<table>
<tr><td rowspan="6">Methodist Health Services Corporation & UnityPoint Health Methodist

Laboratory</td><td>Page # Page 1 of 16</td><td>Section: HEMATOLOGY</td><td>Policy #: HEM0 - 20</td></tr>
<tr><td colspan="2">Approved by: see signature block at end of document</td><td>Date: 6/13/17</td></tr>
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<tr><td colspan="3">Policy Submitted by: Ron Fitzgerald</td></tr>
<tr><td colspan="3">JCAHO Standard: NA</td></tr>
<tr><td colspan="4">POLICY ON: Complete Blood Count: Whole Blood and Body Fluid Analysis on the Sysmex XN-3000 Automated Hematology Analyzer</td></tr>
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I. POLICY STATEMENT:
Standard Operating Procedure for Sysmex XN-3000 Automated Hematology Analyzer

II. PURPOSE:
To provide operating procedures for using the XN-3000 analyzer.

III. SCOPE:
All Hematology technologists will follow this policy when performing patient testing.

IV. PRINCIPLE:
The Sysmex XN-3000 is an integrated system that incorporates two hematology analytical modules as well as an automated slidemaker/stainer.

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 are 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-10 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 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 the cassette at 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.

V. Clinical Significance

Analysis of these numerical and/or morphological findings is useful in the diagnosis of such disease states as anemia, leukemia, allergic reactions, viral, bacterial and parasitic infections.

VI. SPECIMEN:

A. Required specimen
   1. Whole blood collected in K2 EDTA preferred.
   2. Sodium Citrate may be used when EDTA platelet clumping or platelet satellitism is noted on the EDTA specimen. Use Sodium Citrate results for the platelet count and WBC count. Multiply instrument PLT AND WBC result by 1.1 to correct for anticoagulant dilution.
   3. Serous and synovial fluids should be collected in EDTA-2K anticoagulant.
   4. The use of anticoagulant with CSF specimens is neither required nor recommended.

B. Specimen volumes required
   1. Optimal draw is a 12 x 75 tube filled to capacity. The collection tube should be filled to a minimum on one-half full for acceptable results. EXCEPTION: a 2.5ml EDTA tube filled less than one-half full is unacceptable. An EDTA micro-container filled above the 250ul line is adequate for testing in the open mode. Specimen should be well mixed after venipuncture.
   2. Manual analysis whole blood mode
      a. Closed tube – 2 mL
      b. Open tube – 300 µL
      c. Open microtube – 160 µL
   3. Manual analysis body fluid mode
      a. Closed tube – 2 mL
      b. Open tube – 300 µL
      c. Open microtube – 160 µL
   4. Manual analysis – SP-10
      a. Closed tube smear and staining – 2 mL is optimal, 200 µL is aspirated.
      b. Microtainer mode – 300 µL minimum volume, 60 µL is aspirated.

C. Unacceptable specimens including those listed below must be redrawn:
   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.

D. Characteristics that may affect test results: lipemia, icterus, and cold agglutinins.

E. Stored Specimen Stability
   1. Stored at 4-8^{o}C, EDTA blood samples with normal results may be analyzed up to 48 hours without significant loss of differential stability. Stored for 7 days and then disposed of.

2. 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.
3. Allow refrigerated samples to come to room temperature and mix well before analysis.

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

WARNING:	All patient specimens 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 part 1910.1030. Follow specimen handling as outlined by laboratory safety policy.
Recommended:	Wear gloves and a lab coat. Wear safety glasses if there is a risk of splashing.

VII. SUPPLIES & REAGENTS

A. Supplies

1. De-ionized water
2. Lint-free plastic lined lab wipes
3. Gauze
4. Test tubes
5. Plastic squeeze bottles
6. CELLCLEAN® AUTO
7. Sysmex reagents
8. Commercial controls; XN CHECK™, XN CHECK™ BF
9. Alcohol prep pads, isopropyl. Used to clean SP-10 spreader glass
10. Microscope slides, frosted with rounded / clipped corners
    a. 76 x 26 mm; 0.9 – 1.2 mm thick

B. 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.

XN REAGENTS	OPEN EXPIRATION
CELLPACK DCL	60 Days
CELLPACK DST	60 Days
CELLPACK DFL	60 Days
SULFOLYSER	60 Days (1.5L)
	90 Days (5.0L)
Lysercell WNR	60 Days
Fluorocell WNR	90 Days
Lysercell WDF	90 Days
Fluorocell WDF	90 Days

Fluorocell RET	90 Days
Fluorocell PLT	90 Days

SP REAGENTS

Stain – Wright PROTOCOL WRIGHT STAIN]	NA, stable until expiration date printed on the container
Buffer – pH 6.8 [SAS-100 BUFFER SOLUTION]	NA, stable until expiration date printed on the container
CELLPACK DCL	60 Days

A description of each of the Sysmex Reagents is available in the "XN-3000 Instructions for Use" manual (chapter 5) and in the "SP-10 Instructions for Use" manual (chapter 4) located in the back bookshelf in Hematology.

C. XN Reagent Replacement
   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]
         i. Remaining Reagent Volume indicator appears
   3. Replacing a new diluent / hemolytic agent
      a. Display the [Reagent Replacement] dialog box
      b. Remove the cap from the new reagent container
         i. Confirm the reagent has not expired
      c. Input the reagent code (barcode)
         i. Place the cursor in the reagent code field
         ii. Scan the reagent code on the outer box of the new reagent with the hand-held barcode reader or manually enter the reagent code
         iii. Select [OK]
      d. Remove the cap from the old reagent container.
      e. Pull out the dispensing set straight up.
      f. Insert the dispensing set straight into the new container.
      g. Close the cap.
      h. Select [Execute]
         i. Reagent replacement starts. When complete, the dialog box closes automatically.
   4. Replacing CELLPACK DST on the RU-20
      a. Display the RU-20 Maintenance menu.
      b. Select [Replace Reagent]
      c. Remove the cap from the new reagent container.
         i. Confirm that reagent has not expired
      d. Input the reagent code (barcode)
         i. Place the cursor in the reagent code field.
         ii. Scan the reagent code on the outer box of the new reagent with the hand-held barcode reader or manually enter the reagent code.
         iii. Select [OK]
         iv. Remove the cap from the old reagent container
         v. Pull out the dispensing set straight up.

vi. Insert the dispensing set straight into the new reagent container.
vii. Close the cap
viii. Select [Execute]
1) 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.
      i. 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.
      i. 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 holder
      i. Make 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.
      ii. 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.
      i. When the cover is pulled down, the Help dialog box closes automatically.
      ii. The ID of the new reagent is read automatically and the information is registered.
   h. Close the top front cover.
      i. Reagent replacement starts.
      ii. When complete, the reagent replacement window closes automatically.
6. SP-10 Reagent Replacement

The following is a list of replacement messages and the reagent requiring replacement:

Message	**Reagent**
*DCL not filled	CELLPACK DCL
*Stain 1 not filled in Chamber 1	Stain
*Stain 1 not filled in Chamber 2	Stain
*Rinse water not filled (internal chamber not filled)	Deionized water
Replace Rinse water (external container empty)	Deionized water
Replace buffer	Buffer

* 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. Avoid placing spout kit or sensor on a contaminated surface.

* After replacing reagents, run one level of XN CHECK to validate the reagents. Edit the reagent log with a comment stating that QC has been performed.

VIII. 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 shall verify calibration on a continual basis to ensure accuracy of system. Calibration verification is also required if one or more of the following occur:

A. Critical parts are replaced.
B. Controls show an unusual trend or are outside of acceptable limits and cannot be corrected by maintenance or troubleshooting.
C. When advised by Sysmex Field Service Representative.

An on going verification of calibration is done by hematology technical staff by review 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. If a potential problem is noted, re-calibration will be performed by following the procedure located in the Sysmex "XN-3000 Instructions for Use" manual (chapter 12).

IX. QUALITY CONTROL

A. 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. It should be noted that for troubleshooting purposes, additional control runs may be necessary. To QC the SP-10, examine a stained smear from the routine workload for smear and stain quality on a daily basis. XN CHECK Commercial Controls Instructions for Use

1. Remove vials from refrigerator and allow them to come to room temperature (18-25^{o}C), 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.

B. XN CHECK BF Commercial Body Fluid Controls Instructions for Use

1. Remove vials from refrigerator and allow them to come to room temperature (18 – 25^{o}C) 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.

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.

C. Frequency of Control use and review

1. XN CHECK control levels 1, 2, and 3 will be run daily by 1st shift in the Sampler mode after the routine cleaning procedure and as needed by technical staff to check patient or instrument problems and to validate reagents after they have been changed.
2. XN CHECK BF control levels 1 and 2 will be run by technical staff in the Manual BF mode as needed before patient samples are processed.
3. SP-10 slide quality will be evaluated daily on an ongoing basis.
4. The lead technologist will review commercial and X-BarM charts weekly.

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

E. 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.
   a. Select [QC Analysis]
   b. From the list of QC files, select the file to be analyzed. Judgment dialog box will open automatically.
   c. Place thoroughly mixed vial in tube holder, press start switch.
   d. 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.

F. Reviewing Quality Control Results
1. QC File screen
   a. Allows for review of the latest QC results in Radar Chart format for the QC file that is selected in the list.
   b. Any point exceeding the upper or lower limit is marked with a red "X".
2. QC Chart screen
   a. Allows for review of detailed graph data of all QC runs for selected file.
   b. Analysis data is plotted cumulatively and displayed in the chart area as a line graph.
   c. Any point exceeding the upper or lower limit is marked with a red "X".
   d. User must scroll up and down through the chart to view all parameters for each run. 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.

G. 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:
   a. Statistical computations (SD, Mean, CV)

b. Variable target computation
c. 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 will need to be added to the QC data selected by the cursor.
   a. Select [Input Any Comment] to input a free text comment.
   b. Select [Fixed Comments] to use a comment from a list of preset comments in the QC settings menu.
   c. Select [OK]
   d. A comment bubble will be displayed when a comment exists for a QC run.
   e. The comment will be visible in the comment display area when the cursor is placed on the QC run.

H. Verify that all parameters fall within the assigned assay range. If controls fall outside the range call the Sysmex Technical Assistance Center at 1-888-879-7639 to investigate possible control material failure and to obtain troubleshooting recommendations.
   1. Corrective Action for out of range QC Results
      a. Rerun the same vial of control; possible "random error".
      b. If still out, use a new vial of the same lot number of control.
      c. If control is still flagged, run the other two levels of controls.
      d. If 2 out of 3 levels of controls are in; system is in control. Assess all previous results for validity.
      e. Document any trouble shooting actions taken on log sheet.
      f. Control results must meet acceptable criteria prior to reporting patient results.

I. SP-10 Daily QC Slide Review
   1. Review the blood smears macroscopically for acceptability:
      a. Smears are sufficient length (greater than half the length of the unfrosted portion of the slide).
      b. The feathered edge becomes gradually thinner without streaks, holes, or tails.
      c. Even, consistent staining of blood smear.
   2. 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:
         i. RBC's should be pink to orange. There should be good differentiation between normochromic, hypochromic, and polychromatic cells.
         ii. Lymphocytes will display dark purple nuclei with varying shades of blue cytoplasm.
         iii. Neutrophils will display dark purple nuclei, with light pink cytoplasm and lilac granules.
         iv. Monocytes will show lighter purple nuclei. The cytoplasm of the monocytes will be gray-blue with reddish granules.
         v. Eosinophils show bright orange granules in the cytoplasm.
         vi. Basophils display dark blue granules in the cytoplasm.
         vii. 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.

X. Operating Procedure

A. Start-Up Procedure

1. Checks prior to turning on
   a. Visual inspections of analyzer / system / reagents
      i. Place completed samples into final storage area for the lab
      ii. Remove any items that may interfere with operations
      iii. Gather and re-locate all empty racks to designated processing or sample loading area
      iv. If applicable, verify waste container is empty
      v. Verify network / host connections are properly working
      vi. Ensure that the towers (slide supply cassettes) have sufficient slides. Fill with glass slides.
         (A) Remove the tower to be filled.
         (B) Remove the metal insert from the end of the tower.
         (C) 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.
         (D) Replace the metal insert and replace the tower with the frosted end of the slides towards the back of the analyzer.
      vii. Verify sufficient reagent supply is nearby
      viii. 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.
2. Turning ON the entire system
   a. Verify that all power switches for each device are in the ON position
   b. Press the green start-up switch on the sampler unit (left - SP side) to power ON the entire system
3. Log on to the XN-IPU
   i. When the logon dialog box appears, enter Logon name: " xn ".
4. Analyzers and SP-10 self-checks
   a. 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 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

   b. SP-10: System check to evaluate internal stored data files; shutdown check to determine whether shutdown was performed properly, a mechanical initialization sequence.
5. Analyze Quality Control Material
   a. Daily Cleaning

CELLCLEAN AUTO is used to clean the system. Refer to the XN-3000 *Instructions for Use* for detailed, illustrated procedures.

i. Make sure the XN analyzer is in the "Ready" state.
ii. Click the analyzer menu button.
iii. Select "Maintenance".
iv. Select "Cleaning".
A message will appear on screen and the tube holder will slide out.
v. Place a new vial of CELLCLEAN AUTO in the sample tube holder
vi. Press the blue start switch.
NOTE: Cleaning will take approximately 20 minutes. The cleaning process will conclude with a BACKGROUND CHECK and the analyzer will return to the "READY" state in the manual mode.
7. Remove the empty tube of CELLCLEAN AUTO and discard.
8. Return to step 1. and perform "Cleaning" on the other XN analyzer.
* Analyze Quality Control Material.

b. Shutdown – performed weekly

CELLCLEAN AUTO is used to shut down the entire system. Refer to the XN-3000 *Instructions for Use* for detailed, illustrated procedures.

vii. Confirm analyzers, sampler unit and SP-10 are at ready.
viii. Confirm tube holders are retracted into the analyzers.
ix. Obtain 2 empty racks
Place one tube of CELLCLEAN AUTO in rack one, position 8. This rack will shut down the SP-10.
Place 2 tubes of CELLCLEAN AUTO in rack two, positions 9 and 10. This rack will shut down the XN's.
x. Place racks on sampler unit, sampler unit will auto-start.
After shutdown is complete, the SP-10 will power off, the XN-IPU will shutdown, the XN analyzers will power off . Remove the racks.
Wait 30 seconds before performing the start up procedure.
xi. XN on-board maintenance history will auto-populate.
xii. Document shutdown on the SP maintenance log.
* Analyze Quality Control Material.

c. SP-10 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 Daily
Perform Shutdown 1 (every other day)
i. Touch [Conv.int.] on the menu screen
ii. Touch [interrupt]
iii. Touch [Return]
iv. Touch [Shutdown]
v. Touch [Shutdown 1]
vi. Place a tube of CELLCLEAN AUTO in the 10^{th} position of a sample rack.
vii. Place the rack so that the tube is lined up with tube gripper.
viii. Press [OK].
ix. When the process completes, the SP-10 turns off automatically.
x. To restart the SP-10, press the green button on the right side.

2. Perform Shutdown 2 (every other day)
a. Press [SHUTDOWN] on the main screen.
b. Press [Shutdown 2].
c. The shutdown screen displays the number of cassettes and amount of methanol required for the shutdown process. Ensure that required amounts are available.
d. Place a tube of CELLCLEAN AUTO in the 10^{th} position of a sample rack.
e. Place the rack so that the tube is lined up with tube gripper.
f. Press [OK].
g. When the process completes, the SP-10 turns off automatically.
h. To restart the SP-10, press the green button on the right side.

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

i. Press [Maint.] on the main screen. (Maintenance button is not available during routine operation.)
ii. Press [Spreader Glass] and the "Spreader Glass Replace" screen displays.
iii. Press [OK] to move the smear unit forward.
iv. Remove the left tower for easier access to the spreader glass.
v. Wipe the spreader in one direction with an alcohol prep pad.
vi. Replace the tower so that the frosted end of the slides are towards the back of the analyzer.
vii. Press [OK] to return the smear unit to the home position.
viii. Press [OK] to reset the spreader glass cycle counter or [CANCEL] to allow the cycle count to continue.
ix. Press [RETURN].
x. Clean Single Cassettes
xi. Place cassettes in a bin with open end up.
xii. Pour methanol over the cassettes, filling them.
xiii. Swish the methanol and pour off into designated container for reuse.
xiv. Invert cleaned cassettes on absorbent material to dry.

Note: *Methanol may be reused for cleaning cassettes up to three (3) times. Discard when appropriate.*

4. Clean DI water/Buffer containers (Weekly)
   i. If re-usable containers for deionized water and/or buffer are used, empty weekly.
   j. Rinse with methanol and allow to dry.
   k. Fill with fresh deionized water or buffer.
5. As Needed Maintenance
   Refer to the XN-3000 *Instructions for Use* for detailed and illustrated instructions for performing as needed maintenance.

d. Patient Sample Processing

c. System Analysis (sampler analysis) - XN

i. Make sure the analyzer and the sampler are in READY state
ii. Check that tube holder has retracted into the analyzer, press mode button if necessary.
iii. Place sample(s) in rack(s) in right sampler pool (analyzer side)
iv. Rack(s) will auto-start.
v. Samples will run, results will be displayed in the IPU.
vi. On-Board rules engine will determine repeat or reflex testing
vii. Rack will run in reverse to perform repeat or reflex testing.
viii. If smear is required, rack will be transported to SP-10 via analysis line and samples will be aspirated by SP-10.
ix. If no smears are required, rack will be transported to the left sampler pool without stopping at the SP-10.
x. Remove the rack from the left sampler pool when analysis in completed.

Manual Analysis – XN.

WARNING: Potential biohazard exposure when handling open patient specimens. Follow Standard precautions outlined by laboratory safety guidelines.
Recommended: Wear gloves, a lab coat and safety glasses. Use plastic lined gauze when opening.

xi. Check the status of the analyzer. Confirm the analyzer is ready.
xii. Press the mode switch to eject the tube holder.

xiii. Select the Change Analysis Mode button on the control menu
xiv. Select analysis mode
[Whole blood] is selected when whole blood is being analyzed
[Low WBC] Select this to perform low WBC analysis on whole blood
[Pre-Dilution] select when running 1:7 pre-diluted blood.
xv. Select [OK]
xvi. Select Manual Analysis button on the control menu
xvii. Input sample ID or select [Read ID]
xviii. Select [OK]
xix. Properly mix the specimen and place in the tube holder
If running microtainer, remove the cap using caution to avoid splattering.
xx. Press the start switch on the analyzer
The tube holder will slide in and the sample will be aspirated
When the analysis is complete, the tube holder slides out
xxi. Remove the sample, repeat steps for additional samples
xxii. Review results in IPU to determine whether repeat or reflex testing is required. Rerun sample if required. Make smear if required.

d. Body Fluid Analysis – XN

i. Check the status of the analyzer. Confirm the analyzer is ready.
ii. Press the mode switch to eject the tube holder.
iii. Select the Change Analysis Mode button on the control menu.
iv. Select [Body Fluid]
v. Select [OK]
The analyzer will automatically perform a background check up to three times.
vi. Select the Manual Analysis button on the control menu
vii. Input the sample ID or select [Read ID]
viii. Select [OK]
ix. Properly mix the specimen and place in tube holder.
If running microtainer, remove the cap using caution to avoid splattering
x. Press the start switch on the analyzer
The tube holder will slide in and the sample will be aspirated
When the analysis is complete, the tube holder slides out
xi. Remove the sample
xii. Perform Background check prior to running additional samples if indicated
xiii. Return analyzer to Whole Blood mode prior to running whole blood samples.

e. 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.
i. Press mode switch on the sampler
ii. Verify sampler is in READY state
iii. Place the rack in the right pool of the sampler for the analyzer that you wish to use.
iv. Transport begins automatically
v. Remove the rack after analysis is complete
vi. Press the mode switch on the sampler

f. SP-10 Manual Mode – Smear and Stain
i. Press [Conv. Int.] on the SP-10 main menu screen
ii. Press [Interrupt]
iii. Select [Return]
iv. Select [Manual] on the SP-10 main menu screen
v. Op Mode is set to [Smr + Sta], Smpl. Tube is set to [Closed]
vi. Input Specimen information, Sample ID, HCT, select number of slides to be made
vii. Thoroughly mix the sample and place in 10^{th} rack position

viii. 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
ix. Select [Start]
Analysis will begin.
When the tube is returned to the rack, remove the rack.
x. Press [Return] [Conv. Int.] [Stop Int.]

g. SP-10 Manual Mode – Stain Only
i. Select [Manual] mode
ii. Select [Op. Mode], [Stain]. Do not proceed until [START] button is green.
iii. 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.
iv. Press [Start]
v. 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.

h. SP-10 Micro Mode
i. Select [Manual]
ii. Choose [Op. Mode], [Smr.+Stain] and set [Smpl. Tube] to [micro]
iii. Input Specimen information, Sample ID, HCT
iv. Place the thoroughly mixed uncapped microtainer in the micro collection sample tube holder.
v. Select [Start]
vi. Micro tube will be lowered into position and sample will be aspirated.
vii. When aspiration is complete, micro tube will be returned to home position and should be removed.

i. SP-10 Smear Only – No staining occurs

Smear mode may be used in System, Single, or Manual Modes. To access Smear Mode:
a. Press **[Settings]** on the main screen. (A password may be required.)
b. Press **[Select]**, **[Cond.]**, **[Mode]**.
c. Press **[Op. Mode]** and select **[Smear]**. Press **[RETURN]** and **[YES]** to accept the settings.
d. 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.

e. 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

f. 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]
a. Analysis will begin

b. When the tube is returned to the rack, remove the rack

10. Press [Return] [Conv. Int.] [Stop Int.]

11. Remove the rack when sampling is complete.

Return Setting to SMEAR + STAINING

Press **[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.*

IX. REPORTING RESULTS

j. Adult Reference Ranges (18 years and greater)

Parameter	Reference Range	Parameter	Reference Range
WBC:	3.6 – 9.2	Neut %	45 – 80%
RBC	male: 4.38 - 5.58	Lymph %	16 – 45%
	female: 3.70 – 5.14	Mono %	2 – 12%
HGB	male: 13.7 – 17.3	Eo %	0 – 6%
	female: 12.0 – 15.5	Baso %	0 – 2%
HCT	male: 39.0 – 49.0	IG %	0 – 5%
	female: 35.0 – 46.0	Neut #	1.6 – 7.4 x 10_3 / µl
MCV	80.0 – 96.0	Lymph #	1.0 – 4.0 x 10_3 / µl
MCH:	28.0 – 33.5	Mono #	0.1 – 1.1 x 10_3 / µl
MCHC:	32.0 – 37.0	Eo #	0.0 – 0.6 x 10_3 / µl
RDW	11.2 – 15.0	Baso #	0.0 – 0.2 x 10_3 / µl
PLT:	140 – 400		

k. Reportable (Linear) Ranges --- values verified by linearity studies performed March 2014.

2. For Pediatric Reference Ranges (Hematology of Infancy & Childhood, Nathan, 1987) – See Hematology Policy #8.

IX. LIMITATIONS OF PROCEDURE

A. XN-Series Manufacturer Stated Linearity for Whole Blood

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

1. Parameters that exceed these limits are flagged with @ beside the result. The sample must be diluted, rerun and multiplied by the dilution factor. When using a diluting fluid you must run a background count on that

fluid prior to running the diluted specimen. This will ensure that there is nothing that will interfere with the count.
2. Note the use of dilution for linearity on the patient report.

B. XN-Series Manufacturer Stated Linearity for Body Fluids

Parameter	Range	Units
WBC-BF	0.003-10.000	$x10^3/\mu L$
RBC-BF	0.003-5.000	$x10^6/\mu L$

1. Sample results with Error related to WBC-BF, RBC_BF and TC-BF# parameters should not be used.
2. WBC_BF counts <0.003 x 10^3/uL should be reported as <0.003..
3. RBC-BF counts < 0.003 x 10^6/uL should be reported as <0.003..
4. Bronchial Lavage and clear colorless spinal fluid specimens cannot be run on the XN-3000.

C. Possible Sample Interferences

1. 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. There are different methods for handling samples with platelet clumping or “platelet satellitism”. These methods include vortexing 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.
8. Abnormal paraproteins found in Multiple Myeloma patients can falsely increase the HGB. To correct HGB perform plasma replacement.
9. Severely icteric samples may falsely elevate the HGB value and related indices. Make a 1:5 dilution with CELLPACK.
10. Rocking specimen excessively, may affect the WBC differential.
11. Megakaryocytes may falsely increase WBC counts on automated hematology analyzers.

X. REFERENCES

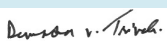
1. Sysmex XN-3000 *Instructions for Use* (North American Edition), Sysmex Corporation, Kobe, Japan.
2. Sysmex XN series *Administrator’s Guide* (North American Edition), Sysmex Corporation, Kobe, Japan
3. Sysmex SP-10 *Instructions for Use* (North American Edition), Sysmex Corporation, Kobe, Japan.

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.
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.

V. MAINTENANCE AND STORAGE:

A. All policies and procedures are reviewed every two years by Laboratory Administration and or the Medical Director of the Laboratory or designee.
B. The Laboratory Administration and Medical Director review policies and procedures when there are changes in practice standards, or requirements.
C. All policies and procedures are reviewed every two years by staff or at the time new or revised ones are put in effect.
D. All policies are retained 8 years after being discontinued or revised.
E. All procedures are retained 2 years after being discontinued or revised.

MMCI Laboratory is a CAP accredited facility, as of 7/1/11 the responsibility of new and/or substantially revised policies and procedures will be restricted the Laboratory Director whose name appears on the CLIA certificate, whose signature appears below. The biennial review will be completed by the Administrative Director.

POLICY CREATION :			
Author:	***Ron Fitzgerald***		***October 10, 2014***
Medical Director:	***Devendra Trivedi, MD***		***October 16, 2014***

MEDICAL DIRECTOR		
DATE	NAME	SIGNATURE
February 11, 2017	Elizabeth A. Bauer-Marsh, M.D.	Elizabeth A. Bauer-Marsh MD
SECTION MEDICAL DIRECTOR		
October 16, 2014	Julia Adams, M.D.	Julia Adams, M.D.

REVISION HISTORY (began tracking 2011)			
Rev	**Description of Change**	**Author**	**Effective Date**
0	New procedure	R. Fitzgerald	10/10/14
1	In body fluid section removed counts <0.003 should be confirmed using alternative method (validated linerarity of the analyser is 0.001). Added to report values less than 0.003 as <0.003.	K. Turpin	4/29/2015
2	Changed acceptable volume to match lab manual, added clear colorless spinal fluids as specimens that cannot be ran on the XN's.	K. Paige	3-14-17
3	Changed name of stain used, pH of buffer used ,days specimens are stored, removed documentation of stain quality, and added a statement referring to the fact that you must do a background count on all diluting fluid prior to running a diluting specimen	K. Paige	6-11-17

REVIEWED BY

Lead	Date	Coordinator/Manager	Date	.1.1.1.1.1 Medical Director	Date
R. Fitzgerald	10/10/14	Kathy L. Turpin	10/16/14	James Adams, M.D.	10/16/14
R. Fitzgerald	5/4/15	Kathy L. Turpin	4/29/15	James Adams, M.D.	5/21/15
Kim Paige	3/14/17	June Bombenek	3/14/17	James Adams, M.D.	3/14/17
Kim Paige	6/11/17	June Bombenek	6/12/17	James Adams, M.D.	6/13/17