Beaumont Laboratory
Royal Oak

Effective Date: 12/23/2019
Supersedes: 04/06/2017
Related Documents:
RC.HM.PR.004 Hematology General Directives
RC.HM.PR.008 Criteria for Review of Peripheral Blood and Body Fluid Smears
RC.HM.WF.010 Suspected Peripheral Blood & Body Fluid Intracellular Microorganisms
RC.HM.WF.028 Resulting Body Fluid Cell Counts and Differentials

DIFFERENTIAL CELL COUNT
CEREBROSPINAL FLUID AND OTHER BODY FLUIDS
CYTOSPIN PREPARATION

RC.HM.PR.023.r10

Principle

The cytocentrifuge concentrates cells by the principle of low speed (1000 rpm) centrifugation from a fluid onto a compact area on a microscope slide. Using a small amount of fluid, the cells are deposited in a uniform mono-layer in a circular area of 6 mm. The slides are air dried and stained with Wright's stain.

See Hematology General Directives for directive on when to perform body fluid differential counts.

Specimen Collection and Handling

Type:	CSF or other body fluids (e.g., pleural, peritoneal, synovial, pericardial, etc.)
Anticoagulant:	None for CSF; K_2EDTA preferred for other body fluids that have a high probability of clotting.
Amount:	Minimum sample size is 0.2 mL Optimum sample size is 0.5 mL
Special Handling:	Specimen must be well mixed before preparing cytospin smears.
Timing:	Specimen is stable for one hour at room temperature.
Criteria for Unacceptable Specimens:	Specimens that are totally clotted are unacceptable and must be recollected.

DIFFERENTIAL CELL COUNT CEREBROSPINAL FLUID AND OTHER BODY FLUIDS – CYTOSPIN PREPARATION

Supplies

Equipment:

1. (Shandon) Thermo Scientific Cytospin 4 Centrifuge
2. Brown or white filter cards
3. Microscope Slides
4. Plastic cytospin sample chambers
5. Wax pencil
6. Dispo pipettes

Reagents:

1. 22% Bovine Albumin – Purchased from Sigma. No manufacturer's expiration date listed. Per known stability and frequency of use, reagent expiration is 12 months from opening or until expected performance is not achieved (i.e. cloudiness, color change, etc.). If expected performance is not achieved, discard and open new vial of reagent.
2. 50% Bleach (Alternatively, a hospital-approved disinfectant may be used in the appropriate dilution).
3. Cidex™ disinfectant
4. Alconox™ detergent

Quality Control

1. Strict adherence to procedure is the only adequate means of quality control.
2. Check timer and RPM's of cytocentrifuge quarterly.

Procedure

1. For very low counts, concentrate cells by sedimentation before making slides.

2. Label the frosted end of two glass slides with computer-generated CSF diff labels.

3. Position a **brown** filter card with flat side against each glass slide (See Note #1).

4. Position plastic cytospin chamber on top of filter paper.

5. Load cytospin set-ups into cytocentrifuge.

   NOTE: Check that hole in filter paper is in alignment with outlet port in cytospin chamber. Cytocentrifuge **must** be properly balanced.

6. Add 2 drops of 22% bovine albumin (stored in 2-8°C refrigerator).

7. **Gently** add specimen to bottom of chamber (not along sides) with a Dispo pipette. (If specimen is added with too much pressure or force, it may cause damage to the cells and loss of cells in filter card.) (See Notes #2 **and** #4.)

   A general guide for amount of specimen to be added is as follows:

10-20 cells	Minimum of 12 drops
21-40 cells	Minimum of 8 drops
41-80 cells	Minimum of 4 drops
100 cells	Minimum of 2 drops

NOTE: Fluid may be diluted with saline or CellPack™ if very cellular.

8. Lock lid into position and start cytocentrifuge **immediately.** A delay will result in a loss of cells.

9. Centrifuge for 5 minutes at 1000 RPM.

10. Carefully remove slides **immediately** after spinning stops. Do not let un-spun fluid contact slide when chamber is disassembled.

11. Place filter card in biohazard bucket. Place chamber in pan of bleach/soap water or Alconox™. Place in Cidex™ disinfectant for maximum of 45 minutes. Rinse in water.

12. Air dry slides; circle cellular area with a wax pencil on **back** of smear.

13. Stain in usual manner with Wright's or Wright-Giemsa stain.

14. Scan **all slides** at 20X for the presence of malignant cell clumps. (See Note 10.)

    NOTE: If any suspicious cells or cell clumps are seen (*EXCEPT those composed of neutrophils, lymphocytes, or macrophages*), forward to pathologist following Path Review Protocol.

15. Perform 100 cell differential in usual manner including percentages of mesothelial cells and macrophages.

Expected Values

CSF:	Normal CSF is essentially clear, colorless and contains only infrequent cells, mostly small lymphocytes; few monocytes; rare neutrophils.
Serous fluids:	Few mononuclear and mesothelial cells.
Synovial fluid:	Mostly mononuclear cells: monocytes, macrophages, and synovial cells. Neutrophils less than 25%.
Bronchoalveolar Lavage (BAL):	Mostly macrophages; NO mesothelials. Lymphocytes 10-12%; neutrophils less than 1% (Non-smokers.) (See Notes 7 and 8 below.)

Reportable Range

1. For CSF report percentage of the following cell types: Poly, Lymph, Monocyte, Macrophage, and Others.

2. For serous fluids report the percentage of the following cell types: Poly, Lymph, Monocyte, Macrophage, Eos, Mesothelial and Others. Mesothelial cells are not found in CSF or synovial fluids.

3. For differentials with lower WBC counts where it is difficult to find 100 cells, count as many cells as possible and include the comment:

   "Insufficient cells for accurate differential - only "X" counted".

4. A scan for blasts must be performed on all pediatric CSFs (regardless of WBC count). If no blasts are seen, ensure that "no blasts seen on scanning cells" is reported:

   "Insufficient cells for accurate differential. No blasts seen on scanning cells."
   -OR-
   "Insufficient cells for accurate differential. ____ blasts seen on scanning a total of cells."

5. All CSFs will have a cytospin smear made, stained, and reviewed. Refer to Resulting Body Fluid Cell Counts and Differentials procedure.

6 The LIS/HIS is reviewed on suspicious fluid morphology for malignancy. This is to: (1) verify Cytology received a specimen and (2) to compare interpretations and see if in agreement.

Notes

1. Use **brown** filter cards for CSF fluids and with small volumes of other body fluids. These cards are thinner than the white cards and will not absorb as much of the overall sample fluid. The use of white filter cards with small volumes will cause loss of cells in the card.

2. Pipette sample **into** chamber. Do not run it down the side of the cone.

3. Viscous synovial fluids can be centrifuged and cells resuspended in saline before preparing cytospin prep.

4. *If a fluid contains clots, cytospin slides should be prepared from a suspension of the clotted material (agitate with wooden sticks) as well as the supernatant to increase possibility of detecting malignant cells.*

5. Report the presence of bacteria **only** when they are intracellular. Refer to "Suspected Intracellular Peripheral Blood & Body Fluid Microorganisms" **Workflow**.

6. Mesothelial cells are seen only in serous fluids, **NOT** in CSF or BALs.

7. Bronchoalveolar lavage (BAL) specimens may contain epithelial cells. For these BAL differentials, report the epithelial cells as *"Non-hematologic cells".* (Do not send for Path Review as Cytology will identify these cells.)

8. The presence of high fluorescence in the Sysmex XE-5000 body fluid channel may indicate the presence of malignant cells.

9. Expected BAL values for smokers: Macrophages greater 90%; lymphocytes 1-5%; neutrophils 1-4%. Many inclusions may be present in macrophages of smokers.

10. All three editions of the Kjeldsberg body fluid morphology atlases are available at the morphology and/ or student benches.
11. When generating a slide label, ensure that the label has printed properly. Also, write the fluid source on the label after printing.

12. After reviewing the cytospin smear, verify cell count by answering YES to CELCK field. If in disagreement with count, repeat the cell count or remake cytospin smears. Failure to answer this field will result in the specimen remaining on the pending log.

13. The distinguishing features of mesothelial cells and malignant cells are as follows:

MESOTHELIAL CELLS (BENIGN)	MALIGNANT CELLS
General	
- Distinguishable borders in cell groups	- Lack of poorly defined margins to cells in clump
- Cell groups on a flat plane - not ball-like	- Ball-like arrangement of cells (spherical aggregates)
- No or rare cells within cells	- Cannibalism (cells within cells)
- Never as large as malignant cells	- Often extremely large, bizarre, monstrous
Nucleus	
- Uniformity of size and structure	- No uniformity of size and structure (large in relation to cytoplasm)
- Round or oval shape	- Varied shapes - often bizarre
- Smooth nuclear membrane	Irregular nuclear membrane
- Even chromatin pattern	- Uneven chromatin pattern (fine and lumpy in same cell)
- Multinucleated with uniform size	- Multinucleated with varied sizes and dissimilarity
- Nucleoli regular	- Large nucleoli, abnormally shaped
- No nuclear molding	- Nuclear molding or mosaic pattern
- Normal mitosis	- Abnormal mitosis
- No herniation of nucleus	- Herniation of nuclear membrane into cytoplasm
- No nuclear clefts (unless artifactural)	- Nuclear clefts
Cytoplasm	
- Uniform character	- Irregular character
- Vacuoles	- Vacuoles may be over the nucleus
- No aberrant nuclear material	
- Aberrant nuclear material	

References

1. Kjeldsberg CR, Krieg AF. Cerebrospinal fluid and other body fluids. In: Davidsohn I, Henry J, eds. Clinical diagnosis by laboratory methods, 17th ed. Philadelphia: WB Saunders. 1984: 459-488.

2. Procedure furnished by Shandon Scientific Company, Ltd, 65 Pound Lane, Willecden, London, England.

3. Kjeldsberg CR, Knight JA. Body fluids, 2nd and 3rd eds. Chicago: ASCP. 1986: 157-161 and 1993: 321-324.

4. Rabinovitch A, Cornbleet, PJ. Body fluid microscopy in US laboratories. Arch Pathol Lab Med, January 1994, Vol. 118,13-17.

Attachments

Attachment A – INSTRUCTIONS FOR CYTOSPIN 4 OPERATION
Attachment B – HISTORIC MEMOS
Attachment C – BODY FLUID REVIEW POLICY

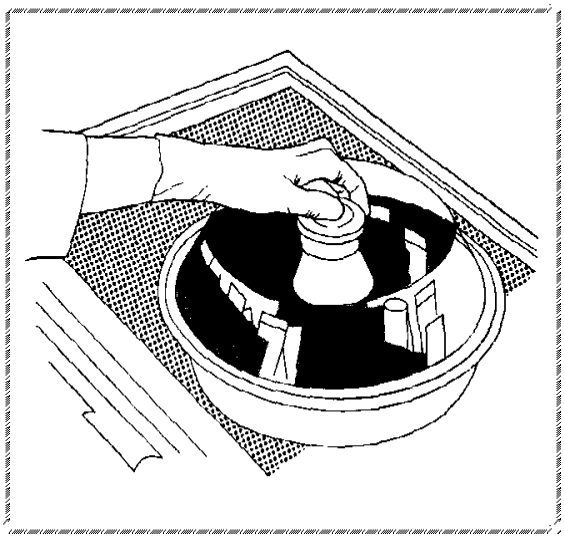
Authorized Reviewers

Medical Director, Hematology

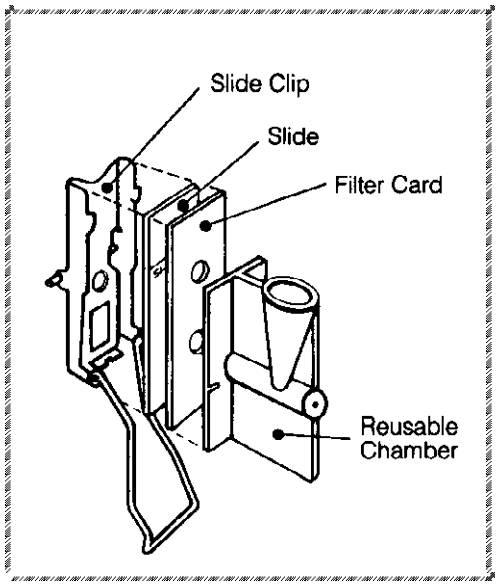
Attachments **ATTACHMENT A - INSTRUCTIONS FOR CYTOSPIN 4 OPERATION**

1. **WARNING: NEVER OPEN THE SEALED HEAD WHEN IT IS IN THE CENTRIFUGE!!** You **MUST** remove the head from the Cytospin centrifuge **BEFORE** opening or closing it!!

2. To open the sealed centrifuge head, press the release button in the center of the sealed head by holding the lid with one hand and pulling the release button with the other. (See Figure 1.) Note how the two seals fit into place: The top seal is just under the release button, and the lid seal is the large seal running around the outside of the bowl.



3. With Cytoclip slide clip in the open position, fit glass slide, filter card and sample chamber against the Cytoclip slide clip. (See Figure 2.) Bring spring clip up and secure under the two retaining hooks.



4. Place chambers and clip assemblies in slots provided in bowl. Make sure that each is free to move forward to an upright position and tilt when released.

5. Load sample in usual fashion, depending on cellularity, **maximum** of 10 drops (0.5 mL).

   NOTE: When sample is placed into the chamber, the clip assembly should be tilted back toward the center of the head so the sample will not run to the front of the chamber and start to be absorbed by the filter card.

6. Replace the lid of the bowl and lock by pushing down on the button in the center of the lid. **REMINDER: The sealed head should always be opened and closed while it is outside the instrument.**

7. Cytospin centrifugation speed should be 1000 RPM and time should be 5 minutes. These will be programmed under Program '1'. Verify these times. If correct values are not in program '1', set from the Program Mode Panel as follows:

   a. Using ↑ and ↓, select Program 1.
   b. Using ↑ and ↓, select speed of 1000 (RPM).
   c. Using ↑ and ↓, select time of 5 (min).
   d. Acceleration speed should be MED.
   e. Press "Save Settings".

8. From Control Mode Panel, press <START>.

9. When the Cytospin centrifuge has stopped, an audible end-of-cycle signal sounds for approximately 3 seconds. This alerts the operator that the head may be removed so that the slides may be removed as soon as possible.

10. Remove bowl from Cytospin centrifuge. Place bowl on counter next to Cytospin centrifuge.

11. Remove lid by pulling up on release button while holding down bowl with opposite hand.

12. Check the sample chambers to see if all of the specimen has spun onto the slide.

13. When disassembling slide clip/slide/filter card, let filter card fall away from assembly. **Care must be taken not to brush the slide along the filter card.**

14. Air dry slides before placing in stainer.

15. Clean cytospin cups and clips in soapy water (1 tablespoon Alconox™ per 1/2 gallon {1/2 pink bucket} of water). There is no time limit needed here.

16. Disinfect cytocups by placing in Cidex™ solution for a maximum of 45 minutes.

    NOTE: Cidex™ comes in a gallon container from Stores and has an attached activator which must be placed in the Cidex™ before initial use. Activate the Cidex™ solution by adding the entire contents of the activator vial to the gallon container. **Shake well.** Date the Cidex™ container. The solution is active for 14 days. It should be a greenish color when you use it.

17. Rinse the cytospin cups in distilled water and lay to dry.

3/09 NP

REFERENCE: Shandon Cytospin 4 Operator Guide, Thermo Electron Corporation, 2004.

ATTACHMENT B

Beaumont® **Inter-department Communication**

William Beaumont Hospital
Royal Oak, MI 48073

TO: Hematology Staff DEPARTMENT: Hematology
FROM: Joan C. Mattson, M.D. DEPARTMENT: Chief, Hematopathology
DATE: December 19, 1991

SUBJECT: Pediatric CSF Differentials

For Pediatric Hematology Oncology CSF specimens with less than 5 cells/mm^3, accession cytospin prep as a "Slide Review" (182) and select "CSF" as specimen type (6).

Scan cytospin prep for blasts and report according to previous memo dated 10-19-90 (see below).

JM/nef

Beaumont® **Inter-department Communication**

William Beaumont Hospital
Royal Oak, MI 48073

To: Hematology Staff Dept:
From: Joan C. Mattson, M.D. Dept: Chief, Hematopathology
Date: October 19, 1990

Re: CSF Differentials

It is the policy of the Hematology Division that CSF differentials are automatically performed on all pediatric patients when greater than 5 cells/mm^3 are present. We have recently been getting requests from Pediatric Hematology / Oncology to perform CSF differentials when counts are less than 5 cells/mm^3. It is likely that these requests are to rule out the presence of blasts. Clearly, when counts are less than 5 cells/mm^3, it may not be possible to find enough cells to give an accurate differential. When such a request is received, a cytospin prep should be scanned for blasts and reported as either:

"Insufficient cells for accurate differential. No blasts seen on scanning cells."

-OR-

"Insufficient cells for accurate differential. _____ blasts seen on scanning a total of _____ cells."

ATTACHMENT C

Beaumont®

Inter-department communication

William Beaumont Hospital

To:	Clinical Pathology Pathologists, Residents, Fellows & Hematology Laboratory Staff	**Department:**	Clinical Pathology
From:	Joan C. Mattson, M.D. Chief, Hematopathology	**Department:**	Clinical Pathology
Date:	Monday, January 19, 2004		

Subject: Body Fluid Review Policy

This is a reminder to all Hematopathologists, residents, and fellows that the following policy has been in place since 2002.

In order to comply with **CAP requirements** as indicated in the Hematology checklist questions: **#** HEM.39850 "If a body fluid specimen is examined in more than one area of the laboratory, is there a mechanism to compare the data and interpretations from these different areas, particularly when a diagnosis of malignancy is rendered?", we are changing our body fluid pathologist review as follows:

1. All body fluids saved for pathologist review must have as part of the HDS comment entered by residents/fellows/pathologists a statement indicating

    a) Whether a sample was received by cytology and
    b) Whether our findings agree with cytology

This will provide a real time comparison of our cases saved for review with cytology samples.

The random sampling of "normal" or non-suspicious body fluids has been discontinued as our fluid review criteria are very broad and includes a large number of random normal samples from diverse sites.

JCM:cg

S:cpadmin/Mattson/Corresp/Blood Fluid Review Policy Mon 01-19-04

DIFFERENTIAL CELL COUNT CEREBROSPINAL FLUID AND OTHER BODY FLUIDS – CYTOSPIN PREPARATION

Document Control

Location of Master: Hematology Procedure Manual
Master electronic file stored on the Clinical Pathology server:
S:\HEMACOAG\Document Control\Hematology\Procedure\Master Documents\bfdiff.doc
Number of Controlled Copies posted for educational purposes: 0
Number of circulating Controlled Copies: 0
Location of circulating Controlled Copies: NA

Document History

Signature	Date	Revision #		Related Documents Reviewed/ Updated
Prepared by: Nancy Ramirez, MT (ASCP)SH	12/1987			
Approved by: Joan C. Mattson, MD	12/29/1987			
Reviewed by: (Signature)	**Date**	**Revision #**	**Modification**	**Related Documents Reviewed/ Updated**
Joan C. Mattson, MD	02/20/1989		OK	
Joan C. Mattson, MD	03/12/1990		Updated and retyped in NCCLS format	
Joan C. Mattson, MD	01/29/1992		Updated Ped Hem criteria pg. 1 and 3	
Nancy Ramirez, MT (ASCP)SH	12/29/1992		Added BAL info. – normals and notes 8, 9; retyped review page.	
Joan C. Mattson, MD	12/30/1992		See attached for previous	
Joan C. Mattson, MD	12/27/1993		OK	
Nancy Ramirez, MT (ASCP)SH	02/1994		Added exceptions to principle; updated steps 14 and 15 to LIS.	
Joan C. Mattson, MD	12/12/1994		Above noted; OK	
Joan C. Mattson, MD	12/22/1995		Added report results #5	
Noelle Procopio, MT(ASCP)SH	02/13/1997		No change	
Joan C. Mattson, MD	12/29/1997		Deleted ref. to older model cytospin in step 4 of procedure; note 4 added, scan all slides	
Noelle Procopio, MT(ASCP)SH	04/21/1998		Reworded exception 1 under principle; incorporated Appendix A	
Noelle Procopio, MT(ASCP)SH	01/04/1999		No change	
Joan C. Mattson, MD	01/24/2000		No change	
Joan C. Mattson, MD	03/22/2000		Added “Wright – Giemsa”; # 13, pg. 3	
Noelle Procopio, MT(ASCP)SH	12/04/2001		No change	

Noelle Procopio, MT(ASCP)SH	12/30/2002		No change	
Joan C. Mattson, MD	02/21/2003		Updated note # 5, pg. 4 regarding bacteria notification/follow through	
Noelle Procopio, MT(ASCP)SH	12/22/2003		No change	
Joan C. Mattson, MD	01/14/2004		Updated to K_2EDTA, pg. 1; updated note # 5, pg. 4 (names and directive); resident/fellow responsibility to correlate findings, Cytology added in 2003 (see reporting results # 5 pg.4)	
Joan C. Mattson, MD	01/26/2004		Added Appendices C And D	
Noelle Procopio, MT(ASCP)SH	01/10/2005		No change	
Joan C. Mattson, MD	02/19/2005	00	Standardized procedure format	
Joan C. Mattson, MD	11/10/2006	01	Updated note #5 regarding intracellular microorganisms; referred to appropriate "process" for further information; pg. 4	
Ann Marie Blenc, MD	07/02/2007		No change; new director.	
Ann Marie Blenc, MD	09/05/2007	02	Updated frequency of centrifuge RPM check pg. 2; added refrigerator temp. range, pg. 2; updated Cidex soak, pg. 3; combined notes, pg. 4; moved directives for when to perform differentials to the General Directives procedure.	
Ann Marie Blenc, MD	02/26/2008		No change	
Ann Marie Blenc, MD	4/02/2009	03	Updated to Cytospin 4; removed procedure step #16 re performing iron stain if macrophages appear to contain iron; added Cidex and Alconox to reagents.	
Ann Marie Blenc, MD	03/31/2010	04	Clarified mesothelial cells in body fluids (Reportable Range).	
Ann Marie Blenc, MD	07/25/2011	05	Added statement that cytospin smears will be made on all CSFs; updated centrifuge RPM and timer to quarterly.	OK
Ann Marie Blenc, MD	07/29/2013	06	Added Notes re slide label printing and fluid source and answering CELCK field; reworded resident/fellow responsibility for suspicious fluid morphology to reflect current practice; removed original Att C memo re body fluid review policy (redundant).	OK

Ann Marie Blenc, MD	12/15/2014	07	Added Note regarding high fluorescence on XE-5000; updated "process" to workflow; updated format; added Attachments section to body of procedure.	OK
Ann Marie Blenc, MD	05/19/2016	08	Removed references to Verkon and Sparquat disinfectants; added hospital-approved disinfectant.	OK
Ann Marie Blenc, MD	04/06/2017	09	Updated logo; updated verbage regarding performing scan for blasts on all pediatric CSFs, regardless of WBC count.	OK
Elizabeth Sykes, MD	02/02/2018			
Peter Millward, MD	01/30/2019		New Medical Director	
Ann Marie Blenc, MD	12/23/2019	10	Clarified LIS/HIS to be reviewed for suspicious fluid morphology for malignancy.	OK