Policy Statement	Core Laboratory Personnel are responsible for insuring the specimen submitted for testing is acceptable and the procedure for performing this assay is not violated.
Purpose	This procedure provides technical instruction for acceptable performance of the "Body Fluid Analysis" on the Sysmex XE-Series" analyzer.
Scope	This procedure applies to testing personnel authorized to perform testing using the Sysmex XE-5000 analyzer.
Responsibility	All authorized personnel are responsible for following procedural guidelines and insuring good laboratory practice is followed.
Related Documents	Sysmex Operators XE-5000 Main Manual Sysmex Operators XE-5000 IPU Manual Sysmex Training Manual

I. PRINCIPLE

The Body Fluid Analysis Mode of the Sysmex XE-5000 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 total nucleated (TC-BF) counts, **mononuclear (MN) cells *(includes lymphocytes, monocytes, and other mononuclear cell types*)** and **polymorphonuclear (PMN) cells (*includes neutrophils, eosinophils, and basophils*)** counts and percentages. The direct current (DC) detection method is used to determine the RBC (RBC-BF) count. Examination of the numerical and/or morphological findings in the body fluid are useful in the diagnosis of disease states, such as meningitis, hemorrhage, malignancy, inflammation, viral, bacterial, and parasitic infections.

II. SPECIMEN

A. Requirements

1. Specimen collection:
   a. The recommended collection tube is potassium EDTA. Plain Red top tube is acceptable if no visible clot is noted.

b. Cerebrospinal (CSF) does not require anticoagulant and is usually collected in special CSF collection tubes.
c. All specimens collected should be free of clots.
d. Extremely viscous fluids can be treated with a very small amount of hyaluronidase added to an aliquot of the sample.

B. Specimen volume required

1. A minimum of 1 ml of sample is required for analysis.

C. Unacceptable specimens:

1. Samples that do not contain sufficient volume refer to Core 6556 R Body Fluid Cell Counts – Manual Method.
2. Clotted samples should not be run on the analyzer. Any sample containing fibrin clots should be performed manually. Refer to Core 6556 R Body Fluid Cell Counts-Manual Method.

D. Characteristics that may affect test results:

1. Clotted samples.
2. Synovial samples that contain uric acid crystals or have a high viscosity.

E. Stored Specimen Stability

1. All fluids should be tested as soon as possible after collection. CSF should be tested within 4 hours of collection. All other body fluids should be tested within 24 hours of collection.

III. SUPPLIES & REAGENTS

A. Supplies

1. De-ionized water
2. Lint-free plastic lined lab wipes
3. Gauze
4. Test tubes
5. Clorox™ bleach (use when CELLCLEAN is indicated)
6. Sysmex reagents
7. Tri-level commercial controls, *e*-CHECK(XE)
8. Hyaluronidase (bovine testes) store at -20°C

B. Sysmex® Reagents

The same instrument is used for peripheral blood counting. No additional reagents are required for body fluid testing. See procedure Complete Blood Count of Whole Blood on the Sysmex XE-5000.

C. Calibration/Quality Control

The same analyzer is used for peripheral blood counting. No additional calibration or quality control is required for body fluid testing. See procedure Complete Blood Count of Whole Blood on the Sysmex XE-5000, CORE 6501R and Core 6035 R.

IV. OPERATING PROCEDURE

A. Start-Up Procedure

1. Check physical status.
2. Check and drain pneumatic trap chamber. (Refer to section 9.2 of the XE-5000 Instructions for Use for detailed information).
3. Check reagent boxes for sufficient run volume.
4. Check printer paper supply.
5. Power-Up Sequence
   a. Press power switch on IPU – Information Processing Unit. Must log on before powering up the Main unit.
   b. Log-on the IPU
   c. XE-5000 program log-on box appears. Type User Name, **HEME** and press **[ENTER]**.
6. Press the power switch on Main Unit. The Pneumatic Unit is controlled by the Main unit, so Pneumatic Unit power is left on. The instrument automatically performs self check on the:

Microprocessor	Mechanical parts
Temperatures	Background counts

7. Press the power switch on the printer.
8. When the “Logon Name and Password” screen displays, log on to the Main Unit.
   a. Press **[NUM/ALPH]** on the Main Unit to display alpha characters (if alpha characters are used for Logon Name).
   b. Press the appropriate keys on the Main Unit keypad, **HEME** for Logon Name.
   c. Press **[ENTER]** for the password.

9. Check background check and trap fluid level on maintenance log (See chart of background acceptable limits below).

XE-5000 Acceptable Background Counts	
Parameters	**Acceptable Limit**
WBC	0.1 x 10^3/ µL
Diff – WBC	0.2 x 10^3/ µL
IMI – Total	0.3 x 10^3/ µL
IMI#	0.005 x 10^3/ µL
NRBC-WBC	0.2 x 10^3/ µL
RBC	0.02 x 10^6/ µL
HGB	0.1 g/dL
PLT	5 x 10^3/ µL
PLT-O	10 x 10^3/ µL

10. Analyze Quality Control, if required.

B. Patient Sample Processing

1. MANUAL MODE **(130 µL sample volume)**
   a. Make sure the instrument is in the Ready status. The Ready LED light should be lit.
   b. Press **[MANUAL]** key on the Main Unit keypad.
   c. Enter the specimen number (up to 15 alpha/numeric characters), using the Main Unit keypad or read the barcode using the handheld barcode scanner.
   d. Press **[▼]** to change the setting parameter to "Mode" and then **[◀]** or **[▶]** keys to set Manual if it is not already selected.
   e. Press **[▼]** to change the setting parameter to "Discrete" and then **[◀]** or **[▶]** keys to select the following:
      ● **3 -** CBC + DIFF
   f. Press **[▼]** to change the setting parameter to "Sample" and then **[◀]** or **[▶]** keys to set to **3:** Body Fluid.
   g. After all the settings are complete press **[ENTER]** to confirm.
   h. When **[ENTER]** key is pressed, a background check is initiated. The background check analysis is repeated three times for the

background check. Results must be less than or equal to the values in the table below:

WBC-BF	0.001 x 10^3/µL
RBC-BF	0.003 x 10^6/ µL

i. If the background does not fall below these limits, the message "Background Error" will be displayed and the sample explorer screen will show the "**F"** with a red background in front of the background run. Select **[AUTORINSE]** on the function menu of the Background Check screen to perform an automatic cleaning. See Chapter 10 for additional information on resolving Background Errors.
j. If the background check is acceptable, the READY LED lights and the Main Unit displays body fluid analysis ready status.
k. Mix the contents of the test tube gently but thoroughly. Uncap the tube.
l. Place sample under the aspiration pipette so that the tip of the pipette is at the bottom of the sample.
m. Press **[Start]** switch. Remove the sample when 2 beeps sound, or the green Ready LED stops blinking. Patient results will print as samples are completed if auto-output is selected.
n. After the sample results are transmitted to the Sysmex computer screen the results must be validated using the **Validate** icon. This can only be done if the Last 20 icon is turned OFF.
o. The results are then transmitted to the Meditech computer system. When resulting the test, indicate the background check results in the appropriate result field in Meditech.
p. When the analyzer RBC count is <1000, perform a manual RBC count according to Core 6556 R Body Fluid Analysis-Manual Method.

CAUTION: If either of the analysis parameters is high:
WBC-BF > 1000 / µL or RBC-BF > 1,000,000/ µL,
there is the possibility of carryover on the analysis results of the next sample and the "Execute Background Check" error is displayed. Analyze the next sample <u>after</u> pressing the [B-check] function menu on the Main Unit. Failure to do so will result with the sample being flagged with an "F" with a red background on

the IPU in the Explorer and Browser screens. Samples flagged with a red "F" may have counts which are falsely increased.

q. Once body fluid analysis is complete, return the analyzer setting to:
   - **i.** Sample No: 1
   - **ii.** Mode: 1 Manual
   - **iii.** Discrete: 7 – CBC +DIFF + NRBC + RET
   - **iv.** Sample: 1 Normal

C. REPORTABLE RANGE

1. Cerebrospinal Fluid

Parameter	Reference Range	
	Neonate	Adult
WBC-BF	0 – 30 /µL	0 – 5 /µL
RBC-BF		
MN %		
PMN %	0 - 8 %	2 – 4 %
MN #		
PMN #		
TC-BF		

2. Synovial Fluid

Parameter	Reference Range
WBC-BF	0 – 200 / µL
RBC-BF	0 / µL
MN %	
PMN %	<25 %
MN #	
PMN #	
TC-BF	

3. Serous Fluid

Parameter	Reference Range	
	Pleural	Peritoneal
WBC-BF	<1000 / µL	<300 / µL
RBC-BF		
MN %	90 - 100 %	

PMN %		
MN #		
PMN #		
TC-BF		

PROCEDURE NOTES

A. Pay special attention to the RBC-BF result. The RBC counts are ONLY reported out in increments of 1000 (i.e. 1000, 2000, 3000, 4000, etc.). If the CSF analyzer RBC count is <1000, a manual RBC count must be performed using the hemacytometer method.
B. Results analyzed in the Body Fluid Mode are not subject to automatic validation, regardless of the setting. Check the display status of the "F" mark and perform validation carefully.
C. Do not place samples on a mechanical rocker. Excessive mixing may induce platelet clumping and alter white cell membranes resulting in false interpretive messages.
D. Clorox, a filtered bleach is recommended for use in cleaning. If Clorox is not available, generic bleach may be used but must be 5% Sodium Hypochlorite concentration and be free of particles that may cause background contamination when used on the analyzer. Clorox must be diluted to a 5% Sodium Hypochlorite concentration before preparing further dilutions recommended for maintenance.
E. For troubleshooting specifics refer to the Sysmex XE-5000 Instructions for Use.

LIMITATIONS OF PROCEDURE

A. XE-5000 MANUFACTURER STATED LINEARITY

Parameter	Range	Units
WBC-BF	0-10,000	/µL
RBC-BF	0-5,000,000	/µL

1. Parameters that exceed these limits are flagged with @ beside the result. The sample must be diluted, rerun and multiplied by the dilution factor.

   Reportable Result = Analyzer result x dilution factor

2. Note the use of dilution for linearity on the patient report.

B. POSSIBLE SAMPLE INTERFERENCES

1. Results may be compromised with clotted samples and synovial samples that contain uric acid crystals or have high viscosity.
2. All fluids should be tested as soon as possible after collection.
3. Further testing should be performed if more specific or sensitive limitation information is needed.

C. MAINTENANCE

The same instrument is used for peripheral blood counting. No additional maintenance is required for body fluid testing. See procedure for Complete Blood Count of Whole Blood on the Sysmex XE-5000.

D. REFERENCES

1. Sysmex XE-5000 *Instructions for Use* (North American Edition), Sysmex Corporation, Kobe, Japan, July, 2007.
2. Sysmex XE-5000 *Software Guide* (North American Edition), Sysmex Corporation, Kobe, Japan, July, 2007.
3. Clinical and Laboratory Standards Institute (CLSI). *Laboratory Documents: Development and Control; Approved Guideline*; Fifth Edition. (GP2-A5, 2006).
4. XE-5000 CLSI Procedure – Body Fluid Analysis Doc. #:1013-LSS, Rev.02. March 2013
5. College of American Pathologists (CAP) Hematology-Coagulation Checklist, current.
6. Body Fluids, 3rd ed.(1993), ed. by Kjedsberg and Knight, ASCP Press