HEM. SYSMEX.16.0 Body Fluid Analysis by the Sysmex XN

PRINCIPLE

The XN body fluid analysis mode performs automated cell counts and a limited differential. The same technologies used in whole blood analysis are employed. White cell counts are determined by fluorescent flow cytometry with scattered light measure and red cell counts employ the direct current detection method. The Body Fluid mode generates values for Total Count (all nucleated cells), WBC-BF, RBC-BF. The differential consists of absolute and relative values for polymorphonuclear (PMN) and mononuclear cells (MN).

A manual differential is performed on all applicable specimens. Reports and slides are submitted to the pathologist to screen for abnormal cells.

DOCUMENT OWNER
Manager, Regional Hematology

RELATED DOCUMENTS
HEM.SYSMEX.11.0
HEM.BF.2.0

SPECIMEN COLLECTION AND STORAGE

A. Anticoagulant of choice is EDTA. Other anticoagulants may alter results. Specimens received in Lithium Heparin tubes should be performed manually via Hemocytometer method.
   Note: Anticoagulant is not required or recommended for CSF fluid.

B. Add a minimum of 1 mL of body fluid to an EDTA (lavender top) tube to prevent specimen from clotting.

C. Specimens received in a syringe or sterile cup should be placed in EDTA as soon as possible. All specimens should be processed ASAP to prevent cell deterioration.

D. Fluids should be transported and tested at room temperature. Store at 2-8°C following testing.

E. Fluids tested on the XN-1000 include: Synovial fluids (examples include: wrists, ankles, knee, elbow, finger, thumb, hip and shoulder) CSF and Serous fluids (pleural, peritoneal, ascites, pericardial, etc.).

REAGENTS, SUPPLIES, AND EQUIPMENT

A. Sysmex analyzer and associated reagents (see HEM.SYSMEX.11.0)

B. Hyaluronidase solid used for thinning viscous synovial fluids. Store at < 0° C in freezer. Reagent is stable until expiration date on bottle or 1 year after opening.

C. Wooden applicator sticks

D. Cellpak (DCL)-Diluent

SPECIMEN PREPARATION

A. <u>All fluid samples</u>:

1. Examine macroscopically for clots, fibrin, tissue and debris. If possible, remove clots or other debris with applicator sticks or transfer pipettes prior to sampling on the XN-1000. A disclaimer should be added to results stating the presence of clots, fibrin or debris may affect the accuracy of cell counts.

NOTE: Specimens with clots, fibrin or tissue present <u>should not</u> be processed on the XN-1000. All specimens <u>must be "fluid consistency" (non-sticky liquid, without clots)</u> to be sampled in Manual Mode. Body Fluid samples should be capable of being aspirated freely. Specimens that cannot be sampled by the XN-1000 may be analyzed using hemocytometer method. Aspiration of clotted, sticky or thick fluids can result in damage to internal components of the cell counter and extended downtime for repairs.

B. <u>Joint Fluids:</u>

1. Add powdered hyaluronidase (dust amount) to an aliquot of specimen. Mix thoroughly.
2. Specimen should pipette freely and not form strings. If not, repeat Step 1 until desired fluid consistency achieved.
3. Treated specimen should be used to make cytospin slides also.
4. **Do not aspirate untreated joint fluids into the XN-1000.**

C. <u>Spinal Fluid:</u>

Spinal fluid cell counts may be reported from the XN-1000 as long as the results fall within the Reportable Range. Most clear fluids will require a manual cell count.

NOTE: Do not report CSF Red Cell counts as <10,000. If RBC count is below reportable range, a manual count must be done.

QUALITY CONTROL AND CALIBRATION

XN CHECK BF Commercial Body Fluid Controls Instructions for Use

A. Remove vials from refrigerator and allow them to come to room temperature (18 – 25°C) for approximately 15 minutes.
B. Mix vials by gentle end-to-end inversion until the cell button in the bottom of the vial is completely suspended.

Two levels of XN CHECK BF will be run with every 8 hour of patient testing.

1 XN CHECK BF Analysis
   a. Check the Status indicator LED on the analyzer to confirm analyzer is in ready state.
   b. If the tube holder is not ejected, press the mode switch. Tube holder will slide out.
   c. Select the Change Analysis Mode button on the control menu.
   d. Select [Body Fluid] mode. Analyzer will automatically perform Autorinse (BACKGROUND CHECK).
   e. Select [OK]
   f. Place thoroughly mixed vial in tube holder, press start switch.

Note: If vial barcode is unreadable, select the analyzer menu button on the control menu.

1. Scan Barcode label.
2. Place thoroughly mixed vial in tube holder, press start switch.
3. 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.

The XN is calibrated every six months, after major repairs, or as results warrant.

PROCEDURE

A. The XN-1000 should be in Ready status.
   1. Press the mode switch to eject the tube holder.
   2. Select the Change Analysis Mode button on the control menu.
   3. Select [Body Fluid].
   4. Select [OK].
      a. The analyzer will automatically perform a background check up to three times.
      b. If background count is unacceptable, perform testing on the back up analyzer or by manual method.
   5. Select the Manual Analysis button on the control menu.
   6. Input the sample ID or select Read ID and scan the bar code.
   7. Select [cap open] on Manual Analysis screen.
   8. Select [OK].
   9. Properly mix the specimen and place in tube holder.

10. Remove cap from specimen. Body fluids will be run without cap.
11. Press the start switch on the analyzer.
    a. The tube holder will slide in and the sample will be aspirated.
    b. When the analysis is complete, the tube holder slides out.
12. Remove the sample.
13. Perform Background check prior to running additional samples if indicated.
14. Return analyzer to Whole Blood mode prior to running whole blood samples.
15. If the sample flags as above linearity, (varies by site-see initial startup studies) repeat testing after diluting the specimen with DCL and the results will be multiplied by the dilution factor.

B. Printouts should be labeled with patient name (last, first), medical record and specimen numbers, and fluid type. LIS labels may be used.
C. Prepare slides using the cytocentrifuge (see procedure HEM.BF.2.0).
D. Perform a manual differential on fluid specimen.

REPORTING RESULTS

A. Refer to computer entry manuals at bench, worksheets are site specific.

B. Cell counts are entered as whole numbers per cubic millimeter(mm3)

C. For Sysmex automated counts:
    a. Mutiply Sysmex WBC by 1000
    Example: Sysmex WBC count 9.62 x10^3 cells /uL
    Report as 9620 cells/cumm

    b. Multiply Symex RBC by 1,000,000
    Example: Sysmex RBC Count 1.12 x 10^6 cells /uL
    Report as 1,120,000 cell/cumm

    c. Analytical Measurement Range (AMR)
        1. WBC 0.01 -400 x 10^3 cells/uL
        2. RBC 0.01-8.40x 10^6 cells/uL
        3. If WBC is 0.01 x 10^3, report ≤ 10 cells/uL
        4. If RBC is 0.01 x 10^6, report ≤10,000 cells/uL

D. RBC values less than 10,000 may be reported as "<10,000/µL" for all specimens **except Spinal Fluids.** All CSF specimens with either RBC and/or WBC counts outside reportable range should have a manual count performed. If a numeric count is requested by the physician for any other fluid, use of manual method is required.

E. WBC values below linearity should be reported from a hemocytometer count. Do not report counts of zero from the XN-1000.

F. Any value exceeding the high linearity (refer to initial studies performed at each site) should be diluted. Dilute all samples with DCL. Multiply results by dilution factor prior to reporting.

NORMAL VALUES AND DISCUSSION REFERENCE RANGES

Serous Fluids (Pleural, Pericardial, Peritoneal)

WBC	<10/μL
RBC	<10,000/μL

Pleural, Peritoneal, Pericardial-

In transudates, the WBC count is usually >1000 /uL. Classifying an effusion as either a transudate or exudate is important because this can help the doctor identify its cause, such as a systemic disease or inflammatory process.

Synovial Fluids:

Color	Yellow
Character	Clear
RBC	<10,000/μL
WBC	<150/μL

These four fluid types are the most common ones given to the lab for testing. For other fluids, refer to one of the body fluid textbooks.

PROCEDURE NOTES

A. Perform a WBC differential on all CSF specimens with a WBC of 5 or more cells.
   1. For patients <18 years of age, a WBC differential must be performed on all samples, even those where WBC count is between 0-5.
   2. All CSF oncology cell count specimens must have a WBC differential performed and be reviewed by a pathologist.

C. Any questionable slides reviewed by a pathologist and confirmed for atypical cells should be resulted with the pathologist's comment and the pathologist's name.

D. Currently, all body fluid differentials are left for the lead technologist or designee to review at Regional Hematology. Other sites should refer to lead technologist or Pathologist for consultation when needed.

E. **Synovial joints** are characterized by the presence of an articular capsule between the two joined bones. Bone surfaces at synovial joints are protected by a coating of articular cartilage. Synovial joints are often supported and reinforced by surrounding ligaments, which limit movement to prevent injury. There are six types of synovial joints:

(1) **Gliding** joints move against each other on a single plane. Major gliding joints include the intervertebral joints and the bones of the wrists and ankles.

(2) **Hinge** joints move on just one axis. These joints allow for flexion and extension. Major hinge joints include the knee, elbow and finger joints.

(3) A **pivot** joint provides rotation. At the top of the spine, the atlas and axis form a pivot joint that allows for rotation of the head.

(4) A **condyloid** joint allows for circular motion, flexion, and extension. The wrist joint between the radius and the carpal bones is an example of a condyloid joint.

(5) A **saddle** joint allows for flexion, extension, and other movements, but no rotation. In the hand, the thumb's saddle joint (between the first metacarpal and the trapezium) lets the thumb cross over the palm, making it opposable.

(6) The **ball-and-socket** joint is a freely moving joint that can rotate on any axis. The hip and shoulder joints are examples of ball and socket joints.

F. When reporting results in Sunquest, type in "HIDE" to parameters not observed. For example, if you did not see any basophils in the differential, type "HIDE" next to BASO so that it will not show up as pending. For Toplab, type "DNR".

LIMITATIONS

A. Specimens with clots may have compromised cell count results. A text comment should be appended to the cell count results indicating the presence of these.

Example comments:

If specimen is completely clotted, cell counts are not performed. Coded comment is **CLTD**. The clotted material is resuspended and a smear is made, stained, and a WBC differential is performed.

If specimen is partially clotted, cell counts are performed and noted on the report. The possibility of inaccurate results due to clotting is noted with the coded comment **PCLOT**.

B. Synovial fluids with crystals or high viscosity may have compromised results. All body fluids with crystals should be performed manually on a hemocytometer.

C. Samples with function errors relating to the RBC or WBC counts should not be reported. Specimens with "blanked out" PMN and MN automated counts may be reported as long as no other errors are present.

REFERENCES

A. Sysmex XN-1000 or XN-2000 *Instructions for Use* (North American Edition), Sysmex Corporation, Kobe, Japan.

B. Sysmex XN series *Administrator's Guide* (North American Edition), Sysmex Corporation, Kobe, Japan.

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