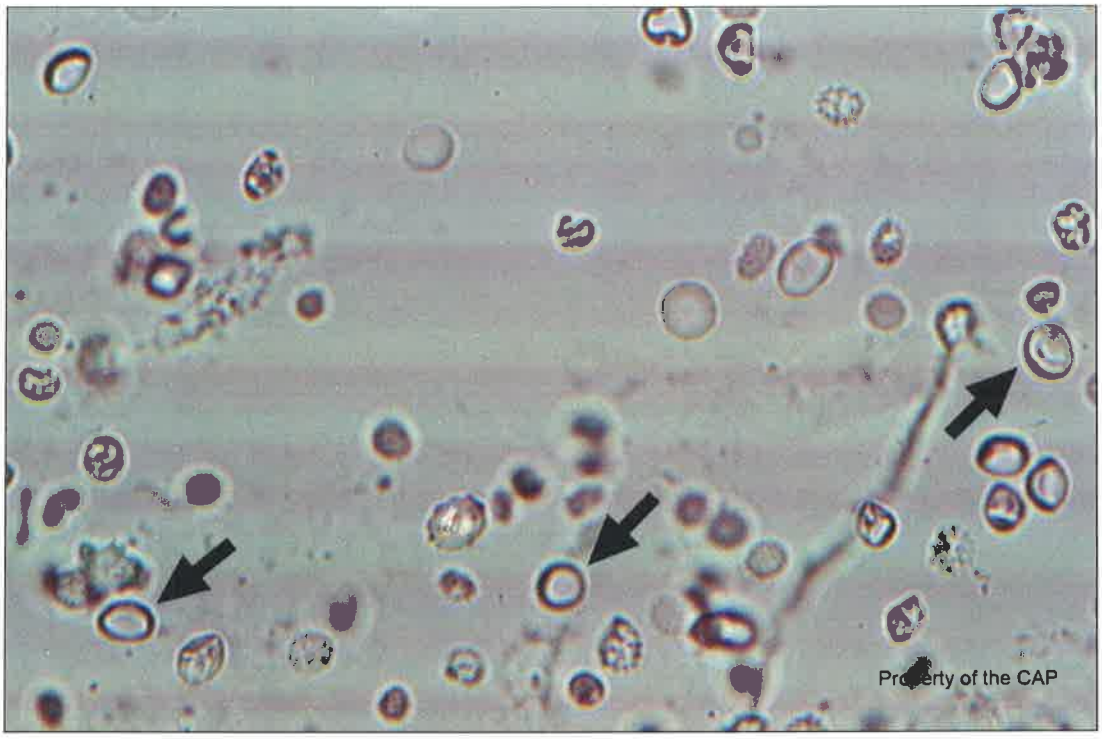
Urine Sediment Photographs

Case History CMP-13 through CMP-15

This urine sample is obtained from a 34-year-old woman. Laboratory data include: specific gravity = 1.025; pH = 7.0; ketones, glucose, protein, blood, nitrite, and leukocyte esterase = positive; bilirubin and urobilinogen = negative.

CMP-13



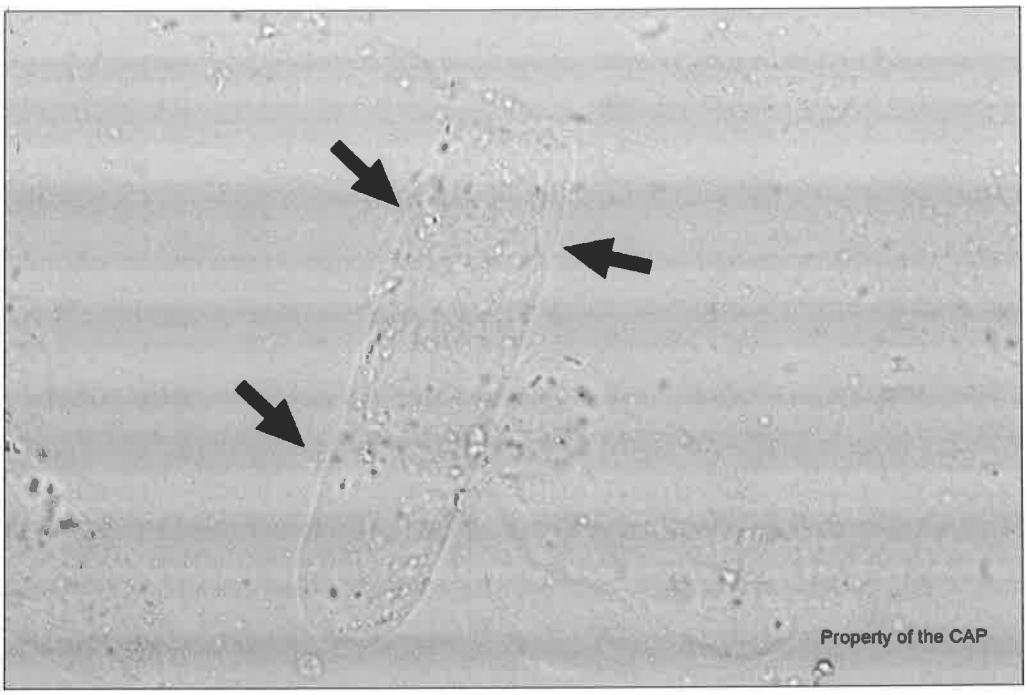
(URINE, UNSTAINED, 40X OR HIGHER POWER)

Identification	CMP Participants No.	%	Performance Evaluation
Erythrocyte	6024	97.7	Good

The arrowed object(s) are erythrocytes, as correctly identified by 97.7% of participants. The red blood cells, (RBCs) in this unstained wet preparation are identified by their size and biconcave disc shape. Other cells in this field are identified as crenated red cells that appear shrunken with or without surface projections that could be mistaken for granules. They are differentiated from granulocytes by their size, which in this case are approximately the same as the intact red cells. Crenated cells are formed by osmotic dehydration in hypertonic urine over time. The high specific gravity of 1.025 is consistent with hypertonic urine that encourages the formation of crenated red cells. Dysmorphic red cells that have fixed distorted shapes due to membrane loss while passing through glomeruli are not seen in this image. Identification of red cells by microscopic exam has clinical implications for bladder cancer screening. Asymptomatic Microhematuria (AMH) is defined as >/= 3 RBC/HPF in the absence of an obvious benign cause. Positive blood by dipstick does not define AMH. Since 4% of patients with AMH on initial work-up have a malignancy, guidelines recommend a urologic work-up, such as a cystoscopy for all patients aged 35 years and older.
https://www.auanet.org/guidelines/asymptomatic-microhematuria-(amh)-guideline

Urine Sediment Photographs

CMP-14



(URINE, UNSTAINED, 40X OR HIGHER POWER)

Identification	CMP Participants No.	%	Performance Evaluation
Hyaline cast (includes non-hemoglobin pigmented cast)	5997	97.2	Good

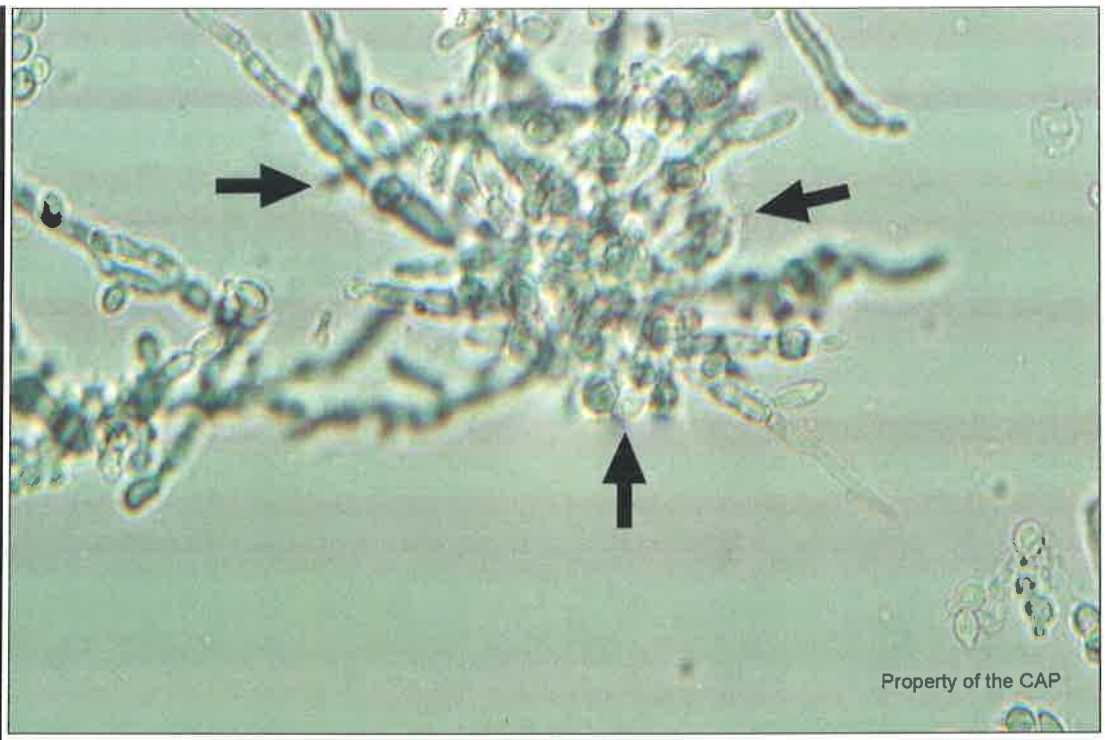
The arrowed object(s) is a hyaline cast, as correctly identified by 97.2% of participants. Hyaline casts form by solidification of the Tamm-Horsfall protein in acidic concentrated urine in the renal tubules. These conditions arise in a variety of normal and disease states such that hyaline casts are non-specific. If other elements are present in the renal tubule when casts form, such as red cells, white cells, renal tubule cells, then these cast components provide specific information and the casts are considered pathological. Hyaline casts by themselves are neither pathological nor associated with specific disease states. When there are no other elements within the cast, hyaline casts are translucent and colorless under bright-field microscopy having a refractive index very near to water. Under certain conditions, hyaline casts are essentially invisible by bright-field microscopy and will be under counted. Depending on the exact conditions in the renal tubules when hyaline casts form, bright field microscopy may be less sensitive to hyaline casts than flow cytometry. In other conditions, mucus may appear in the same light scatter cluster as hyaline casts (casts without inclusions), thus, falsely elevating the flow cytometry hyaline cast count. Empirically, flow cytometry detects more hyaline casts than bright-field microscopy in certain patients and the correlation (agreement) between the methods is poor.

A literature search reveals no clinical situation where the finding of hyaline casts in the urine provides informative diagnostic information associated with specific clinical recommendations that will impact the However, hyaline casts are found in high numbers > 10 casts/LPF more frequently in abnormal urines than in normal urines. Hyaline casts </= 10 casts/LPF are considered normal.

1. European Confederation of Laboratory Medicine (ECLM); European Urinalysis Guidelines. *Scand J Clin Lab Invest*. 2000;60:1-96.
2. Erdbruegger U, Okusa, M. *UpToDate*: Etiology and diagnosis of prerenal disease and acute tubular necrosis in acute kidney injury (acute renal failure); Apr 01, 2016.

Urine Sediment Photographs

CMP-15



(URINE, UNSTAINED, 40X OR HIGHER POWER)

Identification	CMP Participants No.	%	Performance Evaluation
Yeast/Fungi	6129	99.4	Good

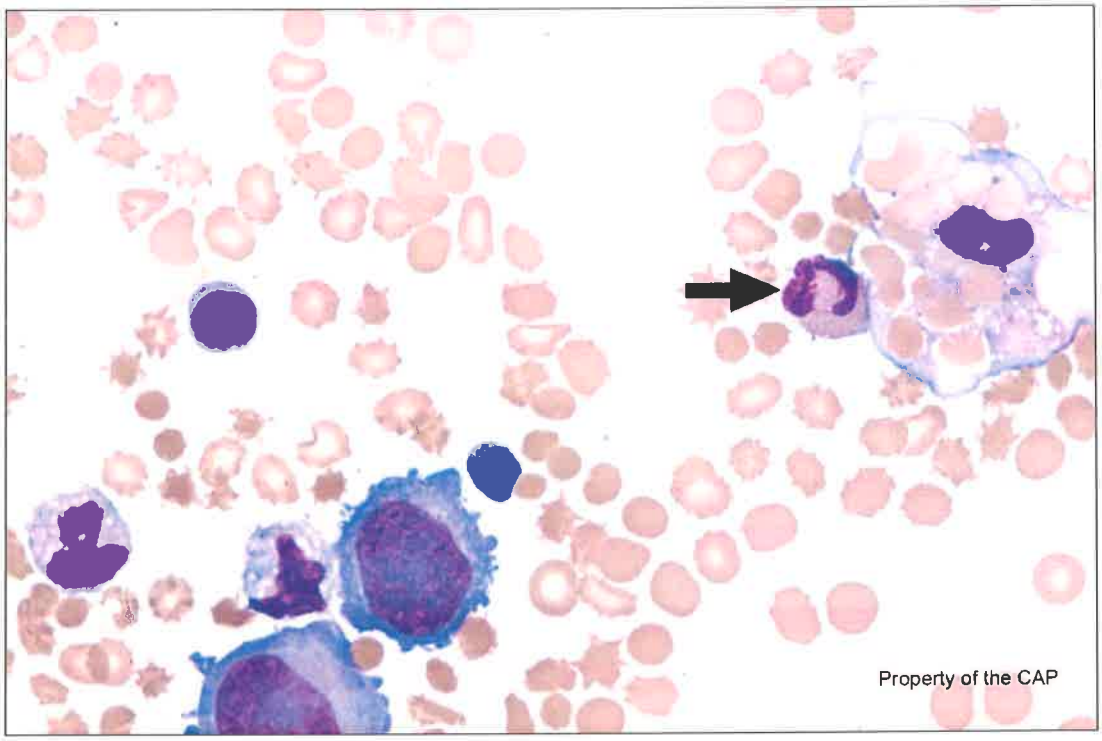
The arrowed object(s) are yeast/fungi, as correctly identified by 99.4% of participants. Yeast are identified as having a thick cell wall by its smooth retractile appearance. These are distinguished from red blood cells by their ovoid shape and the presence of budding forms. The very elongated forms with branching and budding are yeast pseudohyphae. The appearance of these yeast forms with numerous clumped pseudohyphae are typical of *Candida albicans* infections in immunosuppressed patients. Yeast that is contaminating the urine sample from vaginal sources tend to be limited to predominantly ovoid forms with rare or no pseudohyphae.

Body Fluid Photographs

Case History CMP-16 through CMP-18

This patient is a 93-year-old woman with a history of bladder cancer admitted to the hospital with worsening fatigue, weight loss, and bone tenderness. Pleural fluid data shows: Total nucleated cells (TNC) = 900/µL (0.900 x 10E3/µL) and RBC count = 100,000/µL (100.000 x 10E3/µL).

CMP-16



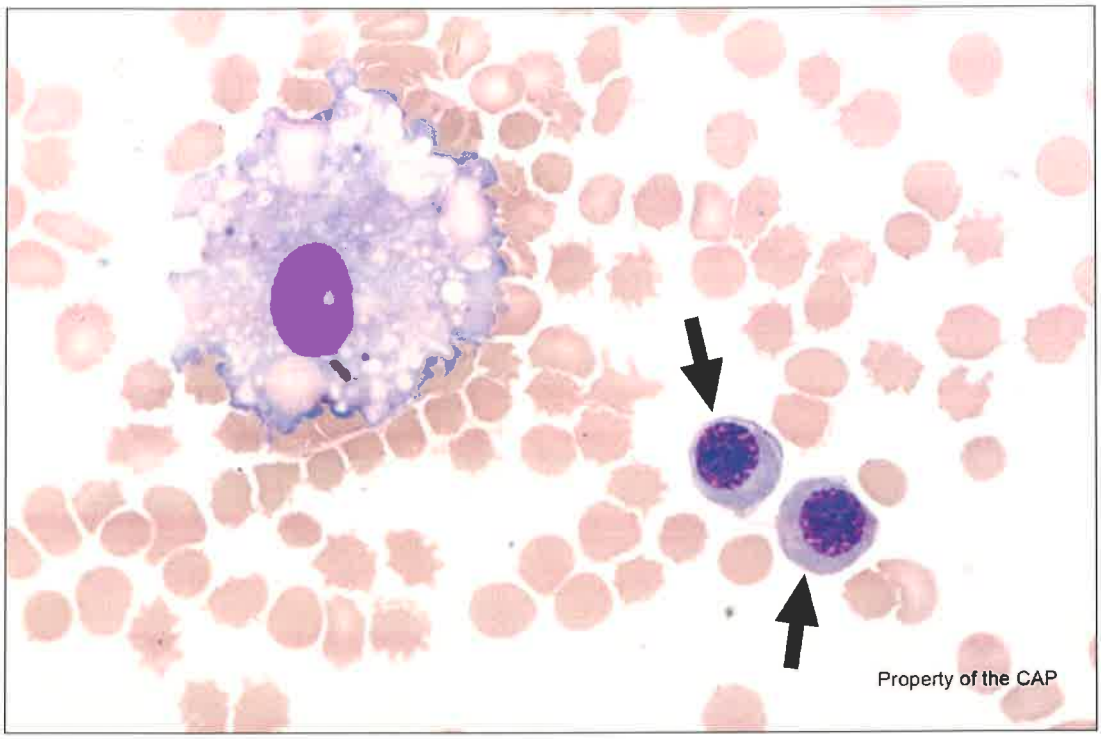
(PLEURAL, CYTOCENTRIFUGE, WRIGHT-GIEMSA, 100X)

Identification	CMP Participants No.	%	Performance Evaluation
Neutrophil, segmented or band	3737	98.2	Good

The arrowed cell is a neutrophil, segmented or band, as correctly identified by 98.2% of participants. The band is round-to-oval and 10 to 18 µm in diameter. The nuclear to cytoplasmic ratio is 1:1.5 to 1:2, and the nuclear chromatin is condensed. The nucleus is indented to more than half the distance to the farthest nuclear margin, but the chromatin is not condensed to a single filament. Segmented neutrophils have similar size to a band neutrophil (ie, 10 to 15 µm in diameter), as well as comparable shape (round to oval), and cytoplasmic appearance (pale pink cytoplasm with specific granules). The nuclear to cytoplasmic ratio is 1:3, and the nuclear chromatin is highly condensed. The nucleus is segmented or lobated (with a normal range of three to five lobes). The lobes are connected by a thin filament that contains no internal chromatin, giving it the appearance of a solid, dark, thread-like line. The presence of these thread-like filaments is the basis for distinguishing the segmented neutrophil from the band neutrophil. In body fluids, the segmented or band neutrophil is usually easily recognized. Often, the nuclear lobes appear eccentric in cytocentrifuge preparations.

Body Fluid Photographs

CMP-17



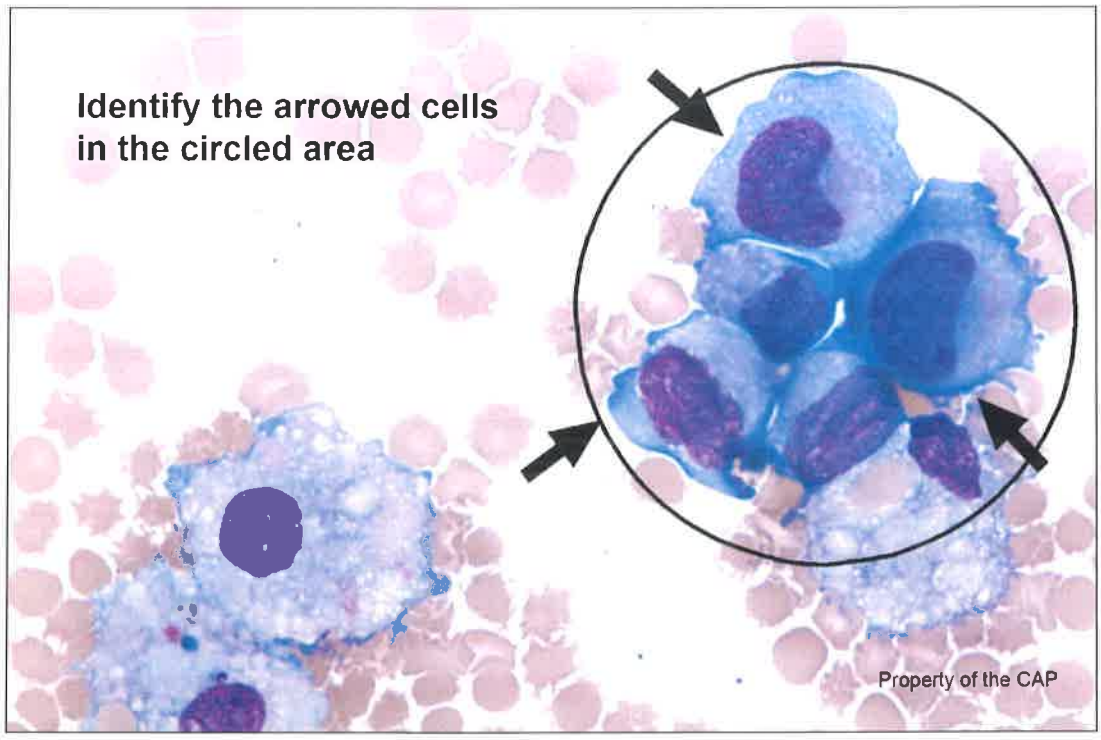
(PLEURAL, CYTOCENTRIFUGE, WRIGHT-GIEMSA, 100X)

Identification	CMP Participants No.	%	Performance Evaluation
Erythrocytes, nucleated	3536	92.9	Good

The arrowed cells are erythrocytes, nucleated, as correctly identified by 92.9% of participants. These cells are found uncommonly in body fluids and are usually derived from peripheral blood contamination in which circulating nucleated red blood cells are present. Occasionally, they may arise from accidental aspiration of the bone marrow in an infant, or adult with osteoporosis. When the nucleated red blood cells are a result of accidental marrow contamination, they are at earlier stages (ie, polychromatophilic and basophilic normoblasts) and may also be associated with immature myeloid cells, whereas nucleated red blood cells due to peripheral blood contamination tend to be at a later stage of development (ie, orthochromic normoblasts).

Body Fluid Photographs

CMP-18



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

Identification	CMP Referees No.	CMP Referees %	CMP Participants No.	CMP Participants %	Performance Evaluation
Malignant cells (non-hematopoietic)	26	47.3	1807	47.5	Non-consensus
Mesothelial cell	12	21.8	820	21.6	
Monocyte/macrophage	8	14.6	540	14.2	
Immature/abnormal cell, would refer for identification	5	9.1	257	6.8	
Lymphocytes, reactive	2	3.6	159	4.2	
Blast cell	-	-	99	2.6	

The arrowed cells are malignant cells (non-hematopoietic) as correctly identified by 47.5% of participants. A variety of neoplastic cells may be found in body fluids. Their morphology is dependent on that of the primary underlying malignancy. Malignant cells may be numerous and clustered or appear as rare single cells. Cytologic features of malignant cells on cytocentrifuge preparations include high nuclear to cytoplasmic ratio, increased cell and nuclear size, irregularly shaped nuclei, atypical nuclear chromatin patterns, large nucleoli, and a tendency to form large clusters, frequently with nuclear molding. The circled cluster of cells shows malignant features such as cellular enlargement, irregular nuclear contours, prominent nucleoli, pleomorphism, and focal nuclear hyperchromasia.

6.8% of the participants identified the cells as immature/abnormal cells, would refer. This is an appropriate choice as a pathologist, etc. would subsequently review the cells for identification.

21.6% of participants incorrectly identified the circled cells as mesothelial cells. The mesothelial cell (20 to 50 µm) normally lines pleural, pericardial, and peritoneal surfaces. These cells can be shed individually or in

CMP-18 (cont.)

clusters. When found in pairs or clusters, mesothelial cells have articulated or coupled cell borders with a discontinuous outer border (clear spaces or "windows") between many of the cells. The nucleus is round to oval in shape with a definitive nuclear membrane and regular contour. Chromatin varies from dense to fine, but it is evenly distributed. Multiple nuclei may occur and the nuclei may overlap; however, the nuclei remain of approximately equal size and shape. One or more nucleoli may be present. The nuclear-to-cytoplasmic ratio is low (less than 1:1), and the nucleus may be central or eccentrically placed. The cytoplasm is light to dark blue and may have a grainy texture, typically dense grainy basophilia or even a crystalline/ground glass appearance to the perinuclear area. With some staining techniques, the periphery and perinuclear cytoplasmic regions may appear as very lightly stained areas. With degeneration, additional small vacuoles may occur throughout the cell. Cytoplasmic budding or fragmentation may also occur. In chronic effusions or during inflammatory processes, mesothelial cells proliferate and become very large. Mitotic figures occasionally are seen within mesothelial cells. The chromatin is less condensed and nucleoli may be prominent; however, the nucleus still retains a definitive, smooth, nuclear membrane. The irregular nuclear contours of the circled cells reveal their malignant nature. Moreover, the clinical history of urothelial carcinoma prompts one to be suspicious of the cells.

14.2% of participants incorrectly identified the circled cells as monocytes/macrophages. Monocytes are bone marrow-derived cells that circulate in the blood. Macrophages arise from bone marrow-derived cells that migrate into tissues and evolve morphologically. Monocyte/macrophage morphology in fluids is quite variable, ranging from the typical monocyte of the peripheral blood to a vacuolated, activated stage with the morphology of a typical macrophage. Monocytes are usually large (12 to 20 µm) with abundant blue-gray cytoplasm and often containing sparse azurophilic granules. The nucleus is round to oval and may show indentation, giving it a kidney bean or horseshoe shape. The chromatin is lacy and small nucleoli may be apparent. Macrophages are larger cells (15 to 80 µm) with abundant cytoplasm showing evidence of active phagocytosis. This includes ingested material such as other blood cells or bacteria, hemosiderin, fungi, and remnants of digested materials as well as cytoplasmic vacuoles post ingestion. One or more round to oval nuclei are present and occasionally prominent nucleoli may be seen. Macrophages can at times be difficult to differentiate from mesothelial cells. Mesothelial cells are usually larger than monocytes/macrophages and usually show a biphasic staining cytoplasm and surface microvilli. Again, the irregular nuclear contours of the circled cells reveal their malignant nature. Moreover, some of them have increased nuclear to cytoplasmic ratio, which would not be typical of benign monocytes/macrophages. Compare these circled cells to the nearby large cells, which are benign macrophages.

4.2% of participants incorrectly identified the cells as lymphocytes, reactive. A variety of reactive lymphocytes have been described. These round to ovoid to irregular cells range from 10 to 25 µm in size with a nuclear to cytoplasmic ratio that varies from 3:1 to 1:2. Immunoblasts and immunoblastic-like reactive lymphocytes are large cells (15 to 20 µm) with round to oval nuclei. They have finely to moderately dispersed chromatin with abundant parachromatin and one or more prominent nucleoli. These may resemble lymphoma cells or blasts. Their cytoplasm is moderately abundant and stains deeply basophilic. The nuclear to cytoplasmic ratio is high (3:1 to 2:1). These reactive lymphocytes correspond to Downey type III cells. Although reactive lymphocytes may become quite large, comparing the circled cells to nearby red blood cells reveals their exceptional large size. This cell size exceeds what would be typical for lymphocytes, even reactive lymphocytes. 0.9% of participants identified the cells as lymphoma cells. Again, the size of the cells excludes the possibility of lymphoid cells, even lymphoma cells.

2.6% of participants identified the circled cells as blast cells. A blast is a large, round to oval cell, 10 to 20

CMP-18 (cont.)

µm in diameter, with a high nuclear to cytoplasmic ratio. The blast often has a round to oval nucleus, but it is sometimes indented or folded. The nuclear chromatin is typically fine, lacey, or granular, and one or more nucleoli may be present. Nucleoli are more prominent in cytocentrifuge slides. The cytoplasm is basophilic and often agranular; however, when cytoplasmic granules occur, they are more easily visualized in the cytocentrifuge slide than in peripheral blood or bone marrow smears. Again, although blast cells may be large, comparing the circled cells to nearby red blood cells reveals their exceptional large size. This cell size exceeds what would be typical for blasts. Finally, 1% of participants identified the circled cells as neutrophil, immature. Again, the size of the cells excludes the possibility of granulocytic lineage cells. Moreover, the cells lack typical granules seen in neutrophil precursors.

Case Presentation:

This patient is a 93-year-old woman with a history of bladder cancer admitted to the hospital with worsening fatigue, weight loss, and bone tenderness. Pleural fluid data shows: Total nucleated cells = 900/µL (0.900 x 10E3/µL); RBC count = 100,000/µL (100.000 x 10E3/µL).

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

Case Discussion: Malignant Pleural Effusion – Urothelial Carcinoma

This case is an example of malignant pleural effusion due to metastatic urothelial carcinoma (bladder cancer). The pleural cavity is typically lined by a single flat layer of mesothelial cells, and normally contains only a minimal amount of clear yellow fluid between the lungs and the chest wall to minimize friction between the surfaces during respiration. This fluid is an ultrafiltrate of plasma. In disease states, more fluid may accumulate creating a pleural effusion. When a pleural effusion is present, fluid can be obtained by thoracentesis (inserting a needle into the pleural space). This fluid is often submitted for laboratory evaluation including chemistry, hematology, and cytology as well as other ancillary testing, as needed.

Based on evaluation, effusions are frequently classified as either a transudate or exudate by Light's criteria. Transudates are typically clear and yellow in appearance and show a fluid to serum protein ratio of ≤ 0.5, fluid to serum lactate dehydrogenase (LDH) ratio of ≤ 0.6, and fluid LDH < 0.67 x the upper limit of normal for serum LDH. Transudates are generally caused by a systemic process, such as congestive heart failure, liver cirrhosis, hypoproteinemia, or nephrotic syndrome and require treatment of the underlying systemic condition. In contrast to transudates, exudates most often appear cloudy, turbid, purulent, or bloody grossly. Exudative fluids are usually due to localized conditions, such as malignancy, trauma, pulmonary infarction, or infection.

Primary tumors of the serosal surface, such as mesothelioma and primary effusion lymphoma, are rare, and metastatic tumors are far more common, with metastatic adenocarcinoma being the most common. 80% of malignant pleural effusions are due to lung carcinoma, breast carcinoma, lymphoma, ovarian carcinoma, and gastric carcinoma with lung carcinoma being the most frequent.

Microscopic evaluation of pleural fluid is critical, especially in exudative effusions that may be due to malignancy. Effusion specimens are especially hardy and can be refrigerated without compromising morphology for 2 weeks or longer. Estimates of sensitivity for detecting serosal malignancy via cytology vary from 58% to 71%, but cytology is known to be more sensitive than blind biopsy. Moreover, the specificity of a malignant diagnosis is quite high with < 1% false-positive rate.

Pleural fluids frequently contain mesothelial cells that must be distinguished from malignant cells. In comparison to mesothelial cells, non-hematopoietic malignant cells often show increased cell size, more pleomorphism, higher nuclear to cytoplasmic ratio, irregularly shaped nuclear contours, coarse/irregular nuclear chromatin patterns, nuclear hyperchromasia, and large, sometimes multiple, nucleoli. Sometimes morphology alone cannot accurately distinguish reactive mesothelial cells from malignancy and immunohistochemical stains may be employed on a cell block, if prepared. The history of metastatic bladder cancer in our patient's clinical history as well as clinical features suggestive of malignancy such as weight loss help support the malignant nature of these atypical, large cells in this fluid.

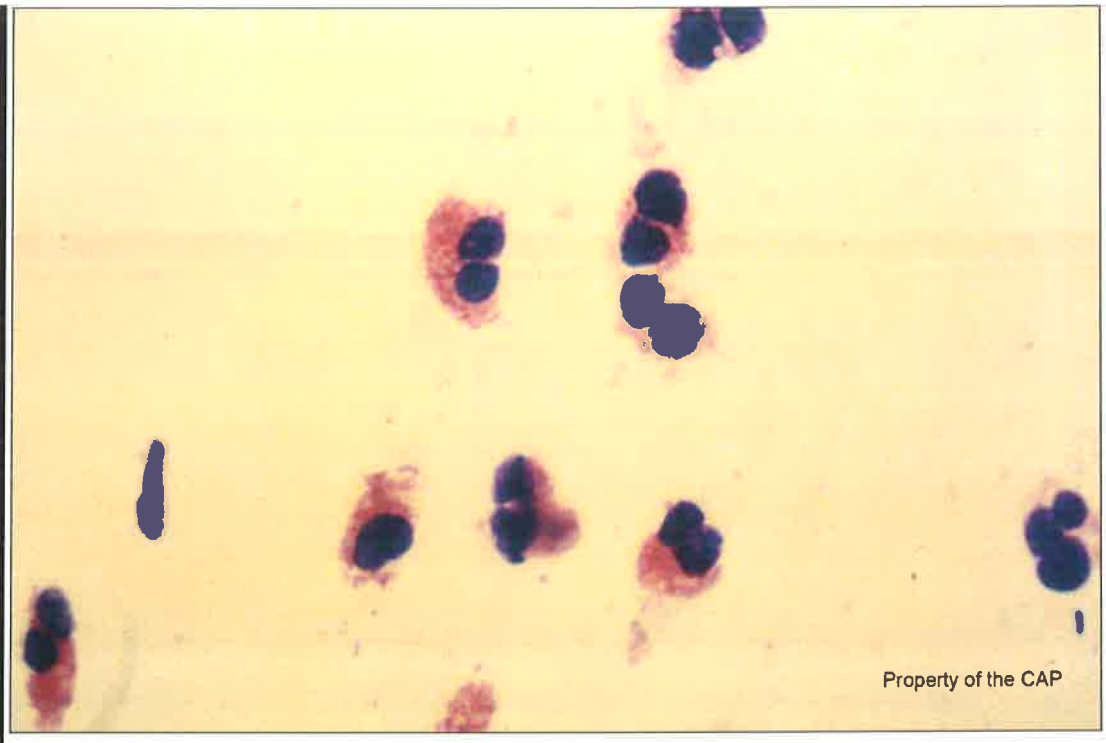
Natasha M. Savage, MD
Hematology and Clinical Microscopy Committee

References:

1. Galagan KA, Blomberg D, Cornbleet PJ, Glassy EF. *Color Atlas of Body Fluids: An Illustrated Field Guide Based on Proficiency Testing.* College of American Pathologists; 2006.
2. Cibas ES, Ducatman BS. *Cytology: Diagnostic Principles and Clinical Correlates.* Saunders Elsevier; 2009.
3. Sundling KE, Cibas ES. Ancillary studies in pleural, pericardial, and peritoneal effusion cytology. *Cancer Cytopathol.* 2018;126 Suppl 8:590-598.

CMMP – Clinical Microscopy Miscellaneous Photographs

CMMP-32



(NASAL, WRIGHT-GIEMSA) High power magnification

Identification	CMMP Participants No.	%	Performance Evaluation
Eosinophils are present	1994	98.9	Good

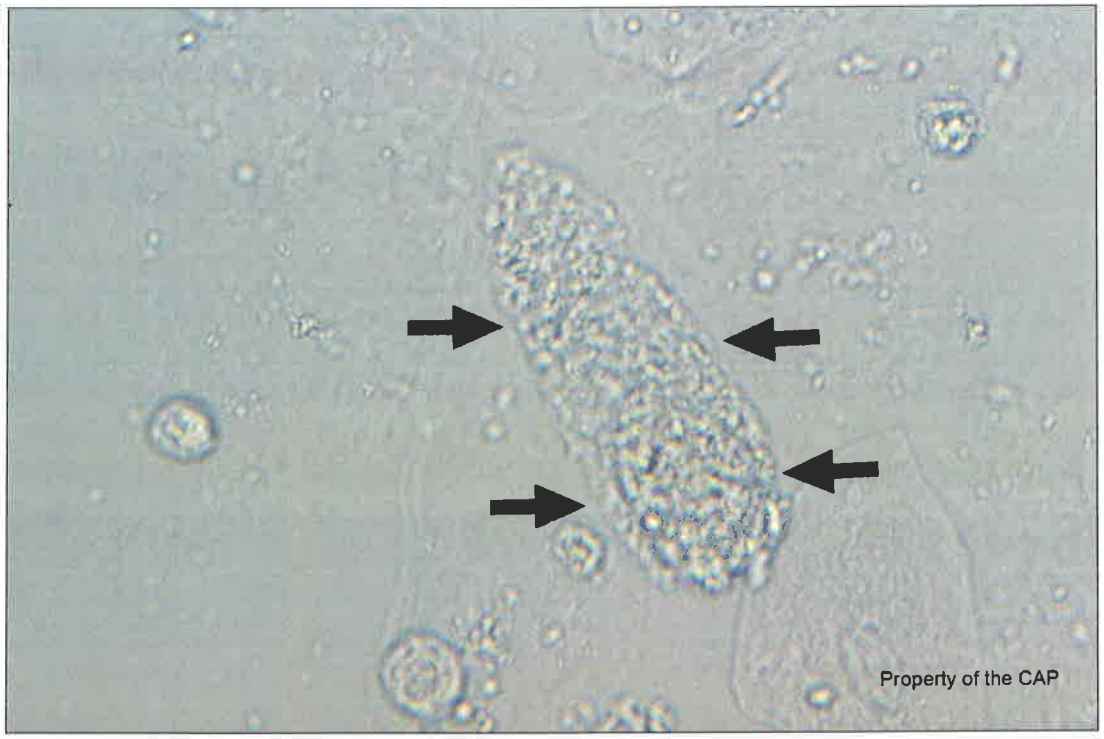
This photomicrograph demonstrates a Wright-Giemsa stained nasal smear. Several eosinophils with bright orange-red spherical granules are seen. These cells typically have two nuclear lobes separated by a thin filament, although there can be three lobes. The finding of nasal eosinophils is supportive of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

CMMP – Urine Sediment Color Photographs

Case History USP-04 through USP-06

This urine sample is from a 37-year-old man with advanced kidney disease, admitted through the emergency room for lower abdominal discomfort and painful urination. Laboratory data include: specific gravity = 1.017, pH = 5.0; ketones, glucose, protein, blood, nitrite, and leukocyte esterase = positive.

USP-04



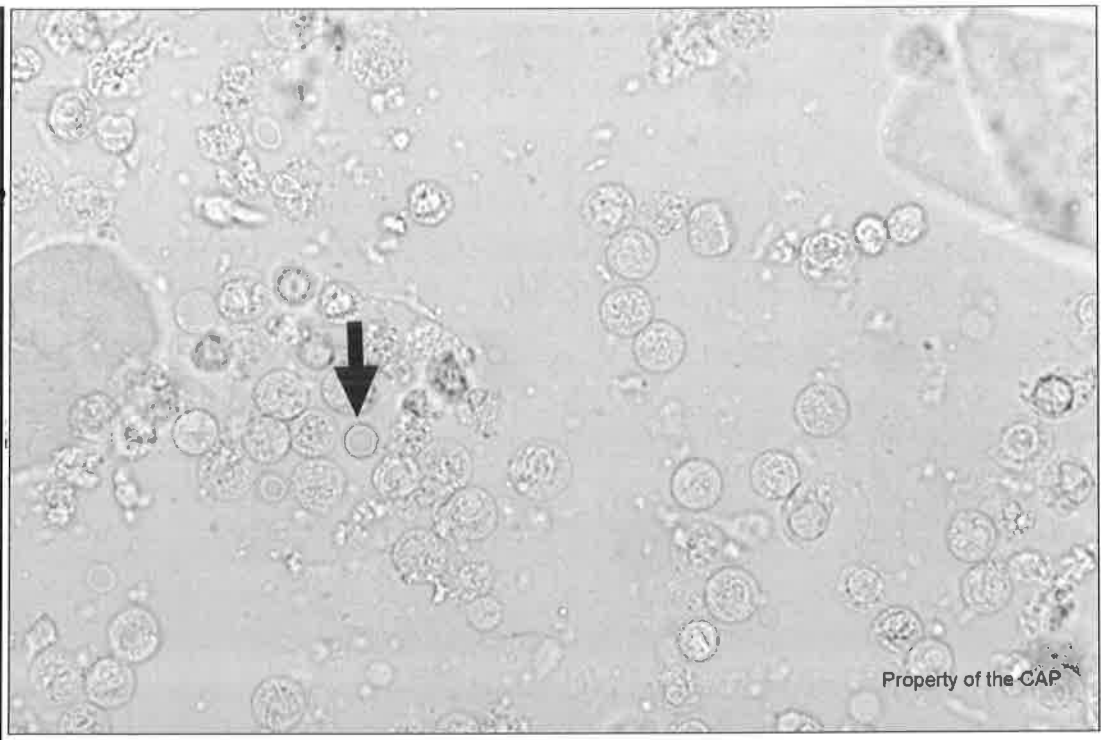
(URINE, UNSTAINED, 40X OR HIGHER POWER)

Identification	CMMP Participants No.	%	Performance Evaluation
Granular cast	3829	94.1	Good

The arrowed object(s) is a granular cast, as correctly identified by 94.1% of participants. The granular contents of this cast may be degenerated cells or protein complexes that were present while the cast formed by precipitation of the Tamm-Horsfall protein in the renal tubule. Granular casts may or may not be normal and their clinical significance requires interpretation of the other findings in the microscopic urinalysis.

CMMP – Urine Sediment Color Photographs

USP-05



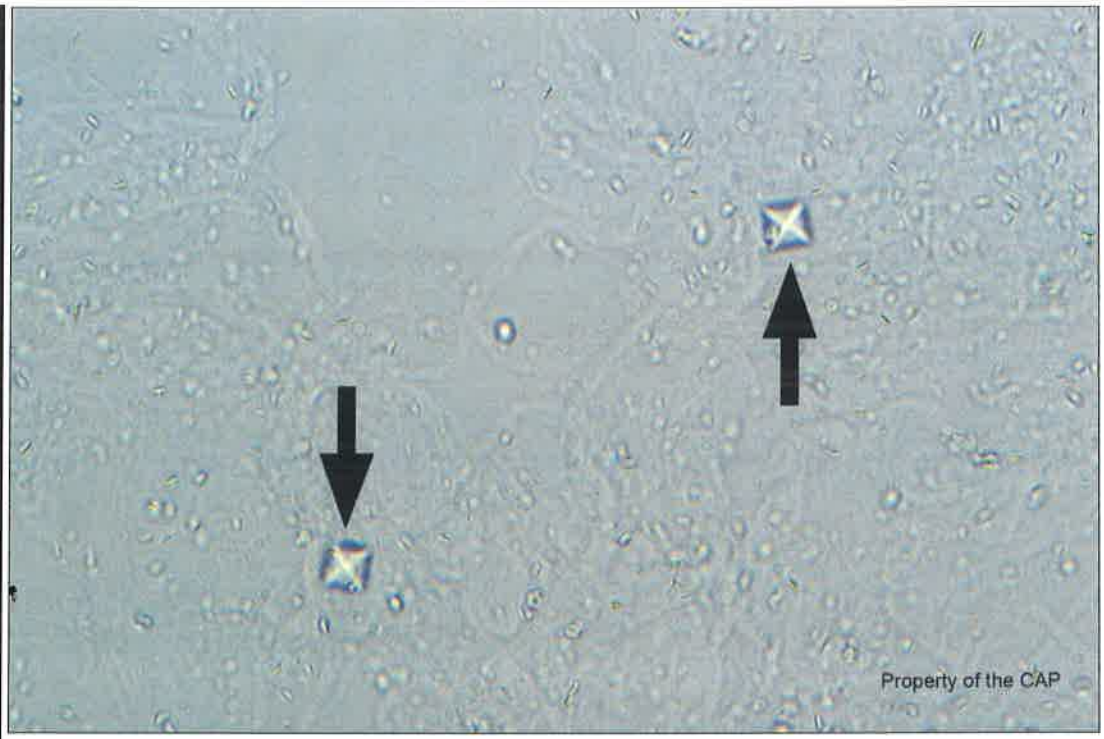
(URINE, UNSTAINED, 40X OR HIGHER POWER)

Identification	CMMP Participants No.	%	Performance Evaluation
Erythrocyte	3971	97.6	Good

The arrowed object(s) is an erythrocyte, as correctly identified by 97.6% of participants. The erythrocyte is identified by its smaller size (7 to 8 µm in diameter) relative to the surrounding white blood cells, orange color, and round shape with refractile outer circumference. The 3-dimensional biconcave disc shape of red cells can be assessed by refocusing up and down with the microscope's fine adjustment focus. The 3-dimensional shape is not apparent in this static photograph. The presence of numerous white blood cells in this field suggests possible urinary tract infection (UTI). Red blood cells are a common feature of urinary tract infections including uncomplicated cystitis. The absence of bacteria and concomitant finding of renal casts suggests possible kidney disease rather than UTI.

CMMP – Urine Sediment Color Photographs

USP-06



(URINE, UNSTAINED, 40X OR HIGHER POWER)

Identification	CMMP Participants No.	%	Performance Evaluation
Calcium oxalate crystals	4057	99.7	Good

The arrowed object(s) are calcium oxalate crystals, as correctly identified by 99.7% of participants. These calcium oxalate crystals have an octahedral shape variably described as envelopes or squares with a cross. This is the most common shape of the dihydrate form. Calcium oxalate crystals can take several other shapes such as ovoid, dumbbell, or elongated rectangle with pyramidal ends. Commonly used image-based automated urine microscopy analyzers will typically misclassify calcium oxalate crystals as red blood cells. Therefore, these automated analyzers cannot be used to autoverify urine red blood cells without manual review of the images prior to validation. Upon finding calcium oxalate crystals on image review, the technologist must consider correcting the red blood cell classification of the automated instrument.

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