BAL (BRONCHIAL ALVEOLAR LAVAGE) CELL COUNT

☒ St. Joseph Medical Center, Tacoma, WA	☒ St. Anthony Hospital Gig Harbor, WA	☐ Harrison Medical Center, Bremerton, WA
☒ St. Francis Hospital, Federal Way, WA	☒ St. Elizabeth Hospital Enumclaw, WA	☐ Harrison Medical Center, Silverdale, WA
☒ St. Clare Hospital Lakewood, WA	☒ Highline Medical Center Burien, WA	☐ PSC

PURPOSE

To provide instructions for performing the cell count on bronchial alveolar lavage (also called bronchial washing) fluid samples.

BACKGROUND

Clinical Significance

The evaluation of cellular components of BAL specimens can be useful in diagnosis of patients with airway diseases, interstitial diseases, sarcoidosis, malignancies, infections, and suspected alveolar hemorrhage.

Methodology

Nucleated cells are manually counted microscopically by the hemocytometer method. Differential counting of nucleated cells is done from a Wright-Giemsa stained smear made by cytocentrifugation.

RELATED PROCEDURES

R-W-HEM-1401	Body Fluid Cell Count
R-W-HEM-1406	Cytocentrifuge Use
R-W-HEM-1405	Hemocytometer Counts
J-W-HEM-1416	Midas III Stainer. See J-W-HEM1424 for stain preparation.
R-PO-HEM-0108	Pathologist Review of Blood and Body Fluids-Criteria

SPECIMEN REQUIREMENTS

Type of Specimen

A minimum of 4 mL of BAL fluid, collected in a cup-shaped vacuum container. No anticoagulant is needed or recommended. Record total volume and site of collection. Multiple samples may be submitted.

Specimen Storage and Stability

Fluid should be transported immediately to the laboratory, as cells begin to disintegrate soon after collection. Specimens from outside laboratories should be held at 2-8^{o}C and transported as soon as possible. Specimens are stable up to four hours at room temperature or refrigerated. Refrigeration is preferred. Cytospin slides should be prepared within the 4 hour stability window.

Criteria for Unacceptable Specimens

Specimens greater than four hours old are likely inadequate for evaluation. Delayed specimens will have testing performed but the comment BALOLD should be added, which reads “Specimen >4 hours old. Cell count may be affected/inaccurate.” Evaluation of such specimens should be made on a case by case basis with supervisor/manager/pathology approval. See Specimen Rejection/Cancellation Protocol

EQUIPMENT / SUPPLIES

- Hemocytometer (Improved-Neubauer): glass or disposable
- Wright-Giemsa stain, slide stainer
- Cytocentrifuge, cytospin chambers, filters and slides
- Hematology diluent, WBC or Turk’s Diluting Fluid, as needed
- 22% Albumin

QUALITY CONTROL

1. One Level of manual body fluid QC is performed every 8 hours of patient testing. Results are recorded in the LIS or the location according to your site.

2. Hemocytometer is inspected for integrity and cleanliness. Results are documented in the LIS or on the worksheet.

3. Certified pipettes are used to dilute specimens. This is documented in the LIS or on the worksheet.

4. WBC or Turk’s Diluting Fluid may be used to hemolyze RBCs for cell counting and to enhance the nucleus of nucleated cells, when the distinction between RBCs and nucleated cells is difficult.

5. Quality check of diluting fluids. Diluting fluids, stains and lysing agents are visually inspected under the microscope each day of use for clarity, cellular elements, and debris. The background count must be less than 3 cellular elements and free of debris. Results are recorded in the LIS or on the worksheet.

6. Cell counts are performed in duplicate by counting both sides of the chamber. Counts must agree within 20%. This is documented in the LIS or on the worksheet.

7. Cells are evenly distributed in the hemocytometer chamber. This is documented in the LIS or on the worksheet.

8. Cytospin slides are submitted for pathologist review. Refer to Pathologist Review of Blood and Body Fluids-Criteria.

PROCEDURE STEPS

Preparation of Specimen for Testing

At SJMC: BAL specimens are delivered directly to Microbiology for culture. Micro will prepare an aliquot of the specimen for delivery to hematology.

Warning: For personal safety, always use a face mask / shield or work under the hood while handling BAL fluids.

1. Check that the specimen source has been entered in the LIS, noting location of specimen collection if this information is available; eg. RML = right middle lobe, LUL = left upper lobe. **Note:** This is important if multiple specimens have been collected. Ensure specimen source details are added.

2. Record the total volume (in mL) of specimen in the LIS or on the worksheet.

3. Record the fluid appearance and color in the LIS or on the worksheet.

4. Visually inspect the fluid in the aliquot tube and record in the LIS or on the worksheet any clots or visible cell clumping. When a result of "Yes" is entered in the result field "Check specimen integrity- Clots Present?, the LIS will automatically append the comment BFCLOT, which reads "Body Fluid specimen has clots present."

Cell Count (hemocytometer)

1. Fill a small capillary tube about ¾ full of well mixed fluid. Vortex specimens if they are highly viscous.

2. Charge both sides of a hemocytometer and allow to settle at least 2 minutes.

3. Scan the counting area on 10X for even cell distribution and presence of cell clumps. Change to 40X lens and count sufficient squares to obtain a count of at least 50 nucleated cells on each side. Use WBC or Turk's Diluting fluid, if necessary, to distinguish between RBCs and nucleated cell types by making a 1:2 dilution or higher (refer to table below of frequently used dilutions). Counts on the two sides must agree within 20%. If not, mix the specimen thoroughly, replate, and recount.
   - If the nucleated count is elevated, estimate the number of cells present and determine an appropriate dilution. See Hemocytometer Counts procedure.
   - By the very nature of its origin, a BAL specimen may contain WBCs, alveolar cells, and bronchial lining cells. All of these nucleated cells are included in the nucleated cell count and in the differential.
   - If large clumps of cells are noted on the 10X scan, additional squares may need to be counted.

Dilutions

1. Manual Dilutions: Make an appropriate dilution as determined by the estimation from the direct plating. Pipette the appropriate volumes of fluid and diluent into a clean, labeled tube. Cap and mix well.

2. Frequently used dilutions:

Ratio	Volume of fluid	Volume of diluent
1:2	100 µL	100 µL
1:5	100 µL	400 µL
1:10	100 µL	900 µL
1:20	50 µL	950 µL
1:50	20 µL	980 µL
1: 101	20 µL	2 mL

   - Dilutions are plated on both sides of a hemocytometer, counted in duplicate, and must agree within 20%. If not, mix tube thoroughly, replate, and recount. Record all results and calculations in the LIS or on the worksheet.

Differential

1. Make slides, within the 4 hour stability, using albumin and the cytocentrifuge and allow to dry.

2. Stain slides with Wright-Giemsa Stain.

3. Assess slide quality. Scan the slide on 10x for general cell distribution. Cells should be adequate in number, intact, evenly distributed and with good stain color. If not, remake the slides.

4. Scan the slide on 10x for the presence of clumps of abnormal or possibly malignant cells or fungal elements.

5. Correlate the number and proportion of cells on the cytospin slide with the manual cell count results.

6. Count 100 nucleated cells and document in the LIS or on the worksheet, which includes the following result fields:
   - Fluid Polys %
   - Fluid Lymphs %
   - Fluid Monos %: Includes Monocytes and Macrophages
   - Fluid Eosinophils %
   - Fluid Basophils %
   - Alveolar Macrophage %: Large cells with dark staining cytoplasm similar in appearance to mesothelial cells and may contain phagocytized material such as hemosiderin or carbon.
   - Bronchial Lining Cells %: columnar in shape and may be ciliated.
   - Fluid Other Relative: Use for cells not in one of the listed categories, epithelial cells, unidentified cells, or suspected malignant cells.
   - Comments: Use for comments on cell inclusions, bacteria, or fungal elements.
   - Path Review Y/N: Submit all BAL cytospin slides to pathology.

If the WBC count is greater than or equal to 5 cells/mm^3, a differential will automatically be added on in the LIS.

If the WBC Count is less than 5 cells/mm^3, do not perform a differential. The LIS will automatically append the comment BF DIF, which reads, “WBC=5 or less per microliter. Differential not performed” and the differential will not be added on.

CALCULATIONS

1. Enter cell count data in the LIS for auto-calculation.

   - **WBC Count Side 1 and Side 2**: enter the number of nucleated cells counted on each side of the hemocytometer

   - **Dilution used for count**: enter dilution used or enter “1” if sample was not diluted

   - **# SQs counted**: enter the <u>total</u> number of large squares counted (# of large squares counted on each side of the hemocytometer added together). For example, if you counted 4 large squares on each side of the hemocytometer, you would enter “8”.

   Note: If manual calculation is necessary, see work instruction: Hemocytometer Counts.

PERFORMANCE CHARACTERISTICS

Expected Values: Cell concentrations are dependent on the amount of saline used and recovered in the lavage process. Appearance will vary depending on the concentration and type of cells present.

Reference Ranges:

WBC Count: 0-300 cells/cmm

Fluid Polys %: 0-25
Fluid Lymphs %: 0-100
Fluid Monos %: 0-100

ORDERING / RESULTING

- All reportable results and comments are documented in the LIS or on the worksheet.
- Body Fluid Path Review will be ordered by rule in the LIS when the test is resulted. Submit all BAL cytospin slides to pathology including counts with <5 nucleated cells.

TECHNICAL NOTES

1. Dilutions:
   - Use the smallest possible dilution, so that the total number of nucleated cells counted on the hemocytometer is between 50/mm^3 and 450/mm^3.

2. BAL specimens often contain mucus and cell clumps. Brief vortexing may help to break up clumps. Some clumps may remain, so it may be necessary to count additional squares on the hemocytometer to include a representative sampling of the cells present as determined by the 10x scan.

3. If the specimen contains clots but can still be pipetted, perform the analysis including the differential.

4. If the specimen contains clumped cells upon microscopic evaluation, use LIS phrase: BF CLUMP (Body fluid specimen has cell clumps present).

5. If the sample contains debris, result "*Debris present*" under the Comment field, as free text.

6. Cell Identification confirmation techniques:
   - Turks Diluting Fluid or WBC Diluting Fluid may be used to better distinguish between RBCs and nucleated cell types. Both enhance the cell nucleus for better identification. If used, you must initially count all cells, then repeat the count using the lysed fluid sample. Final WBC counts should match within 20%, after taking into account the dilution/calculations.
   - Diluted Methylene blue stain may be used. If used, the count should be compared in number and proportion to the cells on the cytospin preparation.
   - For staining or lysing techniques, refer to reference manuals.

REFERENCES

Glossary of Terminology for CSF and Body Fluids. CAP survey material.
Todd and Sanford. Clinical Diagnosis, 17th ed. WB Saunders Co, Philadelphia: 1984, pp 475-488, 564-565, 569.

Collins, R. Douglas. Illustrated Manual of Laboratory Diagnosis, 2nd ed. JB Lippincott Co, Philadelphia: 1975, pp 193-197.

Kjeldsberg & Knight. Body Fluids. ASCP, Chicago: 1982.

Ross and Neely. Textbook of Urinalysis and Body Fluids. Appleton-Century-Crofts, Connecticut: 1983.

Strasinger,S.K. and Shaub Di Lorenzo,M., Urinalysis and Body Fluids, 2008. E A Davis Co. Philadelphia.