OLIVE VIEW-UCLA MEDICAL CENTER
DEPARTMENT OF PATHOLOGY
POLICY & PROCEDURE

NUMBER: 2054
VERSION: 4

SUBJECT/TITLE: HEMA-15 SYNOVIAL FLUID CRYSTALS

POLICY:

1. Examination of synovial fluid for crystals should be performed as soon as possible. Delaying examination may result in false-positive or false-negative identification of crystals.

2. The specimen should first be examined by brightfield light microscopy followed by polarized microscopy to search for birefringent crystals. When birefringent crystals are detected, the red plate compensator should be used to differentiate MSU (monosodium urate) from CPPD (calcium pyrophosphate dihydrate).

3. Polarized microscopy is primarily used to detect monosodium urate (MSU) and calcium pyrophosphate dihydrate (CPPD) crystals. Calcium oxalate, lipid crystals, cholesterol, steroids, starch, and artifacts may also be detected by polarized microscopy.

4. The presence of clinically significant crystals should be reported and described as intracellular and/or extracellular. The following synovial crystals are considered clinically significant:

   Apatite (hydroxyl)
   Calcium oxalate
   Cholesterol
   CPPD (calcium pyrophosphate dihydrate)
   Hematin
   Lipid
   MSU (monosodium urate)
   Steroids

5. Clots and/or fibrin strands seen should be included in the patient's report with the comment "Result may be inaccurate due to the presence of clots and/or fibrin strands".

6. Clumps of cells, fibrin clots, and debris should also be examined because crystals may be trapped in these areas.

7. After testing, the specimen for synovial crystal analysis are stored in refrigerator (#12) for 7 days and then moved to freezer (#15) for three months. Cytospin slides are to be kept for 3 months.

PURPOSE:

CLINICAL COMMENTARY

The identification of crystals is one of the few pathognomonic laboratory tests in the study of arthritides. Improper examination for crystals can result in incorrect patient therapy and unnecessary permanent patient disability. It is important, therefore, that all synovial fluids be carefully examined for crystals. The crystals that may be seen in synovial fluid include monosodium urate (MSU), calcium pyrophosphate dihydrate (CPPD), cholesterol, steroids, apatite (hydroxyl) and other phosphates, oxalate, hematin, and artifacts.

Crystal	Disorder
Apatite (hydroxyl)	Calcinosis, osteoarthritis, rheumatoid arthritis
Calcium oxalate	Renal dialysis, primary oxalosis
Cholesterol	Chronic synovial effusion
Calcium pyrophosphate Dihydrate (CPPD)	Pseudogout
Hematin	Synovial effusions with hemarthrosis
Lipid	Chronic effusions, chylous effusions
Monosodium urate (MSU)	Gout
Steroids	Following joint injections

Monosodium urate (MSU) and calcium pyrophosphate dihydrate (CPPD) crystals in the body may precipitate in or around joints and cause symptoms of clinical arthritis.

Monosodium urate crystals are identified in about 90% of patients with acute urate gout and in about 75% of patients between acute attacks. MSU crystals are also seen in patients with urate gout plus septic arthritis; therefore, the presence of MSU crystals does not exclude coexisting bacterial arthritis. Intracellular MSU crystals are characteristic of acute urate gout.

Calcium pyrophosphate dihydrate crystals are characteristic of a group of conditions referred to as “pseudogout” or CPPD crystal deposition disease. These crystals may be seen in patients with degenerative arthritis, and in arthritides associated with hypothyroidism, hyperparathyroidism, and hemochromatosis.

DEPARTMENTS: PATHOLOGY

DEFINITIONS:

TECHNICAL COMMENTARY

A brightfield clinical microscope with a simple non-rotating analyzer plus first order red compensator inserted into the barrel of the microscope and a simple polarizer that fits over the filter mount at the light exit on the microscope base is used to examine a wet preparation of synovial fluid. The first order red plate compensator retards light one full wave length. When a MSU crystal is oriented so that its long axis (fast ray) is parallel to the short axis (slow ray, z ray) of the compensator, retardation is subtracted, making the crystal appear yellow. When the substage polarizer is rotated 90°, the slow ray of the compensator is added to the slow ray of the MSU crystal and the color becomes blue. The reverse is true for calcium pyrophosphate.

MSU crystals appear as rods or needles from 2-20 μm long. They may be intracellular and/or extracellular and are strongly birefringent when polarized.

Calcium pyrophosphate crystals appear as rods, rectangles, or rhomboids varying from 2-20 μm long and up to 4 μm wide. These crystals may be intracellular and/or extracellular and they are weakly birefringent when polarized (i.e., they do not appear very bright when polarized).

PROCEDURE:

SPECIMEN REQUIREMENTS

Specimen Required:

1. 0.1 mL (minimum) of synovial fluid should be collected in a green top tube (heparinized). Red top tube (no anticoagulant) and sterile cup are also acceptable.
2. The synovial fluid should be properly labeled and delivered to the laboratory as soon as possible and within 4 hours of specimen collection.
3. Sample submitted in syringe should be transferred in a green top tube by a CLS and should be labeled properly.

Criteria for Specimen Rejection:

1. Specimens collected in tubes containing EDTA or calcium oxalate are unacceptable because EDTA and calcium oxalate crystals are birefringent and can be confused with urate or calcium pyrophosphate crystals.
2. Completely clotted specimens.
3. Specimens submitted in syringes **with needle**.
4. Unlabeled specimens (to be discarded).
5. Mislabeled specimens (to be returned to Specimen Processing for clarification).
6. Specimens delivered to the laboratory beyond the maximum allowable time interval as specified above.

Unacceptable specimen should be rejected, the floor or clinic should be notified, and the notification should be properly documented in the patient's report.

Specimen Storage:
After testing, the specimen is stored in the refrigerator (#12) for 7 days and then moved to freezer (#15) for three months. Cytospin slides are to be kept for 3 months.

REAGENTS AND SUPPLIES

1. Brightfield microscope equipped with Polarizing Filter Set model consisting of the following components:
    a. An analyzer with red plate compensator which fits into the microscope barrel.
    b. A polarizer placed over the filter mount at the light exit on the microscope base.
2. Slides (3" x 1"), plain or with frosted end
3. Coverslip, 22 x 22 mm
4. Disposable transfer pipettes
5. Alcohol pads
6. Lens paper
7. Positive control slide: Previously positive patient

PROCEDURE

1. Note and report the following:
   a. Appearance (clarity) of the specimen
   b. Specimen source
   c. Observe for clots and fibrin strands. If present, note in the report the possibility of inaccurate results due to clots and/or fibrin strands.

2. If needed, clean the slide and the coverslip with alcohol pad and carefully dry it with lens paper to eliminate birefringent dust particles that may cause confusing artifacts.

3. Thoroughly mix the specimen using repeated gentle inversion. Observe for clots and fibrin strands and, if present, note in the report the possibility of inaccurate results due to clots and/or fibrin strands.

4. Put a drop of the well-mixed specimen on a slide and place a coverslip over it.

5. Allow wet mounts of slightly viscous specimens to stand 5 minutes before examining under the microscope

6. Dilute markedly viscous specimens with a small amount of saline to make the sample easier to handle. Recheck the fluid for viscosity before proceeding.

7. If the crystals are clumped upon review on wet mount, dilute the specimen with a small amount of saline (up to technologist's discretion), to make the crystals easier to identify.

8. Examine the wet mount prep first.

9. If wet mount is negative or rare crystals are seen, make two slides by cytocentrifugation preparations. Set cytospin at 700 rpm for 5 minutes, stain one cytospin slide and leave the other cytospin slide unstained. Examine both slides. Crystals, especially MSU, may be dissolved by the alcohol in the Wright's staining procedure.

10. Check to make sure the analyzer is pushed all the way into the barrel between the microscope head and the objectives and the red plate compensator is off.

11. Place the slide in position on the microscope stage and focus with the 10x objective. The field appears bright and without color. Switch to the 40x objective, refocus, and examine the specimen.

12. Place polarizer into the filter mount at the light exit on the microscope base and rotate until the field of view appears dark. Turn the light source to 2-4 setting.

13. Carefully examine the specimen. When birefringent rods, needles, rectangles or rhomboids are detected against the dark background, push the red plate compensator into the barrel of the microscope. The field will appear bright pink (magenta) in color. Note the markings (the perpendicular arrows on the right side) on the compensator. The vertical arrow is the z ray and the

horizontal arrow is the x ray.

14. Determine the crystal's long axis orientation to the compensator, i.e., whether it is parallel or perpendicular to the z ray.

16. Identify the crystal using the following guidelines:

    Urate: Negatively birefringent crystal (rod or needle-shaped) is yellow when its long axis is parallel to the z ray and blue when parallel to the x ray. The ends of the crystal are tapered to a sharp point. The central part of the crystal is thicker. It appear intracellular and/or extracellular.

    Calcium Pyrophosphate: Positively birefringent crystal (rod or needle shaped, or occasionally rectangle or rhomboid) is blue when its long axis is parallel to the z ray and yellow when parallel to the x ray. It may appear intracellular and/or extracellular.

For a complete list of synovial fluid crystals and their major characteristics, see table:

Characteristic Features of Synovial Fluid Crystals.

Crystal	Code	Interpretation
No clinically significant crystals (MSU, CPPD, cholesterol, hematin, etc.)	XNO	No clinically significant crystals observed
Urate Crystals	INUR	Intracellular birefringent crystals consistent with urate crystals observed
Urate Crystals	EXUR	Extracellular birefringent crystals consistent with urate crystals observed
Calcium Pyrophosphate	INCA	Intracellular birefringent crystals consistent with calcium pyrophosphate crystals observed
Calcium Pyrophosphate	EXCA	Extracellular birefringent crystals consistent with calcium pyrophosphate crystals observed
Urate Crystals and Calcium Pyrophosphate	INURCA	Intracellular birefringent crystals consistent with urate and calcium pyrophosphate crystals observed
Urate Crystals and Calcium Pyrophosphate	EXURCA	Extracellular birefringent crystals consistent with urate and calcium pyrophosphate crystals observed
Urate Crystals	INEXUR	Intra and extracellular birefringent crystals consistent with urate crystals observed
Calcium Pyrophosphate	INEXCA	Intra and extracellular birefringent crystals consistent with calcium pyrophosphate crystals observed
Urate Crystals and Calcium Pyrophosphate	INEX2C	Intra and extracellular birefringent crystals consistent with both urate and calcium pyrophosphate crystals observed

17. Record results in the Miscellaneous Hematology Log Book and enter results in the computer.

NOTES

1. The unstained specimen is first examined using brightfield light microscopy to detect the presence or absence of globules or chunks of material that may represent apatite (hydroxyl) crystals. Cells, fibrils, lipids, hematin, CPPD, and other crystals may also be seen. Initial observation of fluids by brightfield light microscopy may help in correlating findings with compensated polarized light microscopy. For example, hematin crystals may be mistaken for CPPD crystals by compensated polarized light microscopy. This is less likely to occur if the yellow hematin crystals are initially recognized with brightfield light microscopy. Lowering the microscope condenser will aid in finding crystals with brighfield light microscopy.

2. Birefringent crystal appears as a bright area on a dark background. With polarized light microscopy, MSU and CPPD crystals appear white.

3. Polarized light microscopy with a compensator is used to further identify and confirm the presence or absence of MSU and CPPD crystals. The red compensator retards light so that birefringent crystals produce visibly different color changes.

4. Sometimes it takes a careful search to identify CPPD crystals because of their weak birefringence or small size.

5. MSU and CPPD crystals may occur together.

6. Starch granules tend to have irregular outlines while lipid crystals tend to be round.

7. Corticosteroid crystals represent one of the more common synovial fluid crystals that may be mistaken for MSU crystals. Such crystals may be seen for several weeks or months after injection of corticosteroids into joint cavities or surrounding connective tissue. The combination of large numbers of crystals, irregular shapes, variable sizes, combined with strong birefringence, should suggest the presence of corticosteroid crystals.

8. Hematin is a degradation product of hemoglobin. Red blood cells in synovial effusions are phagocytosed by macrophages. Enzymatic degradation of hemoglobin may result in the production of hematin crystals. They may be mistaken for CPPD crystals because of their rhombohedral shape. Hematin crystals are not blue when parallel to the compensator.

9. Clinically significant crystals should not have irregular outlines or broken and jagged edges.

10. In cases where it is difficult to determine the type or nature of crystalline material present, the review of another slide or consultation with the supervisor or designee may be helpful.

QUALITY CONTROL

Control:
Positive control (previously positive specimen) should be tested whenever an order for synovial fluid crystal analysis is received.

Check for Clerical Errors:
1. The specimen tubes, log sheet, and the computer entries will be checked to ensure consistency.
2. At the end of the shift, the CLS assigned in COAG or H2 needs to do clerical check on all sickle cell tests that has been done for the whole shift.
3. Corrective action, when necessary, will be taken and included in the Corrective log.

REFERENCE RANGE

No clinically significant crystals observed.

Crystal	Brightfield Microscopy Appearance	Birefringence	Color when Parallel to Z ray of Compensator	Approximate Crystal Size (µm)	Comments
Apatite (hydroxyl)	Shiny clumps; Globules	Difficult to detect	N/A	0.5 - 1; 2 – 10 wide	Intracellular or extracellular
Artifacts	Variable	Variable	Variable	Variable	Lack definite crystal morphology
Calcium oxalate	Bipyramidal	Variable	N/A	2 – 10	
Cartilage	Irregular	Strong	Variable	10- 50	Lack definite crystal morphology
Cholesterol	Large notched plates	Strong	Variable	10 -100	Needle forms may be confused with MSU crystals; Rhombohedral forms may be confused with CPPD crystals. Cholesterol – rich synovial fluid may grossly resemble pus (scintillating milky yellow to brown color)
CPPD (Calcium pyrophosphate)	Rhombohedral crystals	Weak	Blue	2 – 20 long; 1 – 4 wide	Intracellular and/or extracellular localization
Hematin	Rhombohedral; appear gold			2 – 8	Presence suggests significant joint hemorrhage
Lipids	Round clumps	Intense	Red and blue Maltese cross	5 – 30	Maltese cross appearance
MSU (urates)	Needlelike crystals	Strong	Yellow	2 – 20 long; 0.2 – 1 thick. Central part is thicker	Intracellular and/or extracellular localization
Steroids	Blunt, jagged edges	Strong	Variable	1 – 15	Lack definite crystal morphology
Starch	Round with central depression	Strong	Red and Blue Maltese cross	5 - 30	Obtained from surgical gloves; Maltese cross appearance

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