UnityPoint Health
Methodist | Proctor

Effective Date: April 20,1994
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Serous and Synovial Fluid Analysis

Hematology Analyzer

Manual Methodologies

FCNTY

I. Purpose

Examination of the numerical and/or morphological findings in the body fluid are useful in the diagnosis of disease states, such as hemorrhage, malignancy, inflammation, viral, bacterial, and parasitic infections.

II. Synovial Fluid

Analysis of synovial fluid is used to classify joint disorders in terms of their pathological origin.

III. Serous Body Fluids

The closed cavities of the body, namely, the pleural, pericardial, and peritoneal cavities, are each lined by two membranes referred to as the serous membranes. One membrane lines the cavity wall (parietal membrane) and the other covers the organs within the cavity (visceral membrane). The fluid between the membranes, which provides lubrication as the surfaces move against each other, is called serous fluid. Normally only a small amount of serous fluid is present because production and reabsorption take place at a constant rate. Fluids for laboratory examination are collected by needle aspiration from the respective cavities. These aspiration procedures are referred to as thoracentesis (pleural), pericardiocentesis (pericardial) and paracentesis (peritoneal).

IV. Principle

The Body Fluid Analysis Mode of the Sysmex XT-4000*i*™ adds a quantitative automated procedure for analyzing cerebrospinal fluid, serous fluid, and synovial fluid. The 4-DIFF scattergram utilizes fluorescent flow cytometry using lateral scattered light and lateral fluorescent light in a specialized analysis sequence to calculate and display the WBC-BF, total nucleated (TC-BF) count, mononuclear (MN) cell/ polymorphonuclear cell (PMN) counts and percentages. The direct current (DC) detection method is used to determine the RBC (RBC-BF) count.

V. Materials

Reagents:
- Hyaluronidase
- Saline/Cellpack
- 2% acetic acid
- Albumin

Supplies:
- Hemocytometer
- Applicator stick
- Plastic pipette
- Glass slide with coverslip

Equipment:
- Areospray slide stainer
- Shandon Cytospin
- Microscope, bright field and phase
- XT-4000i

VI. Sample

Serous body fluid or Synovial (joint) fluid placed in an EDTA tube for a cell count and differential.
The tubes should not be overfilled and the minimum fill volume of the 3.0 EDTA tube is 1.0 ml.

VII. Clotted Sample

A quantitative cell count cannot be done on a completely clotted specimen.
The differential may still be able to be done depending on the severity of the clot. The technologist and/or Pathologist will need to make a decision on how to handle the WBC differential. For cell counts on completely clotted specimens, follow the procedure for Cell Count on Mucoid Body Fluid in this procedure manual. Append a comment to the tests-"Specimen unsuitable for quantitative cell count due to clotted specimen.

VIII. Limitations

1. Results may be compromised with clotted samples and synovial samples that contain uric acid crystals or have high viscosity.
2. Automated Sample results with errors related to WBC and RBC parameters should not be used.
3. All fluids should be tested as soon as possible after collection.

4. Parameters that exceed the limits below are flagged with @ beside the result. The sample must be diluted, rerun and multiplied by the dilution factor. Note the use of dilution for linearity on the patient report.
5. Because it is nearly impossible to get the background count down to 0.000 in the RBC chamber, RBC counts that are 0.002 or below will be reported as < 2,000/ uL.
6. RBC counts of 0.003 or greater can be turned out as long as there are no error messages.

Parameter	Range	Units
WBC-BF	0.000- 9.487	$x10^3/\mu L$
RBC-BF	0.001 – 3.251	$x10^6/\mu L$
TC-BF#	0.000 – 9.494	$x10^3/\mu L$

IX. Quality Control

WBC counts greater than 0.000 and RBC counts of 0.003 can be turned out from the XT 4,000 as long as there are no error messages. Commercial QC is run every day.

We use procedural Quality Control for manual body fluids counts.

1. Check the diluting fluid (if making a dilution) for contaminants.
   a. Saline and Cellpack can be run through the hematology analyzer like a specimen or plate on a Hemocytometer and do a count. Acceptable performance is 0.0
   b. 2% acetic acid should be checked by placing a drop on a glass slide and coverslip. Look for contamination. No contamination is acceptable performance.
   c. If the diluting fluid is contaminated, it should be discarded and replaced.
   d. Record the results on the worksheet provided.
2. The WBC and RBC counts are counted in duplicate and must match within 20%.
3. Review the ratio of RBC to WBC on the cytospin. This is a gauge to see if you identified your cells correctly.

X. Procedure

1. Tests

Tests done in Hematology on synovial and Serous fluids include:
Volume, Color, Clarity, RBC count, WBC count, Differential

2. Source Code

Make sure to put the exact site using right or left. A left hand bracket ([) and the site will give you a list of codes. A ";" and free text is also acceptable.

3. **Worksheet**
   Use the body fluid worksheet to record all of the information needed for processing and resulting the tests in a body fluid profile. See a copy at the end of this procedure.
4. **Synovial Fluid Color and Clarity**
   Evaluate the color and clarity. Normal synovial fluid appears clear and pale yellow. The color becomes deeper yellow in the presence of inflammation and may have a greenish tinge with bacterial infection. As with CSF, the presence of blood from hemorrhagic arthritis must be distinguished primarily by observing the uneven distribution of blood in the specimens obtained from a traumatic aspiration. Turbidity occurs when the cell count is elevated and is usually proportional to the number of cells present.

   A milky fluid may indicate the presence of crystals.

   Serous Fluid Color and Clarity
   Serous body fluids are normally yellow and clear.
5. **Volume**
   If the specimen is received in a bag or syringe, measure the volume before it is dispersed to all sections of the lab
6. **Crystals**
   a. Make a wet preparation for polarizing microscopy by adding 1 drop of synovial fluid to patient labeled slide and add 1 drop of saline. Place cover slip over drop.
   b. Place slide in moist container to prevent drying.
   c. Take to pathologist for polarized microscopic review for crystals.
   d. If no pathologist is available (evenings or weekends) to perform microscopic for crystals, place synovial specimen in Hematology's refrigerator after testing is complete. Leave the body fluid worksheet and a note in Hematology for tech to complete the next normal working day.

7. **Looking for Cell Clumping**
   Place a drop of the body fluid on a glass slide and cover-slip. Look for clumps or debris that would give falsely high or low results. If no clumps or debris are seen, proceed. If clumps or debris are seen, make sure to append a comment-"results my be inaccurate due to partial clotting, cell clumps or debris" whichever is appropriate. From this slide you can get an estimate of the number of cells in the fluid and what type of dilutions will be

necessary.

8. **Hyaluronidase: Found in the – 70 Freezer**
   If the specimen is very viscous, add hyaluronidase to an aliquot (up to 1.0 ml) of specimen. This is done by dipping a wooden applicator stick into the hyaluronidase and stirring it into an aliquot of the EDTA preserved synovial fluid. Mix the specimen well.
9. **Automated RBC and WBC Count**
   Do not run clotted specimens on any analyzer.
   A. Press the **[F2]** key on the keyboard or click on the **[Manual]** icon on the IPU.
   B. Enter the specimen number or read the barcode using the handheld barcode scanner.
   C. Click **"Body Fluid"** for "Mode" to set the analysis mode.
   D. "Discrete" testing should display CBC + DIFF.
   E. After all the settings are complete, press **[ENTER]** or click **[OK]** to confirm.
   F. A background check is automatically initiated. The background check analysis is repeated three times. Results must be 0.000 for WBC and less than 0.0020 for the RBC. See Chapter 10 in the Operator's Manual for additional information on resolving Background Errors.
   G. A regular Auto Rinse can be performed from the Main Menu When attempting to get the Body Fluid parameters background
   H. Mix the contents of the test tube gently but thoroughly. Uncap the tube.
   I. Place sample under the aspiration pipette so that the tip of the pipette is at the bottom of the sample and press the start switch.
   J. Retrieve the printout from the Analyzer and the Research Screen.

   K. RBC counts that are 0.002 or below will be reported as <2,000 RBC counts that are 0.003 or higher will be reported. e.g. a printout that shows 0.007 will be reported as 7,000.
   L. We use the TC-BF# (Total cell count) for the WBC result.

11. Glass Hemacytometer

    #RBC x dilution x 10
    10 squares counted

    #WBC x dilution x10

10 squares counted

If the automated results have asterisks or error codes or the sample has clots, plate the sample on the hemocytometer both sides. Count the RBCs on one hand held counter and the WBCs on another in all 9 large squares and 1 large square on the other side. This is the total RBC or WBC count/mm3. Use the Phase Microscope to help differentiate RBC's from WBC's.
Do all counts in duplicate.

12. Counts that need Diluted

For RBC or WBC counts that need diluted, see the Manual RBC and Manual WBC procedures in this manual.

13. Duplicate counts should match within 20%. Show how all dilutions were made and all math on your worksheet.

14. Make a cytospin preparation for the differential. See the procedure in this manual. Stain on the Aerospray on regular rapid stain 5+ setting. Make sure to label the slide with the patients name, accession number, type of body fluid and what dilution was used, if applicable.

15. Differential

1. Do a 100 cell differential
2. Function: Differential Result Entry Keyboard: YFLD
3. Enter Accession No.
4. Identify the cells as Neutrophil, Lymphocyte, Macrophage/Monocyte, Eosinophil, Mesothelial Cell, Synovial Fluid Lining Cell or Other. Other cells include: Basophils/Mast Cell, Blasts, Lymphoma Cells, Plasma Cells or any Unidentified cell.
5. Begin at the top left of the first circle and scan all fields of both circles to find enough cells to do a 100 cell diff. If there are not 100 cells, do a % of 100 by pressing the = key on the keyboard when done. This is the count termination key and it will automatically give you a percent.
6. If you see crystals, order FCRYST and have the pathologist look at the slide to confirm and result the finding.

16. Pathology Review

Body fluid slides that have “other cells” or any cells that look suspicious are sent to Pathology. Print a cumulative report and place the slide in a holder. Leave fluids from 2nd and 3rd shift for the supervisor to review.

XI. Calculations

Plastic Hemocytometer WBC or RBC = $\frac{\text{Averaged Count X Dilution X 90}}{\text{\# of small squares counted}}$

XII. Reference Intervals

See back of the profile sheet on next page.

XIII. Result Reporting

1. Function: Result Entry
2. Result Mode: Manual
3. Configuration: YHEM PRP Hematology Manual
4. Result
5. Enter the results for Body Fluid Type, Color, Clarity, Volume, Red blood cell count, White blood cell count. If no WBC's are seen on count, but cells are seen in the cytospin, report out as "1".
6. Do not report out the RBC or WBC count without verifying the count with the Cytospin.
7. If there are "other" cells, ask the Pathologist to help with identification. Do not forget to append comments to the cell counts if necessary. Ask if the pathologist wants an interpretation ordered. If yes, order a PATHSM to the accession number.
8. Print a cumulative report and leave the slides, with calculations for Supervisor review.
9. If cytology is ordered, make an extra cytospin to be sent to Methodist along with the sample and the cumulative report.

10. **References**

1. Strasinger, S. Urinalysis and Body Fluids, pp 162-168.
2. CAP Color Atlas of Body Fluids, pp92-95
3. Kjeldsberg and Knight, Body Fluids, 2nd Edition, pp 129-149.
4. Sysmex XT-4000*i*™ *Instructions for Use* (North American Edition), Sysmex Corporation, Kobe, Japan, July, 2009.
5. Clinical and Laboratory Standards Institute (CLSI). *Laboratory Documents: Development and Control; Approved Guideline*; Fifth Edition. (GP2-A5, 2006).

6. College of American Pathologists (CAP) Hematology-Coagulation Checklist, 7/28/2015

11. Related Procedures

Cytospin Procedure
Plastic Hemocytometer Procedure
Manual WBC Procedure
Manual RBC Procedure

Policy Created by: _____ Date: _____

Medical Director Approval: _____ Date: _____

Change of Medical Director: _____ Date: _____

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REVISION HISTORY			
Rev	**Description of Change**	**Author**	**Effective Date**
0	Initial Release	Cindy Schroeder	1/22/16
1	Body fluid slides will only be sent to pathology if they appear to contain abnormal cells. An extra slide will need to be made for all fluids if cytology is ordered. Crystal analysis will be performed by pathology.	Sheanea LaCock	09/06/2017

Reviewed by

Lead	**Date**	**Coordinator/ Manager**	**Date**	Medical Director	**Date**