CHRISTUS SPOHN Health System

CHRISTUS Spohn Laboratory
Clinical Laboratory – Policy and Procedure
Complete Blood Count: Whole Blood on the Sysmex XN-3100, XN-2000 and XN-1000 Automated Hematology Analyzer
RH-0010

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Department:	Hematology	**Effective Date:**	5/7/2019
Policy, Procedure, or Both:	Policy &Procedure	**Revision Date(s):**	12/29/2021
Author:	K. Clark, R.J. Bernshausen	**Version:**	3.0

Applicable Standards	
Standard	Organization
HEM.22000	CAP
HEM.22050	CAP
HEM.22150	CAP
HEM.22200	CAP
HEM.22625	CAP
HEM.25700	CAP
HEM.25760	CAP
HEM.25780	CAP
HEM.25850	CAP
HEM. 33519	CAP
HEM. 35338	CAP
HEM. 35340	CAP
HEM. 35347	CAP
HEM. 35376	
HEM. 35395	
Related Documents	

Version History		
Version	Effective Date	Retired Date
1.0	5/7/2019	12/29/2021
2.0	12/29/2021	

Review History (Up to the Last 15 Occurrences)			
Date	Version	Revision Type	Review By
12/29/2021	2.0	Minor changes	
05/16/23	3.0	Major changes	

Distribution	
Spohn Beeville	Spohn Shoreline
Spohn Kleberg	Spohn South
	Spohn Alice

This policy is followed at the following respective sites:

- *Spohn Alice Hospital*
- *Spohn Beeville Hospital*
- *Spohn Kleberg Hospital*
- *Spohn Shoreline Hospital*
- *Spohn South Hospital*

Principle:

The Sysmex XN-3100 is an integrated system that incorporates two hematology analytical modules as well as an automated Slide maker/Stainer. The XN-2000 consists of two hematology analytical modules.

The analytical module (XN-10) is a quantitative automated hematology analyzer for *in vitro* diagnostic use in determining 31 whole blood diagnostic parameters and 7 body fluid diagnostic parameters. Examination of the numerical and/or morphological findings of the complete blood count by the physician is useful in the diagnosis of disease states such as anemias, leukemias, allergic reactions, viral, bacterial, and parasitic infections.

The analyzer performs hematology analysis according to the hydrodynamic focusing (DC Detection), flow cytometry method (semiconductor laser), and SLS-hemoglobin method.

The device counts and sizes red blood cells (RBC) and platelets (PLT) using electronic resistance detection. Hematocrit (HCT) is measured as a ratio of the total RBC volume to whole blood using cumulative pulse height detection. Hemoglobin (HGB) is converted to SLS-hemoglobin and read photometrically.

The white blood cell (WBC) count, differential (DIFF), reticulocytes (RET) nucleated red blood cells (NRBC) and fluorescent platelets (PLT-F) 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 the cell interior such as the size of the nucleus. Lateral fluorescent 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 staining. Fluorescent light is emitted in all directions. The XN detects the fluorescent light that is emitted sideways.

The Sysmex SP-50 is a fully automated hematology slide preparation and staining system. Whole blood specimens are mixed and aspirated, and a wedge type blood smear is prepared applying hematocrit information from the host computer (if available) to determine optimum smearing criteria. Prepared and labeled smears are shuttled to stain area where they will then be transferred through the various sections to be stained, rinsed, and then dried. The intervals within each section of the

staining process are laboratory defined. Completed smears from staining process are placed into slide magazines ready for review. *(Shoreline and South campus only).*

The system also provides a manual mode smear and stain operation where sample volumes and/or tubes that do not meet requirements for rack operations can be placed in the manual position of the instrument to be aspirated for smear and stain process. In addition, the system also allows for pre-made smears to be routed to the staining process. The unit is self-monitoring and alarms sound when operation is interrupted.

Slides prepared by the Sysmex SP-50 are used for differentiation and morphologic evaluation of cellular elements of whole blood.

Specimen Requirements:

Peripheral Blood

- Whole blood is to be collected in EDTA-2K or EDTA-3K anticoagulant
  NOTE: Smear preparation on specimens older than 4 hours may exhibit a loss of cellular integrity. Please follow laboratory protocol for smear preparation and review.

Specimen volumes required:

- Optimal draw is a 12 x 75 tube filled to capacity. A minimum of 1 mL of whole blood is required for sample analysis
- Manual analysis whole blood mode – XN-3100 & XN-2000
  1. Closed tube – 1 mL minimum sample volume, 88 µL is aspirated
  2. Open tube – 300 µL minimum sample volume, 88 µL is aspirated
  3. Open micro tube – 160 µL minimum sample volume, 88 µL is aspirated
  4. RBT (Raised B - 250µ minimum sample volume, 88µ is aspirated
- Manual analysis – SP-50
  1. Closed tube smear and staining – 500 µL minimum sample volume, 70 µL is aspirated.
  2. Open tube smear and staining - 300 µL minimum sample volume, 70 µL is aspirated
  3. Raised bottom tube - 250 µL minimum sample volume, 70 µL is aspirated Open micro tube - 110 µL minimum sample volume, 38 µL is aspirated

Unacceptable specimens:

The following specimens listed below should be rejected:

1. 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.
2. Grossly hemolyzed samples
3. Samples drawn above an IV line

Characteristics that may affect test results

- Lipemia
- Icterus
- Cold agglutinins.

Stored Specimen Stability:

- Stored at 4-8°C, EDTA blood samples with normal results may be analyzed up to 48 hours without significant loss of differential stability.
- 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.

- Allow refrigerated samples to come to room temperature and mix well before analysis.

  NOTE: *Do not place CBC and Diff samples on a mechanical rocker. Constant rocking may alter white cell membranes, resulting in false interpretive messages.*

Supplies and reagents:

Supplies:

1. CELLCLEAN® AUTO
2. Sysmex reagents
3. Commercial controls; XN CHECK™, XN CHECK™ BF

Sysmex Reagents:

1. Sysmex reagents and CELLCLEAN AUTO are used on the Sysmex XN-Series modules.
2. All reagents are used at room temperature and are to be used within the manufacturer's expiration date on each container.
3. Record date received and date opened on container.
4. All reagents are azide free and are intended for *in vitro* diagnostic use only. Do not ingest.
5. See tables below for reagent stability, storage and usage.

Storage and Stability:

Reagent	Storage		Open exp
Cellpack DCL	2-35°C	if frozen, thaw and mix before use, discard if cloudy	60 days
Cellpack DST	2-35°C		60 days
Cellpack DFL	2-35°C	discard if cloudy or discolored	60 days
Lysercell WNR	2-35°C	all to equilibrate to 15-30 degrees prior to use, do not use if discolored or colored or suspected as being frozen	60 days
Lysercell WDF	2-35°C	all to equilibrate to 15-30 degrees prior to use, do not use if discolored or colored or suspected as being frozen	90 days
Fluorocell WNR	2-35°C	do not use if item has been frozen	90 days
Fluorocell WDF	2-35°C	do not use if item has been frozen	90 days
Fluorocell RET	2-35°C	do not use if item has been frozen	90 days
Flurocell Plt	2-35°C	do not use if item has been frozen	90 days
Sulfolyser	1-30°C	discard if cloudy or discolored	60 days
CellClean Auto	1-25°C		discard after use
XN CHECK controls	2-8°C	do not use if item has been frozen or exposed to excessive heat.	7 days refrig.
XN CAL	2-8°C	do not use if item has been frozen or exposed to excessive heat.	4 hours

Reagent	Reagent Usage
Cellpack DCL	Whole blood diluent (XN) used to rinse spreader, glass sample pipette and piercer (SP-50)
Cellpack DST	Concentrated version of DCL.
Cellpack DFL, Fluorocell RET, Fluorocell PLT	DFL Combines with Fluorcell RET for reticulocyte analysis or Fluorcell PLT for analysis of Platelets by flow cytometry
Lysercell WNR, Fluorocell WNR	WNR Lysercell combines with Fluorcell WNR to hemolyze red cells and non-basophil WBC, basophils and NRBC by flow cytometry.
Lysercell WDF, Fluorocell WDF	WDF Lysercell combines with Fluorcell WDF to hemolyze red cells and stain the WBC so that counts and percentages of neutrophils, immature granulocytes, lymphocytes, monocytes and eosinophils are analyzed by flow cytometry.
Sulfolyser	Lyses RBC to release hemoglobin for measurement.
Cleancell Auto	Strong alkaline detergent is used to remove lysing reagents, cellular residuals and blood proteins remaining in the hydraulics of the analyzer.
XN CHECK controls	Three levels of controls are used to monitor performance of the XN analyzers for RBC and associated indices, WBC and leukocyte differential (both % and #), Platelets and reticulocyte count.
XN CAL	Used to calibrate WBC, RBC, HGB, HCT, PLT and RET

Document all reagent changes on the appropriate log.

Calibration and Precision

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 Field Service Representative.

Calibration verification may be performed by reviewing and documentation of commercial control and X-BarM QC data, proficiency testing results and patient control testing results. The operator may calibrate the following parameters using XN CAL and XN CAL PF calibrator: WBC, RBC, HGB, HCT, PLT, PLT-F and RET.

Before calibration, ensure that the XN is both clean and precise.

1. Precision Check

a. Perform routine maintenance on the analyzer and perform a background count to ensure counts are within acceptable limits.
b. Verify that there is sufficient volume of all reagents. Precision and Calibration procedures will be aborted if the XN runs out of reagent.
c. Obtain a sample of fresh normal whole blood. Do not use commercial controls or calibrators for precision. Note that when remote calibration is performed by Sysmex, the calibrator is used for precision testing. The blood donor specimen should:
    i. Be from a healthy person who is not taking any medication
    ii. Have morphologically and numerically normal CBC.
    iii. Be drawn in a potassium EDTA anticoagulant tube using proper collection technique.
    iv. Have a minimum of 2.5 mL of sample.
d. On the main unit, check the Status indicator LED. Confirm the LED is green indicating the analyzer is Ready
e. If the tube holder has not ejected out, press the mode switch
f. Select the Change Analysis Mode button on the control menu and select Whole Blood
g. Select [OK] to close the dialog box
h. Select the Analyzer menu button on the control menu
i. Select [Calibration] – [Precision Check]
j. Mix the vial containing the sample – 10 end-over-end inversions confirming cell button is dispersed
k. Place the vial in the sample tube holder
l. Press the start switch on the analyzer
m. The analysis is automatically performed 11 times consecutively with the tube holder pulled into the analyzer
n. The tube holder will slide out when analysis is complete
o. The results are displayed in the [Precision Check] analysis dialog box.
    i. If the analysis results do not satisfy conditions for normal results, or if results are outside acceptable limits, the test numbers of the tests that must be repeated are displayed. Select and redo the manual analysis.
p. When all analysis results satisfy the conditions, select [OK] in the dialog box.
q. Select [Yes] to record passing precision results in the precision check history.

NOTE: If an error occurs during analysis and the analysis can no longer continue, stop precision check. Once the error is cleared, redo the manual analysis.

2. Calibration – XN CAL

a. On the main unit, check the Status indicator LED. Confirm the LED is green indicating the analyzer is Ready
b. If the tube holder has not ejected out, press the mode switch
c. Select the Change Analysis Mode button on the control menu and select Whole Blood
d. Select [OK] to close the dialog box
e. Select the Analyzer menu button on the control menu
f. Select [Calibration] – [Calibrator Calibration]
g. Mix the vial containing the calibrator according to package insert
h. Place the vial in the sample tube holder
i. Press the start switch on the analyzer
    i. The analysis is automatically performed 11 times consecutively with the tube holder pulled into the analyzer

ii. The tube holder will slide out when analysis is complete

j. The results are displayed in the [Calibrator Calibration] analysis dialog box.
k. If the analysis results do not satisfy conditions for normal results, or if results are outside acceptable limits, the test numbers of the tests that must be repeated are displayed. Select and redo the manual analysis.
l. When all analysis results satisfy the conditions, select [Calibration] in the dialog box.
m. Select [OK] to display results in the [Calibrator Calibration] execution dialog box.
n. Select the check box to include the calibration parameter in the calibration exercise, clear the check box to exclude the parameter in the calibration exercise. If a parameter meets all of the following criteria, the check box will automatically be selected:
    i. 80% $\leq$ New Rate $\leq$ 120%
    ii. New Rate – Current Rate $\leq +5$
    iii. Range Value $\leq$ Max Range
    iv. Acceptable Limit $\leq$ Delta Percent $\leq$ Service Limit

    If a parameter meets all of the conditions and the Delta Percent is less than the Acceptable Limit, it is excluded from calibration as there is no need for calibration.
    If a parameter does not meet all of the conditions and the Delta Percent is greater than the Acceptable Limit, the calibration cannot be performed. Calibration is performed with the parameter excluded.
    Selecting the check box enables you to manually enter a value in [New Rate (%)]. A range of 80% to 120% may be entered.
o. Select [OK] to update the compensation rates. The calibration process is logged in the calibrator calibration history.

3. Calibration – XN CAL PF

a. On the main unit, check the Status indicator LED. Confirm the LED is green indicating the analyzer is Ready
b. If the tube holder has not ejected out, press the mode switch
c. Select the Change Analysis Mode button on the control menu and select Whole Blood
d. Select [OK] to close the dialog box
e. Select the Analyzer menu button on the control menu
f. Select [Calibration] – [Calibrator Calibration (PLT-F)]
g. Mix the vial containing the calibrator according to package insert
h. Place the vial in the sample tube holder
i. Press the start switch on the analyzer
    i. The analysis is automatically performed 11 times consecutively with the tube holder pulled into the analyzer
    ii. The tube holder will slide out when analysis is complete
j. The results are displayed in the [Calibrator Calibration (PLT-F)] analysis dialog box.
k. If the analysis results do not satisfy conditions for normal results, or if results are outside acceptable limits, the test numbers of the tests that must be repeated are displayed. Select and redo the manual analysis.
l. When all analysis results satisfy the conditions, select [Calibration] in the dialog box.
m. Select [OK] to display results in the [Calibrator Calibration (PLT-F)] execution dialog box.
    i. Select the check box to include the calibration parameter in the calibration (PLT-F) exercise, clear the check box to exclude the parameter in the calibration exercise. If the parameter meets all of the following criteria, the check box will automatically be selected:
    ii. 80% $\leq$ New Rate $\leq$ 120%
    iii. New Rate – Current Rate $\leq +5$
    iv. Range Value $\leq$ Max Range

v. Acceptable Limit ≤ Delta Percent ≤ Service Limit
vi. If the parameter meets all of the conditions and the Delta Percent is less than the Acceptable Limit, it is excluded from calibration as there is no need for calibration.
vii. If the parameter does not meet all of the conditions and the Delta Percent is greater than the Acceptable Limit, the calibration cannot be performed. Selecting the check box enables you to manually enter a value in [New Rate (%)]. A range of 80% to 120% may be entered.

n. Select [OK] to update the compensation rate. The calibration process is logged in the calibrator calibration history.

NOTE: If an error occurs during analysis and the analysis can no longer continue, stop precision check. Once the error is cleared, redo the manual analysis.

Quality Control:

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. Quality control should be run in accordance with licensing agency regulations. It should be noted that for troubleshooting purposes, additional control runs may be necessary. To QC the SP-50, examine a stained smear from the routine workload for smear and stain quality on a daily basis. Document results on appropriate log. (Shoreline and South campus only)

1. XN CHECK Commercial Controls Instructions for Use
   a. Remove vials from refrigerator and allow them to come to room temperature (18-25°C), for approximately 15 minutes.
   b. Mix vials by gentle end-to-end inversion until the cell button in the bottom of the vial is completely suspended.
2. Frequency of Control use and review
   All three levels of controls XN CHECK or equivalent will be run every 12 hours, or 2 rotating levels of controls will be run every 8 hours as determined by each facility.
   SP-50 QC slide will be evaluated daily on day shift.

   The supervisor reviews commercial and X-Bar charts every week.
3. Registering and modifying a QC file – lot information input
   a. Select [QC File] Icon
   b. Select TAB for analyzer from bottom of QC File screen
   c. Select File number to be registered.
   d. Select [Register] button on toolbar
   e. Enter lot information
      i. Material
      ii. Lot Number
      iii. Expiration Date

   f. Select [Restore]
      i. Browse XN QC Limits folder on XN-IPU Desktop
      ii. Select file for QC to be registered
      iii. Select Open. Sysmex Range Limit %'s will automatically upload to the file

   g. Repeat for each level of XN CHECK, XN CHECK BF to be registered and for each module in the XN configuration
   h. To modify an existing QC File, select the QC File and [Modify] from the toolbar. Update the Lot No, Exp. Date as appropriate.

i. Perform parallel studies between production lot and new lot prior to production lot expiration.

4. XN CHECK QC Analysis
   i. Place the vial containing control blood in the rack.
   ii. Place rack on sampler unit; sampler unit will auto-start.
   iii. Results will be plotted on the L-J Chart as well as the Radar Chart for review.

5. Auto set Targets

   a. 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 sets the targets.

      i. Select QC Chart
      ii. Select [Range] and set cursors so that every data point is included
      iii. Select [Register]
      iv. Highlight all parameters and select [Auto Setting]
      v. Confirm that the check box for TARGET ONLY is set. Do not select the check box for LIMIT.
      vi. Select [OK]; the target for each parameter will be calculated and set for the duration of the QC lot.

   b. Repeat steps for each new lot of QC being moved into production. Confirm the target set falls within the range of means provided on the XN Check assay sheet provided.

6. Reviewing Quality Control Results

   a. QC File screen

      i. Allows for review of the latest QC results in Radar Chart format for the QC file that is selected in the list.
      ii. Any point exceeding the upper or lower limit is marked with a red "X".

   b. QC Chart screen

      i. Allows for review of detailed graph data of all QC runs for selected file.
      ii. Analysis data is plotted cumulatively and displayed in the chart area as a line graph.
      iii. Any point exceeding the upper or lower limit is marked with a red "X".
      iv. Users must scroll up and down through the chart to view all parameters for each run.
      v. 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.

      - QC charts may be overlaid on top of each other for comparison.

      i. Select [Compare QC Files] to view QC charts registered to a single analyzer. This will compare the new lot with the current lot.
      ii. Select [Compare Analyzers] to compare QC files for the same material registered to different analyzers.

   c. Follow laboratory protocol for troubleshooting Quality Control results exceeding the upper or lower limit of acceptability.

i. If 3 levels of controls were run and 2 levels are within range, but the 3rd has a parameter that is > 2 SD from mean but less than 3 SD from mean, release the controls and proceed with patient testing.

ii. If only 2 levels of control were run and one level has a parameter that is out of range, or 3 levels of control were run and 2 or more levels have parameters that were out of range go to steps below.

iii. Rerun control that was out of range. If the results are acceptable, proceed with patient testing.

iv. If the repeated result was still out of range, open a new vial of control and repeat testing. If the results are acceptable, proceed with patient testing.

v. If results are not within range, contact Sysmex technical support for guidance on how to proceed.

7. Quality Control Management
   a. From the QC Chart view, select the [Manage] button on the toolbar.
   b. Specify whether a QC run should be excluded from quality control
   c. Select [Not Manage] to exclude data from the following:
      i. Statistical computations (SD, Mean, CV)
      ii. Variable target computation
      iii. Number of data points = n
   d. 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.
   e. A comment may be added to the QC data selected by the cursor
      i. Select [Input Any Comment] to input a free text comment.
      ii. Select [Fixed Comments] to use a comment from a list of preset comments in the QC settings menu.
      iii. Select [OK]
      iv. A comment bubble will be displayed when a comment exists for a QC run.
      v. The comment will be visible in the comment display area when the cursor is placed on the QC run.

8. *Insight*™ Quality Assurance Program (QAP) (The *Insight program* is for XN analyzers only)
   a. Each lot has 2 data submission dates, approximately every 30 days for the 84-day dated product.
   b. Data may be managed in the XN-IPU and/or in *Insight*. See *Insight* User Manuals.
   c. The XN IPU's of all CHRISTUS Spohn Laboratories are connected to Sysmex via the internet.
   d. At a networked PC, establish connection with the *Insight* program via www.sysmex.com/us and submit the data. Contact the *Insight* team with questions at: 1-888-879-7639 (1-888-8SYSMEX).

9. X-barM Moving Patient Averages. See policy/procedure for X-barM

Operating Procedure

1. Start-Up Procedure
   a. Checks prior to turning on
   b. Place completed samples into final storage area for the lab
      i. Remove any items that may interfere with operations.

ii. Gather and re-locate all empty racks to designated processing or sample loading area.
iii. If applicable, verify waste container is empty.
iv. Verify network/host communications are properly working.
v. Verify sufficient reagent supply.
vi. Ensure that all power switches are in the "on" position.

c. Press and release the green master switch on the XN-3100 & XN-2000 sampler unit.
d. The status indicator LED will flash green.
e. The XN-IPU will automatically turn on.
f. The SP-50 will begin start-up (if present).
g. Enter the username and password on the IPU keyboard. The username admin and password m116m gives full user access.
h. Each XN analyzer will begin start-up
i. The XN screen will display the logon
j. Analyzer self-checks
   i. Initialization of the mechanical parts;
   ii. Rinsing of the hydraulic units
   iii. Temperature stabilization
   iv. Background Check (up to 3 times)

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

k. Analyze Quality Control Material

2. Patient Sample Processing

System Analysis (sampler analysis)

a. Make sure the analyzer and the sampler are in READY state
b. Check that tube holder has retracted into the analyzer, press mode button if necessary
c. Place sample(s) in rack(s) in right sampler pool (analyzer side)
d. Rack(s) will auto-start.
e. Samples will run, results will be displayed in the IPU.
f. On-Board rules engine will determine repeat or reflex testing
g. The rack will run in reverse to perform repeat or reflex testing.
h. Remove the rack from the left sampler pool when analysis is completed.

Manual Analysis - XN

a. Check the status of the analyzer. Confirm the analyzer is ready.
b. Press the mode switch to eject the tube holder.
c. Select the Change Analysis Mode button on the control menu
d. Select analysis mode
   i. [Whole blood] is selected when whole blood is being analyzed
   ii. [Low WBC] Select this to perform low WBC analysis on whole blood
   iii. [Pre-Dilution] select when running 1:7 pre-diluted blood.

e. Select [OK]
f. Select Manual Analysis button on the control menu
g. Input sample ID or select [Read ID]
h. Properly mix the specimen and place in the front tube holder
    i. If running microtainer, remove the cap using caution to avoid splattering, select CAP OFF, and place in the rear tube holder
    ii. If running an RBT [Raised bottom tube] select the [Raised Bottom Tube] radio button and place specimen in the front tube holder with *CAP ON*
i. Select OK
j. Press the start switch on the analyzer
    i. The tube holder will slide in, and the sample will be aspirated
    ii. When the analysis is complete, the tube holder slides out
k. Remove the sample, repeat steps for additional samples
l. Review results in IPU to determine whether repeat or reflex testing was performed, or smear review is required.

Maintenance:

- Daily:

Shutdown: Shutdown can be performed either in the Sampler Mode or Manual Mode. Shutdown can also be performed on the entire system or on individual analyzers if the laboratory desires to always have one analyzer available.

1. Shutdown entire system – Sampler Mode

    a. Confirm analyzers, sampler unit is ready.
    b. Confirm tube holders are retracted into the analyzers.
    c. Obtain 2 empty racks. Place 2 tubes of CELLCLEAN AUTO in rack two, positions 9 and 10. This rack will shut down the XNs.
    d. Load the racks onto the right sampler pool (analyzer side).
    e. Shutdown is performed automatically.
    f. The analyzers and the IPU will automatically power off once the Shutdown sequence is complete (approximately 15 minutes).
    g. Remove the rubes of CELLCLEAN AUTO from the racks.

2. Daily Cleaning – Manual Mode - XN Analyzers only
    a. Daily "Cleaning" can be used as an alternative to the daily "Shutdown" procedure to keep one analyzer up and running at all times and to allow for rack flow to the alternate analyzer.
    b. Make sure the analyzer is in the "Ready" state.
    c. Click the analyzer menu button.
    d. Select "Maintenance".
    e. Select "Cleaning".
    f. The tube holder will slide out.
    g. Place a vial of CELLCLEAN AUTO in the sample tube holder.
    h. Press the blue start switch.
    i. Remove the tube of CELLCLEAN AUTO from the rack and discard.

3. Empty waste container (if applicable)
    Empty the waste container daily or when the HELP dialog box appears [Waste containers full]
    a. Prepare an empty waste fluid tank and remove the cap

b. Remove the cap from the waste fluid tank that has become full. Pull the float switch and tubing from the bottle
c. Insert the float switch and tubing into the new waste fluid tank
d. Secure the cap
e. If the HELP dialog box appeared, touch [Execute]. The error will clear, and the dialog box will close

Procedural Notes and Calculations:

1. If making a dilution of a patient specimen and running in XN Whole Blood mode, multiply the parameters by the dilution factor
2. If correcting the HGB or HCT due to interfering substances, recalculate and correct the affected indices:
   a. MCHC = HGB / HCT x 100
   b. MCH = HGB / RBC x 10
   c. MCV = HCT / RBC x 10
3. Current on-board rules should be exported and saved on an external storage device. A printout of the rules should be inserted in the XN-Series Resource Manual.
4. Do not place samples on a mechanical rocker. Excessive mixing may alter white cell membranes resulting in false interpretive messages.
5. For troubleshooting specifics refer to the Sysmex XN-3100 & XN-2000 *Instructions for Use*

Reporting Results:

Reference Ranges are reported with every result. Please refer to the table below:

ANALYTE	AGE	NORMAL RANGE BOTH SEXS	MALE	FEMALE	UNITS
S					
WBC	< 1 DAY	9.0-30.0			X10~3 uL
	1D-6D	9.0-34.0			X10~3 uL
	7D-13D	5.0-21.0			X10~3 uL
	14D-29D	5.0-20.0			X10~3 uL
	1M-11M	5.0-19.5			X10~3 uL
	1YR-1YR 11M	6.0-17.5			X10~3 uL
	2YR-3YR 11M	6.0-17.0			X10~3 uL
	4YR-5YR 11M	5.5-15.5			X10~3 uL
	6YR-7YR 11MO	5.5-14.5			X10~3 uL

	8YR-15YR 11MO	4.5-13.5			X10~3 uL
	16YR-17YR 11MO	4.5-13.0			X10~3 uL
	ADULT	4.8-10.8			X10~3 uL
RBC	0-2 DAYS	3.90-5.50			X10~6/uL
	3-6DAYS	4.00-6.60			X10~6/uL
	7-13 DAYS	3.90-6.30			X10~6/uL
	14-29 DAYS	3.60-6.20			X10~6/uL
	1MO - 59 DAYS	3.00-5.40			X10~6/uL
	2MO-5MO	2.70-4.90			X10~6/uL
	6MO-23MO	3.70-5.30			X10~6/uL
	2YR-5YR	3.90-5.30			X10~6/uL
	6YR-11YR	4.00-5.20			X10~6/uL
	12YR-17YR		4.50-5.30	4.10-5.00	X10~6/uL
	18-20		4.50-5.20	4.00-5.20	X10~6/uL
	ADULT		4.70-6.10	4.20-5.40	X10~6/uL
HGB	0-2 DAYS	13.5-19.5			g/dL
	3-6DAYS	14.5-22.5			g/dL
	7-13 DAYS	13.5-21.5			g/dL
	14-29 DAYS	12.5-20.5			g/dL
	1MO - 59 DAYS	10.0-18.0			g/dL
	2MO-5MO	9.0-14.0			g/dL
	6MO-23MO	10.5-14.5			g/dL

	2YR-5YR	11.5-13.5			g/dL
	6YR-11YR	11.5-15.5			g/dL
	12YR-17YR		13.0-16.0	12.0-16.0	g/dL
	18-20		13.5-17.5	12.0-16.0	g/dL
	ADULT		14.0-18.0	12.0-16.0	g/dL
HCT	0-2 DAYS	42-60			%
	3-6DAYS	45-67			%
	7-13 DAYS	42-66			%
	14-29 DAYS	39-63			%
	1MO - 59 DAYS	31-55			%
	2MO-5MO	28-42			%
	6MO-23MO	33-39			%
	2YR-5YR	34-40			%
	6YR-11YR	35-45			%
	12YR-17YR		37-49	36-46	%
	18-20		41-53	36-46	%
	ADULT		42-52	37-47	%
MCV	0-2 DAYS	98-118			fL
	3- 6 DAYS	95-121			fL
	7-13 DAYS	88-126			fL
	14-29 DAYS	86-124			fL
	1MO - 59 DAYS	85-123			fL
	2MO-5MO	77-115			fL

	6MO-23MO	70-86			fL
	2YR-5YR	75-87			fL
	6YR-11YR	77-95			fL
	12YR-17YR		78.98	78-102	fL
	18-20		80-100	80-100	fL
	ADULT		80-94	81-99	
MCH	0-6 DAYS	31-37			pg
	7-59 DAYS	28-40			pg
	2MO-5MO	26-34			pg
ANALYTE	AGE	NORMAL RANGE BOTH SEXS	MALE	FEMALE	UNITS
	6MO-23MO	23-31			pg
	2YR-5YR	24-30			pg
	6YR-11YR	25-33			pg
	12YR-17YR	25-35			pg
	ADULT	26-34			pg
MCHC	0-2 DAYS	30-36			g/dL
	3-6DAYS	29-37			g/dL
	7-29 DAYS	28-38			g/dL
	1MO-5M0	29-37			g/dL
	6MO-23MO	30-36			g/dL
	2YR-ADULT	31-37			g/dL
RDW	ALL		35.1-43.9	36.4-46.3	fL
PLT	ALL	130-400			X10~3 uL

MPV	ALL	7.4-10.4			fL

ANALYTE	NORMAL RANGE	UNITS
Neut%	40.0-78.0	%
Im Gran%	0.0-5.0	%
Lymph%	15.0-48.0	%
Mono%	0.0-12.0	%
Eos%	0.0-7.0	%
Baso%	0.0-3.0	%
NRBC%	0-0.2	%
Neut#	1.9-8.0	X10^3/uL
Im gran#	0.0-0.4	X10^3/uL
Lymph#	0.9-4.5	X10^3/uL
Mono#	0.15-1.10	X10^3/uL
Eos#	0.00-1.01	X10^3/uL
Baso#	0.00-0.30	X10^3/uL
Retic#	M: 0.026-0.095 F: 0.0164-0.0776	X10^6/uL
Retic%	0.4-2.2	%
Retic-imm%	M: 2.3-13.4 F: 3.0-15.9	%
CHR-Retic	28.8-35.7	pg

Reporting Abnormal Results:

- Reflex manual differentials will be performed every 48 hours (applies to inpatients and outpatients)

1. Criteria for performing a Manual Differential

WBC	<2.0 or >35.0 X10^3/uL *
Neutrophil Absolute #	>25.0 X10^3/uL **
Lymphocyte Absolute #	>5.0 X10^3/uL
Monocyte Absolute #	>2.0 (1000) X10^3/uL
Eosinophils Absolute #	>2.0 (1000) X10^3/uL
Basophils Absolute #	>1.0 (1000) X10^3/uL
Immature Granulocytes %	>5.0 % X10^3/uL

* If Monocyte comprise ≥10% of the Sysmex differential, perform manual differential. 48 hours rule does not apply to this scenario.

** If the analyzer has a Blast/Abnormal lymphocyte Flag, then perform manual differential.

2. Criteria for review of RBC Morphology:
   The first specimen received by laboratory on an admission will have an RBC morphology performed if the following criteria are met:
   a. Hct: <25%
   b. MCV <75 or >105
   c. RDW >22
   d. Hypo, Aniso, Micro, Macro analyzer flags.
   All subsequent specimens will have the comment that the RBC Morphology has been previously reviewed unless the original morphology was performed longer than 7 days prior in which case it will be repeated.

Limitations of Procedure:

1. XN-Series Manufacturer Stated Linearity

Parameter	Range	Units
WBC	0-440.0	$x10^3/\mu L$
RBC	0-8.60	$x10^6/\mu L$
HGB	0-26.0	g/dL
HCT	0-75.0	%
PLT, PLT-F	0-5000	$x10^3/\mu L$
RET%	0-30	%
NRBC%	0-600	/100 WBC

   a. Parameters that exceed these limits are flagged with @ beside the result. The sample must be diluted, rerun, and multiplied by the dilution factor.
   Note: the use of dilution for linearity on the patient report.

2. Possible Sample Interferences
   a. Specimens must be free of clots and fibrin strands.
   b. 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.
   c. Red cell fragments, microcytic RBCs, or white cell cytoplasmic fragments may interfere with automated platelet counts. A fluorescent platelet may be performed to avoid this interference.
   d. Cold agglutinins produce spurious macrocytosis, elevated MCH's MCHC's, falsely decreased RBC counts and HCT's. Rare, warm agglutinin produces the same spurious results as cold agglutinin.
   e. Extremely elevated WBCs may cause turbidity and falsely increase the hemoglobin, in addition to RBC and HCT values.
   f. Severely hemolyzed samples (*in vitro*) falsely decrease RBC and hematocrit. Recollect hemolyzed specimens.
   g. 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. There are different methods for handling samples with platelet clumping or "platelet satellitism". These methods include vertexing of the original sample and reanalyzing

or adding amikacin to the original sample and reanalyzing. Laboratories should define and validate the method(s) used by their facility.

h. Abnormal paraproteins found in Multiple Myeloma patients can falsely increase the HGB. To correct HGB perform plasma replacement.
i. Severely icteric samples may falsely elevate the HGB value and related indices. Make a 1:5 dilution with CELLPACK.
j. Rocking specimen excessively, may affect the WBC differential.
k. Megakaryocytes may falsely increase WBC counts on automated hematology analyzers.

3. Flagging and Action Messages

Abnormal samples on the SN-Series are identified using flagging systems to alert the user of a possible abnormality.

a. Suspect flags generate a message (e.g., Atypical Lymphocyte, WBC Abnormal Scattergram). Numerical results will display an asterisk and the specimen result will display as "Positive"
b. Analyzer generated error codes (e.g., DIFF channel errors). Error will display in both the Browser and Explorer screens
c. User defined flags (e.g., leukocytosis, anisocytosis). These flags are programmable by the customer in the settings menu. When threshold limits are exceeded, a message appears, and the specimen result will display as "Positive"
d. Action Messages (e.g., Difference between WNR and WDF. Check the results) _The results are displayed in the Browser Screen

Refer to the Sysmex XN-Series Automated Hematology Systems Flagging Interpretation Guide for additional information on flagging

References:

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3. Sysmex SP-50 *Instructions for Use [3 volumes]* (North American Edition), Sysmex
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.

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9. Cornbleet J., *Spurious results from automated hematology cell counters. Lab Medicine.* 1983; 8:509-514.
10. Sysmex Reagents of America, Inc. MSDS sheets and reagent product inserts.
11. College of American Pathologists (CAP) Hematology-Coagulation Checklist, July 2012.
12. Stewart, Charles and Koepke, John. *Basic Quality Assurance Practices for Clinical Laboratories*, Van Nostrand Reinhold, 1989, p 189.
13. Gulati GL, Asselta A, Chen C. *Using vortex to disaggregate platelet clumps*, Laboratory Medicine, 28:665, 1997.
14. 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.

15. Sysmex XN-Series Automated Hematology Systems Flagging Interpretation Guide, Document Number: 1166-LSS, Rev 3, January 2017