Children's
Hospitals and Clinics
of Minnesota

BAL Counting WBCs in Bronchoalveolar Lavage

Purpose   This procedure provides instructions for COUNTING WBCS IN BRONCHOALVEOLAR LAVAGE, using a Neubauer counting chamber, and to prepare cytoprep slides for BAL cytology when required.

Policy Statements
- This procedure applies to all laboratory technologists performing hematology, microbiology, and histology, section supervisor, and pathologist.

Materials

Equipment	Reagents/Solutions	Supplies
• Neubauer counting chamber • Wescor Aerospray model 7152 with Cytopro rotor (St.Paul), Wescor Cytopro Cytocentrifuge model 7621 (Mpls.)	• 3% acetic acid or Turks Solution - Store at room temperature in Hematology • Certified Blood Bank Saline - Available in Blood Bank • Cell Chex Two Level Body Fluid Control (Streck # 212425, L1-UC, L2 12x2ml, cat. # B3160-206) • RPMI Medium 1640, (Gibco, Ref# 11875-101), 100ml, supplied by Histology.	• 22 x 30 coverslips • Pipetters with tips • 12 x 75 test tubes • Marker - Moist Mark Plus – Cancer Diagnostics, chc # 22062, or lead pencil. • 15 mL sterile disposable centrifuge tubes • Microscope slides, 1/2G, uncoated for Cytopro. Part # SS-117. • Wescor sample chambers with fast, white Cytopads, caps, pk 48. Part # SS-113. • Wescor sample chambers with slow, tan Cytopads, caps, pk 48. Part # SS-114. • Disposable pipettes

Sample   **Note: All specimens are taken to Microbiology first.**

1. Aliquot of pooled, thoroughly mixed, BAL fluid provided by Microbiology.

2. Minimum amount: 0.5 mL

3. Anticoagulant: None required.

4. Storage: DO NOT store. Deliver to lab immediately after collection.

5. Tube must be labeled with patient's name, medical record number and total volume of fluid collected (provided by Micro).

6. Reject specimens which are not labeled or which have insufficient quantity.

Quality Control   1. A procedural control is performed on each shift by performing a cell count on either

the Level 1 or Level 2 Cell Chex Two Level Body Fluid control manufactured by Streck #212425, cat.# B3160-206.

a.) Controls have a closed vial stability of 6 months, once opened controls are stable for 30 days. Controls should be run in an alternating manner, if the previous shift ran the Level 1 control the next shift should run the Level 2 control.

b.) Warm the selected control to room temperature. Mix the control by rolling the vial in the palm of the hand and then inverting gently several times.

c.) Mount the control on the Hemocytometer as you would a patient, allow to sit for 10 minutes, perform counts.

d.) Enter calculated results for test FWBC (RBC's not reported) in function MEM in Sunquest C-BFL (Low Control), C-BFH (Hi Control).
Be sure to modify the method to match the lab in which you are working. (BF Worksheet, MANL method for Mpls., BFS worksheet, MANB method for St.Paul).

2. Check saline, Turks Solution, or 3% glacial acetic acid for contamination before using as a body fluid diluent:

- If particles are present, obtain new diluent.
- Document the diluent used on the Hematology Fluid Logsheet as well as the results of the background check as acceptable (O.K.) or not.

Procedure   Follow the activities in the table below for COUNTING WBCS IN BRONCHOALVEOLAR LAVAGE.

Step	Action	Related Document
1	Notify Histology when a BAL count is ordered. On weekends, off shifts or holidays this includes paging the Histology Tech on call. The Histology tech would then contact the Pathologist if necessary.	
2	All specimens are taken to Microbiology first. To insure the sterility of the sample Microbiology will set up requested cultures before the sample is received in Hematology. NEVER PROCESS BEFORE MICROBIOLOGY. a. Record the total volume on the request form from Cerner or BRONCHOSCOPY/AIRWAY ASPIRATION TEST REQUESTS FORM. Bronchcoscopy - Airway Aspiration Test Request Form b. Order test code BRON if a cell count has been requested and aliquot a portion for hematology use. An accession number and labels will print for hematology. c. Order test code BAAH in order to track specimens that are having slides made for histology requests. d. Order test code EBRON in order to track specimens that are having extra slides made without a formal histology request. These slides will be held in a box with the hematology slides and will be available in the event histology testing is requested in the future.	

3	Cell Count: Record the appearance, sample site (RUL, RML, RLL, LUL, LLL) and total volume of the specimen in the Body Fluid Logbook: a. Color: BALs are normally colorless b. Clarity – see Table D – Clarity Definitions.	
4	If specimen is **clear and colorless**, plate in Neubauer counting chamber.	
5	If specimen is **not clear and colorless**, make at least a 1:2 dilution with 3% acetic acid or Turks Solution plate in Neubauer counting chamber. Allow the chamber to sit undisturbed for at least 10 minutes before counting cells.	
6	Count all white cells in all 18 squares: a. Use 40x objective to distinguish between cell types. b. Do not count squamous or ciliated epithelial cells. c. The range of cells will be large, from small lymphocytes to gigantic macrophages.	
7	Record results on the Body Fluid Logsheet. Include all calculations.	
8	Enter raw count of WBCS in the computer using the worksheet BRON; the computer completes the final calculation.	
9	The computer will convert the raw WBC count to WBC per µL using the following formula: $$\text{\# cells counted}/\mu L = \frac{\text{\# cells x dilution x depth}}{18 \text{ (area counted)}}$$	
10	The computer will convert the WBC/µL to WBC/total volume with the following formula: # cells/µL x volume in mL x 1000 = # WBC Thou/total volume	
11	CSF or Body Fluids that have been collected from Shunts or Drainage reservoirs can contain a marked amount of cellular debris and/or breakdown products, making an accurate cell count impossible. Result these samples in SmartTerm in function MEM by appending the code ECD (Unable to perform cell count due to extreme cellular degeneration) to CSFC (CWBC,CRBC); BFC (FWBC,FRBC); CYTM (CYWB,CYRB); BRON (BWBC).	

| 12 | After the cell count has been completed prepare slides for Histology. Evaluate the number of stains ordered to insure that an adequate number of cytospin preparations are made; one slide per stain is the minimum. Extra slides should be made incase of breakage or additional stain requests.
As a general rule 6-8 slides should be made with every sample.
Label slides with patient name, date and accession number.
Use RPMI as the dilution medium. In this way left over fluid can be stored in the refrigerator.
Clear fluids may not need to be diluted at all or at a dilution of 1:1. A cloudy fluid will need to be diluted 1:5 or more. Samples that are thick or that contain debris can be vortexed and the debris removed with wooden applicator sticks. If filtering is necessary use filter paper or gauze that has been dampened with RPMI to prevent cell loss or damage.
   ** This requires experienced judgment as the process is dependent upon the amount of specimen and amount of slides needed. Be careful not to over dilute the specimens. There are examples of cytopreps at the bench (one is acceptable, one is to thick) to use as a guideline.
Refer to HEM 3.11 Cytocentrifugation of Body Fluids if necessary.
Deliver slides with paperwork and an Interim Report (function IRA in Sunquest) to Histology. | |
| 13 | Additional Notes:

1. Count all squares when the first large square has 5 cells or less.

2. Count an equal number of squares on each side of the hemacytometer.

3. The number of cells counted on each side of the hemocytometer should agree ± 10%.

4. Computer codes for color, clarity and location:

Table C – Sunquest GUI Color and Clarity Result Codes

Table D – Clarity Definitions
5. Leftover fluid is to be labeled clearly, dated and stored in the refrigerator for seven days. | |

14	In SmartTerm:
	
	Function: MEM
	Worksheet: BRON
	Test-1: `<CR>`
	Accept or Modify: A `<CR>`
	Accn. No.: Enter Accn # `<CR>`
	BAA: Enter codes for appearance and location if provided. Separate codes with a hyphen (-). See Table: [Table AN - BAL Source Code Descriptions]
	BAV: Enter total volume of mLs using 1 decimal place
	BWBC: ENTER Cells Counted: xxx
	                Dilution: xxx
	                Area: xxx
	                xxx (result pops up, enter on Hematology Manual Log Sheet, use scroll bar if result is bypassed)
	BTWM: Calculated by computer
	BTWV: Calculated by computer
	(A)ccept, (M)odify or (R)eject: A	

15	Enter result for BAAH: Function: MEM Test- 1: BAAH Accept or Modify: A `<CR>` Accn No.: Enter Accn # `<CR>` BAAH: Enter COPA (Results will remain pending if not answered)	
16	Enter result for EBRON: Function: MEM Test- 1: EBRON Accept or Modify: A `<CR>` Accn No.: Enter Accn # `<CR>` EBRON: Enter HIDE (Results will remain pending if not answered)	

References

1. Colombo, J.L., Hallberg TK: Recurrent aspiration in children: lipid laden alveolar macrophage quantitation. Pediatr Pulmonol 1987; 3: 86-89.

2. Henry, J.: Clinical Diagnosis by Laboratory Methods, 15th edition, W.B. Saunders Co., Philadelphia, PA, p. 124.

3. Thermo-Shandon Cytospin® 4 Operator Guide, Pittsburgh, PA, 2002.

4. Streck Cell Check Body Fluid Cell Count Control product insert Cat.# B3160-206. 7002 S. 109 Street Omaha, NE 68128 USA, www.streck.com

Appendices

1. Bronchoscopy/Airway Aspiration Test Requests form sent with specimen from surgery (see form at end of procedure).

2. Body Fluid Log Book

Historical Record

Version	Written/Revised by:	Effective Date:	Summary of Revisions
1	Julie Schulte	12/26/1991	Initial Version
2	Laura Carmack	07/06/1995	Updated MIN and STP versions
3	Laura Rachford	09/2000	New STP version
4	Laura Rachford	07/2003	System procedure
5	Laura Rachford	04/2004	Added procedure notes 1 –3
6	Al Quigley	12/2008	Updated/rewritten
7	Al Quigley	05/2010	Added coded comment for samples containing degenerating cells
8	Al Quigley	06/01/11	Revised, reformatted (renamed from Heme.B.25)
9	Al Quigley	01/2012	Added Streck Body Fluid Cell Count for use as procedural control
10	Al Quigley	07/12/12	Slides to be prepared by Histology staff at both sites.
11	Al Quigley	02/05/13	Revised to include preparing slides for Histology.
12	Al Quigley	02/03/14	Cytopro Application
13	Al Quigley	10/05/15	Added test code BAAH to allow for specimen tracking.
14	Al Quigley	04/11/16	Added test code EBRON to allow for specimen tracking on samples without histology requests.