CLS500 Routine Hematology Tests

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Routine Hematology Tests

- All tests have institution-established reference ranges
  - 'Normal' varies with age, sex, altitude and/or testing method

- Tests included in the CBC & Differential are the most frequently requested
  - A battery of cell count measurements
  - EDTA anticoagulated whole blood

- Testing is automated or done manually (e.g, microscopic exam of blood smear) to verify automated results
 - Manual testing delays test turnaround time (TAT)

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Routine Hemo Tests Objectives

- Discuss specimen requirements, reference ranges and significance of the following tests:
  - Complete Blood Count (CBC)
    - WBC count
    - RBC count
    - Hemoglobin
    - Hematocrit
    - RBC Indices (MCV, MCH, MCHC, RDW)
    - Platelet count

  - Differential
    - WBC differential and cell morphology

  - Reticulocyte count

  - Erythrocyte Sedimentation Rate (ESR)

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Use of Anticoagulants
Whole blood, Plasma or Serum

- Coagulation rxn

**Platelets/Clotting Proteins + calcium → thrombin which…
…converts fibrinogen → fibrin**

  - Fibrinogen is used up & cells are trapped in fibrin clot

EDTA whole blood
Citrated plasma
Serum
Clot
Normal heparinized plasma
Hemolyzed Plasma
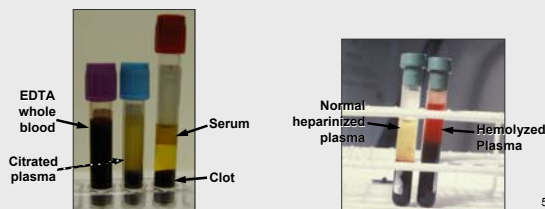
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Routine Hemo Tests Objectives

- Describe pre-analytical errors, i.e., specimen collection errors that can cause inaccurate results.

- Discuss quality control and checks used to assure test validity and prevent erroneous laboratory results due to analytical errors.

- Evaluate CBC and Differential results to detect the presence of a Hematologic disorder or associated condition:
  - Recognize deviations from normal blood cell concentrations for age and sex
  - Identify significant abnormalities in cellular morphology
  - Correlate results with clinical information to guide further testing

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Potential Sources of Error

- The most fundamental responsibility of a laboratory is to *ensure quality test results.*

- Pre-analytical errors are the most common cause of inaccurate results
  - Testing process begins with sample collection
  - Blood collection errors must be recognized and the sample redrawn

- Sources of blood collection error:
  - Wrong patient ID or tube labeling
  - Partially clotted blood or hemolysis
  - Hemodilution or hemoconcentration
  - Wrong tube drawn or insufficient fill
  - Improper handling

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Quality Control

- Control samples with *known values* are used to check the reliability of patient results
  - Control values must fall within established allowable limits
  - Controls monitor the actual testing process including equipment function, reagents and testing technique
  - Controls detect invalid results caused by analytical errors

- Other checks done to prevent erroneous results:
  - Critical values are confirmed, called &/or redrawn
  - Patient results are compared to previous results
  - 'Flagged' or inconsistent data is verified

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WBC Count

- WBC count - total # of white cells reported in thousands/uL; does not distinguish WBC types

WBC Count Reference Ranges	
Vary with age and testing procedure	
Adult	4.0-11.0 x 10^3/uL
Newborn	9.0-30.0 x 10^3/uL
Children (1-10 yo)	6.0-17.0 x 10^3/uL

- Significance
  - ↓ WBC count….Leukopenia
    - Decreased production, increased use
  - ↑ WBC count….Leukocytosis
    - Increased production, shift/pseudo

- Critical values: WBC<1.0 or >40.0 K/uL

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Control samples are run to assure the reliability of test results and can detect:

A. Pre-analytical errors

B. Wrong patient identification

C. Analytical errors

D. A partially clotted EDTA sample

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RBC Count, HGB, HCT

- RBC count – total # of red cells reported in millions/uL

- Hemoglobin - photometric measurement of Hgb concentration in red cells reported in g/dL
  - Corrected Hgb value if lipids or bilirubin interfere
  - NOT the same as plasma Hgb

- Hematocrit – percentage (%) of red cells in a known volume of whole blood

- RBC count, HGB and HCT measurements <u>parallel</u> each other

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Adult CBC

Complete Blood Count (CBC without Diff/Hemagram)		
7.2	WBC x 10^3/uL	4-11 K/uL
5.03 x3=15.1	RBC x 10^6/uL	
15.0 x3=45.0	HGB g/dL	11-17 g/dL
45.4	HCT %	
86.8 =RBC size	MCV fL	82-98 fL
28.8	MCH pg	
33.1	MCHC %	
12.8	RDW %	
202.0	PLT x 10^3/uL	150-450 K/uL

RBC Parameters

RBC Indices

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RBC Count, HGB, HCT

RBC Count, Hemoglobin, Hematocrit Reference Ranges			
Vary with sex, age, altitude and testing procedure			
	RBC Count	HGB	HCT
Males	4.40-5.80 million/cmm	13.0-17.0 g/dL	37.0-51.0 %
Females	3.80-5.20 million/cmm	11.5-15.5 g/dL	35.0-46.0 %
Newborns	5.00-6.50 million/cmm	14.0-25.0 g/dL	44.0-64.0 %
Children (1-10 yo)	3.70-5.50 million/cmm	11.0-14.0 g/dL	34.0-42.0 %

- Significance
  - ↓ RBC, HGB and/or HCT values….Anemia
    - Decreased production, increased loss/destruction
  - ↑ RBC, HGB and/or HCT values….Polycythemia
    - Increased production, fluid loss

- Critical values: HGB <7.0 or >18.5 g/dL

- Relationship: HGB x 3 = HCT *if red cells are normal in size and hgb content*

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Evaluation:

3 adults

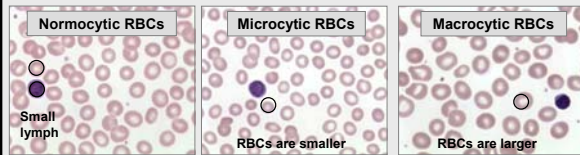| CBC parameters:		(1)	(2)	(3)
WBC	thousands/cmm	6.9	20.4	4.2
RBC	millions/uL	4.61	1.95	7.05
HGB	g/dl	13.7	5.8	18.8
HCT	%	41.2	17.2	58.4
MCV	fl	89.4	87.8	82.8
MCH	pg	29.6	29.6	26.6
MCHC	%	33.1	33.7	32.2
RDW	%	11.4	13.5	12.1
PLT	thousands/cmm	250.	180.	340.

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RBC Indices

- MCHC = average hgb concentration per RBC
  - Normal 32-36% = normochromic red cells
  - MCHC <32% = red cells may be hypochromic

- RDW = index of RBC size variation
  - Normal 10-14% (low RDW) = uniform RBC size
  - A high RDW >14% is caused by variations in RBC size called *anisocytosis*

- Indices are average values
  - Less meaning in heterogeneous RBC populations with wide size variations
    - A normal MCV value with a high RDW >22% may not mean the red cells are normocytic
    - Review RBC morph findings on differential report to judge reliability of MCV
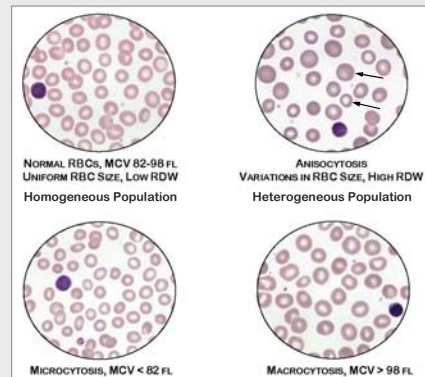
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RBC Indices

- Define size and/or hgb content of red cells
- Include:
  - MCV, mean cell volume
  - MCH, mean cell hemoglobin
  - MCHC, mean cell hemoglobin concentration
  - RDW, red cell distribution width

- Used to classify anemia….MCV most useful

Normocytic RBCs — Small lymph | Microcytic RBCs — RBCs are smaller | Macrocytic RBCs — RBCs are larger

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RBC Indices

NORMAL RBCs, MCV 82-98 FL
UNIFORM RBC SIZE, LOW RDW
Homogeneous Population

ANISOCYTOSIS
VARIATIONS IN RBC SIZE, HIGH RDW
Heterogeneous Population

MICROCYTOSIS, MCV < 82 FL

MACROCYTOSIS, MCV > 98 FL

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RBC Indices

- MCV = average RBC size, varies with age
  - Normal adult MCV 82-98 fL = normocytic red cells
  - MCV <82 fL = microcytic red cells
  - MCV >98 fL = macrocytic red cells
  - Normal newborn ~110 fL; normal children ~77 fL

Normocytic RBCs MCV 82-98 fL | Microcytosis MCV <82 fL | Macrocytosis MCV >98 fL
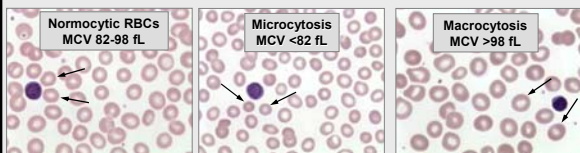
- MCH varies with RBC size and Hgb content
  - Adds little information

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Evaluation:

3 adults

| CBC parameters:		(1)	(2)	(3)
WBC	thousands/cmm	1.0	16.8	8.0
RBC	millions/uL	2.90	3.57	2.35
HGB	g/dl	8.8	8.2	10.0
HCT	%	26.9	25.7	27.2
MCV	fl	92.8	72.0	124.0
MCH	pg	30.2	23.0	42.6
MCHC	%	32.7	31.8	34.4
RDW	%	13.0	17.2	11.6
PLT	thousands/cmm	25.	602.	200.

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Evaluation: This blood smear shows
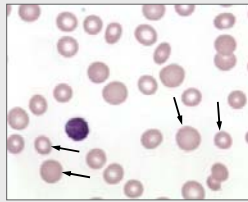
A. A homogeneous population of red cells with uniform RBC size and a low RDW value

B. A heterogeneous population of red cells with wide variations in RBC size and a high RDW value

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Platelet Count

- Hardest cell type to count

- All critical and/or flagged PLT counts are verified microscopically (blood smear exam)

- A major source of error is platelet clumping
  - Caused by partially clotted EDTA blood or heparinized blood
  - Sample must be redrawn to obtain accurate results

Blood smear

Platelet

Platelet clumps
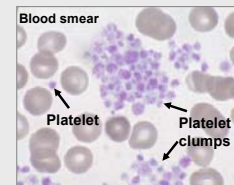
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Evaluation: The red cells shown are
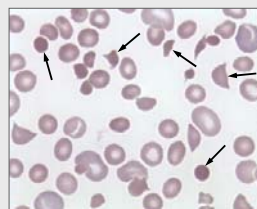
A. Deformable and normal in shape

B. Rigid/damaged and survival will be shortened

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CBC & Automated Differential

AGE: 23Y		SEX: F	'Normal' reference range for age and sex	
CBC & DIFFERENTIAL				
	WBC COUNT	5.1	[4.0-11.0]	X10E3/uL
	RBC COUNT	4.47	[3.80-5.20]	X10E6/uL
	HEMOGLOBIN	13.5	[11.5-15.5]	g/dL
CBC	HEMATOCRIT	39.0	[33.0-45.0]	%
	MCV	87.3	[82.0-98.0]	fL
	MCHC	34.6	[32.0-36.0]	%
	RBC DISTRIB WIDTH	11.2	[10.0-14.0]	%
	PLATELET	344	[150-450]	X10E3/uL
	TYPE OF DIFF DONE	AUTOMATED		
	NEUTROPHIL	61	[45-75]	%
	LYMPHOCYTE	31	[15-45]	%
	MONOCYTE	7	[1-12]	%
	EOSINOPHIL	1	[0-6]	%
DIFF WBC Types	BASOPHIL	0	[0-2]	%
	ABSOLUTE NEUTROPHIL	3.1	[1.8-7.5]	K/uL
	ABSOLUTE LYMPHOCYTE	1.6	[1.0-3.4]	K/uL
	ABSOLUTE MONOCYTE	0.4	% x WBC/uL [0.1-0.9]	K/uL
	ABSOLUTE EOSINOPHIL	0.0	[0.0-0.4]	K/uL
	ABSOLUTE BASOPHIL	0.0	[0-0.2]	K/uL
	NEUTROPHIL COMMENT	Automated Diff...Neutrophils include Segmented and Band Forms		

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Platelet Count

- PLT count - total # of platelets reported in thousands/uL

- Reference range
  - 150,000-450,000/uL

- Significance
  - ↓ PLT count….Thrombocytopenia
    - Decreased production, increased use/destruction
  - ↑ PLT count….Thrombocytosis
    - Increased production, post-splenectomy

- Critical values: PLT <40,000 or >1 million/uL

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CBC & Manual Differential

AGE: 17Y		SEX: M	Parameters with * are outside reference range for age & sex	
CBC				
	WBC COUNT	12.4	[4.5-13.5]	X10E3/uL
	RBC COUNT	2.68*	[4.40-5.80]	X10E6/uL
	HEMOGLOBIN	8.1*	[13.0-17.0]	g/dL
	HEMATOCRIT	24.5*	[37.0-51.0]	%
	MCV	97.0	[82.0-98.0]	fL
	MCHC	33.1	[32.0-36.0]	%
	RBC DISTRIB WIDTH	29.0*	[10.0-14.0]	%
	PLATELET COUNT	365	[150-450]	X10E3/uL
DIFFERENTIAL				
	TYPE OF DIFF DONE	MANUAL DIFF		
	NEUTROPHIL SEG	50	[43-77]	%
	NEUTROPHIL BAND	5	[0-10]	%
	LYMPHOCYTE	27	[15-45]	%
Blood Smear	MONOCYTE	10	[1-12]	%
	EOSINOPHIL	6	[0-6]	%
	BASOPHIL	2	[0-2]	%
	NUCLEATED RBC	4*	[0]	/diff
	ABSOLUTE DIFFERENTIAL#s NOT SHOWN			
	RBC MORPH	Marked Anisocytosis		
		Marked Polychromasia		
		Marked Poikilocytosis		
Significant variations from normal are noted		Few Target Cells		
		Sickle Cells Noted		

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Differentials

- Automated or manual if significant patient abnormalities exist
  - Manual differentials require a blood smear exam
- Differential includes:
  - WBC differential that classifies WBC types
    - Reported in % and absolute # (% x WBC count)
    - % easier to evaluate but absolute # more reliable
  - Cell morphology (blood smear)
    - Significant variations from normal appearance of red cells, white cells and platelets are noted or quantitated on report
    - Specific abnormalities can provide *clues* to the cause of a condition/disorder

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Reticulocyte Count

- Reference range varies with age
  - Adult 0.5-2.0%   25-100,000/cmm Absolute#
  - Newborn 2.0-6.0%
- Significance
  - ↓ absolute Retic count….Reticulocytopenia
    - Decreased RBC production
  - ↑ absolute Retic count….Reticulocytosis
    - Increased RBC production (↑ EPO stimulus)
    - Good indicator for hemolytic anemias

AGE: 45Y	SEX: F		
RETIC COUNT			
RBC COUNT	4.00	[3.80-5.20]	X10E6/uL
RETIC %	1.0	[0.5-2.0]	%
RETIC ABSOLUTE	40	[25-100]	K/cmm

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Differentials

- Reference percentage ranges vary with age

Differential Reference Percentage Ranges

	Adult	Children	
Neutrophils	45-75%		
Segs	43-74%	25-70% (5-16y)	
Bands	0-10%	5-11%	
Lymphocytes	15-45%	45-75% (1-4y)	25-55% (5-14y)
Monocytes	1-12%	1-12%	
Eosinophils	0-6%	0-8%	
Basophils	0-2%	0-2%	

- Significance:
  - Deviations from normal may indicate disease; the cell type involved is often related to function
  - No immature cells should be present
- Critical values: Blasts, other highly abnormal findings; absolute neutrophil # <500/uL

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Erythrocyte Sedimentation Rate

- The ESR or sed rate is a *non-specific* indicator of disease
  - Mainly used to monitor patients with chronic inflammatory disease
- ESR refers to the rate red cells settle as blood stands in a tube
  - In normal persons, sedimentation or falling of red cells is <u>slow</u>
  - Automated or manual
  - Reported in distance the red cells fall in mm/time….mm/hr

Normal | Abnormal | Abn

2 mm/hr | 80 mm/hr | 105 mm/hr

Reticulocyte Count

- Measures rate of RBC production by the bone marrow
  - Retics appear as polychromasia on a Wright's stained blood smear…must order a retic count for number
- Automated or manual using supravital stain
- Reported in % and absolute # (% x RBC count)
  - Absolute # is more reliable than %

NRBC

Polychromasia

Reticulocytes

Wright's stained smear | Supravital stained smear
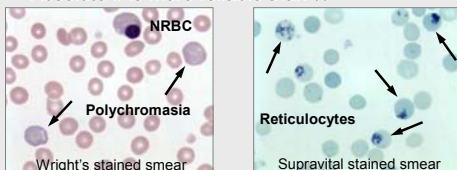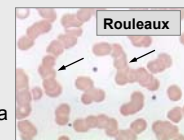
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Erythrocyte Sedimentation Rate

- Reference range varies with age & sex
  - Males 0-10 mm/hr   Males>50y 0-20 mm/hr
  - Females 0-20 mm/hr   Females>50y 0-30 mm/hr
- Significance
  - Under normal conditions, red cells do not form rouleaux….fall slowly
  - High concentrations of certain plasma proteins promote rouleaux = ↑ ESR
  - <u>Fibrinogen</u>, a positive acute phase reactant, is most responsible for an increased/abnormal ESR

Rouleaux

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Erythrocyte Sedimentation Rate

- Significance of increased/abnormal ESR results:
  - Acute and chronic infections…bacterial
  - Chronic inflammatory disorders…RA, SLE
  - Malignancies…cancer, lymphoma, multiple myeloma
  - Tissue necrosis…myocardial infarction
  - Anemia of chronic disease

- Clinical use
  - Monitor response to therapy for rheumatoid arthritis
  - Follow Hodgkin's lymphoma for relapse
  - May help detect occult disease…CRP more sensitive

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Evaluation: What is your next step?

AGE: 27Y	SEX: F		
CBC W/O DIFF			
WBC COUNT	8.7	[4.0-11.0]	X10E3/uL
RBC COUNT	4.09	[3.80-5.20]	X10E6/uL
HEMOGLOBIN	12.2	[11.5-15.5]	g/dL
HEMATOCRIT	36.6	[33.0-45.0]	%
MCV	91.0	[82.0-98.0]	fL
MCHC	33.3	[32.0-36.0]	%
RBC DISTRIB WIDTH	13.4	[10.0-14.0]	%
PLATELET	35*	[150-450]	X10E3/uL

A. Order a differential to identify WBC types and cause for WBC count
B. Request a redraw to confirm platelet count if no signs of bleeding
C. Order a retic count to assess RBC production

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Example of Hemodilution:

Line draw @ 04:45 from ICU patient

AGE: 66Y	SEX: M		
HGB and HCT			
HEMOGLOBIN	6.8*	[13.0-17.0]	g/dL
HEMATOCRIT	20.5*	[37.0-51.0]	%
	Called H&H @ 0500		

Redraw to confirm was requested

AGE: 66Y	SEX: M		
HGB and HCT			
HEMOGLOBIN	10.8*	[13.0-17.0]	g/dL
HEMATOCRIT	32.5*	[37.0-51.0]	%
	Results from 0530 redraw sample		

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Evaluation: What do you suspect?

AGE: 43Y	SEX: M	SOB, jaundice & hemoglobinuria	
CBC & DIFFERENTIAL			
WBC COUNT	12.2*	[4.0-11.0]	X10E3/uL
RBC COUNT	2.53*	[4.40-5.80]	X10E6/uL
HEMOGLOBIN	8.0*	[13.0-17.0]	g/dL
HEMATOCRIT	23.1*	[37.0-51.0]	%
MCV	93.5	[82.0-98.0]	fL
MCHC	34.7	[32.0-36.0]	%
RBC DISTRIB WIDTH	19.6*	[10.0-14.0]	%
PLATELET	228	[150-450]	X10E3/uL
TYPE OF DIFF DONE	MANUAL DIFF		
DIFF Not Shown			
RBC MORPH	Moderate Anisocytosis		
	Moderate Polychromasia		
	Moderate Poikilocytosis		
	Several Schistocytes		
	Few Spherocytes		

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Evaluation: What is your next step?

AGE: 63Y	SEX: M		
CBC W/O DIFF			
WBC COUNT	19.7*	[4.0-11.0]	X10E3/uL
RBC COUNT	4.56	[4.30-5.90]	X10E6/uL
HEMOGLOBIN	14.5	[13.0-17.0]	g/dL
HEMATOCRIT	42.8	[37.0-51.0]	%
MCV	94.0	[82.0-98.0]	fL
MCHC	33.8	[32.0-36.0]	%
RBC DISTRIB WIDTH	12.4	[10.0-14.0]	%
PLATELET	183	[150-450]	X10E3/uL

A. Order a differential to identify WBC types and cause for WBC count
B. Request a redraw to confirm platelet count if no signs of bleeding
C. Order a retic count to assess RBC production

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Evaluation: What do you suspect?

AGE: 60Y	SEX: M	Fever	
CBC & DIFFERENTIAL			
WBC COUNT	49.5*	[4.0-11.0]	X10E3/uL
Critical WBC called to Ann in AICU @1025 by ls			
RBC COUNT	3.47*	[4.40-5.80]	X10E6/uL
HEMOGLOBIN	10.8*	[13.0-17.0]	g/dL
HEMATOCRIT	31.1*	[37.0-51.0]	%
MCV	89.5	[82.0-98.0]	fL
MCHC	34.6	[32.0-36.0]	%
RBC DISTRIB WIDTH	14.0	[10.0-14.0]	%
PLATELET	173	[150-450]	X10E3/uL
TYPE OF DIFF DONE	MANUAL DIFF		
NEUTROPHIL SEG	44	[43-77]	%
NEUTROPHIL BAND	45*	[0-10]	%
LYMPHOCYTE	3*	[15-45]	%
MONOCYTE	3	[1-12]	%
EOSINOPHIL	1	[0-6]	%
BASOPHIL	0	[0-2]	%
METAMYELOCYTE	2*	[0]	
MYELOCYTE	2*	[0]	
ABSOLUTE SEG	21.8*	[1.5-7.5]	K/uL
ABSOLUTE BAND	22.2*	[<1.0]	K/uL
ABSOLUTE LYMPH	1.5	[1.0-3.4]	K/uL
ABSOLUTE MONO	1.5*	[0.1-0.9]	K/uL
ABSOLUTE EOS	0.5*	[0.0-0.4]	K/uL
ABSOLUTE BASO	0.0	[0-0.2]	K/uL
ABSOLUTE META	1.0	[0]	K/uL
ABSOLUTE MYELO	1.0	[0]	K/uL
RBC MORPH	Moderate Polychromasia		
WBC MORPH	Toxic Granulation and Dohle Bodies noted		

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