STEM CELL LABORATORY (STCL)

DOCUMENT NUMBER: STCL-EQUIP-023

DOCUMENT TITLE:

Sysmex XN-450 Hematology Analyzer Automated Blood Count Procedures for the STCL

DOCUMENT NOTES:

Document Information

Revision: 01	**Vault:** STCL-Equipment-rel
Status: Release	**Document Type:** STCL

Date Information

Creation Date: 19 Mar 2025	**Release Date:** 20 Mar 2025
Effective Date: 20 Mar 2025	**Expiration Date:**

Control Information

Author: WATER002	**Owner:** WATER002
Previous Number: None	**Change Number:** STCL-CCR-562

STCL-EQUIP-023
SYSMEX XN-450 HEMATOLOGY ANALYZER AUTOMATED BLOOD COUNT PROCEDURE FOR THE STCL

1 PURPOSE

1.1 The purpose of this procedure is to provide users with basic instructions regarding the proper use and operation of the Sysmex XN-450 Automated Hematology Analyzer.

1.2 Users should consult the *Sysmex XN-450 Instructions for Use Manual,* as needed, for additional information

2 INTRODUCTION

2.1 The Sysmex XN-450 is a quantitative automated hematology analyzer for in-vitro diagnostic use for determining 24 hematological parameters. The analyzer directly measures the WBC, RBC, HGB, HCT, RDW-SD, PLT-I, NEUT%, LYMPH%, MONO%, EO%, BASO % and IG%. The remaining parameters are calculated or derived: MCV, MCH, MCHC, RDW-CV, MPV and differential #.

2.2 The Sysmex XN-450 counts and sizes red blood cells (RBC) and platelets (PLT) using hydrodynamic impedance counting (sheath flow DC method). At the same time the hematocrit (HCT) is measured as the ratio of the total RBC volume to whole blood using pulse height detection method. Hemoglobin is converted to SLS-hemoglobin and is read photometrically.

2.3 White Blood Cell (WBC) count and differential are evaluated using flow cytometry with a semiconductor laser utilizing scattered light and fluorescence to determine the differences in cell size, complexity and RNA/DNA content. The WBC differential channel classifies neutrophils, lymphocytes, monocytes, eosinophils, and basophils by cellular complexity and nucleic acid content. The differential cell placement is then enhanced utilizing Adaptive Cluster Analysis.

2.4 The Sysmex XN-450 performs all aspirations using a single aspirator.

3 SCOPE AND RESPONSIBILITIES

3.1 The Stem Cell Laboratory co-medical directors, Stem Cell Laboratory manager, and employees of the Stem Cell Laboratory who use this equipment are responsible for ensuring the requirements of this procedure are successfully met.

3.2 STCL reports only WBC, HCT, and manual differentials (*when ordered*) as reflected on the CAP's Laboratory Accreditation Program Laboratory Activity Menu (SU:1393603).

4 DEFINITIONS/ACRONYMS

4.1	WBC	White Blood Cell
4.2	RBC	Red Blood Cell
4.3	HGB	Hemoglobin

4.4	HCT	Hematocrit
4.5	PLT	Platelet
4.6	NEUT	Neutrophil
4.7	LYMPH	Lymphocyte
4.8	MONO	Monocyte
4.9	EOS	Eosinophil
4.10	BASO	Basophil
4.11	IG	Immature Granulocyte
4.12	MCV	Mean Corpuscular Volume
4.13	MCH	Mean Corpuscular Hemoglobin
4.14	MCHC	Mean Corpuscular Hemoglobin Concentration
4.15	RDW-SD	Red Cell Distribution Width-Standard Deviation
4.16	RDW-CV	Red Cell Distribution Width – Coefficient of Variation
4.17	MPV	Mean Platelet Volume
4.18	RNA/DNA	Ribonucleic Acid / Deoxyribonucleic Acid
4.19	EDTA	Ethylenediaminetetraacetic Acid
4.20	CPD	Citrate Phosphate Dextrose
4.21	ACD-A	Anticoagulant Citrate Dextrose Solution A
4.22	STCL	Stem Cell Laboratory
4.23	˚C	Degrees Celsius

5 MATERIALS

5.1 Supplies

5.1.1 Test tubes

5.1.2 CELLCLEAN AUTO: Detergent for fully automated hematology analyzer. This is a strong alkaline detergent used to remove lysing reagents, cellular residuals, and blood proteins remaining in the hydraulics of the analyzer on XN-L Series automated hematology analyzers.

5.1.2.1 CELLCLEAN AUTO Storage and Stability

5.1.2.1.1 Store at 1-30^{o} C, away from direct sunlight.

5.1.2.1.2 Unopened, stable until expiration date printed on the container

5.1.3 XN-L CHECK commercial controls, 3 levels (Low, Normal, and High) consist of human red and white blood cells with a platelet component suspended in fluid medium.

5.1.3.1 Store vials at 2-8˚ C. Do not freeze or expose vials to excessive heat. Unopened and properly stored XN-LCHECK is stable until the expiration date reflected on the vial.

5.1.3.2 Open vial stability is 15 days when promptly refrigerated after each use. Record the open date, open expiration date and technologist's initials upon vial opening.

5.1.3.3 Heat or freezing can damage XN-L CHECK without gross visible changes. Moderate hemolysis can be normal. Deterioration is suspected when the mean of the control results is not within the assay expected ranges after appropriate troubleshooting. If deterioration is suspected, call the Sysmex Technical Assistance Center at 1-888-879-7639 (1-888-8SYSMEX).

5.2 Reagents

5.2.1 Four Sysmex reagents are used on the Sysmex XN-450.

5.2.2 All reagents are used at room temperature and are to be used (unopened) within the manufacturer's expiration date on each container.

5.2.3 When container is placed on the instrument, record the date the reagent was placed into use, the open expiration date and the performing technologist's initials on the container.

5.2.4 All reagents are azide free, and they are intended for in vitro diagnostic use only.

5.2.5 Refer to *STCL-EQUIP-023 JA2 Sysmex XN-450 Hematology Analyzer Reagent Replacement* for instructions for replacing reagents on the analyzer.

5.2.6 Reagent Verification is not a stand-alone documented process. Several systems are used together to verify reagent consistency from cube to cube and from lot to lot. The following programs validate the reagent/instrument system: 1) daily background checks, 2) the IPU reagent log, 3) the moving average QC file (selected parameters used primarily by the service representative), 4) the commercial control files which are compared across peer groups using ILQC and Insight programs, 5) the XS-450 problem log.

Reagent	Usage Temperature	Storage Temperature	Open Expiration
CELLPACK DCL	15 - 35˚ C	2 - 35˚ C	60 days
Lysercell WDF	15 - 35˚ C	2 – 35˚ C	60 days
Fluorocell WDF	15 - 35˚ C	2 - 35˚ C	90 days
SULFOLYSER	15 - 35˚ C	1 - 30˚ C	60 days

5.2.7 CELLPACK DCL is a clear and colorless whole blood diluent for use in the determination of hemoglobin and impedance counting and sizing of blood cells. It also forms a laminar sheath flow around the diluted sample for hydrodynamic focusing of the RBC and PLT. Do not use it if there are signs of reagent contamination or color change. If the reagent is frozen, thaw, mix thoroughly and allow bubbles to disperse before use.

5.2.8 Lysercell WDF: Reagent product to be combined and used with Fluorocell WDF. By hemolyzing red blood cells with Lysercell WDF and dying the white blood cell component with Fluorocel WDF, the counts and percentages of neutrophils, lymphocytes, monocytes, eosinophils and basophils are analyzed. Do not use reagent if frozen or there are signs of contamination or color change.

5.2.9 Lysercell WDF: used to stain leukocytes in diluted and lysed blood samples for the determination of the 5-part differential including NEUT, LYMPH, MONO, EO and BASO. Do not use it if frozen or there are signs of contamination or color change.

5.2.10 SULFOLYSER: lysing reagent that releases the hemoglobin to be measured by the SLS hemoglobin method. Do not use it if there are signs of reagent contamination or color change. If the reagent is frozen, thaw and warm reagent in a 30˚ C water bath to dissolve ingredients completely and mix thoroughly before use.

5.3 Specimen

5.3.1 Samples may be comprised of peripheral whole blood, umbilical cord blood, granulocytes, bone marrow or peripheral blood progenitor cells collected in EDTA, Heparin, CPD, Sodium Heparin or ACD-A anticoagulants.

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

5.3.3 The white counts from these samples are verified by comparison with the flow cytometer count.

5.3.4 Alert values and general hematology, corrective action and follow-up procedures are not applicable in STCL given the nature of the cellular products being tested.

6 EQUIPMENT

6.1 Sysmex XN-450 Hematology Analyzer (*or equivalent*)

7 SAFETY

7.1 Wear all applicable personal protective equipment when handling potentially infectious blood and body fluids to include, but not limited to, gloves, lab coat, etc.

NOTE: Do not ingest. Avoid skin and eye contact. In case of skin contact, wash immediately with plenty of soap and water. In case of contact with eyes, flush with plenty of water immediately. Consult with a physician in case of ingestion and/or eyes contact.

8 PROCEDURE

8.1 Calibration

8.1.1 Initial calibration is performed during installation by the Sysmex Service Engineer. Calibration compensates for any bias inherent to the pneumatic, hydraulic and electrical system that may affect the accuracy of results. Calibrators traceable to reference methods are used in the calibration of the instrument. WBC differential parameters are calibrated in the factory prior to shipment and verified by the field representative upon installation.

8.1.2 Calibration of Sysmex hematology analyzers does not expire and is not reagent lot dependent. Per the XN-L Series IFU, calibration should be performed only when indicated.

8.1.3 Calibration is also required if one or more of the following occur:

8.1.3.1 Critical parts are replaced such as manometers, apertures or detector circuit boards.

8.1.3.2 Calibration Verification fails (Controls show an unusual trend or are outside of acceptable limits and cannot be corrected by maintenance or troubleshooting.)

8.1.3.3 When advised by Sysmex representative.

8.1.4 Calibration verification generally required at least every 6 months by regulatory agencies may include review and documentation of acceptable performance on all three levels of commercial control and XM-L QC data, proficiency testing results, inter-laboratory patient control testing and periodic exchange of donor bloods.

8.2 Start Up

8.2.1 Check reagent boxes for sufficient run volume.

8.2.2 Check printer paper supply.

8.2.3 Power Up Sequence

8.2.3.1 Verify that all power switches for the devices are in the ON position. Press the Green power button on the front of the XN-450 to power ON the entire system

8.2.3.2 Log on to the XN-450 IPU. Log on the IPU with your Username (STCL, our Lab pneumonic) and press [ENTER]. No password is required.

The instrument automatically performs self-check on the microprocessor, mechanical parts, temperatures and background counts.

Sysmex X*N-450* Acceptable Background Counts	
Parameters	**Acceptable Limit**
RBC	0.02 x 10^{12}/ L
HGB	0.1 g/dL
PLT	10 x 10^{9}/ L
WBC – C	0.1 x 10^{9}/ L
WBC – D	0.1 x 10^{9}/ L

8.2.4 Press the power switch on the printer, if necessary.

8.3 Analyze Commercial Quality Control Materials

8.3.1 All three levels of XN-L CHECK (L, N, and H) are tested at the beginning of each shift or are run once every eight hours of analyzer use.

8.3.1.1 Remove vials from the refrigerator and allow them to come to room temperature for approximately 15-30 minutes.

8.3.1.2 Mix vials by gentle end to end inversion until the cell button in the bottom of the vial is completely suspended.

8.3.1.3 Confirm the analyzer is in a Ready state.

8.3.1.4 If the sample tube holder is not ejected, press the sample tube holder open/close switch.

8.3.1.5 Touch [Manual] on the bottom right of screen

8.3.1.6 Scan the barcode on the QC vial

8.3.1.7 Touch [OK]

8.3.1.8 Place thoroughly mixed vial in tube holder, press start switch.

8.3.1.9 When analysis is complete, analysis results are displayed. Users 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.

8.3.1.10 Complete form *STCL-EQUIP-023 FRM2 Sysmex XN-450 Maintenance Log*

8.4 Reviewing Quality Control Results in BeyondCare Quality Monitor

8.4.1 Log into BCQM Application

8.4.1.1 The laboratory user should log into the BCQM software so that the program is visible while running Quality Control samples. The BCQM software application is accessed at: https://bcqm.sysmex.com.

8.4.2 Enter your Customer Resource Center (CRC) credentials using your complete email and password. Select LOG IN to continue to the BCQM home screen.

8.4.3 Select the correct laboratory site in the drop-down menu on the left-hand side of the screen.

8.4.4 Refer to STCL-EQUIP-023 JA1 Sysmex XN 450 Automated Hematology Analyzer Quality Control Management.

8.5 Review QC L-J or Radar Charts (**Used if the BCQM software is down)**

8.5.1 Allows for review of detailed graph data of all QC runs for selected files

8.5.2 Analysis data is plotted cumulatively and displayed in the chart area as a line graph or Radar Chart

8.5.3 Results outside of acceptable limits are displayed with a red "X" on the chart. The parameter name and the result value will be displayed with a red background.

8.5.4 Scroll through the screens to view all parameters by using the scroll bar on the right of the screen or press the down arrow.

8.5.5 Select [Range] to set a main cursor and a sub-cursor so that data between the two cursors can be manipulated.

8.5.6 Statistics may be analyzed over any selected range.

8.5.7 Targets may be auto set for the selected range

8.5.8 To cancel range mode, select [Range] on the toolbar again or exit QC Chart mode.

8.5.9 Verify that all parameters fall within established limits. If a parameter falls outside the established limits, do not test or report patient results. For troubleshooting refer to and complete *STCL-EQUIP-023 FRM1 Sysmex XN 450 QC Review Log - DAILY.*

8.6 Weekly/Monthly Review of QC

8.6.1 Print L-J graphs for each control level weekly. The date range should be set from the date of QC implementation to the current date.

8.6.2 Graphs will be analyzed and any significant findings documented for each control level directly on the printout.

8.6.3 A significant finding would include the following:

8.6.3.1 Three consecutive days outside of + 2 SD for a particular analyte or assay.

8.6.3.2 Trends identified where a particular control runs along the upper or lower limits instead of the mean.

8.6.3.3 Multiple assays reported outside of + 2SD in the same control level repeated over two consecutive days.

8.6.3.4 Significant findings will be reported to the laboratory manager and/or manufacturer technical support as needed.

8.6.3.5 Complete *STCL-EQUIP-023 FRM3 Sysmex XN 450 QC Review Log – Weekly/Monthly*.

8.7 Patient Sample Processing

8.7.1 **MANUAL Analysis –** (25 µL aspirated sample volume) minimum of 300 µL in tube. **NOTE**: STCL samples typically contain ~500 µL.

8.7.1.1 Check the status of the analyzer. Confirm the analyzer is ready.

8.7.1.2 If the sample tube holder is not ejected, press the sample tube holder open/close switch.

8.7.1.3 Select analysis mode

8.7.1.3.1 [Whole Blood] is selected when whole blood is being analyzed.

8.7.1.4 Select **[OK]**.

8.7.1.5 Select Manual Analysis button on the control menu.

8.7.1.6 Input sample ID or use handheld barcode reader to scan sample ID

8.7.1.6.1 Patient information- touch Input to enter patient ID.

8.7.1.6.2 Aspiration Sensor- If a sample is suspected of having low hemoglobin, turn off the aspiration sensor in the Manual Analysis dialog box (for dilutions)

8.7.1.6.3 Cap Open- Select this checkbox to perform analysis with the sample tube cap open.

8.7.1.6.4 Mix the sample appropriately.

8.7.1.6.5 Place the sample in the sample tube holder. If the sample has a cap, it is not necessary to remove it unless it is non-pierceable.

8.7.1.6.6 Press **Start** switch on the analyzer.

8.7.1.6.7 The tube holder will slide in, and the sample will be aspirated. When the analysis is complete, the tube holder slides out.

8.7.1.6.8 Remove the sample, repeat steps for additional samples.

8.8 Calculations

8.8.1 If making a dilution of the patient specimen and running in the XN 450 Whole Blood mode, multiply the parameters by the dilution factor. CELLPACK DCL should be used as the diluent.

8.8.2 Limitations of Procedure

8.8.2.1 Sysmex XN-450 Linearity

Parameter	Range	Units
WBC	0.04 - 440.0	$x10^3$/uL
RBC	0.02 - 8.60	$x10^6$/uL
HGB	0.1 - 26.0	g/dL
HCT	0.2 – 74.5	%
PLT	2 - 5000	$x10^3$/uL

8.8.2.2 Values > upper limit:

8.8.2.2.1 Parameters that exceed these limits are flagged with @ beside the result. The sample must be diluted, rerun and multiplied by the dilution factor. Dilutions may be run immediately following preparation but within 30 minutes of dilution.

8.8.2.2.2 Note the use of dilution for linearity.

NOTE: WBC counts above 100 $x10^9$/L may falsely elevate the hemoglobin measurement. They may also falsely increase the RBC count, MCV and Hct.

8.8.2.3 Values < lower limit:

8.8.2.3.1 Check sample for clots or fibrin. (Request recollection of specimen, if applicable.)

8.8.2.3.2 Perform background check to ensure that background is at a minimum.

8.8.2.3.3 Check previous results when available.

8.8.2.3.4 Perform film review and count estimates if low count is PLT and is less the 100 x 109/L.

8.8.2.4 Clotted Samples or Sample with Fibrin Strands

8.8.2.4.1 Samples with clots or fibrin are unacceptable. Some counts or all counts may be affected by the clotting process.

8.8.2.4.2 Call the appropriate collection site and request that the sample be recollected if applicable. If the sample is unable to be recollected, consult with STCL co-medical directors for further instruction.

8.8.3 Acceptable Reporting Format

8.8.3.1 Reporting Abnormal Results to Physicians

8.8.3.1.1 Abnormal/unexpected results are reported to the co-medical directors and/or attending physician as appropriate. Results may require follow-up action, based upon co-medical directors or designee instruction, prior to release of product.

8.8.3.2 Alert Values/Corrective Actions:

8.8.3.2.1 Alert values and general hematology, corrective action and follow-up procedures are not applicable in STCL given the nature of the cellular products being tested. If the STCL instrument is being used as a backup by another laboratory, they should refer to their own procedures regarding value reporting.

8.8.3.3 Instrument Flags:

8.8.3.3.1 When histogram and/or instrument flags are obtained, appropriate follow-up action to confirm results is required before reporting patient's results.

NOTE: Every instrument ERROR Message requires follow up action. See instrument manual troubleshooting section.

8.8.3.4 Unusual results found on blood film examination may require review by the STCL co-medical directors or patient attending physician.

8.9 Shut Down - Performed Daily (*to clean the detector and dilution lines*)

8.9.1 Confirm analyzer and sample units are ready

8.9.2 If the sample tube holder is not ejected, press the sample tube holder open/close switch.

8.9.3 If any tubes remain in holder, remove".

8.9.4 Touch [Menu] on Toolbar

8.9.5 Touch [Shutdown] Touch [OK]

8.9.5.1 XN on board maintenance history will auto-populate Shutdown.

8.9.5.2 IPU will automatically shut off at the conclusion

8.9.5.3 Press Green power button to restart IPU

8.10 XN -450 Routine Cleaning- performed weekly. CELLCLEAN AUTO is used to shut down the entire system.

8.10.1 Confirm the analyzer is ready

8.10.2 Touch the [Maintenance] Icon in the Menu screen.

8.10.3 Touch [Rinse instrument]

8.10.4 Touch [Routine Cleaning]

8.10.4.1 If the sample tube holder is not ejected, press the sample tube holder open/close switch and place CELLCLEAN AUTO in the tube holder.

8.10.4.2 Press start switch

8.10.4.3 XN-450 on-board maintenance history will auto-populate Routine Cleaning.

8.11 Procedure Notes

8.11.1 Analysis of the specimen on the Sysmex XN-450 is recommended before removing the cap to make a smear.

8.11.2 DO NOT PLACE samples on a mechanical rocker. Excessive mixing may induce platelet clumping and alter white cell membranes resulting in false interpretive messages.

8.11.3 For troubleshooting specifics refer to the Sysmex XN-Series troubleshooting manual.

9 RELATED DOCUMENTS/FORMS

9.1 STCL-EQUIP-023 FRM1 Sysmex XS-450 QC Review Log - DAILY

9.2 STCL-EQUIP-023 FRM2 Sysmex XS-450 Maintenance Log

9.3 STCL-EQUIP-023 FRM3 Sysmex XS-450 QC Review Log – Weekly / Monthly

9.4 STCL-EQUIP-023 JA1 Sysmex XS-450 Automated Hematology Analyzer Quality Control Management

9.5 STCL-EQUIP-023 JA2 Sysmex XN-450 Hematology Analyzer Reagent Replacement

9.6 STCL-EQUIP-023 JA3 Sysmex XN-450 Hematology Analyzer Dilution Protocol

9.7 STCL-EQUIP-023 JA4 Sysmex XN 450 Hematology Analyzer As Needed Maintenance

9.8 STCL-EQUIP-023 JA5 Sysmex XN 450 Hematology Analyzer Precision and Calibration Checks

10 REFERENCES

10.1 Sysmex XN-L Series Basic Operation (North American Edition), Sysmex Corporation, Kobe, Japan

10.2 Sysmex XN-L Series General Information (North American Edition), Sysmex Corporation, Kobe, Japan

10.3 Sysmex XN-L Series Troubleshooting (North American Edition), Sysmex Corporation, Kobe Japan.

10.4 Koepke, John. Practical Laboratory Hematology. Churchill Livingstone Inc. 1991. p.24-25, 36-39.

10.5 Clinical and Laboratory Standards Institute (CLSI). Laboratory Documents: Development and Control; Approved Guideline; Fifth Edition. (GP2-A5, 2006)

10.6 Sysmex Reagents of America Inc., Mundelein, IL. XN CAL, Hematology Calibrators: Calibrators for Sysmex Hematology XN-L Series Analyzers, package insert.

10.7 Sysmex America, Inc., Lincolnshire IL. XN-L Check Hematology Control insert sheet for Sysmex XN-L Series Analyzers package insert.

10.8 Sysmex America, Inc., Mundelein, IL. Sysmex e-CHECK Insight™ Overview Guide, Appendix A-Xm Quality Control, Version 1.0a, 14-September-00.

10.9 Sysmex America, Inc., Lincolnshire, IL. Sysmex Insight™ Overview Guide

10.10 Stewart, Charles and Koepke, John. *Basic Quality Assurance Practices for Clinical Laboratories*, Van Nostrand Reinhold, 1989, p 189

10.11 Cornbleet J. Spurious results from automated hematology cell counters. Laboratory Medicine. 1983:8:509-514.

10.12 Sysmex America Inc., Lincolnshire, IL. XN-L Applications Manual

10.13 Sysmex Reagents of America, Inc. SDS sheets and reagent product inserts.

10.14 College of American Pathologists (CAP) Hematology and Coagulation Checklist.

10.15 BeyondCare^{SM} Quality Monitor for Hematology Instructions for Use

10.16 BeyondCare^{SM} Quality Monitor for Hematology Inspection Guide

11 REVISION HISTORY

Revision No.	Author	Description of Change(s)
01	B Waters-Pick / Kara Hallyburton	New Document

Signature Manifest

Document Number: STCL-EQUIP-023 **Revision:** 01

Title: Sysmex XN-450 Hematology Analyzer Automated Blood Count Procedures for the STCL

Effective Date: 20 Mar 2025

All dates and times are in Eastern Time.

STCL-EQUIP-023 Sysmex XN-450 Hematology Analyzer Automated Blood Count Procedures for the STCL

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Quality

Name/Signature	Title	Date	Meaning/Reason
Bing Shen (BS76)	Associate Director, Quality Assurance	20 Mar 2025, 12:47:31 PM	Approved

Document Release

Name/Signature	Title	Date	Meaning/Reason
Amy McKoy (ACM93)	Document Control Specialist	20 Mar 2025, 01:28:39 PM	Approved