UnityPoint Health Pekin
Department of Pathology
Pekin, IL 61554

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Bronchial Lavage

Manual Cell Count and Differential
BALFCT

I. PRINCIPLE

A bronchial lavage is performed to obtain fluid from the lining of the epithelial surface of the lower respiratory tract. The cells present on the epithelial surface of the palveoli are believed to represent the inflammatory and immune system of the entire lower respiratory tract.

II. CLINICAL SIGNIFICANCE

Patients with interstitial pulmonary fibrosis have an increased number of neutrophils. Patients with idiopathic pulmonary fibrosis demonstrate lymphocyte percentages in the same range (10%) as normal controls and decreased percentages of alveolar macrophages.

III. SPECIMEN

A. Washing from bronchial lavage. Stable at 2-8°C for 24 hours.
B. Stored 2-8°C for 7 days in urinalysis/body fluid refrigerator.

IV. REAGENT

A. Wright's Stain
B. Nerl saline
C. Spinalscopics control

V. EQUIPMENT

A. Neubauer hemocytometer
B. Cytocentrifuge
C. Cytocentrifuge Disposable Funnels
D. Disposable Pipettes
E. Glass Slide
F. Hematek Slide Stainer

VI. QUALITY CONTROL

A. Manual counts on a Hemocytometer [HE-0101]:
    1. Hemocytometer techniques are monitored by commercial materials.
    2. Techs performing manual counts must analyze quality controls, obtain results within the acceptable ranges and document results.
    3. Two levels of commercial Spinalscopics control materials must ran every 8 hours of patient testing, per tech when a specimen is received.
B. Procedure for manual analysis of Spinalscopics control material:

1. Remove the controls from the refrigerator and allow them to come to room temperature (18-25°C) for 10-15 minutes but no more than 20 minutes. Mix the controls thoroughly by inverting the bottles several times, squeezing the bulb in the cap, aspirating and expelling the control through the glass dropper attached to the cap at least 10 times immediately prior to use to assure homogeneity of the contents. Thorough mixing with each use is important in order to obtain reproducible results. Avoid foaming.
2. Using glass dropper, charge both sides of the hemocytometer chamber with level 1. Repeat with level 2. Each control level must be tested in duplicate.
3. Immediately recap the controls. The Spinalscopics Controls should be stored tightly capped refrigerated (2-8°C) when not in use. Do not freeze. Once opened, the controls are stable for six months when stored at 2-8°C between uses.
4. Allow the cells to settle by placing the hemocytometer in a humidifier chamber for 10-15 minutes and no longer than 30 minutes before counting.
5. Count the RBC's and WBC's in the 9 squares on each side. The two sides must agree within 20% or 4 cells on a low count. Average the two sides. Divide average by 0.9 (or multiply by 1.1) and round to the nearest whole number. Result must be within that level's acceptable range or a two-sided re-plate is required for that level.
    - $\frac{\text{Average \# cells counted}}{0.9}$ = Total Cells/µL
    - Average # cells counted X 1.1 = Total Cells/µL
6. Document results on the Hemocytometer Control clipboard
7. Expected Ranges:
    a. The expected ranges are based on the average number of cells per µL counted in the nine large gridded squares on both sides of the hemocytometer counting chamber.
    b. Check commercial control package inserts for expected ranges.

C. If performing a dilution, background checks must be performed on the Nerl saline at the time of the counts at least once per day of testing or with a change in lot number or the saline. Charge a hemocytometer with the Nerl Saline and observe for contamination. Document on Body Fluid Cell Count form.

VII. PROCEDURE:

A. Cell Counts:

Cell Counts will not be done unless specifically ordered by the physician. If the cell count is specifically ordered, the WBC and RBC can be resulted in LIS by removing "HIDE" and entering results.

1. Mix specimen well.
2. Observe the clarity of the specimen to aid you in determining dilution to use.

a. If clear, charge both sides of the hemocytometer with undiluted fluid
b. If cloudy, perform a dilution using Nerl Saline.

3. Charge both sides of the hemocytometer.
4. Perform a WBC and RBC with a hemocytometer [refer to HE-0101]:
    a. Mix the specimen well.
    b. Using a disposable pipette, charge both sides of the hemocytometer chamber with specimen, taking care not to overfill the chamber.
    c. Allow the cells to settle by placing the hemocytometer in a humidified chamber for 10-15 minutes, but no longer than 30 minutes, before counting.
    d. Count cells in the 9 squares on each side.
    e. The two sides must agree within 20% or within 4 cells on a low count
    f. If count is extremely high, dilute the sample with Nerl Saline
    g. Average the two sides. Divide average by 0.9 (or multiply by 1.1) and round to the nearest whole number. Result must be within that level's acceptable range or a two-sided re-plate is required for that level.
        - $\frac{\text{Average \# cells counted}}{0.9}$ = Total Cells/µL
        - Average # cells counted X 1.1 = Total Cells/µL

    h. Calculate the total number of red and white blood cells per µL.
    i. If fibrin or other debris causes cell clusters, repeat the count. Report with a comment that the cell count maybe inaccurate due to debris or interferences.
5. Document results on the Body Fluid worksheet and the Bronchial Analysis form.

B. Differential
1. Prepare 2 cytospin slides from well-mixed specimen according to the cytospin procedure
2. Allow cytospin slides to dry thoroughly.
3. Stain with Wright's staining using the Hema-Tek Stainer
4. Check the slide quality under the microscope.
5. Take the slide and patient's Bronchial Analysis form, to the pathologist to perform the manual differential.
6. If a pathologist is not available, place the patient's slide and Bronchial Analysis form on counter next to refrigerator. The first shift hematology tech should follow up on this ASAP when pathologist is available

C. If requested, Report "Inflammatory Cell Ratio" (% lymphs versus % neutrophils). Please use the formula below to calculate the ICR.

VIII. CALCULATIONS

EXAMPLE: 200 Cell Differential: Neutrophils- 30
Lymphocytes- 90
Histocyte- 80

90 + 30 = 120
90/120 = 75% Lymphocyte
30/120 = 25% Neutrophils
ICR = Neutrophils 25%; Lymphocytes 75%

IX. REPORTING RESULTS

A. Report Results in LIS
   1. WBC and RBC as #/µL
   2. Differential as percentages
   3. Inflammatory Cell Ratio as percent

B. Reference Intervals: Normal Adult Values
   1. Alveolar macrophages 90-96%
   2. Lymphocytes 6-8%
   3. Neutrophils 1%
   4. Eosinophils 1%
   5. Basophils 1%

X. PROCEDURAL NOTES/PROBLEM-SOLVING TIPS

A. Footnote any body fluid that has a clot, clumped cells, or other debris present and indicate that counts may be inaccurate.
B. Place cell counts on worksheet.
C. Order Path Review only if requested.
D. If cytology is also ordered on the specimen inform the clinical pathologist that a body fluid count was also ordered and performed.
E. For RBC and WBC counts, a reference interval has not been established for this test on the supplied specimen type. Please integrate test results into the context of the patient's clinical condition for interpretation.

XI. REFERENCES

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B. "Basic Principles and Techniques of Bronchoalveolar Lavage", Talmadge E, King Jr, MD. wwwuptodate.com. "Bronchoalveorlar Lavage", Baughman RP

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3. www.aafp.org/afp/2005/0315/afp20050315p1153-f1.jpg

POLICY CREATION :	***Date***
Author: ***Kelly Hall, MLS (ASCP)***	***August 20, 2018***
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Reviewed by:

Lead	Date	Coordinator/ Manager	Date	Medical Director	Date
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Rev	Description of Change	Author	Effective Date