BAL (BRONCHIAL ALVEOLAR LAVAGE) CELL COUNT

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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 Count
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 0.5 mL of BAL fluid, collected in a cup-shaped vacuum container. No anticoagulant is needed or recommended.

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.

Criteria for Unacceptable Specimens

Specimens greater than four hours old are likely inadequate for evaluation. Delayed specimens will have testing performed offline. 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

QUALITY CONTROL

1. One Level of body fluid QC is performed every 8 hours of patient testing.

2. WBC or Turk's Diluting Fluid may be used to hemolyze RBCs for cell counting when the distinction between RBCs and nucleated cells is difficult.

3. 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.

4. Cell counts are performed in duplicate counting both sides of the chamber. Counts must agree within 10%.

5. Cytospin slides are submitted for pathologist review. Refer to Pathologist Review of Blood and Body Fluids-Criteria R-PO-HEM0108.

PROCEDURE STEPS

Preparation of Specimen for Testing

Note: 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 while handling BAL fluids.

1. Record the appearance and color on the worksheet.

2. Record source, 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.

3. Record the volume of specimen on the worksheet.

4. Visually inspect the fluid in the aliquot tube and note on the worksheet any clots or visible cell clumping.

Cell Count (hemocytometer)

1. Fill a small capillary tube about ¾ full of well mixed fluid.

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

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. Counts on the two sides must agree within 10%. If not, mix the specimen thoroughly, replate, and recount.

- If the nucleated count exceeds 250/mm^2, estimate the number of cells present and determine an appropriate dilution.
- 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 to include them in proportion to their numbers.

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
1:5	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	2.00 mL

- Dilutions are plated on both sides of a hemocytometer, counted in duplicate, and must agree within 10%. If not, mix tube thoroughly, replate, and recount. Record all results and calculations on the worksheet.

Differential

1. Make slides using the cytocentrifuge.

2. Stain slides with Wright-Giemsa Stain on the manual slide stainer.

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.
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 on the worksheet. Note: The worksheet template includes the following result fields:
   - BF POLYS
   - BF LYMPHS
   - BF MONONUC: Includes Monocytes, and Macrophage
   - EOS%
   - BASO%
   - BF OTHER: Use for cells not in one of the categories, unidentified cells, or suspected malignant cells.
   - BF Comment: Use for comments on cell inclusions, bacteria, or fungal elements.
   - BF PR
   - 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.

If the WBC Count is less than 5 cells/mm^3, do not perform a differential. Result the BF COMMENT as SEE COM and add the phrase footnote: BF DIF (WBC=5 or less per microliter. Differential not performed). Result the remaining cell groups in LIS as N/A.

CALCULATIONS

1. Enter cell count data in the LIS for auto-calculation. Note: If manual calculation is necessary, see work instruction: Hemocytometer Counts (R-W-HEM 1405).

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.

ORDERING / RESULTING

- All reportable results and comments are documented on the worksheet.
- Enter results in LIS using order code: CELL BAL
- BF PR (Body Fluid Path Review) will be ordered by rule when the test is resulted. Submit all BAL cytospin slides to pathology.

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^2 and 250/mm^2.

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, use LIS phrase footnote: BF CLOT (Body fluid specimen has clots present) or BF CLUMP (Body fluid specimen has cell clumps present).

5. If the sample contains debris, result "*Debris present*" under BF Comment, 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.
   - 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

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