St.JosephHealth Queen of the Valley

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References:	*Laboratory*

Body Fluid - Synovial Fluid - Count & Crystals

Description

Synovial fluid is a plasma dialysate secreted by the synovial membranes found in joint cavities. It functions to lubricate joint space and transport nutrients to articular cartilage. The presence of hyaluronate differentiates synovial fluid from the other body fluids and provides the normal viscosity. Various disorders produce changes in the type of cell population present.

Purpose

Synovial fluid is examined to help determine the cause of joint disease. In some cases it may provide a better reflection of the events in the articular cavity than blood tests do.

Specimen Collection

Synovial fluid is aspirated by the physician via arthrocentesis. The syringe used for collection should be coated with **Sodium Heparin** (25 units/ml of Synovial fluid). Lithium heparin and EDTA should **NOT** be used because they can produce artifacts that can interfere with microscopic examination for crystals.

Reagents & Supplies

IRIS IQ200
IRIS Lysing Reagent
IRIS Diluent
IRIS Body Fluid Rack
IRIS Body Fluid Tubes
IRIS Body Fluid Labels
Neubauer Improved C-Chip Disposable Hemocytometer
Lyophilized Hyaluronidase Powder – store in freezer (<0°C)
Isotonic Saline (0.9 % NaCl)
10% Acetic Acid
BSA – 22 % Bovine Albumin Solution (Store refrigerated at 2 – 10 °C)
Streck Cell-Chex body fluid cell count control (Store refrigerated at 2 – 10 °C)
Cytoseal 60 Mounting Medium

Specimen Handling

Normal Synovial fluid viscosity is comparable to raw egg white. Synovial fluids that are thick, viscous and should be treated with Hyaluronidase to increase cell recovery prior to performing a manual cell count. For viscous samples with high cell populations, reduce viscosity with normal saline. Highly viscous samples may need additional time (30 minutes) for the cells to settle when plated onto a Hemacytometer. All synovial fluids placed on the IQ200 should be treated with hyaluronidase prior to placing on the analyzer. Place a small amount of hyaluronidase powder, collected using two wooden sticks like chopsticks, into an aliquot of synovial fluid. Mix the fluid well by gentle inversion and allow to sit for 10-15 minutes or until specimen flows easily in drops from a pipette. Untreated specimen should be used for crystal identification and manual differential as the enzyme may produce artifact.

Quality Control

Refer to Body Fluids IQ200 for QC procedure.

Each CLS performing a manual body fluid cell count will count one level of commercially produced manual body fluid control every eight hours of patient testing. These QC results will be logged in the Manual Body Fluid Control binder and reviewed by supervisor at least monthly.

Insufficient Sample

If insufficient Synovial fluid is submitted (QNS) for all requested tests, Microbiology orders and examination for Crystals should be given priority.

Count and Differential Procedure

1. ***Gross Examination***
   Thoroughly mix the fluid and record the specimen type, total volume received, appearance & color on the Body Fluid Worksheet form. Aliquot specimen as needed and label each aliquot with the appropriate LIS-generated label. Distribute aliquots to the laboratory sections where testing is to be performed.
   Note: Grossly bloody Synovial fluid should be centrifuged and the color/clarity of the supernatant reported. *Turbidity is reported as the inability to read newsprint through the fluid.*
2. ***Automated Total Cell Count-*** *Refer to Body Fluids IQ200 Procedure*
3. ***Manual Total Cell Count***
   - Mix the synovial fluid by inversion. Load a small amount of the undiluted synovial fluid to each side of the chamber of a clean hemacytometer. Allow the counting chamber to sit covered with a moistened filter paper in half of a Petri plate for 5 min to allow the cells to settle.
   - Using a phase microscope, scan the entire hemacytometer on 10X (low power) to ensure there is an even distribution of cells and determine if dilution will be required.
   - Switch to the 40X objective to count RBC and WBC. Only cells touching the same two sides of each square are counted along with the cells contained completely within the square.
   - Count five large squares on each sides of the chamber for a total of 10 large squares. (The corner squares and the center square of each side are traditionally counted.)
   - Counts from each side of chamber should agree within 20%.
   - The volume in each large square (9 per side) = 0.1 mm^3. **The count in ten large squares**

represents the total cell count per/mm^3 (10 X 0.1 = 1mm^3).

- If the cells are too numerous to count accurately, dilute the specimen using a calibrated pipette and isotonic saline, mix, charge, and recount. Note the dilution factor and calculation on the worksheet.
- RBCs can be lysed by making dilution of 1 drop 10% acetic acid to 9 drops synovial fluid. Allow mixture to stand for 5 min, mix, charge and recount. Erythrocytes should be either absent or ghost cells. The nucleus of segmented neutrophils will be bright, while the lymphocyte nucleus will be round.
- If less than 10 large squares are counted use the following calculation:

$$\frac{\text{\# Cells Counted X dilution factor}}{\text{\# large squares counted X 0.1 mm}^3} = \text{total cell count per/mm}^3$$

Example: The fluid is diluted 1:10 and 45 WBC are counted in 9 squares

$$\frac{45 \text{ X } 10}{9 \text{ X } 0.1} = 500 \text{ WBC/ mm}^3$$

4. ***Differential Leukocyte Cell Count***
   - Prepare at least one slide using the Wescor Aerospray Slide Stainer & Cytocentrifuge procedure
   - Following cytocentrifugation of specimen, allow slides to air-dry completely.
   - Stain slides in the Wescor Aerospray Slide Stainer, utilizing the same stain used for Differentials on blood smears.
   - Perform and report the WBC Differential count using the manual differential keypad and record the results on the Body Fluid Worksheet. Record any Diff comments in the area provided.

Expected Values

Normal Synovial fluid is very viscous, light yellow, clear or slightly cloudy, and does not clot. An aspirated volume of more than 3.5 ml from the knee is considered abnormal. Turbidity usually indicates leukocytosis, cartilage debris, or the presence of crystals.

WBC count in normal samples ranges from 0 – 200 cells/µL (with < 25 % PMNs)
RBCs and Crystals should be absent.

LIS Reporting

Use the Enter Results screen to enter the results from the body fluid worksheet.

- Examine color of fluid. Report as:
  - Amber (AMB)
  - Brown (BR)
  - Colorless (COL)
  - Green (GR)
  - Light Yellow (LT)
  - Pink (P)
  - Red (RED)
  - Yellow (YEL)
- Examine appearance (clarity) of fluid. Report as:

- Clear (CLE)
- Cloudy (CLO)
- Clotted (CLOT)
- Moderately Cloudy (MO)
- Slightly Cloudy (SLC)
- Turbid (T)

- Indicate Volume in mls (Total of all tubes)
- Report any clotting observed
- Report Total WBC count in cells/ mm^3
  - Enter a comment stating "Total Nucleated Cells"
- Report Total RBC count in cells/ mm^3
- Report each cell type seen in the WBC Differential in percentages (%)
  - Calculate the percentages if fewer than 100 cells are counted.
- Indicate # of cells counted (100) for the differential
- Review results for accuracy prior to verifying

Examination for Crystals

1. Place a drop of specimen on a clean slide, covering with a coverslip. ("clear" specimens should be concentrated by spinning an aliquot of fluid for 10 minutes in the UA centrifuge.)
2. Scan by ordinary light (with reduced light) or phase microscopy on low power (10X).
3. Examine with polarized light using the high dry objective (40X). **(See page 5 for instructions)**

The types of crystals that may be seen in Synovial fluid include monosodium urate, calcium pyrophosphate dihydrate, cholesterol, steroid, and Hydroxyapatite, other phosphates, oxalate and artifacts.

Quality Control

A known uric acid control slide will be used to confirm patient's results by comparison of crystal color and orientation. Document control results on worksheet.

Expected Values

Using a polarizing microscope, monosodium urate crystals (MSU) appear as strongly birefringent with needle or rod shapes. They may be intracellular (don't forget to look carefully for this) or extracellular. Calcium pyrophosphate dihydrate crystals (CPPD) appear as weakly birefringent and can also be needle shaped, although more often appear short and chunky. The presence of MSU is a characteristic finding of patients during acute attacks of gout. CPPD crystals are characteristic of pseudogout. MSU and CPPD have distinct, but opposite, characteristics when viewed with color compensated polarized light. The color of MSU and CPPD crystals will change from blue to yellow and vice versa as the compensator is rotated. MSU crystals are yellow when the long axis of the crystal is parallel to the slow wave of the compensator (Z'). CPPD crystals will have the opposite orientation.

LIS Reporting

- (N) No crystals seen under polarized light
- (**P**) Birefringent crystals resembling Uric Acid seen under polarized light.

NOTE: (P) Positive for Uric Acid results are verified by a second CLS before reporting.

- If another crystal form is seen it may be entered using free text.

Reference:

1. Ringsrud, Karen; Linne, Jean: "Urinalysis and Body Fluids, A ColorText and Atlas", Mosby-Year Book Inc. 1995.
2. Nikon Eclipse E400 Instruction Manual. 2003.
3. Turgeon, Mary Louise, Clinical Hematology, Lippincott, Williams & Wilkins, Third Edition, 1999.
4. Kjeldsberg & Knight, Body Fluids, Second Edition, 1986.
5. Freeman, James A. and Beeler, Myrton F, Laboratory Medicine/Urinalysis and Medical Microscopy, Second Edition, Lea & Febiger, 1983.

Polarized Light Microscopy Instructions

A polarizing microscope with a first-order red compensator is used. The polarizing filter is placed between the light source and the specimen. A second polarizing filter is situated above the specimen. One of the filters is rotated so as to be at right angles to the other which produces a black field because all light waves are cancelled.

Certain crystals have the ability to rotate or polarize light so they are visible when viewed through crossed polarizing filters. This property is known as birefringence. Objects exhibiting this ability are termed weakly or strongly birefringent depending on how completely they polarize the light. Strongly birefringent crystals appear bright white against the dark background whereas weakly birefringent crystals appear less bright. The use of the red order compensator will further identify the crystal due to the properties exhibited.

Equipment:

1. Olympus BX43 Light Microscope
2. Olympus U-POT and U-GAN polarizer attachments
3. Positive Uric Acid control slides

Procedure:

1. Make sure microscope is on ***bright field*** setting (select "O" setting on phase turret).
2. Place U-POT polarizer on the field diaphragm with white hash mark facing up.
3. Place a slide on the microscope slide stage and engage the 40x objective.
4. Slide the U-GAN analyzer, labeled surface up, fully into the slot directly above the lenses until it clicks into place.
5. Set the λ-plate rotation lever of the U-GAN to the center position (•). Look into the eyepiece and rotate the U-POT polarizer to the darkest position.
6. Push the λ-plate rotation lever towards the back of the microscope (counterclockwise). The background should now be magenta colored.
7. In this position, locate crystals running parallel with the arrow (parallel with Y-axis). Rotating the lever to the position towards the front of the microscope will result in those crystals changing to the opposite color (yellow --> blue, blue --> yellow).
8. Return the U-POT and U-GAN to their boxes when finished with analysis.

Parallel	Perpendicular	Interpretation
Yellow	Blue	Uric Acid
Blue	Yellow	CPPD

Related Procedures:

1. Body Fluids- IQ200
2. Wescor Aerospray Slide Stainer & Cytocentrifuge

Attachments:

U-GAN Insert

Approval Signatures

Step Description	Approver	Date
Laboratory Medical Director	Brady Feliz: Physician, Laboratory Medical Director	12/2019
Laboratory Admin Director	Olive Romero: Administrative Laboratory Director	10/2019
	Lisa Weir: Hematology Supervisor	10/2019