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DIFFERENTIALS & PERIPHERAL SMEAR EVALUATION
Microscopy

I. Principle
A peripheral smear is made and stained with Wright's stain for examination and evaluation of erythrocyte and platelet morphology and the generation of a leukocyte differential count.

II. Clinical Significance
Automated cell counters cannot always recognize atypical or immature leukocytes and abnormalities in the erythrocytes and platelets. This requires a technologist/technician to view the smear microscopically to identify cell types. The quantitation of cell types and the evaluation of erythrocyte morphology forms the basis in diagnosing leukocytic, erythrocytic and thrombocytic disorders.

III. Specimen

Preferred Specimen	K2 EDTA anticoagulated whole blood required.
Storage/Retention	2-8° C for 4 days
Sample Stability	48 hours
Rejection Criteria	Clotted specimens or those containing fibrin strands. Improper volume collected. Improperly labeled samples. Grossly hemolyzed. Samples suspected of intravenous fluid contamination. Samples exceeding stability requirements.

IV. Reagent
Immersion Oil

V. Instrumentation/Equipment/Calibration
A. Stained peripheral blood smear
B. Light microscope with 50X & 100X oil immersion lenses
C. Device for counting cells(computer/cell counter)

VI. Quality Control
Every peripheral smear is evaluated at time of differential performance. Smears not found to have appropriate cell distribution, proper stain appearance, or be free of precipitate are considered unacceptable and will require new slide preparation.

VII. Procedure
A. Perform and report out the manual differential on all newborn CBC's with differential. Make sure that you report bands (even if they are "0").
B. If any of the following criteria are found on automated result, perform action(s) as described:

WBC Count	<2.0 x 10^3/µL		Prepare buffy coat, make 2 Wright's stains, perform manual differential, order Abnormal Pathology Review.
PLT Count	≤ 50 x 10^9/L		Perform platelet estimation from smear. Order Abnormal Hematology Review.
	> 600 x 10^9/L		
Basophil %	> 4.0%		Perform manual differential. If the absolute basophil count is >0.2 order Abnormal Pathology Review.
@ flag next to result	WBC	>440.00 x 10^3/µL	Perform dilution and rerun: a. Make 1:5 dilution with DCL Cell Pack, rerun in manual mode, calculate the result by multiplying the result by 5. b. Make alternate dilution with Cell Pack, rerun in manual mode, calculate result by multiple count by dilution factor c. Note dilution on patient report.
	RBC	>8.60 x 10^6/µL	
	HGB	>26.0 g/dL	
	HCT	>75.0%	
	PLT	>5000 x 10^3/µL	
	RETIC %	>30.00%	
	nRBC%	>600 /100 WBC	
RET Abn Scattergram flag	This IP Message indicates that the instrument has detected increased activity in the RET-THR (threshold) area, the RET scattergram or increased activity in the RET-UPP (Upper Particle Plateau) area on the RET-EXT scattergram. Follow suggested Action steps in Sysmex XN flagging Guide		
WBC abn Scattergram flag	1. If dashes (— —) are in place of numeric data: □ Verify differential results by performing the following. o repeating the sample o performing a manual differential 2. If asterisk (*) are next to results: □ Verify differential results by performing the following. o scanning the slide for abnormal cells and to estimate the WBC count o performing a manual differential if abnormal cells are observed □ If no abnormalities are found when reviewing the smear and the WBC estimate matches the analyzer reported WBC, the results with asterisks (*) may be reported.		
Neutropenia flag	Perform peripheral smear evaluation. If smear correlates with automated results, verify. If not, perform manual differential. If absolute count is less than 1.0 x 10^3/uL, order Abnormal Pathology Review.		
Lymphocytosis flag	Perform peripheral smear evaluation. If smear correlates with automated results, verify. If not, perform manual differential. If patient is ≥ 40 years and # Lymph > 5.0 x 10^3/uL OR patients 16-39 years with # Lymph >7.5 x 10^9/L, order Abnormal Pathology Review.		
Monocytosis flag	Perform peripheral smear evaluation. If smear correlates with automated results, verify. If not, perform manual differential. If absolute count is greater than 2.5 x 10^3/uL, order Abnormal Pathology Review.		
Eosinophilia flag	Perform peripheral smear evaluation. If smear correlates with automated results, verify. If not, perform manual differential. If absolute count is greater than 2.0 x 10^3/uL, order Abnormal Pathology Review.		
Basophilia flag	Perform peripheral smear evaluation. If smear correlates with automated results, verify. If not, perform manual differential. If count is greater than 0.2 x 10^3/uL, perform manual differential and order Abnormal Pathology Review.		

Suspect, Blast / Abn Lympho? flag	The Blast / Abn Lympho? IP message indicates that the analyzer has detected abnormal clustering in the region for blasts and abnormal lymphocytes in the WDF scattergram. An asterisk (*) appears next to the Neutrophil, Lymphocyte and Monocyte % and #. The asterisk (*) indicates these results may be unreliable and should be confirmed. 1. Perform a peripheral smear evaluation for the presence of: • blasts – lymphoblasts, myeloblasts, and myelomonoblasts • immature granulocytes – promyelocytes, myelocytes, metamyelocytes • atypical or immature lymphocytes • other abnormal cells NOTE: Reviewing the feathered edge and sides of the peripheral smear is suggested as blasts and other large cells may migrate to this area during smear preparation. 2. If no abnormalities are found, the results with the asterisk (*) may be reported. 3. If abnormal cells are present, perform manual differential. a. If smudge cells seen, prepare albumin slide. b. Any blasts, promyelocytes, plasma cells, cells suspicious for malignancy and unclassifiable cells are seen; Order Abnormal Pathology Review. c. **If a new acute leukemia is suspected after normal business hours, report blasts and report the critical result. The slide should be saved for first AM review by the clinical pathologist. The pathologist on call should be notified when the technologist is uncertain regarding the presence or absence of leukemia (>20% blasts).**
Suspect, Left Shift? flag	The Left Shift? IP message indicates that the analyzer has detected abnormal clustering in the region for left shift (bands) in the WDF scattergram. An asterisk (*) appears next to the Neutrophil and Eosinophil % and #. The IG% and IG# may also have an asterisk. The asterisk (*) indicates these results may be unreliable and should be confirmed 1. Perform a peripheral smear evaluation for the presence of: • band cells in increased numbers • toxic granulation or vacuolation of neutrophils • other abnormal cells 2. If no abnormalities are found, the results with the asterisk (*) may be reported. 3. If abnormal cells are present, perform manual differential
IG Present Message flag	1. Scan/ Perform a peripheral smear evaluation for the presence of: • immature granulocytes – promyelocytes, myelocytes and metamyelocytes • band cells in increased numbers >10% perform a manual differential • toxic granulation or vacuolation of neutrophils • other abnormal cells 2. If abnormal cells are present,blasts, pros, or plasma cells perform manual differential 3. Any IG% greater than 5% , perform a manual differential.
Suspect, Atypical Lympho? flag	The Atypical Lympho? IP message indicates that the analyzer has detected significant clustering in the region for atypical lymphocytes that is located in the upper left lymphocyte region on the WDF scattergram. An asterisk (*) appears next to the Neutrophil, Lymphocyte, Monocyte, Eosinophil and Immature Granulocyte % and #. The asterisk (*) indicates these results may be unreliable and should be confirmed. 1. Perform a peripheral smear evaluation for the presence of:

	• atypical or variant lymphocytes • abnormal or atypical monocytes • immature lymphocytes, such as seen in ALL or CLL • immature monocytes • smudge cells • other abnormal cells 2. If no abnormalities are found, the results with the asterisk (*) may be reported. 3. If abnormal cells are present, perform manual differential a. If smudge cells seen, prepare albumin slide. b. Any blasts, promyelocytes, plasma cells, cells suspicious for malignancy and unclassifiable cells are seen; Order Abnormal Pathology Review.
NRBC Flag	Note: The XN-Series analyzers identify and count NRBCs simultaneously while counting WBCs. No further correction of the WBC count is required. Perform peripheral smear evaluation. a. If none present, correct automated count to zero. b. If greater than one present: 1. Perform manual differential. 2. Correlate manual count to automated count. If results correlate report automated count. 3. Order abnormal pathology review for any adult with ≥ 3 NRBC present, or newborns ≤ 3days with > 15 seen.
Suspected RBC agglutination flag	Asterisks (*) appear next to the RBC, HGB, HCT, MCV, MCH, MCHC and RET # parameters. The asterisk (*) indicates these results may be unreliable and should be confirmed. 1. Scan the peripheral smear for the presence of agglutinated RBC's. Visually check the sample tube for agglutination. 2. If agglutination is present warm specimen for 15-30 minutes in 37°C waterbath. Reanalyze the warmed sample in the manual mode after mixing by manual inversion 10 times. Make a new peripheral smear from the warmed sample if agglutination is severe and WBCs and PLTs cannot be accurately assessed. 3. In cases with high cold agglutinin titers, a plasma replacement using warm CELLPACK® DCL may be necessary to reduce the interference from the antibody. Further warming post-plasma replacement may also be necessary. a. To perform a plasma replacement i. Centrifuge an aliquot of blood from the primary tube to separate the cells from the plasma. ii. Using a pipette, remove a measured amount of plasma removing as much plasma as possible without disturbing the buffy coat. iii. Add back the same amount of CELLPACK DCL as the volume of plasma removed in step ii. (Example: If 0.5 mL of plasma is removed then add back 0.5 mL of CELLPACK DCL.) iv. Cap the tube and mix the sample by manual inversion until the cells are fully resuspended in the CELLPACK DCL. v. Reanalyze the sample in the manual mode. 4. In cases where a warm-reacting antibody has caused agglutination, a plasma replacement may reduce the interference from the antibody. Room temperature CELLPACK DCL may be used to replace the plasma.

	5. If the plasma replacement does not work perform a manual HCT. Confirm that the HCT result meets the "Rule of Three" (HGB x 3) ± 2%. "NA" RBC & Indicies and footnote " cannot be reported due to heavy cold agglutinin". Report WBC, HGB, Manual HCT, Plt count and WBC differential if ordered.	
RBC Lyse Resistance flag	Check HGB/HCT ratio. a. If "Rule of Three" is met, report out results. b. If "Rule of Three" is not met: 1. Perform manual hematocrit to confirm automated hct. 2. Report HGB as "N/A, Unable to Determine Hemoglobin Value"	
Turbidity/HGB Interference Flag	The Turbidity/HGB Interference? IP Message occurs when the MCHC is >37.5 g/dL and indicates that turbidity may be present in the diluted and lysed sample. This turbidity could interfere with the HGB detection light path and falsely increase the HGB value. Other interfering substances or conditions may impact the hematocrit and also cause an MCHC >37.5 g/dL. Asterisks (*) appear next to the HGB, MCH and MCHC parameters. The asterisk (*) indicates these results may be unreliable and should be confirmed NOTE: An MCHC up to 37.5 g/dL may indicate a normal specimen on the high end of normal range in which case no action is needed. This may occur more often in samples with higher hemoglobin and hematocrit results. Consider the MCHC and the MCV together when evaluating results and the reasons for the interference. Refer to the following table for possible interferences and corrective actions.	
Turbidity/HGB Interference Flag (Continued)	Pattern of Results: • Low or Normal MCV High MCHC (>37.5 g/dL)	Encountered in: • Hemolysis • Plasma electrolyte abnormalities (i.e., low sodium) affecting hematocrit results • Severe lipemia • Icterus • Severe leukocytosis affecting hemoglobin measurement • Abnormal plasma protein precipitation affecting hemoglobin measurement Refer to Troubleshooting Chart
Turbidity/HGB Interference Flag (Continued)	Pattern of Results: High MCV High MCHC (>37.5 g/dL)	Encountered in: • RBC Agglutination • Rouleaux Refer to Troubleshooting Chart

Troubleshooting Chart for Turbidity/HGB Interference Flag

Low Sodium Affecting Hematocrit?	RBC Agglutination?	Severe Lipemia, Icterus, Abnormal Protein or Leukocytosis Affecting Hemoglobin Measurement or Hemolysis?
1. Perform a 1:5 dilution of sample with CELLPACK DCL 2. Allow the dilution to equilibrate for ten to fifteen minutes	1. Prewarm at 37°C for fifteen to thirty minutes then rerun 2. Severe cold agglutinins or rouleaux may require dilution or plasma	1. Perform a 1:5 dilution of sample with CELLPACK DCL 2. Repeat diluted sample 3. Correct results for dilution factor

3. Rerun after equilibration 4. Correct results for dilution factor prior to reporting. NOTE: MCV, MCH, MCHC, RDW-SD, RDW-CV, MPV and differential percent results are unaffected by dilution and do not require correction.	replacement with CELLPACK DCL. 3. For severe cold agglutinins, additional incubation at 37°C may be necessary following dilution or plasma replacement.	prior to reporting. Lipemia or Icterus Only Perform a plasma replacement procedure Hemolysis: Recollect a new sample.

HGB Defect Flag	Perform peripheral smear evaluation. Correlate RBC parameters with RBC morphology. If defect present, order Abnormal Hematology Review.
Abnormal, RBC Abn Distribution Flag	Perform peripheral smear evaluation for the presence of: • increased anisocytosis • multiple RBC populations • fragmented RBCs • rouleaux or RBC agglutination (refer to suggested action for "RBC Agglutination?") 1. Report out any abnormal morphology according to our reportable grading system and correlate results between RBC parameters and morphology seen.. 2. If no abnormalities are found, the results with the asterisk (*) may be reported. 3. If dashes (— —) are in place of numeric data, repeat testing of specimen. 4. If the RBC morphology is normal and the MCHC is abnormal (<30 or >37.5 g/dL) an interfering substance or condition may be present. Refer to the suggested guidelines for the HGB/Turbidity Interference? IP Message.
Abnormal, Dimorphic Population Flag	Follow the quide for "Abnormal, RBC Abn Distribution" above. If two RBC populations are seen on the peripheral smear and dashes -- -- are present for the RDW result, report comment "Dimorphic red cell population; unable to calculate RDW."
Aniso/Micro/Macro	Perform peripheral smear evaluation. a. Correlate RBC morphology to automated RBC parameters. Order Pathology Review when: 1. MCV < 70 fL or > 115 fL. 2. Severe RBC morphologic abnormalities (3+) schistocytes or spherocytes.
Erythrocytosis	If HGB > 19 g/dL, order Abnormal Pathology Review.
Iron Deficiency Flag	Perform peripheral smear evaluation. Correlate MCV and MCHC with RBC morphology.
Fragments Flag	Perform peripheral smear evaluation. If ≥ 3+ schistocytes present, order Abnormal Hematology Review. If red cell fragments, microcytic RBC's, or WBC cytoplasmic fragments are found: a. Make sure the analyzer performed a PLT-F. If the analyzer did not, manually run a PLT-F on the specimen. b. If there is no asterisk (*) next to the PLT-F result; report automated PLT-F results. c. If there is an asterisk (*) next to the PLT-F result perform a platelet estimate on the peripheral smear. d. If estimate correlates with automated count within ±50,000 on counts over 100,000 OR ±20,000 on counts under 100,000, report automated value. If it does not correlate, re-estimate, re-analyze, or recollect.

Thrombocytopenia/Thrombocytosis Flag	Perform platelet estimate on peripheral smear. a. Make sure the analyzer performed a PLT-F. If the analyzer did not, manually run a PLT-F on the specimen. b. If there is no asterisk (*) next to the PLT-F result; report automated PLT-F results. c. If there is an asterisk (*) next to the PLT-F result perform a platelet estimate on the peripheral smear. d. If estimate correlates with automated count within ±50,000 on counts over 100,000 OR ±20,000 on counts under 100,000, report automated value. e. If estimate does not correlate, re-estimate, re-analyze, or recollect. f. Order Pathology Review when $\leq 50 \times 10^3$/uL or $> 600 \times 10^3$/uL platelet counts.
Abnormal, PLT Abn Scattergram	The PLT Abn Scattergram IP Message can only be generated when a PLT-F count is performed. This IP Message occurs when clustering in the platelet and IPF area on the PLT-F Scattergram is abnormal. The PLT-F and IPF are reported with an asterisk (*). Dashes may appear in place of data for the MPV or the MPV may be reported with an asterisk (*).The asterisk (*) indicates these results may be unreliable and should be confirmed. 1. Perform peripheral smear evaluation for the presence of: • large or giant platelets • platelet clumps • fragmented RBCs • microcytic RBCs • parasites If abnormal RBC, PLT or other morphology is noted, report the abnormalities according to our grading system. 2. Perform a manual platelet estimate. 3. If platelet estimate confirms accuracy of analyzer count, it may be reported. 4. If platelet clumps have interfered, perform one of the alternate procedures recommended in the section Suggested Actions for PLT Clumps? IP Message.

Suspect, PLT Clumps? flag	The PLT Clumps? IP Message is determined by abnormal clustering in the WNR, WDF or PLT-F scattergrams. In the WDF and PLT-F scattergrams the FSC-W measurement is also used to identify platelet clumps. Asterisks (*) will appear next to the PLT and MPV. The asterisk (*) indicates these results may be unreliable and should be confirmed. 1. Check the sample for the presence of clots. a. If a clot is present reject specimen. 2. Scan the peripheral smear, especially the feathered edge, for the presence of abnormal morphology including: • fibrin strands • platelet clumps i. If any of the above are present, verify the WBC and PLT by a manual slide estimate. ii. If the WBC and PLT estimates match the analyzer counts, report the results. iii. If the estimates do not match the analyzer counts, refer to the next step to obtain an accurate count. 3. If platelet clumps or fibrin strands have interfered, perform one of the following alternate procedures to obtain an accurate count: a. Re-draw specimen in EDTA and sodium citrate tubes if possible. Analyze re-drawn EDTA tube. If the repeat run has no PLT Clumps? IP Message, report these results. b. If there is still a PLT Clumps? IP Message and platelet clumps are present on smear review, it could be an in vitro reaction with EDTA. Analyze the sodium citrate tube. Obtain only the WBC and PLT counts from the sodium citrate tube as sodium citrate alters RBC morphology and indices. c. Multiply the PLT results from the sodium citrate tube by 1.1. d. If recollection is not possible or if platelet clumps persist when using sodium citrate, estimate the platelet count and report as decreased, adequate or increased and comment on the platelet clumps.

B. Perform peripheral smear evaluation (if required)
1. Scan smear on minimum of 5-10 fields at 50X magnification.
2. Review WBC, RBC, and platelet abnormalities or instrument flag notations.
3. If smear is normal, or agrees with automated results, verify automated report.
4. If abnormalities are found and do not correlate with automated results, continue with differential or other troubleshooting techniques. If any of the following elements are present, perform manual differential:

Band	>10%
IG flag	>5%
	>1%
Basophil	>4%
Plasma Cell	Any Seen
Blast/Immatures	Any Seen
NRBC's/100	>1%

C. Using LIS Result Entry, order a manual differential if required.
D. Verify name on slide correlates with analyzer histogram.

E. Perform a WBC estimate on 50X oil in the area which differential will be performed on.
   1. Estimate the number of leukocytes you see per field by scanning 5-10 fields.
   2. Multiple that number by 2.
   3. Estimate should compare to within ± 2.0-3.0 of WBC.
      a. If WBC does not match within limits, investigate cause by either re-estimating WBC, performing another automated WBC count, or preparing new slide.
      b. If estimate correlates to analyzer WBC count, continue with next step.
      c. Document that you performed the WBC estimate by placing number value on the LIS result entry.

F. Perform a 100 cell leukocyte differential on 50X oil using Diff counter ICON.

G. While performing differential, document abnormal WBC morphology and report as follows:

WBC Morphology Element	Quantity required to report	Report as:
Auer Rods	ANY	PRESENT
Dohle Bodies	ANY	PRESENT
Hypersegmented Neutrophils	>10% of WBC Differential	PRESENT
Immature eosinophils and/or basophils	ANY	PRESENT
Pelgeroid cells (hyposegmented or bilobed neutrophils)	ANY	PRESENT
Reactive lymphocytes	>10% of Lymph Differential	PRESENT
Smudge Cells (perform albumin slide evaluation)	≥10% of WBC Differential	PRESENT
Toxic granulation	ANY	PRESENT
Vacuolated Neutrophils	ANY	PRESENT

H. Perform RBC morphology using 100X oil in a thin area where red blood cells are evenly spaced.
   1. If indices, RDW, and scan appear normal, report RBC morphology NORMAL.
   2. Confirm Hematocrit value fits the "Rule of Three" = HCT = (HGB X 3) ± 2%.
      a. If Rule is found true, continue with step 8.c.
      b. If not found to be true, possible lipemia, cold agglutinins, osmotic matrix effect, or instrument malfunction may be present. Troubleshoot according to Complete Blood Count: Whole Blood and Body Fluid Analysis on the Sysmex XN-3000 Automated Hematology Analyzer
      c. Grade as follows: Determine percent by estimating number of cell types on 100X oil field

Percent Cell Type	AVERAGE CELLS/HPO	Modifier
< 3%	< 7	Occ
3-5%	7-11	1+
6-10%	12-22	2+
11-15%	23-55	3+
>25%	>55	4+

   3. Report any morphology as described.

RBC MORPHOLOGY:	QUANTITY REQUIRED TO REPORT	REPORT AS:
ACANTHOCYTES	GRADED ≥ 2+	GRADE

ANISOCYTOSIS (RDW >20.0)	GRADED ≥ 2+	GRADE
BASOPHILIC STIPPLING	ANY	GRADE
BURR CELLS	GRADED ≥ 2+	GRADE
HELMET CELLS	GRADED ≥ 1+	GRADE
HGB CRYSTALS	ANY	PRESENT
HOWELL JOLLY BODIES	ANY	GRADE
HYPOCHROMIA	>1/3 Central Pallor Seen	PRESENT
OVALOCYTES	GRADED ≥ 2+	GRADE
OVALOMACROCYTES	ANY	PRESENT
POLYCHROMASIA	GRADED ≥ 2+	GRADE
ROULEUAX	ANY	PRESENT
SCHISTOCYTES***	GRADED ≥ 2+	GRADE
***if > 2+ schistocytes are present, perform platelet estimation.		
SICKLE CELLS	ANY	GRADE
SIDEROCYTES	ANY	GRADE
SPHEROCYTES	ANY	GRADE
TARGET CELLS	GRADED ≥ 2+	GRADE
TEAR DROP CELLS	ANY	GRADE

I. NUCLEATED RBC'S:
   a. If 5 or more nrbc's are seen on peripheral smear.
      1) Correlate manual count to automated count.
      2) If counts correlate Report number of NRBC's and WBC counts from the instrument.
      3) If they do not match, investigate the problem either by performing another manual count, performing another instrument count or drawing a new sample.
      4)

J. PLATELETS: If PLT CT flag or when count is <100 X 10^9/L or > 600 X 10^9/L, perform scan on entire slide, especially the edges on all three sides, on 100X oil in thin area where red cells do not touch.
   a. If platelet clumps found, have specimen redrawn. If platelet satellitism present, redraw into Sodium Citrate. Run specimen and multiply results by 1.1.
   b. If no clumps appear, perform a platelet estimate and scan platelet morphology.
      1) Count the platelets in 10 fields and multiply by 2,000 to estimate.
         i. Compare estimate against the automated platelet count; the two should compare within ±50,000 for normal or elevated counts and ± 20,000 on decreased counts.
         ii. If they do not match, investigate the problem either by re-estimating, performing another instrument count or drawing a new sample.
         iii. Document that you performed the PLT estimate by placing number value on the analyzer print-out.
   c. Report out platelet morphology including giant platelets and hypogranular platelets as PRESENT.
   d. If giant platelets are seen in moderate amounts, perform a manual WBC.

VIII. Procedural Notes:

1. At the discretion of the technical staff, a 200 cell differential may be performed when manual differential differs from automated differential or previous patient differential, or in instances of questionable results that may require another tech to perform differential to confirm accuracy.

2. Lipemic or Grossly Hemolyzed Specimen:
   a. Lipemia, icterus, and/or hemolyzed samples may cause an erroneous HGB, MCH and MCHC when performed on automated analyzer. Lipemic and/or grossly hemolyzed specimens will be recognized by the failure of the 3Xs rule for HGB/HCT, an increased MCHC and "HGB Turbidity?" flag. The MCH and MCHC will also be erroneous since the hemoglobin is used in both calculations.
   b. Icteric samples may falsely elevate HGB. Perform 1:5 dilution with DCL Cell Pack and rerun in capillary mode.
   c. Confirmation of lipemia can then be made by spinning an aliquot of blood and/or a micro-hematocrit to observe cloudy plasma sample.
   d. Perform a plasma replacement procedure or use a plasma blank and recalculate.
      1) Plasma Replacement Procedure
         i. Pour an aliquot of well-mixed whole blood into a test tube and spin for 2 minutes.
         ii. Pipette off as much plasma as possible without disturbing RBCs. Note the amount that was pipetted off.
         iii. Using cell pack reagent, replace the exact amount that was pipetted off.
         iv. Mix aliquot sample well and rerun. Run within 15 minutes of preparing sample
         v. Compare RBCs of original specimen to that of the replacement specimen to insure an accurate count (RBCs must be within 5%). Check MCHC to see that it is within expected range.
         vi. If either RBCs and/or MCHC do not correlate, repeat procedure making new aliquot solution.
         vii. Recalculate the MCH & MCHC using corrected HGB. (See Indices Procedure for formulas).
         viii. Enter results into LIS.
      2) Plasma Blank
         i Spin down an aliquot of blood. Aspirate off the plasma and perform a HGB on the plasma sample.
         ii Calculate the corrected hemoglobin using the following formula.

$$\text{Corrected HGB} = \text{Whole Blood HGB} - \frac{[\text{Plasma HGB} \times 100 - \text{HCT}]}{100}$$

         iii Recalculate the indices using corrected HGB. (See Indices Procedure for formulas).

3. Osmotic Matrix Effect: When a patient has a highly elevated glucose and/or sodium level, the MCV, HCT, & MCHC may be erroneous when performed on an automated cell analyzer. This is called the osmotic matrix effect. When RBC have a high concentration of either sodium and/or glucose and are diluted with saline, the cells are swell causing a spurious macrocytosis, which given an erroneous high HCT> This elevated HCT will then cause an erroneous MCV. The osmotic matrix effect will be recognized by the failure of the 3Xs rule for HGB/HCT and a decreased MCHC with HYPO flag. To correct this effect:
   a. Make a 1:5 dilution and allow to set for 15 minutes.
   b. Run the dilution on manual Mode.

c. If still appears erroneous, spin a micro HCT and recalculate MCV, MCH & MCHC.

4. Platelet Satelliting: Platelet Satellism is when platelets adhere to the WBC and form a satellite around the cell. This will greatly decrease the instrument platelet count. Platelet satelliting can be recognized on a Wright's stained peripheral smear. When this occurs, perform the following procedure:
   a. Redraw the sample in a blue top sodium citrate tube:
   b. Run the sample through automated analyzer to obtain a PLT CT.
   c. Before reporting results, platelet count must match platelet estimate from smear (± 50.0 on count > 100.0 and ± 20.0 on counts <100.0).

5. Correction of WBC for micro megakaryocytes: WBC counts must be corrected for the presence of Micromegakaryocytes since manual hemocytomer methods and automated instruments cannot differentiate between the two. If greater than 5 micromegakaryocytes seen on the differential, correct the WBC using the following formula.

$$\frac{\text{WBC} \times 100}{\text{Micromegakaryocytes} + 100} = \text{Corrected WBC}$$

IX. Reporting Results

1. Before verifying results correlate differential with hemogram for aberrant results.
2. Verify results in LIS.
3. Determine if pathologist review is required according to Policy: Criteria Used For Ordering Abnormal Hematology Reviews, HEMO-01; if so, follow up accordingly.

X. References

1. Koepke, "PRACTICAL LABORATORY HEMATOLOGY", Churchill Livingstone Inc. 1991

POLICY CREATION :		
Author:	***Theresa Mikolajczyk***	***August 31, 2010***
Medical Director:	***Horea Baila, M.D.***	***September 7, 2010***

MEDICAL DIRECTOR		
DATE	NAME	SIGNATURE
February 11, 2017	Elizabeth A. Bauer-Marsh, M.D.	Elizabeth A. Bauer-Marsh MD
SECTION MEDICAL DIRECTOR		
December 16, 2011	Wei Liu, M.D., PhD	
July 2, 2014	Julia Adams, M.D.	Julia Adams, M.D.

REVISION HISTORY (began tracking 2011)			
Rev	**Description of Change**	**Author**	**Effective Date**
1	Minor formatting changes, incorporated abnormal flag/action section from HST procedure, and specimen troubleshooting information.	T. Mikolajczyk	8/31/10
2	Updated notation in 9.d Morphology Table to require platelet estimate for > 2+schistocytes, in place of manual platelet count.	T. Mikolajczyk	10/28/10
3	Updated process for manual differentials to reflect with the new LIS. Changes on the troubleshooting techniques correlating with the criteria established by the pathologist for abnormal peripheral smear reviews.	N. Rutledge	10/31/11
4	Added to procedure suggested Action steps for RET ABN Scattergram flag. Spelling corrections	N. Rutledge	5/31/12
5	Added under procedure promyelocytes, plasma cells, cells suspicious for malignancy and unclassifiable cells., system logo updated	Kathy Turpin	1/21/14
6	Changed all of the flagging guide to reflect the new XN 3000 flagging guide	Kathy Turpin	3/27/15
7	Added A.) Perform manual differentials on all newborn CBC's with Diff's.	Kathy Turpin	9/28/15
8	Clarified language on IG flag and manual differential	Kim Paige	12/1/16
9	Clarified pathology protocol for suspected leukemias.	June Bembenek	03/03/17

Reviewed by

Lead	Date	Coordinator	Date	Manager	Date	Medical Director	Date
			3/31/10				3/30/10
			9/7/2010				9/7/10
		Theresa R Mikolajczyk	10/28/10				10/28/10
			11/7/11				12/16/11
			6/12/12				6/8/12
		Kathy L. Turpin	1/21/14				2/4/14
R. Fitzgerald	3/30/15	Kathy L. Turpin	3/27/15				4/28/15

R. Fitzgerald	9/28/15	Kathy L. Turpin	9/28/15			Julie Adams, M.D.	2/1/16
K. Paige	1/20/17	June Bambenek	1/20/17	Kathy L. Turpin	1/20/17	Julie Adams, M.D.	1/20/17
K. Paige	3/3/17	June Bambenek	3/3/17	Kathy L. Turpin	3/3/17	Julie Adams, M.D.	3/6/17