CBC, DIFFERENTIAL AND RETICULOCYTE SYSMEX XN-3100

RC.HM.PR.080.r00

Principle

The Sysmex XN-3100 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 hydrodynamic focusing (DC Detection), flow cytometry (semiconductor laser), and the SLS-hemoglobin method. 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-10 detects the fluorescent light that is emitted sideways.

The Sysmex SP-50 is part of the XN-3100. It 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-10 to determine optimum smearing criteria. The dried smear is automatically advanced to the staining area. The system also provides a manual mode operation where pre-made smears may be stained. The unit is self-monitoring and alarms when operation is interrupted. Slides prepared by the Sysmex SP-50 are used for differentiation and morphologic evaluation of cellular elements in whole blood. Refer to the separate SP-50 procedure for complete instructions for use, maintenance, etc.

In addition, the XN-3100 also has a separate RU-20 water system that is connected to the laboratory DI water system. The RU-20 continually monitors the reverse osmosis water provided to the XN-3100. Using the Type II DI water and Cellpack DST reagent. One RU-20 will supply Cellpack to a maximum of 2 XN-10 analyzers.

The XN-3100 also includes the and software which directs the sample racks from module to module based on orders received from the host.

Specimen Collection and Handling

Type:	Whole blood collected in a 4 mL Vacutainer® tube. This is the preferred sample. OR Capillary blood collected in an EDTA Microtainer® tube [preferably a Raised Bottom Tube (RBT)]
Anticoagulant:	K_2EDTA or Heparinized samples may be used for HGB & HCT. Sodium Citrate may be used for samples that exhibit platelet clumping. Multiply PLT and WBC results by 1.1
Amount:	Whole blood: - Minimum sample size is 2.0 mL. - Optimum sample size is 4.0 mL. Capillary blood - Minimum sample size is 250 µL. - Optimum sample size is 500 µL.
Special Handling:	Closed Mode: The XN-3100 automatically mixes the specimen. Sysmex recommends that samples greater than 4 hours old be gently mixed prior to placing on XN-3100 startyard. Do not place specimen on mechanical rocker. Constant rocking may cause PLT clumping and altered white cell membranes resulting in false interpretative messages. Rim all Microtainer® specimens with wooden applicator sticks to check for clots prior to analysis. Samples containing hemolysis, lipemia, icteria, cold agglutinins or cryoglobulins may give false results (see *CBC Correction Procedure*).
Timing:	**CBCND and CBCWD** Specimen is stable for 8 hours at room temperature and for 72 hours if refrigerated at ~4°C. Allow all samples to come to room temperature before analysis. **RETIC** Specimen is stable for 8 hours at room temperature and for 72 hours if refrigerated at ~4°C. Allow all samples to come to room temperature before analysis.
Criteria for Unacceptable Specimens:	Specimens containing clots, or inappropriate volumes or drawn above the IV, or that have been frozen are unacceptable and must be redrawn.

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, lab coat, and goggles.

Reagents

XN Reagents	Open Expiration	Temperature
Cellpack DCL	60 Days	Room
Cellpack DST	60 Days	Room
Cellpack DFL	60 Days	Room
Sulfolyser SLS	60 Days (1.5L) 90 Days (5.0L)	Room
Lysercell WNR	60 Days	Room
Fluorocell WNR	90 Days	Room
Lysercell WDF	90 Days	Room
Fluorocell WDF	90 Days	Room
Fluorocell RET	90 Days	Room
Fluorocell PLT	90 Days	Room

1. **Cellpack DCL**: Whole blood diluent for use in measuring the numbers and sizes of RBC and platelets for HGB concentration determination and as a Sheath fluid for FCM detector.
    a. Store at 2º-35ºC, away from direct sunlight
    b. If frozen, thaw and mix thoroughly before using
    c. Is clear and colorless
        i. If it is showing signs of contamination or instability such as cloudiness or discoloration, replace
    d. Unopened, it is stable until expiration date printed on the container.
        i. Opened, stable for 60 Days

2. **Cellpack DST** (DST): Concentrated diluent of reagent for use in hematology analyzers and to be used with the RU-20 water system.
    a. Store at 2º-35ºC away from direct sunlight
    b. If frozen, thaw and mix thoroughly before using
    c. CELLPACK DST is clear and colorless
        i. If it is showing signs of contamination or instability such as cloudiness or discoloration, replace
    d. Unopened, it is stable until expiration date printed on the container.
        i. Opened, stable for 60 Days

3. **Cellpack DFL** (DFL): Whole blood diluents for use in hematology analyzers; used in combination with Fluorocell™ RET for the analysis of reticulocytes, or with Fluorocell PLT for the analysis of platelets by flow cytometry method using a semiconductor laser
    a. Store at 2º-35ºC, away from direct sunlight

   b. Do not use the reagent if it is suspected to have been frozen
   c. Replace the reagent if it is showing signs of contamination or instability such as cloudiness or discoloration
   d. Unopened, it is stable until expiration date printed on the container
      i. Opened, stable for 60 Days

4. **Sulfolyser (SLS)**: Reagent for the automated determination of hemoglobin concentration of blood. Sulfolyser is a lysing reagent that releases the hemoglobin to be measured by the SLS hemoglobin method.
   a. Store at 1º-30ºC away from direct sunlight.
   b. Allow the container to equilibrate to environmental temperature (15-30°C) prior to use.
   c. Do not use the reagent if is suspected to have been frozen.
   d. Replace the reagent if it is showing signs of contamination or instability such as cloudiness or discoloration.
   e. Unopened, it is stable until expiration date printed on the container.
      i. Opened, stable for 60 Days (1.5L) or 90 Days (5L).

5. **Lysercell WNR**: Reagent product to be combined and used with Fluorocell WNR. By hemolyzing red blood cells with Lysercell WNR and by differentiating white blood cells (non-basophil), basophils, and nucleated red blood cells with Lysercell WNR and Fluorocell WNR, the white blood cell count, basophil count, basophil percentage, nucleated red blood cell count, and nucleated red blood cell percentage are analyzed.
   a. Store at 2º-35ºC, away from direct sunlight
   b. Allow the container to equilibrate to environmental temperature (15-30°C) prior to use
   c. Do not use the reagent if it is suspected to have been frozen
   d. Replace the reagent if it is showing signs of contamination or instability such as cloudiness or discoloration
   e. Unopened, it is stable until expiration date printed on the container
      i. Opened, stable for 60 Days

6. **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 Fluorocell WDF, the counts and percentages of neutrophils, immature granulocytes, lymphocytes, monocytes, and eosinophils are analyzed.
   a. Store at 2º-35ºC, away from direct sunlight
   b. Allow the container to equilibrate to environmental temperature (15-30°C) prior to use
   c. Do not use the reagent if it is suspected to have been frozen
   d. Replace the reagent if it is showing signs of contamination or instability such as cloudiness or discoloration
   e. Unopened, it is stable until expiration date printed on the container.
      i. Opened, stable for 90 Days

7. **Fluorocell WNR**: Used to stain the nucleated cells in diluted and lysed blood samples for determination of white blood cell count, nucleated red blood cell count and basophil count in blood
   a. Store at 2º-35ºC in a dark place
   b. Do not use the reagent if it is suspected to have been frozen

   c. Unopened, it is stable until expiration date printed on the container
      i. Opened, stable for 90 Days

8. **Fluorocell WDF**: Used to stain the leukocytes in diluted and lysed blood samples for determination of differential count in blood
   a. Store at 2º-35ºC in a dark place
   b. Do not use the reagent if it is suspected to have been frozen
   c. Unopened, it is stable until expiration date printed on the container
      i. Opened, stable for 90 Days

9. **Fluorocell RET**: Used to stain the reticulocytes in diluted blood samples for the assay of reticulocyte count, reticulocyte percent and platelet count in blood.
   a. Store at 2º-35ºC in a dark place
   b. Do not use the reagent if it is suspected to have been frozen
   c. Unopened, it is stable until expiration date printed on the container
      i. Opened, stable for 90 Days

10. **Fluorocell PLT:** Used to stain the platelets in diluted blood samples for the assay of platelet counts in blood
    a. Store at 2º-35ºC in a dark place
    b. Do not use the reagent if it is suspected to have been frozen
    c. Unopened, it is stable until expiration date printed on the container
       i. Opened, stable for 90 Days

11. **Cellclean Auto**: Detergent for fully automated hematology analyzers. To be used as a strong alkaline detergent to remove lysing reagents, cellular residuals, and blood proteins that may remain in the hydraulics of the analyzer. Use as a cleaning fluid for the hematology analyzers and the SP-50.
    a. Store at 1-25ºC, away from direct sunlight
    b. Do not use the reagent if it is suspected to have been frozen
    c. Unopened, it is stable until expiration date printed on the container
       i. Discard after each use.

Calibration and Maintenance

1. Calibration procedure can be found in Attachment A. The service engineer sets the differential measurement devices for optimum performance.
2. Startup/maintenance procedures can be found in Attachment B.

Quality Control

Sysmex has developed model-specific control range limit percent's (%) to help better manage and/or identify changes in control results through the introduction of Six Sigma based ranges. These range limit percents (%) have been developed using Six Sigma methods, which use evidence such as, parameter-specific performance goals, bias, and CV. These changes will allow the operator to identify changes in analyzer performance, and detect appropriate error detection with minimal false rejections.
Sysmex Evidence-Based control limits are calculated using a performance goal of 4 Sigma, Insight™ average parameter bias, and analyzer variability (CV). The HCT, MCV, and MCHC control limits are calculated using 3.5 Sigma goals due to slight RBC swelling during the use of

the lot number. The established control limit provides a percent limit for each parameter that includes control performance, analyte test availability, allowable bias and performance goal. The Sysmex Evidence-Based control limits were derived from 6 cumulative Insight™ reporting periods to calculate the limits. Using cumulative data incorporates analyzer and control material performance throughout the lot life of controls.
It is recommended that action be taken when Insight™ comparisons indicates a parameter bias +/- 3 SDI, and/or parameter CV significantly higher than historic or group recovery.

COMMERCIAL CONTROL MATERIAL

XN Check

1. Quality control is performed in order to monitor an analyzer's performance over time and should be run in accordance with licensing agency regulations.
    a. It should be noted that for troubleshooting purposes, additional control runs may be necessary.
2. XN Check is a whole blood commercial control used to monitor performance of XN analyzers. It is manufactured by Sysmex and is available as a tri-level package with each vial containing 3 mL of control material.
3. XN Check consists of human red and white blood cells with a platelet component suspended in fluid medium. The sample includes cellular components for evaluating neutrophils, lymphocytes, monocytes, eosinophils, basophils, immature granulocytes (including bands, metamyelocytes, myelocytes and promyelocytes), nucleated RBCs, and reticulocytes.
4. Stability:
    a. Store vials at 2-8ºC. Unopened and properly stored, XN CHECK is stable until the expiration date printed on the unopened vial (84 days). Open vial stability is 7 days when promptly refrigerated after each use. Record the expiration date on each vial upon opening or cap piercing.
    b. Do not freeze or expose to excessive heat.
    c. Heat or freezing can damage XN CHECK without gross visible changes.
        i. Moderate hemolysis can be normal.
        ii. Deterioration is suspected when the mean of the control results is not within the assay expected ranges after appropriate troubleshooting.
5. If deterioration is suspected, call the Sysmex Technical Assistance Center. 1-888- 879-7639 (1-888-8SYSMEX)

Special Safety Precautions

WARNING: POTENTIALLY INFECTIOUS MATERIAL
The human blood used in the 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.

Procedure for Specimen Processing

Run online with the WAM. Resulting is performed in the WAM. Results are then transmitted to the laboratory information system (LIS).

PATIENT PROCESSING (AUTOMODE)

1. Place specimens in a rack. Ensure that labels are smooth with no loose edges and that samples contain appropriate volume.
2. Place the rack onto the feeder section of the XN-3100 start yard, ensuring that the groove on the bottom of the rack is properly seated in the feeder. The system will recognize the rack and automatically transport the rack to the BT-40 barcode terminal. Here the host computer is queried for the sample number and the analysis order. The rack barcode is also read here.
3. The rack will then be transported to the appropriate analyzer. The system is set to load balance the volume of work and distribute the samples across the 2 XNs, as they are available. Once sample analysis is complete, the rack will travel and pool at the stock yard.
4. DO NOT remove racks or samples that are being processed by the system.
5. The XN automatically mixes the sample 8 times, aspirates 88 µL of whole blood, and analyzes the sample according to the barcode discrete order.

IF BARCODE READ ERRORS OCCUR:
Once samples have been analyzed, correct the sample number in the XN Sample Explorer.

1. Click on [SAMPLE EXPLORER] in the tool bar. Verify “LAST 20” icon is de-selected.
2. Highlight the line with the sample that requires correction of sample order number.
3. Click on [VALIDATE] in tool bar to unvalidate the data. (“V” disappears in the first column of Sample Information Tab.)
4. Click on [MODIFY] on the tool bar.
5. Enter the correct accession number in the [Sample No.] field.
6. Click on [OK] to save new ID.
7. Click on [VALIDATE]. The sample results will be sent to WAM automatically.

SEND RESULT TO LIS:
NOTE: Unvalidated data may not be reprinted or retransmitted.

1. Click on [SAMPLE EXPLORER] in the tool bar. Verify “LAST 20” icon is de-selected.
2. Highlight sample(s) by ID number to reprint or retransmit.
3. Click [OUTPUT] in tool bar.
4. To reprint to GP, choose [Report (GP)].
5. To retransmit to LIS, choose [Host Computer (HC)].

PATIENT PROCESSING (MANUAL MODE WITH BARCODE)

1. Press [Mode Switch] button on the analyzer. Make sure analyzer is in Ready state.
2. Touch the [Manual Analysis] button on the control menu.
3. Confirm that ‘Read ID’ is checked.
    a. If running an RBT, ensure RBT is checked.
4. Mix the sample thoroughly, place in appropriate sample tube holder
    a. Front sample holder is for **capped** full size EDTA tubes and RBT.
    b. Back sample holder is only for **small uncapped** Microtainer tubes that do **NOT** have the raised bottom.
5. Press Start switch.
6. Sample barcode will be read and the appropriate testing will be run according to the order.
7. After aspiration, the tube holder will slide out. Remove the sample.

8. Press the [Mode Switch] button to return to Sampler mode.

PATIENT PROCESSING (MANUAL MODE WITHOUT BARCODE)

1. Press [Mode Switch] button on the analyzer. Make sure analyzer is in Ready state.
2. Touch the [Manual Analysis] button on the control menu
3. Uncheck 'Read ID' box.
4. Scan sample barcode in making sure that 'Query the Host' is checked.
    a. **Note**: If you are running a dilution or body fluid, check the **'Cap Open'** box.

WARNING: Potentially biohazardous exposure when handling open patient specimens. Follow *Body Substance Isolation Procedures* outlined by Laboratory Safety Guidelines. Wear gloves, lab coats, and goggles. Use gauze when opening.

5. **Mix the sample thoroughly**, place in appropriate sample tube holder
    a. Front sample holder is for **capped,** full size EDTA tubes and RBT.
    b. Back sample holder is only for **small uncapped** Microtainer tubes that do **NOT** have the raised bottom.
6. Press 'Start switch.
7. After aspiration, the tube holder will slide out. Remove the sample.
8. Press the [Mode Switch] button to return to Sampler mode.

RESULTING

1. Resulting is performed in the WAM.
2. Results are then transmitted to the LIS. Samples are autoverified when all of the following criteria are met:
    a. WBC 2.0-20.0 (inclusive) AND
    b. HGB 8.1-18.0 (inclusive) AND
    c. MCV 66-114(inclusive) AND
    d. RDW-CV <25.0 AND RDW-SD <70.0 AND
    e. PLT 75-1000 (inclusive) AND
    f. MCHC 30.0-38.0 (inclusive) AND
    g. NRBCs <464.0
    h. Neuts ≥0.5 AND
    i. Lymphs 0-10.0 (inclusive) AND
    j. Monos ≤3.0 AND
    k. Eos ≤4.0 AND
    l. IG ≤10.0% AND
    m. Retic ≤23% AND
    n. No delta checks (within 72 hours) AND
    o. No instrument flags
3. Refer to the WAM Op Alerts for operator actions to be taken when resulting.
4. Other actions/(guidelines) to be taken:
    a. **WBC, neut, or lymph count less than 0.1**: Report as "<0.1".
    b. **LEFT SHIFT:** No action needed.
    c. **HGB DEFECT:** No action needed.
    d. **RET ABN SCATTERGRAM:** Send specimen and printout to Core Lab for verification. Do NOT use the Pre-dilute mode on the XN.

e. **THROMBOCYTOPENIA:** This flag occurs when the impedance platelet count is less than 75 bill/L. A fluorescent PLT will be reflexed. If first occurrence, order a manual smear in WAM Action Box and place the rack back on the line so a smear will be made by the SP-50. Alternately, make a smear manually and stain it in manual mode on the SP-50. **Always check for clot when PLT <75, regardless if patient has been running that way.** Document as an internal comment in WAM Action box:
    i. PLT counts <75 with no clot and **no** flags may be accepted by the HST tech if PLTS <75 were previously verified by smear.
    ii. The comment: "PLT verified by alternate method" will automatically drop into the PLT comment field.
    iii. If there are no previous results, refer to morphology bench for verification with a stained smear.

f. **Asterisks next to parameter:** The WAM has been programmed to recognize XN results associated with asterisks. Refer to morphology bench for review. Check stained smear for NRBCs, clumped/giant platelets, or cryoglobulins. If specimen is (has):
    i. **Clotted:** Request redraw. Cancel specimen in LIS and in WAM.
    ii. **Clumped platelets:** Refer to morphology bench for verification with stained smear after first **checking for a clot**. If "Clumped in EDTA" for PLT estimate is selected in the WAM, the numerical PLT value will automatically be replaced with "See comment". (See CBC Corrections Procedure for example).
    iii. **Giant platelets:** If stained smear estimate is in disagreement with instrument count, add comment: "WBC may be inaccurate due to WBC/PLT interference" to the WBC field.
    iv. **Nucleated RBCs:** Correct WBC count (manually or XN reflex). (See CBC Corrections Procedure).
    v. **IG Asterisk Error:** If result matches previous history, no scan is required. If there is no history or there are other flags present that warrant a slide review, the slide must be reviewed.

g. **High take-off on WBC:** Indicative of cryoglobulins. Send to Core Lab for verification. Do NOT use the Pre-dilute mode on the XN.
    i. **NOTE**: Verify WBC and PLT estimate on smear. Falsely increased WBC and PLT results have been known to be caused by cryoglobulins.

h. **Voted out differentials** (-------): The XN-3100 will automatically send the specimen to the SP-50 for a slide.

i. **Results are voted out (-------):** Data suppressed due to analysis or function error. Check specimen for clots or QNS. If no clot or not QNS, rerun. If clotted or QNS, request redraw. Cancel specimen in LIS and in WAM.

j. **MCHCs less than 30:** SUSPECT AGED SPECIMEN. Check for accompanying low MCV.
    i. **NOTE:** If MCHC are consistently outside the 30.0-37.0 range sample after sample, suspect an instrument problem.

k. **Turbidity/HGB Interference:**
    i. **MCHC >38.0 –** Check previous results (HGB, if previously lipemic/ icteric/ possible cold agglutinin) Send specimen and printout to Core Lab. Do NOT use the Pre-dilute mode on the XN.

l. **PLT abnormal distribution flag: If no other flags-** No action needed.

m. **PLT delta check:**

i. **If decrease in PLT count: Check for a clot.** Review if results are trending or if this is a one- time occurrence. Review smear if first time <75, and/or if platelet count is <101. If no clot found, report platelet results and add the following comment: "*Results in question; suggest repeat if not compatible with clinical picture*".
ii. **If increase in PLT count:** Check if results are trending or if this is a one-time occurrence. If results appear to be out of context, verify patient B#, check if patient received any PLT.

n. **PLT count greater than 5000:** Send specimen and printout to Core Lab. Do NOT use the Pre-dilute mode on the XN.
o. **Suspect Flag "Fragments?":** Sample will be sent to the reflex analyzer for fluorescent PLT. The XN judges if the fluorescent PLT count should be reported.
p. **Elevated MCV with decreased MCHC:** Check chart review to see if patient is hyperglycemic (Glucose >600 mg/dL). If so, send specimen and printout to Core Lab. Do NOT use the Pre-dilute mode on the XN.
q. **WBC count greater than 497.51:** Send specimen and printout to Core Lab. Do NOT use the Pre-dilute mode on the XN.
r. **RBC count greater than 9.1:** Send specimen and printout to Core Lab. Do NOT use the Pre-dilute mode on the XN.
s. **HGB value greater than 27.0:** Send specimen and printout to Core Lab. Do NOT use the Pre-dilute mode on the XN.
t. **HCT value great than 78.0:** Send specimen and printout to Core Lab. Do NOT use the Pre-dilute mode on the XN.
u. **Delta Check HGB:** If no previous results, check for clots and put an internal comment in WAM. If clotted, request redraw and cancel in LIS and WAM. Verify B number on the tube, or if patient was transfused, etc. Enter comment: "*Rechecked and verified*". A comment must be documented for all delta checks.
v. **Delta Check for MCV:** Check history and enter comment next to MCV value: "*Rechecked and verified*" (HE01), result in question (HE08), or result change from previous (HE38). A comment must be documented for all delta checks in WAM.

Expected Values

1. Refer to the "Hematology Normal Ranges" procedure for current normal ranges.
2. Refer to the "Critical Values and Response for Hematology Results" procedure for current critical values.

Limitations

1. **WBH XN-SERIES ESTABLISHED LINEARITY**

Parameter	Range	Units
WBC	0-497.5	$x10_3$/mcL
RBC	0-9.14	$x10_6$/mcL
HGB	0-26.9	g/dL
HCT	0-77.6	%
PLT	0-5487	x103/mcL
PLT-F	0-5960	x103/mcL
RET %	0-31	%
RET Abs #	0-470	bil/L

2. Parameters that exceed the manufacturer's limits are flagged with @ beside the result. The sample must be diluted, rerun in manual mode and multiplied by the dilution factor. Send specimen and printout to Core Lab. Do NOT use the Pre-dilute mode on the XN.

Interfering Substances

1. Specimens must be free of clots and fibrin strands. Clotted specimens may exhibit decreased PLT, WBC, RBC, HGB, and HCT results.
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 do not interfere with Sysmex automated platelet counts due to instrument moving thresholds.
4. Cold agglutinins produce spurious macrocytosis, elevated MCHs, MCHCs, falsely decreased RBC counts and HCT's. Rare warm agglutinins produce the same spurious results as a cold agglutinin.
5. Severely hemolyzed samples (in vitro) falsely decrease RBC and hematocrit. Recollect hemolyzed specimens.
6. Platelet clumping and/or "platelet satellitism" can occur in specimens collected in EDTA and may falsely increase the WBC and falsely reduce the platelet count. Specimens collected in Sodium Citrate anticoagulant may be analyzed in conjunction with a K2EDTA specimen if requested by physician. Multiply instrument result obtained on the sodium citrate tube by 1.1 dilution factor. Validate results with smear reviews, on slides made from both lavender and blue top tubes.
7. Abnormal paraproteins found in Multiple Myeloma patients can falsely increase the HGB. Send specimen and printout to Core Lab. To correct HGB, core lab will perform plasma replacement or plasma blank procedure.
8. Lipemia falsely elevates the HGB and MCHC. Send specimen and printout to Core Lab, where a plasma replacement or plasma blank procedure will be performed. Do NOT use the Pre-dilute mode on the XN.
9. Severely icteric samples may falsely elevate the HGB value and related indices. Make a 1:2 dilution with Cellpack DCL and run in manual mode. Place dilution factor in WAM rerun tab and results will automatically be multiplied by dilution factor.
10. Megakaryocytes may falsely increase WBC counts on automated hematology analyzers.
11. Unknown interferences may also adversely affect results obtained from the instruments. It may be necessary to use a combination of correction procedures to obtain valid results, as indicated in the *CBC Corrrections* procedure. Send specimen and printout to Core Lab for analysis.

Notes

1. If any type of unexpected result is obtained, investigate thoroughly (Blood Bank query, previous result tab in WAM, LIS test history, admitting diagnosis, other lab work,

possible contamination, contact nurse/physician, etc.) before determining whether those results should be released or canceled.

2. Samples stored at room temperature may exhibit an increase in MCV after 8 hours; the increase may be minimized by refrigeration. Allow all samples to come to room temperature before analysis.

3. Keep bloods at 4°C after testing.

4. Type: “Repeated” as an internal WAM comment if rerunning a specimen to check a parameter that differs greatly from previous results.

5. When RDW results are (-----), the WAM will automatically enter #nm which translates to “Unable to Perform” in LIS. This flag is due to a dimorphic RBC population. Scan slide if differential is ordered and no previous CBCWD. Report applicable RBC morphology.

6. Nucleated RBC’s: All samples run on the XN will have a NRBC count performed and WBC corrections are automatically made. To view the uncorrected (WBC + NRBC) count, locate patient # in the IPU, go to Browser, and then click on the Service tab, WNR. The uncorrected WBC is the TNC-N count.

7. Megakaryocytes: When megakaryoctyes are present, perform a fluorescent platelet count and a WBC and PLT estimate.

8. IRF of 0.0: Report as “<0.02”. Do not report the IRF if there is an instrument flag (RET Abnormal Scattergram) message.

9. Analysis of the specimen on XN-SERIES is recommended before removing the cap to make a smear.

10. For troubleshooting specifics refer to the *Sysmex XN-3100 Instructions for Use* Manual.

Special Notes

“CBC-Differential” Specimens
Slides must be scanned when there is a **first time occurrence** of the following:

1. PLT # <75
2. Neut # <0.5 bil/L
3. >60% Lymph (age >12 months and <18 years)
4. Lymph # >15.0 bil/L
5. Mono **#** ≥3.0 bil/L
6. MCV <66 or >114

Slides must **always** be scanned when:

1. Any IP flag (Blast/Abn Lymph?, Atypical/Abnormal Lympho, etc.) **Exception: Left Shift, PLT Abn Dist (with no other flag) or Atypical Lympho flag with lymphocytes <50.0%.**
2. Previously reported blasts.
3. Platelet clumps

NOTE: For differential specimens requiring a scan, the WAM will validate only the CBC parameters.

NOTE: Some differential specimens will be automatic manual diffs such as incomplete differential computation, etc.

NOTE: For results in question, samples that require dilution, or further verification- notify Core Lab Hematology and send the sample and XN print-out to the Core Lab Hematology.

Attachments

Attachment A – Sysmex XN-3100 Calibration
Attachment B – Sysmex XN-3100 Startup/Maintenance
Attachment C – Quality Control
Attachment D – Reagent Replacement
Attachment E – Scan Guidelines

References

- Microtainer and Vacutainer are registered trademarks of Becton, Dickinson and Company.
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Authorized Reviewers

Chair, Pathology and Laboratory Medicine
Medical Director, Hematology

ATTACHMENT A
Sysmex XN-3100 Calibration

Calibration

Initial calibration is performed during installation by the Sysmex Field Service Representative. Calibrations are also performed on an ongoing basis by a Sysmex Field Engineer, only with direction from Sysmex will the Supervisor or designee do this.
Calibration is performed 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 will also be 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.
- Reagent changes (change in type of reagent from same manufacturer or change to a
- different vendor)

Calibration verification may be performed 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

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.

Calibrators:

XN CALTM: for use in calibrating the analyzer for WBC, RBC, HGB, HCT, PLT and RET

a. Store the calibrator in a dark refrigerator at 2-8ºC
b. Unopened and properly stored, XN CAL is stable until the expiration date printed on the unopened vial.
c. Open vial stability is 4 hours

XN CALTM PF: for use in calibrating the analyzer for PLT-F (platelet count obtained from the PLT-F channel)

a. Store the calibrator in a dark refrigerator at 2-8ºC
b. Unopened and properly stored, XN CAL PF is stable until the expiration date printed on the unopened vial
c. Open vial stability is 4 hours

Precision Check:

1. Perform daily and weekly maintenance on the analyzer.
   a. Check background count to ensure counts are within acceptable limits
   b. Verify that there is sufficient volume of reagents. Precision and Calibration procedures will be aborted if the XN runs out of reagent.
2. Obtain a sample of fresh normal whole blood. Do not use commercial controls or calibrators for precision. The blood donor specimen should:
   a. Be from a healthy person who is not taking any medication.

b. Have a morphologically and numerically normal CBC.
c. Be drawn in a potassium EDTA anticoagulant tube using proper collection technique.
d. Have a minimum of 2.5 mL of sample.

3. On the main unit confirm the LED is green indicating the analyzer is ready.
    a. If the tube holder has not ejected, press the mode switch.
4. Select the Change Analysis Mode button on the control menu and select Whole Blood
5. Select [OK] to close the dialog box
6. Select the Analyzer menu button on the control menu
7. Select [Calibration] – [Precision Check]
8. Mix the vial containing the sample
    a. 10 end-over-end inversions confirming cell button is dispersed
9. Place the vial in the sample tube holder
10. Press the start switch on the analyzer
    a. The analysis is automatically performed 11 times consecutively with the tube holder pulled into the analyzer
    b. The tube holder will slide out when analysis is complete
11. The results are displayed in the [Precision Check] analysis dialog box.
    a. 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.
    b. Select and redo the manual analysis
12. When all analysis results satisfy the conditions, select [OK] in the dialog box.
13. 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.

Calibration – XN CAL

1. On the main unit confirm the LED is green indicating the analyzer is Ready.
2. If the tube holder has not ejected, press the mode switch
3. Select the Change Analysis Mode button on the control menu and select Whole Blood
4. Select [OK] to close the dialog box
5. Select the Analyzer menu button on the control menu
6. Select [Calibration] – [Calibrator Calibration]
7. Mix the vial containing the calibrator according to package insert
8. Place the vial in the sample tube holder
9. Press the start switch on the analyzer
    a. The analysis is automatically performed 11 times consecutively with the tube holder pulled into the analyzer
    b. The tube holder will slide out when analysis is complete
10. The results are displayed in the [Calibrator Calibration] analysis dialog box.
11. 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.
    a. Select and redo the manual analysis.
12. When all analysis results satisfy the conditions, select [Calibration] in the dialog box.
13. Select [OK] to display results in the [Calibrator Calibration] execution dialog box.

14. 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:
    a. 80% < New Rate < 120%
    b. New Rate – Current Rate < +5
    c. Range Value < Max Range
    d. Acceptable Limit < Delta Percent < Service Limit
        1) 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.
        2) 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.
            a) Calibration is performed with the parameter excluded.
        3) Selecting the check box enables you to manually enter a value in [New Rate (%)].
            a) A range of 80% to 120% may be entered.
15. Select [OK] to update the compensation rates.
16. The calibration process is logged in the calibrator calibration history.

Calibration – XN CAL PF

1. On the main unit confirm the LED is green indicating the analyzer is Ready.
2. If the tube holder has not ejected, press the mode switch.
3. Select the Change Analysis Mode button on the control menu and select Whole Blood.
4. Select [OK] to close the dialog box.
5. Select the Analyzer menu button on the control menu.
6. Select [Calibration] – [Calibrator Calibration (PLT-F)].
7. Mix the vial containing the calibrator according to package insert.
8. Place the vial in the sample tube holder.
9. Press the start switch on the analyzer
    a. The analysis is automatically performed 11 times consecutively with the tube holder pulled into the analyzer.
    b. The tube holder will slide out when analysis is complete.
10. The results are displayed in the [Calibrator Calibration (PLT-F)] analysis dialog box.
11. 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.
    a. Select and redo the manual analysis.
12. When all analysis results satisfy the conditions, select [Calibration] in the dialog box.
13. Select [OK] to display results in the [Calibrator Calibration (PLT-F)] execution dialog box.
14. 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:
    a. 80% < New Rate < 120%
    b. New Rate – Current Rate <+5
    c. Range Value < Max Range
    d. Acceptable Limit < Delta Percent < Service Limit

15. 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.
16. 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.
17. Selecting the check box enables you to manually enter a value in [New Rate (%)].
    a. A range of 80% to 120% may be entered.
18. Select [OK] to update the compensation rate.
    a. The calibration process is logged in the calibrator calibration history.
19. All levels of QC should be performed after calibration to verify the calibration procedure.
    a. If controls do not fall within limits, the calibration should be repeated.
    b. Do not process patients unless the calibration has passed with acceptable QC performance.
    c. Print and place QC results on supervisor's desk.
20. After 10 runs post calibration, re-autoset all QC parameters.

NOTE: It is the belief of the Hematology and Clinical Microscopy Resource Committee (HCMRC) that verification with a single calibrator sample, coupled with appropriate background count, should be sufficient to indicate that an instrument is performing accurately over the entire patient reportable range. The HCMRC also believes that calibration is confirmed if the quality control results are within their limits. They also believe that it is not necessary to use the new hematology linearity materials available commercially to verify calibration of hematology analyzers. Our Laboratory also has the same beliefs and is following above.

WARNING: POTENTIALLY INFECTIOUS MATERIAL.
The human source materials from which this product was derived has been found non-reactive for HBsAG, HCV and HIV using FDA specified techniques. However, no current tests can assure the absence of these pathogens. SCS-1000 calibrator must be handled like human blood.

References

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- College of American Pathologists (CAP): *Hematology Checklist*.
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ATTACHMENT B
Sysmex XN-3100 Maintenance

I. DAILY MAINTENANCE

Each XN analyzer will be shutdown daily by the midnight shift.

Pre-operational Checks

1. Refill printer with paper.
2. Check Pneumatic Unit trap chamber for fluid.

A. XN Shut Down/Start Up Procedure

1. Make sure that the analyzer and the sampler are in the ready state.
2. Make sure the tube holder is retracted into analyzer.
3. Shutdown may be performed in the manual mode or sample mode. Routinely shutdown will be performed in manual mode.
    1. **Manual Mode**:
        a. At the IPU, click on the analyzer menu button for the analyzer you will be shutting down. A menu appears. Choose Shutdown.
        b. The analyzer will automatically eject the sampler. Place a new CELLCLEAN AUTO tube in the front sampler and press blue start button.
        c. Percent progress of the Shutdown displays on the IPU in the respective display box.
        d. Once Shutdown is complete, the IPU will display a Restart prompt. Click on it. Once complete, the analyzer will display the ready state. IPU will prompt you when to remove the CELLCLEAN AUTO tube from the sampler.
    2. **Sampler Mode**:
        a. Place a tube of CELLCLEAN AUTO Position 9 and 10 of a sample rack. Both analyzers will go through shutdown.
        b. Once the barcode is read, Shutdown is performed automatically.
        c. Percent progress of the Shutdown displays on the IPU in the respective analyzer display box.
        d. Once Shutdown is complete, the analyzer will display the ready state.
4. Document all maintenance performed in the XN3100 maintenance logs.

Note: The analyzers will be shutdown daily at separate times ensuring that at least one analyzer is operational at all times.

Note: Acceptable XN Background count values-

Checked Parameter	Acceptable Values	Explanation
WBC-N	0.10 x 10^3/µL or less	WBC counted I the WNR channel
WBC-D	0.10 x 10^3/µL or less	WBC counted I the WNR channel
WBC-P	0.10 x 10^3/µL or less	WBC counted I the WNR channel

RBC	0.02 x 10^6/µL or less	-
HGB	0.1 g/dL or less	-
PLT-I	10 x 10^3/µL or less	PLT counted in the RBC/PLT channel (PLT particle size distribution)
PLT-F	3 x 10^3/µL or less	PLT counted in the PLT-F channel

II. WEEKLY MAINTENANCE

Once per week, the XN3100 will be completely shutdown per recommendations from Sysmex. At this time, the IPU's and the CT-90 controller transporter system will be shutdown.

A. **Full Shutdown Procedure of XN-3100**

1. **IPU Shutdown:** Power down both of the IPU's:
   a. Click on 'Exit IPU' in Main menu
   b. Log off of Windows program by clicking on the start icon in left corner of IPU and click on 'Shutdown'.
   c. Wait for the IPU to completely power down. Allow system to stay powered down for 2-5 minutes.

Start Up Procedure

a. After 2-5 minutes, press and release the green master start up switch.
b. Each attached conveyor will start (indicated by the green status indicator LED)
c. The IPU's will automatically turn on.
d. An audible alarm will sound and display an error "RU has stopped supplying reagents". This error resolves itself as the analyzer completes the start-up process.
e. If any XN was in a shutdown status, it will begin start up.
f. The XN IPU's will display the log on screen.
g. Log on username: xn and Password: none
h. Touch 'OK'

III. As Needed

Occasionally, the following maintenance procedures may need to be performed. The procedures can be found in the Sysmex XN Series Instructions for Use Manual, Chapter 13-Performing Maintenance of Instrument and Replacing supply parts.

a. Rinse flow cell
b. Remove air bubble from flowcell
c. Drain the waste chamber
d. Adjusting the pressure (0.25 MPa, 0.16 MPa, 0.07 MPa)

ATTACHMENT C
Quality Control

Quality assurance includes routine maintenance and service in conjunction with the use of controls and calibrators. The combination of these methods provides the assurance of complete quality control. For both the CBC and CBC/Diff. parameters, it uses the established technique of commercial controls. The purpose of running commercial controls is to verify instrument accuracy and to identify any shifts or trends in parameters. In addition, the use of low, normal and high controls will verify the instrument's linearity.

A new XN-Check Lot is shipped every 56 days and an Insight Interlaboratory Quality Assessment Report is generated every month.

A. CONTROL MATERIAL

XN-Check is a tri-level whole blood commercial control for use with the Sysmex XN-series hematology analyzers.

1. **Ingredients**

   XN-Check consists of human red and white blood cells with a platelet component suspended in fluid medium. The sample includes cellular components for evaluating Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils, Immature Granulocytes (including Bands, Metamyelocytes, Myelocytes and Progranulocytes), Nucleated RBC's and Reticulocytes.

   Each vial contains 3.0 mL of control material. XN-Check is provided in 3 levels: Abnormal Low, Normal, and Abnormal High concentration.

2. **Storage**

   Vials should be stored in the upright position, at 2-8 degrees Celsius. DO NOT FREEZE or expose to excessive heat.

3. **Stability**

   The unopened product is stable until the expiration date stated on the control. Opened vial stability is 7 days if handled properly. Heat or freezing can damage *e*-Check without gross visible changes. Moderate hemolysis can be normal. Deterioration is suspected when the laboratory mean of several days control results is not within the assay expected range. If deterioration is suspected, call Hotline at 1-888-879-7639.

WARNING: POTENTIALLY INFECTIOUS MATERIAL
The human blood used in XN Check is not-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.
Recommended: wear gloves, lab coat, and goggles.

B. PROCESSING CONTROLS

Remove vials from refrigerator. Allow at least 15 minutes to warm to room temperature. Mix vial by gentle end to end inversion until the cell button in the bottom of the vial is completely suspended (approximately 20 inversions). Do not mix mechanically.
Control materials are run in the same manner as patient samples.
Patient results cannot be reported until any out of range control situation is resolved. Return vials to refrigerator as soon as possible, preferably within 30 minutes.

C. FREQUENCY OF CONTROL USE

Three levels of the XN-Check controls are run each day.

DAYSHIFT	PM SHIFT	MN SHIFT
L1	L2	L3

D. ESTABLISHING COMMERCIAL CONTROL LIMITS

Sysmex has developed model-specific control range limit percent's (%) to help better manage and/or identify changes in control results through the introduction of Six Sigma based ranges. These range limit percent's (%) have been developed using Six sigma methods, which uses evidence such as parameter-specific performance goals, bias and CV. These changes will allow the operator to identify changes in analyzer performance, and detect appropriate error detection with minimal false rejections.

Sysmex Evidence-Based control limits are calculated using a performance goal of 4 Sigma, Insight average parameter bias, and analyzer variability (CV). The HCT, MCV, and MCHC control limits are calculated using 3.5 Sigma goals due to slight RBC swelling during the use of the lot number. The established control limit provides a percent limit for each parameter that includes control performance, analyte test availability, allowable bias and performance goal.

The Sysmex Evidence-Based control limits were derived from 6 cumulative Insight reporting.

E. PREPARING QC FILES

1. **Entering Lot Information for a New Lot of Controls**

File Setup
a. Select [QC file] Icon
b. Select TAB for analyzer from bottom of QC File Screen
    i. The files must be setup on all XN's (both the XN-R and XN-L at the IPU).
c. Select File number to be registered
d. Select [Register] button on toolbar
e. Enter lot information
    i. QC level from drop down
    ii. Lot Number
    iii. Expiration Date
f. Select [Restore]
    i. Browse XN QC Limit % REV 02 folder on XN IPU desktop folders
    ii. Select file for QC to be registered

iii. Select Open
iv. Sysmex Range Limit %'s will automatically upload to the file.
g. Repeat steps a-f to enter lot information for the other levels of control and repeat for each analyzer.

Run each level of the new lot once into the designated QC file and check that all parameters fall within the package assay range. If they fall outside the range, call the hotline – may be due to product damage.

2. **Establishing Target Means for New Control Lots (LOT PARALLEL)**

A. Run the 3 levels of QC once per shift for 5 days prior to the previous lot number expiring.
B. After a minimum of 10 data points are accumulated, auto set the targets
a. Select QC chart
b. Double click File to be targeted
c. Select [Range] and drag cursors so that every data point is included.
d. Select [Modify]. This opens up the QC file set up window
e. Highlight all parameters by clicking on WBC and dragging to the last parameter and select [AutoSetting].
f. Confirm that the check box for TARGET ONLY is set. Do not select the check box for LIMIT.
g. Select [OK]; the target for each parameter will be calculated and set for the duration of the QC lot.
h. Repeat steps for each new lot of QC being moved to production.
i. Confirm the target set falls within the range of means provided on the XN Check assay sheet provided.
j. Notify the supervisor if he laboratory mean for any parameter is not within the manufacturer's assay range. Call the hotline.

F. RUNNING CONTROLS

A. Remove vials from refrigerator. Allow at least 15 minutes to warm to room temperature. Mix vial by gentle end to end inversion until the cell button in the bottom of the vial is completely suspended (approximately 20 inversions). Do not mix mechanically.
B. Place the QC vial(s) in a sample rack
C. Place rack on sampler unit, sampler unit will auto-start
D. Results will be plotted on the L-J chart as well as the Radar chart for review.
1. Use the scroll bar on the right of the charts to view all the parameter.
E. Controls must be within acceptable ranges prior to accepting patient results.
1. Reruns of any out of range QC points should be performed in the manual mode.

G. EVALUATION OF DAILY CONTROL RESULTS

A. Daily review of QC:
1. The XN operator for the day will open the QC file screen.
2. Choose level of QC to review
a. Radar chart format only allows for review of the latest QC results.

3. Double click line item or click QC Chart icon at top of screen to reveal all data points in that QC level.
    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.

B. Daily QC Management (Out of control QC)

1. Open L-J graph on out of control level
2. Select [Manage] to document actions taken on the QC run that has fallen out of range.
    a. A comment must be added to the QC data selected by the cursor.
        1. Select [Input Any comment] to input a free text comment
        2. Select [Fixed Comments] to use a comment from a list of preset comments in the QC settings menu.
        3. Enter or choose appropriate comment
        4. Select [OK].
    b. A comment bubble will be displayed when a comment exists for a QC run.
    c. The comment will be visible in the comment display area when the cursor is placed on the QC run.
        1. Double click the bubble to view the comment, revise or add more information.
3. After troubleshooting both QC and reagent, run all 3 levels of control and if control(s) is still out contact Sysmex TAC and notify supervisor or designee.
    a. Do not use instrument for patient results until QC issue is resolved.
    b. Determine if the quality control failure(s) are significant enough to possibly alter the results of the previously resulted patients by checking the XBarM graph in the QC file and looking at OpAlerts that have occurred since previous acceptable QC run.
    c. Document on the XN QC problem log actions taken to resolve QC issue.

Note: Patient test results obtained in an analytically unacceptable test run or since the last acceptable test run must be re-evaluated to determine if there is a significant clinical difference in patient/client results. Re-evaluation may or may not include retesting patient samples, depending on the circumstances.

C. Additional QC Evaluation Functions

1. QC charts may be overlaid on top of each other for comparison, either lot to lot on the same analyzer or analyzer to analyzer.
    a. Open QC file to analyze.
    b. Open [Ref]
        i. Select [Compare QC Files] to view QC charts registered to a single analyzer. This will compare the new lot with the old/current lot.

ii. Select [Compare Analyzers] to compare QC files for the same material registered to different analyzers. This will overlay instruments QC files (on the same wagon) over each other to check for how the instruments correlate with each other.

J. END OF REPORTING PERIOD

Quality control from the XN analyzers is submitted to the Insight program dynamically for peer group comparison. Our laboratory maintains an SNCS connection to the Sysmex Insight QAP program. Each QC result will transmit automatically to Insight after each run. Laboratory QC data will be compared to peer group data using the Control Limit % established by Sysmex. Data that exceeds a parameter bias of +/- 3SDI will be reviewed, or when the parameter CV is significantly higher than historic or group recovery. Managed QC points will be evaluated weekly as to whether they should be included for evaluation in peer group data. Outliers that are within the manufacturer's assay range will be included.
The Insight team can be contacted with questions at: 1-888-879-7639.

Managing Insight Data

1. Select the Windows icon in the lower left corner of the IPU screen of the XN.
2. Select the shortcut button to the Windows desktop located in the lower right hand corner of the same screen.
3. Select the Insight icon
4. Enter your login and password.
5. View the opening page and determine if our account has the message that we have QC Exceptions.
6. Locate the Report Center menu on the left side of the page.
7. Select Customer QC Reports
8. Locate QC data menu and select Review QC data.
9. Using the drop down arrows, select the analyzer, level and lot number of QC to be reviewed.
10. Click on "Review Your Data", this will display the QC runs for this selection.
11. Using the range printout, determine if the outliers displayed should be Managed (included in the peer group comparison data) or Not Managed (excluded from the peer group comparison data).
    a. Generally, values within the manufacturer's assay ranges will be included (Managed).
        i. Select the outlier to be Managed/Not Managed
        ii. View the screen and select the radial button for the desired action (Managed/Not Managed)
        iii. Select from the drop down arrow box the canned message that is appropriate.
        iv. Document in the second box any other needed comment.
        v. Click on Save Comment. The pop-up "Your comment was successfully saved" will display.
        vi. Click "Back to Review Data"
        vii. Notice the MA or NM next to the display of the run you just documented.
        viii. Continue this process for each outlier that has occurred.

12. QC Levy-Jenning reports for each period will be printed after Insight Data is managed and before the Period deadline established by Sysmex.

K. REVIEW OF PEER GROUP PERIOD AND END OF LOT REPORTS

Sysmex Insight Period 1 and Period 2 reports will be reviewed by the department Medical Director and supervisor/designee when reports are available. Laboratory values should fall within the range of the peer group comparison report without SD's greater than 2.0. Review the bolded lab SD and CV values. Bold face values will indicate a variance form the peer group data. This lab comparison data can be retrieved at any time and does not need to wait for the end of the specified Period dates.

The SDI represents the number of SD's by which the laboratory mean value for a parameter differs from the peer group. Values outside the +/- 3 SDI range will be flagged in the notes column. An SDI may be a statistically significant accuracy bias and will be flagged with a P (Positive bias). A quick review of this column will readily reveal any potential problems.
The CV measurement is a comparison of precision/imprecision of the analyzer to the peer group. These should be investigated. When a lab's CV is greater than 1 ½ times the groups', it will appear in bold type. It does not necessarily mean that our precision is unacceptable. Both SD and CV should be evaluated to assess the analyzers performance. Contact Sysmex TAC for any necessary help or review the Insight Participant Guide for further troubleshooting tips.

L. PATIENT MOVING AVERAGES – XBARM (XM)

Batch size is set for 20 patient samples.
After every 20 valid samples, the XN will plot a point on the Xm quality control graph. This can be monitored on lieu of a retained patient sample for a longitudinal control (if 100 or more patients are run each day).
Notify supervisor of any discrepancies. Supervisor will review Xm graphs every month.

M. ESTABLISHING XM TOLERANCE LIMITS

Historical limits will be established for Xm by collecting a total of 200 data point representing 4000 samples in 20 patient size batches.
After 200 points are plotted on the Xm chart, auto-set the limits %'s and targets for the Xm chart. Data will be collected over multiple reagent lots and over
Refer to *XE-5000 IPU Operator's Manual*, section 4 for procedure for autosetting.
Print the data (LP-Data). Save this printout for documentation of the new historical limits for Xm. These 180 data points should not be collected in less than one month and not more than 3 months.

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- NCCLS: *Clinical Laboratory Technical Procedure Manuals* – Third Edition; Approved Guideline. (GP2-A3, 1996).

ATTACHMENT D
Reagent Replacement

XN-3100 will alarm and replacement message will be displayed. Determine which analyzer is alarming and which reagent to change. Do not use reagent that is believed to have been frozen.

REAGENT	ABBREVIATION	OPEN EXPIRATION
Cellpack DST	DST	60 days
Cellpack DCL	DCL	60 days
Cellpack DFL	DFL	60 days
Lysercell WBR	WNR	60 days
Lycercell WDF	WDF	90 days
Sulfolyser	SLS	90 days
Flourocell WNR	F-WNR	90 days
Flourocell WDF	F-WDF	90 days
Flourocell RET	F-RET	90 days
Flourocell PLT	F-PLT	90 days

REPLACING THE REAGENT

A. XN Reagent replacement when alarmed.

1. Alarm will sound when reagent replacement is necessary. Do not replace reagents until alarmed to do so.

2. Click “Reset Alarm” to silence alarm and ‘OK’, this also opens the replacement screen.

3. Replace reagent.
    1. Click on reagent to replace
    2. Place new reagent on board and label appropriately.
    3. Place a check in the ‘Replace reagent’ box
    4. With curser in ‘Replace reagent’ box, scan in new reagent. Scan barcode on **top** of box of reagent.
    5. Select [Execute]. Reagent will automatically replenish and a progress box will appear.
    6. When replacement is complete, XN will return to ready state.

B. Reagent replacement from Analyzer Menu

a. Open Analyzer Menu
b. Select Reagent Replacement
c. A pictogram will appear with all of the reagent options and remaining test count.
    1. Select reagent to be replaced by clicking or touching anywhere on the icon for that reagent.
    2. Select [Execute] to open reagent window.
d. Place new reagent on board, place a check in the ‘Replace reagent’ box.
e. With the cursor in the “Reagent Code” box, scan in new reagent.
f. Select [Execute] to replenish new reagent. A progress box will appear and XN will return to ready state once replacement is complete.

C. Reagent Replacement from Status Bar

a. Click on reagent status bar at bottom of instrument status bar (the color coded bar graph)

b. A pictogram will appear with all of the reagent options and remaining test count.

1. Select reagent to be replaced by clicking or touching anywhere on the icon for that reagent.
2. Select [Execute] to open reagent window

c. Place new reagent on board, place a check in the 'Replace reagent' box.

d. With the cursor in the "Reagent Code" box, scan in new reagent.

e. Select [Execute] to replenish new reagent. A progress box will appear and XN will return to ready state once replacement is complete.

D. Replacing Cellpack DST with an RU-20 on the IPU

a. When the Cellpack DST runs out, an alarm sounds. Press [OK] to acknowledge the alarm.

b. On the IPU, click on DST icon at the lower right side of screen.

c. Replace Reagent screen pops up showing the current lot and volume information.

d. Click anywhere on the DST icon and a new lot information screen will appear.

e. Check 'Reagent Replace' box.

f. Place cursor in Reagent Code box and scan in new cube of Cellpack DST.

g. Replacement will begin, a progress box will appear and the XN will return to the ready state once replacement is complete.

E. Replacing Cell DST on the RU-20 Unit

a. Press [Reagent] on the status screen on the RU-20 unit itself.

b. Press [Regist]

c. Scan in the Reagent Code (long barcode on top of reagent cube). Note: If you have difficulty deciphering the letters and numbers of the barcode, do the following:

1. Go to PC and open start menu
2. Choose programs
3. Select Notepad
4. Using Soft barcode reader, scan Reagent Code from DST cube, it will be displayed on the document in a more user friendly font.

d. Press [OK] to register the reagent information.

e. Press [Replace].

f. Replace with new cube of Cellpack DST and press [OK].

F. Replacing Fluorocell Dye Cartridges

a. XN alarms and Help box appears on IPU

b. Reset the alarm and click 'Close'. In the status bar, the yield sign will flash until a new cartridge is in place.

c. Prepare the new reagent cartridge and check expiration on box.

d. Open the XN top front cover.

e. Pull up the cover from the reagent that is to be replaced.

f. Remove old reagent.

g. Replace new reagent ensuring that the color of the label on the new reagent cartridge matches the color of the dye cover.

1. The fluorocell reagents have a radio frequency ID chip (RFID) in the form which the XN retrieves all of the information relating to that specific cartridge.
2. If you remove and replace the same cartridge, the XN will recognize the ID chip so it is not possible to put an empty cartridge on or mix up any of the flourocell dye cartridges.

h. Pull down the cover on the reagent you replaced until you hear a click.

1. When the cover is pulled down, the Help dialog box closes automatically. The ID of the new reagent is automatically read and the information is registered and the test quantity is reset.
2. Close the top front cover.

Reference

- *Sysmex XN-3100 Instructions for Use Manual*. Sysmex Corporation, Kobe, Japan, March 2017.

ATTACHMENT E
Scan Guidelines

1. Using 20x dry objective, scan smear until at least 100 WBCs are reviewed. (If leukopenic specimen, scan for at least 90 seconds).

2. Estimate the approximate cell percentages and verify against hematology analyzer percentages. Scan for abnormal cells (larger and bluer) and confirm at higher power.

3. A **manual** diff must be performed by a medical technologist if:
   a. any blasts are seen
   b. Disagreement between IG% and combined metas / myelos / promyelos seen on scan
   c. 10% or more reactive lymphs are seen
   d. Scattergram has "----" for any diff parameter except basos
   e. Scan does not agree with hematology analyzer
   f. 5 or more plasma cells are seen

4. **For *"Immature Gran?"*** IP flags: scan for metas, myelos or earlier granulocytes in addition to toxic granulation, Döhle bodies and/or vacuoles.

5. **For "Blast/Abn Lymph?"** IP Flag: scan for blast, immature granulocytes, atypical/immature lymphocytes or other abnormal cells. If no abnormalities are found, the instrument data can be reported after review by a secondary review tech. Follow WAM Workflow guidelines.

6. **For "PLT clumps?" or "PLT Clumps(S)?"** scan for fibrin strands, PLT clumps, giant PLT. Refer to the CBC Correction Procedure or WAM Workflow manual for reporting guidelines.

7. **If pancytopenia in unknown patient, suspect MDS/leukemia.**

8. If hematology analyzer did not detect immature grans (IG), but occasional meta, myelo or promyelo is seen upon scan, accept the hematology analyzer diff and add the Diff Comment "*Occ meta/myelo seen on scan".*

9. For ***"Atypical Lympho?"*** IP flag: Not necessary to review slide unless accompanied by lymphocytosis of >49.9%. If less than 10% reactive lymphs are seen, accept the hematology analyzer diff and add the Diff comment, *"Occ. reactive lymphs seen on scan."*

10. If hematology analyzer did not detect NRBCs, but occasional NRBC is seen upon scan, accept the hematology analyzer diff and add the Diff Comment *"Occ. nRBC seen on scan."*

11. If less than 5% plasma cells are seen, accept the hematology analyzer diff and add the Diff Comment add, *"Occ. plasma cell seen on scan."*

12. If a manual differential is performed and it agrees with the hematology analyzer, **accept the hematology analyzer** diff and select the LIS comment *"Verified".*

13. Report PLT estimate (adq, inc, dec) regardless of which type of differential (scan or manual) is performed.

14. RBC morphology must be reported, even when normal. Use the comment *"Unremarkable"* when normal RBC morphology is seen.

ATTACHMENT F
SUMMARY OF SYSMEX FLAGS

DEFINITIVE MESSAGES

			WBH Limits
WBC:	Neutropenia	NEUT#	< 0.5
	Lymphocytosis	LYMPH#	> 10.0
	Lymphocytosis	LYMPH%	> 59.9
	Monocytosis	MONO#	> 3.0
	Eosinophilia	EOSIN#	> 4.0
	Leukocytosis	WBC	> 20.0
RBC:	Anemia	HGB	< 8.0
	Anisocytosis	RDW-SD	> 70
		RDW-CV	> 25
	Microcytosis	MCV	< 66
	Macrocytosis	MCV	> 114
PLT:	Thrombocytopenia	PLT	< 75
	Thrombocytosis	PLT	>1000

CBC, DIFFERENTIAL AND RETICULOCYTE
SYSMEX XN-3100

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Document History

Signature	Date	Revision #		Related Documents Reviewed/ Updated
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