LABORATORY 10: Body Fluids Differential

**Note** Students should review and bring their hematology cell identification notes to Body Fluid Differential lab(s) and are expected to review the corresponding information in the lecture guide/Powerpoints, classroom notes and textbook information. Study questions at the end of this lab will require a review of notes and course resources on seminal and amniotic fluids and other body fluids well as body fluid cells.

**Points** Points are awarded for Admission Tickets, skills, including general lab requirements, as well as successful and timely completion of Study Questions. Study Questions are due by the end of the next lab period, or as designated by the lab instructor. Student results are expected to be within ± 20% of instructor's values.

**Objectives**
Using criteria set by the instructor, the student will:
1. Using appropriate resources and reference materials perform differential cell counts on two prepared cytospin Wrights stained slides obtaining results within ± 20% accuracy of instructor's values.
2. Scan at least 1 cytospin slide and identify a possible malignant cell.
3. Scan at least 1 cytospin slide and identify intracellular bacteria.
4. Scan at least 1 cytospin slide and identify erythrohages, siderophages, & leukophages.
5. Scan at least 1 cytospin slide to identify inclusions (crystal, organisms, etc.) seen in the macrophages.
6. Use appropriate recording format & applicable quality control measures to determine the acceptability of test results.
7. Answer all pre-test and study questions using related information found in the textbook, lecture guide, and this lab procedure and submit the results to the instructor by the due date.

**Principles and Related Information**
Cell differentials, along with the WBC and RBC cell counts provide extremely important information for the diagnosis and treatment of diseases involving CSF, serous, and synovial cavities. Infections, hemorrhages, and malignancies are of primary concern. In addition to basic cell identification, cells must be examined for the presence of inclusions that provide information concerning disease possibilities or state.

The body fluid differential count is performed on a stained smear. According to an article published in CAP Today (January 2012), ‘body fluid differential counts are most accurate when performed on cytospin preparations, or by a similar concentrating system’. Using a concentrating method allows the technician to view a much larger number of cells and more efficiently. In addition to centrifugation and cyto-centrifugation, other methods employed to concentrate the cells - include: sedimentation, and filtration. Due to time constraints, sedimentation and filtration are not routinely used; however, they produce less cell distortion. If the specimen is grossly bloody, a wedge / push smear may be useful.

The body fluid specimen collected for cell count and differential must be collected in an anticoagulant tube, preferably EDTA. In a standard centrifugation preparation, the tube is spun for approximately 5 - 10 minutes, the supernatent removed and saved for chemical testing, if needed. The sediment is placed on a clean slide, allowed to air dry and stained with Wright / Wright-Giemsa stain or Gram's stain, if bacteria are suspected.

Cytocentrifuge is an instrument that uses centrifugal force to direct the cells onto a small filter circle on a slide. The body fluid specimen is transferred to a funnel-type of assembly and spun for 1000 RPM for 10 minutes. As with the traditional centrifugation method, cells are likely to become distorted during the process. Cellular distortion may result in the presence of cytoplasmic vacuoles, nuclear clefting, prominent nucleoli, absent or unclear nuclear and cytoplasmic boarders, etc. Adding a small amount of albumin to the specimen before processing may reduce the amount of distortion. Note: Synovial fluid may need to have hyaluronidase added prior to making the slide to reduce the viscosity and make the slide more uniform.

Consult the textbook for normal and abnormal cells that can be seen in the various body fluids. In general, any nucleated cell that is normally seen in peripheral blood can also be found in a body fluid. Abnormal cells, including plasma and LE cells can also be seen in some conditions. Cells that normally line the various body cavities can occasionally also be found and must be appropriately classified by type: mesothelial cells found in pleural,
peritoneal or pericardial fluids and synovial lining cells found in synovial fluid. Macrophages containing inclusion(s) in its cytoplasm are classified by the type of inclusion.

- Erythrophages contain one or more red cells
- Siderophages contain hemosiderin/siderotic bodies or hematin crystals
- Leukophages contain white blood cell(s)
- Lipophage contains ingested fat/lipid

Malignant cells can also be found in body fluid aspirates and depending on the clinical site protocol may be initially classified as “Other” “Unclassified” or “Suspicious”. These slides must be reviewed by a cytologist/pathologist who will classify the cells.

Note: in MLT courses, any ‘suspicious cell’ should be brought to the attention of a supervisor.

Synovial fluid/joint fluid is produced by cells of the synovial membrane. This fluid provides nutrients and serves as a lubricant to the joint. The very thick/viscous consistency of normal synovial fluid is due to the presence of hyaluronic acid as well as mucopolysaccharides in the fluid.

When performing cell counts on synovial fluid, it is critically important to remember that any dilution of the fluid must be done with normal saline. The use of diluted acids, or other diluents may cause the hyaluronic acid and mucopolysaccharides to form clots which will entrap the cells and cause falsely decreased results.

Consult the lecture guide for additional information regarding reasons for and procedure used in the collection of synovial fluid as well as expected results and crystals that may be found.

**Evaluation of other Body Fluids**

Laboratory analysis of seminal fluid is most often performed in the evaluation of a couple’s fertility, in the preparation for artificial insemination or to determine the effectiveness of a vasectomy. If the analysis is to evaluate the quantity and quality of the sperm, three day abstinence is required before the collection period. The collected sample must be kept at room temperature and delivered to the lab ASAP. It is important that the time of the collection is noted.

Other reasons for sperm analysis include the evaluation of an unknown fluid to identify it as semen (forensic analysis).

Consult with your textbook and lecture guide for expected normal semen analysis results and testing procedures.

Consult with the lecture guides and your textbook for information regarding the analysis of sweat, amniotic fluid, gastric and fecal analysis.

**Equipment and Supplies**

1. Microscope
2. Differential Cell counter

**Supplemental References**

Kiechle, Frederick L., *Q & A CAP Today*, January 2012


Examination of body fluids: preparation of slides and cell morphology. On-line article, American Society for Clinical Laboratory Science, Wintr 2009, Volume: 22, Source Issue 1)

Current hematology course textbook.

Current UA / BF course textbook(s)

Lecture and Lab Guides & related course Powerpoints.

Web resources

**Agenda**

1. Classroom discussion, overview of procedures, activities, and expectations with Q & A.
2. Perform two (2) body fluid differentials on cytospin slides provided.
3. Scan resource cytospin slides for erythrophages, siderophages, leukophages, possible malignant cells, intracellular bacteria, and other inclusions/crystals.
4. Use lecture, lab and textbook materials to aid in answering study questions.
**Procedure**

1. Scan the entire cytospin prepared slide using 10x objective. Check for cell distribution and note cell clumps.

2. In an area of the slide where the cells appear evenly distributed, change to the 100x oil objective to begin the differential. As you move through the slide, use a battlement track to ensure that you are not back-tracking over a previously counted area.

3. Count and differentiate cells 100 nucleated cells. Include the following cell populations in the differential:
   - **neutrophils/segs**
     - Generally have the same characteristics as in peripheral blood. Increased numbers indicate an inflammatory response, such as acute bacterial meningitis (in CSF) and sepsis in otherwise normally sterile cavities.
   - **lymphocytes**
     - Although there can be significant individual variation, BF lymphocytes often have same characteristics as those in peripheral blood. Reddish blue to deep blue stained nucleus with clumped smudged chromatin. Lymphocytes have a small amount of light to moderate blue staining smooth cytoplasm and generally maintain a smooth boarder. Reactive lymphocytes with clumped chromatin and increased dark blue cytoplasm are often seen among normal looking lymphocytes during viral infections. Note: cytospin technique may cause the lymphocyte’s nucleus to appear lobed or as a ‘clover-leaf’ and are nucleoli are occasionally seen.
     - Increased numbers of mononuclear cells (lymphocytes and monocytes) have been associated with viral, tubercular and fungal infections / meningitis.
   - **monocyte/macrophages**
     - Like lymphocytes, the monocyte will have a reddish blue staining nucleus with pale blue cytoplasm. The chromatin of the monocyte’s nucleus is much less dense that that of the lymphocyte and it is not uncommon to see the nuclei. The nucleus of the monocyte is much less regular than that of the lymphocyte and it often appears bent into a ‘horseshoe’ shape. The boarders of the nucleus and the cell cytoplasm are often irregular and have small blebs or pseudopod projections. The cytoplasm will have a smooth, slightly grainy appearance - often referred to as ‘ground glass’, and maybe a bit grayer in color. In addition, the cytoplasm often contains vacuoles, something that would not be expected in lymphocytes.
     - For all practical purposes, monocytes and macrophages are the same cell. A monocyte leaves the peripheral blood, enters a body fluid, primarily for the purpose of scavenging and removing old dying cells and foreign substances. As the monocyte becomes activated for this purpose, it often undergoes structural changes. The nucleus may be pushed to one side and the cytoplasm becomes more vacuolated.
     - Erythophage – a macrophage that has engulfed one or more RBCs following a bleed into an area normally free of blood. This process can begin as soon as several hours post bleed.
     - Siderophage – as the RBCs in the erythrophage are digested, the breakdown products are seen as darkly staining granular deposits of iron-rich hemosiderin deposits and the cell is more appropriately named a siderophage. Further degradation will result in iron-free hematoidin (similar to bilirubin) crystals.
   - **eosinophils**
     - Often identified by the characteristic red-orange cytoplasmic granules, in a well stained smear. Increased numbers are often associated with allergic response. Reactions to fungal and parasitic infections and response to neural shunts, X-ray dyes, etc.
   - **basophils**
     - Characteristic deep-blue / black cytoplasmic granules make this cell easily identified.
   - **ependymal cells** – rarely seen cavity lining cell found only in CSF. Increased numbers can follow a traumatic brain injury, pneumonencephalography, surgery, etc.
   - **mesothelial cells** – cavity lining cells sometimes seen in in serous fluids.
   - **synovial lining cells** – cavity lining cells sometimes seen in synovial fluid.
4. “Other” or “Unclassified” cells to be identified by a pathologist must be noted in “Comments” AND brought to the attention of the instructor.

5. Nucleated RBCs are not counted as part of the differential, but should be brought to the attention of the instructor and a note made in “Comments”.

6. Macrophages with inclusions are part of the differential and are counted as macrophages. They MUST be noted in the “Other Cells” area. (Example: 2 erythropages and 1 siderophage seen during the differential). Please bring these cells to the attention of the instructor.

7. Intracellular bacteria or hematoidin crystals must be brought to the instructor’s attention. If verified, it is reported in “Comments”.
<table>
<thead>
<tr>
<th>Specimen</th>
<th>1</th>
<th>2</th>
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</thead>
<tbody>
<tr>
<td>Patient Name</td>
<td></td>
<td></td>
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<tr>
<td>Patient / Slide ID #</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specimen type</td>
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<td>Lymphocytes</td>
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<td>Macrophages</td>
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<td>Eosinophils</td>
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<td>Inclusions (specify type)</td>
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<td>Comments</td>
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</tbody>
</table>

Testing performed by: | Date:
Laboratory Exercise #10: Study Questions

Student Name ______________________ Date _____________________ / 20 points

Instructions: Answer the following questions using lecture notes, reading assignments, and information presented in the laboratory. Each question is worth one point unless otherwise stated. Laboratory study questions are due by the end of the following lab period, unless otherwise stated by the course instructor.

1. (5 pts, ½ pt each)
   1. What would be the significance, if any, of the following the following in the listed respective fluids?

<table>
<thead>
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<th>CSF Findings:</th>
<th>Significance</th>
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<tbody>
<tr>
<td>↑ lymphocytes</td>
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<tr>
<td>↑ neutrophils</td>
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<tr>
<td>↑ eosinophils</td>
<td></td>
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<tr>
<td>↑ monocytes</td>
<td></td>
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<tr>
<td>erythrophages</td>
<td></td>
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<tr>
<td>several ependymal cells</td>
<td></td>
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<tr>
<td>blasts</td>
<td></td>
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<tr>
<td>Other Body Fluid Findings:</td>
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<tr>
<td>several mesothelial cells in ascites</td>
<td></td>
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<tr>
<td>several synovial lining cells</td>
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<tr>
<td>LE cells</td>
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</tbody>
</table>

2. (3 pts) List three (3) reasons for performing semen analysis?

3. (3 pts) Provide three (3) special requirements or instructions for the proper collection of semen for analysis?

4. (2 pts) State the normal values for the following semen procedures.
   a. volume -  
   b. motility -  
   c. morphology -  
   d. sperm count -  

5. (2 pts) List four (4) reasons for performing an amniocentesis?

6. What is an L/S ratio and how is it used?

7. Why is a sweat test ordered?

8. (2 pts) State 2 reasons for performing a gastric analysis.

9. What hormone stimulates the production of gastric HCl?