TRAINING UPDATE

Lab Location:	All Sites	**Date Distributed:**	3/28/2021
Department:	Core lab	**Due Date:**	4/28/2021

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Sysmex XN Series Operation for CBC and Reticulocytes (AHC.H963 v10)

Description of change(s):

1. **Section 10.6:** added a rule to flag H&H that don't meet the "Rule of 3s criteria (HCT not within 3x HB (+/-5))

2. **Addendum 9 step 19**: If the H&H does not match the Rule of 3s criteria. DI will display the error, "Rule of 3s. Warm to 37 degrees for 30 min, rerun. Investigate issue if unresolved. See section 10.6 of SOP".

The above changes are highlighted in the attached SOP revision.

This revised SOP will be implemented on March, 2022

Document your compliance with this training update by taking the quiz in the MTS system.

Technical SOP

Title	**Sysmex XN Series Operation for CBC and Reticulocytes**
Prepared by	Ashkan Chini Date: 5/17/2017
Owner	Robert SanLuis Date: 5/17/2017

Laboratory Approval	**Local Effective Date:**	
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		

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1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Hemogram *(WBC, RBC, HGB, HCT, MCV, MCH, MCHC ,RDW PLT, MPV)*	Sysmex XN Series	CBCND
Hemogram & diff (*WBC,RBC,HGB,HCT,MCV,MCH,MCHC,RDW, PLT, MPV, differential)*		CBC
Differential count only		DIFF
Platelet Count		PLTC
Reticulocyte (*Percent, Absolute, RET-He)*		RCOUNT

Abbreviation	Term	Abbreviation	Term
WBC	White Blood Cell	MCHC	Mean Corpuscular Hemoglobin Concentration
RBC	Red Blood Cell		
HGB	Hemoglobin	RDW	Red Cell distribution Width
HCT	Hematocrit	DIFF	Differential Count
MCV	Mean Cell Volume	PLT	Platelet
MCH	Mean Corpuscular Hgb	MPV	Mean Platelet Volume
RETIC	Reticulocyte Count	IPF	Immature Platelet Fraction
RET-He	Reticulocyte Hgb Equivalent	IG	Immature Granulocytes

Department
Hematology

2. ANALYTICAL PRINCIPLE

The RBC detector counts the RBC and PLT via the Hydro Dynamic Focusing. At the same time, the hematocrit is calculated via the RBC pulse height detection method.
The RBC is calculated as a particle count between lower and upper discriminators, which are automatically setup in the ranges of 25 – 75 fL and 200 – 250 fL. The particle size distribution is checked for abnormal relative frequencies at each discriminator level existence of more than one peak and abnormal distribution width.
The PLT count is calculated as a particle count between lower and upper discriminator, which are automatically set up in the ranges of 2 – 6 fL and 12 – 30 fL. PLT particular size distributions are checked for abnormalities, including abnormal relative frequencies at the lower discriminator, abnormal distribution widths, and existence of more than one peak.
Flow Cytometry is used to analyze physiological and chemical characteristics of cells and other biological particles. It is also used to analyze those cells and particles as they are passed through extremely small flow cells.
The WNR Channel uses flow cytometry to create a scatter gram and is primarily used to count the white blood cells and nucleated RBC. This scatter gram displays groups of nucleated RBC, basophil, non-basophil WBC, hemolyzed RBC and platelets.

The WDF Channel uses flow cytometry to create a scatter gram and is primarily used for classifying WBCs. This scatter gram displays groups of lymphocytes, monocytes, eosinophils, basophils and neutrophils.
The WPC Channel uses flow cytometry to create a scatter gram and is used for detecting immature WBCs such as myeloblasts and abnormal lymphocytes. This scatter gram displays groups of immature/abnormal WBCs and mature WBCs.
Sysmex XN does not use the formula HCT = (RBC x MCV)/10; instead it directly measures hematocrit by adding up the cumulative number and heights of the pulses determined during the RBC counting process.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	N/A
Special Collection Procedures	N/A

3.2 Specimen Type & Handling

Criteria	
Type -Preferred **-Other Acceptable**	K_3EDTA or K_2EDTA Whole Blood Sodium Citrate – for platelet count only
Collection Container	Lavender Top Tube or Microtainer Blue Top Tube (Sodium Citrate)
Volume - Optimum **- Minimum**	Full Tube Adult: 1.0 mL, Microtainer: 0.5 mL
Transport Container and Temperature	Collection container at room temperature
Stability & Storage Requirements	Room Temperature: 48 Hours
	Refrigerated: 48 Hours
	Frozen: N/A
Timing Considerations	N/A
Unacceptable Specimens & Actions to Take	Specimens that are unlabeled, improperly labeled, or those that do not meet the stated criteria are unacceptable. Notify the attending nurse or physician and request a recollection and credit the test with the appropriate LIS English text code for "test not performed" message. Example Quantity no sufficient-QNS; Wrong collection-UNAC. Document the request for recollection in the LIS. Refer to section 13.11 for Icterus & Lipemic specimens
Compromising Physical Characteristics	Gross hemolysis: Refer to section 13.11
Other Considerations	Cancel clotted specimens, notify the attending nurse or physician and request a redraw.

NOTE: Labeling requirements for all reagents, calibrators and controls include: (1) Open date, (2) Substance name, (3) Lot number, (4) Date of preparation, (5) Expiration date, (6) Initials of tech, and (7) Any special storage instructions. Check all for visible signs of degradation.

4. REAGENTS

The package insert for a new lot of kits must be reviewed for any changes before the kit is used. A current Package Insert is included as a Related Document.

4.1 Reagent Summary

Reagents / Kits	Supplier & Catalog Number
Cell Clean Auto	Sysmex Corporation, Cat. No. CF579595
Cell Pack DCL	Sysmex Corporation, Cat. No. DCL-300A
Cell Pack DFL	Sysmex Corporation, Cat. No. BT965910
Fluorocell PLT	Sysmex Corporation, Cat. No. CD994563
Fluorocell RET	Sysmex Corporation, Cat. No. BN337547
Fluorocell WDF	Sysmex Corporation, Cat. No. CV377552
Fluorocell WNR	Sysmex Corporation, Cat. No. CP066715
Lysercell WDF	Sysmex Corporation, Cat. No. ZA900001
Lysercell WNR	Sysmex Corporation, Cat. No. ZA900002
Sulfolyser SLS	Sysmex Corporation, Cat. No. BJ350971
Wright-Giemsa Stain	Sysmex Corporation, Cat. No. ACC-SP5741
Phosphate Buffer Solution, pH6.8	Sysmex Corporation, Cat. No. ACC-SP5548
Methyl Alcohol Absolute	Medical Chemical corporation, Cat. No. 107B
NERL Reagent Grade Water	Thermo Fisher Scientific, Cat. No. 9800-4

4.2 Reagent Preparation and Storage

Reagent	Cell Clean Auto
Storage	Store at 1 - 30°C. Avoid exposing to direct sunlight
Stability	This reagent is for single use only. Once the product stopper is punctured, the remaining reagent must be thrown away after each use.
Preparation	None

Reagents	Cell Pack DCL, Cell Pack DFL
Storage	Store at 2 - 35°C. Avoid exposing to direct sunlight
Stability	Once in use, these reagents remain stable for 60 days.
Preparation	None

Reagents	Fluorocell PLT, Fluorocell RET, Fluorocell WDF, Fluorocell WNR, Lysercell WDF
Storage	Store at 2 - 35°C. Avoid exposing to direct sunlight
Stability	Once in use, this reagent remains stable for 90 days.
Preparation	None

Reagent	Lysercell WNR
Storage	Store at 2 - 35°C. Avoid exposing to direct sunlight
Stability	Once in use, this reagent remains stable for 60 days.
Preparation	None

Reagent	Sulfolyser SLS 1.5 L
Storage	Store at 1 - 30°C. Avoid exposing to direct sunlight
Stability	Once in use, this reagent remains stable for 60 days.
Preparation	None

Reagent	Wright – Giemsa Stain
Storage	Store at 15 - 30°C
Stability	Unopened and opened until the expiration date on the container.
Preparation	None

Reagent	Phosphate Buffer Solution, pH 6.8
Storage	Store at 15 - 30°C
Stability	Unopened and opened until the expiration date on the container.
Preparation	None

Reagent	Methyl Alcohol Absolute
Storage	Store at 15 - 35°C
Stability	Unopened and opened until the expiration date on the container.
Preparation	None

Reagent	NERL Reagent Grade Water
Storage	Store at room temperature
Stability	Reagent will remain stable for 30 days after opening
Preparation	None

Auto Rinse needs to be run after every reagent change; to run an Auto Rinse:

- Click the **Analyzer Menu** button
- Select **Auto Rinse**
- Once complete, [Auto Rinse] disappears and the background check begins
- Background check performs analysis without aspirating the sample to verify the effects of the auto rinse
- When Background check is finished, from the **Main Menu**

- Select **Sample Explorer**
- High light the background check which was just run
- Click **Validate**
- Report prints
  Note: There should be no values on the report; only zeros and dashes should appear. If any value other than zero appears on the report, that means the background check has failed and the instrument will flag the failure.
- Review and sign the printed report and place in the Sysmex Maintenance binder.

To get reagent details, such as when it was placed onboard, lot number, expiration date and volume; From **Main Menu** select **History**, then click on **Reagent Replacement Log** tab.

To scan a reagent:

- From **Main Menu** either click on the reagent picture or click on **Analyzer Manu** then select **Reagent Replacement**.
- Select the desired reagent
- Select **Replace the Reagent**
- Click on the blank space below **Reagent Code**, make sure the cursor is blinking, then scan the barcode of the reagent
- Click on **Execute**

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier& Catalog Number
XN CAL™	Sysmex 213527
XN CAL™ PF	Sysmex 213536

5.2 Calibrator Preparation and Storage

Calibrator	XN CAL™ and XN CAL™ PF
Storage	Store at 2-8°C in a dark refrigerator.
Stability	Unopened: Manufacturer's Expiration Date Opened: 4 hours
Preparation	Calibrator is supplied ready to use. Bring to room temperature prior to testing. Mix per manufacturer's guidelines.

5.3 Calibration Criteria and Procedure

Criteria	Special Notations
Frequency	• Assay calibration must be performed when the instrument is first placed in service and at least every 6 months thereafter. This process is managed by Sysmex Customer Support Center. 1) First half of the year: Field Service Engineer (FSE) will perform the calibration. 2) Second half of the year: Customer Support Center will ship the calibrator ahead of the time. They will contact the Insight account holders prior to the calibration due date to inform them of the date and time for the calibration. • In addition, calibration verification is required (regardless of the length of time since last performed) immediately if any of the following occurs: 1) When control data indicates a significant shift in assay results and cannot be corrected by maintenance or troubleshooting. 2) After major maintenance, service or replacement of critical parts. 3) When advised by Sysmex Field Service Representative.
Tolerance Limits	Sysmex will provide a calibration report: 1) Review Reagents Information and Calibration Materials to verify all products used for calibration are in-date. 2) On the Background Counts chart, the Count for each parameter must be less than the Limit. 3) On the Precision Open Mode chart, the CV% of each parameter must not exceed the % Limit. 4) On the Sensitivity Verification, each parameter's Mean must fall between Lower and Upper Limit. 5) On the Calibration of Directly Measured Parameters – a. The first run is always eliminated. b. The Mean of each parameter must fall between Lower and Upper Limit. c. If the current coefficient of variation is not the same as the new one, 10 points of QC must be run and reviewed and the QC ranges adjusted if necessary.
Procedure	• Calibration is performed by Sysmex FSE or • Calibration is performed by Sysmex through remote access. Customer must contact Sysmex to order calibration material, for any calibrations that are done outside of major maintenance by FSE or the Sysmex calibration schedule. • Refer to Chapter 12 of the Instructions for Use manual – Performing Calibration – The Customer will be guided by the Sysmex representative to run the calibrators and the values will be updated by the Sysmex representative.

Criteria	Special Notations
	• If calibration factors (compensations rates) have been changed, calibration verification must be performed by running all levels of commercial control. • At the completion of the calibration, a certificate of calibration will be printed by the Sysmex FSE that must be reviewed by a Group Lead/ Supervisor or designee.

6. QUALITY CONTROL

6.1 Controls Used

Controls	Supplier and Catalog Number
XN CHECK, Levels 1, 2 & 3	Sysmex Corporation, Cat. No. 213499

6.2 Control Preparation and Storage

Control	XN CHECK
Preparation	None
Storage	Store at 2 - 8°C
Stability	Unopened: manufacturer's expiration date Opened: 7 days when stored at 2 - 8° C after each use.

6.3 Frequency

All three levels of control must be run on all Sysmex XN instruments every 8 hours of patient testing. QC must also be performed after shutdown, maintenance or instrument repairs.

Refer to addenda 4 for instructions to perform parallel testing for new lots of QC materials.

6.4 Tolerance Limits and Criteria for Acceptable QC

A. Tolerance Limits

The Hematology QC program is monitored in the instrument and should be set up using the Evidence-based QC Limit % Range specific for XN analyzers. These limits are provided by Sysmex and are intended to ensure reasonable error detection capability and minimal false rejection rates. Target values for each level of control will be calculated based on the data collected in the new lot evaluation.

B. Criteria for Acceptable QC

- All Controls must be within the acceptable range.
- Controls and patient data must be reviewed for acceptability and for atypical or unexpected results or trends prior to reporting patient results.

- DO NOT release results from runs with unacceptable controls or with unusual patterns, trends or distribution in patient values.

C. Corrective Action

- All rejected runs must be effectively addressed and include the following documentation:
  - Control(s) that failed and/or atypical or unexpected patient results
  - Actions taken
  - Statement of what was done with the patient samples from the affected run/batch,
  - Date and initials of the person recording the information.
- Patient samples in failed analytical runs must be reanalyzed.
- **Precision Statistics:** When there is a significant shift/bias on QC data, the root cause of the increased imprecision must be investigated and a resolution needs to be considered immediately. All of these actions must be documented including an evaluation of whether or not this affected patient care.

NOTE: The laboratory director or designee may override rejection of partial or complete runs. Justification for the override must be documented in detail.

6.5 Documentation

- QC tolerance limits are programmed on the instrument; it calculates cumulative mean, SD and CV and stores all information for easy retrieval.
- Quality control records are reviewed daily at the bench, weekly by the Lead Technologist or designee, and monthly by the Supervisor/Manager or designee.
- Refer to complete policies and procedures for QC documentation and for record retention requirements in the Laboratory QC Program.

6.6 Quality Assurance Program

- Training must be successfully completed and documented prior to performing this test. This procedure must be incorporated into the departmental competency assessment program.
- The laboratory participates in CAP proficiency testing. All proficiency testing materials must be treated in the same manner as patient samples.
- Monthly QC must be presented to the Medical Director or designee for review and signature.
- QC is submitted to Sysmex for peer group comparison as it is run. Refer to addenda 4 and 5
- Consult the Laboratory QC Program for complete details.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

Sysmex XN Series
Sysmex Automated Slide Preparation Unit SP – 10 (SG/WOMC only)

7.2 Equipment

Microscope
Refrigerator

7.3 Supplies

Immersion Oil
Applicator sticks
Glass Slides
Lens Paper
Sysmex Glass Slides for Stainer (SG/WOMC only)
Ribbon for Stainer (SG/WOMC only)
Glass Plate Spreader (SG/WOMC only)

8. PROCEDURE

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

8.1	Maintenance
1	Perform required instrument maintenance. Refer to addendum 2 for details.
2	Perform required stainer maintenance (SG/WOMC). Refer to addendum 3 for details.

8.2	Test Run
1	Place the racks in the right side feed conveyor. Pre- mixing of samples is not required.
2	Racks will automatically feed to the analyzer. Tubes are rotated to read barcode.
3	On-board IPU rules will determine repeat or reflex testing. Rack will run in reverse to perform the repeat or reflex test.
4	When load is completed, remove racks from left side feed conveyor.

8.3	Special Handling
1	To load a STAT specimen while the instrument is analyzing other samples: Press the mode switch button, the tube holder (for manual/pediatric samples) slides out forward; then run the STAT specimen in manual mode.
2	Aspiration sensor must remain ON all the time. The only times that Aspiration Sensor is turn off are: • When hemoglobin is < 4.0 • When Platelet Poor Plasma study is in progress

NOTE: In the event that the test system becomes inoperable, notify supervision or designee for further direction. Patient specimens must be stored in a manner that maintains the integrity of the specimen.

9. CALCULATIONS

The following calculations are performed automatically by the instrument. The formulas used to calculate MCV, MCH and MCHC are:

- MCV: (HCT/RBC) x 10
- MCH: (HGB/RBC) x 10
- MCHC: (HGB/HCT) x 100

The following calculations are performed when screening a slide and/or performing a differential (refer to Sections 10.6 and 13 for details)

WBC Estimate:
Calculate the average WBC in 10 fields using the 50X objective and multiply by 3,000. If WBC estimate does not agree with what the instrument has reported within ± 20%, repeat the estimate focusing on the feathered edge of the smear. If counts still do not agree, consult with the supervisor or tech in charge.

Platelet Estimate:
Calculate the average PLT in 10 fields using the 100X objective and multiply by 20,000. If PLT estimate does not agree with what the instrument has reported within ± 20%, repeat the estimate focusing on the feathered edge of the smear. If counts still do not agree, consult with the supervisor or tech in charge.

Megakaryocytes could potentially interfere with the WBC count.
If the WBC estimate does not correlate with the automated WBC count in the presence of five or more megakaryocytes, then correct the WBC count use the formula below. To get the uncorrected WBC, **Main Menu** – **Data Browser** – select **Service** tab – select **WNR**.
TNC – N has the uncorrected WBC count.

Corrected WBC = (Uncorrected WBC x 100) / (100 + # of megakaryocytes)

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

The relationship of the RBC, HGB and HCT to MCV, MCH and MCHC			
Parameter Affected	**MCV**	**MCH**	**MCHC**
RBC ↓	↑	↑	No change
RBC ↑	↓	↓	No change
HGB ↓	No change	↓	↓
HGB ↑	No change	↑	↑
HCT ↓	↓	No change	↑
HCT ↑	↑	No change	↓

10.2 Rounding

Any result rounding is performed at the interface level.

10.3 Units of Measure

Parameter	Units	Decimal	Parameter	Units	Decimal	Parameter	Units	Decimal
WBC	x10(3)/mcL	1	RBC	$10^6/\mu L$	1	HGB	g/dL	1
HCT	%	1	MCV	fL	1	MCH	pg	1
MCHC	g/dL	1	PLT	x10(3)/mcL	0	MPV	fL	1
RDW	%	1	Diff, auto	%	1	Retic	%	1
RET-He	pg	0	Diff manual	%	0	IPF	%	0
Retic absol	x10(6)/mcL	4	Diff absol	x10(3)/mcL	2	IG	%	1

10.4 Analytical Measurement Range (AMR)

Parameter	Sysmex XN Series
WBC	0 - 440 x 10^3
RBC	0.00 - 8.60 x 10^6
HGB	0 - 25 g/dL
HCT	0 - 75%
PLT, PLT-F	0 - 5,000 x 10^3
RET%	0 - 30.0%

10.5 Review Patient Data

- Review patient results for unusual patterns, trends or distribution.
- Report atypical or unexpected results or trends for this test to appropriate supervisory personnel, prior to releasing results.

10.6 Repeat Criteria and Resulting

Dilution:

Sysmex XN is not capable of doing any dilutions. Refer to WBC and RBC sections for dilution details, and then follow these steps to program them on the instrument:

- Manually program the accession number by typing the corresponding alpha character equivalent of the day of the week then followed by the number. **Verify the characters are set in a way that the DI will not auto verify the result**.
- Run the sample in manual mode.
- When the sample is resulted, multiply the value by the dilution factor used to make the dilution.

WBC		
Condition	**Action 1**	**Action 2**
< 0.5	Check for a clot, re-analyze sample in LW (Low WBC) mode.	SCAN
≤ 2.0	• Check Sample for a clot • If unable to evaluate 100 cells, do a 50 cell Diff and multiply results by 2. • Re-analyze and verify the count is within ± 15%. Add comment code RVT (reviewed by Technologist)	DIFF
≥ 30.0	Scan to verify count and rule out the increase due to presence of Giant platelets or abnormal protein/cryoglobulin	SCAN
≥ 440.0	Re-analyze using dilution factor 2 (use DCL Cell Pack as diluent); if the diluted result is still > 440.0, then report as > 880.0 , refer to ≥30.0, add comment code REP (results confirmed, test repeated)	SCAN

RBC		
Condition	**Action 1**	**Action 2**
≥ 8.60	Re-analyze using dilution factor 2 (use DCL Cell Pack as diluent); if the diluted result is still > 8.60 then report as > 17.2, scan to verify morphology and add comment code REP	SCAN

HGB		
Condition	**Action 1**	**Action 2**
< 4.0	Refer to section 8.3	N/A
≤ 6.0	Check for a clot	N/A
≥ 20.0 Excludes Neonates	Check coagulation sample if HCT ≥ 55, add REP comment.	N/A
≥ 25.0	Re-analyze, if the result remains ≥25.0, then report as ≥25.0 and add RVT comment. For neonates, if there is not sufficient sample to re-test, report as ≥25.0	N/A

MCV		
Condition	**Action 1**	**Action 2**
≤ 50.0	Check for a clot, re-analyze and verify morphology, add RVT comment.	SCAN
≥ 130.0	Warm specimen to 37°C for 30 minutes then re-analyze, verify morphology, denote any rouleaux or RBC agglutinins	SCAN

Platelet		
Condition	**Action 1**	**Action 2**
< 50	Check for a clot, re-analyze using optical mode if not already performed.	N/A
50-100	When patient is <30 days: Check for a clot, perform platelet estimate, and add RVT comment.	SCAN
> 5000	Re-analyze using dilution, perform platelet estimate, add REP comment.	SCAN

Lymphocyte		
Condition	**Action 1**	**Action 2**
≥ 70	If differential agrees with auto count, Pathologist review is required	DIFF
Note: For patients <12 years old perform DIFF if the Lymphocyte count is greater than Neutrophil		

Neutrophil		
Condition	**Action 1**	**Action 2**
≥ 90 No flags	Scan for any bands, hyper segmentation or toxic granulation	SCAN
≥ 90 With flags	Rule out immature Neutrophils	DIFF

Monocyte		
Condition	**Action 1**	**Action 2**
> 25	Rule out immature Monocytes	DIFF

Eosinophil		
Condition	**Action 1**	**Action 2**
≥ 35	Scan to verify Eosinophils. Rule out presence of parasites.	SCAN

Basophil		
Condition	**Action 1**	**Action 2**
≥ 3.5	Mix for 5 minutes then re-analyze, if still elevated scan to verify cells	SCAN

Reticulocyte		
Condition	**Action 1**	**Action 2**
> 30.0%	• Check Sample for a clot, Vortex the sample for 1 – 2 minutes and reanalyze • If same result is obtained with no flag - check patient's history, check with the attending physician/nurse to confirm the situation and result, inform the supervisor/tech in charge and with their permission report as > 30.0 %. • If same result is obtained with a flag, refer to section 13.9	Repeat
> 6.0%	• When patient is greater than 30 days old, hold for scan	SCAN

Differential Timing
If a patient had a differential done in the past 48 hours while remaining admitted, there is no need to do another differential if CBC parameters are improving (moving toward normal). Exceptions are: • If blasts flag • Physician request • If Asterisk appears next to results

H and H Mismatch
On samples where hemoglobin and hematocrit results differ significantly using the rules of 3s - for example HGB value of 12.5 and HCT value of 1.6 (HCT not within (3 x HGB) +/- 5)), do the following: • Check for a clot • Incubate the sample for 30 minutes at 37 °C and reanalyze. If the H and H mismatch remains proceed to next step • Spin the sample and check for hemolysis, lipemia or icteric. If either one is detected, refer to section 13.11 • Contact attending physician / nurse and ask for patient history, medication and a redraw if the current result seems questionable.

Supervisor (or designee) / Pathologist slide review		
Abnormality	**Supervisor**	**Pathologist**
Prolymphs > 5%	X	
Reactive and/or atypical lymphocytes >20%	X	
Bands > 25%	X	
Meta/Myelos/Promyelo >10%	X	
Any blast cell	X	X
Any unidentifiable cell	X	X
Any parasite or microorganism (reviewed by microbiology also)	X	
Lymphocyte > 75% in patients < 17 years of age	X	
Lymphocyte > 70% in patients > 17 years of age	X	X

NOTE: The above guidelines are for new and recurring patients performed initially and over each subsequent hospital encounter (ED visit, OP visit or admission).

Handling and Resulting Pathologist Reviewed Slides

A. Technician/Technologist will append English Text code SFPR (Submitted for path review) to one of the cell counts and then release / report results of their differential

B. Technician/Technologist will submit slides for pathologist review as follows:
   1. Add order code HPATH to the accession via REI or GUI Order Entry.
   2. Ensure slide is of acceptable quality for pathology review; appropriate smear, adequate staining, and properly labeled.
   3. Cover-slip the slide
   4. Complete Pathologist Slide Review Request form
   5. Attach analyzer print-out

C. Technician/Technologist will enter the Pathologist differential results into the LIS via SmartTerm. Note: This should include the pathologist's comments or assessment regarding the diff count that has already been reported. The original report does NOT need to be corrected.

Physician Request for Manual Differential
If a physician requests a manual differential, then a new order (accession number) must be created. Utilize the same collect date and time as the sample and order test code DIFF.

11. EXPECTED VALUES

11.1 Reference Ranges

See Addendum 1.

11.2 Critical Values

Parameter	Age	Critical Low	Critical High
HGB	1 month and older	< 6.1	> 19.9
HGB	0-29 days	< 6.1	> 23.9
WBC	all ages	< 2.1	> 29.9
Platelet	all ages	< 31	> 899

11.3 Standard Required Messages

SGMC only: If patient is in NICU (3C), then call platelet count ≤ 75 x10(3)/mcL

12. CLINICAL SIGNIFICANCE

- **CBC –** The quantitative and qualitative analysis of the cellular elements of blood will identify imbalance between cell production, cell release, cell survival, or cell loss. This information increases the accuracy and specificity of diagnosis based on pathogenesis and is also used to monitor the effectiveness of therapy.
- **Automated Differential –** The Differential distribution of white blood cells will, when correlated with absolute white cell count, identify imbalances between cell production, cell release, cell survival and/or cell loss. This information increases the accuracy and specificity of diagnosis based on pathogenesis and is also used to monitor the effectiveness of therapy.
- **Platelet Count –** Platelets must be present in adequate numbers and have proper function to aid in hemostasis. A normal bleeding time is dependent on adequate platelet number and function.
- **Reticulocyte Count -** The enumeration of reticulocytes provides an effective means of determining red cell production and regeneration. Elevation is seen in patients with hemolytic anemia, hemorrhage (acute and chronic), treatment of iron-deficiency anemia and megaloblastic anemias and uremia. Decreased counts may be seen in aplastic anemia, aplastic crisis of hemolytic anemias and ineffective erythropoiesis as seen in thalassemia, pernicious anemia and sideroblastic anemia.
- **IPF – Immature Platelet Fraction:** Platelets are produced in the bone marrow and are normally not released into the bloodstream until they have matured. When platelet numbers in the blood are low (thrombocytopenia), it stimulates the bone marrow to produce platelets faster. When the need is great and when production cannot keep up with

demand, then an increased number of immature platelets will be released into the blood stream. The IPF may be used to help a healthcare provider determine the likely cause of a person's thrombocytopenia, that is, decrease in production by the bone marrow (IPF is low) versus increased loss of platelets in the blood (IPF is higher).

- **RET-He – Reticulocyte Hemoglobin Equivalent:** It is one way to measure the hemoglobin inside of reticulocyte. Reticulocytes are "young" red blood cells that are released by the bone marrow before they become fully mature. The amount of hemoglobin inside of reticulocytes can help determine if there has been enough iron available, to be incorporated into hemoglobin production and then into red blood cell production in the bone marrow. This makes the test useful in identifying functional iron deficiency.
- **IRF – Immature Reticulocyte Fraction:** It is the ratio of immature reticulocytes to the total number of reticulocytes. This parameter provides a very early and sensitive index of marrow erythropoietic (RBC production) activity.
- **Immature Granulocytes (IG):** This instrument has a 6-part differential that is comprised of Neutrophil, Lymphocyte, Monocyte, Eosinophil, Basophil and Immature Granulocyte. The Immature Granulocyte results include metamyelocyte, myelocyte and promyelocyte.
- **Platelet Fluorescent:** The platelet measurement is done using a nucleic acid stain specific for platelet organelles and flow cytometry. The PLT-F result will have "&F" to the left of the result indicating the result was obtained in the PLT-F channel.

13. PROCEDURE NOTES

- **FDA Status:** FDA Exempt/Cleared or Approved with modification(s).
- **Validated Test Modifications:** Sample room temperature stability extended. Quest Corporate Validation on file, see the Technical document index.

This section explains the Interpretive Program (IP) messages generated by the Sysmex XN analyzer and the corrective actions.

13.1	WBC Abn Scattergram
Cause: Clustering in the WNR or WDF scattergrams is abnormal; meaning analyzer cannot separate the cell population with confidence.	
Corrective Action: 1. If dashes appear in place of data: a. Repeat the sample b. If dashes still remain, perform a manual differential count 2. If Asterisk appears next to data: a. Scan the slide for abnormal cells and NRBC b. Perform a manual differential if abnormal cells are observed c. If no abnormal cells are found, then report the result	

13.2	NRBC Present
The analyzer identifies and counts NRBCs simultaneously while counting WBCs (both are counted separately). **No further correction of the WBC count is required.** If NRBCs are greater than 0.01/100 WBC, the lymphocyte count is automatically corrected.	

13.3	IG Present (>5% flag)

Cause: Presence of cells (metamyelocyte, myelocyte and promyelocyte) accurately quantitated by the analyzer

Corrective Action:

For IG >5%, this flag appears in red. Perform a manual differential count and scan the peripheral smear for the presence of the following:

- Promyelocytes, myelocytes and metamyelocytes
- Band cells in increased numbers
- Toxic granulation or vacuolation of neutrophils
- Other abnormal cells

13.4	Blast/Abn Lymph

Cause: The analyzer has detected abnormal clustering in the region for blasts and abnormal lymphocytes in the WDF (differential) scattergram.

Corrective Action:

1. If dashes appear in place of data:
   a. Repeat the sample
   b. If dashes still remain, perform a manual differential count
2. If Asterisk appears next to data, perform a manual differential count and scan the peripheral smear for the presence of the following:
   a. Blasts – lymphoblasts, myeloblasts, and myelomonoblasts
   b. Immature Granulocytes – promyelocytes, myelocytes, and metamyelocytes
   c. Atypical or immature lymphocytes
   d. Other abnormal cells

13.5	Left Shift

Cause: The analyzer has detected abnormal clustering in the region for the left shift (bands) in the WDF (differential) scattergram.

Corrective Action:

1. If dashes appear in place of data:
   a. Repeat the sample
   b. If dashes still remain, perform a manual differential count
2. Perform a manual differential count and scan the peripheral smear for the presence of the following:
   a. Band cells in increased numbers
   b. Toxic granulation, hyper-segmentation or vacuolation of neutrophils
   c. Other abnormal cells

13.6	Atypical Lymph

Cause: The analyzer has detected significant clustering in the region for atypical lymphocytes that is located in the upper left lymphocyte region on the WDF (differential) scattergram. An asterisk appears next to the Neutrophil, Lymphocyte, Monocyte, Eosinophil and Immature Granulocyte % and #.

13.6	Atypical Lymph

Corrective Action:
Perform a manual differential count and scan the peripheral smear for the presence of the following:

- Atypical or variant lymphocytes
- Abnormal or atypical monocytes
- Immature lymphocytes
- Immature monocytes
- Smudge cells
- Other abnormal cells

13.7	RBC Abn. Distribution

Cause: When the histogram pattern from the RBC channel is abnormal or when RBC is < 0.50 x $10^6/\mu L$. Dashes may appear in place of results for the RDW-CV. This message may cause certain RBC parameters to be marked with an asterisk.

Corrective Action:
Scan the peripheral smear for the presence of abnormal RBC morphology such as those listed. (**Only note if findings are ≥ 2+)**:

- Increased anisocytosis
- Multiple RBC population
- Fragmented RBC
- Poikilocytosis
- Rouleaux or RBC agglutination

If the RBC morphology is normal and MCHC is abnormal (<30 or >37.5 g/dL) refer to guidelines for the HGB/Turbidity Interference? IP Message, section 13.11
If RDW remains uncalculated, result as UNC (unable to calculate)

Note: For consistent morphological reporting, the following criteria are recommended. Review 10 fields using the 100X objective to calculate this mean range.

Variation	Normal	1+	2+	3+
Poikilocytosis	0	1 – 5	6 – 15	> 15
Microcytosis or Macrocytosis	0	1 – 5	6 – 15	> 15
Anisocytosis	0 – 5	6 – 15	15 – 30	> 30
Polychromasia	0 – 2	3 – 4	5 – 6	> 6
Hypochromia	0 – 5	6 – 15	16 – 30	> 30

Cell Type	Normal	1+	2+	3+
Spherocyte, Acanthocyte, Sickle cell, Rouleaux	0	1 – 5	6 – 15	> 15
Helmet cell	0 – 1	1 – 5	6 – 15	> 15
Tear drop, Target cell, Schistocyte, Ovalocyte, Elliptocyte, Burr cell, Stomatocyte, Blister cell	0 – 2	3 – 4	5 – 6	> 6

13.8	Dimorphic Population

Cause: When there are multiple peaks in the RBC histogram pattern. Refer to section 13.7 for corrective action.

13.9	RET Abn Scattergram

Cause: The analyzer cannot separate the cell population with confidence. There is a possibility of cell overlap. Asterisks appear next to the RET%, RET#, IRF and RET-H*e* parameters.

Corrective Action:

1. Vortex 1-2 minutes and repeat.
2. If the flag is not eliminated, or the RBC count is <0.50 x 106/µL review the peripheral smear for the presence of polychromasia, parasites, NRBCs, Howell-Jolly Bodies, Heinz bodies or basophilic stippling. If any of those abnormal cells are present, report the results with comment "results may be affected by the presence of interfering substances".

13.10	RBC Agglutination

Corrective Action:

1. Warm the sample at 37º C for 15 - 30 minutes. 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 measured. See section 9 for WBC and PLT estimate calculations.
2. Sometimes agglutination can be so severe that warming the sample does not enable accurate analysis. In this case perform a plasma replacement:
   a. Centrifuge an aliquot of blood from the primary tube to separate the cells from the plasma.
   b. Using a pipette, remove a measured amount of plasma removing as much plasma as possible without disturbing the buffy coat.
   c. Add back the same amount of CELLPACK DCL as the volume of plasma removed in step "b".
   d. Cap the tube and mix the sample by manual inversion until the cells are fully re-suspended in the CELLPACK DCL.
   e. Reanalyze the sample in the manual mode.

13.11	Turbidity/HGB Interference

If the MCHC is ≤ 30.0 or ≥ 37.5, repeat to rule out random error.

- If MCHC is ≤ 30.0 a slide should be made and scanned to look for potential causes of spuriously low MCHC, example: marked sickle cells or target cells.
- If the MCHC is greater than 37.5, a slide should be made and examined as well as visual inspection of the sample to determine the integrity of the specimen. The smear review / visual inspection should indicate to the technologist which category the specimen falls into – cold agglutinin, lipemia, hemolysis, icterus or the situation where the results are accurate due to the presence of spherocytes.

Corrective Action:

1. If Spherocytes are noted on the slide:
   a. Report the MCHC with a comment reflecting the presence of spherocytes as 1+, 2+ or 3+
2. If significant RBC agglutination is noted on the slide, warm specimen in a 37°C heat block for 30 minutes and rerun. If not resolved, continue warming and rerun every 15 minutes continuing incubation after each run, not to exceed one hour. If necessary, make a warmed slide for morphology evaluation.
   a. If MCHC is within normal range, Report results with the LIS code SWCG which translates to "Specimen was prewarmed to 37ºC to obtain results. Cold agglutinin/cryoglobulin suspected."
   b. If MCHC is still greater than 37.5 after one hour of incubation, perform plasma replacement. Refer to section 13.10

13.11	Turbidity/HGB Interference

3. If hemolysis is suspected, examine the specimen for visual hemolysis. If gross hemolysis is observed, cancel the specimen with the appropriate comment: -HMT
4. If lipemia or icterus is suspected, perform plasma replacement. Refer to section 13.10

13.12	Fragments

Cause: Due to size comparison of certain RBC or Platelet population, the analyzer cannot separate the cell population with confident. There is a possibility of cell overlap.

Corrective Action: Scan the peripheral smear for the presence of fragmented RBCs and other poikilocytosis

13.13	PLT Abn Distribution

Cause: Indicates that the analyzer has detected abnormal size and population of platelets. Dashes may appear in place of data for the MPV or the MPV may be marked with an asterisk.

Corrective Action:

GEC: Vortex the specimen for 1 – 2 minutes and repeat. If the same flag appears, then scan the peripheral smear to estimate the platelet count and review for the presence of abnormal RBC or PLT morphology such as:

a. Giant platelets
b. Platelet clumps
c. Fragmented RBCs
d. Microcytic RBCs
e. Parasites

If platelet estimate confirms accuracy of analyzer count, report the result.
If MPV remains uncalculated, result as UNC (unable to calculate)

SGMC, WOMC & FWMC: Vortex the specimen for 1 – 2 minutes and reanalyze in PLT-F mode. If the same flag appears, then scan the peripheral smear to estimate the platelet count and review for the presence of abnormal RBC or PLT morphology such as:

a. Giant platelets
b. Platelet clumps
c. Fragmented RBCs
d. Microcytic RBCs
e. Parasites

If platelet estimate confirms accuracy of analyzer count, report the result.
If MPV remains uncalculated, result as UNC (unable to calculate)

13.14	PLT Abn Scattergram (SGMC, WOMC & FWMC Only)

Cause: When clustering in the platelet and IPF area on the PLT – F Scattergram is abnormal. The PLT – F, IPF %, and sometimes MPV are reported with an asterisk. Dashes may appear in place of data for the MPV.

Corrective Action: Follow the steps in section 13.13 under SGMC and WOMC.

13.15	PLT Clumps

Cause: Abnormal clustering of platelets in the WNR, WDF or PLT – F scattergrams.

Corrective Action:

1. Check the sample for the presence of clots:
   - If a clot is detected, cancel the current specimen and request a redraw.
   - If no clot is detected,
     SGMC, WOMC & FWMC: vortex the specimen for 1 – 2 minutes and repeat in PLT – F mode.
     GEC: vortex the specimen for 1 – 2 minutes and repeat.
2. If the flag appears again, then scan the peripheral smear for the presence of abnormal morphology including:
   - Fibrin strands
   - Platelet clumps

Note:

If either fibrin or clumps are present, verify the WBC and PLT by a manual slide estimate (refer to section 9). If the WBC and PLT estimates match the analyzer counts, report the results.

If the estimates do **not** match the analyzer counts, proceed to next step.

3. Recollect a new sample in sodium citrate (blue top tube).
   - Manually program the accession number by typing the corresponding alpha character equivalent of the day of the week then followed by the number and then followed by "-blue" or "-purple". **Verify the characters are set in a way that the DI will not auto verify the result**.
   - Run the blue and purple sample in manual mode.
   - When the sample is resulted, multiply the platelet count from the blue top tube value by 1.1 (to account for the different blood to anticoagulant ratio) and report this in DI
   - The MPV is also reported from the blue top tube, but no correction factor is applied because MPV is not affected by dilution.
   - The rest of the CBC and Diff should be reported from the purple top tube.
4. If the platelet clumps still remain and the platelet count is ≤130, remove the platelet count number and result with these two comments:
   - Unable to report due to significant platelet clumping
   - Platelet estimate comment (decreased, increased or normal)

13.16	Giant Platelets – GEC only

Corrective Action:

Refer to section 13.15 steps 1 and 2

If PLT count is ≤130 with significant giant platelets found during morphology review, then release the result and append the LIS code GPINF which translates to "The WBC count and platelet count may be altered due to interferences caused by the presence of significant numbers of large giant platelets".

Note: Fluorescent platelet functionality will resolve this at SGMC and WOMC.

14. LIMITATIONS OF METHOD

14.1 Precision

Precision is assessed by analysis of peripheral blood. The data appears consistent and all parameters have a low CV%.

14.2 Interfering Substances

Parameter	Causes of Spurious Increase	Causes of Spurious Decrease
WBC	PLT clumps, Cryoprotein, Cryoglobulin, Fibrin, Giant platelets (Platelets > 1,000,000/µL)	Leukocyte aggregation
RBC	Leukocytosis (>100,000µL) Giant platelets (Platelets > 1,000,000/µL)	Erythrocyte aggregation Microerythrocytes Fragmented RBCs
HGB	Leukocytosis (>100,000µL) Lipemia, Abnormal protein	
HCT	Leukocytosis (>100,000µL) Severe diabetes, Uremia, Spherocytosis	Erythrocyte aggregation, Microerythrocytes, Fragmented RBCs
MCV	Spuriously increased HCT Spuriously decreased RBC	Spuriously decreased HCT Spuriously increased RBC
MCH	Spuriously increased HGB Spuriously decreased RBC	Spuriously decreased HGB Spuriously increased RBC
MCHC	Spuriously increased HGB Spuriously decreased HCT	Spuriously decreased HGB Spuriously increased HCT
Platelets	Cryoglobulin, Cryoprotein, Fragmented WBCs, Fragmented RBCs, Microerythrocytes	Giant PLT, PLT Clumping PLT Satellitosis
Retic	Erythrocyte aggregation, giant platelets, clumped platelets, fragmented leukocytes, malaria, Howell –Jolly bodies	None specified

14.3 Clinical Sensitivity/Specificity/Predictive Values

Patient results may vary with sample condition and setting of instrument to review criteria established by the laboratory. Results may also vary due to disease abnormalities, overall patient population and institutional review criteria.

15. SAFETY

Cell Clean Auto reagent causes severe skin burns and eye damage.

Refer to your local and corporate safety manuals and Safety Data Sheet (SDS) for detailed information on safety practices and procedures and a complete description of hazards.

16. RELATED DOCUMENTS

- Safety Data Sheets
- Sysmex XN Reference Manual
- Critical Values (Lab policy)
- Quality Control Program policy
- Quest Diagnostics Records Management Program
- Laboratory Safety Manual
- Data Innovations Instrument Manager, Laboratory Policy

- Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls
- Pathologist Slide Review Request (AG.F127)
- Sysmex XN Maintenance Log (AG.F377)
- Sysmex Stainer SP–10 Maintenance Log (AG.F378)
- Automated Stainer Differential Comparison and Stain Quality Log (AG.F379)

17. REFERENCES

1. Quest Diagnostics Best Practice Sysmex XN Series Operation for CBC SOP, revised 04/03/2017
2. Quest Diagnostics Best Practice Sysmex XN Series Operation for Reticulocytes SOP, revised 01/09/2017
3. Sysmex Hematology Analyzer XN Series Instruction for use, revised 07/2015
4. Sysmex Stainer SP – 10 Series Instruction for use, revised 02/2013
5. Sysmex XN – 3000 Automated Hematology System Quick Guide, revised 01/2013
6. Sysmex XN Check Quality Control Package Insert, revised 12/2014
7. Roehrl, M.H., et al. Age-Dependent Reference Ranges for Automated Assessment of Immature Granulocytes and Clinical Significance in an Outpatient Setting. Arch Pathol Lab Med. 2011, 135(4):471-7.
8. Kickle, T.S., et al. A Clinical Evaluation of High Florescent Platelet Fraction Percentage in Thrombocytopenia. Am J Clin Pathol 2006;125:282-287
9. Reticulocyte hemoglobin content. Alan Mast, Morey Blinder, Dennis Dietzen. Blood: Vol 83, Issue 4, 307-310

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
0	8/25/17	8.2	Add stainer maintenance	L Barrett	R SanLuis
0	8/25/17	10.3	Add missing parameters	L Barrett	R SanLuis
0	8/25/17	10.4	Correct upper limit for Hgb	A Chini	R SanLuis
0	8/25/17	10.6	Update repeat criteria	R SanLuis	R SanLuis
0	8/25/17	13.7	Add micro & macrocytosis criteria	R SanLuis	R SanLuis
0	8/25/17	13.9.13.11	Delete dilution	R SanLuis	R SanLuis
0	8/25/17	19	Add addenda 6 and 7	L Barrett	R SanLuis
0	8/25/17	Add 1	Standardize decimal places	L Barrett	R SanLuis
0	8/25/17	Add 3	Add QC requirements	L Barrett	R SanLuis
0	8/25/17	Add 4	Add QC outlier documentation steps	Z Morrow	R SanLuis
0	8/25/17	Add 5	Add detail to data management	Z Morrow	R SanLuis
0	8/25/17	Footer	Corrected pages 28-36	L Barrett	R SanLuis
1	3/13/18	10.6	Add physician request for manual diff	L Barrett	R SanLuis
1	3/13/18	19	Add addendum 8	A Chini	R SanLuis
1	3/13/18	Add 1	Add peds values for IG %, extend adult age to include 13-18 yrs.	L Barrett	R SanLuis
2	5/2/18	10.3	Add decimal places for each analyte	L Barrett	R SanLuis

Version	Date	Section	Reason	Reviser	Approval
2	5/2/18	Add 1	Change IG% value to one decimal place	M Sabonis	R SanLuis
3	7/9/18	10.6, Add 7	Add action for plt between 50-100; add scan if retic >6.0% for ages >30 days	L Barrett	R SanLuis
3	7/9/18	10.6	Slide review process changed to release tech results and order manual diff for path review	L Barrett	R SanLuis
4	1/16/19	Header	Updated facility	L Barrett	R SanLuis
4	1/16/19	10.6	Update HGB ≥25.0 process; revise path review ordering and resulting	L Barrett	R SanLuis
4	1/16/19	Add 2	Deselect profiles and Purge data added	H Genser	R SanLuis
4	1/16/19	19	Added addendum 9, include no demographics flag	L Barrett D Concepcion	R SanLuis
5	6/4/19	13.15	Added detail for reporting blue top result	H Genser	R SanLuis
5	6/4/19	Add 9	Updated Release/Reject results, Order of release, Morphology with auto diff	D Concepcion	R SanLuis
6	4/27/21	Header, all	Changed WAH to WOMC	L Barrett	R SanLuis
6	4/27/21	4.2	Updated storage temp for Sulfolyser	L Barrett	R SanLuis
6	4/27/21	Add 2	Added record values of vacuum pressure	L Barrett	R SanLuis
7	9/19/21	Header	Deleted specific sites, added All Labs	L Barrett	R SanLuis
7	9/19/21	13.13-.15	Added FWMC	L Barrett	R SanLuis
7	9/19/21		Added FWMC cell counter	L Barrett	R SanLuis
7	9/19/21	Footer	Updated prefix to AHC	L Barrett	R SanLuis
8	2/8/22	6.3	Changed QC frequency from every 4 hours of use to every 8 hours of use	R SanLuis	N Cacciabeve
9	3/23/21	10.6	Added Rule of 3s to H & H mismatch.	D Concepcion	R SanLuis
9	3/23/21	Add. 9 .19	Added DI flag with rule of 3s comment	D Concepcion	R SanLuis

ADDENDA

Addendum	Title
1	Reference Ranges
2	Sysmex XN Maintenance and Quality Control
3	Sysmex Stainer SP–10 Maintenance
4	QC Instructions on the Sysmex XN
5	QC Instructions on Insight
6	Smear Review and Manual Differential
7	CBC DIFF/SCAN Action and Repeat Criteria
8	Sysmex Rules Management
9	DI (Data Innovations) Information and Actions

Addendum 1:

Adult Reference Ranges

Parameter / Units of Measure	Female		Male	
	13 – 18 years	> 18 years	13 – 18 years	> 18 years
WBC / x10(3)/mcL	4.5 – 13.0	4.0 – 10.0	4.5 – 13.0	4.2 – 9.1
RBC / 10^6/µL	4.10 – 5.10	3.93 – 5.22	4.50 – 5.30	4.63 – 6.08
HGB / g/dL	12.0 – 16.0	11.2 – 15.7	13.0 – 16.0	13.7 – 17.5
HCT / %	36.0 – 46.0	34.1 – 44.9	37.0 – 49.0	40.1 – 51.0
MCV / fL	78.0 – 102.0	79.4 – 94.8	78.0 – 102.0	79.0 – 92.2
MCH / pg	25.0 – 35.0	25.6 – 32.2	25.0 – 35.0	25.7 – 32.2
MCHC / g/dL	32.0 – 37.0	32.2 – 35.5	32.0 – 37.0	32.3 – 36.5
RDW / %	11.5 – 14.0	11.7 – 14.4	11.5 – 14.0	11.6 – 14.4
PLT / x10(3)/mcL	150 – 450	182 – 369	150 – 450	163 – 337
MPV / fL	7.2 – 11.1	9.4 – 12.3	7.2 – 11.1	9.4 – 12.4
Absolute Neutrophil / x10(3)/mcL	2.10 – 11.52	1.56 – 6.13	2.10 – 11.52	1.78 – 5.38
Absolute Lymphocyte / x10(3)/mcL	0.77 – 5.85	1.18 – 3.74	0.77 – 5.85	1.32 – 3.57
Absolute Monocytes / x10(3)/mcL	0.14 – 1.30	0.24 – 0.86	0.14 – 1.30	0.30 – 0.82
Absolute Eosinophil / x10(3)/mcL	0 – 0.78	0.04 – 0.36	0 – 0.78	0.04 – 0.54
Absolute Basophil / x10(3)/mcL	0 – 0.26	0.01 – 0.08	0 – 0.26	0.01 – 0.08
NRBC / 100 WBC	0	0	0	0
IG / %	0.0 – 0.8	0.0 – 0.8	0.0 – 0.8	0.0 – 0.8
RETIC / %	0.6 – 2.7	0.5 – 1.7	0.6 – 2.7	0.5 – 1.8
Absolut RETIC / x10(6)/mcL		0.0164 – 0.0776		0.0260 – 0.0950
RET-He / pg		> 28		> 28
IPF / %		0 - 3		0 - 3

Sources:

- Sysmex Hematology Analyzer XN Series Instruction for use, revised 07/2015
- Roehrl, M.H., et al. Age-Dependent Reference Ranges for Automated Assessment of Immature Granulocytes and Clinical Significance in an Outpatient Setting. Arch Pathol Lab Med. 2011, 135(4):471-7.
- Kickle, T.S., et al. A Clinical Evaluation of High Florescent Platelet Fraction Percentage in Thrombocytopenia. Am J Clin Pathol 2006;125:282-287
- Reticulocyte hemoglobin content. Alan Mast, Morey Blinder, Dennis Dietzen. Blood: Vol 83, Issue 4, 307-310

Addendum 1 (continued): Pediatric Reference Ranges

Pediatric CBC and Differential Reference Ranges											
Parameter/Units of Measurement	**0d**	**2d**	**3d**	**2w**	**1m**	**2m**	**3m**	**6m**	**1y**	**2y**	**6y – 12y**
WBC/ x10(3)/mcL	19.0–25.0	9.0-29.9	9.0-29.9	9.0-29.9	5.0-19.5	5.0-19.5	5.0-19.5	6.0-17.5	6.0-17.5	6.0-17.0	5.0-16.0
RBC/ 10^6/µL	4.00-6.60	3.90-5.90	3.90-5.90	3.90-5.90	3.10-5.30	3.10-5.30	2.70-4.50	3.10-5.10	3.90-5.50	3.90-5.50	3.90-5.50
HGB/ g/dL	14.5-22.0	13.4-19.9	13.4-19.9	13.4-19.9	10.7-17.1	9.1-14.0	9.1-14.1	9.5-14.1	11.3-14.1	11.3-14.1	11.5-14.0
HCT/ %	45.0-65.0	42.0-65.0	42.0-65.0	42.0-65.0	33.0-55.0	28.0-42.0	29.0-41.0	29.0-41.0	31.0-41.0	31.0-41.0	34.0-42.0
MCV/ fL	95.0-121.0	88.0-123.0	88.0-123.0	88.0-123.0	88.0-123.0	91.0-112.0	74.0-108.0	74.0-108.0	70.0-86.0	70.0-86.0	73.0-87.0
MCH/ pg	31.0-37.0	31.0-37.0	31.0-37.0	31.0-37.0	27.0-36.0	27.0-36.0	25.0-35.0	25.0-35.0	23.0-31.0	23.0-31.0	24.0-30.0
MCHC/ g/dL	29.0-37.0	28.0-36.0	28.0-36.0	28.0-36.0	28.0-36.0	28.0-36.0	28.0-36.0	30.0-36.0	30.0-36.0	30.0-36.0	31.0-36.0
RDW/ %	11.5-14.0	13.0-18.0	13.0-18.0	11.5-16.0	11.5-16.0	11.5-16.0	11.5-16.0	11.5-16.0	11.0-15.0	11.0-15.0	11.0-15.0
PLT/ x10(3)/mcL	150-450	150-450	150-450	150-400	150-400	150-400	150-400	150-400	140-400	140-400	140-400
MPV/ fL	7.2-11.0	7.5-11.6	7.5-11.6	7.5-11.6	7.5-11.6	7.5-11.6	7.5-11.6	7.5-11.6	7.5-11.6	7.5-11.5	7.5-11.5
Absolute Neutrophils/ x10(3)/mcL	11.59-18.75	5.31-21.90	4.41-18.30	2.97-12.30	1.50-7.02	1. 50-7.02	1.65-8.00	1.98-7.18	1.98-7.18	2.22-7.65	2.10-11.52
Absolute Lymphs/ x10(3)/mcL	3.61-5.75	2.43-11.40	2.61-12.00	4.68-20.70	2.75-14.04	2.75-14.04	2.80-12.29	3.30-12.60	2.88-11.03	2.88-10.71	1.75-7.68
Absolute Monocytes/ x10(3)/mcL	0.95-3.75	0.00-1.50	0.36-2.40	0.18-1.80	0.10-1.17	0.15-1.95	0.15-1.95	0.18-1.75	0.18-1.75	0.18-1.70	0.15-1.60
Absolute Eosinophils/ x10(3)/mcL	0.00-1.50	0.00-1.80	0.00-1.80	0.00-1.80	0.00-1.17	0.00-1.17	0.00-1.17	0.00-1.05	0.00-1.05	0.00-1.02	0.00-0.96
Absolute Basophils/ x10(3)/mcL	0.00-0.50	0.00-0.60	0.00-0.60	0.00-0.60	0.00-0.39	0.00-0.39	0.00-0.39	0.00-0.35	0.00-0.35	0.00-0.34	0.00-0.32
Nucleated RBC/ 100 WBC	0	0	0-8	0	0	0	0	0	0	0	0
IG / %	0.0-6.0	0.0-6.0	0.0-0.4	0.0-0.4	0.0-0.4	0.0-0.4	0.0-0.4	0.0-0.4	0.0-0.4	0.0-0.4	0.0-0.4
Retic / %	1.0-7.5	1.0-7.5	1.0-7.5	0.6-2.7	0.6-2.7	0.6-2.7	0.6-2.7	0.6-2.7	0.6-2.7	0.6-2.7	0.6-2.7

The reference ranges should be interpreted as from and including the age specified in the title of the column

Addendum 1 (continued):

Pediatric and Adult % Differential Reference Ranges

Parameter/Units of Measurement	0d	1d	2d	3d	4d	6d	8d	15d	1m	2m	3m	4m	7m	1y	13m	3y	4y	5y	6y	7y	12y+
Neutrophils/ %	61 - 75	60 -74	59-73	49-61	45-55	37-45	33-41	29-35	30-36	30-36	33-41	33-41	35-43	35-43	37-45	39-47	42-52	44-54	42-72	42-72	42-72
Bands / %	1-15	1-15	1-15	1-15	1-15	1-15	1-15	1-15	1-15	1-15	1-15	1-15	1-15	1-15	1-15	1-15	1-15	1-15	1-15	1-15	1-15
Myelocyte/ %	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Metamyelocytes/ %	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1
Promyelocytes/ %	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Blast / %	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lymphocytes/ %	19-23	22-28	27-33	29-35	32-40	40-48	46-56	52-64	55-67	55-67	56-68	55-67	52-64	48-58	48-58	43-53	42-52	39-47	35-43	34-42	17-45
Atypical Lymphocytes / %	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5
Monocyte / %	5-15	5-15	4-8	4-8	4-8	4-8	4-8	2-6	2-6	3-10	3-10	3-10	3-10	3-10	3-10	3-10	3-10	3-10	3-10	3-10	3-10
Basophil/ %	0-2	0-2	0-2	0-2	0-2	0-2	0-2	0-2	0-2	0-2	0-2	0-2	0-2	0-2	0-2	0-2	0-2	0-2	0-2	0-2	0-2
Eosonophil/ %	0-6	0-6	0-6	0-6	0-6	0-6	0-6	0-6	0-6	0-6	0-6	0-6	0-6	0-6	0-6	0-6	0-6	0-6	0-6	0-6	0-6
Plasma Cell/ %	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1

Reference: % Cell Differential were obtained from Sunquest Quality Assurance's Database

Addendum 2:

Sysmex XN Maintenance

1. Both Daily and Weekly shutdowns will take place between 3 and 4am.
2. To record the Pressure Vacuum:
   Analyzer Mode – Maintenance – Pressure Adjustment
   Record the numeric values on the Sysmex XN Maintenance Log.

3. If for any reason daily shutdown cannot be done on a Sysmex XN, Daily Cleaning can be used as an alternative to the shutdown to keep one analyzer up and running at all times.

Daily Cleaning	
1.	Verify the analyzer is in the Ready state
2.	Click the **Analyzer Menu** button
3.	Select **Maintenance**
4.	Select **Cleaning**
5.	The tube holder will slide out
6.	Place a vial of CELLCLEAN AUTO in the sample tube holder
7.	Press the blue start switch.
Note: Cleaning will take approximately 20 minutes. The cleaning process will conclude with a background check and the analyzer will return to the Ready state in the Manual Mode.	

Instrument Startup after a Complete Shutdown	
1.	Verify the power switches on each device is turned on
2.	Press the Startup switch (green switch) on the samples
3.	Turn on the IPU and log in
4.	Once the power on the instrument turns on, a self-test automatically runs for about 10 minutes doing initialization of the mechanical parts, rinse & temperature stability

Sysmex XN Manual Shut down	
1.	Make sure the Status indicator LED on the analyzer is green
2.	Go to Menu
3.	Select Shut down and the tube holder (for manual/pediatric samples) slides out forward
4.	Place a CELLCLEAN AUTO in the tube holder
5.	Press the Start switch

Addendum 2:

Sysmex XN Maintenance (continued)

Sysmex XN Automatic Shut down	
1.	Make sure both the analyzer and the sampler are in the ready state
2.	Make sure that the tube holder (for manual/pediatric samples) is retracted into the analyzer
3.	**GEC:** Place a CELLCLEAN AUTO in a regular patient rack in position 10; load the rack on the instrument. **SGMC & WOMC:** Place a CELLCLEAN AUTO on a regular patient rack and load the rack on the instrument: a. Position 9: Analyzer Left b. Position 10: Analyzer Right You can shut down one particular analyzer by placing CELLCLEAN AUTO only in the position for that analyzer.
4.	If the sampler auto – start function is ON, conveying automatically starts when the rack is loaded. If the sample auto – start function is OFF, click the sampler analyzer button in the control menu and then click start
5.	Shut down is performed automatically
6.	When the instrument is ready to be initialized the message **Press Start SW** will appear. Press the green button to initialize

Weekly IPU Shut down	
	SG & WOMC, Note: This shut down does not affect the SP – 10 Stainer, so the unit could remain ON.
1.	Make sure XN analyzer(s) is/are in ready mode
2.	Open the front hood cover, use the black switch to turn the analyzer(s) OFF
3.	From **Main Menu** select **Exit IPU**
4.	From Windows screen restart the computer
5.	When the computer is back ON, log back in to IPU
6.	Now turn the analyzer(s) back ON
7.	Deselect the RETIC and PLT-F profiles after powering ON

Weekly Purge Patient Data from Sysmex XN	
1.	Go to the **Explorer menu**
2.	Click on **Sample Info Tab**
3.	Scroll to the bottom of the list and highlight the patients you wish to purge. Only choose patient test results that are older than one week.
4.	Click on **Delete**.
5.	Document data purge on the Sysmex XN Maintenance Log.

Addendum 3:

Sysmex Stainer SP–10 Maintenance and Quality Control

1. Stain quality and differential comparison for the SP-10 stainer is performed each day of use. Refer to the Automated Stainer Differential Comparison and Stain Quality Log for criteria.
2. Both Shutdown 1 and Shutdown 2 will be done by day shift.
3. Daily Shutdown Automatically:

Stainer SP – 10 Automatic Shutdown (Shutdown 1)	
1.	Before the daily shut down is initiated, verify the following: a. The instrument is in a ready mode and there are no racks/samples on board b. There is at least 12 single cassettes on the cassette supply table c. There is at least 450 mL of methanol in the methanol bottle
2.	Place a cell clean Auto in position 8 of a sample rack. Load the rack onto the right sampler pool (analyzer side)
3.	Shutdown is performed automatically

4. Daily Shutdown Manually:

Stainer SP – 10 Manual Shutdown (Shutdown 1)	
1.	Before the daily shut down is initiated, verify the following: a. The instrument is in a ready mode and there are no racks/samples on board b. There is at least 12 single cassettes on the cassette supply table c. There is at least 450 mL of methanol in the methanol bottle
2.	Select **Conv.int.** on the menu screen – **Interrupt** – **Return** – Select **Shutdown** on the menu screen – Select **Shutdown 1 (daily)**
3.	Place a cell clean Auto in position 10 of a sample rack
4.	Place the rack on the sampler (SP-10 side), lining the tube up with the gripper. Use the mark on the sampler as a guide for the left edge of the rack. Press **OK**.

5. Weekly Shutdown:

Stainer SP – 10 Shutdown (Shutdown 2)	
1.	Before the daily shut down is initiated verify the following: a. The instrument is in a ready mode and there are no racks/samples on board b. There is at least 9 single cassettes on the cassette supply table c. There is at least 800 mL of methanol in the methanol bottle
2.	Select **Conv.int.** on the menu screen – **Interrupt** – **Return** – Select **Shutdown** on the menu screen – Select **Shutdown 2 (weekly)**
3.	Place a tube of CELLCLEAN AUTO in the 10th position of a sample rack. Place the rack on the sampler (SP-10 side), lining the tube up with the gripper. Use the mark on the sampler as a guide for the left edge of the rack. Press **OK**.
4.	When shutdown ends, the device will automatically turn off. Slide the rack to the left on the analysis line, and then remove.

Addendum 3:

Sysmex Stainer SP–10 Maintenance (continued)

6. To load a sample in a Manual Mode:
   Note: This mode could be used for STAT specimens.

Manual Mode	
1.	Select **Conv.int.** in the SP-10's **Main Menu** screen
2.	Select **Interrupt** – **Return** - **Manual** in the main menu screen
3.	Select tube type and touch **Closed.** Enter Sample ID
4.	Mix the sample tube and place it in position 10 of a sample rack
5.	Set the rack so that its left end matches up with the label on the conveyor, select **START**
6.	Wait until the smear is prepared and the sample is returned to the rack then remove the rack
7.	Select **Return** in the manual screen - Select **Conv.int.** in the main menu screen – Select **Stop int**

7. Daily Maintenance:

Clean Spreader Glass	
1.	Select **Maint.** on the menu screen - **Spreader glass – OK**
2.	When prompted to replace the spreader glass, open the top cover - Clean the surface of the spreader glass with gauze moistened with reagent grade water
3.	Close the top cover, then select **OK** – Select **Cancel**
Note: Do NOT reset the counter after cleaning. If the counter is reset, the message to replace the glass will appear at the wrong time.	

Clean Cassettes	
1.	Remove the used cassettes from the instrument and place them in the assigned container
2.	Fill the container with Methanol until the tops of the cassettes are covered
3.	Take the cassettes out of the container **immediately**. Do **NOT** allow the cassettes to sit in Methanol for more than 5 minutes
4.	Allow the cassettes to air dry

8. Monthly Maintenance:

Monthly Maintenance	
1.	Use a damp gauze and wipe and clean the sample racks
2.	Use a damp gauze and wipe and clean the right and left sampler rack pools
3.	Use a damp gauze and clean the measurement line

Addendum 4:

QC Instructions on the Sysmex XN

To document outliers of daily control runs:

1. Click on **QC File**
2. Double click on the **QC level with the outlier** to view the Levy Jennings Chart
3. From the top menu select **Manage**
4. Under the Specify Excluded Section, select **Not Managed**
5. Under the Comments Settings, click the **drop down arrow** and choose a comment that qualifies with the outlier
6. Click on **OK** to save

To register a new lot:

1. From **Main Menu** Select **QC File**
2. High light one empty row then click **Register**
3. Update Control Level, Lot number and Expiration date
4. High light all methods from top to bottom then click on **Variable Target**
5. Click on **Restore**
6. Select the correct file for desired QC level, then select **OK**

Parallel test new controls by analyzing each level of control a minimum of twice a day for 5 days on each analyzer prior to expiration of previous lot. After a minimum of 10 data points are accumulated on each analyzer, auto set the targets using the steps below:

1. From **Main Menu**, select **QC File**
2. Select desired lot, then click **Modify**
3. Highlight all methods from top to bottom, then click on **Auto Setting**
4. Confirm that the check box for **Target** is set. Do **NOT** select the check box for Limit.

To adjust QC ranges:

1. From **Main Menu**, Select **QC File**
2. Double click on the desired lot
3. Click on **Range**
4. Drag the graph to cover the most recent shift/bias
5. Select **Modify**
6. Select the desired method, click on **Auto Setting**
7. Confirm that the check box for **Target** is set. Do **NOT** select the check box for Limit.

To be able to see two different lots of QC on the same page or to see the same QC of both instruments on the same page:

1. From **Main Menu** Select **QC File**
2. Open up desired QC Levey Jennings chart, on top click on **Reference**
3. Choose either **Compare QC Files** or **Compare Analyzers**

Addendum 5:

QC Instructions on Insight for Supervisor or Designee

Insight is the website used to submit and review QC data. All QC data is submitted to insight automatically.

To log onto Insight go to www.sysmex.com/Insight

Each lot of QC has two periods; QC needs to be reviewed and submitted by the Supervisor or Designee before the due date for each period, otherwise the lab's data will not be included in the peer group study.

Note: If for some reason the due date is missed, QC can still be submitted and a report will be generated, but the data will not be included in peer group.

Since QC is submitted automatically, Supervisor or Designee must log onto Insight to manage the data. If the outliers are not excluded before the due date of each Period, the QC report will include the data points that should be excluded such as QNS runs.
It is recommended that the supervisor or designee log onto Insight and check each lot to make sure data has been transmitting, before the due date for each Period.

To exclude/manage outliers:

- Log onto Insight
- On the Home Page, on the left side of the screen locate **QC data**
- Select **Review QC Data**
- Select the desire analyzer, lot and QC
- Click on **Review your data** – this page will show every run including date and time
- To exclude a run, click on **Manage Data**
- Select **Not Managed**
- Select a pre-typed comment from **Select Comment Type** or under **Enter Comment Description** free text a comment
- Save comment – back to review data

To pull a report or review weekly/monthly QC:

- Log onto Insight
- From Home Page, under **Report Center** – Select **Customer QC Report**
- When new page opens, click on **View All QC Reports (including Lot – to – Date Reports)**. This page will list all the lot numbers up to 2 years. Note that each lot appears three different times; Period 1, Period 2, and Cumulative.
- Cumulative has the data from Periods 1 and 2 included with peer group.

Reviewing the report:

The report includes every method's average run, Assay Mean and Group Mean. The following flags will appear under notes, depending on how the QC performs:

W: Warning
P: Positive Bias
N: Negative Bias

If Warning appears for a method once, that means to check the instrument and do maintenance to make sure it is working properly.

If two consecutive Warnings, Positive Bias, or Negative Bias appear, call technical support and trouble shoot with them. They will examine the situation and might send service over to do a calibration and other trouble shooting.

To find the Insight calendar:

- Log onto Insight –
- From the Home Page, under **Reference/Documents** – Select **Lot Calendars**
- Select the **XN CHECK Calendar**. This provides information such as:
  - When each Period begins
  - When each Period ends
  - Due date to submit data

Addendum 6:

Smear Review and Manual Differential

A. General Instruction/Notes
   1. All smears for WBC differential must be made with the wedge pull film technique. A properly made Wright stain blood smear should have the following characteristics:
      a. RBC - Pink with central pallor
      b. NRBC - Dark purple nucleus.
      c. WBC
         - Neutrophil - Dark purple nuclei with light pink cytoplasm dotted with lilac granules.
         - Lymphocyte - Dark purple nucleus. Cytoplasm with varying shades of blue (robin egg blue).
         - Monocyte - Cytoplasm of monocytes stains a faint bluish gray tinge.
         - Eosinophil - The eosinophilic granules, bright red to orange.
         - Basophil - The basophilic granules very dark bluish purple.
   2. Prepare blood films within 4 hours of the blood collection in EDTA.
   3. Stain the film within one hour of preparation with Wright stain.
   4. The leukocytes must be well preserved, and anticoagulant effects such as excessive vacuolization or changes in nuclear shape must be minimal. Less than 2% of the leukocytes may be smudged, except in some lymphoproliferative disorders.
   5. There should be sufficient working area with minimum 2.5 cm in length terminating at least 2 cm from the end of the slide.
   6. Acceptable morphology within working area and no artifact introduced by the technique. Also there should be minimum distributional distortion.
   7. A far end that becomes gradually thinner, without growing streaks, troughs, or ridges, all of which indicate an increased number of leukocytes carried into this area.

B. Prepare a blood smear and Scan:
   1. Transfer a small drop of blood (2-3mm) to a pre-cleaned slide, frosted side up. The blood is placed in the centerline of the slide just past the frosting.
   2. A second slide is used as a spreading slide. The “pusher” slide is placed at an angle of 30-45 degrees to the slide containing the blood drop. The slide is moved back to make contact with the drop, allowing the blood to spread the entire width of the slide.
   3. The “pusher” slide is then quickly and smoothly pushed forward to the end of the smear, creating a wedge smear. It is important that the whole drop is picked up and spread quickly. Moving the pusher slide too slowly accentuates poor leukocyte distribution by pushing larger cells to the very end and the slides of the smear.
   4. The drop of blood should be of such a size that the film is about 30mm in length. (If necessary repeat this process until an acceptable slide is obtained.)
   5. Label the slide using a sharp pencil. On the frosted side, record the patient's last name, or as much as will fit on the slide and accession or barcode. Allow the slide to air dry. Load the slide on the slide stainer.
   6. Use the 50x oil immersion objective and examine the smear microscopically. Check the smear to see that it is well made, the distribution of the cells is uniform and the staining is satisfactory.

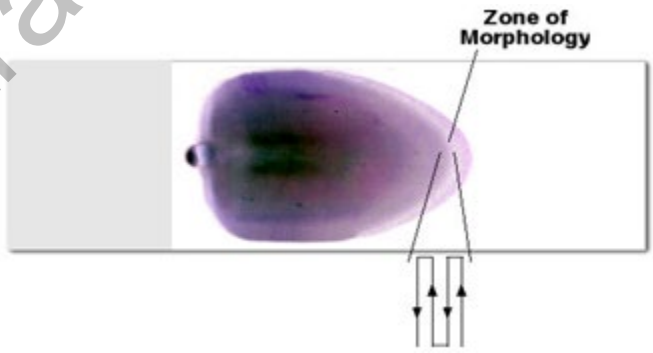
Criteria for a well-stained smear include:

a. No precipitated stain should be seen.
b. The erythrocytes are orange or pink.
c. The nuclei of the leukocytes are purplish-blue.
d. Neutrophilic granules are reddish to pink-Iilac.
e. Eosinophilic granules are red to orange.
f. Basophilic granules are very dark bluish-purple.
g. Platelets stain dark blue-purple.

7. Scan the slide and look for abnormal or suspicious cells that may be in disproportionately low numbers. Look for nucleated red cells, immature cells, atypical lymphocytes and platelet clumps or large platelets. Estimate the white cell count to see if there is any gross error in the instrument count. This could also detect a clotted specimen or perhaps a mix-up in blood specimens.
8. If no significant abnormalities are noted the comment "*Smear review agrees with automated differential. No significant red cell or platelet abnormalities are noted"* should be entered.
9. If a smear review shows a discrepancy with the automated differential or reveals any significant abnormalities or problems for which a manual differential is deemed medically necessary, a manual differential will be performed.

C. Differential Counting Procedure

1. Use the "battlement" tract for this examination. Each identified cell must be classified by cell type. Cells found in a Differential are: neutrophil, segmented; neutrophil, band; lymphocyte, normal; lymphocyte, variant or atypical; monocyte; eosinophil; basophile; other nucleated cells (except nucleated red cells). Include distorted cells that are clearly identifiable in the appropriate classification. While counting the cells, make a note of any abnormalities present in the cells. It is important to examine cellular morphology and to count leukocytes in areas that are neither too thick nor too thin.



2. On each slide, 100 leukocytes should be counted. If the blood is leukopenic, process additional slides in parallel. Exceptions to the 100 cell differential are as follows:

If	Then
WBC greater than 25,000/MM^3	Perform a 100 cell differential count and compare counts to the automated counts. Perform a 200 cells count if difference is greater than 10%.

If	Then
WBC of greater than 2,000 and less than 25,000	A 100 cell differential count is to be performed
WBC less than 2,000	Perform a 100 cell count if possible; however, a 50 cell differential may be performed, insure correct percentage is entered in LIS.
WBC less than 500	Stain 2-3 slides and perform classifying as many cells as possible, ensure correct percentage is entered in LIS.

Note: When the standard 100 cell Differential is not performed, the number of cells counted must be noted in the LIS.

3. Express the results of the differential count as a percentage of all the leukocytes counted.

4. Count nucleated red blood cells present and report the result as the number per 100 leukocytes counted.

5. Examine the red cell morphology in a thin area of the slide where the red cells either do not overlap or lightly overlap. They should have a central pallor. In most cases an abnormality must be a consistent finding in order to be significant. Note any variations from normal and classify them according to section 13.7.

   - All clinically significant findings such as specific cell types, inclusions, polychromasia, etc., will be reported from the smear evaluation.

IF	Then
NO clinically significant findings to be added to a patient report.	Result as Normal.
ANY additions to the patient report, such as RBC morphology, cell differential, PLT morphology, etc.	Report all clinically significant findings using the Diff key board in the LIS or using the cell counter in DI

6. Examine the smear for platelets morphology and number. Find a thin area where red cells are not overlapping. Perform a PLT estimate and use the following if comparing to automated count.

IF	Then
In the presence of a platelet flag, a platelet estimate must be performed. Using the 100X objective	Count the PLT in each of 10 microscopic fields in areas of the slide where the RBCs are evenly dispersed. Divide the total # of platelets by 10 to establish the mean and multiply by 20,000.
The Sysmex platelet count and the platelet estimate do not agree within ± 20%	Repeat the platelet estimate and/or platelet count. If counts still do not agree, consult the supervisor or designee.

D. **Germantown Emergency Center**: differentials that are to be reviewed by the Pathologist will be sent via courier to Shady Grove Medical Center Hematology section with a Pathologist Slide Review Request. SGMC staff will take the slide and paperwork to the pathologist for review.

NOTE: If malaria is observed in the blood smear: Call the patient's physician and report your finding. The physician may request a malaria smear review and identification.

Addendum 7:

CBC DIFF/SCAN Action and Repeat Criteria

Criteria	Criteria Action Limit	Action 1	Action 2
WBC	< 0.5	Check for a clot, re-analyze sample in LW mode	SCAN
WBC	≤ 2.0	Check for a clot	DIFF
WBC	≥ 30.0	Check for Giant Platelets	SCAN
WBC	≥ 440.0	Re-analyze using dilution	SCAN
RBC	≥ 8.60	Re-analyze using dilution	SCAN
HGB	< 4.0	Re-analyze with the Aspiration Sensor OFF	None
HGB	≤ 6.0	Check for a clot	None
HGB	≥ 20.0	Re-analyze, check Coag sample if HCT ≥ 55	None
HGB	≥ 25.0	Re-analyze, if still ≥25.0, then report as ≥25.0 and add RVT comment. Neonate: if QNS for repeat, then report as ≥25.0	None
MCV	≤ 50.0	Check for a clot, re-analyze and verify morphology	SCAN
MCV	≥ 130.0	Warm sample for 30 min, re-analyze, verify morphology	SCAN
PLT	< 50	Check for a clot, re-analyze	None
PLT	50-100	If patient <30 days/old - Check for a clot, perform PLT estimate, and add RVT comment.	SCAN
PLT	> 5000	Re-analyze using dilution, perform PLT estimate	SCAN

Criteria	Criteria Action Limit	Action 1	Action 2
Lymph	≥ 70	Pathologist review for patient >17 y/old	DIFF
Neutro	≥ 90 no flags	Scan for bands, hyper-segmentation, toxic granulation	SCAN
Neutro	≥ 90 & flags	Rule out immature Neutrophils	DIFF
Mono	> 25	Rule out immature Monocytes	DIFF
Eos	≥ 35	Scan to verify Eosinophils. Check for parasites	SCAN
Baso	≥ 3.5	Mix for 5 min, re-run. If still elevated SCAN	SCAN
Retic	>30.0%	Check for a clot, Vortex 1 -2 min, re-analyze	Repeat
Retic	>6.0%	Scan for patient >30 days/old	SCAN

Criteria	Action
WBC Abn Scattergram	If dashes appear: re-analyze, if dashes still remain perform a DIFF If asterisk appears: scan, perform a DIFF if abnormal cells observed
NRBC Present	Release the result. No further correction of WBC is required
IG Present >5%	Perform a DIFF and check for the presence of: Promyelocytes, Metamyelocytes and Myelocytes
Blast/Abn Lymph	If dashes appear: re-analyze, if dashes still remain perform a DIFF If asterisk appears: perform a DIFF, check for blasts & immature granulocytes
Left Shift	If dashes appear: re-analyze, if dashes still remain perform a DIFF and check for bands, toxic granulation, hyper-segmentation, vacuolation of neutrophils
Atypical Lymph	Perform a DIFF
RBC Abn Distribution	SCAN, Perform a morphology (only note if findings are ≥2+)
Dimorphic Population	SCAN, Perform a morphology
RET Abn Scattergram	Vortex 1 -2 min, re-analyze. If flag remains, scan smear for abnormal cells

Criteria	Action
RBC Agglutination	Warm & re-analyze. If flag still remains perform plasma replacement
Turbidity/HGB interference	Re-analyze, if flag remains SCAN. Check for the presence of Spherocytes. If RBC agglutination is noted warm sample and re-analyze. If lipemic or Icteric, perform a plasma replacement.
Fragments	SCAN for fragmented RBCs & other poikilocytosis
PLT Abn Distribution & PLT Abn Scattergram	GEC: Vortex 1 – 2 min, then re-analyze FWMC, WOMC & SGMC: Vortex 1 – 2 min, re-analyze in PLT-F mode All sites: If flag remains, SCAN and perform a morphology
PLT Clumps	Check for clots. If present, cancel and request re-draw. If no clots, then GEC: Vortex 1 – 2 min, then re-analyze FWMC, WOMC & SGMC: Vortex 1 – 2 min, re-analyze in PLT-F mode All sites: If flag remains, SCAN and perform a morphology If fibrin or clumps are present, recollect a new sample in sodium citrate

Addendum 8:

Sysmex Rules Management

	To add or modify a rule
1.	From the Main Menu select **Rule**
2.	For Sysmex Instrument rules – select the **Rerun/Reflex/Comment Rule** tab For the Stainer – select **SP Rule** tab
3.	High light the desired rule, then select **Modify** on top of the screen
4.	To set up a rule, for example RBC >= 8.6, a. Under **Function**, select **item value** b. Under **Analysis items**, select RBC c. Use the keyboard on the screen (not the PC keyboard) to add desired rule d. Click on Equation to check the formula e. Under **Action/Comment**, choose the repeat criteria and type the desired comment

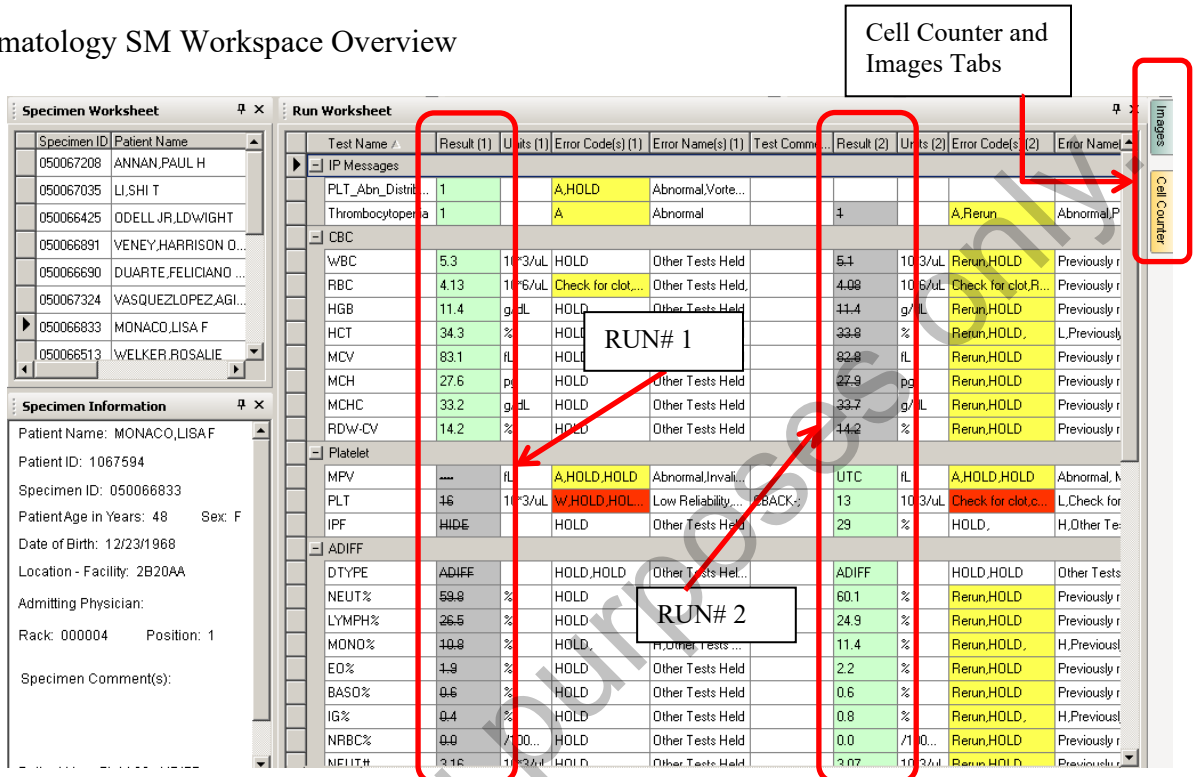
	To import rules from a flash drive
1.	Insert the flash drive
2.	From the Main Menu, select **Rule**
3.	Select **File** on top of the screen
4.	Select **Restore**, locate the desired file on flash drive and accept to save.

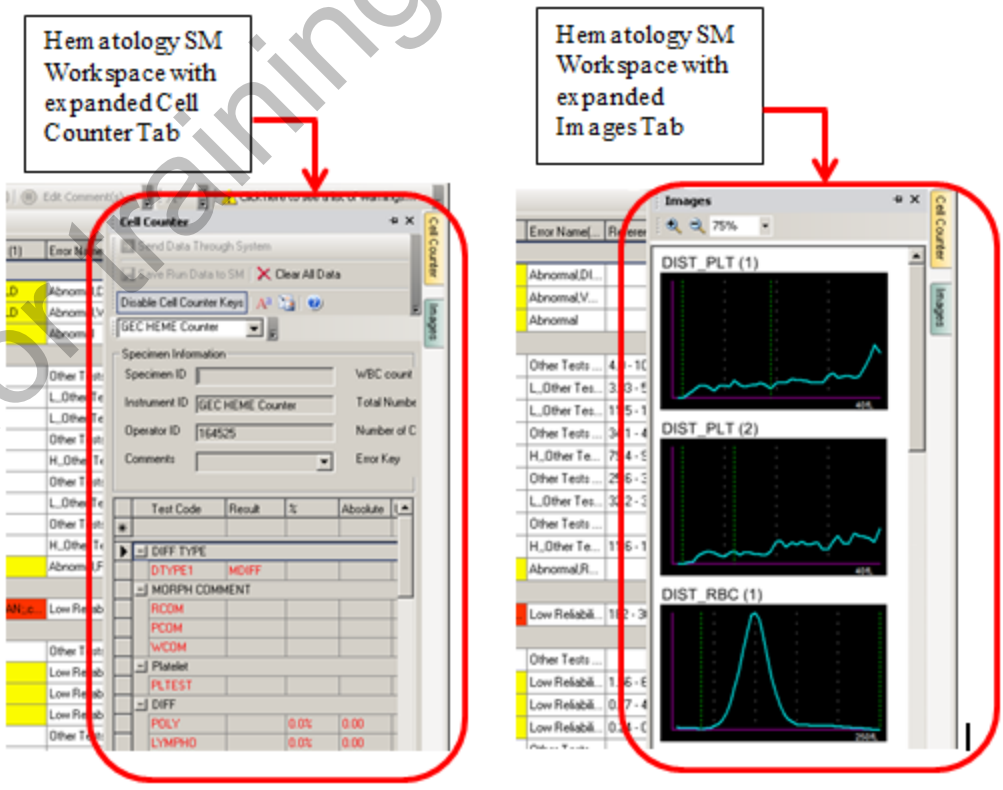
	To export rules from a flash drive
1.	Insert the flash drive
2.	From the Main Menu, select **Rule**
3.	Select **File** on top of the screen
4.	Select **Back Up**, save the file on flash drive and accept to save.

Addendum 9:

DI (Data Innovations) Information and Actions

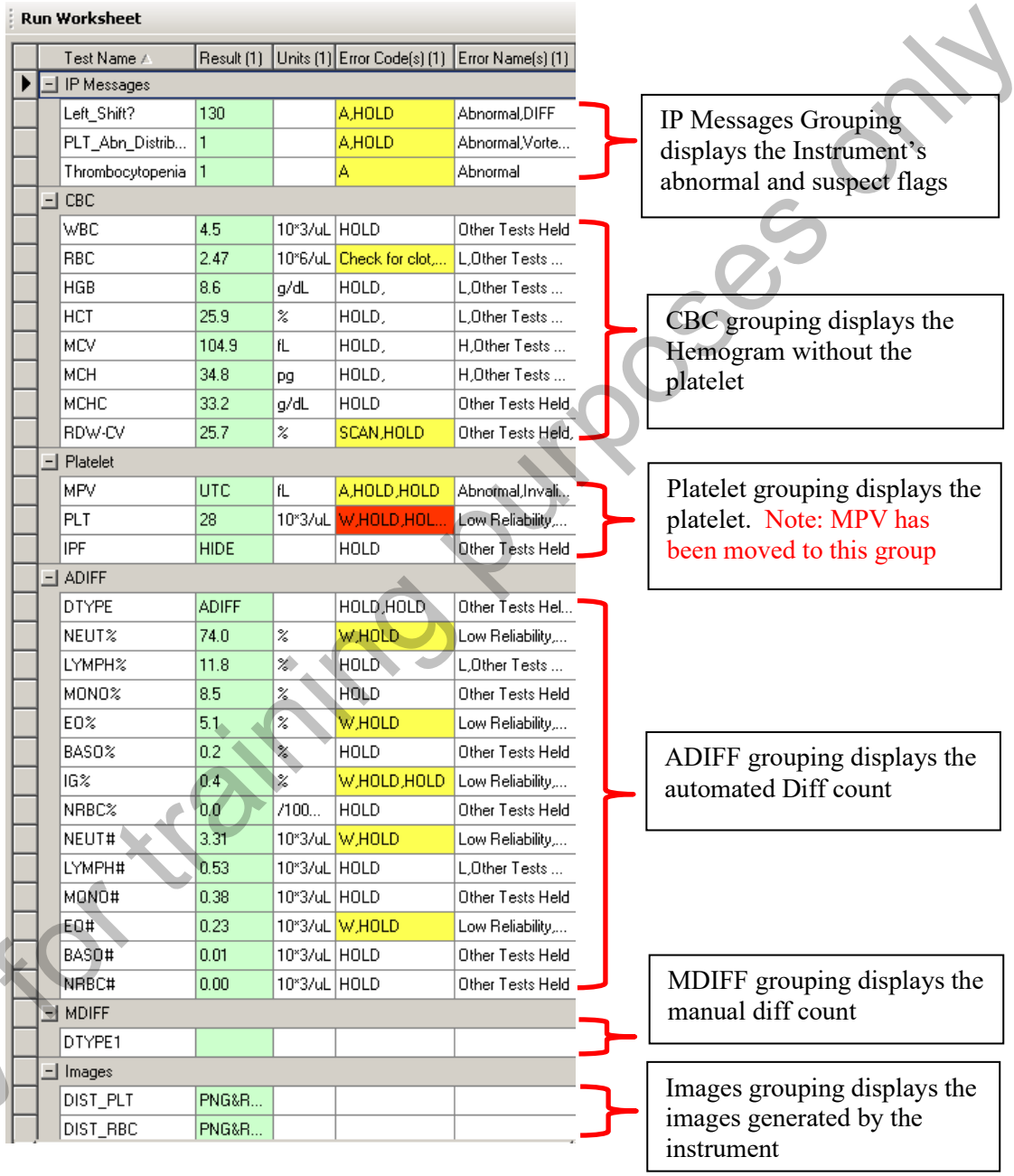
1. Hematology SM Workspace Overview





2. Hematology RunWorksheets Test Grouping

The Hematology RunWorksheet is divided into six test groups. The groups are IP Messages, CBC, Platelet, ADIFF, MDIFF and Images. See the description of each group below.



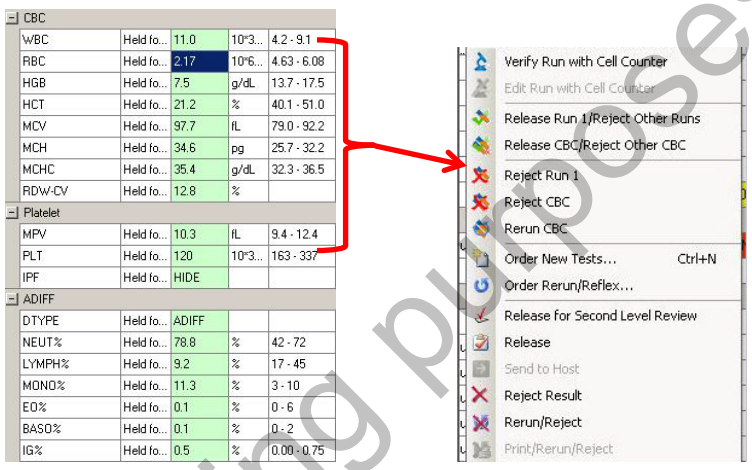
Run Worksheet

Test Name	Result (1)	Units (1)	Error Code(s) (1)	Error Name(s) (1)
IP Messages				
Left_Shift?	130		A,HOLD	Abnormal,DIFF
PLT_Abn_Distrib...	1		A,HOLD	Abnormal,Vorte...
Thrombocytopenia	1		A	Abnormal
CBC				
WBC	4.5	10*3/uL	HOLD	Other Tests Held
RBC	2.47	10*6/uL	Check for clot,...	L,Other Tests ...
HGB	8.6	g/dL	HOLD,	L,Other Tests ...
HCT	25.9	%	HOLD,	L,Other Tests ...
MCV	104.9	fL	HOLD,	H,Other Tests ...
MCH	34.8	pg	HOLD,	H,Other Tests ...
MCHC	33.2	g/dL	HOLD	Other Tests Held
RDW-CV	25.7	%	SCAN,HOLD	Other Tests Held,
Platelet				
MPV	UTC	fL	A,HOLD,HOLD	Abnormal,Invali...
PLT	28	10*3/uL	W,HOLD,HOL...	Low Reliability,...
IPF	HIDE		HOLD	Other Tests Held
ADIFF				
DTYPE	ADIFF		HOLD,HOLD	Other Tests Hel...
NEUT%	74.0	%	W,HOLD	Low Reliability,...
LYMPH%	11.8	%	HOLD	L,Other Tests ...
MONO%	8.5	%	HOLD	Other Tests Held
EO%	5.1	%	W,HOLD	Low Reliability,...
BASO%	0.2	%	HOLD	Other Tests Held
IG%	0.4	%	W,HOLD,HOLD	Low Reliability,...
NRBC%	0.0	/100...	HOLD	Other Tests Held
NEUT#	3.31	10*3/uL	W,HOLD	Low Reliability,...
LYMPH#	0.53	10*3/uL	HOLD	L,Other Tests ...
MONO#	0.38	10*3/uL	HOLD	Other Tests Held
EO#	0.23	10*3/uL	W,HOLD	Low Reliability,...
BASO#	0.01	10*3/uL	HOLD	Other Tests Held
NRBC#	0.00	10*3/uL	HOLD	Other Tests Held
MDIFF				
DTYPE1				
Images				
DIST_PLT	PNG&R...			
DIST_RBC	PNG&R...			

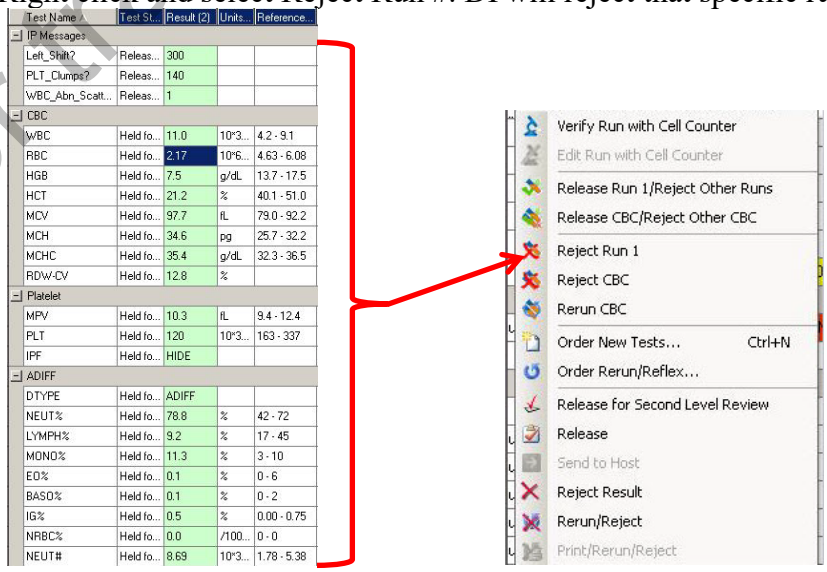
3. Releasing and Rejecting Results

- Release by Group
  a. Identify the group that needs to be released first. See Order of Release
  b. Left click on one of the results within the group.
  c. Right click and select Release CBC/Reject Other CBC to release by group. DI will release the CBC and reject other CBC from a different run. Repeat steps to release other groups within that run

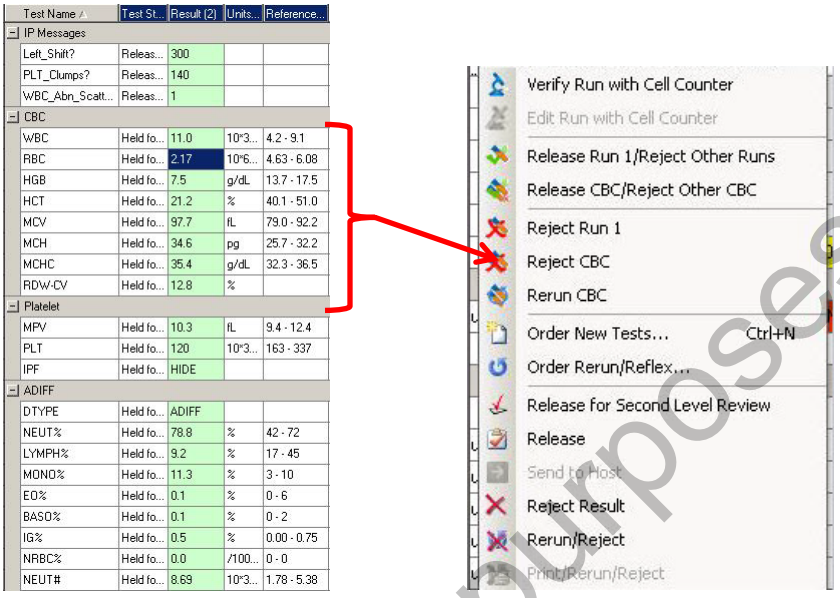
  Note: The following methods of releasing results such as Release Run # and Release (noted as Check Mark) are not recommended. Selecting Release will release all of the different runs.



- Reject by Run #
  a. Identify the run that needs to be rejected
  b. Left click on one of the results within the group.
  c. Right click and select Reject Run #. DI will reject that specific run



- Reject by Group
  a. Identify the group that needs to be rejected
  b. Left click on one of the results within the group.
  c. Right click and select Reject CBC to reject by group. Repeat steps to reject the other groups within that run

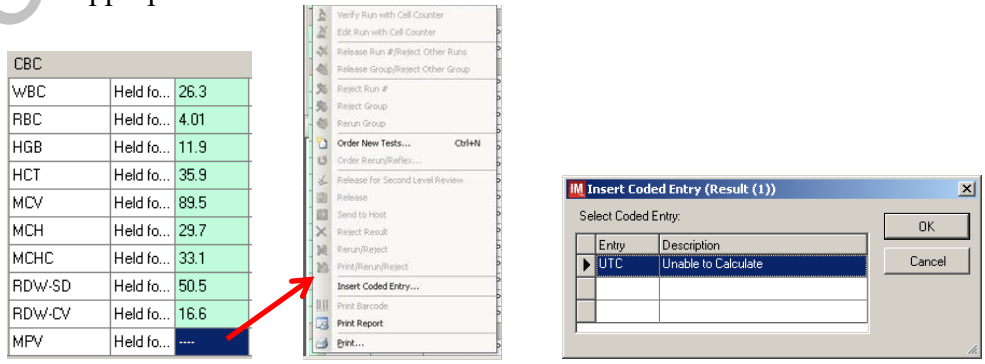


4. Order of Release

   Releasing results by group in a specific order is important. Always release the groups from top to bottom order. Never release the Automated Diff or Manual Diff group first before CBC group. For example, release the CBC group, Platelet Group, then followed by Automated DIFF or Manual Diff group.

5. To Insert Coded Entry within the Hematology Run Worksheet

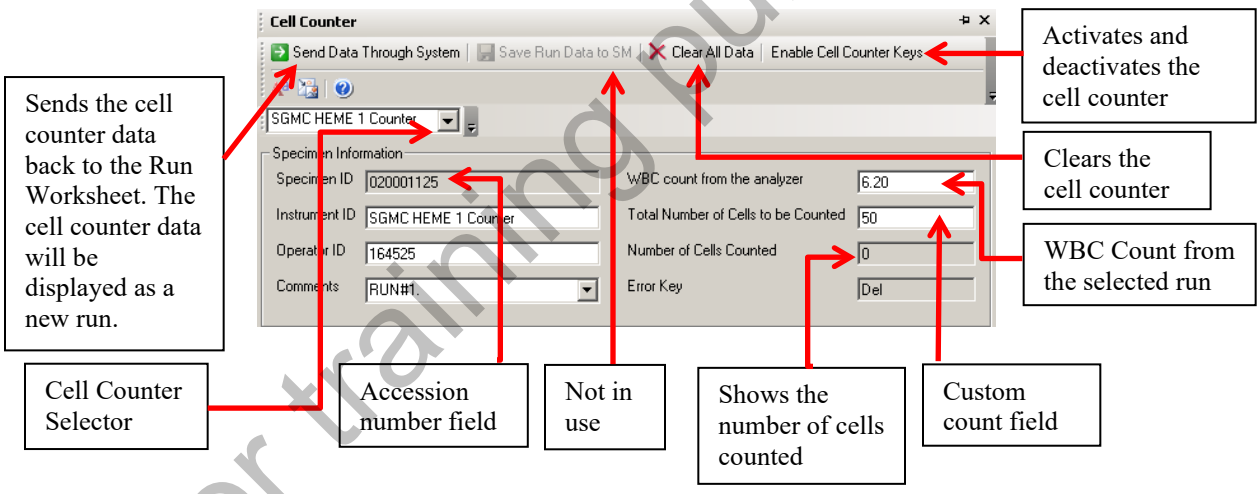
   a. Right click on the result field to be edited
   b. Select "Insert Coded Entry"
   c. Select the appropriate code and click OK to close the window



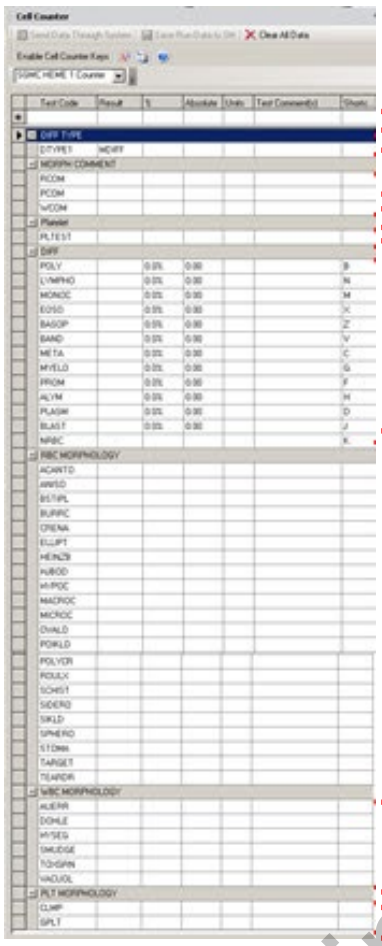
6. To see the previous MDIFF performed
   Go to the DTYPE1 result field and scroll to the right until the most recent previous result for MDIFF is displayed. If there is one, check the resulting date and time.

7. Overview of the the Cell Counter

   a. Cell Counter Functions and Fields



b. Cell Counter Grouping



Displays the default MDIFF result for DTYPE1

The section used to enter the results for RCOM, PCOM and WCOM. To result any of these fields, select "BELOW" from the drop down menu and enter the comment under the Test Comment(s) field preceded with ";"

When this PLT Estimate field is not empty, DI will add "RCM" as a comment on the RunWorksheet.

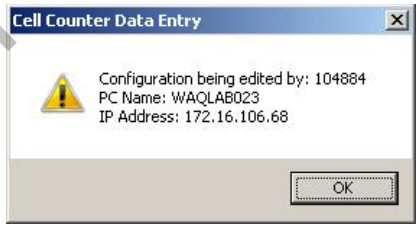
This is where the WBC Differential cells are counted. The manual cell count can be entered by first activating the cell counter and entering the count by using the assigned shortcut keys. Another way of entering the manual count is by deactivating the cell counter and using the number keypad to enter the count.

This is where the RBC morphology cells are entered. To enter morphology, deactivate the cell counter and select the result field of the cell type observed. A drop down list will display the different results for the selected morphology.

This is where the WBC morphology cells are entered. To enter morphology, deactivate the cell counter and select the result field of the cell type observed. A drop down list will display the different results for the selected

This is where the PLT observations are entered

c. There is one cell counter (per site) at GEC and FWMC. There are two each at SGMC and WOMC. Each cell counter can only handle one active user. If another user tries to access the same cell counter that is already in use, DI will display an error that says "configuration is being edited by: 123456"



Location	Cell Counter Assignment
FWMC	FWMC Heme Counter
GEC	GEC Heme Counter
SGMC Microscope #1	SGMH Heme 1 Counter
SGMC Microscope #2	SGMH Heme 2 Counter
WAH Microscope #1	WAH Heme 1 Counter
WAH Microscope #2	WAH Heme 2 Counter

8. Using the Cell Counter

   a. To Enter the Accession number for the Cell Counter

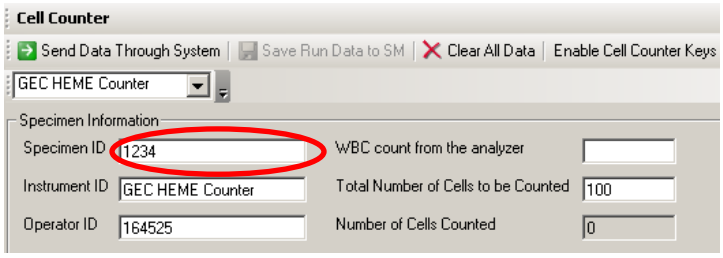
   There are two ways of entering the accession number for use by the Cell Counter. Once the accession number has been entered, the WBC Count_from_the_Analyzer field will be populated with the WBC count from the selected run

   i. By using the "Verify Run with Cell Counter"
   - Right click on the specific result column that you would like to be verified by the cell counter
   - Select the "Verify Run with Cell Counter"

   Note: When the verify Run with Cell Counter is grayed out, do the following:

   - go to the cell counter tab and select a cell counter that is not in use (see section 7c)
   - go back to the run worksheet

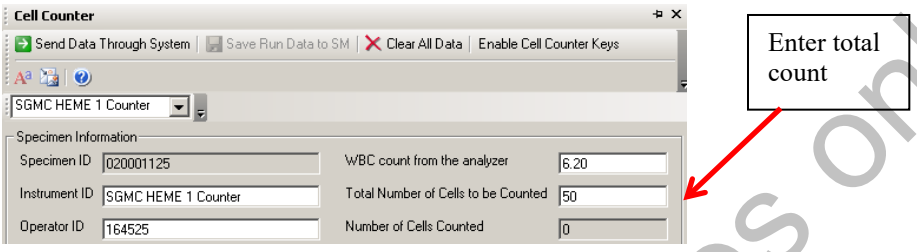
   ii. By manually typing the specimen ID within the Cell Counter
   - Select the appropriate cell counter for your location
   - Enter the accession number within the Specimen ID field and press the TAB key



b. Setting the Total Number of Cells to be Counted field

To change the Total Number of Cells to be Counted:

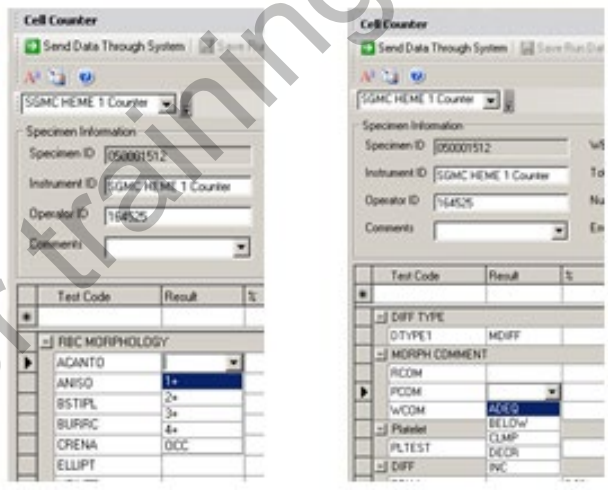
- Disable the Enable Cell Counter Keys button.
- Enter the desired total count on the "Total Number of Cells to be Counted" field. The default count is 100



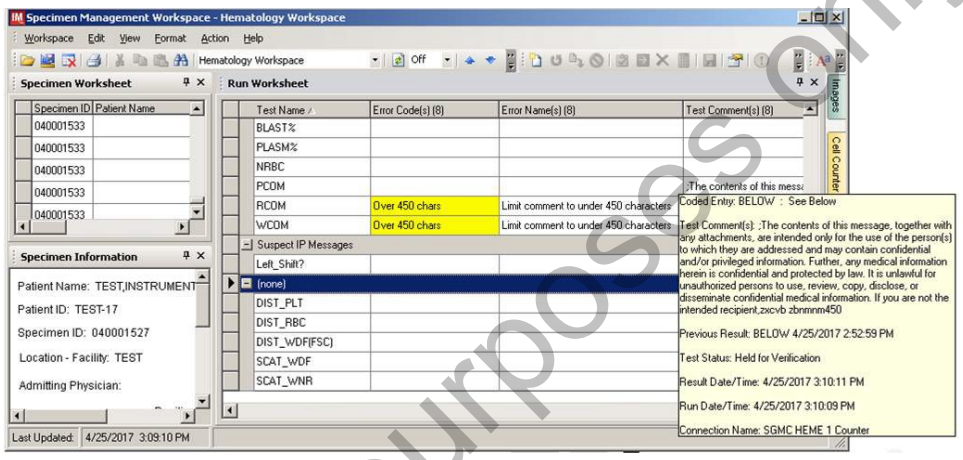
c. To Insert Coded Entry within the Cell Counter

- Toggle the "Enable Cell Counter" button to disable it
- Click on the result field to show the drop-down menu arrow
- Click on the drop-down menu arrow
- Select the appropriate code

Note: The coded entry only works for Morph Comment, Platelet, RBC Morphology, WBC Morphology and PLT Morphology groups



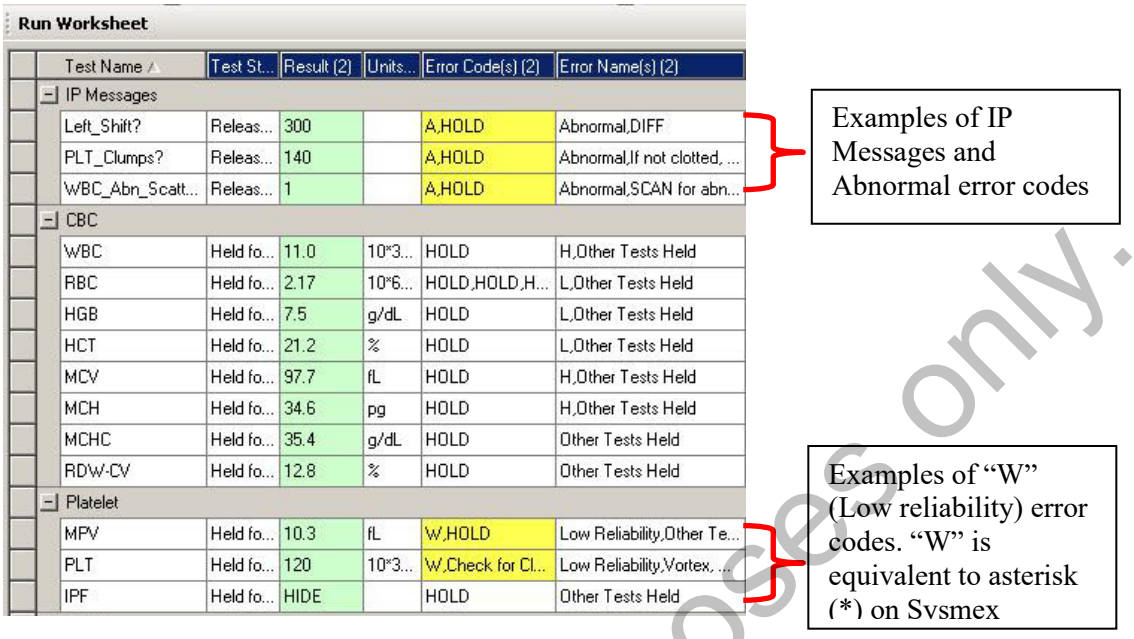
d. Resulting WCOM (Path Review)
   - To enter the narrative from the Path Review, click on the WCOM result field and select "BELOW" from the drop down list. Under the WCOM Test Comment field, enter the path review narrative.
   - There is a limit of 450 characters for WCOM. This limit also applies to RCOM and PCOM fields. DI will display an error message of "Over 450 characters" when the comment entered on this field is over the limit



9. Error and IP (Interpretive Program) Messages

Error Code	Error Name	Description
A	Abnormal	Abnormal result due to analysis or hardware error
W	Low Reliability	This error code is equivalent to asterisk (*) on Sysmex printout

IP Message	Work Instruction
Atypical_Lympho?	DIFF- Perform diff
Blasts/Abn_Lympho?	DIFF- Perform diff
Dimorphic_Population	Scan smear for abnormal RBC morph
Fragments?	Scan smear for abnormal RBC morph
IG_Present	DIFF- Perform diff
Left_Shift?	DIFF- Perform diff
NRBC_Present	SCAN. Remove if not seen on smear
PLT_Abn_Distribution	Vortex and Rerun
PLT_Abn_Scattergram	Vortex and Rerun
PLT_Clumps(S)	If not clotted, Vortex. Rerun in PLT-F mode. If unresolved, SCAN
PLT_Clumps?	If not clotted, Vortex. Rerun in PLT-F mode. If unresolved, SCAN
RBC_Abn_Distribution	Scan smear for abnormal RBC morph
RBC_Agglutination?	Warm at 37, rerun. If unresolved, perform plasma replacement
RET_Abn_Scattergram	Perform 1:5 dilution
Sample_Mixing_Failure?	Vortex and Rerun
Turbidity/HGB_Interference?	If gross hemolysis, cancel with HMT. If lipemic, do saline replacement
WBC_Abn_Scattergram	SCAN for abnormal cells and NRBC



Run Worksheet

Test Name	Test St...	Result (2)	Units...	Error Code(s) (2)	Error Name(s) (2)
IP Messages					
Left_Shift?	Releas...	300		A,HOLD	Abnormal,DIFF
PLT_Clumps?	Releas...	140		A,HOLD	Abnormal,If not clotted, ...
WBC_Abn_Scatt...	Releas...	1		A,HOLD	Abnormal,SCAN for abn...
CBC					
WBC	Held fo...	11.0	10*3...	HOLD	H,Other Tests Held
RBC	Held fo...	2.17	10*6...	HOLD,HOLD,H...	L,Other Tests Held
HGB	Held fo...	7.5	g/dL	HOLD	L,Other Tests Held
HCT	Held fo...	21.2	%	HOLD	L,Other Tests Held
MCV	Held fo...	97.7	fL	HOLD	H,Other Tests Held
MCH	Held fo...	34.6	pg	HOLD	H,Other Tests Held
MCHC	Held fo...	35.4	g/dL	HOLD	Other Tests Held
RDW-CV	Held fo...	12.8	%	HOLD	Other Tests Held
Platelet					
MPV	Held fo...	10.3	fL	W,HOLD	Low Reliability,Other Te...
PLT	Held fo...	120	10*3...	W,Check for Cl...	Low Reliability,Vortex, ...
IPF	Held fo...	HIDE		HOLD	Other Tests Held

Examples of IP Messages and Abnormal error codes

Examples of "W" (Low reliability) error codes. "W" is equivalent to asterisk (*) on Svsmex

10. Release/Hold Conditions

Condition	CBC	PLT	DIFF	RET	Release/Hold
1	Normal	Normal	Normal	Normal	Release CBC, PLT, DIFF & RET
2	Normal	Normal	Normal	Abnormal	Release CBC, PLT, DIFF. Hold RET
3	Normal	Normal	Abnormal	Normal	Release CBC, PLT & RET. Hold DIFF
4	Normal	Normal	Abnormal	Abnormal	Release CBC & PLT. Hold DIFF & RET
5	Normal	Abnormal	Normal	Normal	Release CBC & RET. Hold PLT & DIFF
6	Normal	Abnormal	Normal	Abnormal	Release CBC. Hold PLT, DIFF & RET
7	Normal	Abnormal	Abnormal	Normal	Release CBC & RET. Hold PLT & DIFF
8	Normal	Abnormal	Abnormal	Abnormal	Release CBC. Hold PLT, DIFF & RET
9	Abnormal	Normal	Normal	Normal	Release RET. Hold CBC, PLT, DIFF
10	Abnormal	Normal	Normal	Abnormal	Hold CBC,PLT,DIFF & RET
11	Abnormal	Normal	Abnormal	Normal	Release RET. Hold CBC, PLT, DIFF
12	Abnormal	Normal	Abnormal	Abnormal	Hold CBC,PLT,DIFF & RET
13	Abnormal	Abnormal	Normal	Normal	Release RET. Hold CBC, PLT, DIFF
14	Abnormal	Abnormal	Normal	Abnormal	Hold CBC,PLT,DIFF & RET
15	Abnormal	Abnormal	Abnormal	Normal	Release RET. Hold CBC, PLT, DIFF
16	Abnormal	Abnormal	Abnormal	Abnormal	Hold CBC,PLT,DIFF & RET

11. Coded Entry

The following are added to all the components of the CBC and Platelets:

HIS: Result consistent with patient history
PTR: Patient transfused
SREV: Smear reviewed
UTC: Unable to calculate
CLMP: Clumped **Added to platelet group only
OCC-CLMP: Occasional Clumping **Added to Platelet group only
1+, 2+, 3+, 4+, OCC: Possible results for GPLT (Giant Platelets)
INC, DEC,ADEQ, BELOW: Possible results for PCOM

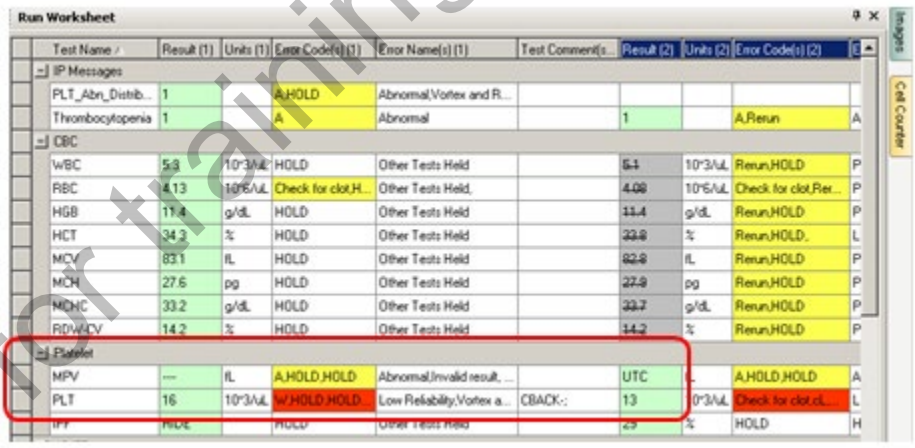
12. Clumped Platelets

Action: Depending upon quantity of clumping noted

a. If platelets clumping is more than occasional, then report PLT as CLMP and result MPV as UTC.
b. If platelets clumping is occasional, then add comment of OCC-CLMP in the Test comment field associated with PLT.

13. MPV

If on the first run MPV is resulted as "---" DI will display an error code to rerun. If the rerun is still "---" DI will automatically replace "---" with code UTC



14. SCAN indicated in DI

Action:
Review slide for abnormal cells. If no abnormal cells are seen, then enter SREV [Smear Reviewed] under the Test Comment field for the test that is associated with the SCAN comment.

15. Performing Morphology for CBC with Automated Diff

    a. Change DTYP1 result to ADIFF
    b. Enter morphology results via the Cell Counter
    c. Click on "Send Data Through System." The morphology will be displayed as a new run within the Specimen Workspace.
    d. Release the results by group from top to bottom order

16. Platelet Estimates

    a. Use Cell Counter to result platelet estimate.
    b. Click on "Send data through system"
    c. Platelet estimate results will display in a new run along with the MPV results.
    d. Release the platelet group with the platelet estimate (it will automatically reject the other platelet groups)

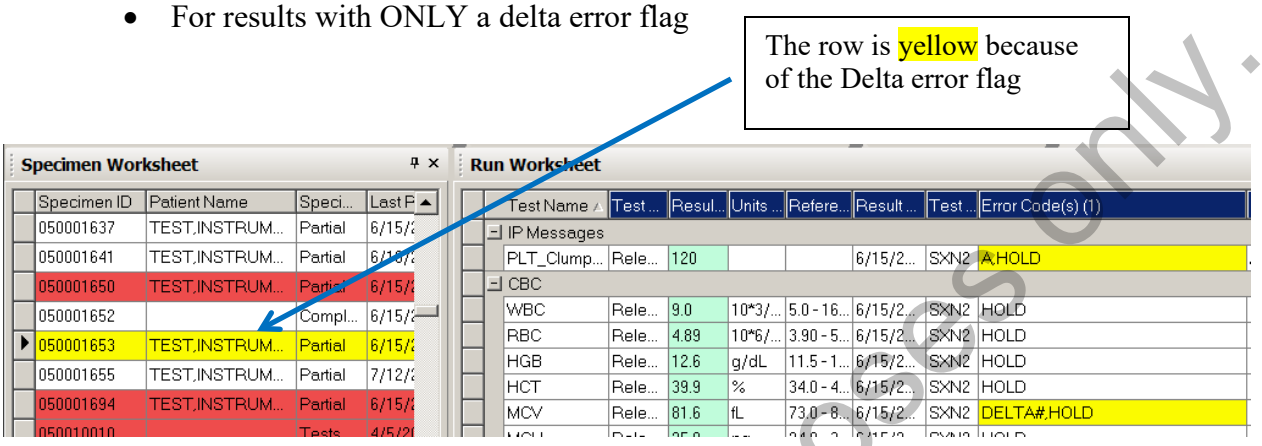
Note: if MPV result is "---" then result MPV in Cell Counter as UTC

Run Worksheet

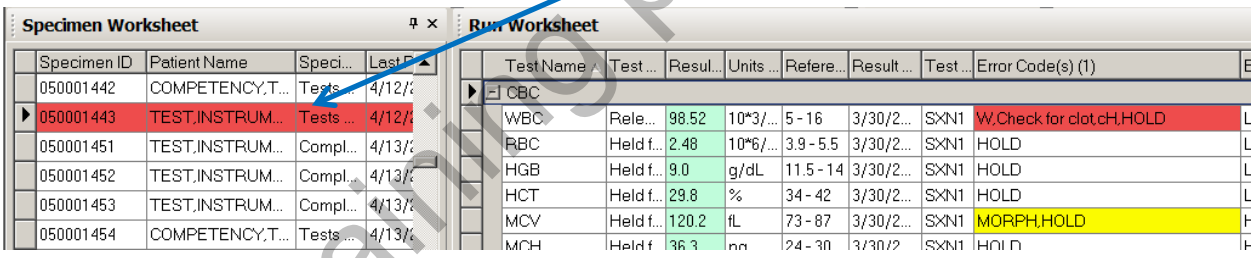
Test Name	Result (9)	Units (9)	Reference ...	Test I...	Error Code(s) (9)	Test Comment...
IP Messages						
WBC_Abn_Scattergram						
CBC						
WBC						
RBC						
HGB						
HCT						
MCV						
MCH						
MCHC						
RDW-CV						
MPV						
PLT						
Platelet						
MPV	8.7			SXN1		
PLT						
IPF	HIDE			SXN1		
PLT Estimate	400	10^3/uL	140 - 400	SXN1		RCM

17. Review of Results with Critical, and/or Delta Error Flags
Color flagging of results with critical and/or delta error flags(s) will display on both the Specimen and Run Worksheets.

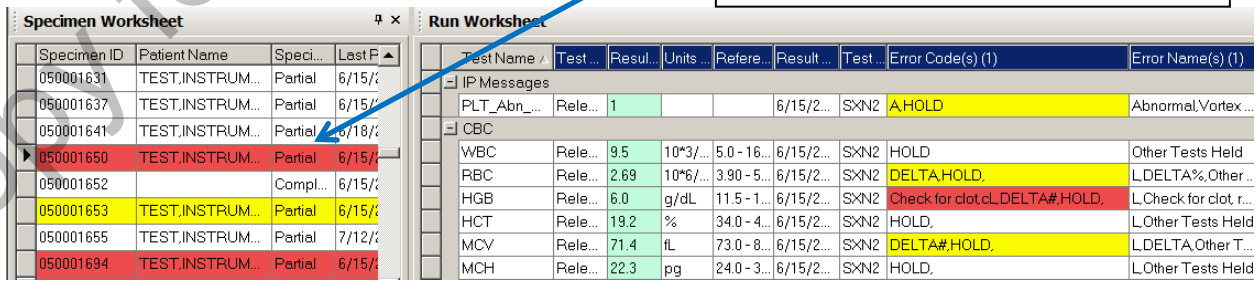
- For results with ONLY a delta error flag



- For results with ONLY a critical error flag



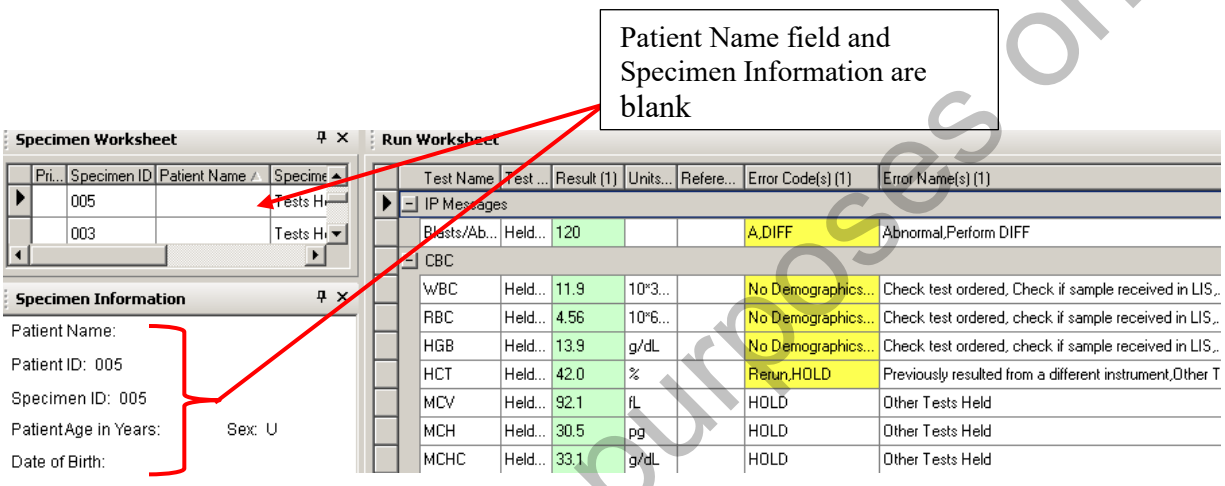
- For results with critical and delta error flags



18. Orders without Demographics

Patient demographic information will not be available when an un-received specimen or a specimen with a non-Sysmex test is run on the Sysmex (ie., EST or BNP). If an un-received sample is processed and resulted, the LIS will default the result date/time into the collect date/ time fields and provide erroneous information to clinical caregivers.

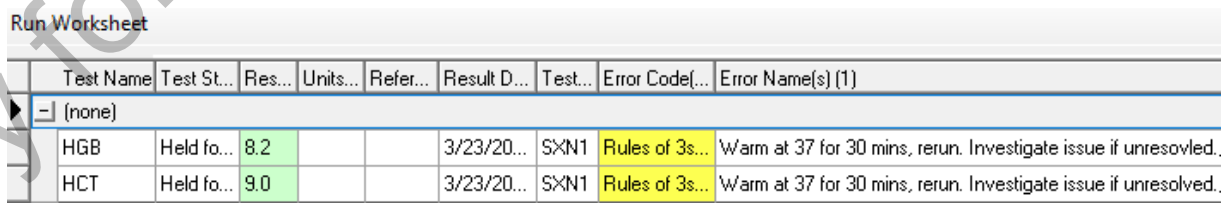
DI will display error code **No Demographics** and instructions "Check test ordered, Check if sample received in LIS"



- For a specimen that is not received in LIS
  Action: Check to see if correct test is ordered. If correct test is ordered, then receive specimen in LIS and rerun

- For a specimen that is received in LIS, but incorrect test ordered (non-Sysmex test ie. ESR, BNP)
  Action: Check the test printed on the label and deliver specimen to the correct section of the lab. Reject run on DI.

19. H and H Mismatch using the Rules of 3s



- H and H mismatch uses the following Rules of 3s formula: HCT = (3 x HGB) +/- 5
- DI will display an error of "Rules of 3s. Warm at 37 degrees for 30 mins, rerun. Investigate issue if unresolved. See Section 10.6 of SOP."