|  |
| --- |
| **Complete Cell Count of Whole Blood – Sysmex XN 3000** |
| **Purpose** | This procedure provides instructions for performing a complete cell count on whole blood (CBC). |
| **Principle** | The Sysmex 3000 in an integrated system that incorporates two hematology analytical modules as well as an automated slide maker / stainer.The analyzer performs hematology analysis according to the hydrodynamic focusing (DC Detection), flow cytometry method (semiconductor laser), and SLS-hemoglobin method.Red blood cells and platelets are counted using electronic resistance detection. Hematocrit is measured as a ratio of the total RBC volume to whole blood using cumulative pulse height detection. Hemoglobin is converted to SLS-hemoglobin and read photometrically.The WBC count, differential, reticulocytes, nucleated RBC’s, and fluorescent platelets are all evaluated using flow cytometry with a semiconductor laser exploiting the differences in cell size, complexity and RNA/DNA content. Forward scattered light provides information on blood cell size and lateral scattered light provides information on cell interior such as the size of the nucleus. Lateral light intensity increases as the concentration of the stain becomes higher. By measuring the intensity of the fluorescence emitted, information is obtained on the degree of blood cell staning. Fluorescent light is emitted in all directions. The XN detects the fluorescent light that is emitted sideways.The Sysmex SP-10 is a fully automated hematology slide preparation and staining system. Whole blood specimens are mixed and aspirated and a blood smear is prepared using hematocrit information from the Sysmex XN to determine optimum smearing criteria. The dried smear is automatically loaded into an individual slide cassette and is then advanced to the staining area. In the staining area, stain and buffer are dispensed into operator defined intervals. The system also provides a manual mode operation where pre-made smears may be added to be stained. The unit is self monitoring and alarms when operation is interrupted.Slides prepared by the Sysmex SP-10 are used for differentiation and morphologic evaluation of cellular elements of whole blood. |
| **Policy Statements** | * This procedure applies to all laboratory technologists performing hematology testing, section supervisor, and pathologist.
 |
| **Materials** | **Equipment** | **Reagents** | **Supplies** |
| **Special Safety****Precautions** | * **Sysmex XN 3000 Analyzer:** analyzer, personal computer, printer and associated non-disposable parts
* **SP-10 Slide Preparation Unit:** analyzer, printer and associated non-disposable parts
 |  **● Cell Pack DCL:**Reagent used for measuring the numbers and sizes of RBC’s and Platelets by hydrodynamic focusing With the addition of specified lyse reagent for Hemoglobin determination. Also as sheath fluid for FCM detector.On the SP-10 slide maker / stainer it is used as a cleaning agent.Open expiration date 60 days.Store at 2-35°C.Chc# 30239.● **Cell Pack DST:**Concentrated diluent to be used by connecting to a reagent dilution unit (RU-20).Reagent used for measuring the numbers and sizes of RBC’s and Platelets by hydrodynamic focusing With the addition of specified lyse reagent for Hemoglobin determination. Also as sheath fluid for FCM detector.Open expiration date 60 days.Store at 2-35°C.Chc# 30240.**● Cell Pack DFL:**Reagent used in combination with Fluorocell RET for the analysis of reticulocytes or with Fluorocell PLT for the analysis of of platelets by flow cytometry method using a semiconductor laser.Open expiration date 60 days.Store at 2-35°C.Chc# 30234.**● Sulfolyser:**Reagent used for determination of hemoglobin concentration in the blood.Open expiration date 90 days.Store at 1-30°C.Chc# 30241.**● Lysercell WNR:**Reagent used in combination with Fluorocell WNR. RBC’s are hemolyzed and WBC’s are differentiated into non-Basophils, Basophils, and Nucleated RBC’s. This allows for a WBC count, Basophil count, Basophil percentage, NRBC count and NRBC percentage.Open expiration date 60 days.Store at 2-35°C.Chc# 30243.**● Lysercell WDF:**Reagent used in combination with Fluorocell WDF. RBC’s are hemolyzed and dyeing the WBC component with Fluorocell WDF the counts and percentages of Neutrophils, Lymphocytes, Monocytes and Eosinophils are analyzed.Open expiration date 90 days.Store at 2-35°C.Chc# 30242.**● Fluorocell WNR:**Reagent used to stain nucleated cells for the determination of WBC count, NRBC count and Basophil count.Open expiration date 90 days.Store at 2-35°C.Chc# 30237.**● Fluorocell WDF:**Reagent used to stain the WBC’s for the determination of a four part differential count.Open expiration date 90 days.Store at 2-35°C.Chc# 30238.**● Fluorocell RET:**Reagent used to stain the Reticulocytes for the assay of Reticulocyte count, Reticulocyte percentage and Platelet count.Open expiration date 90 days.Store at 2-35°C.Chc# 30233.**● Fluorocell PLT:**Reagent used to stain Platelets.Open expiration date 90 days.Store at 2-35°C.Chc# 30235.**● CellClean Auto:**Cleaning solution used to remove cellular residuals, and blood proteins in the Sysmex analyzer and the SP-10 slide preparation unit.Single use 4ml vials.Store at 1-30°C.Chc# 30236.**● Control blood (XN CHECK/XN CHECK BF):**Control used to monitor performance on XN analyzers.Open expiration date:XN CHECK - 7 daysXN CHECK BF - 30 days Store at 2-8°C.XN CHECK:Chc# 30230.XN CHECK BF:Chc# 30231.**● Calibrator (XN CAL/XN CAL PF):**XN CAL - Calibrator used for WBC, RBC, HGB, HCT, PLT, and RET.XN CAL PF – Calibrator used for Platelet Count (PLTF). Store at 2-8°C.Open expiration date: 4 hours.**For Hazard risks please see MSDS on line on Childrens Star Net;** [MSDS Search | MSDSonline](https://msdsmanagement.msdsonline.com/a07dc954-23d8-42a9-b591-ef5763cdfd33/ebinder/?nas=True)**WARNING:POTENTIALLY INFECTIOUS MATERIAL.**The human blood used in XN CHECK is non-reactive for Hepatitis B Surface Antigen and negative for antibodies to HIV-1, HIV-2, and Hepatitis C Virus using FDA specified techniques. However, no current tests can assure the absence of these pathogens. XN CHECK should be considered potentially infectious and must be handled with precautions used for human blood as described in CDC recommendations and in compliance with the Federal OSHA Bloodborne Pathogen Standard, 29CFR, 1910.1030.  | **●** Sample racks for 2ml tubes and raised bottom tubes (RBT).● De-ionized water● Alcohol prep pads, isopropyl. Used to clean SP-10 spreader glass.● Microscope Slides, frosted with rounded / clipped corners76 x 26mm; 0.9 - 1.2 mm thickChc# 30455.● SP Wrights StainChc# 30458.● SP Buffer pH 6.8Chc# 30456.● MethanolUsed for cleaning of the staining system and cassettesChc# 11223. |
| **XN Reagent****Replacement****Sample****Calibration** **and Precision****(Remote** **Calibration)****Calibration** **And Precision****(Onsite** **Calibration)****Quality Control****Operating****Procedure****Maintenance****Resulting in****Sunquest****Limitations** **Of****Procedure****Procedural** **Notes****References** | 1.When the reagent runs out during analysis, the analysis is paused and an error message appears in the analyzer area of the Control menu. 2. Display the [Reagent Replacement] dialog box to replace the reagent. a.) Select the help button on the control menu  b.) Select [Execute]  Remaining Reagent Volume indicator appears **3. Replacing a new diluents / hemolytic agents** a.) Display the [Reagent Replacement] dialog box b.) Remove the cap from the new reagent container Confirm the reagent has not expired c.) Input the reagent code (barcode) Place the cursor in the reagent code field Scan the reagent code on the outer box of the new reagent with the hand-held barcode  reader or manually enter the reagent code Select [OK] Remove the cap from the old reagent container. Pull out the dispensing set straight up. Insert the dispensing set straight into the new container. Close the cap. Select [Execute] Reagent replacement starts. When complete, the dialog box closes automatically.**4.) Replacing CELLPACK DST with an RU-20** a.) Display the RU-20 Maintenance menu. b.) Select [Replace Reagent] c.) Remove the cap from the new reagent container.Confirm that reagent has not expired d.) Input the reagent code (barcode) Place the cursor in the reagent code field. Scan the reagent code on the outer box of the new reagent with the hand-held barcode reader  Select [OK] Remove the cap from the old reagent container Pull out the dispensing set straight up. Insert the dispensing set straight into the new reagent container. Close the cap Select [Execute] Reagent replacement starts. When complete, the dialog box closes automatically.**5.) Replacing Dye** a.) Display the [Reagent Replacement] dialog box. b.) Prepare the new reagent cartridge. Confirm the reagent has not expired. c.) Open the top front cover. d.) Pull up the cover from the reagent that is to be replaced. When the dye solution cover is pulled up, a Help dialog box appears in the IPU screen.  e.) Remove the old reagent cartridge from its holder f.) Install the new reagent cartridge into the holderMake sure the color of the label on the new reagent cartridge matches the color of the dye cover and install. Analyzer will beep as confirmation of new reagent installation.If the wrong reagent is installed, the analyzer beeps repeatedly and the Help dialog box appears in the IPU screen. g.) Pull down the cover on the reagent until you hear a click. When the cover is pulled down, the Help dialog box closes automatically. The ID of the new reagent is read automatically and the information is registered. h) Close the top front cover. Reagent replacement starts. When complete, the reagent replacement window closes automatically.**SP-10 Reagent Replacement**The following is a list of replacement messages and the reagent requiring  **Message** **Reagent**\*DCL not filled CELLPACK DCL\*Stain 1 not filled in Chamber 1 Stain\*Stain 1 not filled in Chamber 2 Stain\*Stain 2 not filled 2nd stain (if using 2 stain method)\*Rinse water not filled Deionized water (internal chamber not filled) Replace Rinse water Deionized water (external container empty) Replace buffer Buffer Replace methanol Methanol\* Reagents with internal chambers. Other reagents use bottle sensors. a.) When a reagent container is empty, an alarm sounds and a dialogue box displays.  Press **[OK]** to silence the alarm and close the dialogue box. b.) Press **[Help]** icon and follow the corrective action message. c.) When replacing a reagent with an internal chamber, press **[OK]** to clear the action message  and reset. For reagents with bottle sensors, the error clears when the reagent is replaced or  filled. d.) Replace reagent using clean technique. The spout kit should not be placed on any potentially contaminated surface. The spout kit should be removed from the old container and put directly into the new container that contains the fresh reagent. **Document all reagent changes on the appropriate log.**1. Required Specimen; Whole blood should be collected in EDTA-2K or EDTA-3K anticoagulant. Serous (peritoneal and pleural) and synovial fluids should be collected in EDTA-2K anticoagulant. It is not necessary to use anticoagulant with CSF specimens.2. Required sample volumes XN3000; a.) Sampler analysis 2ml EDTA whole blood, aspirated vol. = 88ul, required sample vol. = 1ml. b.) Sampler analysis Raised Bottom Tube (RBT) whole blood, aspirated vol. = 88ul, required  sample vol. = 250ul. c.) Body fluid open red top microtainer, aspirated vol. = 88ul, required sample vol. = 160ul. d.) Diluted blood (1:7), aspirated vol. = 70ul, required sample vol. = 140ul. e.) **6ml or larger EDTA tubes cannot be loaded on the analyzer, an aliquot should be**  **removed and analyzed in a red top microtainer with the cap removed.**3.) Required sample volumes SP-10; a.) Sampler analysis 2ml EDTA whole blood, aspirated vol. = 200ul, required sample vol. = 1ml. b.) Sampler analysis Raised Bottom Tube (RBT) whole blood, aspirated vol. = 200ul, required  sample vol. = 500ul.  c.) Manual mode smear and staining – 1ml is optimal, 200ul is aspirated. d.) Manual mode (red top microtainer) – 300ul minimum volume, 60ul is aspirated.4.) Unacceptable specimens including those listed below must be redrawn; a.) Clotted samples or those containing clots, fibrin strands, or platelet clumps. All specimens will  be checked visually for obvious clots prior to sampling by the analyzer. b.) Grossly hemolyzed samples. c.) Samples drawn above an IV line.  5.) Characteristics that may affect test results: lipemia, icterus, and cold agglutinins. 6.) Stored Specimen Stability; a.) Stored at 4-8oC, EDTA blood samples with normal results may be analyzed up to 48 hours without significant loss of differential stability. b.) Sample stability at room temperature is 24 hours. Samples stored at room temperature may  exhibit an increase in MCV after 24 hours, which may be minimized by refrigeration. c.) Allow refrigerated samples to come to room temperature and mix well before analysis.  d.) Do not place CBC and Diff samples on a mechanical rocker. Constant rocking may alter white  cell membranes, resulting in false interpretive messages.Initial calibration is performed during installation by the Sysmex Field Service Representative. Perform calibration as needed, e.g., when QC data is fluctuating. However, if the abnormality in the QC analysis data was caused by an error in the analyzer, degradation of the reagent, or degeneration of the control blood, do not perform calibration. Calibrators traceable to reference methods are used in the calibration of the analyzer. The laboratory must verify calibration every six months or on an "as-needed" basis to ensure accuracy of system. Calibration verification is also required if one or more of the following occur:* Critical parts are replaced.
* Controls show an unusual trend or are outside of acceptable limits and cannot be corrected by maintenance or troubleshooting.
* When advised by Sysmex Service Engineer (SE).

Calibration should only be completed when troubleshooting indicates that there is no major underlying Problem with the analyzer, reagents or quality control materials.Calibration verification may be performed by review and documentation of commercial quality control results, and X-BarM QC data, proficiency testing results or patient control testing results. Calibration verification may also be accomplished by processing a commercial calibrator and comparing results to those published on the calibrator assay sheet.Calibration verification procedures may be done by a Sysmex SE on site, or remotely through the Sysmex Network Communications System ( SNCS ) with the Sysmex Calibration Specialist. The following items are completed by the Sysmex representative during the calibration verification process;1.) Documentation and review of the analyzer service history.2.) Documentation and review of QC testing results.3.) Documentation and review of historical Sysmex *Insight* reports. 4.) Analyzing the Sysmex calibrator accoeding to the manufacturers recommendations to verify  precision and calibration (accuracy) of the analyzer.5.) Documentation of calibration verification results and generation of a calibration verification  certificate for laboratory records.A. Remote Procedure 1.) A Sysmex representative will contact the laboratory prior to the expiration date of the existing calibration certificate to schedule the Managed Calibration event. 2.) Sysmex will ship the calibrator to arrive before the scheduled Managed Calibration event. 3.) Sysmex will send a reminder email to the laboratory representative prior to the scheduled  event. The email contains the schedule date, time and instructions for the Managed Calibration event.  4.) Follow the instructions in the email to prepare for the Managed Calibration event. 5.) On the pre-arranged day, a Sysmex representative will contact the laboratory representative. SNCS is used to remotely connect with the analyzer. 6.) Sysmex will ask the operator to log off the Information Processing Unit (IPU). Sysmex will then remotely log into the IPU with a user name and password that prevents viewing or access to patient data.7.) Sysmex will review the analyzer’s historical and current QC recovery with the laboratory  representative. During this analysis, if indicated by abnormal QC recovery, Sysmex may  discontinue the Managed Calibration event and schedule an on-site service visit for analyzer troubleshooting and calibration verification. 8.) Using SNCS, Sysmex will prepare the IPU and analyzer for calibration verification. 9.) Prepare the calibrator as described in t he calibrator package insert when instructed by the Sysmex representative. 10.) Analyze the calibrator when instructed by the Sysmex representative. Data is captured automatically and analyzed by Sysmex. 11.) Sysmex will compare the recovery of the initial analyses to the calibrator package insert ranges and discuss the recovery with the laboratory representative. 12.) If the calibrator and QC recovery indicate a calibration adjustment is required, Sysmex will recommend adjusting the calibration using SNCS. During this analysis, if indicated by abnormal calibrator recovery, Sysmex may discontinue the Managed Calibration  event and schedule an on-site service visit for analyzer troubleshooting and calibration  verification. 13.) If an adjustment is made, Sysmex will prepare the IPU and analyzer to verify the calibration  adjustment using SNCS. Sysmex will instruct the operator to analyze the calibrator 6 times. 14.) After calibration verification passes, Sysmex will prepare the IPU and analyzer to run at least 2 levels of QC. 15.) Mix and analyze the QC material when instructed by the Sysmex representative. 16.) Once the calibration verification and the QC recovery meets manufacturer specifications Sysmex will retrieve data from the IPU using SNCS to prepare a certificate of calibration  verification. Sysmex will send the completed certificate of calibration verification to automatically print on the IPU printer. This certificate contains information about reagent  and calibrator lot numbers, expiration dates, precision data calibrator and QC recovery and acceptable ranges for calibrator recovery. 17.) Sysmex will review the certificate and QC recovery with the operator and log off the IPU. The SNCS remote event will be terminated and Sysmex will instruct the operator to log onto the IPU. 18.) Sign the certificate of calibration verification and retain it for our records.   B.) Onsite Calibration NOTE: The following steps are performed by the Sysmex SE 1.) Precision Check a. Verify that there is sufficient volume of all reagents and reagents are within expiration dates. b. Perform routine maintenance on the analyzer, if required, and perform an Autorinse  to ensure background counts are within acceptable limits. c. Prepare the calibrator as described in the calibrator product insert. d. Upload current IPU data into Sysmex Evidence – Based Calibration (EBC) application Set Up and Instrument Recovery tabs. e. Analyze calibrator 10 times in the primary (manual) sampling mode. i. Upload the results from the 10 calibrator runs into the EBC application Precision tab. ii. Review the results from the 10 calibrator runs and ensure the coefficient of variation (CVs) are within the specifications contained in the EBC application. The EBC application will flag any parameters that failed the precision check. iii. If the precision fails, do not continue with calibration until corrective action has  been completed and acceptable precision results are obtained. 2.) Calibration (Accuracy) verification a. Prepare the calibrator as described in the calibrator product insert. b. Analyze calibrator 6 times in the primary (manual) mode.  c. Upload the results from the 6 calibrator runs into the EBC application. d. The EBC application will disregard the first analysis and calculate a mean, standard deviation (SD) and CV for the results of the remaining 5 calibrator runs. The mean  of the 5 calibrator runs is compared to assay sheet ranges. The EBC application will flag the mean of any parameter that is outside of the calibrator assay sheet ranges. e. Adjust the Count of Correction (COC) of any parameter flagged as being outside of  of the calibrator assay sheet ranges. f. If COC was adjusted rerun the calibration verification procedure starting from step 2. NOTE: Sysmex XS-Series and XN-Series use a common aspiration probe and pathway  for open and closed sampling and analysis. Mode to mode calibration is not required because of this common aspiration pathway. Quality control is performed in order to monitor an analyzer’s performance over time. XN CHECK and XN CHECK BF is the material used to monitor the performance  of the XN analyzer. To QC the SP-10, examine a stained smear from the routine workload  for smear and stain quality on a daily basis. Document results on appropriate log.1. XN CHECK Commercial Controls Instructions for Use
2. Remove vials from refrigerator and allow them to come to room temperature (18-25oC),

for approximately 15 minutes.1. Mix vials by gentle end-to-end inversion until the cell button in the bottom of the vial is completely suspended.
2. XN CHECK BF Commercial Body Fluid Controls Instructions for Use
	* + 1. Remove vials from refrigerator and allow them to come to room temperature (18 – 25oC) for approximately 15 minutes.
			2. Mix vials by gentle end-to-end inversion until the cell button in the bottom of the vial is completely suspended.
3. Frequency of Control use

 a. Three levels of XN CHECK control ( LO, NORC, HI ) will be run on each shift. b. Two levels of XN CHECK BF control will be run daily on the day shift.1. Registering and modifying a QC file – lot information input ( Dayshift )
	1. Select [QC File] Icon
	2. Select TAB for analyzer from bottom of QC File screen
	3. Select File number to be registered.
	4. Select [Register] button on toolbar
	5. Enter lot information
		1. Material
		2. Lot Number
		3. Expiration Date
	6. Select [Restore]
		1. Browse XN QC Limits folder on XN-IPU Desktop
		2. Select file for QC to be registered
		3. Select Open.
		4. Sysmex Range Limit %’s will automatically upload to the file
	7. Repeat for each level of XN CHECK, XN CHECK BF to be registered and for each module in the XN configuration
	8. To modify an existing QC File, select the QC File and [Modify] from the toolbar. Update the Lot No, Exp. Date as appropriate.
	9. Perform parallel studies between production lot and new lot prior to production lot expiration.
2. XN CHECK QC Analysis
	1. Place the vial containing control blood in the rack.
	2. Place rack on sampler unit; sampler unit will auto-start.
	3. Results will be plotted on the L-J Chart as well as the Radar Chart for review.
3. XN CHECK BF Analysis
	1. Check the Status indicator LED on the analyzer to confirm analyzer is in ready state.
	2. If the tube holder is not ejected, press the mode switch. Tube holder will slide out.
	3. Select the Change Analysis Mode button on the control menu.
	4. Select [Body Fluid] mode. Analyzer will automatically perform Autorinse.
	5. Select [OK]
	6. Place thoroughly mixed vial in tube holder, press start switch.
	7. If vial barcode is unreadable, select the analyzer menu button on the control menu.
		1. Select [QC Analysis]
		2. From the list of QC files, select the file to be analyzed. Judgment dialog box will open automatically.
		3. Place thoroughly mixed vial in tube holder, press start switch.
		4. When analysis is complete, analysis results are displayed. User should review results and either accept or cancel the run. Accepting the run will transfer the results to the L-J Chart and the Radar Chart for review.

  7. Auto set Targets ( Day shift )* 1. Parallel test new controls by analyzing the chosen levels of control, selected per lab policy QC protocol, a minimum of twice a day for 5 days prior to expiration or previous lot. After a minimum of 10 data points are accumulated, auto set the targets.
		1. Select QC Chart
		2. Select [Range] and set cursors so that every data point is included
		3. Select [Register]
		4. Highlight all parameters and select [Auto Setting]
		5. Confirm that the check box for TARGET ONLY is set. Do not select the check box for LIMIT.
		6. Select [OK]; the target for each parameter will be calculated and set for the duration of the QC lot.
		7. Repeat steps for each new lot of QC being moved into production.
		8. Confirm the target set falls within the range of means provided on the XN Check assay sheet provided.

 8. Reviewing Quality Control Results* 1. QC File screen
		1. Allows for review of the latest QC results in Radar Chart format for the QC file that is selected in the list.
		2. Any point exceeding the upper or lower limit is marked with a red “X”.
	2. QC Chart screen
		1. Allows for review of detailed graph data of all QC runs for selected file.
		2. Analysis data is plotted cumulatively and displayed in the chart area as a line graph.
		3. Any point exceeding the upper or lower limit is marked with a red “X”.
		4. User must scroll up and down through the chart to view all parameters for each run.
		5. Select [Range] to set a main cursor and a sub-cursor so that data between the two cursors can be manipulated.
			1. Statistics may be analyzed over any selected range.
			2. Targets may be auto-set for the selected range.
			3. To cancel range mode, select [Range] on the toolbar again or exit QC Chart mode.
		6. QC charts may be overlaid on top of each other for comparison.
			1. Select [Compare QC Files] to view QC charts registered to a single analyzer. This will compare the new lot with the current lot.
			2. Select [Compare Analyzers] to compare QC files for the same material registered to different analyzers.
			3. **Follow laboratory protocol for troubleshooting Quality Control results exceeding the upper or lower limit of acceptability.**

[HEM 10.1 Quality Control in Hematology, Coagulation , Serology, Urinalysis](http://khan.childrensmn.org/Manuals/Lab/SOP/Heme/Heme/198970.pdf) 9. Quality Control Management* 1. From the QC Chart view, select the [Manage] button on the toolbar.
	2. Specify whether a QC run should be excluded from quality control
	3. Select [Not Manage] to exclude data from the following:
		1. Statistical computations (SD, Mean, CV)
		2. Variable target computation
		3. Number of data points = n
	4. An open circle will be displayed on the L-J Chart when the QC run is not managed or excluded and is not connected by a line to the adjacent QC runs.
	5. A comment may be added to the QC data selected by the cursor
		1. Select [Input Any Comment] to input a free text comment.
		2. Select [Fixed Comments] to use a comment from a list of preset comments in the QC settings menu.
		3. Select [OK]
		4. A comment bubble will be displayed when a comment exists for a QC run.
		5. The comment will be visible in the comment display area when the cursor is placed on the QC run.

 10. Recording and Storage of QC Data ( Day Shift ) m. Printing and saving QC Data 1. Select QC Files Icon and highlight file to output. 2. Select QC Chart Icon. 3. Set Range of points to output by clicking [Range] and capturing the points  with the cursors. 4. Select [output] to print the selected chart to either GP or LP. 5. Select [file] to save the data to removable media.11. SP-10 Daily QC Slide Review **a. Review the blood smears macroscopically for acceptability:**1. Smears are sufficient length (greater than half the length of the unfrosted portion of the slide).
2. The feathered edge becomes gradually thinner without streaks, holes, or tails.
3. Even, consistent staining of blood smear.

 **b. Review the blood smears microscopically for acceptability:**1. Relatively even distribution of cellular elements.
2. Acceptable morphology within the working area.
3. None or very little artifact of the cell morphology, (e. g., “punched-out” RBC’s, smashed WBC’s).
4. None, or very little stain precipitate or debris
5. The staining is consistent and imparts the characteristic cytoplasmic color differences and distinct nuclear chromatic patterns of the whole spectrum of blood cells. Acceptable stains will display the following characteristics:
6. RBC’s should be pink to orange. There should be good differentiation between normochromic, hypochromic, and polychromatic cells.
7. Lymphocytes will display dark purple nuclei with varying shades of blue cytoplasm.
8. Neutrophils will display dark purple nuclei, with light pink cytoplasm and lilac granules.
9. Monocytes will show lighter purple nuclei. The cytoplasm of the monocytes will be gray-blue with reddish granules.
10. Eosinophils show bright orange granules in the cytoplasm.
11. Basophils display dark blue granules in the cytoplasm.
12. Platelets will be violet to purple.

If smear quality is unsatisfactory, clean, or if necessary, replace the spreader glass. If still unable to obtain an acceptable smear, refer to the SP-Series Implementation Manual troubleshooting section. If the troubleshooting steps do not resolve the problem, notify the supervisor / key operator when available or call the Sysmex Technical Assistance Center (TAC) 1-888-879-7639. Document all corrective action according to laboratory protocol.12. ***Insight*TM** Quality Assurance Program (QAP)Our laboratories ( Minneapolis and St. Paul ) maintain an SNCS connection. The QC results will transmit automatically to ***Insigh***t after each run. There is no need to batch upload the data to ***Insight***.Our *Insight* identification numbers are; Mpls. 27194, St. Paul 27083.XN Serial Numbers for Mpls; 24041, 24042XN Serial Numbers for St.Paul; 24177, 24178The Technical Specialist in Hematology is responsible for saving the data to a USB memory device and submitting by due date in lieu of an SNCS connection. * 1. Each lot has 2 data submission dates, approximately every 30 days for the 84-day dated product.
	2. Data may be managed in the XN-IPU and/or in ***Insight***. See ***Insight*** User Manuals.
	3. Insert flash drive into USB port on the IPU’s hard drive.
	4. Select the QC file you want to output, click [File], [Output in Sysmex ***Insight***]. Save the file to the flash drive.
	5. Repeat for each file needing ***Insight*** submission.
	6. Properly eject the flash drive from the IPU.
	7. At a networked PC, establish connection with the ***Insight*** program via [www.sysmex.com/us](http://www.sysmex.com/us) and submit the data. Contact the ***Insight*** team with questions at: 1-888-879-7639 (1-888-8SYSMEX).

  * 1. **Start-Up Procedure**
		1. Checks prior to turning on
			1. Visual inspections of analyzer / system / reagents
				1. Place completed samples into final storage area for the lab
				2. Remove any items that may interfere with operations
				3. Gather and re-locate all empty racks to designated processing or sample loading area
				4. If applicable, verify waste container is empty
				5. Verify network / host connections are properly working
				6. Ensure that the towers (slide supply cassettes) have sufficient slides. Fill with glass slides.
1. Remove the tower to be filled.
2. Remove the metal insert from the end of the tower.
3. Fan the slides to prevent them from adhering to each other and place them with the frosted end up and towards the open end of the tower.
4. Replace the metal insert and replace the tower with the frosted end of the slides towards the back of the analyzer.
	* + - 1. Verify sufficient reagent supply is nearby
				2. Fill the cassette supply table with clean, dry single cassettes. The Sysmex logo should be forward and the notch at the bottom must be away from you (or to the left). The supply table holds up to 100 cassettes. A minimum of 8 cassettes are required for start-up.
		1. Turning ON the entire system
			1. Verify that all power switches for each device are in the ON position
			2. Press the start-up switch on the sampler to power ON the entire system
		2. Log on to the XN-IPU
			1. When the logon dialog box appears, enter user name and password

 Mpls - lab1 and labstaff4, followed by xn enter. St. Paul - lab2 and labstp222, followed by xn enter xn enter.* + 1. Analyzers and SP-10 self-checks
			1. XN: Initialization of the mechanical parts; Rinse; Temperature stabilization; Background Check (up to 3 times)

|  |
| --- |
| **XN Acceptable Background Counts** |
| **Parameters** | **Acceptable Limit** |
| WBC-N | 0.10 x 103/ μL |
| WBC-D | 0.10 x 103/ μL |
| RBC | 0.02 x 106/μL |
| HGB | 0.1 g/dL |
| PLT-I | 10 x 103/ μL |
| PLT-F | 3 x 103/ μL |

b. SP-10: System check to evaluate internal stored data files; shutdown check to determine whether shutdown was performed properly, a mechanical initialization sequence.* + 1. Analyze Quality Control Material
	1. **Patient Sample Processing**
		1. System Analysis (sampler analysis)
			1. Make sure the analyzer and the sampler are in READY state
			2. Check that tube holder has retracted into the analyzer, press mode button if necessary
			3. Place sample(s) in rack(s) in right sampler pool (analyzer side)

 **Verify that if sampling from Raised bottom tubes (Microtainers) that racks have a** **yellow stripe.*** + - 1. Rack(s) will auto-start.
			2. Samples will run, results will be displayed in the IPU.
			3. On-Board rules engine will determine repeat or reflex testing
			4. Rack will run in reverse to perform repeat or reflex testing.
			5. If smear is required, rack will be transported to SP-10 via analysis line and samples will be aspirated by SP-10.
			6. If no smears are required, rack will be transported to the left sampler pool without stopping at the SP-10.
			7. Remove the rack from the left sampler pool when analysis in completed.
		1. Manual Analysis - XN
			1. Check the status of the analyzer. Confirm the analyzer is ready.
			2. Press the mode switch to eject the tube holder.
			3. Select the Change Analysis Mode button on the control menu
			4. Select analysis mode
				1. [Whole blood] is selected when whole blood is being analyzed
				2. [Low WBC] Select this to perform low WBC analysis on whole blood
				3. [Pre-Dilution] select when running 1:7 pre-diluted blood.
			5. Select [OK]
			6. Select Manual Analysis button on the control menu
			7. Input sample ID or select [Read ID]
			8. Select [OK]
			9. Properly mix the specimen and place in the tube holder
				1. If running microtainer, remove the cap using caution to avoid splattering.
			10. Press the start switch on the analyzer
				1. The tube holder will slide in and the sample will be aspirated
				2. When the analysis is complete, the tube holder slides out
			11. Remove the sample, repeat steps for additional samples
			12. Review results in IPU to determine whether repeat or reflex testing is required. Rerun sample if required. Make smear if required.
		2. Body Fluid Analysis - XN
			1. Check the status of the analyzer. Confirm the analyzer is ready.
			2. Press the mode switch to eject the tube holder.
			3. Select the Change Analysis Mode button on the control menu.
			4. Select [Body Fluid]
			5. Select [OK]
				1. The analyzer will automatically perform a background check up to three times
			6. Select the Manual Analysis button on the control menu
			7. Input the sample ID or select [Read ID]
			8. Select [OK]
			9. Properly mix the specimen and place in tube holder.
				1. If running microtainer, remove the cap using caution to avoid splattering
			10. Press the start switch on the analyzer
				1. The tube holder will slide in and the sample will be aspirated
				2. When the analysis is complete, the tube holder slides out
			11. Remove the sample
			12. Perform Background check prior to running additional samples if indicated

Return analyzer to Whole Blood mode prior to running whole blood samples* + 1. Off-line analysis; The sampler for the analyzer, or the sampler for the SP-10 is separated from the transport line of the overall system and operated as a standalone device
			1. Press mode switch on the sampler
			2. Verify sampler is in READY state
			3. Place the rack in the right pool of the sampler for the analyzer that you wish to use.
			4. Transport begins automatically
			5. Remove the rack after analysis is complete
			6. Press the mode switch on the sampler
		2. SP-10 Manual Mode – Smear and Stain
			1. Press [Conv. Int.] on the SP-10 main menu screen
			2. Press [Interrupt]
			3. Select [Return]
			4. Select [Manual] on the SP-10 main menu screen
			5. Op Mode is set to [Smr + Sta], Smpl. Tube is set to [Closed]
			6. Input Specimen information, Sample ID, HCT, select number of slides to be made
			7. Thoroughly mix the sample and place in 10th rack position
			8. Place the rack so that the sample aligns with the tube gripper and that the left end of the rack fits the label on the sampler
			9. Select [Start]
				1. Analysis will begin
				2. When the tube is returned to the rack, remove the rack
			10. Press [Return] [Conv. Int.] [Stop Int.]
		3. SP-10 Manual Mode – Stain Only
			1. Select [Manual] mode
			2. Select [Op. Mode], [Stain]. Do not proceed until [START] button is green.
			3. Place labeled, unstained blood films into cassettes at the front of the cassette supply table on the right side of the analyzer. If multiple slides are to be stained, place them in consecutive cassettes.
			4. Press [Start]
			5. The cassettes will be fed to the stain table and the smears will be stained. An empty cassette will follow to indicate the end of the run.
		4. SP-10 Micro Mode
			1. Select [Manual]
			2. Choose [Op. Mode], [Smr.+Stain] and set [Smpl. Tube] to [micro]
			3. Input Specimen information, Sample ID, HCT
			4. Place the thoroughly mixed uncapped microtainer in the micro collection sample tube holder.
			5. Select [Start]
			6. Micro tube will be lowered into position and sample will be aspirated.
			7. When aspiration is complete, micro tube will be returned to home position and should be removed.
		5. SP-10 Smear Only – No staining occurs

Smear mode may be used in System, Single, or Manual Modes. To access Smear Mode:* 1. Press **[Settings]** on the main screen. (A password may be required.)
	2. Press **[Select]**, **[Cond.]**, **[Mode]**.
	3. Press **[Op. Mode]** and select **[Smear]**. Press **[RETURN]** and **[YES]** to accept the settings.
	4. To use Smear Only in System Mode:
		1. Place bar coded samples in a Sysmex rack.
		2. Place the rack in the right pool of the Sampler Unit.
		3. Racks will auto-start.
		4. Racks are transported to the XN analyzer and then to the SP-10 where a smear will be prepared when appropriate criteria are met.
	5. To use Smear Only in Off Line Mode: The sampler for the analyzer, or the sampler for the SP-10 is separated from the transport line of the overall system and operated as a standalone device
		1. Press mode switch on the sampler
		2. Verify sampler is in READY state
		3. Place the rack in the right pool of the sampler for the analyzer that you wish to use.
		4. Transport begins automatically
		5. Remove the rack after analysis is complete
		6. Press the mode switch on the sampler
	6. To use Smear Only in Manual Closed Mode:
		1. Press [Conv. Int.] on the SP-10 main menu screen
		2. Press [Interrupt]
		3. Select [Return]
		4. Select [Manual] on the SP-10 main menu screen
		5. Op Mode is set to [Smear], Smpl. Tube is set to [Closed]
		6. Input Specimen information, Sample ID, HCT, select number of slides to be made
		7. Thoroughly mix the sample and place in 10th rack position
		8. Place the rack so that the sample aligns with the tube gripper and that the left end of the rack fits the label on the sampler
		9. Select [Start]
			+ 1. Analysis will begin
				2. When the tube is returned to the rack, remove the rack

 10. Press [Return] [Conv. Int.] [Stop Int.]* + - 1. Remove the rack when sampling is complete.

Return Setting to SMEAR + STAININGPress **[Settings], [Select], [Cond.], [Mode], [Smr + Sta.]**.Press **[RETURN]** and **[YES]**. **Note:** *If setting is left at* ***Smear****, the system will perform smear only in all modes.** 1. **Shutdown – performed daily**
		1. CELLCLEAN AUTO is used to shut down the entire system. Refer to the XN-3000 *Instructions for Use* for detailed, illustrated procedures.
			1. Confirm analyzers, sampler unit and SP-10 are at ready.
			2. Confirm tube holders are retracted into the analyzers.
			3. Obtain 2 empty racks
				1. Place one tube of CELLCLEAN AUTO in rack one, position 8. This rack will shut down the SP-10.
				2. Place 2 tubes of CELLCLEAN AUTO in rack two, positions 9 and 10. This rack will shut down the XNs.
			4. Place racks on sampler unit, sampler unit will auto-start.
			5. XN on-board maintenance history will auto-populate
			6. Document shutdown on the SP maintenance log.
	2. **Maintenance**

Document all maintenance procedures on the appropriate log sheet for the SP-10. Maintenance performed on the XN will be automatically tracked in the maintenance history.* + 1. SP-10

a. Daily* + - * 1. Clean Spreader Glass: Power must be on to perform this maintenance – may be performed prior to Shutdown, or after Start-up.

Press **[Maint.]** on the main screen. (Maintenance button is not available during routine operation.)Press **[Spreader Glass]** and the “Spreader Glass Replace” screen displays.Press **[OK]** to move the smear unit forward.Remove the left tower for easier access to the spreader glass.Wipe the spreader in one direction with an alcohol prep pad.Replace the tower so that the frosted end of the slides are towards the back of the analyzer.Press **[OK]** to return the smear unit to the home position.Press **[OK]** to reset the spreader glass cycle counter or **[CANCEL]** to allow the cycle count to continue.Press **[RETURN]**.* + - * 1. Clean Single Cassettes

Place cassettes in a bin with open end up.Pour methanol over the cassettes, filling them.Swish the methanol and pour off into designated container for reuse.Invert cleaned cassettes on absorbent material to dry.**Note:** *Methanol may be reused for cleaning cassettes up to three (3) times. Discard when appropriate, according to laboratory policy.* b. Weekly* 1. Perform Shutdown 2 (Weekly)
		1. Press **[SHUTDOWN]** on the main screen.
		2. Press **[Shutdown 2]** (Weekly).
		3. The shutdown screen displays the number of cassettes and amount of methanol required for the shutdown process. Ensure that required amounts are available.
		4. Place a tube of CELLCLEAN AUTO in position 10 of a Sysmex rack.
		5. Place the rack so that the tube is lined up with tube gripper.
		6. Press **[OK].**
		7. When the process completes, the SP-10 turns off automatically.
		8. To restart the SP-10, press the green button on the right side.
	2. Clean DI water/Buffer containers
		1. If re-usable containers for deionized water and/or buffer are used, empty weekly.
		2. Fill new container with fresh deionized water or buffer. Remove the spout kit from the old container and put directly into the fresh reagent.
		3. Rinse old container with methanol and allow to dry, cover any openings with caps or parafilm and store for future use.

 c. Monthly  a. Perform Super Clean Procedure  [HEM-17.2-Sysmex-SP-10-Slide-Maker-Stainer-Super-Clean-Procedure.pdf](http://intranet.childrensmn.org/References/labsop/heme/heme/hem-17.2-sysmex-sp-10-slide-maker-stainer-super-clean-procedure.pdf) d. As Needed MaintenanceRefer to the XN-3000 *Instructions for Use* for detailed and illustrated instructions for performing as needed maintenance. **A. Resulting on line in Sunquest** Function: OEM <CR> Device: XNM (Mpls) or XNS (SP) <CR> Test-1: <CR> Workload: <CR> Start at Cup: <CR> Waiting (ENTER TO EXIT ‘OE’) As results cross the interface, the accession number will appear. 1. Review data on Main Sample Explorer screen; **a. Positive/Negative, Validation result**  A positive result is displayed with a red background and a negative result is displayed with a green background. Positive results will indicate is the result was due to an abnormal blood cell differential value [Diff.], abnormal cell morphology [Morph.], or abnormal blood cell count [Count]. A negative result is displayed with a green background if the sample has no errors, these samples should auto file into Sunquest. **b. Action result** Nothing is displayed if there are no action messages. If there is an action message it is displayed on a red background. The following are Action messages; [Check] There may be a mix up of samples. Otherwise, there is a significant difference in the analysis results. Check the sample. [Review] Channel difference has occurred. Check the analysis results. [Retest] Check the analysis mode, the order and status of the sample, then reanalyze. **c. Error result**  Nothing is displayed if there are no errors. If there is an error message it is displayed on a red background. The following are Error messages; [Func.] An analysis error other than the ID barcode error or [Result] has occurred. [Result] One of the following errors has occurred; [Blood cannot be aspirated], [Insufficient blood volume], [Low count error]. **d. Rule result**Nothing is displayed if there are no samples. Details of the comment are displayed in the [Error /Rule Comments] field in the analysis data pane. The following are Rule/Result comments; [Repeat] The analysis must be repeated due to an error in the first test. [Rerun] Analysis must be repeated for the same item in the first test. The analyzer to t=be used for reanalysis is displayed on the right [Different],[Same], [Any]. [Reflex] Due to results from the first test, analysis must be performed with additional items. The discrete test to be added is displayed on the right. 2. A manual differential is performed with one or more of the following; WBC >25.0 x10³ PMNs <10% or >90% Lymphocytes >80% Monocytes >15% Eosinophils >15% Basophils >3.5% Imm. Gran. (IG) >5.0% Flags (any of the following); WBC Abn Scattergram\*\* \*\* This flag may indicate an invalid differential or invalid WBC count.  Review the WNR scatterplot;  If there are NRBC’s present they should be clearly defined by a cluster of “purple” scatter  to the left of the WBC population. A large blue cluster to the left or a blending of two blue clusters on the scatter may indicate the presence of NRBC’s that were not recognized by  the analyzer. If this is the case perform the following;  Reject the WBC, NRBC%, and NRBCA count in Sunquest and perform a manual  differential in DFW keyboard. In MEM, worksheet CBC, test WBC enter the following; WBC count from XN NRBCA from the XN (may be zero) NRBC counted (from differential) Return to NRBCA (default) keyboard, accept differential which should be in HOLD file, charge for differential (i.e. CBCC). Corrected WBC count will now be reported in Sunquest with the comment “Adjusted for Nucleated RBC’s”.  If the WNR is as expected, with two clearly defined populations the WBC can be accepted  from the XN. The differential will be performed manually.  Automated ANC’s for HOC locations should NOT be reported in these situations. Enter  results as HIDE in Sunquest.   IG Present? Left Shift? Atypical Lympho? Blasts/Abn Lympho?  3. A slide review is performed (Auto Diff in hold) with one or more of the following: MCV <70fl or >100fl, confirm and report the presence of  microcytes/macrocytes MCHC >37.5 gm/dl, check for spherocytes or cold agglutinins RDW >18.0% CV, confirm and report amount of anisocytosis Present PLTC <50.0 or >1,000.0 confirm

|  |  |  |
| --- | --- | --- |
| Step | Action | Related Document |
| 1 | ABC Results:a. If acceptable, press A, <CR>b. If a parameter fails delta, check for transfusion, sample integrity, IV placement, etc., take appropriate actiond. If a parameter fails verify:•Check for previous results and/or critical• Call critical to appropriate nurse or physician when  observed for the first time or when result becomes  critical again, see Critical Values for Laboratory Tests procedure. [Critical Values for Laboratory Tests](https://www.childrensmn.org/departments/lab/pdf/critical-values-and-lab-result-read-back-policy.pdf)  |  |
| 2 | ADIF Resultsa. After ABC is acceptedPENDING TRANSACTIONS, PLEASE WAIT appears onthe screenBilling CBCA (bills an automated diff)The differential % results appearCell Count Absolute (ANC) appearsOther Test DTYP (diff type) appears as AUTO FILE (Y/H/N) ?b. Review the Sysmex printout for flags and abnormal scatterplots.c. Diff acceptable? Press Y (yes).Diff is redisplayed with (A)ccept, (M)odify, or (R)eject.[second chance to review and reject the automated diff ifappropriate].Press A.d. Diff fails criteria? Press N (no).e. Diff requires slide review? Press H (hold).  |  |
| 3 | a. Print the failed or review sample printouts to the differential bench.b. Bill samples that require manual differential or slide review at the differential bench. |  |

A. XN-Series Manufacturer stated linearity \*

|  |  |  |
| --- | --- | --- |
| Parameter | Range | Units |
| WBC | 0 - 440.0 | x 10³/ul |
| RBC | 0 - 8.60 | x 10⁶/ul |
| HGB | 0 - 26.0 | g/dl |
| HCT | 0 - 75.0 | % |
| PLTC,PLTC-F | 0 - 5,000 | x 10³/ul |
| RET% | 0 - 30.0 | % |
| NRBC% | 0 - 600 | /100 WBC |
| WBC-BF | 0.003 - 10.0 | x 10³/ul |
| RBC-BF | 0.002 – 5.00 | x 10⁶/ul |

\* Linearity has been validated for each analyzer.1. Parameters that exceed these limits are flagged with @ beside the result. The sample must be  Diluted with Cell Pack DCL, rerun and multiplied by the dilution factor.2. Note the use of a dilution for linearity on the patient report.B. Possible Sample Interferences1. Specimens must be free of clots and fibrin strands.
2. Marked changes in plasma constituents, (e.g., low sodium, extremely elevated glucose) may cause cells to swell or shrink. The blood to anticoagulant ratio is important.
3. Red cell fragments, microcytic RBC's, or white cell cytoplasmic fragments may interfere with automated platelet counts. A fluorescent platelet may be performed to avoid this interference.
4. Cold agglutinins produce spurious macrocytosis, elevated MCH's MCHC's, falsely decreased RBC counts and HCT's. Rare, warm agglutinins produce the same spurious results as a cold agglutinin.
5. Extremely elevated WBCs may cause turbidity and falsely increase the hemoglobin, in addition to RBC and HCT values.
6. Severely hemolyzed samples (*in vitro*) falsely decrease RBC and hematocrit. Recollect hemolyzed specimens.
7. Giant platelets and clumped platelets may falsely elevate the WBC count and falsely decrease the platelet count. Platelet clumping and/or "platelet satellitism" can occur in specimens collected in EDTA. This may falsely elevate the WBC count and falsely decrease the platelet count.
8. Extremely lipemic samples may falsely elevate HGB and result in a markedly increased MCHC. To correct HGB perform Saline Replacement procedure.
9. Severely icteric samples may falsely elevate the HGB value and related indices. Make a 1:5 dilution with Cell Pack DCL.
10. Rocking specimen excessively, may affect the WBC differential.
11. Megakaryocytes may falsely increase WBC counts on automated hematology analyzers.
12. Erythrocyte aggregation (cold agglutinin), giant platelets, possibility of platelet

clumps, fragmented leukocytes, Malaria, Howell-Jolly bodies may interfere withReticulocyte counts.A. White Blood Cell Count 1. Invalid WBC counts should be checked against a Wright’s stained smear. 2. For counts above the linear range, dilute 1:2 with Cell Pack DCL, multiply result By the appropriate dilution factor. 3. Extremely elevated WBCs may cause turbidity and falsely increase the hemoglobin, in  addition to RBC and HCT values. 4. Patients that are from hospital location HOC should have all automated ANC counts  reported. ANC counts with asterisks (\*) should be entered in MEM. Another ANC  will be calculated from the manual differential (ANM).  5. For WBC counts < 0.6 automated diffs are reported to HOC physicians, DO NOT take the smear to the microscope for review. 6. For WBC counts < 0.1 differentials are not reported, the patient is charged for ABCO only by Sunquest. Result CBC in OEM with two “yes” answers ( see example below):  M78077 (3514) DTYP :AUTD Auto CREDITING AND RESULTING BASED ON CONDITIONS (ADD) TEST-1: DTYP2-OBL Orders for dept: General Lab Test(s): CBC ABCO-OBL DTYP2-OBL ACC. NO: M78077 TEST-1: DTYP2 ‘HIS’ ORDER NO.: C643084-0  TEST-1 ADIF DIFFERENTIAL  ORDERED AS PART OF PACKAGE CBC RESULTS FILED. DELETE ?Y  CREDIT TEST REQUEST B. Red Blood Cell Count 1. For counts above the linear range, dilute 1:2 with Cell Pack DCL. 2. RBCs may be spuriously decreased due to cold agglutinins (MCHC >37.5); warm the  Sample for 10 minutes at 37°C, rerun immediately. 3. Check that the other RBC parameters agree by the “Rule of 3” • 3 x RBC = Hgb +/- 3 • 3 x Hgb = Hct +/- 3C. Hemoglobin 1. Result should agree with the RBC and Hct. 2. If Hgb is to high (MCHC >37.5), run sample on the Hemocue, this will correct for lipemia and  elevated WBC counts. 3. For Hgb’s above the linear range, dilute 1:2 with Cell Pack DCL, multiply result by the appropriate dilution factor. 4. Results should agree with previous values +/- 1.0 g/dl per 24 hrs; check for known bleeding problem, dehydrated patient receiving IV fluids, post surgery, hematology/ oncology patients. D. MCV 1. MCV should agree with previous result +/- 2.0 unless patient has been transfused. 2. MCV may be spuriously high due to cold agglutinins, warm 10 minutes at 37°C, rerun.E. MCHC 1. MCHC >37.5 except in cases of spherocytosis or occasionally sickle cell anemia, Check for lipemia or cold agglutinins. Samples that have extremely high cold agglutinin titers or excessive amounts  of lipemia may require plasma replacement. Perform the following procedure if  the hemoglobin does not correct completely on the Hemocue; **Plasma Replacement Technique**a. Aliquot a portion of the EDTA whole blood into a labeled tube. b. Spin the aliquoted sample in the centrifuge. c. Remove a measured amount of plasma and discard. d. Add an equal volume of warmed Cell Pack DCL  ( repeat steps b and c if still grossly lipemic ). e. Run the well mixed sample on the analyzer, label as “plasma replaced result”. f. Compare the RBC count with the original sample to verify proper dilution, the result should be within a 5%: Original RBC – Replaced RBC Original RBC x 100 = % Difference g. WBC and PLTC should be taken from the original result. h. RBC,HGB,HCT,MCV,MCH,MCHC and RDW should be taken from the  plasma replaced results. Enter corrected results in OEM at the (A)ccept, (M)odify, (R)eject prompt by typing M – specific tests(s). Example; M-HGB, MCH, MCHC  Further warming post plasma replacement may also be necessary.  2. MCHC <32.0 with high RBC and low MCV suggests microcytic anemia, check slide, report.  3. MCHC continuously <32.0 or >37.5 on sequential normal patients suggests an instrument  malfunction, check controls, perform troubleshooting.F. Platelet count 1. Perform slide review on platelet counts that are <50 x 10³. 2. For counts above the linear range, dilute 1:2 with Cell Pack DCL. 3. Checks with previous counts should agree +/- 15% unless patient is bleeding, is in DIC, has a platelet antibody or has been transfused. 4. Routine platelet counts are performed by impedance methodology. When necessary platelet counts will be performed by fluorescence using a nucleic acid stain specific for  platelet organelles and flow cytometry.  With this methodology an Immature Platelet Fraction (IPF) is also available. The IPF indicates the ratio of immature platelets to the total number of platelets in the patients peripheral blood. These immature platelets, newly released from the bone marrow, may contain increased amounts of cytoplasmic RNA which allows them to be differentiated from mature platelets. The reticulated platelet count has been used by clinicians as a measure of thrombopoietic activity of the bone marrow. The following are situations when a fluorescent platelet (PLT-F) count is performed; a. Abnormal, PLT Abn Distribution b. Suspect, PLT Clumps? c. Any routine count <100 x 10³ d. Abnormal, PLT Abn Scattergram\* e. Difference between PLT and PLT-F. Check the results\* \* this message only occurs on platelet counts run as PLT-F. The persistence of asterisks, hyphens or invalid messages requires that a slide review is  performed. Scan the peripheral smear to estimate the platelet count and review for the  presence of abnormal morphology such as: • large or giant platelets • small platelets • platelet clumps • fragmented RBCs • microcytic RBCs • parasites  If the platelet estimate confirms the accuracy of the analyzer count it may be reported. If the platelet estimate does not confirm the accuracy of the analyzer count It may be necessary to confirm it with an alternate method such as a manual platelet count.G. Reticulocyte Count 1. For Reticulocyte counts above the linear range, dilute 1:2 with Cell Pack DCL, multiply result  by the appropriate dilution factor. 2. Abnormal, RET Abn Scattergram. This flag is generated when the analyzer has detected Increased activity in t he RET-UPP ( Upper Particle Plateau ) area on the RET-EXT scattergram. This could be due to the presence of NRBCs, Howell-Jolly Bodies or stress reticulocytes. These are not included in the reticulocyte count. Asterisks appear next to the  reticulocyte parameters. Prepare a 1:5 dilution with Cell Pack DCL and run in the manual mode. If the flag is  eliminated multiply the absolute reticulocyte count by 5 and report the other parameters ( Ret%, IRF, RET- H*e* ) as they are ( no dilution factor necessary ). If the flag is not eliminated It may be necessary to confirm it with an alternate method such as a manual reticulocyte count 3. The RET- H*e* which is a measure of the hemoglobin content of the reticulocyte is  also performed with a reticulocyte count.1. Sysmex XN-3000 *Instructions for Use* (North American Edition), Sysmex Corporation, Kobe, Japan.
2. Sysmex XN series *Administrator’s Guide* (North American Edition), Sysmex Corporation, Kobe, Japan
3. Sysmex SP-10 *Instructions for Use* (North American Edition), Sysmex Corporation, Kobe, Japan.
4. Clinical and Laboratory Standards Institute (CLSI). Laboratory Documents: Development and Control; Approved Guideline; Fifth Edition. (GP2-A5, 2006).
5. Sysmex America Inc., Lincolnshire, IL. XN CAL, XN CAL PF Hematology Calibrators: Calibrators for Sysmex Hematology XN-Series Analyzers, package insert.
6. Sysmex America Inc., Lincolnshire, IL. XN CHECK Hematology Control for Sysmex XN-Series Analyzers package insert.
7. Sysmex America Inc., Mundelein, IL. Sysmex ***Insight***Participant Overview Guide.
8. Koepke, John. *Practical Laboratory Hematology.* Churchill Livingstone Inc. 1991.

p. 24-25, 36-39.1. Cornbleet J., *Spurious results from automated hematology cell counters. Lab Medicine.* 1983;8:509-514.
2. Sysmex Reagents of America, Inc. MSDS sheets and reagent product inserts.
3. College of American Pathologists (CAP) Hematology-Coagulation Checklist, July 2012.
4. Stewart, Charles and Koepke, John.  *Basic Quality Assurance Practices for Clinical Laboratories*, Van Nostrand Reinhold, 1989, p 189.
5. Gulati GL, Asselta A, Chen C. *Using vortex to disaggregate platelet clumps*, Laboratory Medicine, 28:665, 1997.
6. Zhou X, Xiaoli W. *Amikacin Can Be Added to Blood to Reduce the Fall in Platelet Count*, American Journal of Clinical Pathology, 136:646-652, 2011.
7. Flagging Interpretation Guide

Document number 1166-LSS, Rev. 2 August 2016 [Sysmex Flagging Interpretation Guide](http://khan.childrensmn.org/Manuals/Lab/SOP/Heme/Res/212364.pdf) 16. Immature Granulocytes (IG) Frequently Asked Questions  1215-MKT, Rev. 2, February 2016 [Immature Granulocytes FAQ](http://khan.childrensmn.org/Manuals/Lab/SOP/Heme/Res/212365.pdf) 17. Reticulocyte Hemoglobin (RET-He) Frequently Asked Questions 1216-MKT, Rev. 2, February 2016 [Reticulocyte Hemoglobin RET- He FAQ](http://khan.childrensmn.org/Manuals/Lab/SOP/Heme/Res/212367.pdf) 18. Immature Platelet Fraction (IPF) Frequently Asked Questions 1217-MKT, Rev. 2, February 2016 [Immature Platelet Fraction FAQ](http://khan.childrensmn.org/Manuals/Lab/SOP/Heme/Res/212366.pdf) 19. Sysmex Customer Resource Center (CRC) <https://www.sysmex.com/us/en/Pages/Registration.aspx> Mpls. Site ID 10685 St. Paul Site ID 12271 20. Sysmex Quick Guide [Sysmex XN 3000 Quick Guide](http://khan.childrensmn.org/Manuals/Lab/SOP/Heme/Res/212369.pdf) 21. Sysmex Advanced Clinical Parameters,  Clinicians Frequently Asked Questions [Advanced Clinical Parameters FAQ - Clinicians](http://khan.childrensmn.org/Manuals/Lab/SOP/Heme/Res/212368.pdf)   |
| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Al Quigley | 12/13/16 | Initial Version |
|  | 2 | Al Quigley | 9/1/17 | Added weekly disinfecting and replacement of water container on SP-10. Added monthly Super Clean procedure for SP-10. |