Body Fluid Analysis **Technical Procedure #1304.t**

Principle

The pleural, peritoneal and pericardial cavities are lined with serous membranes separated by a small amount of fluid. The accumulation of fluid in these cavities constitutes an effusion. A peritoneal effusion is also called ascites.

Effusions can be further separated into transudates and exudates. In general, transudates indicate that fluid has accumulated because of systemic disease while exudates are ususlly associated with disorders involving inflammation, infection or malignancy. Transudates are usually clear and pale yellow with relatively low protein and WBC counts. Exudates are usually variably turbid, purulent or bloody with higher protein and WBC counts. Cell counts and differentials are useful in determining the nature of the effusion and in evaluating the response to treatment.

A peritoneal lavage is an emergency procedure used to distinguish between patients with significant abdominal injury and those with insignificant injury. In this procedure, one liter of Ringer's lactate solution is infused into the abdominal cavity via peritoneal dialysis catheter and immediately retrieved by gravity. It is then sent to the laboratory for testing.

A bronchoalveolar lavage (BAL) is performed to examine cells present in the lower airway. These fluid specimens are not sterile and may contain epithelial cells and bronchoalveolar macrophages in addition to hematopoietic cells.

Specimen

- All body fluids should be tested on a STAT basis.
- Serous fluids should be ideally collected in multiple containers according to the following protocol:
    - Hematology: 0.5 ml minimum, EDTA or heparin anticoagulant. Non-anticoagulated specimens may be used if not clotted.
    - Chemistry: Non-anticoagulated or heparinized tubes.
    - Microbiology: Sterile container required. Send immediately.
    - Cytology: No additives
    - If the specimen is not collected in multiple containers, the well-mixed specimen should be aliquoted on receipt in Hematology using strict asceptic technique when microbiological testing is requested.

- If a small clot is present perform the cell counts. Add the comment “small clot present, counts may be affected”. If a large clot is present, notify the physician or nurse that the cell counts cannot be performed. A differential is still performed.

- Fluids greater than 4 hours old before testing must have a disclaimer added, "*Specimen greater than 4 hours old; results may be affected.*"

- Any tubes not used, along with the tube used for hematological testing should be stored in the CSF rack in the refrigerator for 1 week after testing is complete.

- All specimens should be stored in the refrigerator for 1 week after testing complete.

Equipment

- Hemocytometer chamber (Neubauer) and #1 thickness coverglass (Riechert Bright Line; Buffalo, New York)

- Test tubes, any size convenient for making dilutions

- Pasteur pipettes, transfer pipettes and Rainin pipettes

- Microscope slides (stores)

- Marking pen, permanent ink

- Cytospin II with slide carriers (Shandon Southern Instruments)

- Disposable Cytospin funnels with built-in filter card (Shandon Southern Instruments)

- Hemastainer and associated reagent system

- Gauze

- Microscope

Reagents

- Saline (0.9 % NaCl) (stores) Store at room temperature, stable for use if no visible turbidity, color change, etc. observed to the noted expiration date.

- Albumin, (22% Bovine) (Gamma Biologicals Inc., Houston Tx) Store at 2-6 degrees C. Stable for use in the absence of growth to the noted expiration date.

- CSF Diluting Fluid (AJP Scientific) Store at room temperature. Stable for use in the absence of growth to the noted expiration date.

- WBC Diluting Fluid (AJP Scientific) Store at room temperature. Stable for use in the absence of growth to the noted expiration date.

Quality Control

- Hemacytometers must be clean and free of scratches, with bright lines easily visible.

- Any diluting fluid used must be examined for contamination the day of use.

- Cell count quality control is provided by duplicate counts and duplicate dilutions on chambers.

- To ensure accurate distinction of erythrocytes from other cell types in the hemocytometer, an additional procedure beyond unstained bright-field microscopy may be performed, such as addition of CSF diluting fluid, or phase microscopy.

- Slides prepared for each fluid are examined by the technologist for:

    - Proper staining
    - An adequate number of undistorted cells within the reading area
    - If cells appear distorted beyond recognition, there are too many cells (packed field), or cells appear imperfectly stained, the slide should be remade with attention to correction of the problem noted.

- The cell counts and differential are correlated, making sure that cell types and quantity seen appear consistent.

- All body fluid cytopreps are submitted for Pathology Review. The result of patient testing is reviewed for completeness, technique and random or clerical error.

Procedure

- Generate a worksheet

    - A worksheet is generated using the pneumonic BF

- Gross Appearance

- Record the color, clarity and volume of specimen received. Comment on any anomalies noted, such as fibrin clots, blood clots, hemolysis or lipemia.

- Cell count

  - Mix specimen well but gently to ensure even distribution of cells.

  - If high cell counts are expected (turbid or bloody specimen), the specimen must be diluted before testing.

  - When a dilution is necessary, it must be diluted and counted in duplicate

  - To avoid errors caused by over- or under-dilution, it may be necessary to prepare separate dilutions for WBC and RBC counts.

  - The choice of diluent depends on the type of fluid and the type of cell that is being counted.

  a. For most dilutions, normal saline will provide the best results as neither RBCs nor WBCs will be lysed or morphologically distorted.

  b. For WBC counts with interference from RBCs, choose a diluent that will lyse the RBC. Such diluents include WBC Diluting Fluid or CSF Diluting Fluid or a hypotonic solution of saline and distilled water mixed in a 1:3 ratio. The commercial products contain variable amounts of acetic acid however, and will precipitate any protein in pleural and peritoneal fluids, interfering with the cell count.

  - Each specimen or dilution must be plated in duplicate, on its separate hemacytometer. Using a Pasteur pipette, plain capillary pipette, or an Oxford pipette set to 10 ul, to plate each dilution on both sides of the hemocytometer chamber. Label chamber with the specimen number and dilution (ie. X10).

  - Place plated chamber on wet gauze and cover with an inverted Petri dish. Allow cells to settle for 5-10 minutes.

  - With condenser completely lowered to increase contrast and using the 40X high dry lens, count the WBCs and RBCs.

  - Under low light, count the WBCs and RBCs present in 5 square millimeters on each side of the counting chamber. If necessary, the phase microscope may be utilized to distinguish WBCs from RBCs.

- Cells in the hemocytometer appear as follows by light or phase contrast microscopy:

  - RBCs: Are anucleate with a distinct cellular outline, peripheral hemoglobin and clear centers. They may be crenated with blunt projections or may be smooth. Turned sideways, they may resemble “sticks”.

  - WBCs: Appear nucleated, granular, and may have an irregular outline. Cytoplasmic processes may be seen. May have a greenish tinge.

- If cell clumps are present on the hemocytometer, do not try to count individual cells within the clump. Qualify the result with a disclaimer: "*Count may not be accurate due to the presence of cell clumps.*"

- The number of squares counted should be determined by the number of cells present in each square. Count enough squares to generate an accurate cell count (200-1000 cells). Counts from each side of the chamber should match within 20%. For cell counts with low numbers (<10) these limits do not apply. Average all counts.

- Calculate the cell counts:

$$\text{Count/mm}^3 = \frac{(\#\text{ of cells on one side} + \#\text{ of cells on one side}) \times (\text{dilution factor})}{(\text{total \# of squares mm})(0.1\ \text{mm}^3)}$$

$$\text{volume of } 1\ \text{mm}^2 = 0.1\ \text{mm}^3$$

NOTE: The WBC count includes "others" such as tissue cells and NRBCs.

- Differential

- Smear preparation

a. Using a permanent marking pen, label a microscope slide with the Hematology number, patient's last name and first initial, unique identifier (bar code) number, and the specimen source.

b. Place the slide (writing slide up) in the Cytospin funnel carrier, place a disposable funnel over it, and close carrier.

c. For low protein body fluids such as lavages, a drop of albumin should be added to enhance adherence to the slide and to provide a protein matrix to prevent cell damage during centrifugation. High protein fluids (pleural and peritoneal fluids) do not need albumin, which may interfere with staining.

- Place 3-10 drops of straight or diluted body fluid specimen into the prepared funnel. Less fluid is needed dependent on the estimated cell count. Technologist experience is essential to minimize repeat smear preparations in order to prepare an acceptable differential slide.

Note:	The number of drops used is also dependent on the bore of the dropper used. Disposable plastic pipettes have a much larger bore than glass Pasteur pipettes and therefore should have a lower number of drops used.

- Place the slide carrier in the cytocentrifuge and run at the following settings:

  i. Time 5 minutes

  ii. Speed 80 X 10 rpm

  iii. Acceleration LOW

Note:	Speed and time may be changed to improve recovery and decrease morphological artifacts, if needed.

- After centrifugation is complete, remove the slide from the carrier, using the barrier of the biological hood to prevent contact with possible aerosolization from the cytocentrifuge, and allow the slide to air dry.

- Stain the slide on the BC slide stainer.

Note:	When many cells are present, the stain may be inadequate and cells appear young and blast-like. In this case, the slide should be put through the stainer a second time, skipping the first bath (methanol).

- Perform a differential on the slide.

  Report the cells according to cell type. Results are reported as a percentage in whole numbers. The number of cells counted should also be reported. When manually entering differential results, calculate the total and verify that results add up to 100%. Cells and other structures seen in body fluids may be:

- Neutrophils
- Lymphocytes
- Histiocytes/Macrophages
- Eosinophils
- Basophils/Mast cells
- Mesothelial cells
- Tumor cells
- Bacteria or yeast. Note whether intra or extra cellular

NOTE: Bronchoalveolar Lavage (BAL) Specimens

BAL fluids may have alveolar macrophages, which are classified as macrophages.

Macrophages laden with carbonaceous material may be seen in patients who smoke tobacco. These cells resemble siderophages in other fluids but the carbonaceous material will be black, brown, or blue-black, and will be more droplet-like. (photo p. 604, Rodak)

Mesothelial cells are NOT seen in BAL fluids since these cells line the body cavities and not the lung lining. They are macrophages.

Ciliated epithelial cells can be seen and should be reported since they indicate that the sample was obtained from the upper respiratory tract instead of deeper in the lung. These are columnar cells with the nucleus at one end of the cell, elongated cytoplasm, and cilia at the opposite end of the cell from the nucleus. They can occur in clusters. If the sample is not aged when the cell count is performed, these cells will be in motion in the hemocytometer since they can be propelled by the cilia. (photo p.603, Rodak)

- If any malignant cells, blasts or "others" are noted, the specimen should be sent to Cytology for correlation. Requisition "CYT" and send specimen to cytology. Note in the LIS, using canned text for "HPRL": "*Preliminary report only: referred for Pathologist review. If immediate response required, please call 734-2490."*
    - Any malignant cells, blasts, or others must be treated as a critical value if present for the first time.

- Call nurse or physician with any findings of bacteria-like organisms.

- Turn the slide in for Pathology Review by placing the slide in the daily path review box and entering the position number in the F/S field (folder/slot).

Reference Ranges

Serous effusions are not considered normal.

In the normal state, serous fluids are present in scant amounts only:

Fluid	Volume
Pleural	<20 ml
Pericardial	10-50 ml
Peritoneal	<50 ml

Serous fluids are clear and pale yellow.

A peritoneal lavage is performed to rule out intra-abdominal injury.

WBC: $<100/mm^3$
RBC: $<1000/mm^3$

Clinical Significance

Effusions of serous fluids form by ultrafiltration. The ultrafiltrates are classified as transudates or exudates. A transudate is an effusion caused by mechanical factors influencing formation or resorption of fluid (decreased plasma albumin or increased venous pressure). An exudate is an effusion caused by damage to the mesothelium (infection, malignancy, etc.). Effusions may also form due to the escape of chyle from the thoracic duct (chylous effusion).

Common causes of effusion in serous fluids:

Pleural:

Transudates	**Exudates**	**Chylous Effusion**
Congestive heart failure Hepatic cirrhosis obstruction of	Neoplasms: Bronchogenic carcinoma Metastatic carcinoma	Damage or thoracic duct

Lymphoma
Mesothelioma
Infection:
- Tubercuolosis
- Primary bacterial peritonitis
- Secondary bacterial peritonitis (ie. appendicitis)

Trauma
Pancreatitis
Bile peritonitis (2^o to ruptured gall bladder or perforation)

Pericardial:

Transudates	**Exudates**
Congestive Heart Failure	Infections:
Hepatic cirrhosis	- Bacterial pericarditis
Hypoproteinemia	- Tuberculosis
	- Fungal pericarditis
	- Viral or mycoplasmal pericarditis
	Neoplasms:
	- Metastatic carcinoma or lymphoma
	Trauma
	Myocardial Infarct
	Hemorrhage effusion
	Metabolic (ie. uremia, myxedema)
	Rheumatoid disease
	Systemic lupus erythematosis

Peritoneal:

Transudates	**Exudates**	**Chylous Effusion**
Congestive Heart Failure	Neoplasms:	Damage or obstruction of thoracic duct
Hepatic cirrhosis	- Hepatoma	
Hypoproteinemia	- Metastatic carcinoma	
	- Lymphoma	
	- Mesothelioma	
	Infections:	
	- Tuberculosis	
	- Primary bacterial peritonitis	
	- Secondary bacterial peritonitis (ie. appendicitis)	
	Exudates (cont'd)	
	Trauma	
	Pancreatitis	
	Bile peritonitis (2^o to ruptured gall bladder or perforation)	

References

- Kjeldsberg, C. and Knight, G.; Body Fluids; 3rd Ed.; pp. 159 - 176; ASCP Press, Chicago; 1993.
- Henry J., Ed.; Clinical Diagnosis and Management by Laboratory Methods; 18th Ed.; pp. 463 - 469; W.B. Saunders Company, Philadelphia; 1991.
- McBride, L. J., Textbook of Urinalysis and Body Fluids, A Clinical Approach, pp. 195 – 265; Lippincott, Philadelphia, 1998.
- Rodak, Bernadette, Hematology Clinical Principles and Applications, 2nd Edition., pp 591-605, WB Saunders, 2002.

Procedure History

Date	Written/Revised By	Revision	Approved Date	Approved By
12/90		Adopted		
9/92	J Jeffries	minor revisions	9/2/92	E Larkin MD
6/94	J Jeffries	add reference ranges, format revision	6/30/94	E Larkin MD
5/97	J Jeffries	IBM,update qc& rgts,error correction	5/21/97	E Larkin MD
		Annual Review	12/30/97	E Larkin MD
		Annual Review	10/28/98	E Larkin MD
		Annual Review	12/7/99	E Larkin MD
		Annual Review	10/03/00	E Larkin MD
		Annual Review	10/17/01	E Larkin MD
11/02	J Cannon	Annual Review	11/5/02	E Larkin MD
		Annual Review	10/17/03	E Larkin MD
9/22/04	J Cannon	Add BAL Ref.	9/22/04	E Larkin MD
11/23/04		Annual Review	11/23/05	E Larkin MD
2/24/05		New Director	2/24/05	K Janatpour MD
11/4/05	J Cannon	Clarify BAL diffs	11/10/05	K Janatpour MD
		Annual Review	09./18/06	D Dwyre MD
		Annual Review	11/05/07	D Dwyre MD
		Annual Review	07/03/08	D Dwyre MD
07/09	L Gandy	Minor Revision	07/23/09	D Dwyre MD
10/09	L Gandy	Minor Revision	11/17/09	D Dwyre MD
		Annual Review	10/15/10	D Dwyre MD

Body Fluid Analysis **Technical Procedure #1304.t**

Date	Written/Revised By	Revision	Approved Date	Approved By
		Biannual Review	8/24/12	D Dwyre MD
11/12	L Freeman	Changed acceptable CV	11/18/12	D Dwyre MD
1/13	L Gandy	Count small clots	Minor	
4/14	L Gandy	Storage changed from 1 month to 1 week	Minor	
		Biannual Review	10/1/14	D Dwyre MD
5/5	L Gandy	Add intra/extra cellular comment	Minor	