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DOCUMENT NUMBER:	RIV-PPP-0456
DOCUMENT TITLE:	Body Fluids Analysis_ Cell Count
DOCUMENT NOTES:	

LOCATION:	RIV-rel	**VERSION:**	06
DOC TYPE:	RIV PPP	**STATUS:**	Release

EFFECTIVE DATE:	14 Mar 2024	**NEXT REVIEW DATE:**	14 Mar 2026
RELEASE DATE:	14 Mar 2024	**EXPIRATION DATE:**	

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BODY FLUID ANALYSIS: MANUAL CELL COUNT

Policy

- Body fluid cell counts should be completed within 1-2 hours of specimen collection.
- Morphologic observation competency assessment performed annually.

Specimen Type

- CSF
- Pleural fluid
- Peritoneal fluid
- Gastric fluid
- Synovial fluid
- Pericardial fluid
- Abdominal fluid
- Ascites fluid
- Bronchoalveolar lavage
- Bronchial lavage
- Thoracentesis fluid
- Other (indicate under comment section if applicable and must be approved by Medical Director)

Storage Requirements

Room Temperature: Must be processed immediately, within 1 hour for CSF and 2 hours for others from time of receipt in the lab.

Refrigerator Temperature: Store specimens at 2°C to 8°C if it cannot be processed right away.

Specimen Storage: Specimens will be kept in the laboratory for at least 7 days.

Slide Storage: Body fluid smears/pathology review smears are retained for a month for possible review or reference.

Specimen Collection

CSF: Sterile Collection Tube (SF10) usually #3 or #4,
Other body fluids: Collected in EDTA or Sodium Heparin Tube.
BAL: Sterile container. Must be processed within 36 hours.

- CSF specimen is collected in tube #3 and #4 sterile screw top tubes numbered in order of collection. Unless specified by physician tube

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number 3 or 4 are used for the cell count and differential. Tube #1 is used for chemistry and serological tests and tube #2 is usually designated for microbiology.

- The other specimens can be collected in sterile tubes containing anticoagulants for cell counts in EDTA or sodium heparin. The large containers must be well mixed, and a small aliquot aseptically removed and placed in a 5 mL heparin tube for cell count. BAL must be processed within 36 hours.

- If no anticoagulant has been added, observe container for fibrin clots or pellicles. If none are seen, immediately mix specimen well and transfer an aliquot to a tube containing an anticoagulant.

- If fibrin clots or pellicles are seen in the specimen submitted for cellular evaluation, perform count as usual then add the comment, "Specimen contains small clots, cell count may not be accurate" or "Specimen contains large clots, cell count may not be accurate.

- If Synovial Fluid is viscous, see procedure below. For the other specimen, if it is completely clotted, do not perform the test. Add the comment, "Specimen completely clotted, unable to perform cell count."

  Note: If there is a prolonged delay in performing the test (e.g. >8 hours), add in the comment, *"Specimen testing was delayed, result may be inaccurate due to cellular degradation."*

- Due to difficulty in obtaining body fluid specimen, effort will be utilized to perform cell count and/or manual differential analysis if possible. Technical judgment is required in the handling of these specimens. Confer with a pathologist or manager if necessary.

- Always use **STERILE TECHNIQUE** when handling body fluid specimens.

Equipment And Reagents

- In CYTO™ C- Chip Disposable Hemocytometer
- Sterile Normal saline diluent
- 22% Bovine Serum Albumin solution
- Slides

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- Sterile pipettes
- Hematek Slide Stainer
- Single Cytology funnels (Cytofunnel)
- Hyaluronidase Type III Bovine Sterile Mixture Sigma # H-3884
- Calibrated MLA Pipettes
- Cytocentrifuge
- Modified Wright's Stain Pack
- 10% Glacial Acetic Acid
- Equipment includes microscope capable of counting at 40X magnification

Procedure

I. Appearance

Step	Action
1.	Examine fluid grossly. Record color and appearance/transparency in "Body Fluid" patient log. **Note:** Use of the term "Xanthochromia," which is produced by RBC lysis, should be limited to CSF, and not used to describe other fluids. If the CSF visually contains blood, centrifuge a small amount of CSF or examine the supernatant of Chemistry specimen.
2.	Observe specimen for fibrin clots and pellicle formation. • If small fibrin clots are detected, perform test as usual and include a comment in report stating that results may not be accurate due to fibrin clot formation. • If specimen is completely clotted, do not perform cell count. Notify provider that the specimen is clotted.
3.	Determine total volume and enter volume in "Body Fluid" patient log.
4.	To reduce viscosity of synovial fluids, add Hyaluronidase to one (1) ml of fluid (stored in the freezer), incubate at 37°C for 10 minutes.

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II. Manual Cell Counts

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1.</td><td>Thoroughly mix specimen. Load by capillary action both sides of hemacytometer chambers with 10uL of undiluted well mixed body fluid specimen.
Note: On high counts a dilution may be necessary.
C-Chip Hemacytometer is for single use only, should be clean, and free of scratches. Discard hemacytomter after use.</td></tr>
<tr><td>2.</td><td>If the fluid is extremely bloody, hazy or cloudy, it may be necessary to dilute specimen with sterile saline. Dilution must be set-up in duplicate. Record dilution in designated column of "Body Fluid" patient log.

DILUTION GUIDE FOR MLA PIPETTES:

<table>
<tr><th>Volume of Sample</th><th>Volume of Diluent</th><th>Dilution Factor</th></tr>
<tr><td>100 µL</td><td>100 µL</td><td>2</td></tr>
<tr><td>50 µL</td><td>100 µL</td><td>3</td></tr>
<tr><td>50 µL</td><td>200 µL</td><td>5</td></tr>
<tr><td>20 µL</td><td>100 µL</td><td>6</td></tr>
<tr><td>10 µL</td><td>100 µL</td><td>11</td></tr>
<tr><td>10 µL</td><td>200 µL</td><td>21</td></tr>
<tr><td>20 µL</td><td>500 µL</td><td>26</td></tr>
<tr><td>10 µL</td><td>500 µL</td><td>51</td></tr>
<tr><td>10 µL</td><td>1000 µL</td><td>101</td></tr>
</table></td></tr>
<tr><td>3.</td><td>Cell counts and calculation of cell counts can be difficult. It is imperative that counts be calculated correctly so when in doubt consult with your co-worker or supervisor.

Note: for Moderate and High WBC counts; prepare 1:20 dilution using 10% Glacial Acetic Acid. Let the diluted specimen stand 3-5 minutes for complete hemolysis. Glacial Acetic Fluid should not be used when counting cells in synovial fluids since it causes the formation of mucin clots.</td></tr>
</table>

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BODY FLUID ANALYSIS: MANUAL CELL COUNT

4.	Allow the cells to settle in a humidified chamber for at least 2 minutes before counting.
5.	With the low-power (10X) objective, quickly scan both ruled areas of the hemacytometer to determine whether cells present are evenly distributed. If cells are unevenly distributed repeat step one.
6.	With the high power (40X) dry objective, count the RBCs and WBCs in the 9 large squares of both chambers. • Record counts in designated columns of "Body Fluid" patient log. • Record numbers of squares counted if not all 9 squares are counted. Note: Count only cells within the ruled area and those cells which rest on the top line and left-hand line of the ruled area. Do not count the cells touching the bottom or right-hand lines.
7.	The counts from each chamber must agree within 10% or the count must be repeated.
8.	Calculate the results: • If specimen were counted undiluted take the average of counts from both chambers and multiply the average by 1.1 (if 9 squares counted). • If count was performed on a dilution, multiply the average count from both chambers by 1.1 (if 9 squares counted) and by the dilution factor. • If the number of squares counted are less than 9, use formula below: $\frac{\text{Number of cells counted x dilution x 10(depth)}}{\text{Number of large squares counted}} = \text{cells}/\mu\text{L}$
9.	If $\leq$ 5 white blood cells are present, the cell differential is omitted. Result as "Differential is not indicated." If specimen contains more than 5 white blood cells proceed to "WBC Differentiation" section of this procedure.

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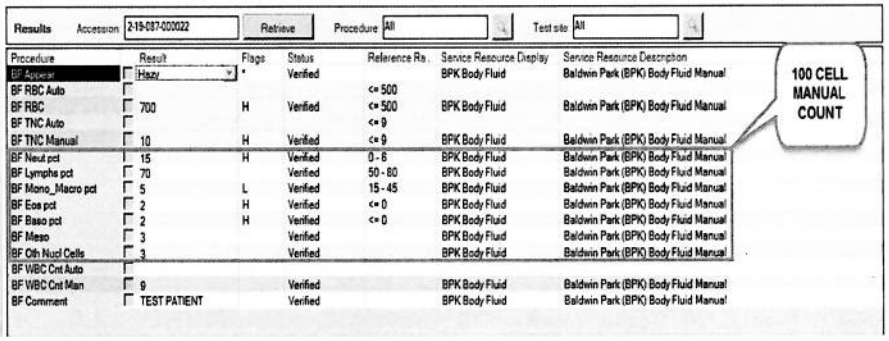
10.	Record results on the Body Fluid patient Log **Note:** Enter results in Cerner as shown in the highlighted fields as shown below.



Procedure	Result	Flags	Status	Reference Ra...	Service Resource Display	Service Resource Description
BF Appear	Hazy	*	Verified		BPK Body Fluid	Baldwin Park (BPK) Body Fluid Manual
BF RBC Auto				<= 500		
BF RBC	700	H	Verified	<= 500	BPK Body Fluid	Baldwin Park (BPK) Body Fluid Manual
BF TNC Auto				<= 9		
BF TNC Manual	10	H	Verified	<= 9	BPK Body Fluid	Baldwin Park (BPK) Body Fluid Manual
BF Neut pct	15	H	Verified	0 - 6	BPK Body Fluid	Baldwin Park (BPK) Body Fluid Manual
BF Lymphs pct	70		Verified	50 - 80	BPK Body Fluid	Baldwin Park (BPK) Body Fluid Manual
BF Mono_Macro pct	5	L	Verified	15 - 45	BPK Body Fluid	Baldwin Park (BPK) Body Fluid Manual
BF Eos pct	2	H	Verified	<= 0	BPK Body Fluid	Baldwin Park (BPK) Body Fluid Manual
BF Baso pct	2	H	Verified	<= 0	BPK Body Fluid	Baldwin Park (BPK) Body Fluid Manual
BF Meso	3		Verified		BPK Body Fluid	Baldwin Park (BPK) Body Fluid Manual
BF Oth Nucl Cells	3		Verified		BPK Body Fluid	Baldwin Park (BPK) Body Fluid Manual
BF WBC Cnt Auto						
BF WBC Cnt Man	9		Verified		BPK Body Fluid	Baldwin Park (BPK) Body Fluid Manual
BF Comment	TEST PATIENT		Verified		BPK Body Fluid	Baldwin Park (BPK) Body Fluid Manual

Procedure **III. WBC Differentiation**

Step	Action
1.	Label one glass slides with the patient's name, date, and specimen source or with specimen barcode.
2.	Place the cytofunnel in the cytospin head. Cytoclip assemblies must be loaded directly opposite from each other. Note: Remove cytospin head from cytospin centrifuge before loading cytofunnels. If cytofunnels are not balanced instrument will not run.
3.	Put 3-6 drops of the fluid into the cytofunnel chamber. <u>Suggested:</u> Maximum cell count/suspension: 500 TNC/µL, 5,000 RBC/µL Amount cell suspension/chamber: 5 drops

WBC Count/µL:	0-100	101-300	301-700	701-1500	1501-3000
Dilution:	none	1:2	1:4	1:10	1:20
Drops of fluid	5	5	3	1	1
Drops of saline	0	5	9	9	19

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	After making the WBC dilution, if the RBC count has not fallen to ≤ 5000, dilute further. **Note**: Overcrowding the preparation distorts cellular morphology.
4.	Place cap on cytofunnel. Add 1 drop of 22% bovine serum albumin into each chamber.
5.	Lock the lid of cytospin head and place into cytocentrifuge. Spin at 1,000 RPM for 6 minutes.
6.	Carefully remove the slides.
7.	Air dry slides.
8.	Stain slides on Hematek slide stainer. Check for the quality of the stained slides to ensure clear differentiation of cells.
9.	Perform a 100 WBC differential count. Separate cells into Neutrophils, Lymphocytes, Monocytes/Macrocytes, Eosinophils, Basophils, and other nucleated cells. **Note:** Body fluids containing large numbers of atypical tissue cells, clumps of tissue cells, and malignant appearing cells or any tissue cell not easily identified as a macrophage or mesothelial should be referred to a pathologist. After hours and weekend shifts should provide the physician with the WBC and RBC count and a comment indicating that a pathology review was requested. Leave the differential resulting section pending.
10.	Slides are saved for a month for possible review or reference.

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Procedure **IV. Cerner Result Entry**

Result Field	CSF	Pleural	Synovial	Body Fluid
Total Vol (in mL)	Record body fluid total volume			
Xanthochromia	Yes or No	Not applicable	Not applicable	Not applicable
Color	Colorless Yellow Pink Red	Colorless Yellow Pink Red		
Appearance	Bloody Clear Cloudy Clotted Hazy Slightly Hazy	Bloody Clear Cloudy Clotted Hazy Slightly Hazy	Bloody Clear Cloudy Clotted Hazy Slightly Hazy	Bloody Clear Cloudy Clotted Hazy Slightly Hazy
RBC	Numeric or specimen clotted	Numeric or specimen clotted	Numeric or specimen clotted	Numeric or specimen clotted
TNC	Numeric or specimen clotted	Numeric or specimen clotted	Numeric or specimen clotted	Numeric or specimen clotted
Neutrophil %	Numeric	Numeric	Numeric	Numeric
Lymphocytes %	Numeric	Numeric	Numeric	Numeric
Monocyte/Macrophage %	Numeric	Numeric	Numeric	Numeric
Eosinophils %	Numeric	Numeric	Numeric	Numeric
Basophils %	Numeric	Numeric	Numeric	Numeric
Mesothelial Cells %	Numeric	Numeric	Numeric	Numeric
Other Nucleated Cells %	Numeric	Numeric	Numeric	Numeric

Comment: Indicate tube number if more than 1 tube is counted.

Xanthochromia of the CSF refers to a yellow color of the supernatant after the CSF has been centrifuged. It is caused by subarachnoid hemorrhage, traumatic tap, jaundice, etc. Centrifuge a sterile tube not used for Cell Count or refer to Chemistry specimen.

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Procedural Notes

A. Perform CSF cell count on last tube collected, either tube #3 or #4. If physician or provider requested on also counting tube #1, perform the cell count per request. Add a body fluid comment "*Cell Count performed on CSF Tube #_*"

B. It is the responsibility of the reporting CLS to ensure the accuracy of result transcribed from Body Fluid Patient Log into Cerner resulting system.

C. If the specimen submitted may be QNS (quantity not sufficient) for the test ordered, notify requesting physician for test prioritization prior to proceeding with the process. Document in Cerner Result Comment field.

D. **Body Fluid Distribution:**
   1. The person logging in is responsible of identifying and distributing all test requested for the submitted sample, unless samples are to be passed from one department to another.

   2. Routinely, body fluids are initially submitted to Hematology, unless no hematology request were submitted. In this case, the person logging in will hand it to the CLS in charge of the department indicated on the request.

   3. If requests are only for send out or pathology and no other areas in the laboratory, sample may be distributed directly to the said department.

Reference Ranges

Refer to SCPMG-PPP-0105 GenLab Reference Range Values on LabNet.

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Criteria - Abnormal Slide for Path Review and Retention

1. Body fluid smears are sent to Pathologists for review based on the following criteria:
   - Any suspected malignant cells seen.
   - Any body fluid smear needing Pathology review as determined by CLS.
   - Ordered by physician.

2. Resulting pathology review:
   - When reporting, perform a corrected report and remove the previous comment (*Slide Sent for Review).*

3. If the Pathologist agrees with the result, enter the Pathologists comment with name and date of the pathologist reviewed the smear.

   Example:

   All the unidentifiable cells are blast.
   Smear has been reviewed by *Pathologist name, date of review.*

   Or

   Some of the unidentifiable cells are blast.
   Smear has been reviewed by *Pathologist name, date* of review.

4. If the Pathologist does not agree with the result, CLS will enter the new result then it will be conveyed to the clinician immediately by the pathologist, designee, or CLS and a corrected report will be issued.

5. CLS will follow up with the Pathology Department if the slide review is pending for more than 2 days.

6. The slides will be retained in the hematology section for a month in an orderly fashion for possible review or reference. Pathology review result will be saved for at least 2 years.

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References

- Kjeldsberg C, Knight J: Body Fluids, 2nd ed.1986.
- Ringsrud KM, Linne JJ: Urinalysis and Body Fluids, 1st ed. 1995
- Smith, J: Body Fluid Cellular Morphology, 1999
- Strasinger, SK: Urinalysis and Body Fluids, 3rd ed. 1994

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End

Signature Manifest

Document Number: RIV-PPP-0456 **Revision:** 06
Title: Body Fluids Analysis_ Cell Count
Effective Date: 14 Mar 2024

All dates and times are in Pacific Standard Time.

Body Fluids Analysis_ Cell Count

Laboratory Manager Approval

Name/Signature	Title	Date	Meaning/Reason
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Rogelio Ang Lee (K149343)	ADA	19 Feb 2024, 02:55:48 PM	Approved
Ruchita Sukhadia (S346951)	ASST DIR OPER AREA LAB	20 Feb 2024, 10:07:46 AM	Approved
Marissa Calilung (Q468002)	Area Lab ManagerMVMC	06 Mar 2024, 12:52:33 PM	Approved

Operations Director Approval

Name/Signature	Title	Date	Meaning/Reason
Annaleah Raymond (Q741709)	Laboratory Operations Director	10 Mar 2024, 04:39:15 PM	Approved

Medical Director Approval

Name/Signature	Title	Date	Meaning/Reason
Mark Taira (P161328)	CLIA Director	12 Mar 2024, 08:45:36 PM	Approved