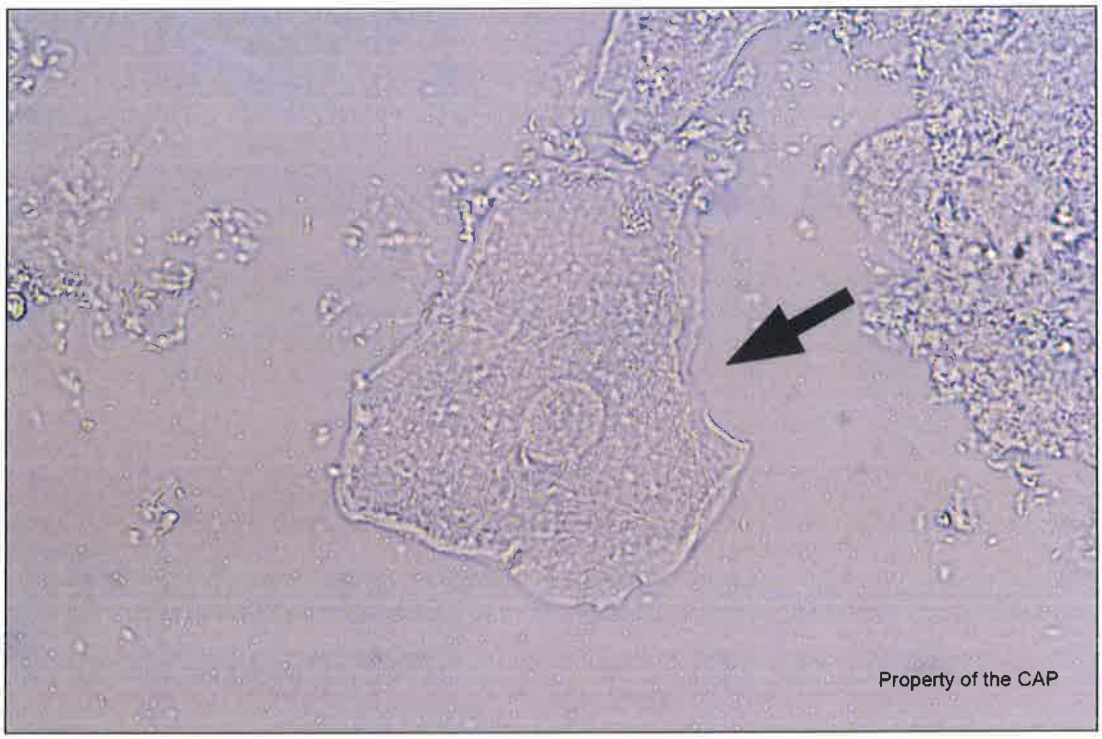
Urine Sediment Photographs

Case History CMP-17 through CMP-20

The patient is a 43-year-old asymptomatic, healthy, athletic woman who participated in a marathon run 2 days prior. Laboratory data include: Specific gravity = 1.012; pH = 5.7; blood, glucose, ketones, nitrite, protein, and leukocyte esterase = negative.

CMP-17



(URINE, UNSTAINED, HIGH POWER)

Identification	CMP Participants No.	%	Performance Evaluation
Squamous epithelial cell	5706	91.9	Good

The arrowed object is a squamous epithelial cell, as correctly identified by 91.9% participants. Squamous epithelial cells are a normal finding in urine because they line or cover the bladder trigone, female urethra, vagina, and distal male urethra. Squamous epithelial cells are the most common type of lining cell seen in urine.

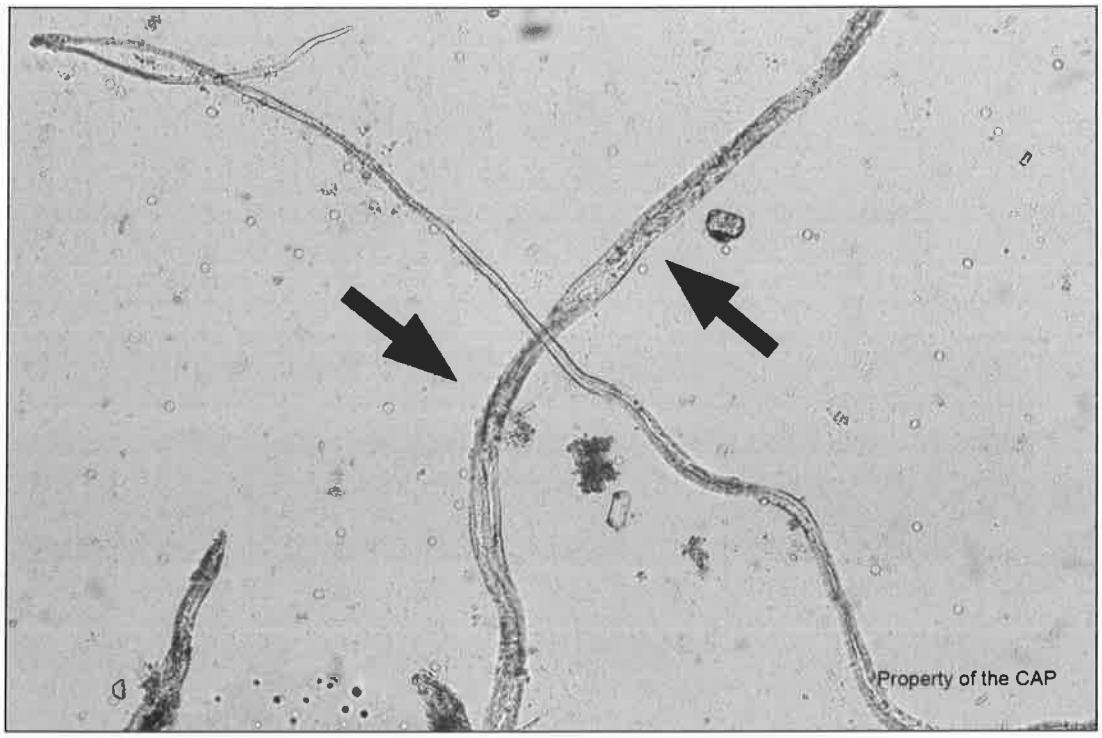
Squamous epithelial cells are large, thin, flat cells with sharp angulated margins, frequently containing keratohyaline granules. Squamous cells measure 30 - 50 microns in diameter and contain a round centrally located nucleus about the size of a red blood cell. Occasionally, squamous cells are found in sheets. Squamous cells act as a protective barrier. If large numbers of squamous epithelial cells are found in a specimen, it indicates that the specimen was not properly collected as a clean-voided midstream collection.

Squamous cells can be differentiated from transitional epithelial cells by their larger size, keratohyaline granules, small pyknotic nuclei and angulated margins.

Renal tubular epithelial cells differ from squamous cells in that they are smaller, elongated and have polar nuclei.

Urine Sediment Photographs

CMP-18



(URINE, UNSTAINED, HIGH POWER)

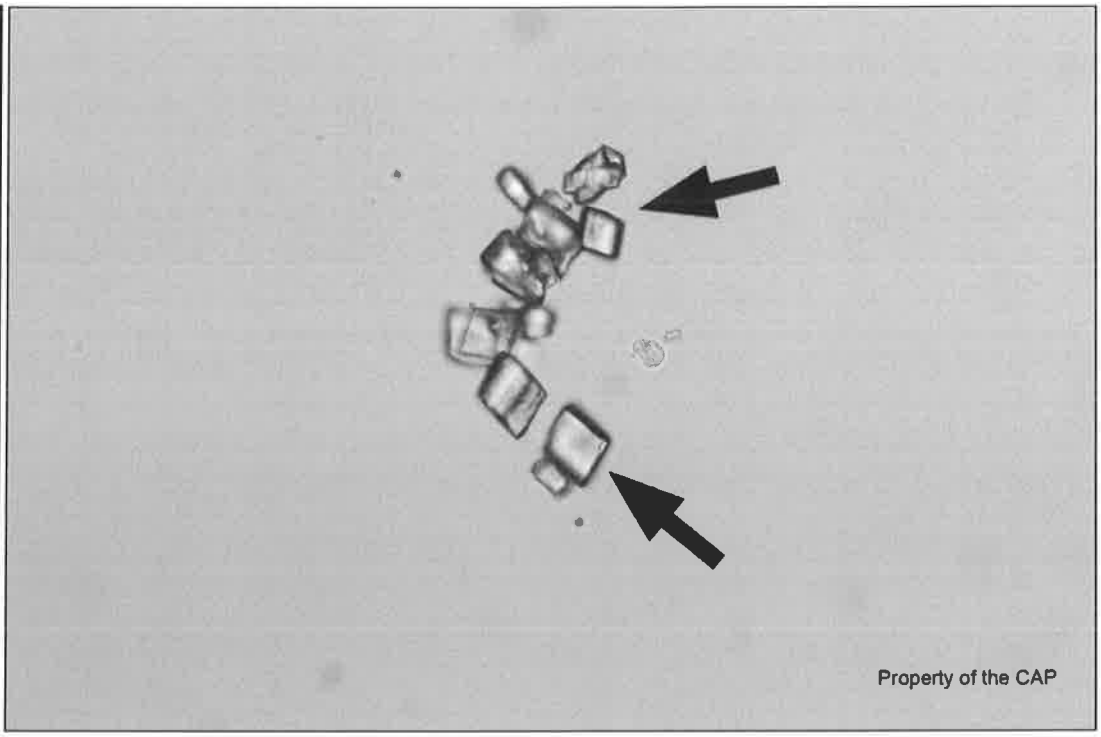
Identification	CMP Participants No.	%	Performance Evaluation
Fibers	5647	91.0	Good

The arrowed objects are fibers, as correctly identified by 91.0% of participants. Fibers are a common contaminant of urine and can be introduced during collection or processing. Fiber size and shape are variable, as the fibers may originate from clothing, hair, dressings or diapers. Fibers tend to be long, are usually birefringent under polarized light and may be refractile. Although the sides of fibers tend to be parallel, the sides may be nonparallel, twisted or frayed. Fibers are not associated with any disease states.

Fibers may be confused with mucous threads. Mucous threads do not have internal structure, are not refractile and do not exhibit birefringence. Fibers might also be confused with casts, particularly those originating in diapers. Fibers from diapers resemble waxy casts. However, most casts do not show birefringence, have an internal structure or have twisted forms. Fatty and crystal casts may have birefringence, but polarization of the fatty casts will show a "Maltese cross" pattern and distinct crystals should be evident in crystal casts.

Urine Sediment Photographs

CMP-19



(URINE, UNSTAINED, HIGH POWER)

Identification	CMP Participants No.	%	Performance Evaluation
Uric acid crystals	6033	97.2	Good

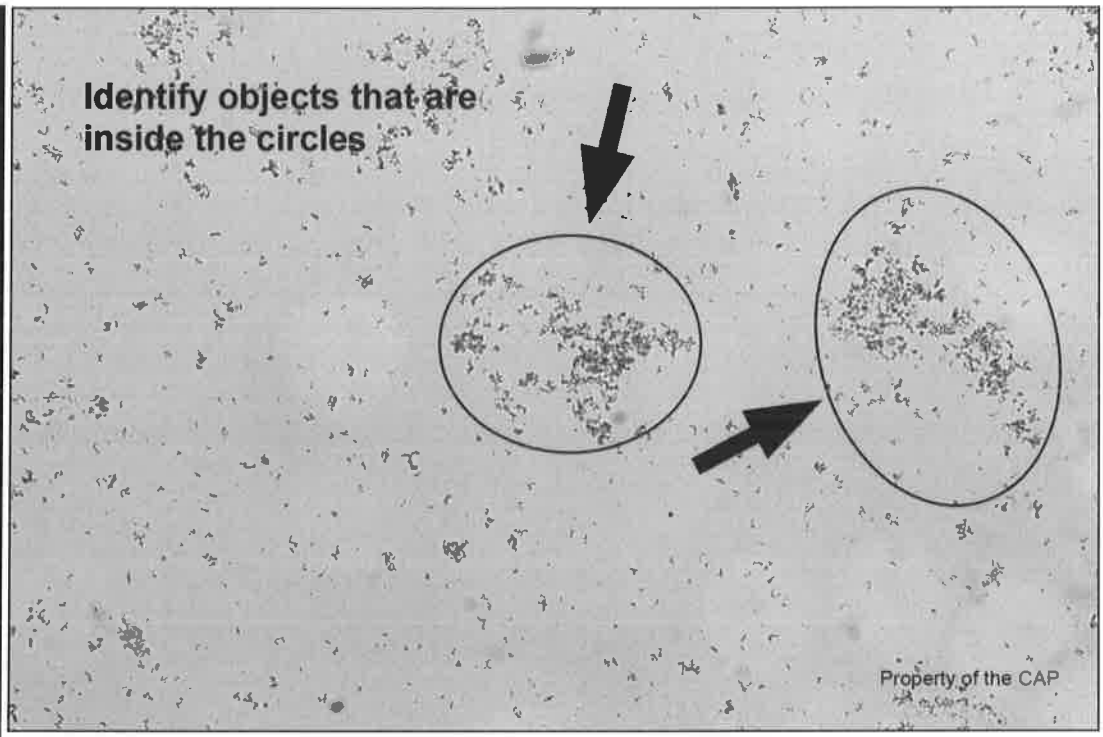
The arrowed objects are uric acid crystals, as identified by 97.2% of participants. Uric acid crystals are found in normal acid (pH < 5.8) urine and also seen in hyperuricemia with or without nephrolithiasis. If nephrolithiasis (urinary tract stone disease) is being considered, the presence of uric acid crystals is only significant if the specimen is fresh. Uric acid crystals may also be increased in gouty nephropathy.

Uric acid crystals assume a variety of shapes including hexagons, stars, barrels, prisms, cubes, rosettes, "whetstones", "drumsticks", "wheat sheaves" and lemons. Uric acid crystals show strong, polychromatic birefringence and may be very pale yellow, amber or yellow-brown.

Uric acid crystals with a hexagonal shape are occasionally confused with cysteine crystals. Uric acid crystals show polychromatophilic birefringence and do not yield a positive nitroprusside test.

Urine Sediment Photographs

CMP-20



(URINE, UNSTAINED, HIGH POWER)

Identification	CMP Participants No.	%	Performance Evaluation
Amorphous urates	6086	98.1	Good

The objects within the arrowed circles are amorphous urates, as correctly identified by 98.1% of participants. Amorphous material is either amorphous phosphates or amorphous urates and is differentiated based on pH. Amorphous phosphates are more common and occur at a pH > 6.3. Amorphous urates occur at a pH < 5.8.

Amorphous material is formless or shapeless precipitates of sodium, potassium, magnesium and calcium salts. Both types of amorphous material increase on standing and cooling. Formation of amorphous material is related to concentration, temperature and age of the urine sample. Amorphous phosphates tend to be white and amorphous urates tend to be reddish (so- called "brick dust") due to their selective absorption of the urinary pigment "uroerythrin".

Differentiation of the two types of amorphous material can be based on pH. Additionally, amorphous phosphates do not dissolve when heated to 60 degrees C and do dissolve in dilute acetic acid.

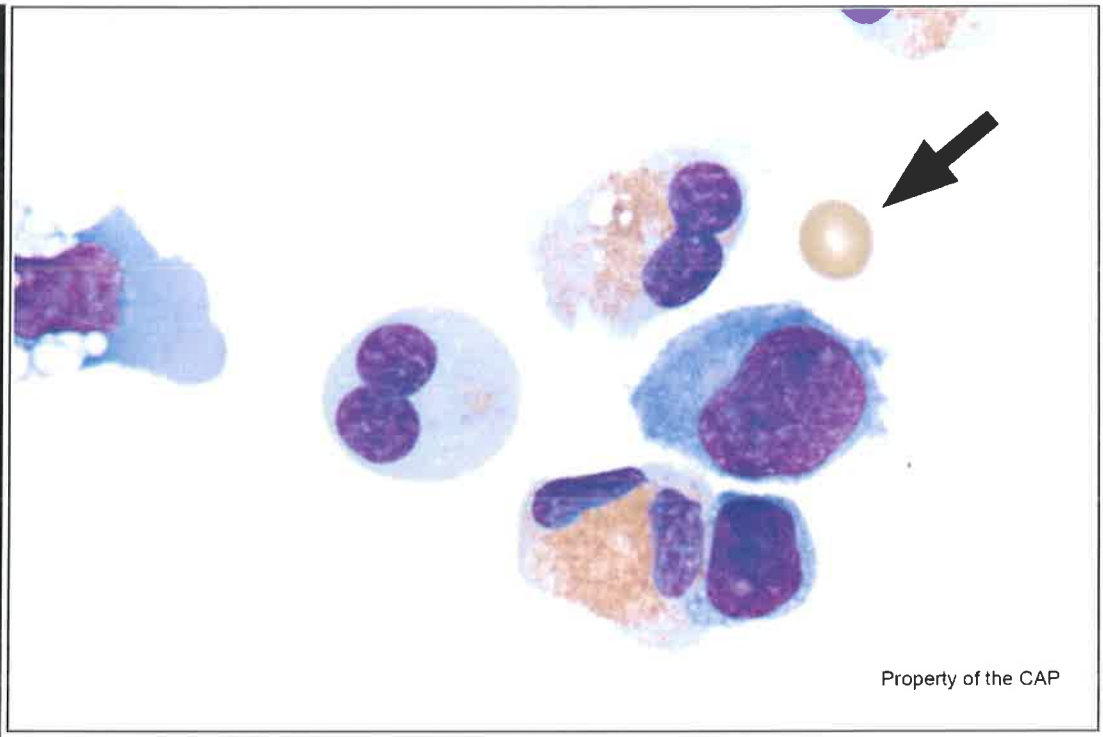
Neither type of amorphous material is directly associated with disease states. However, both types may obscure other microscopic findings if present in larger amounts.

Body Fluid Photographs

Case History CMP-21 through CMP-26

This patient is a 68-year-old woman being seen in the emergency room with a severe headache, stiff neck, nausea, lethargy, and lack of appetite for the past 4 days. Cerebrospinal fluid laboratory data include: WBC = 20/µL (0.020 x 10E3/µL); RBC = 5/µL (0.005 x 10E3/µL).

CMP-21



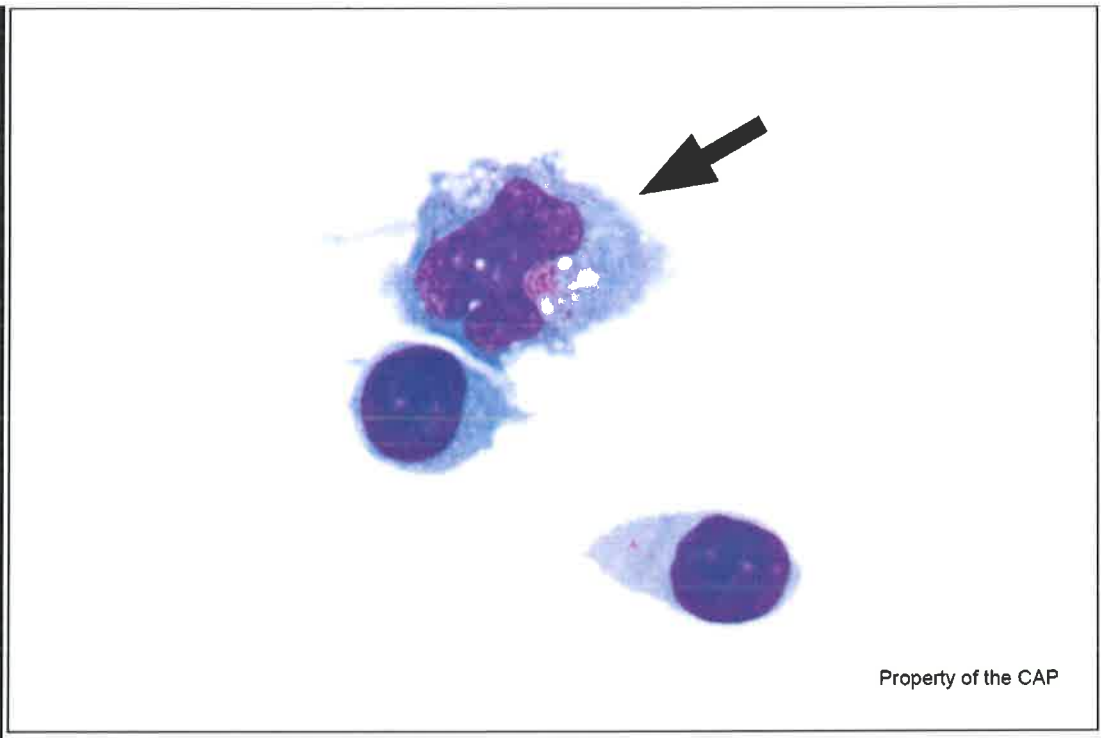
(CSF, CYTOCENTRIFUGE, WRIGHT-GIEMSA, 100X)

Identification	CMP Participants No.	%	Performance Evaluation
Erythrocyte	3663	99.8	Good

The arrowed object is an erythrocyte, as correctly identified by 99.8% of participants. Erythrocytes in body fluids have a similar appearance to those in peripheral blood, lacking nuclei and with hemoglobinized cytoplasm. Erythrocytes are not normally found in body fluids but may be introduced during medical procedures or trauma.

Body Fluid Photographs

CMP-22



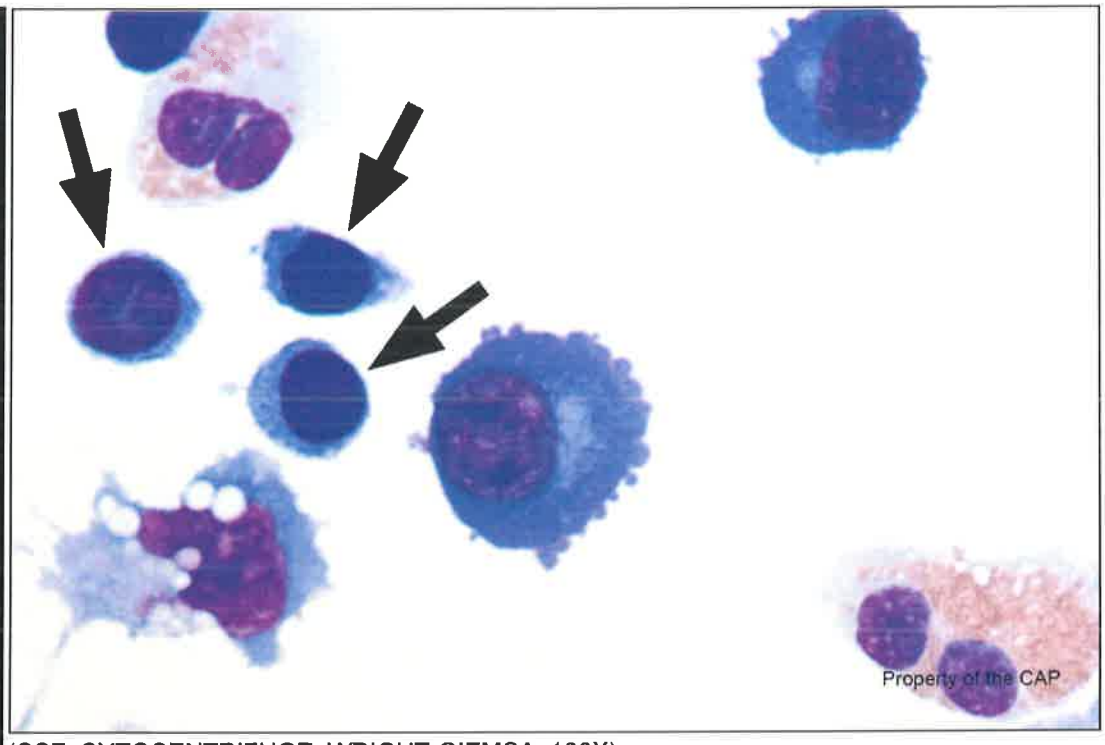
(CSF, CYTOCENTRIFUGE, WRIGHT-GIEMSA, 100X)

Identification	CMP Referees No.	CMP Referees %	CMP Participants No.	CMP Participants %	Performance Evaluation
Monocyte/Macrophage	29	96.7	3411	93.0	Educational

The arrowed object is a monocyte/macrophage, as correctly identified by 93.0% of participants. Monocytes/macrophages in body fluids can show a spectrum of morphology ranging from a monocyte similar to that seen in peripheral blood, to a vacuolated, activated macrophage. Monocytes are 12 - 20 µm in size with abundant gray-blue cytoplasm containing few azurophilic granules. The nucleus can be round or indented.

Body Fluid Photographs

CMP-23



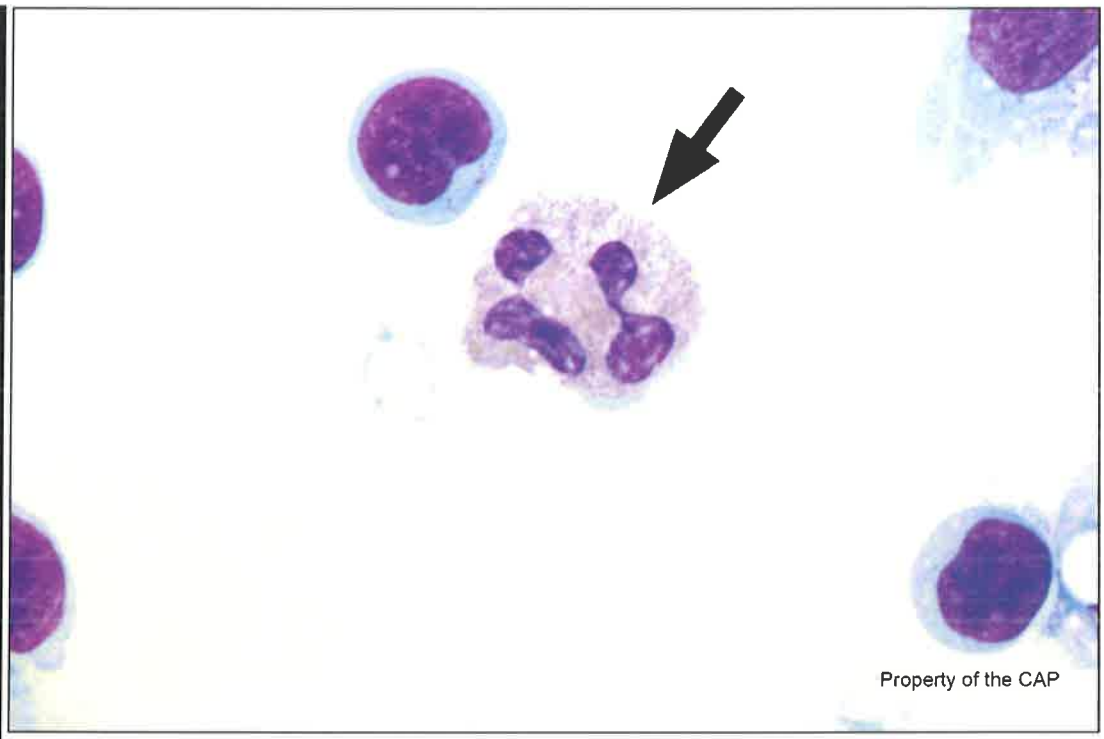
(CSF, CYTOCENTRIFUGE, WRIGHT-GIEMSA, 100X)

Identification	CMP Participants No.	%	Performance Evaluation
Lymphocyte	3620	98.7	Good

The arrowed objects are lymphocyte, as correctly identified by 98.7% of participants. Lymphocytes are smaller than neutrophils and monocytes, but slightly larger than erythrocytes. As in peripheral blood, lymphocytes are mononuclear cells usually with round nuclei with condensed chromatin and scant to moderate cytoplasm. However cytospin preparations can distort the nuclei and cause cytoplasmic spreading or fraying.

Body Fluid Photographs

CMP-24



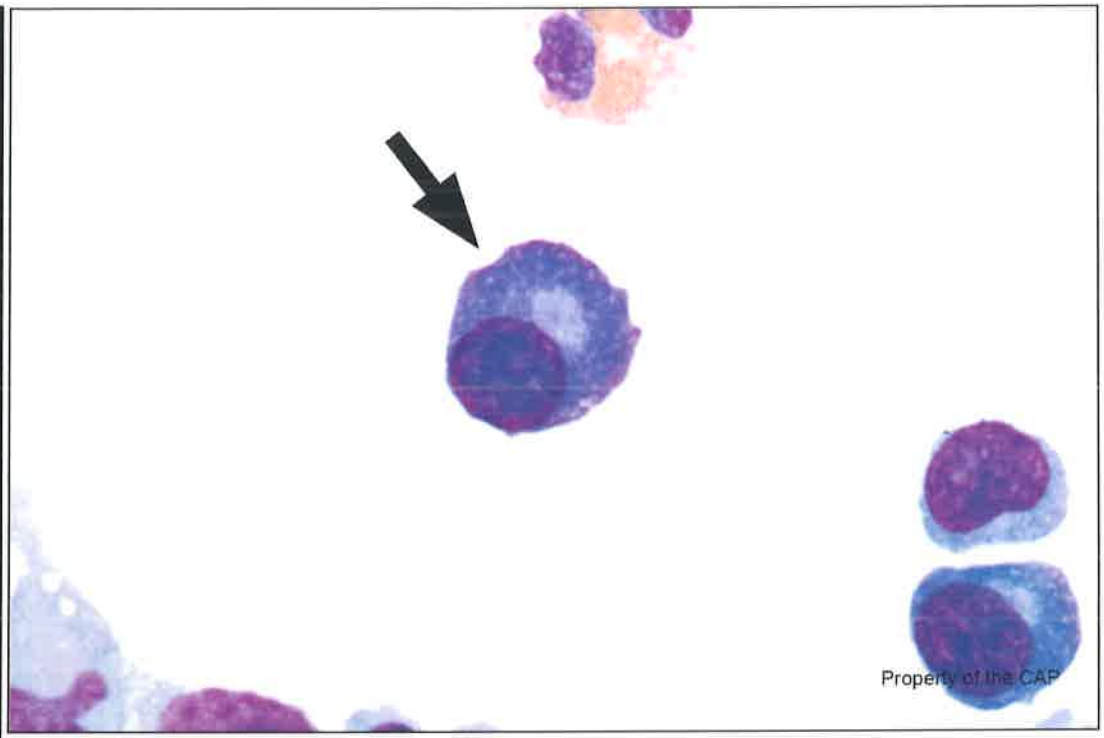
(CSF, CYTOCENTRIFUGE, WRIGHT-GIEMSA, 100X)

Identification	CMP Participants No.	%	Performance Evaluation
Neutrophil, segmented or band	3641	99.2	Good

The arrowed object is a neutrophil, segmented or band, as correctly identified by 99.2% of participants. Mature neutrophils have multilobulated (3-5) nuclei and are 10 - 15 µm in diameter, although this can be distorted in some spin preparations. The cytoplasm contains numerous secondary/specific granules.

Body Fluid Photographs

CMP-25



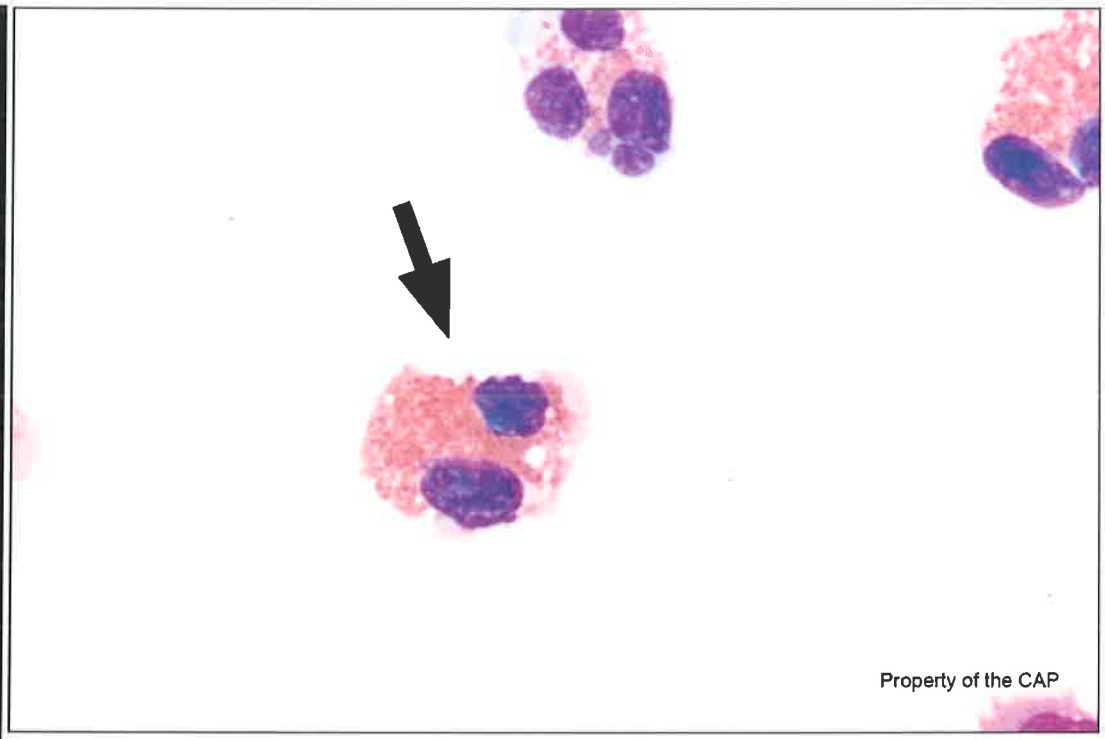
(CSF, CYTOCENTRIFUGE, WRIGHT-GIEMSA, 100X)

Identification	CMP Participants No.	%	Performance Evaluation
Plasma cell	3523	96.1	Educational

The arrowed object is a plasma cell, as correctly identified by 96.1% of participants. Plasma cells are not normally found in body fluids, but can be seen in a variety of disease states. Plasma cells are characterized by an eccentrically placed round nucleus with condensed chromatin and a paranuclear Golgi region (hof) in deeply basophilic cytoplasm. Binucleated forms may be seen.

Body Fluid Photographs

CMP-26



(CSF, CYTOCENTRIFUGE, WRIGHT-GIEMSA, 100X)

Identification	CMP Participants No.	%	Performance Evaluation
Eosinophil	3660	99.8	Good

The arrowed object is an eosinophil, as correctly identified by 99.8% of participants. Eosinophils are identified by their characteristic granules, which are orange-red and larger than neutrophilic granules. The nuclei are usually bilobed. The presence of many eosinophils can be seen as a reaction to foreign bodies, parasites, or introduction of air into a body cavity.

Case Presentation:
This patient is a 68-year-old woman being seen in the emergency room with a severe headache, stiff neck, nausea, lethargy, and lack of appetite for the past 4 days. Cerebrospinal fluid laboratory data include: WBC = 20/μL (0.020 x 10E3/μL); RBC = 5/μL (0.005 x 10E3/μL).

(CSF, CYTOCENTRIFUGE, WRIGHT-GIEMSA, 100X)

Case Discussion: Viral Meningitis

Meningitis is an inflammation of the meninges, the tissues covering the central nervous system. It is relatively uncommon, with an estimated 4 to 30 cases per 100,000 people per year in developed countries. Meningitis can be caused by bacterial infection. Non-bacterial ("aseptic") meningitis can be caused by other infectious agents (viruses, parasites), medication, and neoplasia, although in a significant number of cases a specific etiology may never be found.

Regardless of the underlying cause, most cases of meningitis present with similar symptoms including headache, fever, and signs of meningeal inflammation (stiff neck). Patients may also have sensitivity to light, nausea, and vomiting, as well as other symptoms related to the underlying cause (eg, upper respiratory symptoms with enteroviruses, genital lesions with HSV-2). There are generally few or no focal lesions of the central nervous system seen on imaging studies.

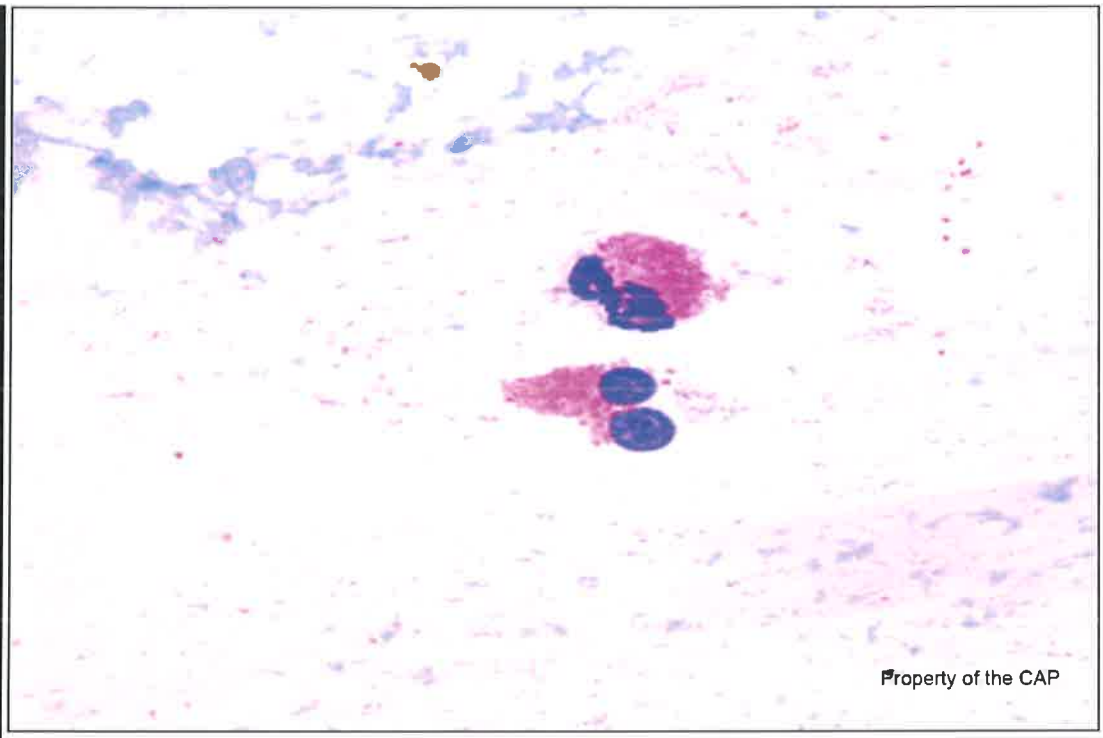
Work-up of patients with suspected meningitis always includes examination of the cerebrospinal fluid. Bacterial meningitis is life-threatening, and is always investigated for, usually by CSF gram stain and culture, and blood culture. If the gram stain is negative, empiric antibiotic treatment may still be given to especially susceptible patients including the elderly and immunocompromised, until the culture is negative. Cases of bacterial meningitis also typically have elevated glucose (< 40 mg/dL), low CSF to serum glucose ratio, high protein (> 200 mg/dL) and significant CSF pleocytosis (> 1000/μL). In contrast viral meningitis is characterized by a relatively mild pleocytosis (WBC < 250/μL), normal glucose, and a modest protein elevation. Significant exceptions include meningitis due to mumps or lymphocytic choriomeningitis virus (LCMV), which can have elevated glucose and a high CSF WBC count (> 1000/μL). The pleocytosis of viral meningitis is usually lymphocytic, while bacterial meningitis usually causes a neutrophilic predominance. However, early enterovirus meningitis has a neutrophilic predominance.

Cases of suspected viral meningitis are increasingly assessed via nucleic acid testing and serologies as opposed to viral culture, which has relatively low sensitivity and requires several days. The prevalence of different viral causes has changed with the advent of modern vaccines. Mumps was the most frequent cause prior to use of the measles, mumps, rubella vaccine, but now is seen infrequently. Enteroviruses (coxsackie, echovirus, etc.) are frequent causes of viral meningitis in the summer or fall. Primary infection with HIV and HSV-2 can also present with meningeal involvement. Arboviruses such as West Nile virus and St. Louis encephalitis virus can also cause meningitis, but more frequently present with encephalitis. Immunocompromised patients are susceptible to meningitis caused by other viruses such as CMV, VZV, HHV-6 and EBV, but these are uncommon in immunocompetent individuals.

In contrast to bacterial meningitis, viral meningitis is usually self-limiting and can be treated with supportive care. Thus early exclusion of a bacterial cause and identification of a viral cause can reduce hospitalizations and overtreatment. One notable exception is HSV-2 meningitis, which is usually treated with antivirals such as acyclovir.

CMMP – Clinical Microscopy Miscellaneous Photographs

CMMP-38



(NASAL, WRIGHT-GIEMSA) High power magnification

Identification	CMMP Participants No.	%	Performance Evaluation
Eosinophils are present	2279	99.8	Good

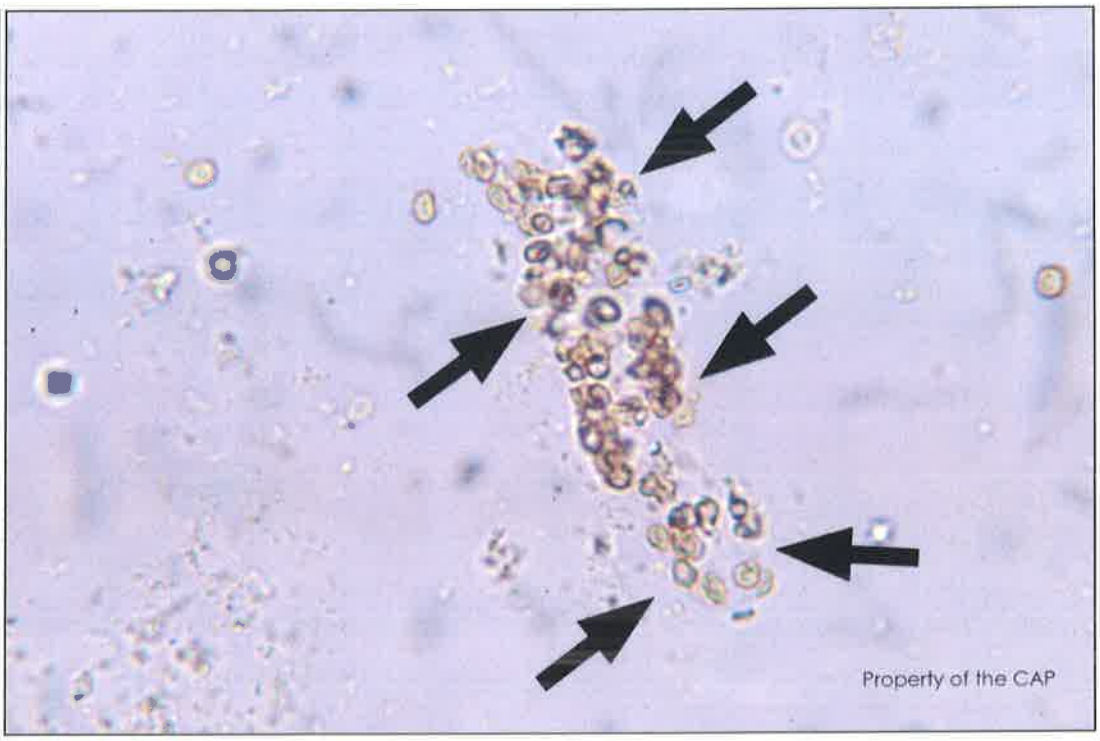
This photomicrograph demonstrates a Wright-Giemsa stained nasal smear. Two eosinophils with bright orange-red spherical granules are seen. The finding of nasal eosinophils is supportive of the diagnosis of allergic rhinitis. These cells have nuclei with two lobes separated by a thin filament. Non-allergic causes of nasal discharge will typically be acellular or show a predominance of neutrophils.

CMMP – Urine Sediment Color Photographs

Case History USP-05 and USP-06

The patient is a 12-year-old girl who was being evaluated for acute nephritis. Laboratory data include: Specific gravity = 1.010; pH = 6.0; blood, leukocyte esterase and protein = positive; glucose, ketones, and nitrite = negative.

USP-05



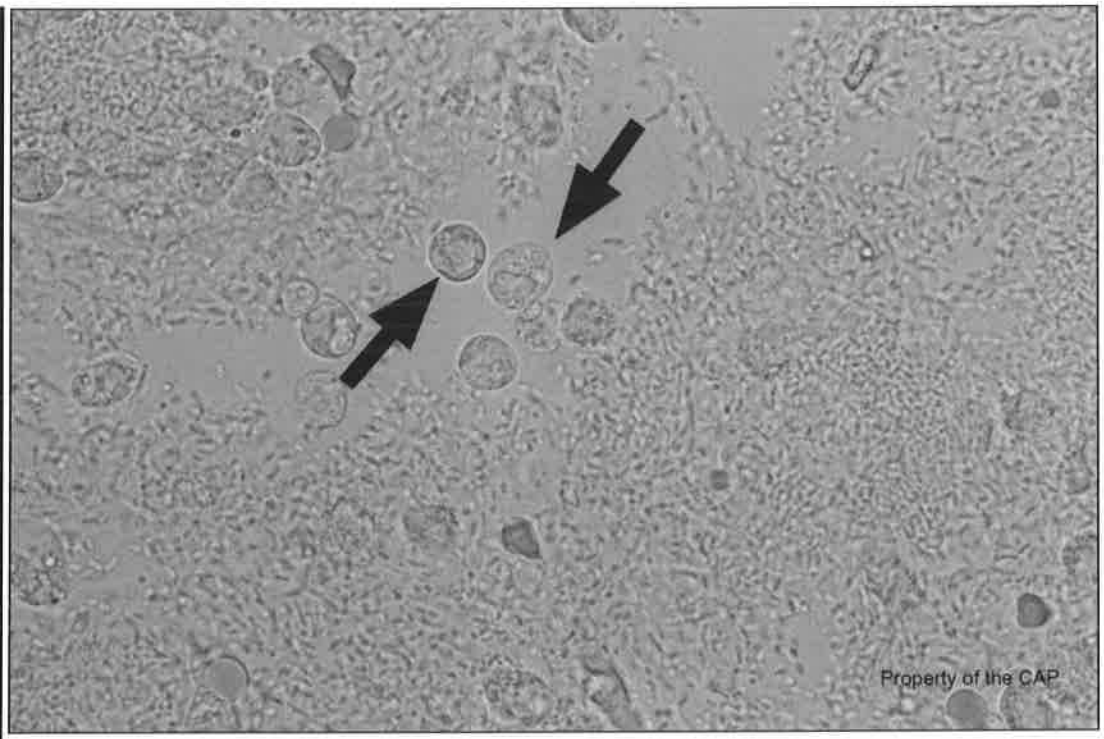
(URINE, UNSTAINED, HIGH POWER)

Identification	CMMP Participants No.	%	Performance Evaluation
Red blood cell casts	3484	96.7	Good

The arrowed object is a red blood cell casts, as correctly identified by 96.7% of participants. These are composed predominantly of intact erythrocytes covering a cast. Although the red blood cells may be shrunken, they can be identified by their yellow to reddish-brown color and the uniformity of the cell size. This is in contrast to a fatty cast, in which the fat globules vary in size. Red blood cell casts are seen with acute nephritis, especially in cases with glomerular injury.

CMMP – Urine Sediment Color Photographs

USP-06



(URINE, UNSTAINED, HIGH POWER)

Identification	CMMP Participants No.	%	Performance Evaluation
Leukocyte	3488	96.8	Good

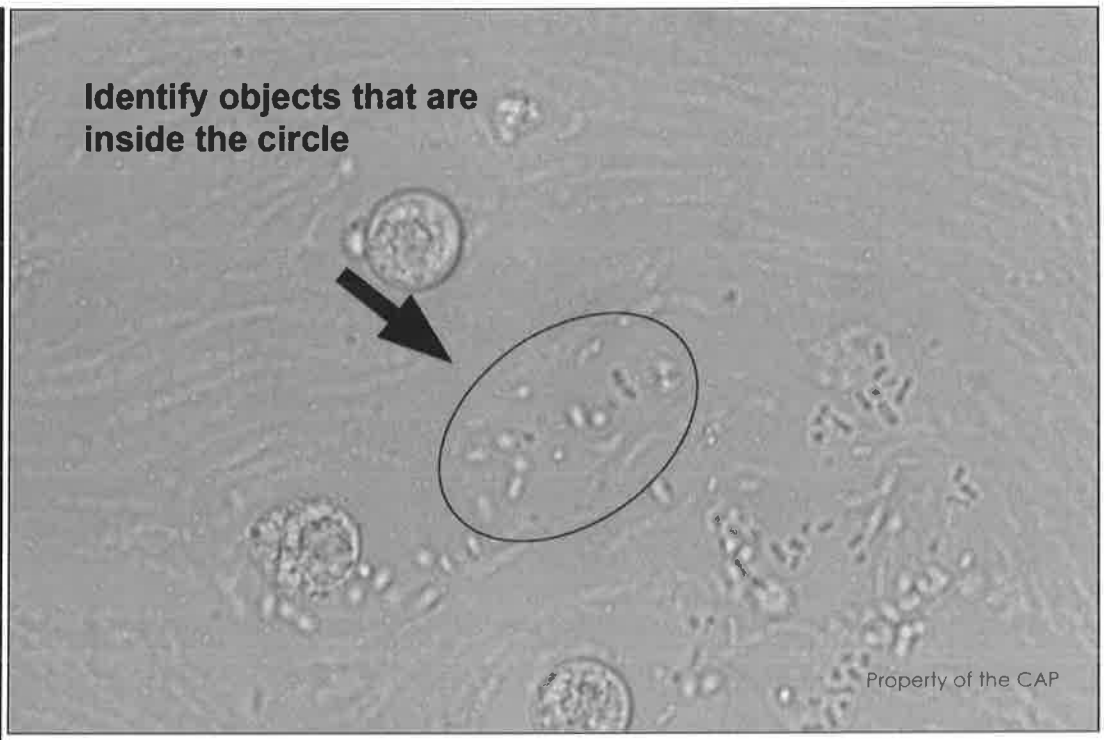
The arrowed objects are leukocytes, as correctly identified by 96.8% of participants. In unstained urine neutrophils are colorless and granular, approximately 10 - 12 µm in diameter. The cells may be larger if they have phagocytized yeast or bacteria, or with swelling in hypotonic urine. In fresh urine, the multilobulated nucleus may be appreciated, however as the cell degenerates the segments fuse and granules are lost. Eosinophils are slightly larger than neutrophils. Lymphocytes are rarely present in urine, and are slightly larger than red blood cells, with round nuclei and non-granular cytoplasm. Increased leukocytes, especially neutrophils, are seen in many urinary tract disorders. Less than 5 neutrophils per HPF can be seen in healthy individuals, however increased leukocytes or clumps of leukocytes are highly suggestive of acute infection.

CMMP – Urine Sediment Color Photographs

Case History USP-07 and USP-08

The patient is a 75-year-old woman who developed urinary incontinence. Laboratory data include: Specific gravity = 1.026; pH = 8.5; leukocyte esterase and nitrites = positive; blood, glucose, ketones, and protein = negative.

USP-07



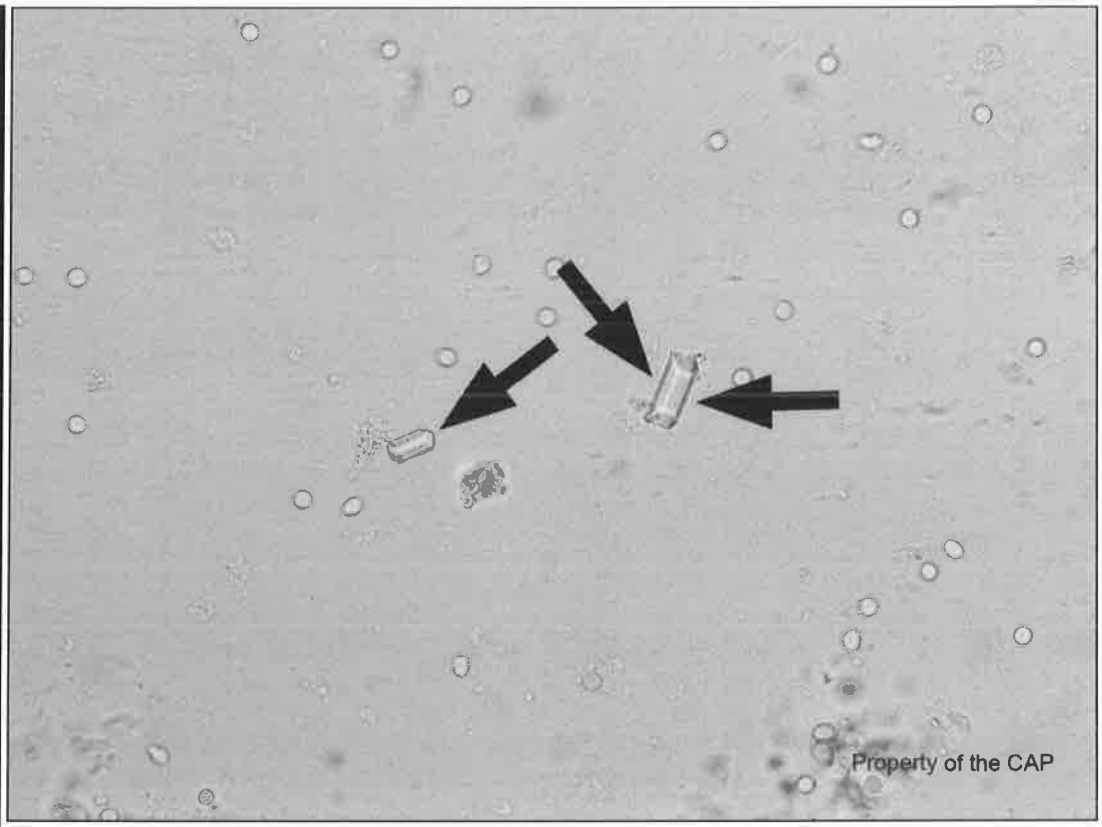
(URINE, UNSTAINED, HIGH POWER)

Identification	CMMP Participants No.	%	Performance Evaluation
Bacteria	3485	96.8	Good

The objects in the circle are bacteria, as correctly identified by 96.8% of participants. Bacteria in urine can be either round (cocci) or rod-shaped (bacilli). They can be present singly or in clusters, pairs, or chains. Bacteria are small in comparison to leukocytes and other cells, and generally have a uniform size and shape. In patients being treated with antibiotics, the bacteria can take abnormally elongated shapes.

CMMP – Urine Sediment Color Photographs

USP-08



(URINE, UNSTAINED, HIGH POWER)

Identification	CMMP Participants No.	%	Performance Evaluation
Ammonium magnesium (triple) phosphate crystals	3566	99.0	Good

The arrowed objects are ammonium magnesium (triple) phosphate crystals, as correctly identified by 99.0% of participants. These crystals are found at neutral to alkaline pH, are clear in color, and characterized by their "coffin-lid" appearance. Triple phosphate crystals are birefringent and are often accompanied by amorphous phosphates and bacteria.

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CM-B 2017: Analysis of Synovial Fluid: A Joint Effort

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This activity is approved for continuing education credit in the states of California and Florida.

Disclosure Statement

The following authors/planners have financial relationships to disclose:
None

The following authors/planners have no financial relationships to disclose:
David Bosler, MD, FCAP

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Learning Objectives

Upon completing the reading and answering the learning assessment questions, you should be able to:

1. Understand the importance of preanalytical issues to accurate evaluation of synovial fluid.
2. Describe the basic components of a synovial fluid evaluation.
3. Know the crystals that cause crystal induced arthropathy and how to identify them and distinguish them from their mimics.
4. Explain the tests relevant to diagnosis of infectious arthritis, their uses and limitations in diagnosing the most common infectious agents.

CM-B 2017: Analysis of Synovial Fluid: A Joint Effort

INTRODUCTION

Joint fluids are analyzed in the laboratory in a variety of specific clinical situations, mostly involving arthritis and unexplained effusions in the joint. It is important for the laboratorian to understand these clinical situations in order to ensure that the testing performed provides valuable information that contributes to answering the clinical question(s) being asked. As is the case with other body fluids, understanding and controlling the preanalytical steps in synovial fluid collection, storage and transport is important to ensuring that the results are valid and meaningful. This educational activity focuses on the clinical indications for testing, the components of the evaluation, relevant findings and their implications. Most testing of synovial fluid provides results that, while contributory, are nonspecific and require correlation with other clinical and laboratory findings to make a diagnosis. Crystal arthropathies and infectious arthritis, two clinical scenarios for which laboratory analysis provides definitive and actionable diagnostic information, are covered in more detail.

Normal synovial fluid

Synovial fluid is contained within the cavities of the body's diarthroses or synovial joints. The articulating surface of the bones in these joints is lined with hyaline cartilage, and the entire joint is enveloped in a joint capsule made of fibrous tissue and synovial lining cells. Synovial fluid lies in the cavity between the capsule and the cartilaginous surfaces[1]. The fluid lubricates and cushions the joints, and supplies oxygen and nutrients to the joint's cartilage and synovial lining. It is largely composed of plasma ultrafiltrate with the addition of hyaluronic acid (produced by Type B synovial cells), which aids the fluid's joint protective function through increased viscosity. Normal synovial fluid is clear and colorless with a viscosity similar to an egg white, and is sterile. Under normal conditions the volume of synovial fluid is minimal. As with many other body fluids, normal cellularity is low, composed of a scant mixture of lymphocytes, monocytes, macrophages and scattered synovial lining cells.

Collection and evaluation of synovial fluid

Synovial fluid may be collected and evaluated primarily as part of the diagnostic work-up for arthritis, painful inflammation of the joint often with associated swelling that can arise from a variety of underlying conditions described in more detail later. These underlying states often cause the normally minimal volume of synovial fluid to expand substantially, allowing for relatively easy collection. Using sterile technique, fluid can be aspirated by inserting a needle into the joint directly through the overlying skin. Adherence to sterile technique is crucial in order to avoid both iatrogenic infection of the joint and contamination of the sample by skin flora. Testing performed on the fluid varies depending on the clinical indication for sampling the fluid. Ideally, three tubes should be drawn, a sterile collection for microbiology culture first, a sodium heparin or EDTA tube for cell counts and microscopic evaluation, and a plain tube for gross evaluation and chemistry testing. Since testing may be limited by the volume that can be aspirated from the joint, having a protocol to prioritize testing in low volume specimens is recommended[1]. Certain anticoagulants, such as oxalate, powdered EDTA and lithium heparin should not be used since they have ingredients that mimic crystals and can cause false positives or complicate the evaluation[2].

Once collected, the fluid should be transported to the lab and evaluated as soon as possible. Delays in processing may affect results such as cell counts and glucose, which can be affected as soon as one hour after collection[3]. In addition, processing delays may reduce the sensitivity of evaluation for crystals[4]. Upon receipt in the lab, the first action should include a clerical quality check. The specimen must have at least the minimum required labeling of two unique identifiers, and the labeling should be consistent across all documentation

associated with that specimen. Orders are checked against the specimen(s) received.

Spotlight on preanalytics

As with other body fluids, preanalytical events, including collection, transport and storage, can have an important impact on the evaluation of synovial fluid and the results of testing. It is therefore important for laboratorians to know enough about a specimen's preanalytical journey to understand how analysis and results may be affected by collection techniques and, storage and transport conditions in order to avoid potential blind spots that could have a negative effect on test accuracy. Laboratories should understand and control specimen storage and transport conditions in order to minimize the potential effects of specimen aging. How are specimens stored and transported, and how long does the process take? Stability for the specimen type under these conditions (eg, ambient vs refrigerated) must be validated, and criteria established for storage and transport that ensure specimen integrity.

Evaluation of synovial fluid typically involves several components. Gross visual inspection should start with a check to ensure that the transport packaging is intact and has not been compromised, and that there are no leaks. The volume, color, clarity and viscosity of the specimen should be noted. Common laboratory testing of fluids may include hematologic (cell count, differential count), microbiology (Gram stain, culture, antigen/molecular detection), and occasionally chemistries (eg, glucose, lactate). The microscopic evaluation typically involves review of a Wright stained slide, and includes scanning the cells and their morphology, as well as scanning for organisms and crystals. The most specific findings in synovial fluid (and therefore the most critical to prioritize and evaluate when suspected) are crystals and identification of infectious organisms.

Cell counts for synovial fluid are often performed using a hemocytometer. Although they can be performed on an automated cell counter, pretreatment with hyaluronidase may be necessary to avoid clogging the instrument and spuriously high counts due to non-WBC particles. Differential counts are most often manual, with slide preparation often also requiring hyaluronidase treatment. Digital image based cell counts and differential counts offer an alternative that can be automated and is not subject to the potential problems created by high viscosity[3]. Synovial fluid WBC count is normally less than 0.15×10^9/L. The normal differential count is composed predominantly of monocytes and macrophages, typically with approximately 20% neutrophils and 15% lymphocytes[3].

Indications for synovial fluid collection and their findings

Synovial fluid is most often aspirated to evaluate joint effusions, which can be caused by a variety of underlying conditions. They can be broadly categorized into five groups: 1) Non-inflammatory, 2) Inflammatory, 3) Septic, 4) Crystal induced, and 5) Hemorrhagic. The primary etiologies for each of these groups are listed in Table 1.

Table 1. Differential Diagnosis by Joint Fluid Groups

Group 1 Non-Inflammatory	Group 2 Inflammatory	Group 3 Septic	Group 4 Crystal-Induced	Group 5 Hemorrhagic
osteoarthritis	rheumatoid arthritis	bacterial	gout	traumatic arthritis
traumatic arthritis	lupus erythernatosus	mycobacterial	calcium pyrophosphate dehydrate (CPPD) crystal deposition disease	hemophiliac arthropathy
osteochondritis dissecans osteochondromatosis	reactive arthritis (Reiter's syndrome)	fungal	apatite-associated arthropathy	pigmented villonodular tenosynovitis
neuropathic osteoarthropathy	reginal enteritis			neuropathic osteoarthropathy
pigmented villonodular tenosynovitis	ulcerative colitis			synovial hemangioma
	ankylosing spondylitis			
	psoriasis			

Galagan KA, Blomberg D, Cornbleet PJ, Glassy EF. Color atlas of body fluids: an illustrated field guide based on proficiency testing.[1]

The categories are descriptive, and significant overlap between them and the findings they present with can exist. An understanding of the general tendencies can however, when combined with the overall clinical picture, help to affirm or redirect the clinical impression and lend guidance to the subsequent elements of the diagnostic work up. Table 2 shows how components of the gross examination, cell count and differential count can be used to help categorize joint effusions. Generally speaking, there is a spectrum of increasing volume and opacity of fluid, with increasing cell count and percentage neutrophils comparing normal to noninflammatory, inflammatory and infectious (septic) categories. The normally high viscosity breaks down with increasing inflammation. Clotting, which is not present in normal synovial fluid given the absence of fibrinogen, is also commonly present in these pathologic states.

Table 2. Common Gross and Cell Count Findings in Synovial Fluid

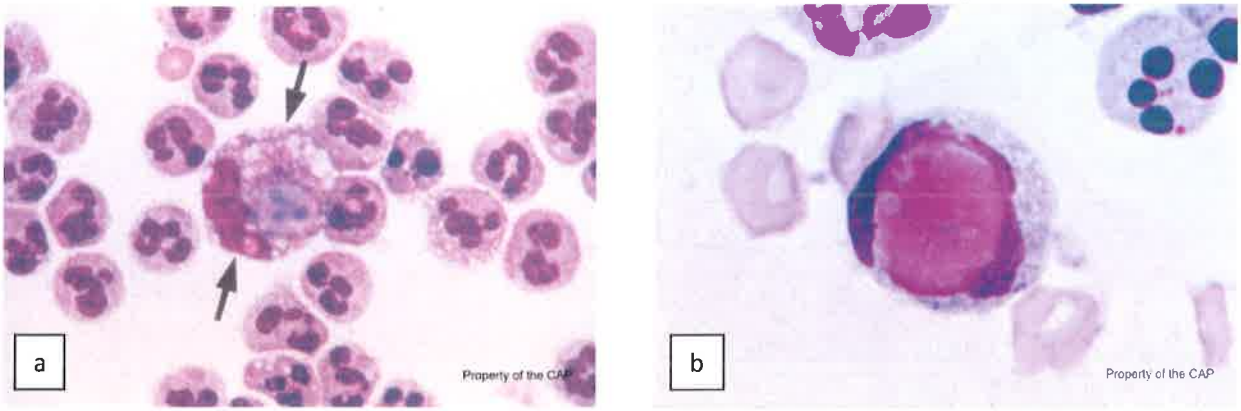
	Normal	Noninflammatory	Inflammatory	Infectious
Volume (mL)	<4	Often >4	Often >4	Often >4
Color/clarity	Clear, colorless	Transparent, pale yellow	Translucent to opaque, yellow to white	Opaque, white
Viscosity	Very high	High	Low	Very low
Fibrin clot	None	Often	Often	Often
WBC x 10^9/L	<0.15	<3.0	3-50	>50
Neutrophils	<25%	<25%	>70%	>90%

Ringsrud KM, Linné JJ. Urinalysis and body fluids a color text and atlas[2]

In addition to the parameters listed in Table 2 that can be useful generally in identifying inflammatory arthritis, a few specific microscopic findings may provide clues to diagnosis in inflammatory or autoimmune arthritides (Figure 1). Reiter's cells, which are macrophages or monocytes containing phagocytized neutrophils, are associated with Reiter's syndrome, an asymmetric reactive inflammatory arthritis triggered by infection outside the joint, most commonly in the gastrointestinal or genitourinary systems[1,5]. LE cells, neutrophils containing denatured

nuclear material with a glassy appearance, historically have been used as a diagnostic adjunct for lupus arthritis. Neither of these cell types is entirely specific however, as either can be seen in rheumatoid arthritis[3]. When present these findings can nonetheless contribute to the overall diagnostic picture.

Figure 1.



Specific findings in inflammatory arthritides: Reiter cell (a) and LE cell (b). Courtesy of College of American Pathologists.[1]

Hemorrhagic joint effusions are associated with a variety of underlying conditions, including trauma, pigmented villonodular synovitis, synovial hemangioma, as well as a hematologic conditions such as hemophilia, thrombocytopenia, anticoagulant therapy, sickle cell anemia and myeloproliferative neoplasms[3]. Hemorrhagic joint fluids are grossly opaque, red-brown or xanthochromic, usually have a relatively low WBC count and abundant red blood cells[3]. Traumatic collection of synovial fluid can sometimes be confused with true pathologic bleeding, and these two possibilities must be distinguished whenever possible. Features such as clearing with centrifugation may point toward a traumatic tap, while xanthochromia and the presence of hemosiderin laden macrophages are more supportive of hemarthrosis. These distinctive properties must be interpreted with caution since they blur over time if evaluation of the specimen is delayed.

Crystal Induced Arthritis

Monosodium urate (MSU) and calcium pyrophosphate dehydrate (CPPD) crystals are the primary findings of clinical relevance in crystal induced arthritides[1,4]. Since the presence of these crystals, particularly intracellularly, is pathognomonic for crystal induced arthritis, their accurate identification is a paramount component of synovial fluid evaluation. MSU and CPPD crystals are associated with gout and CPPD crystal arthropathy (also known as "pseudogout" or chondrocalcinosis), respectively, with the crystals themselves playing a role in pathogenesis of the arthritis.

Gout is the most common inflammatory arthritis in men[4]. It results from hyperuricemia, or increased levels of serum uric acid, which can be due to either increased production or decreased excretion of uric acid or both. Uric acid is a breakdown product of purines, a basic building block for DNA and RNA. Although abnormalities of specific enzymes are identified in a minority of cases, a specific enzymatic abnormality behind overproduction of uric acid is not identified in most cases. Overproduction may be due to high cellular turnover or lysis (such as cellular destruction in chemotherapy). Chronic renal disease may result in decreased uric acid excretion[6]. Hyperuricemia does not invariably lead to gout however. Age and duration of hyperuricemia, alcohol consumption, obesity, lead toxicity, use of certain medications and genetic predisposition are risk factors

associated with conversion of hyperuricemia to gout[6]. MSU crystals form in the setting of super-saturated uric acid within the joint space, inducing the intense inflammatory response responsible for the signs and symptoms of gout[1,6]. Clinically, gout presents with acute and intermittent episodes of a painful joint that is red, warm and tender. It most often begins with a single joint, classically the first metatarsophalangeal joint of the big toe ("podagra"), although other joints can be involved (eg, insteps, ankles, heels, knees, wrists, fingers, and elbows). The episode resolves completely with time, but left untreated recurs. Recurrent episodes occur at shorter intervals and may progress to involve multiple joints. Years of intermittent attacks may progress to chronic tophaceous gout, with tophi composed of aggregates of MSU crystals depositing in the joint space, tendons, cartilage, ligaments and periarticular soft tissue, eventually leading to erosion of the bone and destruction of the joint[6]. Gouty arthritis can be treated by life style modification of the factors that increase risk of attacks, as well as by medications that decrease uric acid production (eg, allopurinol) or increase excretion (eg, probenecid).

Collection and timely analysis of synovial fluid for suspicion of gout can be difficult particularly in the primary care setting since collection can be difficult, particularly from smaller joints, and analysis for crystals requires a polarizing microscope and trained and competent testing personnel. Given these difficulties, the ability to diagnose gout based on clinical criteria would be particularly valuable to clinicians. Various features are incorporated into numerous diagnostic algorithms, including recurrent episodes of acute arthritis, maximum inflammation developing within 1 day, redness, painful or swollen first metatarsophalangeal joint, proven or suspected tophi, and hyperuricemia[7,8]. Although numerous algorithms have been studied, with some yielding sensitivities and specificities approaching 80%, evaluation of joint fluid for the presence of crystals remains the gold standard and should be used particularly in cases where other entities are considered in the differential diagnosis[7].

Calcium pyrophosphate crystal arthropathy occurs in older individuals and equally between the sexes[6]. It may be sporadic and idiopathic, hereditary, or secondary. Secondary CPPD arthropathy may be associated with prior joint surgery or damage, or a variety of conditions such as calcium and magnesium metabolic disorders, hypothyroidism, hyperparathyroidism or hemochromatosis. Hereditary CPPD arthropathy involves mutations in the pyrophosphate transport channel, and is typically associated with earlier onset and severe osteoarthritis[6]. CPPD crystal deposits are formed in abnormal articular cartilage matrix (typically adjacent to chondrocytes), which may rupture into the joint space. Similar to gout, the manifestations of disease in CPPD arthropathy result from the inflammatory response recruited after crystal deposition. Less is known about the factors that precipitate acute attacks of arthritis in CPPD crystal arthropathies. Although the clinical features parallel those of gout, the presentation, severity and course is more variable. There is no treatment that specifically alters crystal formation.

Crystal induced arthropathy has a similar cellular background as inflammatory arthritis of other etiologies (see Table 2), although the inflammation may be more prominent with gout. Crystal analysis may be performed with light microscopy (phase contrast improves sensitivity of detection). The optimal sensitivity and specificity is achieved with polarized microscopy using a high-quality polarizing microscope with a first-order red plate compensator and evaluating the refringence (light refraction) pattern of any crystals identified. The polarizer is placed immediately above the light source and the analyzer (another polarizing filter) is placed between the objective and the oculars oriented 90 degrees from the polarizer in order to create a dark background. The red compensator is then placed between the analyzer and the oculars at a 45 degree angle. MSU crystals are long and needle-like, with sharp tapered ends. They are strongly and negatively birefringent, yellow when parallel with the long axis of the compensator and blue when perpendicular with it[3] (see Figure 2). CPPD crystals are

classically rhomboid in shape and usually thicker, although they may be rod-shaped or rectangular. They are weakly and positively birefringent, blue when parallel with the long axis of the compensator and yellow when perpendicular to it. The sensitivity and specificity of polarized light microscopy is 78% and 79%, respectively for MSU crystals and 12% and 67%, respectively for CPPD crystals[3]. Repeat examination after 24 hours of refrigeration may improve sensitivity of detection. Potential pitfalls of crystal identification include misidentifying other types of crystals (see below), corn starch from glove powder, cartilage fragments and foreign debris from joint prostheses. Corticosteroid crystals may be a particular challenge, potentially mimicking either MSU or CPPD crystals since they show refringence and can be needle shaped. These crystals often show variable appearance (including blunt or jagged ends), which may be a clue in differentiating them from MSU or CPPD, and most often they show positive birefringence[1,3]. They may be present in synovial fluid for up to a month following intra-articular injection, so review of recent history can be important.

Other crystals may be present in synovial fluid, including cholesterol, hematoidin (see Figure 2C & 2D), calcium oxalate, Charcot-Leyden, and lipid crystals[1,3]. While these crystals are not associated with crystal induced arthritis, they are important to recognize either due to potential clinical correlations or to ensure accurate differentiation from MSU and CPPD crystals.

Figure 2.

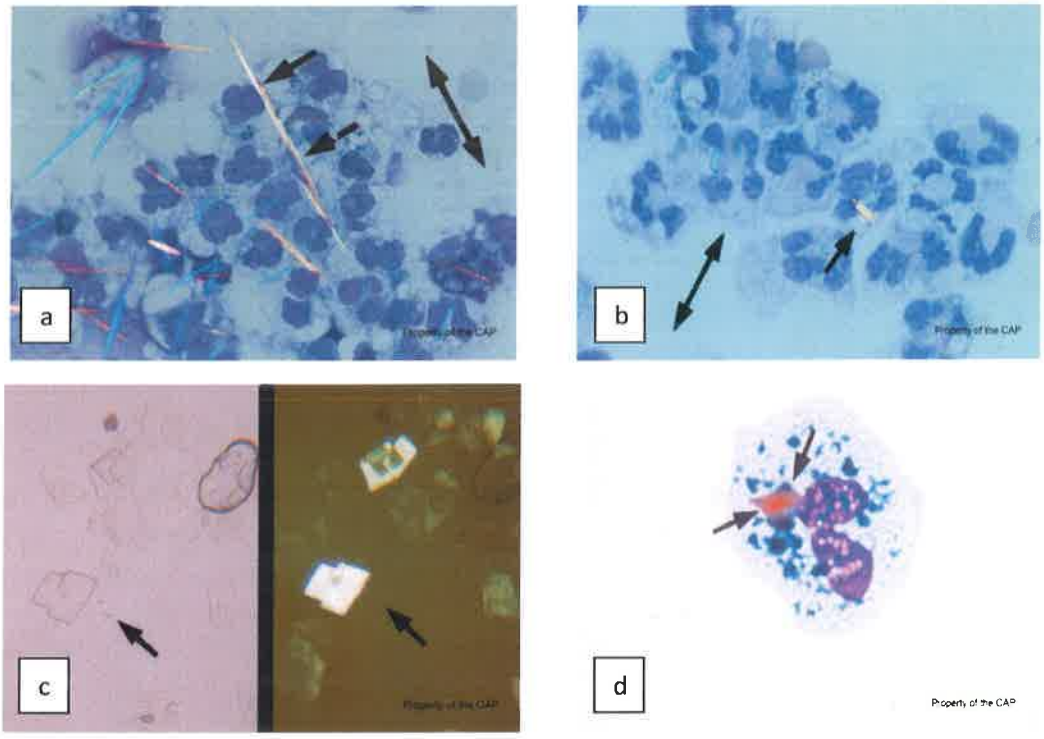


Figure 2: Synovial fluid crystals: a) monosodium urate, negatively birefringent; b) calcium pyrophosphate dehydrate, positively birefringent; c) cholesterol; d) hematoidin.). Courtesy of College of American Pathologists.[1]

The complexity of accurate crystal identification emphasizes the importance of training and competency. One study illustrated this need using online testing of rheumatologists, trainees, lab techs, and other physicians. While

identification of MSU crystals was relatively high (81% getting each of 8 MSU images correct), the percentage correctly identifying all non-MSU images was significantly lower, at only 23%, indicating significant room for improvement[9]. Due to the complexity of evaluation as well as the complexity of technical setup, synovial fluid crystal analysis is categorized as CLIA high complexity testing. Thus, although knowing whether arthritis is crystal induced during a patient's visit may be helpful to patient care, there are significant hurdles (space, equipment and preventative maintenance, personnel, training and competency, proficiency evaluation, patient throughput) that must be overcome to effectively and compliantly implement and maintain synovial fluid crystal analysis at the point of care.

Infectious Arthritis

Infectious arthritis is most often a result of seeding from a disseminated infection, although it can also result from direct extension from adjacent bone infection (osteomyelitis) or a soft tissue abscess. The etiologies may be bacterial, mycobacterial, viral or fungal depending on the clinical setting. The presentation of acute bacterial infectious arthritis is most often monoarticular, acute, painful and swollen, and may be accompanied by signs of systemic infection such as fever and leukocytosis. The most common bacterial pathogens vary with age, with *Haemophilus influenza* most commonly affecting children under two years of age, *Staphylococcus aureus* more common in older children, and the sexually transmitted *Neisseria gonorrhoeae* prominent in adolescents and adults. Host factors such as immune deficiency, debilitating illness, joint trauma, chronic arthritis, and intravenous drug abuse may increase susceptibility to infectious arthritis in the setting of systemic infection[6]. Certain conditions predispose to specific pathogens, such as sickle cell anemia and Salmonella[6], and specific recent travel history and associated geographically endemic dimorphic fungal infections (eg. Southwest US and Coccidioides immitis)[3]. Although appendigeal joints are most commonly affected, axial joints are more susceptible in intravenous drug users.

Mycobacterial, viral, and Lyme disease are also clinically relevant etiologies. Mycobacterial arthritis results from hematogenous seeding or less commonly by direct extension, involving weight bearing joints in patients with tuberculosis. The course is chronic and progressive with worsening pain and often lacking systemic signs and symptoms[6]. It induces a confluent granulomatous inflammatory response that over time becomes erosive and destructive, risking obliteration of the joint space if not effectively treated. Viral arthritides can be caused by a variety of viruses, including alphavirus, parvovirus B19, rubella, Epstein-Barr virus, and hepatitis B and C viruses[6]. The disease course is variable, most often subacute to chronic, and may be a manifestation either of direct pathogen infection or a reactive autoimmune response.

Lyme disease is caused by the spirochete *Borrelia burgdorferi*, transmitted by the bite of an infected Ixodes deer tick. Initial skin manifestations (target lesion) evolve to systemic disease if untreated, with a majority of untreated individuals developing a migrating arthritis that affects one to two typically large joints (such as knees, shoulders, elbows, ankles) at a time[6]. Although mostly responsive to antibiotics, about 10% of individuals develop a chronic arthritis that is refractory to antibiotic therapy and lacks demonstrable signs of the pathogen, possibly auto-immune in etiology.

Rapid diagnosis and treatment of infectious arthritis is important given the potential for worsening infection and destruction of the involved joints. For bacterial arthritis, aspiration of the fluid with identification of the pathogen makes the diagnosis. Aspirated fluid of infected joints is suppurative: opaque and white, with high white blood cell

count and a high percentage of neutrophils (Table 2). Gram stain and culture are typically the first line diagnostic tools. Although Gram stain results are available rapidly, the sensitivity of Gram stain varies by pathogen, from up to 75% for staphylococcal infections to 25% for gonococcal infections[3]. Culture has higher sensitivities, typically 75-90% for untreated infections, although gonococcal infections may be significantly lower. There should be a high index of suspicion for concomitant infectious and crystal induced arthritis given the frequent concurrence. One retrospective study of over 600 cases with septic arthritis found concurrent crystal-induced arthritis in 27%. The false negative rate of initial Gram stain was higher in cases with concurrence, emphasizing the need for high suspicion and follow-up with culture[10].

Targeted testing by PCR can be very useful in rapid detection, and may also be necessary in detection of organisms that are difficult or slow to culture such as mycobacteria, borrelia, and viral. Synovial biopsy including Ziehl-Neelson or Kinyoun special stains may be necessary in patients clinically suspected of *Mycobacterium tuberculosis* infection to evaluate for the background of granulomatous inflammation and to demonstrate the presence of the characteristic acid fast bacilli that indicate mycobacterial organisms in affected tissue.

Periprosthetic joint infection is a relatively common and severe complication of total joint replacement that merits consideration as a unique condition within the context of synovial fluid analysis. The clinical need for early and accurate means to detect periprosthetic joint infection has driven the investigation of alternative diagnostic approaches and algorithms. Consensus diagnostic guidelines have been developed that allow for definitive diagnosis of periprosthetic joint infection, and they have become the gold standard for diagnosis. For example, the International Consensus Meeting on Periprosthetic Joint Infection recommended the following criteria in 2013: either one of two major criteria: 1) Two positive periprosthetic cultures with phenotypically identical organisms, or 2) A sinus tract communicating with the joint; OR THREE of the following minor criteria: elevated serum CRP and ESR, either an elevated synovial fluid WBC count OR ++ reaction on leukocyte esterase test strip, elevated synovial fluid PMN (neutrophil) percentage, a single positive culture, positive histological analysis of periprosthetic tissue[11]. A recent study of patients requiring surgery for either periprosthetic joint infection or aseptic joint loosening confirmed the valuable contributions of synovial fluid WBC count and percent neutrophils, and demonstrated improvement in positive predictive value by adding synovial fluid C-reactive protein and adenosine deaminase[11]. Strip-based leukocyte esterase testing has also been promoted as useful in diagnosing periprosthetic joint infection, partially because it can be performed at the point of care immediately after aspiration. For example, one study evaluating POC leukocyte esterase testing reported 97% positive predictive value and 85% negative predictive value of chemical strip leukocyte esterase ++ values in diagnosing periprosthetic joint infection[12]. Two different test strip systems performed equivalently against gold standard diagnostic criteria. As noted in the above consensus diagnostic criteria, the presence of leukocyte esterase (++) can be used in lieu of elevated WBC count. Use of leukocyte esterase by POC for this indication must be evaluated carefully however for a variety of reasons. The chemical strips available for use are intended for testing urine rather than synovial fluid, and the effects of this different matrix haven't been thoroughly evaluated. Although chemical strips can have CLIA waived status for urinalysis, using them in a manner different from their intended use makes the testing CLIA high complexity and a lab developed test. This designation results in significant requirements for demonstrating clinical and analytical validity prior to implementation, and the regulatory requirements for high complexity testing (including personnel requirements) apply. Risks associated with test user variability inherent in the point of care setting must also be addressed.

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