# SYNOVIAL FLUID CELL COUNT

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|---|---|---|
| ☒ 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 synovial fluid.

## BACKGROUND

Analysis of the synovial fluid may assist in differentiation of septic, inflammatory, and non-inflammatory processes, resulting in an arthritic state.

## RELATED PROCEDURES

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| R-W-HEM-1405 | Hemocytometer Count |
| R-W-HEM-1406 | Cytocentrifuge Procedure |
| M-F-HEM-1186 | Body Fluid Worksheet |
| R-W-HEM-1407 | Synovial Fluid Crystal Identification |
| R-PO-HEM-0108 | Pathologist Review of Blood and Body Fluids-Criteria |
| R-F HEM-0110 | Pathologist Review of Blood and Body Fluids- Worksheet |
| R-F HEM-0109 | Pathologist Review of Blood and Body Fluids-Instructions |

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

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

## SPECIMEN REQUIREMENTS

SPECIMEN TYPE:

- Fluid synovial or joint aspirate. Other fluids not rejected but confirm ordered correctly.
- Semi-gelatinous or calcified joint aspirates. For this sample type, cancel the cell count, but perform the differential and synovial fluid crystal analysis, if possible.
- Joint tissue aspirate: Attempt to perform crystal analysis, if possible.

HANDLING: Deliver ASAP. Store at 2-8°C. Fluid specimens held 1 week. Place specimen into EDTA tube ASAP.

VOLUME: Fluid minimum volume: 0.5 ml.
Fluid or semi-gelatinous aspirate aliquots: EDTA aliquot preferred.
Tissue or solid joint aspirates: may be submitted ASAP after collection in a sterile plastic collection tube.



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STABILITY: 24 hours, if performed from refrigerated EDTA aliquot. Tissue sample aspirates: 24 hours

## SAMPLE LIMITATIONS

- Specimens with clots or fibrin are acceptable. Totally clotted specimens are suitable for crystal analysis. Perform a differential, if possible.
- 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. An appropriate comment should be attached to results if stability limits were compromised.

## 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, if needed.
- Microscope and Tally counter
- Wright's or Wright-Giemsa Stain/Stainer
- Cytocentrifuge, cytospin chambers, filters, slides, 6% albumin
- Microscope with polarizing filter, analyzer, and red-plate compensator.
- Glass slides, coverslips, clear nail polish
- Hyaluronidase

## 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 not normally submitted for pathologist review, but may be submitted if requested.

## PROCEDURE STEPS


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**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 color of the fluid and appearance.

5. Record the volume of the total specimen collection.

6. To test for specimen viscosity, aspirate the fluid in a transfer pipette, release the fluid slowly back into the tube.
   - Normal viscosity: The falling drop of fluid is drawn out into a 2-inch or longer tenacious band.
   - Abnormal or Reduced viscosity: The drops fall like water.

7. Perform pH if requested using the pH paper.

8. Treat highly viscous fluids with Hyaluronidase to reduce viscosity.
   - Confirm viscosity has been performed prior to treatment.
   - Take an applicator stick; put it in the hyaluronidase bottle. Remove the stick and put it in the tube containing an aliquot of the synovial specimen.
   - Mix gently. The few particles adhering to the dry stick are sufficient to lower the viscosity for the cell count.

**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.


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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 |

- Dilutions are plated on both sides of a hemocytometer, counted in duplicate, and must agree within 20%. If not, mix tube thoroughly, replate, and recount. Record all results and calculations in the LIS or on the worksheet.

**Differential**

If the WBC Count is less than 5 cells/mm³, a differential is not necessary. Add the comment BF DIF (WBC=5 or less per microliter. Differential not performed).

Make slides using the cytocentrifuge. If a cytocentrifuge is not available, make a manual slide using sediment from a spun sample if sample volume is sufficient.

Stain with Wright's or Wright-Giemsa stain manually or on the slide stainer.

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.

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.

- For non-liquid or semi-solid specimens which cannot be counted (i.e finger or toe joint aspirates), place a portion of the sample on a slide and gently cover with a cover slip. Seal the edges with clear nail polish. Label with two patient identifiers.
- Scan the sealed slide and note if WBC's or RBC's are present. If present, add COMMENT on the differential. Example: "Unable to perform cell count, few WBC and moderate RBC's present."
- Save this specimen for Crystal analysis, if ordered.

Count 100 nucleated cells and document on the worksheet. Note: The worksheet template includes the following result fields:

- Fluid Polys
- Fluid Lymphs
- Fluid Monos (includes monocytes and macrophages)
- Fluid Mesos- are not present in synovial or joint fluids. Occasionally synovial lining cells will be present.
- 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


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- 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.

Prepare a slide for crystal examination and identification or use the wet mount slide previously prepared in Step 14 See work instruction, Synovial Fluid Crystal Identification.

. Slides are NOT normally submitted for pathologist review, but will be performed if specifically requested by the physician or if requested by the technologist. If requested, 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.
- Send the specimen by courier if routine. Send by cab if the sample is to be treated as a STAT.
- Notify the hematology department that the slides are en-route.

## NORMAL VALUES

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|---|---|
| **Color** | Clear to straw |
| **Appearance** | Clear |
| **Viscosity** | Normal |
| **Volume** | Less than 3.5 mL |
| **Crystals** | Absent |
| **WBC** | 0-200 u/L. |
| **Differential** | Neutrophils: 0-25% |
| | Lymphocytes and mononuclear cells are normal |
| | 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. Caution must be used when Turk's diluent is used with specimens having high protein content, as the glacial acetic acid concentration 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 for the WBC count. Correlate dilution results with cytospin or push slides.


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- 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


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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





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