BODY FLUID CELL COUNT

☒ St. Joseph Medical Center, Tacoma, WA	☒ St. Anthony Hospital Gig Harbor, WA	☐ Harrison Medical Center, Bremerton, WA
☒ St. Francis Hospital, Federal Way, WA	☒ St. Elizabeth Hospital Enumclaw, WA	☐ Harrison Medical Center, Silverdale, WA
☒ St. Clare Hospital Lakewood, WA	☐ Highline Medical Center Burien, WA	☐ PSC

PURPOSE

To provide instruction for performing manual analysis of body fluids

BACKGROUND

Analysis of serous body fluids (pleural, peritoneal, or pericardial) is used to differentiate effusions of unknown etiology and to diagnose inflammatory infiltrates, malignancies, pulmonary disease, and other abdominal conditions. Peritoneal fluids include ascites, paracentesis, and other abdominal fluids. Pleural fluids include thoracentesis fluids, pleural effusions and other chest fluids except those collected from within the pericardial membrane. See related documents below for CSF and Synovial Body fluid work instructions.

RELATED PROCEDURES

R-W-HEM 1405	Hemocytometer Count
R-W-HEM-1406	Cytocentrifuge Use
M-F-HEM-1186	Body Fluid Worksheet
R-W-HEM-1402	Synovial Fluid Cell Count
R-W-HEM-1400	CSF Cell Count
R-F HEM-0110	Pathologist Review of Blood and Body Fluids- Worksheet
R-F HEM-0109	Pathologist Review of Blood and Body Fluids-Instructions
R-PO-HEM-0108	Pathologist Review of Blood and Body Fluids-Criteria

Note: Samples that meet specimen handling and testing requirements can be performed using automated methods. See Related Procedures below:

M-W-HEM-1441	LH Automated Body Fluid Cell Counts
J-W-HEM-1555	DXH Automated Body Fluid

SPECIMEN REQUIREMENTS

SPECIMEN TYPE:

- Peritoneal Fluid includes ascites, paracentesis or other abdominal fluids
- Pleural Fluid includes thoracentesis, pleural effusions and other chest fluids except those collected within the pericardial membrane.

HANDLING: Deliver ASAP. Storage: 2-8°C. Specimens retained for 1 week.

VOLUME: Minimum volume: 0.5 ml EDTA aliquot

STABILITY: 24 hours, if performed from refrigerated EDTA aliquot

SAMPLE LIMITATIONS

Specimens with clots or fibrin are acceptable. Totally clotted specimens are rejected. Specimens received beyond stability limits will be performed and results reviewed by a MT-Coordinator or Lead Tech to evaluate if results can be reported.

EQUIPMENT/ SUPPLIES

- Hemocytometer (Improved-Neubauer): glass or disposable
- Plastic petri dish with moist cloth, calibrated pipette, as needed
- Hematology diluent, WBC or Turk's Diluting Fluid, as needed
- Microscope and Tally counter
- Wright's or Wright-Giemsa Stain/Stainer , TS Meter, pH paper (if needed)
- Cytocentrifuge, cytospin chambers, filters, slides, 6% albumin

QUALITY CONTROL

1. One Level of manual body fluid QC is performed every 8 hours of patient testing. Results are recorded in the LIS or the location according to your site.

2. Hemocytometer is inspected for integrity and cleanliness. Results are documented in the LIS or on the worksheet.

3. Certified pipettes are used to dilute specimens. This is documented in the LIS or on the worksheet.

4. CSF or Turk's Diluting Fluid may be used to hemolyze RBCs for cell counting and to enhance the nucleus of nucleated cells, when the distinction between RBCs and nucleated cells is difficult.

5. Quality check of diluting fluids. 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.

6. Cell counts are performed in duplicate by counting both sides of the chamber. Counts must agree within 20%. This is documented in the LIS or on the worksheet.

7. Cells are evenly distributed in the hemocytometer chamber. This is documented in the LIS or on the worksheet.

8. Cytospin or manual slides are submitted for pathologist review. Refer to Pathologist Review of Blood and Body Fluids-Criteria

PROCEDURE STEPS

Preparation of Specimen for Testing

1. Check that the Body Fluid sample has been correctly ordered before proceeding.

2. Record all results and observations on a Body Fluid worksheet or in the LIS.

3. Visually inspect the fluid in the aliquot tube and record in the LIS and on the worksheet any clots or visible cell clumping. When a result of "Yes" is entered in the result field "Check specimen integrity- Clots Present?", the LIS will automatically append the comment BFCLOT, which reads "Body Fluid specimen has clots present."

4. Determine the type of fluid (source), color and appearance.

5. Record the volume of the total specimen collection.

6. Measure the specific gravity of the specimen using the red-top tube aliquot. If the fluid is purulent or grossly bloody, centrifuge the aliquot prior to SPG testing and perform SPG on the supernatant.

7. Perform pH if a PH BF has been ordered.

Cell Count - only nucleated cells need to be counted.

Note: Samples that meet specimen handling and testing requirements can be performed using automated methods. See Related Procedures above.

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.

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 diluting fluid, if necessary, to distinguish between RBCs and nucleated cell types by making a 1:2 dilution or higher (refer to table below of frequently used dilutions). Counts on the two sides must agree within 20%. If not, mix the specimen thoroughly, replate, and recount. If the nucleated count is elevated, estimate the number of cells present and determine an appropriate dilution.

4. Perform a manual cell count for Total Nucleated Cells (TNC). This includes WBC's, and all other nucleated cells seen on the hemocytometer. The count will be reported in the LIS as WBC's. See work Instruction, Performing a Hemocytometer Count.

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
1:2	100 µL	100 µL
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 mL

Differential

1. If the WBC Count is greater than 5 cells/mm^3, prepare one or more cytospin or push slides. See work instruction, Cytocentrifuge Use. If a cytocentrifuge is not available, make a manual slide using sediment from a spun sample if sample volume is sufficient.

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. Correlate the number and proportion of cells on the cytospin slide with the manual cell count results.

5. Count 100 nucleated cells and document on the worksheet or in the LIS. Note: The worksheet template includes the following result fields:
   - Fluid Polys
   - Fluid Lymphs
   - Fluid Monos (includes monocytes and macrophages)
   - Fluid Mesos
   - Fluid Eosinophils
   - Fluid Basophils
   - Fluid Other: Use for cells not in one of the categories, unidentified cells, or suspected malignant cells. This would also include plasmacytes, tumor cells or nucleated RBC's. Add a footnote identifying other cell types. Atypical cells should have the comment BF ATY, "Atypical Cells, pending pathologist review".
   - Comments: Use for comments on cell inclusions, bacteria, or fungal elements.
   - Path Review
   - If bacteria or fungus is noted (confirm with gram stain or Microbiology), determine if it is intracellular or extracellular. Rule out stain or slide contamination as the cause. Result findings as a comment in the LIS. **NOTE:** The presence of bacteria/fungus (non-contaminant) in certain serous fluids (i.e. pleural) may need to be called to the provider as soon as possible. Contact the microbiology department or the on-call pathologist if you have questions.

Submit Slides for Pathologist Review

Submit the slide for pathologist review (Refer to Pathologist Review of Blood and Body Fluids-Criteria, Pathologist Review of Blood and Body Fluids - Worksheet, Pathologist Review of Blood and Body Fluids-Instructions)

NOTE: If a pathologist is not available at your site to review the body fluid slides:
- Transfer the sample order in the LIS to SJMC
- Send an aliquot of the body fluid along with the cytospin or push slides in case further slides are needed.
- Send a copy of the completed cell count / differential and a copy of your worksheet.
- Send the specimen by courier if routine. Send by cab if the sample is to be treated as a Stat.
- Notify the SJ hematology department that the slides are enroute.

NORMAL VALUES

	Pleural	Peritoneal	Pericardial
Color	Colorless or pale yellow	Colorless or pale yellow	Colorless or pale yellow
Appearance	Clear	Clear	Clear
Specific Gravity	0 – 1.016 for transudates.	0 – 1.016 for transudates.	0 – 1.016 for transudates.
pH	<7.20 suggests infection >7.40 suggests malignancy		
Cell Count: WBC	0-300 u/L	0-300 u/L	None
Neutrophils	Less than 25%	Less than 25%	Less than 25%
Lymphocytes, mononuclear, and mesothelial cells	Normally present in serous fluids.		
Other	Not usually present and may indicate an abnormality		

MANUAL CALCULATION FOR CELL COUNTS

See work instruction, Hemocytometer Count.

TECHNICAL NOTES

- RBC cell counts are performed upon special request of the physician.
- Turks Diluting Fluid or Cerebrospinal Fluid Diluting Fluid (Eng Scientific, Inc.) may be used when RBC's must be lysed to accurately identify and count the nucleated cells. Both enhance the cell nucleus for better identification. Turk's should not be used with specimens having high protein content, as the acetic acid in the diluent may cause cell clumping.
- When using Turk's or CSF Diluting Fluids, you must initially count all cells if RBC's have been requested, then repeat the count using the lysed fluid sample.
- Diluted Methylene blue stain may be used to improve recognition of nucleated cells in the sample. 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 at your specific site.

REFERENCES

1. Susan King Straasinger, "Urinalysis and Body Fluids", Third Edition, 1994, F.A. Davis, Company.
2. Kjeldsberg & Knight. Body Fluids. ASCP, Chicago, 1982.
3. Todd and Sandford. Clinical Diagnosis, 17th ed. WB Saunders Co, Philadelphia: 1984, pp475-488, 564-565, 569.
4. Ross and Neely. Textbook of Urinalysis and Body Fluids. Appleton-Century-Crofts, Connecticut: 1983.
5. Coulter®LH Series Workstation, Body Fluid Application Operator's Guide, 2004