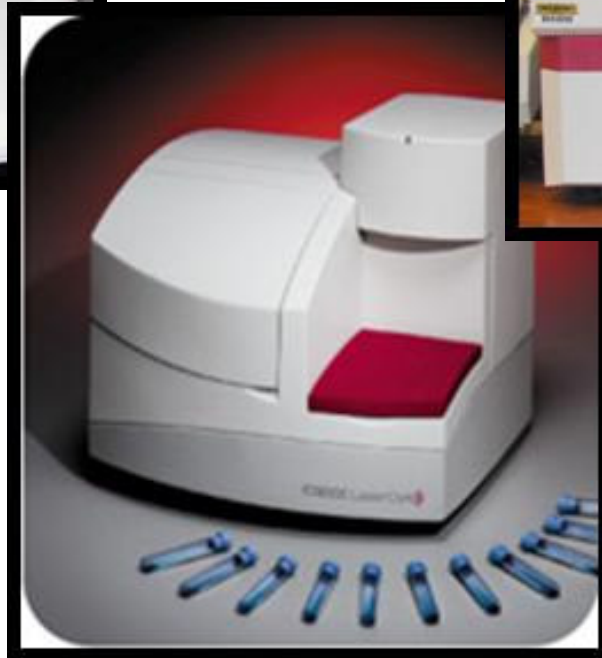
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Advanced Automated Instrumentation



Impedance Analyzers

Reference Lab Analyzers



Flow Cytometry (laser)

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Hematology – Is Blood Film Needed?

- **ABSOLUTELY YES !!**
 - Blood film examination is needed for all ..
 - Low-end hematology analyzers
QBC VetAutoread, Impedance-based instruments
 - High-end hematology analyzers
LaserCyte (in-house)
Cell-Dyn (reference lab)
Advia (reference lab)

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IDEXX
LABORATORIES

Hematology – Is Blood Film Needed?

- **ABSOLUTELY YES !!**
 - Blood film examination is needed for all ..
 - Low-end hematology analyzers
QBC VetAutoread, Impedance-based instruments
 - High-end hematology analyzers
LaserCyte (in-house)
Cell-Dyn (reference lab)
Advia (reference lab)
 - 1-3 minute maximum time on scope
 - Validate data
 - Provide morphology comments

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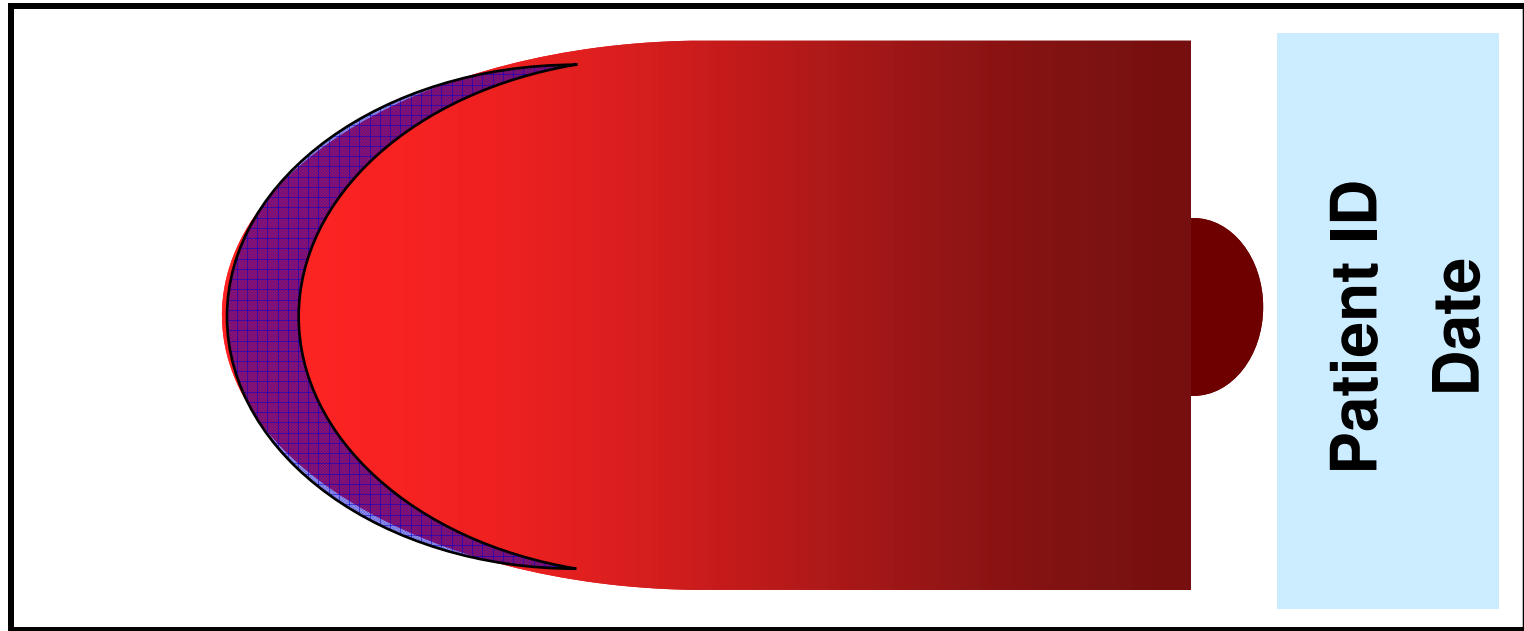
Peripheral Blood Film Preparation

- 30 – 45 degree angle
 - Increased angle with low PCV
 - Decreased angle with high PCV
- Fluid controlled motion
- Results
 - Body
 - Monolayer
 - Feathered edge



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Anatomy of the Peripheral Blood Film

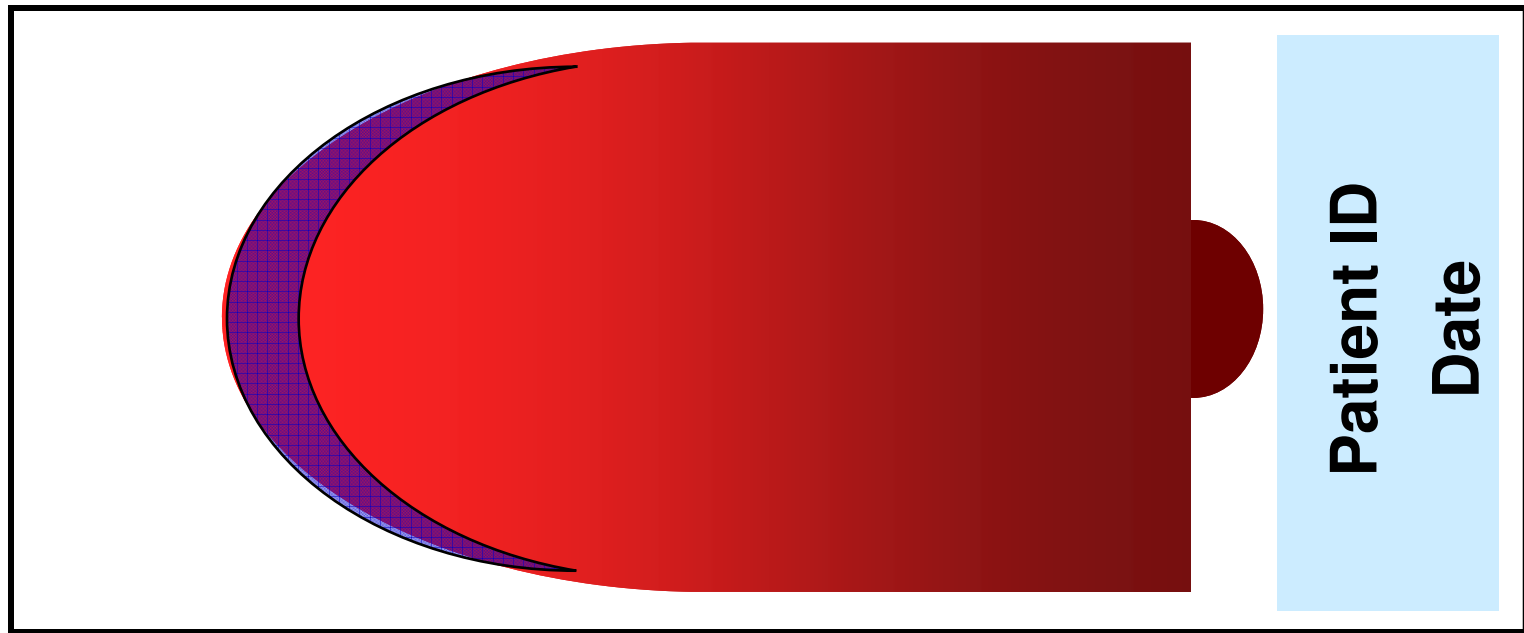


Feathered Edge

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LABORATORIES

Anatomy of the Peripheral Blood Film

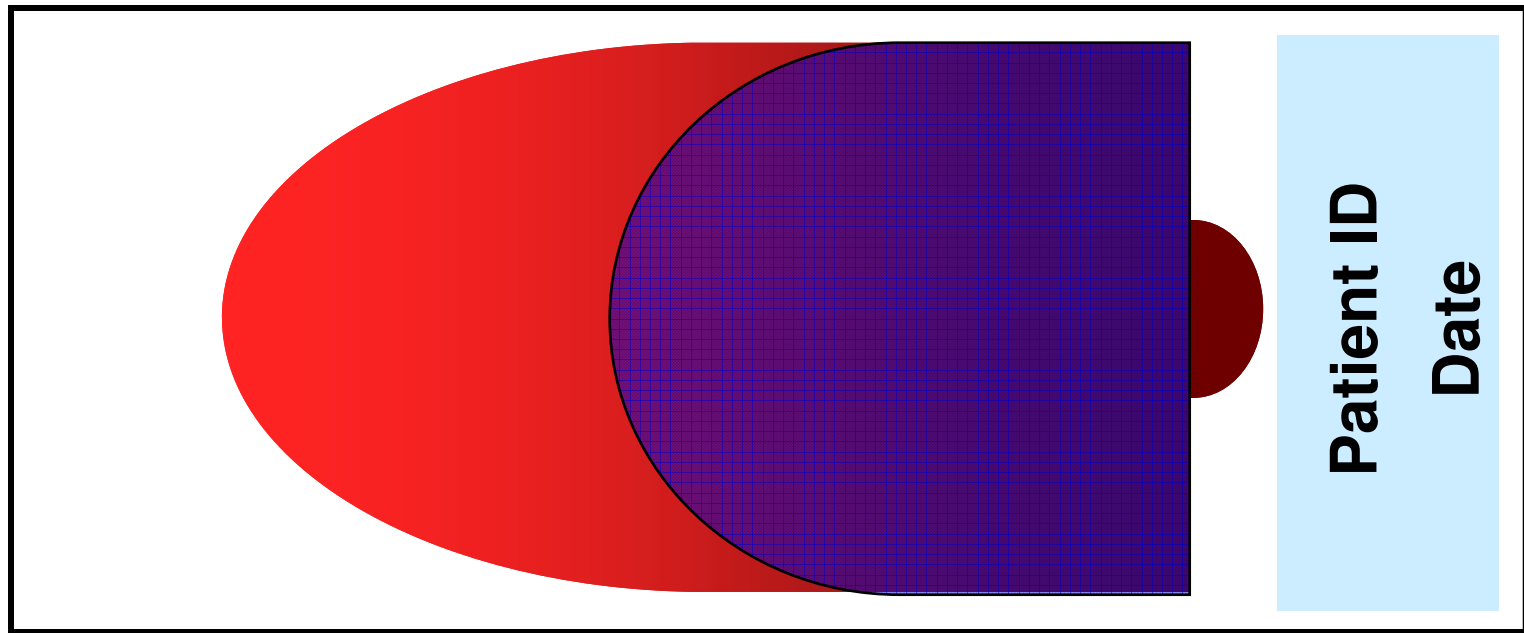


Feathered Edge

- Platelet clumps
- Large cells
- Microfilaria

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Anatomy of the Peripheral Blood Film

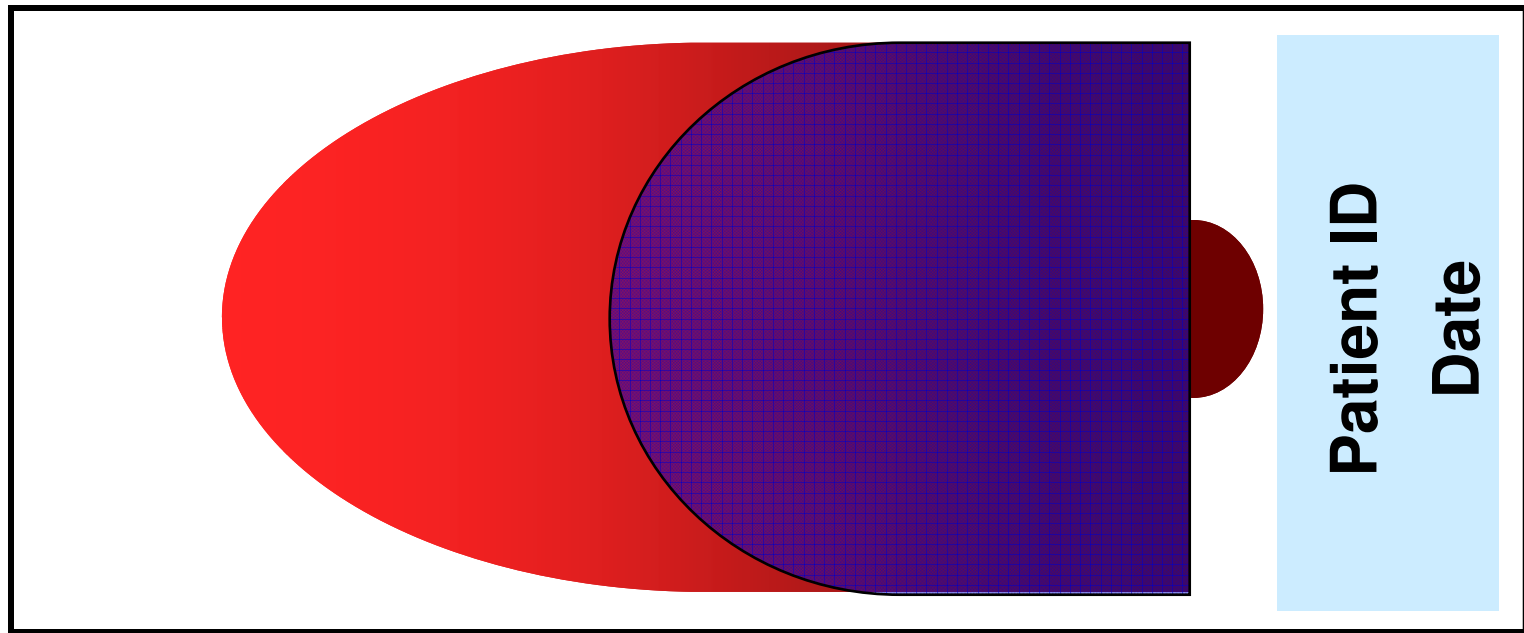


Body

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LABORATORIES

Anatomy of the Peripheral Blood Film

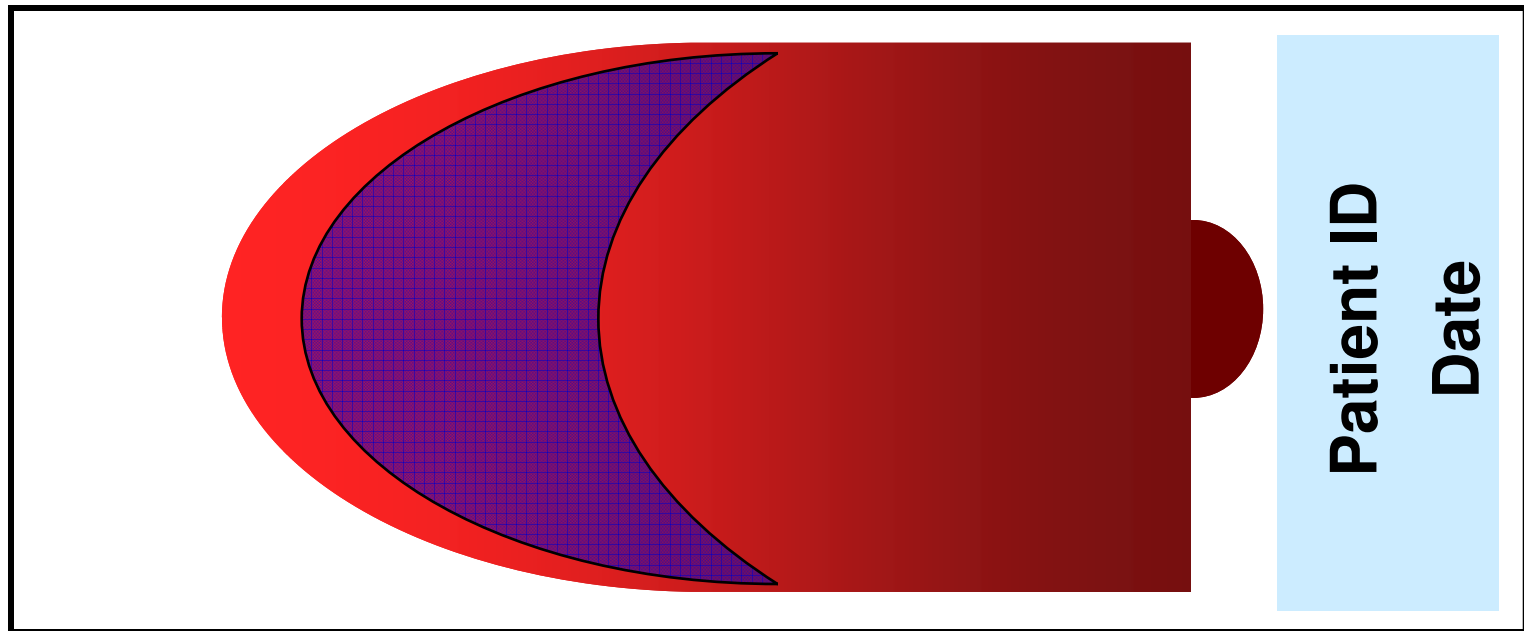


Body

- Rouleaux
- Agglutination

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Anatomy of the Peripheral Blood Film



Monolayer

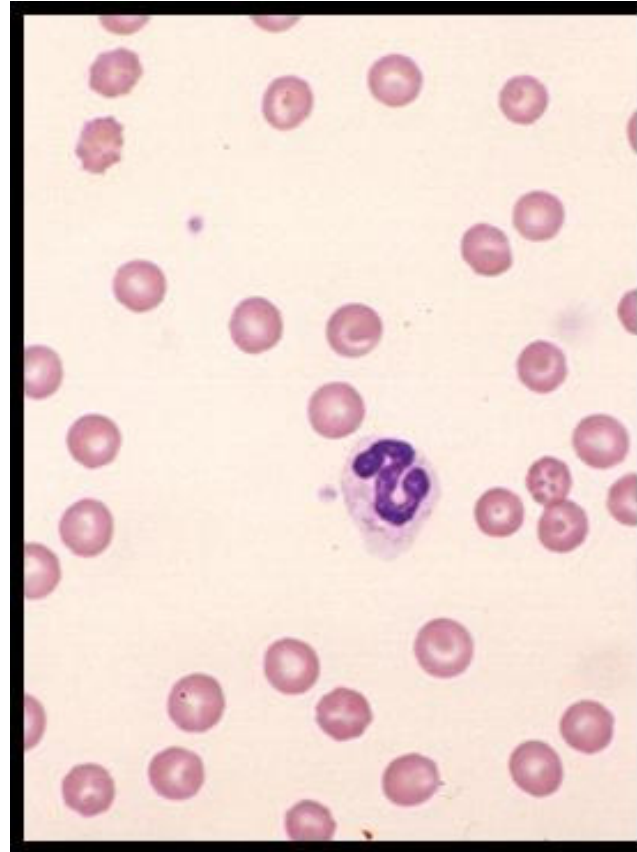
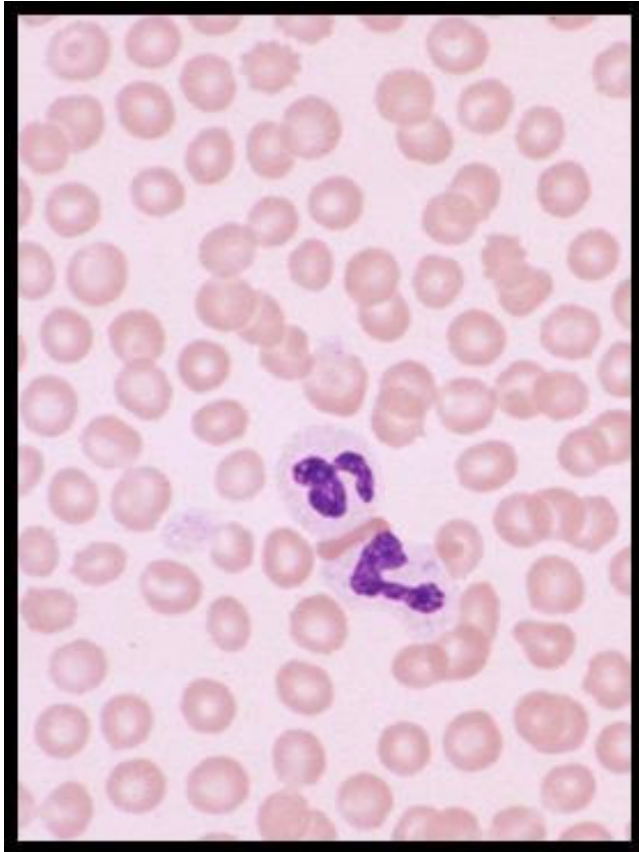
- Platelet number estimation
- Leukocyte number estimation
- Morphologic evaluation
- Data validation

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Erythron – Validate Data

- Validate numerical data generated with instruments
 - Red blood cell mass



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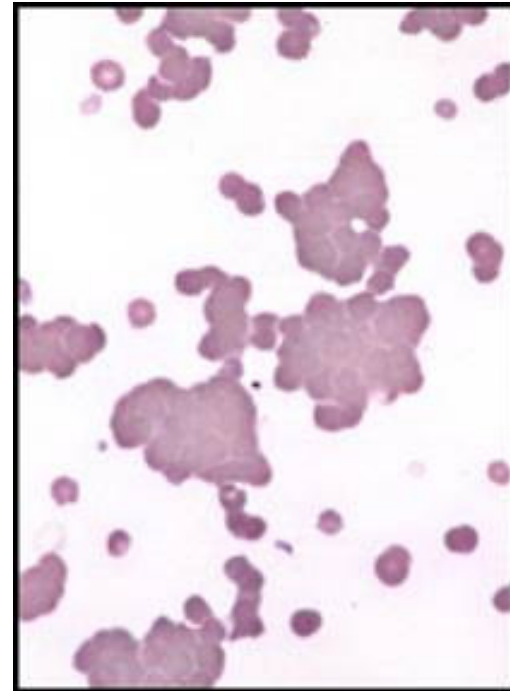
IDEXX
LABORATORIES

Erythron – Validate Data

- Erythrocytes
 - Confirm count – clumping / agglutination



Gross Agglutination



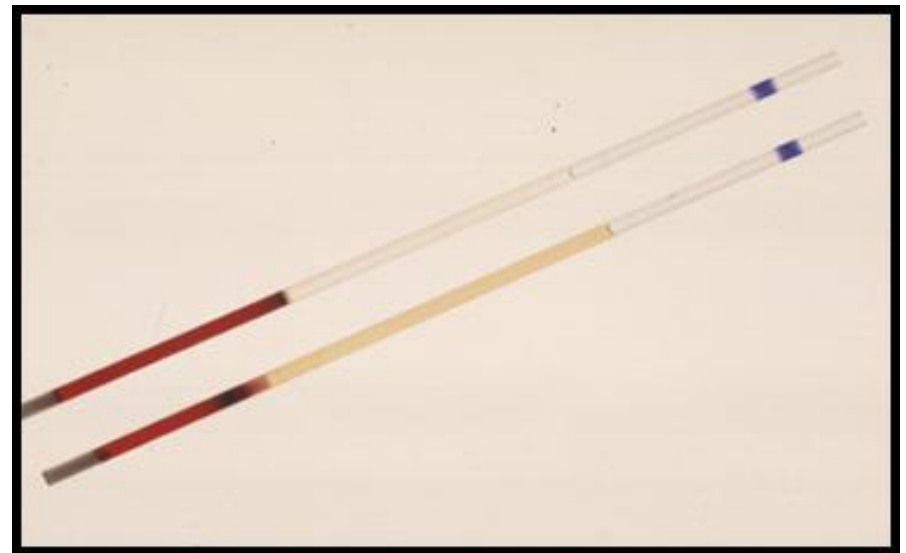
Microscopic
Agglutination

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Erythron

RBC = 3.84 x10¹²/L (5.50 - 8.50)
HCT = 28.5 % (37.0 - 55.0)
HGB = 8.8 g/dL (12.0 - 18.0)

Measure of RBC mass –
severity of anemia



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IDEXX
LABORATORIES

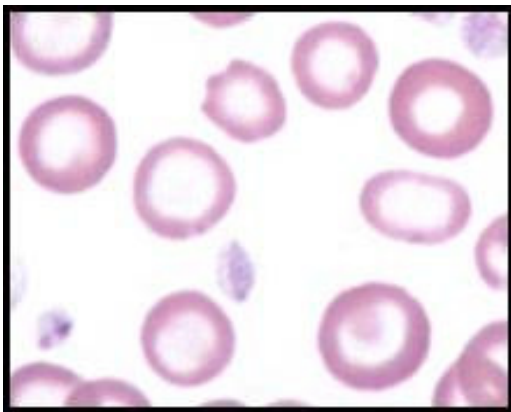
Erythron

RBC	=	3.84	x10 ¹² /L	(5.50 - 8.50)
HCT	=	28.5	%	(37.0 - 55.0)
HGB	=	8.8	g/dL	(12.0 - 18.0)
MCV	=	74.2	fL	(60.0 - 77.0)
MCH	=	23.18	pg	(19.50 - 24.50)
MCHC	=	31.2	g/dL	(31.0 - 37.0)
RDW	=	15.8	%	(14.7 - 17.9)

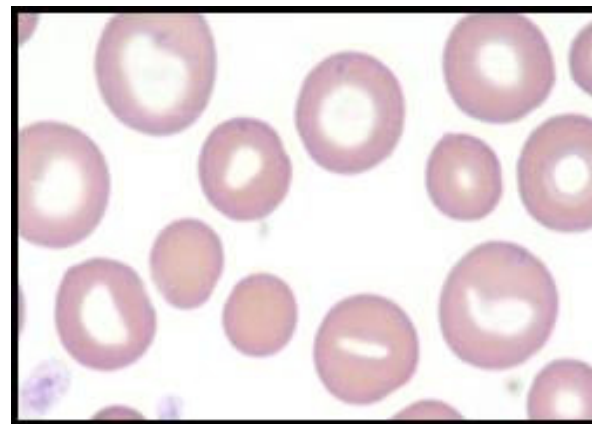
Measure of RBC mass –
severity of anemia

Description of RBC
population

Objective measure of
variation in RBC size



Low MCV, Low MCHC



Anisocytosis

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Erythron

RBC	=	3.84	x10 ¹² /L	(5.50	-	8.50)
HCT	=	28.5	%	(37.0	-	55.0)
HGB	=	8.8	g/dL	(12.0	-	18.0)
MCV	=	74.2	fL	(60.0	-	77.0)
MCH	=	23.18	pg	(19.50	-	24.50)
MCHC	=	31.2	g/dL	(31.0	-	37.0)
RDW	=	15.8	%	(14.7	-	17.9)
%RETIC	=	0.7	%					
RETIC	=	26.9	K/ μ L					

Measure of RBC mass –
severity of anemia

Description of RBC
population

Objective measure of
variation in RBC size

Objective measure of
regeneration

Practice what's possible™

Erythron

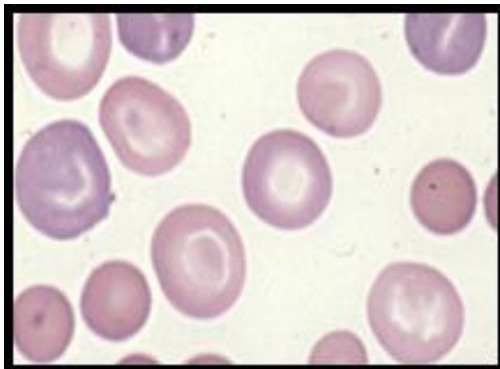
RBC	=	3.84	x10 ¹² /L	(5.50 - 8.50)
HCT	=	28.5	%	(37.0 - 55.0)
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Measure of RBC mass –
severity of anemia

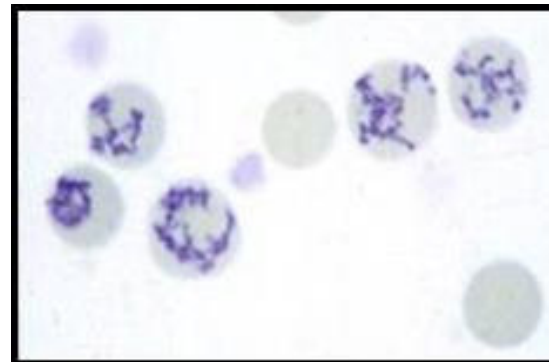
Description of RBC
population

Objective measure of
variation in RBC size

Objective measure of
regeneration



Routine Stain



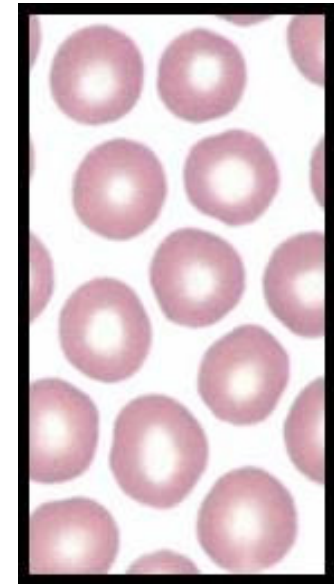
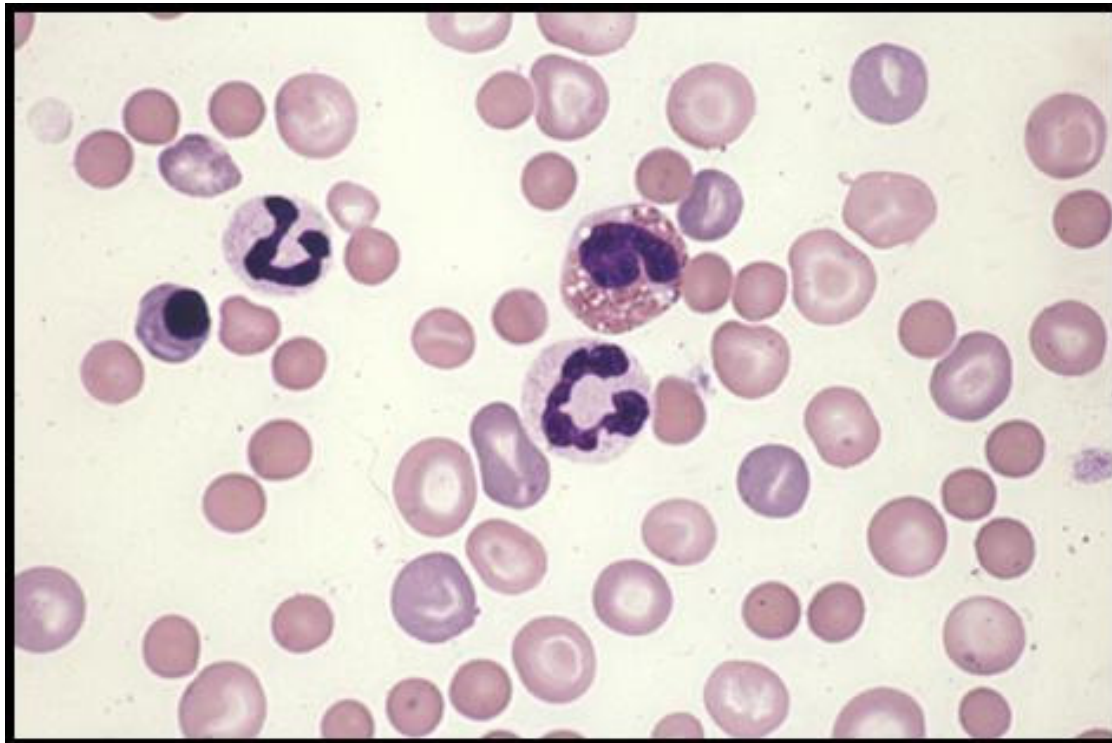
New Methylene Blue

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IDEXX
LABORATORIES

Erythron – Validate Data

- Erythrocytes
 - Confirm count – clumping / agglutination
 - Confirm reticulocyte count with scan



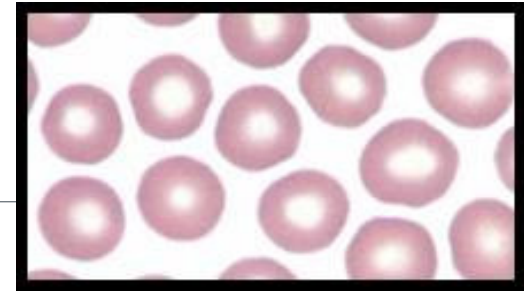
Normal
No significant
Polychromasia

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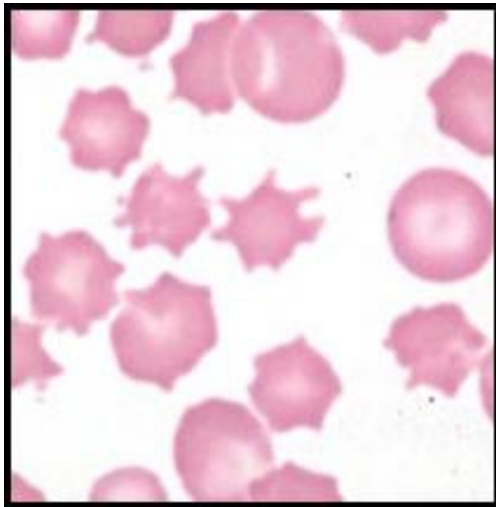
IDEXX
LABORATORIES

Erythron – Validate Data

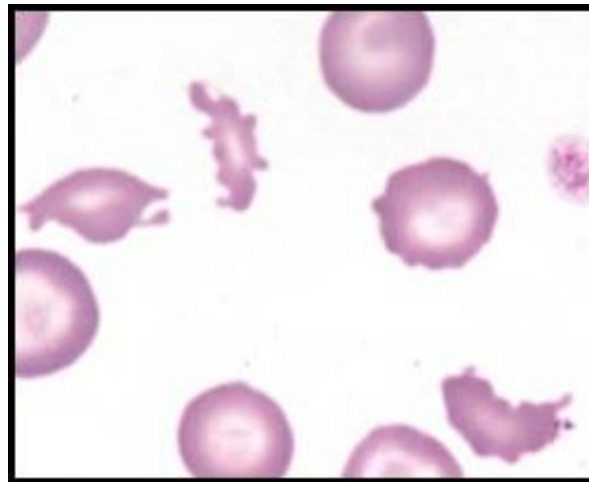
- Erythrocytes
 - Confirm count – clumping / agglutination
 - Confirm reticulocyte count with scan
 - Examine morphology of erythrocytes



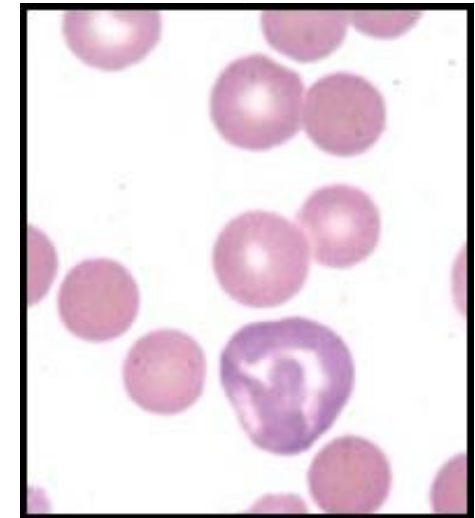
Normal



Acanthocytes



Schistocytes

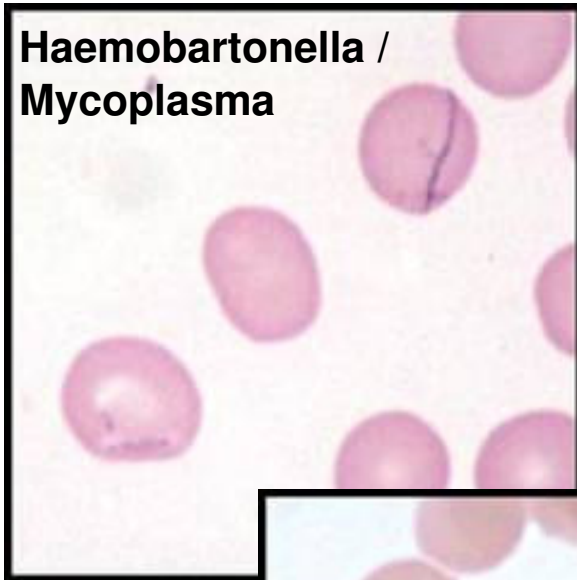


Spherocytes

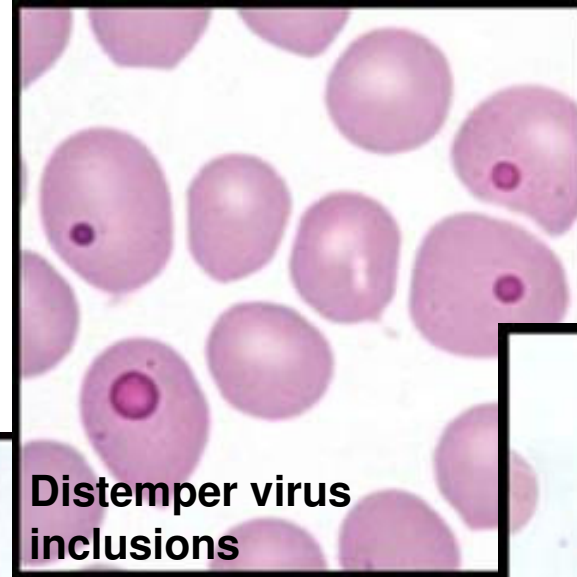
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Miscellaneous Inclusions

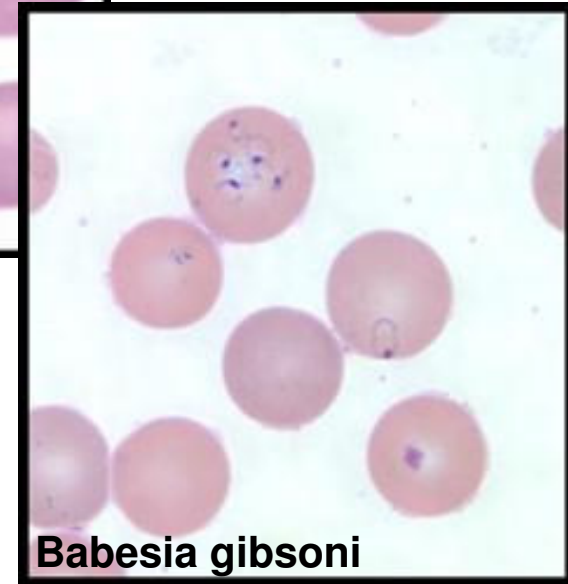
**Haemobartonella /
Mycoplasma**



**Distemper virus
inclusions**



Babesia gibsoni



Basophilic stippling



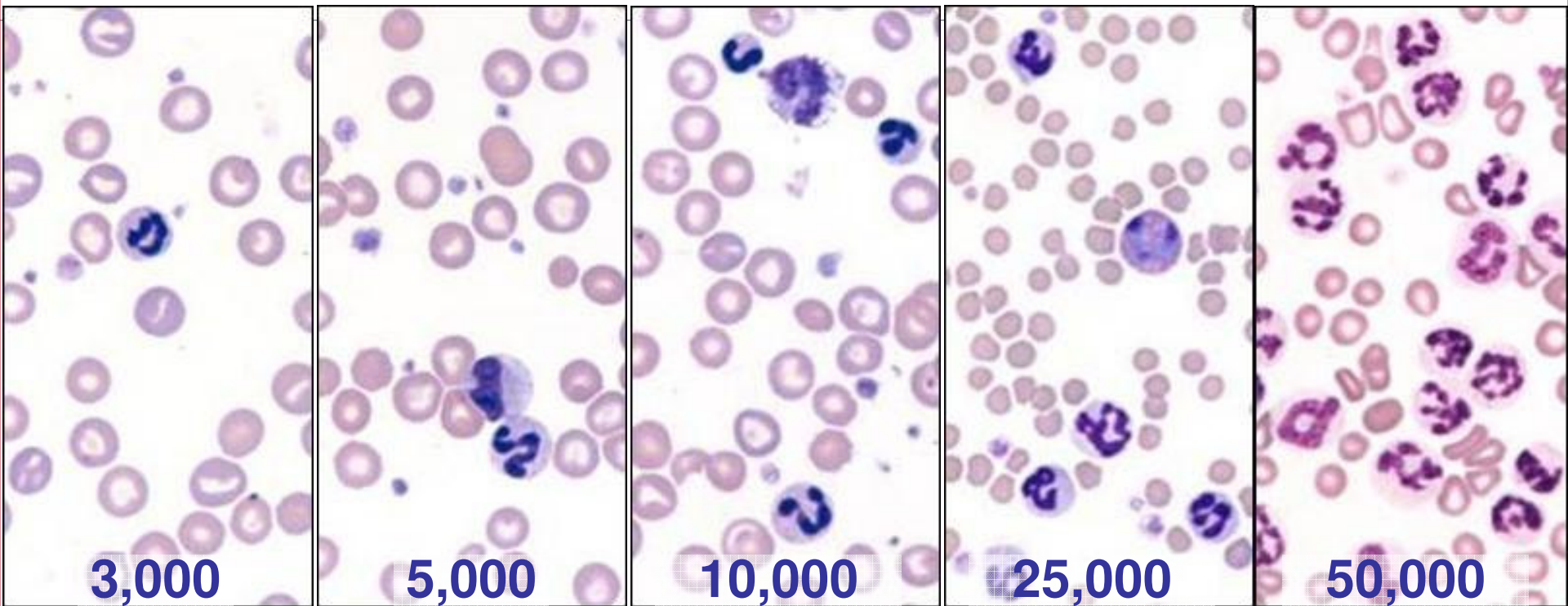
Leukon – Validate Data

- Validate WBC count

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IDEXX
LABORATORIES

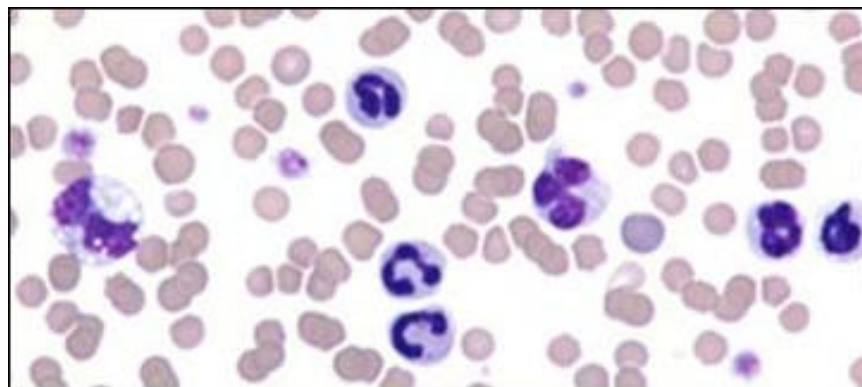
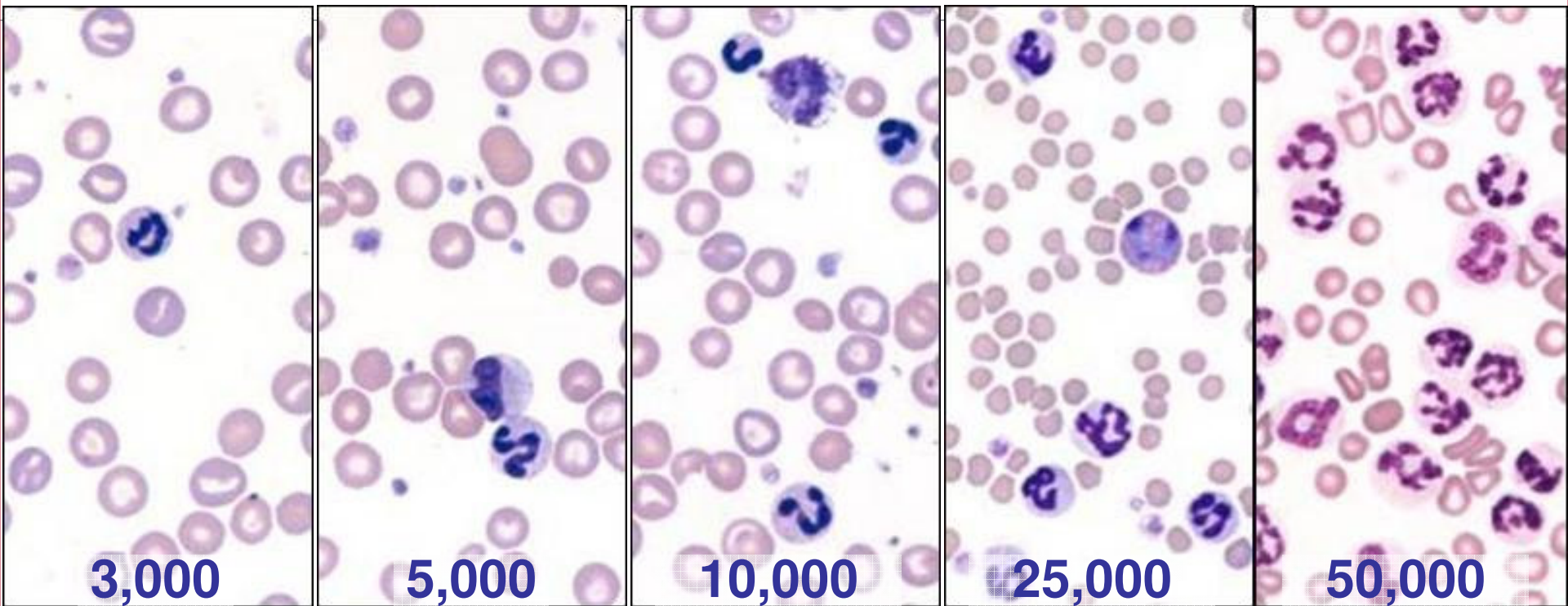
Leukon – Validate WBC Count



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LABORATORIES

Leukon – Validate WBC Count

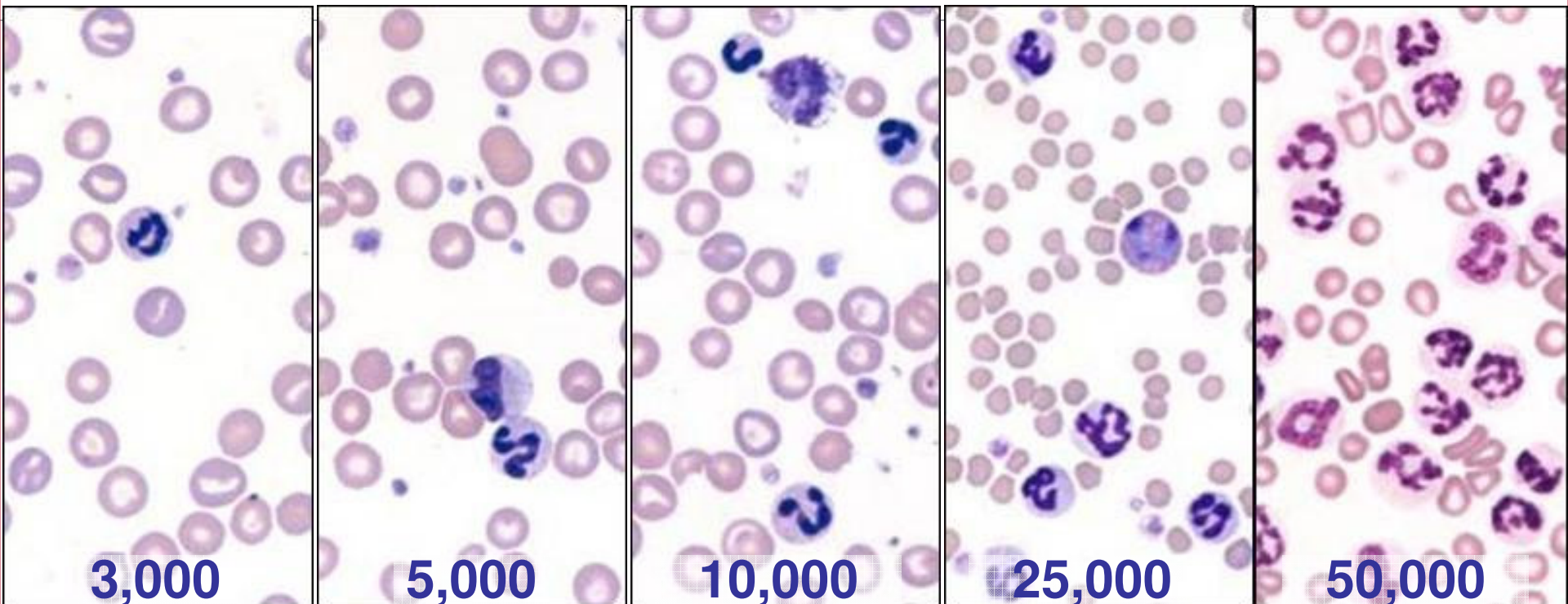


WBC reported
24,000

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Leukon – Validate WBC Count



WBC reported
2,000

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LABORATORIES

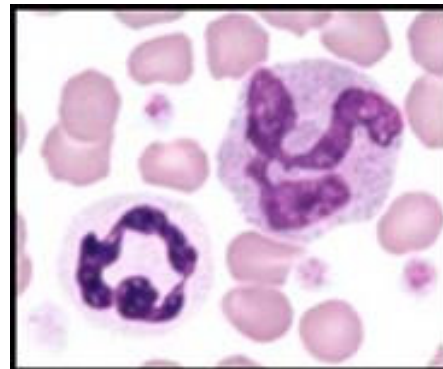
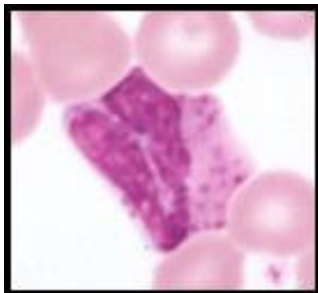
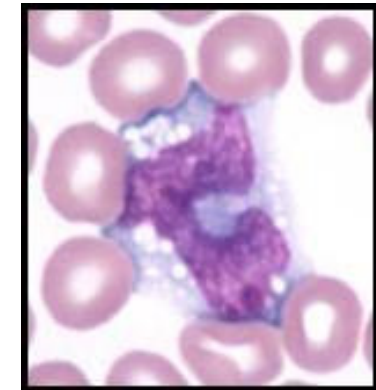
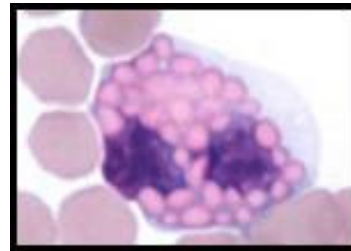
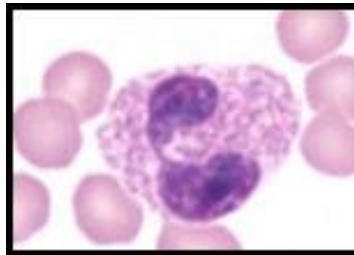
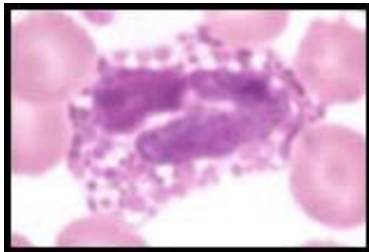
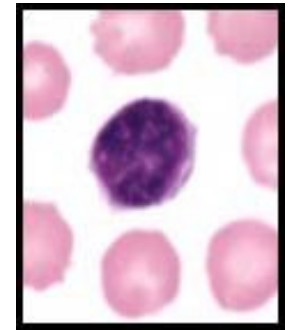
Leukon – Validate Data

- Validate WBC count
- Validate leukocyte distribution

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Five-part Differential

WBC	=	70.60	x10 ⁹ /L	(5.50	-	16.90)
NEU	=	64.25	x10 ⁹ /L	(2.00	-	12.00)
LYM	=	4.94	x10 ⁹ /L	(0.70	-	4.90)
MONO	=	1.41	x10 ⁹ /L	(0.30	-	2.00)
EOS	=	0.00	x10 ⁹ /L	(0.10	-	1.49)
BASO	=	0.00	x10 ⁹ /L	(0.00	-	0.10)



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Inflammatory Leukogram Patterns

Leukocyte Type	Minimal Inflammation	Mild Inflammation	Moderate Inflammation	Established Inflammation	Overwhelming Inflammation
Mature Neutrophil	N	N - ↑	↑ - ↑↑	↑ - ↑↑↑	↓ - ↓↓↓
Band Neutrophil	N	N - ↑	↑ - ↑↑	N - ↑	↑
Lymphocyte	N	N - ↓	↓ - ↓↓	N - ↑	↓↓
Monocyte	N	N - ↑	N - ↑↑	N - ↑	N
Eosinophil	N	N - ↑	↓	N - ↑	↓
Basophil	N	N - ↑	N - ↑	N - ↑	N

Inflammatory Leukogram Patterns

Leukocyte Type	Minimal Inflammation	Mild Inflammation	Moderate Inflammation	Established Inflammation	Overwhelming Inflammation
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Band Neutrophil	N	N - ↑	↑ - ↑↑	N - ↑	↑
Lymphocyte	N	N - ↓	↓ - ↓↓	N - ↑	↓↓
Monocyte	N	N - ↑	N - ↑↑	N - ↑	N
Eosinophil	N	N - ↑	↓	N - ↑	↓
Basophil	N	N - ↑	N - ↑	N - ↑	N

Common Leukogram Patterns

Leukocyte Type	Moderate Inflammation	Glucocorticoids ("Stress")	Epinephrine ("Excitement")
Mature Neutrophil	↑ - ↑↑	↑ - ↑↑	N - ↑
Band Neutrophil	↑ - ↑↑	N	N
Lymphocyte	↓ - ↓↓	↓↓	N - ↑
Monocyte	N - ↑↑	N - ↑	N
Eosinophil	↓	↓	N
Basophil	N - ↑	N	N

Common Leukogram Patterns

Leukocyte Type	Moderate Inflammation	Glucocorticoids ("Stress")	Epinephrine ("Excitement")
Mature Neutrophil	↑ - ↑↑	↑ - ↑↑	N - ↑
Band Neutrophil	↑ - ↑↑	N	N
Lymphocyte	↓ - ↓↓	↓↓	N - ↑
Monocyte	N - ↑↑	N - ↑	N
Eosinophil	↓	↓	N
Basophil	N - ↑	N	N

Confidence Limits for Differential Counts

What range of counts is acceptable when performing a 100 cell manual differential if there are 75% neutrophils?

- A. 74 – 76 %
- B. 72 – 78 %
- C. 70 – 80 %
- D. 65 – 84 %

Confidence Limits - Differential

The 95% confidence limits for various percentages of leukocytes of a given type as determined by differential counts on stained blood smears*†

a	n=100	n=200	n=500	n=1,000
0	0-4	0-2	0-1	0-1
1	0-6	0-4	0-3	0-2
2	0-8	0-6	0-4	1-4
3	0-9	1-7	1-5	2-5
4	1-10	1-8	2-7	2-6
5	1-12	2-10	3-8	3-7
6	2-13	3-11	4-9	4-8
7	2-14	3-12	4-10	5-9
8	3-16	4-13	5-11	6-10
9	4-17	5-14	6-12	7-11
10	4-18	6-16	7-13	8-13
15	8-24	10-21	11-19	12-18
20	12-30	14-27	16-24	17-23
25	16-35	19-32	21-30	22-28
30	21-40	23-37	26-35	27-33
35	25-46	28-43	30-40	32-39
40	30-51	33-48	35-45	36-44
45	35-56	37-53	40-50	41-49
50	39-61	42-58	45-55	46-54
55	44-65	47-63	50-60	51-59
60	49-70	52-67	55-65	56-64
65	54-75	57-72	60-70	61-68
70	60-79	63-77	65-74	67-73
75	65-84	68-81	70-79	72-78
80	70-88	73-86	76-84	77-83
85	76-92	79-90	81-89	82-88
90	82-96	84-94	87-93	87-92
91	83-96	86-95	88-94	89-93
92	84-97	87-96	89-95	90-94
93	86-98	88-97	90-96	91-95
94	87-98	89-97	91-96	92-96
95	88-99	90-98	92-97	93-97
96	90-99	92-99	93-98	94-98
97	91-100	93-99	95-99	95-98
98	92-100	94-100	96-100	96-99
99	94-100	96-100	97-100	98-100
100	96-100	98-100	99-100	99-100

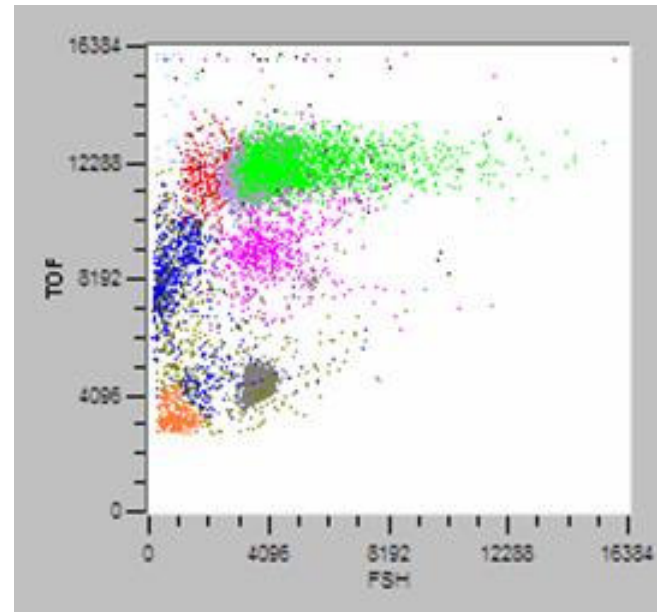
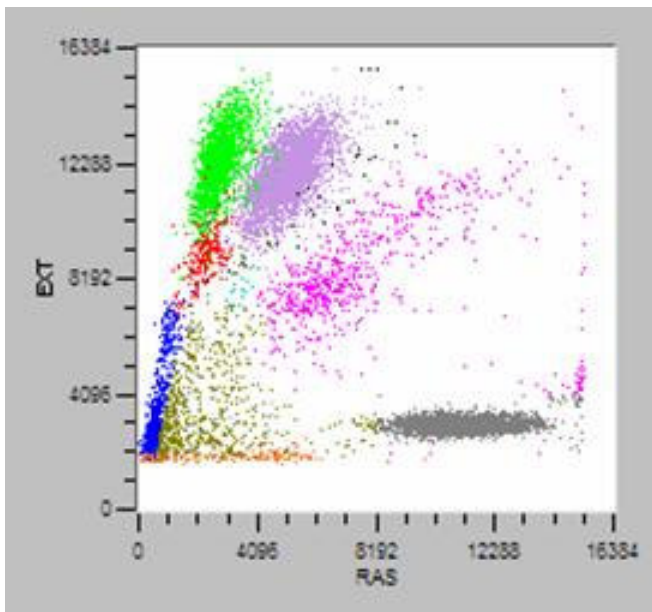
n=	100	200	500	1000
30	21-40	23-37	26-35	27-33
35	25-46	28-43	30-40	32-39
40	30-51	33-48	35-45	36-44
45	35-56	37-53	40-50	41-49
50	39-61	42-58	45-55	46-54
55	44-65	47-63	50-60	51-59
60	49-70	52-67	55-65	56-64
65	54-75	57-72	60-70	61-68
70	60-79	63-77	65-74	67-73
75	65-84	68-81	70-79	72-78
80	70-88	73-86	76-84	77-83
85	76-92	79-90	81-89	82-88
90	82-96	84-94	87-93	87-92
91	83-96	86-95	88-94	89-93
92	84-97	87-96	89-95	90-94
93	86-98	88-97	90-96	91-95
94	87-98	89-97	91-96	92-96
95	88-99	90-98	92-97	93-97
96	90-99	92-99	93-98	94-98
97	91-100	93-99	95-99	95-98
98	92-100	94-100	96-100	96-99
99	94-100	96-100	97-100	98-100
100	96-100	98-100	99-100	99-100

Practice what's possible™

Leukon – Validate Leukocyte Distribution

WBC	=	70.60	x10 ⁹ /L	(5.50	-	16.90)
NEU	=	64.25	x10 ⁹ /L	(2.00	-	12.00)
LYM	=	4.94	x10 ⁹ /L	(0.70	-	4.90)
MONO	=	1.41	x10 ⁹ /L	(0.30	-	2.00)
EOS	=	0.00	x10 ⁹ /L	(0.10	-	1.49)
BASO	=	0.00	x10 ⁹ /L	(0.00	-	0.10)

Leukocyte Distribution
- 5-part differential



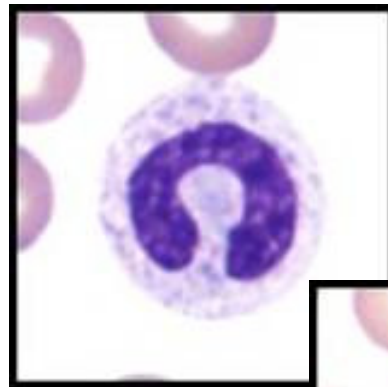
Practice what's possible™

Blood Film – Validate Data

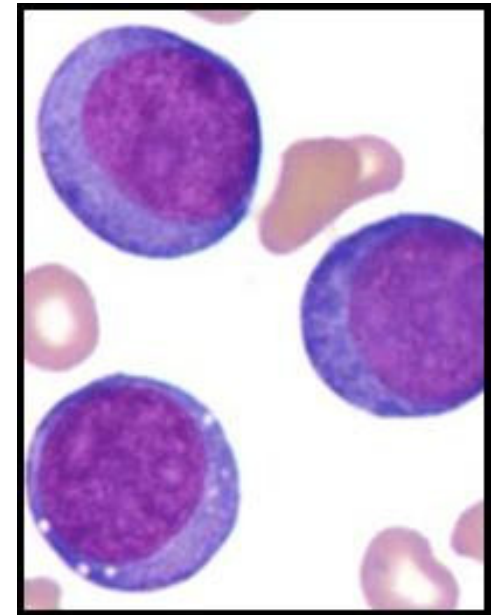
- Validate WBC count
- Validate leukocyte distribution
- Examine WBC morphology



“Left Shift” – Immature Neutrophil Forms



Neutrophil Toxicity



Abnormal Leukocytes

Practice what's possible™

Thrombon – Validate Data

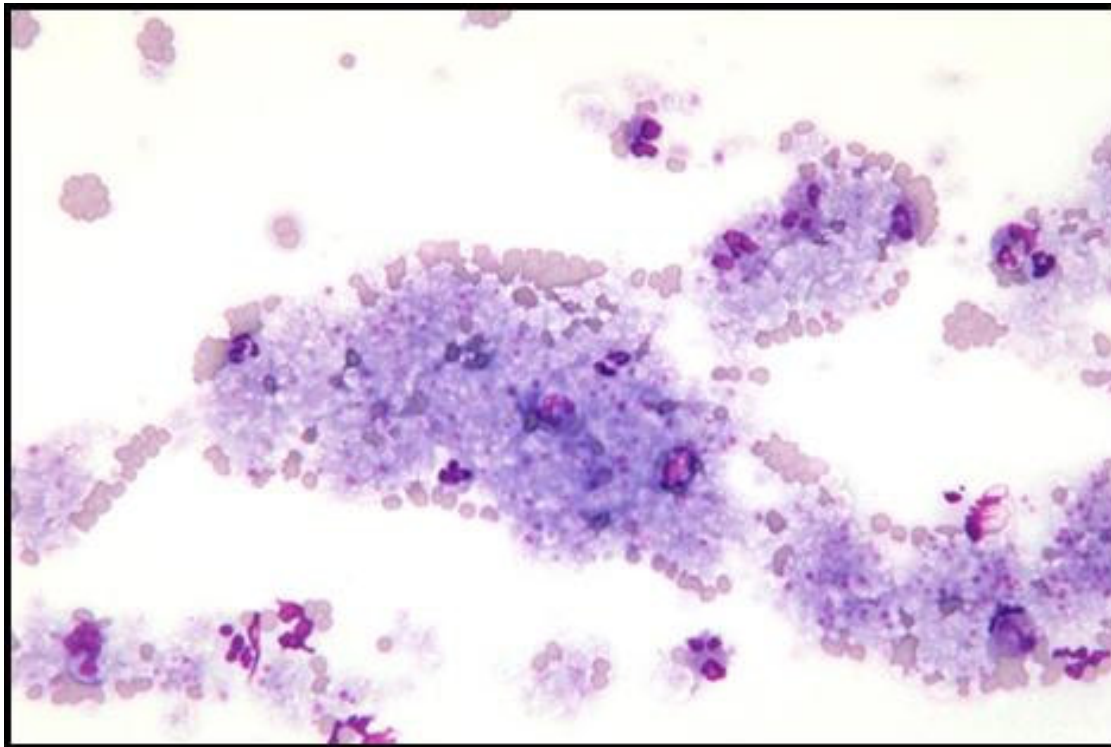
- Platelets
 - Confirm count

Practice what's possible™

IDEXX
LABORATORIES

Thrombon – Validate Data

- Platelets
 - Confirm count
 - Never accept a low platelet count from any analyzer without confirming with blood film

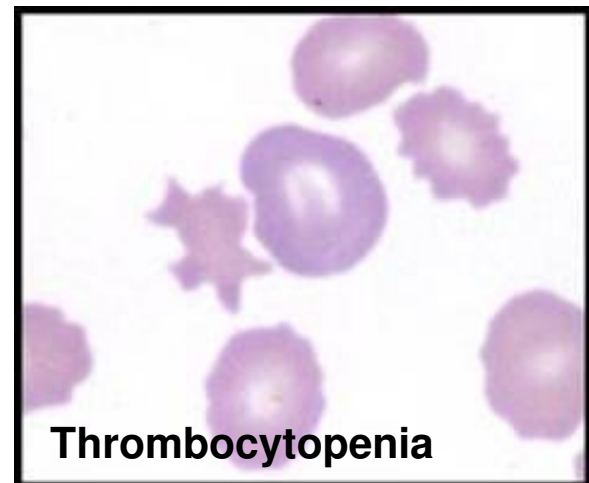
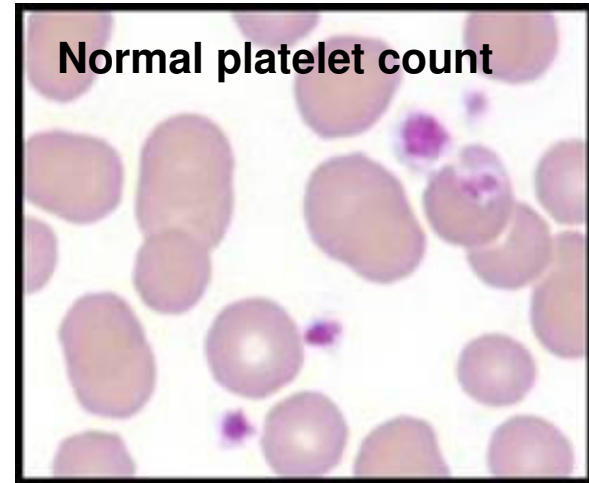


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Platelet Number Evaluation

- Number of platelets per 100x oil objective field of view
 - Minimum: 8 – 10
 - Maximum: 35 – 40
- Potential semiquantitation
 - 20,000 x number of platelets seen per 100x objective field of view



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Thrombon – Validate Data

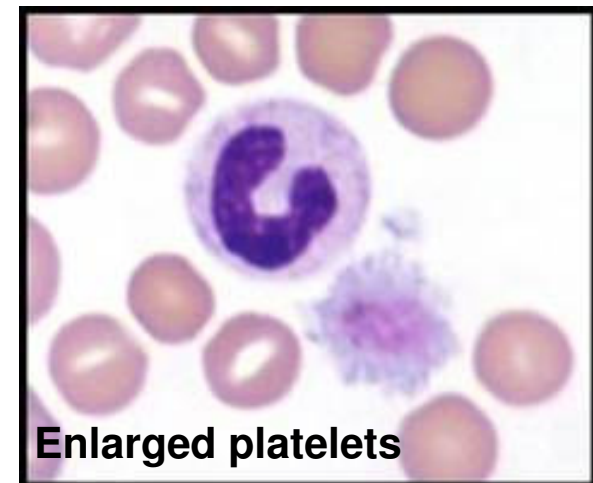
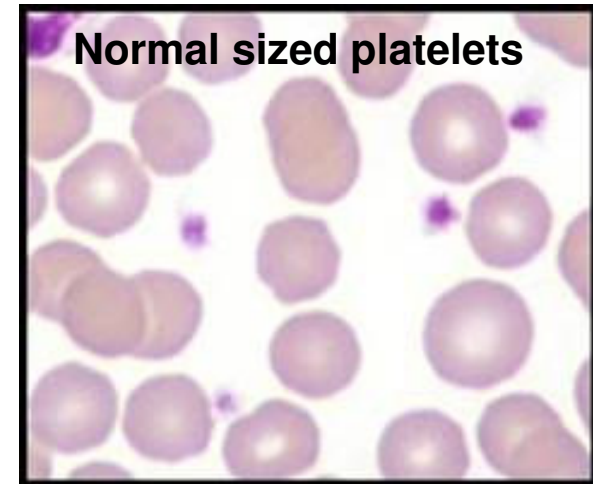
- Platelets
 - Confirm count
 - Evaluate platelet morphology

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Identification Of Enlarged Platelets

- Platelets larger than normal
 - Potential increased MPV from hematology analyzer
- In the cat
 - Usually equivocal finding
- In most other species
 - Indicates marrow response to peripheral demand for platelets
 - Thrombocytopenia not required
 - Inflammation and compensated response by marrow



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Reference Lab Hematology – Needed?

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Reference Lab Hematology – Needed?

- Experience
 - Use the laboratory for complicated cases
 - Potential of a “pathology review”
 - Use the laboratory as a great teaching resource

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Reference Lab Hematology – Needed?

- Experience
 - Use the laboratory for complicated cases
 - Potential of a “pathology review”
 - Use the laboratory as a great teaching resource
- Quality Assurance
 - Use the laboratory to periodically check your in-house system
 - Realize that different instruments produce different results
 - Realize that aged samples are not the same as a fresh sample

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Questions?



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IDEXX
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diagnostic edge

October 2006 Issue



October is National Pet Wellness Month

Establish a baseline, uncover hidden illness and offer better care with annual wellness screening for all your patients.

• [Featured Case Study:](#)

Nine-year-old neutered male dachshund, Bailey, was showing signs of anorexia and lethargy, by Matthew Eberts, DVM

- **Screen for four heartworm and three tick-borne diseases at once—including anaplasmosis**—Increase your parasite-screening power with the [SNAP[®] 4Dx[®] Test](#).

- **Don't miss out on tax savings!** You may be able to take advantage of Section 179 regarding the purchase of capital equipment.

- **Get your test results in just eight minutes** with the [IDEXX StatSpin[®]](#) high-speed centrifuge and the [VetTest[®]](#) Chemistry Analyzer.

- **Quick Tip:** Here's one way to help you get a urine sample from a kitten.

- **Training Opportunities:** Check out the latest list of **CE credit-approved** Seminars available this month from IDEXX

- **October is National Wellness Month!** See why it's important to test even seemingly healthy patients—and why [wellness screening](#) pays off.

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Education

Featured case study:

Nine-year-old neutered male dachshund with anorexia and lethargy

by Matthew Eberts, DVM, Lakeland Veterinary Hospital, Baxter, Minnesota

Bailey



Patient

Nine-year-old male neutered dachshund, Bailey

Presenting complaints

An acute onset of lethargy, drinking normally but eating only sparingly, no history of vomiting or diarrhea, no significant past health problems. Owner insists tick exposure is not possible.

Physical exam

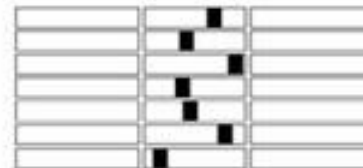
- Unremarkable
- All peripheral joints: no palpable pain
- Weight: 25 kg
- Neck: no palpable pain
- Back: no palpable pain
- Temperature: 102.3° F
- Respiratory rate: 24 rpm
- Heart Rate: 130 bpm
- Abdominal palpation: no pain and no palpable masses

Plan

Because of the acute onset of clinical signs and the vague clinical presentation, a general health screen chemistry profile along with a complete blood count and complete urinalysis were requested in the hope of defining direction for possible further diagnostics.

Hematology

RBC	7.32 M/mL	(5.50 - 8.50)
HCT	49.0 %	(37.0 - 55.0)
HGB	17.8 g/dL	(12.0 - 18.0)
MCV	66.9 fL	(60.0 - 77.0)
MCH	24.32 pg	(18.5 - 30.0)
MCHC	36.3 g/dL	(30.0 - 37.5)
RDW	15.7 %	(14.7 - 17.9)
%RETIC	.5 %	

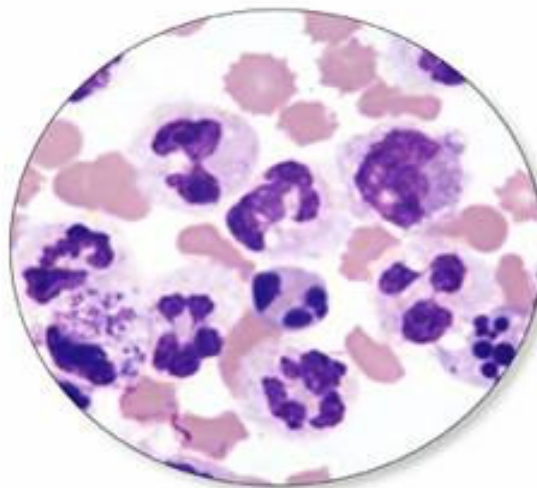


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Interactive Challenge

NOW with FREE CE Credit!

Can you correctly answer the following questions pertaining to this picture of a concentrated cellular preparation of abdominal fluid from a dog with acute abdominal pain (Wright's stain, 100x objective field of view)? Abdominal fluid had a protein content of 3.4 g/dL and a total nucleated cell count of 53,000/ μ L.



- A. Identify the primary nucleated cells present.
- B. Classify the process as transudate, modified transudate, exudate or neoplastic effusion.
- C. Suggest a possible cause or specific diagnosis.

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All fields are required for CE credit records.

A. Identify the primary nucleated cells present.

B. Classify the process as transudate, modified transudate, exudate or neoplastic effusion.

C. Suggest a possible cause or specific diagnosis.

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