SEROUS AND SYNOVIAL FLUID

Automated Body Fluid Cell Counts on Sysmex XN-2000

And Manual Cell Count

FCNTY

I. PRINCIPLE

The analytical module (XN-10) is a quantitative automated hematology analyzer for *in vitro* Diagnostic use in determining 32 whole blood diagnostic parameter and seven body fluid diagnostic parameters. Examination of the numerical and/or morphological findings of the cells found in body fluid are useful in the diagnosis of disease states such as malignancy, inflammation, viral, bacterial and parasitic infections.

The device has a separate Body Fluid mode, which this laboratory will use to analyze serous and synovial body fluids. Red blood cells (RBC) are count using electronic resistance detection. The white blood cell (WBC) counts are evaluated using flow cytometry with a semiconductor laser exploiting the differences in 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 size of the nucleus. The WBC differential for Body Fluids includes polymorphonuclear (PMN) and mononuclear (MN) cell percent and calculated counts. The Total Nucleated Cell count (TC-BF) includes WBC and High Fluorescence Cell count (HFBF), a measurement for cells with large nuclei, such as mesothelial or malignant cells.

Directly Measured Parameters: WBC, RBC, TC-BF, BF-PMN%, BF-MN%

Calculated Parameters: BF-PMN#, BF-MN#

II. CLINICAL SIGNIFICANCE

Synovial Fluids:

Synovial fluid is produced by dialysis of plasma across the synovial membrane and by the secretion of a hyaluronate protein complex by the synovial membrane. Analysis of synovial fluid is used to classify joint disorders in terms of their pathological origin. The Profile consists of Appearance, Volume, WBC and RBC cell count, Differential and Crystal Identification.

Synovial fluid aspirations provide useful information for diagnosis and classification of joint disorders. Normal WBC is less than 200 cells/mm^3. A low WBC with predominately mononuclear cells (<25% polys) suggests a noninflammatory joint fluid such as traumatic arthritis, osteoarthritis, or systemic lupus. A high WBC count suggests an inflammatory disease, and a high WBC (>100,000/mm^3) with a

high proportion of polys strongly suggest infection.

Crystal identification is useful in the differentiation of gout and pseudogout. Monosodium urate crystals are characteristic of gouty arthritis. Calcium pyrophosphate crystals are indicative of pseudogout. Cholesterol crystals are associated with chronic inflammatory conditions.

Serous Fluids:

Fluids between membranes of pleural, pericardial and peritoneal cavities form on the basis of plasma ultrafiltration and is called serous fluid. Fluid formation is simultaneously controlled by four factors (1) the permeability of the capillaries in the parietal membrane; (2) the hydrostatic pressure in these capillaries; (3) the colloid osmotic pressure produced by the presence of plasma proteins within the capillaries; and (4) the absorption of fluid by the lymphatic system.

Many pathologic conditions can cause a buildup (effusion) of serous fluid that is divided into two categories: Transudates and Exudates. Classification is based upon appearance, leukocyte count, total protein concentration, albumin concentration and cholesterol concentration. Transudates result from a mechanical process that disrupts the balance in the regulation of fluid filtration and reabsorption such as changes in hydrostatic pressure caused by congestive heart failure. Exudates are from an inflammatory process. They are produced by conditions that directly involve the membranes of the particular cavity, including infection and malignancies. Hematology performs the leukocyte count and differential. A total nucleated count and red blood cell count are commonly run on the XN analyzer but can be performed by the hemocytometer method if necessary. The differential is performed on a Wright's stained cytocentrifuged specimen.

III. SPECIMEN

A. Synovial and Serous fluids with EDTA-2K.
    1. Serous fluids may include ascites, pleural, pericardial, peracentesis, thoracentesis fluids
    2. CSF and Bronchial specimens should never be run on the XN-10 analyzers. See respective protocols for these specimens.
B. Volume requires
    1. Closed tube – 1mL
    2. Open tube – 300µL
    3. Open microtube – 160µL
C. Body Fluid specimens should be fresh and received in less than one hour from collection.
D. If Serous or Synovial fluids are in a syringe when received, place into 2-3 plain sterile red-top tubes for chemistry and microbiology and in one dry-coated K2

EDTA (lavender) tube for microscopic exam for crystals and cell count.

E. Specimen can be stored at room temperature for 4 hours, but refrigerated up to 24 hours. Specimens will be stored at 2-8°C for 7 days.
F. No specimen should be rejected as they are unique specimens. Note any issues in results (e.g. delay in testing, clotting, etc) they may affect results.

IV. REAGENT

A. Eleven Sysmex reagents are used on the Sysmex XN-2000. Refer to Procedure "Sysmex XN-2000" (HE-0596).
B. All reagents are stored at room temperature and are to be used within the manufacturer's expiration date on each container.
C. Record date received and date opened on container and in the computer record history.
D. All reagents are azide free and intended for in vitro diagnostic use only

REAGENT	EXP.	FUNCTION
CELLPACK DCL*	60 DAYS	Whole blood diluent and used for Body Fluid dilutions
CELLPACK DST	60 DAYS	Concentrated diluent of reagent
CELLPACK DFL	60 DAYS	Whole blood diluent. Used in combination with Fluorocell RET for the analysis of reticulocytes or with Fluorocell PLT for platelet analysis by flow cytometry method.
SULFOLYZER 1.5L	60 DAYS	Lysing reagent that releases the hemoglobin for the determination of hemoglobin concentration of blood.
SULFOLYZER 5.0L	90 DAYS	Lysing reagent that releases the hemoglobin for the determination of hemoglobin concentration of blood.
Lycercell WNR	60 DAYS	Lysing reagent used with Fluorocell WNR to differentiate white blood cells (non-basophil), basophils, and NRBCs.
Fluorocell WNR	90 DAYS	Used to stain and get WBC count and differentiate white blood cells (non-basophil), basophils, and NRBCs.
Lycercell WDF*	90 DAYS	Lysing reagent used to hemolyze RBCs and used with Fluorocell WDF to differentiate white blood cells (PMNs, lymphocytes, monocytes and eosinophils).
Fluorocell WDF*	90 DAYS	Used to stain and differentiate leukocytes (PMNs, lymphocytes, monocytes and eosinophils).
Fluorocell RET	90 DAYS	Used to stain reticulocytes in diluted blood samples for the assay of reticulocyte percent in blood.
Fluorocell PLT	90 DAYS	Used to stain platelets in diluted blood samples for the assay of platelet counts in blood by fluorescence.
XN CHECK BF*	30 DAYS	Body fluid commercial control used to monitor performance.

*Used in Body Fluid Analysis.

E. Wright's stain
F. Nerl Saline
G. CELLPACK DCL
H. Spinalscopics control material
I. Hyaluronidase

J. XN CHECK™ BF controls Levels 1 and 2

V. EQUIPMENT

A. Neubauer hemocytometer
B. Disposable Pipettes
C. Glass Slides and Coverslips
D. Slide Stainer
E. Microscope
F. Cytofunnel sample chambers with filter card
G. Hema-Tek® Slide Stainer
H. Cytocentrifuge
I. Sysmex XN-2000 analyzer, automated cell counters

VI. CALIBRATION

The automated Sysmex XN-2000 calibration is verified every six months or on an "as-needed" basis to ensure system accuracy. Refer to Procedure HE-0596 "Sysmex XN-2000
The hemocytometer is checked for accuracy by manufacturer.

VII. QUALITY CONTROL

A. Sysmex XN-2000 Analyzers: Body fluid control, XN CHECK™ BF control levels 1 and 2 should be run under Manual BF mode once in 24 hours when there is a body fluid to be analyzed.
   1. When analyzer is in ready state, press the mode switch
   2. Select [Body Fluid] under Change Analysis Mode
   3. Select [OK]
   4. The XN-2000 will automatically perform an Autorinse for a background check.
   5. Remove vials from refrigerator and allow them to come to room temperature (18-25°C), for approximately 15 minutes.
   6. Mix vials by gentle end-to-end inversion until the cell button on the bottom of the vial is completely suspended.
   7. Place the mixed vial in the tube holder and press the start switch.
   8. Run both levels, reviewing QC Results.
   9. Only perform body fluid analysis if QC is acceptable.

B. Manual body fluid counts:
   1. Hemocytometer techniques are monitored by commercial materials.
   2. Techs performing manual counts must analyze quality controls, obtain results within the acceptable ranges, and document results
   3. Two levels of commercial Spinalscopics control materials must be run every 8 hours of patient testing, per tech when a specimen is received

C. Procedure for manual analysis of Spinalscopics control material:
   1. Remove the controls from the refrigerator and allow them to come to room temperature (18-25°C) for 10-15 minutes but no more than 20 minutes. Mix the controls thoroughly by inverting the bottles several times, squeezing the bulb in the cap, aspirating and expelling the control through the glass dropper attached to the cap at least 10 times immediately prior to use to assure homogeneity of the contents. Thorough mixing with each use is important in order to obtain reproducible results. Avoid foaming.
   2. Using glass dropper, charge both sides of the hemocytometer chamber with level 1. Repeat with level 2. Each control level must be tested in duplicate.
   3. Immediately recap the controls. The Spinalscopics Controls should be stored tightly capped refrigerated (2-8°C) when not in use. Do not freeze. Once opened, the controls are stable for six months when stored at 2-8°C between uses.
   4. Allow the cells to settle by placing the hemocytometer in a humidified chamber for 10 minutes and no longer than 30 minutes before counting.
      a. In a clean 15mm petri dish, place a square of gauze and slightly wet is with DI water.
      b. Break the plastic handle of an applicator into two pieces and place one on each side of the wetted gauze.
      c. Set the hemocytometer on top of the two applicator pieces, which will keep the hemocytometer off the wet gauze.
      d. Place the lid on the petri dish and let the cells set. The moisture in the chamber will prevent evaporation.
   5. Count the RBC's and WBC's in the 9 squares on each side. The two sides must agree within 20% or 4 cells on a low count. Average the two sides. Divide average by 0.9 (or multiply by 1.1) and round to the nearest whole number. Result must be within that level's acceptable range or a two-sided re-plate is required for that level.
      - $\frac{\text{Average \# cells counted}}{0.9}$ = Total Cells/µL
      - Average # cells counted X 1.1 = Total Cells/µL
   6. Document results on the control clipboard
   7. Expected Ranges: Check commercial control package inserts for expected ranges.
   8. Counts on patient samples should never be done unless QC is within range

D. CELLPACK DCL is to be used for dilutions when specimens are run on the XN analyzer. A portion of this should be run in BF mode prior to analyzing the specimen being diluted to observe for contamination.

VIII. PROCEDURE

A. Record all information needed for processing and resulting the tests in a body fluid profile on the body fluid worksheet. See a copy at the end of this procedure.

B. Initial handling: Hematology department accepts specimen and distributes appropriate amounts to bacteriology, chemistry and hematology
   1. Check all tubes/syringes for proper labeling of patient's name, DOB, and ID number.
   2. Obtain the total volume of all tubes before they are taken to the various departments. Note the volume on patient's Body Fluid Worksheet. Also record color and clarity, including absence or presence of clots
   3. Bacteriology - If bacteriology tests are ordered and there is only a tube or syringe, aliquot the needed volume then distribute remaining to Hematology and Chemistry. A minimum 1 mL, optimum 5 -10 mL, in sterile red top tube.
   4. Chemistry – only if separately ordered tests are listed as the synovial profile does not include chemistry tests. A minimum 1 mL, Optimum > 5 mL in plain red top tube. Promptly centrifuge tube to analyze supernate as cells may alter the chemical composition of the fluid.
   5. Hematology - immediately place a minimum of 1.0 mL in small volume K2 EDTA tube. Slides for crystals analysis in synovial fluids are made from this tube or red top.

C. RBC and Total Cell/WBC count
   1. Before analyzing, determine the quality of the specimen
      a. Place a drop of the body fluid on a slide with a cover slip and examine under a microscope
      b. If no cell clumps are seen and cells are intact run sample on analyzer.
      c. If clumps of cells are observed but cells are intact, a cell count should be done manually on a hemocytometer. A note should be added stating the "results may be inaccurate due to partial clotting, cell clumps or debris".
      d. If deteriorated cells are primarily seen do not run on analyzer or try to count on hemocytometer as cell counts would be inaccurate.
         i. Append a comment – "Specimen unsuitable for quantitative cell count due to low quality of specimen"
         ii. Create a cytospin and determine if a differential can be done on any intact cells.
      e. If a specimen is completely clotted, a count cannot be done.
         i. Append a comment – "Specimen unsuitable for quantitative cell count due to clotted specimen."
         ii. Create a cytospin and do a differential from the cells remaining in the fluid.

f. A cell differential should be done on all specimens whether a cell count can be performed or not.

2. If sample is too viscous to mix well, hyaluronidase (kept in Chemistry freezer) should be added to an aliquot of specimen.
    a. Aliquot a well-mixed sample (~1mL) into a disposable tube.
    b. With a wooden applicator stick, transfer a few crystal bits and stirring it into 1.0 mL of Body Fluid.
    c. Incubate for five minutes, mixing occasionally.
    d. If the fluid is extremely viscous, more hyaluronidase can be added and/or more time can be allowed for the hyaluronidase to work.
    e. Incubation at 37°C for 1-5 minutes may help.

3. Automated counts on the XN-2000:
    a. All synovial and serous body fluids can be run on the XN analyzer provided the sample is not clotted or has cell clumping.
    b. Press the mode switch to eject the tube holder when analyzer is ready.
    c. Select [Body Fluid] under the Analysis Mode button on the control menu.
    d. Select [OK].
    e. The analyzer will automatically perform a background check up to three times.
    f. Select the Manual Analysis button on the control menu.
    g. Input the sample ID or select [Read ID] if sample is barcoded.
    h. Select [OK].
    i. Properly mix the specimen and place in tube holder.
        i. If a secondary tube is used, i.e. for hyaluronidase treatment or dilution, use a 12x75mm tube and run with [Cap Open] selected.
        ii. If running microtainer, remove the cap using caution to avoid Splattering.
    j. Press the start switch on the analyzer.
    k. The print-out of the results has a specific section for body fluid counts.
    l. A Background check is required prior to running additional samples if the analyzer alerts you with the error message "Analysis result is high". The message on the screen:
        i. WBC-BF and TC-BF# > 100.00 x 10^2/uL
        ii. RBC-BF > 100.0 x 10^4/uL
            - Sample will be displayed with Red background in Sample Explorer.
            - Analyzer will prompt user to run an Autorinse. Select [Execute].

m. Samples need only to be run once on the analyzer providing cell ranges are within acceptable linearity limits and no asterisks (*) are on any parameters.
n. Parameters that exceed linearity are flagged with @ beside the result. Body fluid samples with high cells counts may prompt an "Analysis result is high" error.
    i. The sample must be diluted with CELLPACK DCL, rerun and results multiplied by the dilution factor.
    ii. A portion of the CELLPACK DCL should be run in BF mode prior to analyzing the specimen being diluted to observe for contamination. No cells should be detected in the CELLPACK DCL alone. If cells are detected, obtain a fresh aliquot of diluent. Keep the printout of the diluent results with the Body Fluid results.
    iii. Calculate the correct cell counts by multiplying the cell counts by the dilution factor. (E.g. is sample was diluted 1:3, multiply by 3.) Write out calculations on the printout sheet to ensure accuracy, and save.
    iv. Note the use of dilution for linearity on the patient report.
    v. Linearity of XN-2000 for Body Fluids
        1. WBC-BF: 0.003-10.000 $x10^3/\mu L$
        2. RBC-BF: 0.002-5.000 $x10^3/\mu L$
        3. TC-BF#: 0.003-10.000 $x10^3/\mu L$
o. When cell counts are below instrument linearity, the results may be reported as less than (<) the linearity of the analyzer.
    i. TC-BF# & WBC-BF: ≤ 3 cells/µL
    ii. RBC-BF: ≤ 2,000 cells/µL
p. Return analyzer to Whole Blood mode prior to running whole blood samples

4. Manual counting using a hemocytometer:
    A. Mix the specimen well.
    B. Using a disposable pipette, charge both sides of the hemocytometer chamber with specimen, taking care not to overfill the chamber.
        - If specimen is cloudy, perform a dilution using Nerl saline
        - Perform a background check with just the Nerl saline to confirm that no cells or debris is seen prior to making dilution.
    C. Allow the cells to settle by placing the hemocytometer in a humidified chamber for 10 minutes, but no longer than 30 minutes, before counting.
    D. Count the RBC's and WBC's on each side. The two sides must agree within 20% or 4 cells on a low count, or a two-sided re-plate is

required for that level.

E. Calculate the total number of red and white blood cells per µL. Write out calculations on the Body Fluid worksheet to ensure accuracy
F. If fibrin or other debris causes cell clusters, repeat the count. Report with a comment that the cell count maybe inaccurate due to debris or interferences.
G. See the Hemocytometer protocol for further information and calculations

D. Differential
1. Label a slide with patient's full name, date and patient's accession number with a pencil.
2. Make a cytospin for all samples, using a cytocentrifuge. A smear may be made for purulent specimens. See the Cytospin protocol for further information.
3. Perform a 100 cell differential cell count using 50X and/or 100X oil and report percent (%) of each cell seen.
    a. Go to Differential Result Entry, use keyboard: YFLD, and enter the Accession Number
    b. The cell types encountered in pleural, pericardial, and peritoneal fluids are:
        i. Neutrophils
        ii. Lymphocytes
        iii. Monocyte/Macrophage
        iv. Eosinophil
        v. Mesothelial Cell
        vi. Other Cells
            1. Add Comment to indicate the cells present
            2. Basophil, Mesothelial cells, Lining cells, Plasma cells, malignant cells, etc.
    c. If cell count is low in the body fluid and 100 cells are not available, click on the Count Terminate key (Term =) to get a percent of 100.
4. Alert the pathologist if any of the following are found: Blasts, malignant cells, abnormal appearing cells, many large groups of non-white cells, crystals, or organisms. This will allow for appropriate correlation with other ancillary tests (ex. Cytology). The pathologist will guide you on how to report out the specimen.
5. Correlate the number of cells seen on the slide to the cell count.
    a. The proportion of the RBC's and WBC's as well as the number of cells seen are QC checks.
    b. If there is any discrepancy, repeat both the cell count and the preparation of the cytospin.

E. Wet prep for Synovial fluid crystals:
   1. Place a drop of synovial fluid on slide and cover with coverslip.
   2. Place slide in moist container to prevent drying.
   3. Take the slide and the patient's synovial fluid profile form, to the pathologist to look at the sediment at this time for intracellular crystals. If necessary, he/she will use a polarized light microscope.
   4. If a pathologist is not available, place tube in hematology's refrigerator and put a note with the patient's synovial profile form next to the refrigerator or on the hematology bench. The first shift hematology tech should follow up on this ASAP when pathologist is available.

F. Supernatant should be saved and labeled as such from all centrifuged portions, for possible additions of chemistry tests.

IX. REPORTING RESULTS

A. Before resulting, any data flags must be addressed and Troubleshooting done
   1. An asterisk (*) indicates a result may be unreliable, a manual count or dilution may be necessary to obtain accurate results
   2. Counts at or below linearity should be reported as less than or equal to (≤) the lower limit
      a. Total Cell count (TC-BF#): ≤ 3 cells/µL
      b. Red Blood Cell count (RBC-BF): ≤ 2,000 cells/µL

B. Enter results under Result Entry:
   1. Total Collected Volume, including specimen volume used in any other testing
   2. Enter the Type of body fluid being analyzed
      a. Codes for each body fluid type can be found on the Body Fluid Worksheet
   3. Clarity (Clear, Slightly Hazy, Hazy, Cloudy, Turbid, Opaque)
      a. Note any other observations, e.g. present of clots
   4. Color (Colorless, Yellow, Straw, Amber, Pink, Red)
   5. Report the total cell count (TC-BF#):
      a. If automated count is below analyzer linearity, turn out count as ≤ 3/µL.
      b. If clots or debris are present in the specimen, comment that the WBC is approximate due to presence of clots or debris.
   6. Red Blood Cell count
      a. If automated count is below analyzer linearity, turn out count as ≤2,000/µL

C. Perform and result the differential under Differential Result Entry:
      a. Use keyboard option or manually enter counts for Neutrophils, Lymphocytes, Monocytes, Eosinophils, and Other Cells

b. Comment under "Other Cells" the type(s) seen and in what numbers (Basophil, Mesothelial cells, Lining cells, Plasma cells, malignant cells, etc.)

D. Crystals – reported by the pathologist and inputted into LIS by the tech.
E. Note any actions taken to obtain results, e.g. using hyaluronidase, or diluting a sample for linearity.

X. PROCEDURAL NOTES

A. Linearity of Body Fluid Analysis:
   a. WBC-BF: 0.003 – 10.000 $x10^3/\mu L$
   b. RBC-BF: 0.002 – 10.000 $x10^6/\mu L$
   c. TC-BF#: 0.003 – 10.000 $x10^3/\mu L$
B. Add a note to the results for any body fluid that has a clot, clumped cells, or other debris present; indicate that the counts may be inaccurate.
C. To help cells adhere to the slide and prevent cells from disintegration or smudging adding a drop of 22% Albumin to the cytospin
D. A reference interval has not been established for this test on the supplied specimen type.
E. Document manual cell counts on Body Fluid Worksheet. Write out calculations on the sheet to ensure accuracy and save.
F. For Synovial fluids, do not use oxalate, powdered EDTA, or lithium heparin because they can produce artifacts in microscopic examination for crystals.

XI. REFERENCES

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B. Kjeldsberg, C.R. and Knight, J.A., Body Fluids, 3rd Edition, p 265-301, 309-324. ASCP, Chicago.
C. Quantimetrix, Spinalscopics Product Insert, MO46031A 10/16
D. Sysmex XN-1000 or XN-2000 *Instructions for Use* (North American Edition), Sysmex Corporation, Kobe, Japan
E. Sysmex America Inc., Lincolnshire, IL. XN CHECK BF Hematology Control for Sysmex XN-Series Analyzers package insert.
F. College of American Pathologists (CAP) Hematology-Coagulation Checklist, 8/21/2017

POLICY CREATION :	Date:
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