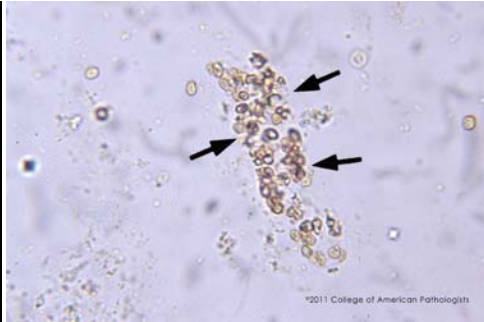


## Urine Sediment Photomicrographs/Photographs

### Case History CMP-17

This urine is from a 12-year-old female who was being evaluated for kidney disease. Laboratory data include: Specific gravity=1.010; pH=6.0; blood, leukocyte esterase and protein=positive; glucose, ketones, and nitrite=negative.



Identification	Referees		CMP Participants		Performance Evaluation
	No.	%	No.	%	
RBC cast	20	100.0	4378	98.3	Good

CMP-17

The arrowed object is a red blood cell (RBC) cast and was correctly identified by 98.3% of participants.

RBC casts are the least common cast seen, and they are among the most clinically significant. They are seen in acute glomerulonephritis, all glomerulopathies and malignant hypertension.

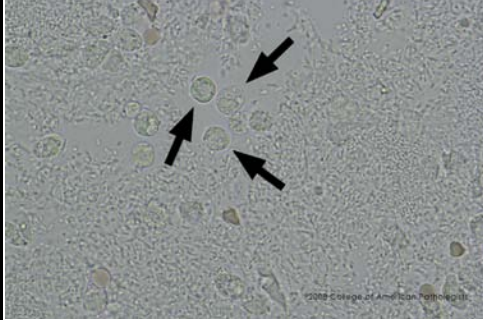
Recognizable red cells cover all or part of the hyaline matrix of the cast. They have a tinge of red or brown color. Because red cells are fragile and degenerate soon after voiding, RBC casts are best seen in freshly voided specimens.

RBC casts can be differentiated from white blood cell casts by the presence of intact red cells. Granular casts lack identifiable intact cells and pigment, and have granules within the cast matrix.

## Urine Sediment Photomicrographs/Photographs

### Case History CMP-18

This urine is from a 12-year-old female who was being evaluated for kidney disease. Laboratory data include: Specific gravity=1.010; pH=6.0; blood, leukocyte esterase and protein=positive; glucose, ketones, and nitrite=negative.



Identification	Referees		CMP Participants		Performance Evaluation
	No.	%	No.	%	
Leukocytes	20	100.0	4372	98.3	Good

CMP-18

The arrowed objects are white blood cells (WBCs) and were correctly identified by 98.3 % of participants.

The majority of leukocytes seen in urine are neutrophils. They measure 10-12 microns, are round, oval or amoeboid. They have nuclei that may be indented or segmented, with coarsely granular or clumped chromatin.

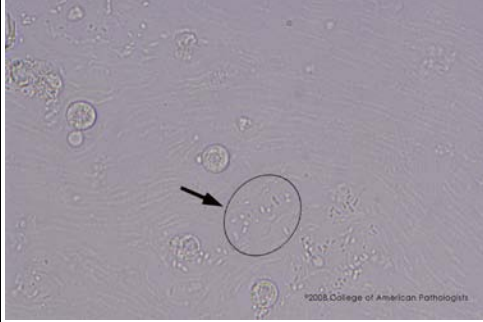
Normal urine contains up to 5 neutrophils per high power field. Increased numbers of neutrophils are associated with urinary tract infections, nephritis, tumors and kidney or bladder stones.

Neutrophils should not be confused with red cells. Even when swollen, which occurs in hypotonic urine, they retain a nucleus and cytoplasmic granules, differentiating them from red cells. They might also be confused with renal tubular epithelial cells, but neutrophils are smaller and have indented or segmented nuclei compared to the typical distinct round or oval, often eccentric, nuclei in renal tubular epithelial cells.

## Urine Sediment Photomicrographs/Photographs

### Case History CMP-19

This urine is from a 75-year-old female nursing home resident 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



CMP-19

Identification	Referees		CMP Participants		Performance Evaluation
	No.	%	No.	%	
Bacteria	20	100.0	4356	97.9	Good

The arrowed objects are bacteria and were correctly identified by 97.9% of participants. Bacteria may be in chains, clusters, pairs or within leukocytes. Urine is normally sterile. Bacteria are seen in urine contaminated during collection or from a patient with a urinary tract infection.

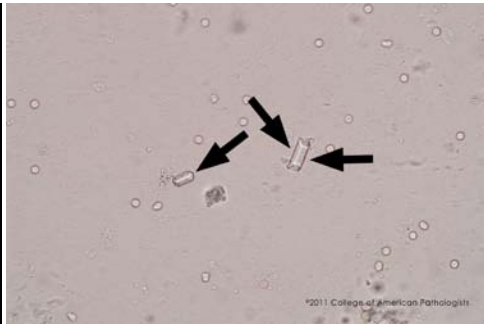
Smaller numbers of mixed morphology bacteria suggests contamination, whereas larger numbers of bacteria with a single morphology suggests infection. Additionally, the presence of white blood cells, a positive leukocyte esterase and/or positive nitrates also suggest infection.

Bacteria may be confused with amorphous urates or phagocytosed debris. A Gram stain will distinguish between these possibilities.

## Urine Sediment Photomicrographs/Photographs

### Case History CMP-20

This urine is from a 75-year-old female nursing home resident 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



Identification	Referees		CMP Participants		Performance Evaluation
	No.	%	No.	%	

Ammonium magnesium phosphate crystals	20	100.0	4435	99.6	Good
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CMP-20

The arrowed objects are ammonium magnesium phosphate crystals and were correctly identified by 99.6% of participants. Also commonly known as “triple phosphate” crystals, they are found at pH of 6.5 or higher. They are colorless prisms, moderately birefringent, soluble in dilute acetic acid, with a morphology classically described as that of a coffin lid. Rare rosette and fern forms have been described, usually admixed with the classic type.

While clinically insignificant, increased numbers of these crystals have been described in women with kidney stones of the staghorn type in particular and in patients with urinary stasis.

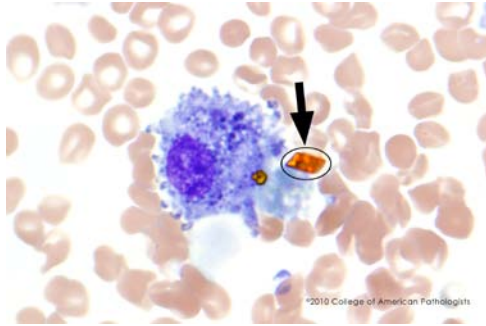
They may be confused with hippuric acid or variant calcium oxalate crystals seen in ethylene glycol poisoning. Hippuric acid crystals are elongated hexagons lacking the “lid” on the coffin. Variant calcium oxalate crystals are usually found with classic forms and lack the “coffin lid” appearance. The clinical history of ethylene glycol poisoning will also help distinguish these crystals.

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Hematology and Clinical Microscopy Resource Committee

## Body Fluid Photomicrographs/Photographs

### Case History CMP-21

The patient is a 66-year-old female with headache, dysphagia, right hemiparesis, and gait instability due to a brain tumor. A cerebrospinal fluid specimen was taken from an ventricular shunt several days after resection of the tumor. CSF sample laboratory findings include: Color = bloody. After centrifugation = xanthochromic; turbidity = 2+. Total nucleated cells =  $0.087 \times 10^3 / \mu\text{L}$ ; RBC =  $0.004 \times 10^6 / \mu\text{L}$ .



CMP-21

Identification	Referees No.    %	CMP Participants No.    %	Performance Evaluation
Hematoidin / hematin crystal	19    100.0	2889    94.1	Good

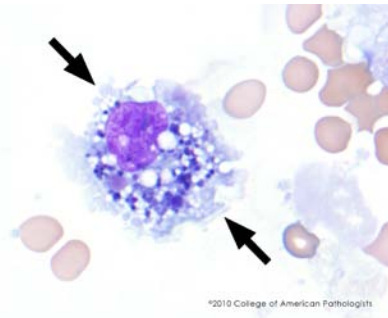
The arrowed object was properly identified as hematoidin / hematin crystal by 94.1% of the participants.

The crystals are the result of breakdown of hemoglobin. They may be recognized in tissues as well as body fluids usually about two weeks after a bleeding /hemorrhagic episode. The crystal may be extracellular as shown in this image or intracellular as shown in image CMP-24. Of interest is that a Prussian blue stain for iron will be negative in these crystals, as opposed to hemosiderin that should stain positive. Hematoidin is chemically very similar to bilirubin and usually presents as yellow–orange rhomboid crystals 2- 8  $\mu\text{m}$  in length. Hematin and hematoidin are used interchangeably to describe these crystals, but chemically, hematin is a porphyrin compound and the result of denatured hemoglobin.

## Body Fluid Photomicrographs/Photographs

### Case History CMP-22

The patient is a 66-year-old female with headache, dysphagia, right hemiparesis, and gait instability due to a brain tumor. A cerebrospinal fluid specimen was taken from an ventricular shunt several days after resection of the tumor. CSF sample laboratory findings include: Color = bloody. After centrifugation = xanthochromic; turbidity = 2+. Total nucleated cells =  $0.087 \times 10^3 / \mu\text{L}$ ; RBC =  $0.004 \times 10^6 / \mu\text{L}$ .



Identification	Referees		CMP Participants		Performance Evaluation
	No.	%	No.	%	
Macrophage containing hemosiderin	14	73.7	2297	74.7	Educational
Macrophage containing abundant small lipid vacuoles/droplets	2	10.5	320	10.4	Educational
Neutrophil/macrophage with phagocytized bacteria	1	5.3	234	7.6	Educational
Monocyte/macrophage	2	10.5	131	4.3	Educational

The arrowed object was properly identified as macrophage containing hemosiderin (siderophage) by 74.7% of the participants.

The cell is a macrophage with dark blue granules which are an iron byproduct coupled with protein as a consequence of digested red cells. The Prussian blue stain can be used for confirmation of the presence of iron /hemosiderin. This pigment should be differentiated from melanin and anthracotic pigment.

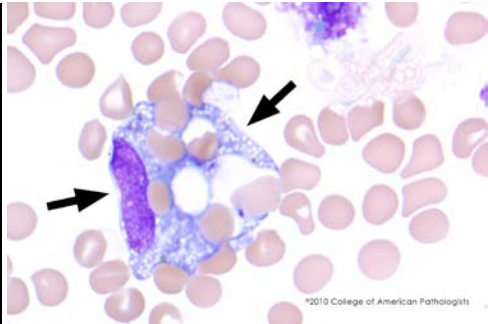
The identification of siderophages in the CSF along with the presence of hematoidin crystals is evidence of previous hemorrhage.

CMP-22

## Body Fluid Photomicrographs/Photographs

### Case History CMP-23

The patient is a 66-year-old female with headache, dysphagia, right hemiparesis, and gait instability due to a brain tumor. A cerebrospinal fluid specimen was taken from an ventricular shunt several days after resection of the tumor. CSF sample laboratory findings include: Color = bloody. After centrifugation = xanthochromic; turbidity = 2+. Total nucleated cells =  $0.087 \times 10^3 / \mu\text{L}$ ; RBC =  $0.004 \times 10^6 / \mu\text{L}$ .



Identification	Referees		CMP Participants		Performance Evaluation
	No.	%	No.	%	
Macrophage containing erythrocytes	17	89.5	2920	95.0	Educational
Macrophage containing abundant small lipid vacuoles/droplets	2	10.5	81	2.6	Educational

CMP-23

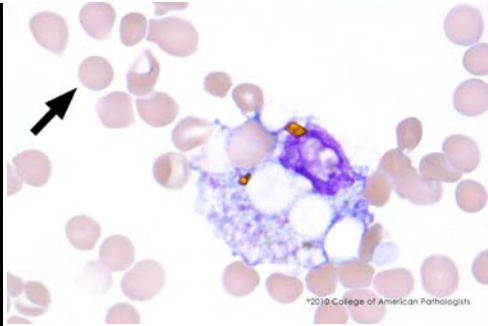
The arrowed object was properly identified as macrophage containing erythrocytes (erythrophage) by 95.0% of the participants.

The erythrophage is a macrophage that has ingested red blood cells as clearly depicted in this image. This usually occurs as a consequence of hemorrhage, precipitated by trauma and /or any other cause. This can also occur in vitro after specimen collection, so in itself it does not represent unequivocal evidence of previous hemorrhage. Erythrophages can also be seen as part of a hemophagocytic syndrome and in the company of macrophages showing leukophagocytosis. Please note that this erythrophage also shows early evidence of probable iron / hemosiderin pigment with several isolated small dark blue granules.

## Body Fluid Photomicrographs/Photographs

### Case History CMP-24

The patient is a 66-year-old female with headache, dysphagia, right hemiparesis, and gait instability due to a brain tumor. A cerebrospinal fluid specimen was taken from an ventricular shunt several days after resection of the tumor. CSF sample laboratory findings include: Color = bloody. After centrifugation = xanthochromic; turbidity = 2+. Total nucleated cells =  $0.087 \times 10^3 / \mu\text{L}$ ; RBC =  $0.004 \times 10^6 / \mu\text{L}$ .



CMP-24

Identification	Referees		CMP Participants		Performance Evaluation
	No.	%	No.	%	
Mature erythrocyte	19	100.0	3053	98.9	Good

The arrowed object was properly identified as a mature erythrocyte by 98.9% of the participants.

When encountered in the CSF, mature erythrocytes are morphologically similar to the ones in the peripheral blood; however, their presence is not normal. Erythrocytes may be the result of contamination by a traumatic lumbar puncture or the manifestation of a pathologic hemorrhagic process as in this case.

Please note the presence of another erythrophage with abundant number of vacuoles, in the image, as well as the presence of small hematoidin crystals. All of these elements in combination represent morphologic evidence of a hemorrhagic process.



## Discussion

The laboratory handling and examination of the cerebrospinal fluid (CSF) provides valuable support for the evaluation and diagnosis of many central nervous system (CNS) conditions. Commonly performed tests on CSF include protein and glucose levels, cell counts, and differential by microscopic examination of cytocentrifuged slides, gram stain and microbiologic evaluation by culture and now molecular testing as well.

Evaluation of the CSF truly starts at the bedside while the specimen is being collected, as the measurement of the opening pressure by the physician can provide very important information. For example, high opening pressure is a clue to the diagnosis of intracranial hypertension, which may be seen in a spectrum of disorders such as meningitis, intracranial hemorrhage and tumors. The confirmation of a high opening pressure at the time of lumbar puncture is also important as the collection should proceed very slowly and with limitations on the amount of fluid that is collected.

In the clinical case presented for this challenge, the diagnosis of a brain tumor was previously established after the patient initially presented with the complaint of headache. At least 1-2 % of patient visits to emergency departments are related to headaches, and this complaint may account for more than 5 million emergency department visits annually in the United States. Many patients suffer from primary headache disorders such as migraine, but in others, headache may be the manifestation of a much serious disorder with significant morbidity and mortality, such as subarachnoid hemorrhage (SAH).

Bleeding into the subarachnoid space may occur secondary to a ruptured brain aneurysm, trauma / head injury, mass occupying lesions and many others. However a bloody CSF may also result from injury to the small veins in the epidural space when performing the procedure. Frequently these two situations have to be clinically differentiated.

This patient had a brain tumor, underwent surgical resection, and the CSF specimen was obtained several days after the surgical resection.

The findings of xanthochromia, active red cell phagocytosis (CMP-23 and 24) with siderophages (CMP-22) and hematoidin crystals (CMP-21 and 24) are consistent with SAH. The hemorrhage most likely dates back to the time of this patient's initial presentation.

Initial clinical evaluation of patients like the one presented here usually includes analysis of the CSF along with radiographic studies such as computed tomography scanning (CT), to confirm or exclude the presence of a CNS disorder.

CSF is an ultra-filtrate of plasma, secreted primarily by the choroid plexus and fills the space within the cerebral ventricles and the subarachnoid space. The CSF is not static and circulates around the cerebral hemispheres and spinal cord and is reabsorbed into the venous sinuses. The CSF protects the nervous system from abrupt changes in pressure and provides a means for the exchange of nutrients and waste for the CNS.

The total volume of CSF is replaced about 5 to 6 times in a 24 hour period. Under normal circumstances, adults have a CSF total volume of 100 to 150 mL, children 60 to 100 mL, and infants 10 to 60 mL.

CSF is obtained by inserting a needle into the lumbar subarachnoid space, a procedure known as lumbar puncture. CSF may also be obtained by cisternal and lateral cervical puncture, through ventricular cannulas, or from shunts as in the presented case.

The normal CSF is clear and of a watery consistency. Abnormal findings include a cloudy, turbid, purulent, bloody or xanthochromic CSF. Turbidity or cloudiness starts to show with WBC counts over 200 cells  $\mu$ /L or RBC of 400  $\mu$ /L. A grossly bloody fluid usually has RBC counts greater than 6,000  $\mu$ /L.

Xanthochromia generally refers to a pale pink to yellow color of the CSF. However this observation should be made in the supernatant of a previously centrifuged CSF and in comparison with a tube of distilled water. Pale pink to orange xanthochromia is related to RBC lysis with release of oxyhemoglobin. This may be the earliest feature supporting the presence of SAH, but as this may also occur as an in vitro phenomenon, it is not specific for the diagnosis of SAH. However, yellow xanthochromia is derived from bilirubin, and its formation can

only occur in vivo as it is an enzyme dependent process. Consequently, this finding is more specific for the diagnosis of SAH.

As the evaluation by the naked eye for yellow xanthochromia is somewhat subjective, many advocate the confirmation of the presence of bilirubin by spectrophotometry. This is a common practice in Europe but uncommon in the United States. Xanthochromia is present in more than 90% of patients within 12 hours of the onset of subarachnoid hemorrhage. However, it may also be present with very high serum bilirubin levels and consequently must be very carefully interpreted in neonates and patients with marked jaundice.

The normal CSF nucleated cell count in adults is 0-5  $\mu$ /L and in neonates 0-30  $\mu$ /L, with decreasing values until adolescence. No RBCs should be present, and their identification is usually an indication of a pathologic process or a traumatic tap. Although RBC counts in themselves have no diagnostic value, they are useful in extrapolation formulas to derive a corrected CSF WBC when there is a coexistent peripheral blood CBC. Protein and glucose CSF results should be interpreted in correlation with the serum patient values. Over 80% of the CSF protein content is derived from plasma. Elevations of total protein are nonspecific, and perhaps one of the most common abnormalities found in the CSF as an indicator of meningeal or CNS disease.

In summary, this case nicely illustrates the whole spectrum of morphologic findings seen as a consequence of bleeding into the subarachnoid space. These findings may be present up to a month after the initial episode, and therefore may be useful as evidence of a SAH when CT scan abnormalities may have disappeared or resolved.

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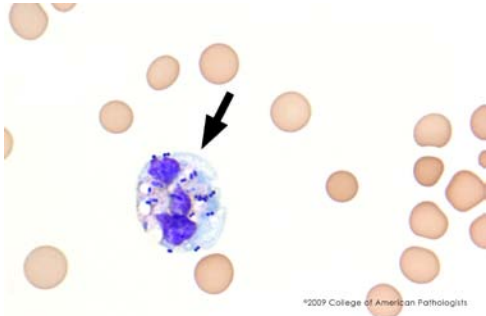
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Hematology and Clinical Microscopy Resource Committee

## Body Fluid Photomicrographs/Photographs

### Case History CMP-25

The patient is a 32-year-old female with a history of systemic lupus erythematosus and end-stage renal disease. She presents in pain with bloody peritoneal fluid. Peritoneal fluid sample laboratory findings include: Total nucleated cells =  $0.063 \times 10^3 / \mu\text{L}$ ; RBC =  $0.05 \times 10^6 / \mu\text{L}$ .



CMP-25

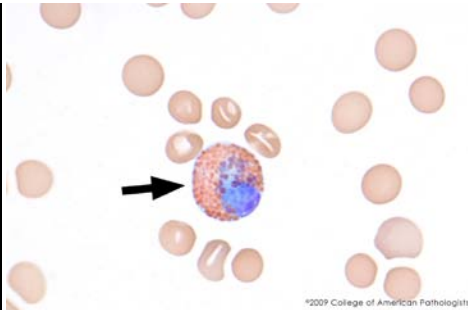
Identification	Referees		CMP Participants		Performance Evaluation
	No.	%	No.	%	
Neutrophil with phagocytized bacteria	18	100.0	2960	99.1	Good

The cell identified by the arrow is a neutrophil with phagocytized bacteria, as correctly identified by 99.1% of the participants. Phagocytized bacteria have a uniform appearance. Depending on the species, bacteria may be round or rod-shaped and present as single organisms, diploid forms or in chains. The bacteria present within this neutrophil are diplococci. Intracellular bacteria should be distinguished from intracellular granules or debris.

## Body Fluid Photomicrographs/Photographs

### Case History CMP-26

The patient is a 32-year-old female with a history of systemic lupus erythematosus and end-stage renal disease. She presents in pain with bloody peritoneal fluid. Peritoneal fluid sample laboratory findings include: Total nucleated cells =  $0.063 \times 10^3 / \mu\text{L}$ ; RBC =  $0.05 \times 10^6 / \mu\text{L}$ .



CMP-26

Identification	Referees		CMP Participants		Performance Evaluation
	No.	%	No.	%	
Eosinophil	18	100.0	2982	99.7	Good

The cell identified by the arrow is an eosinophil, as correctly identified by 99.7% of the participants. The eosinophil has a bilobed nucleus and numerous large orange-red granules. The granules are larger than the primary granules of neutrophils. Large numbers of eosinophils in peritoneal fluid specimens can be related to foreign body reaction, parasitic infection, or to prior abdominal procedures including paracentesis.

## Discussion

Bacterial peritonitis is a bacterial infection of the membranes that line the inner wall of the abdominal cavity and cover the intraabdominal organs. Bacterial peritonitis is most often secondary to perforation of the abdominal wall or rupture of an intra-abdominal organ. Common causes include a ruptured appendix, gastric ulcer, or colon perforation related to diverticulosis or tumor. Perforation of the gastrointestinal tract releases large numbers of fecal organisms into the peritoneal space causing peritoneal infection. Bacteria can also be introduced into the peritoneal cavity through peritoneal dialysis, pancreatitis, biliary infection and trauma. Treatment of secondary bacterial peritonitis usually involves antibiotic therapy and in some cases, surgical removal of the source of infection and infected tissues.

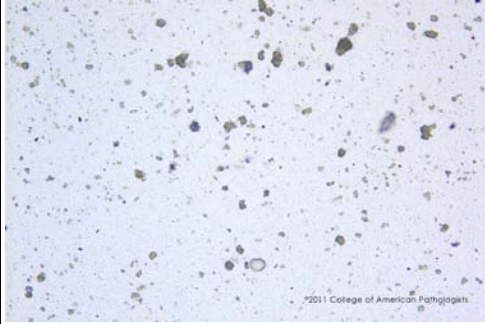
Spontaneous bacterial peritonitis is bacterial infection of the peritoneal tissues without any identifiable secondary source of infection. Spontaneous bacterial peritonitis occurs in patients with cirrhosis of the liver, nephrotic syndrome and in immunocompromised patients. The pathogenesis of spontaneous bacterial peritonitis is uncertain, but is thought to be related to localized immune dysfunction. Spontaneous bacterial peritonitis is a known complication of systemic lupus erythematosus, particularly in patients with ascites. Patients with spontaneous bacterial peritonitis present with fever, abdominal pain and tenderness; however, some patients may have no signs or symptoms. In patients with advanced liver disease, the diagnosis may be masked by hepatic encephalopathy. Laboratory analysis of ascitic fluid obtained by paracentesis is the corner stone of the diagnosis and timely treatment of spontaneous bacterial peritonitis. The presence of greater than 250 neutrophils /uL in the appropriate clinical setting is considered to be consistent with the diagnosis of spontaneous bacterial peritonitis. Spontaneous bacterial peritonitis is treated immediately with broad spectrum antibiotics and antimicrobial therapy may be narrowed after results of bacterial cultures and sensitivities become available.

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# Clinical Microscopy Miscellaneous Photomicrographs/Photographs

CMMP-36



Identification	Referees		CMMP Participants		Performance Evaluation
	No.	%	No.	%	

Ferning absent	12	100.0	1672	99.6	Good
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A vaginal sample collected to evaluate for the presence or absence of ferning. This specimen is negative for ferning in this specimen. Examination of vaginal secretions for ferning is used to detect rupture of amniotic membranes. The “fern test” was initially described in 1955 and its ease of use and clinical utility has been confirmed by multiple published studies.

## Clinical Microscopy Miscellaneous Photomicrographs/Photographs

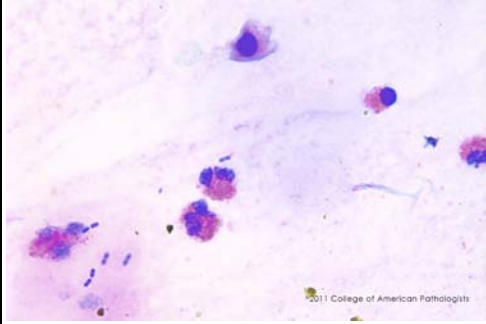


CMMP-37

Identification	Referees		CMMP Participants		Performance Evaluation
	No.	%	No.	%	
Yeast/Fungi present	16	94.0	2563	93.2	Good

The KOH wet preparation demonstrates pseudohyphae which exhibit branching and are consistent with *Candida* species. A vaginal wet prep is often collected in the evaluation of vaginitis. The three most common types of acute vaginitis are bacterial vaginosis, vulvovaginal candidiasis and trichomoniasis. Candidiasis, as demonstrated in this slide, accounts for 15-30% of the cases of acute vaginitis. Uncomplicated vulvovaginal candidiasis is defined as infrequent (three or fewer episodes per year), with mild-to-moderate symptoms, probably caused by *C. albicans*, and occurring in an immunocompetent host. There are many approved topical antifungal treatments and one oral agent, fluconazole (150 mg), in a single dose. 80-90% of women will have symptomatic relief with either the topical or oral therapy.

## Clinical Microscopy Miscellaneous Photomicrographs/Photographs



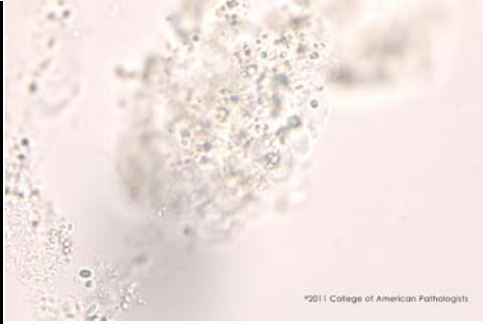
CMMP-38

Identification	Referees		CMMP Participants		Performance Evaluation
	No.	%	No.	%	
Eosinophils present	13	100.0	2162	99.9	Good

This nasal smear has eosinophils present, which exhibit the typical bilobed nucleus and numerous cytoplasmic eosinophilic granules. Nasal smears for eosinophils are an aid to distinguishing allergic rhinitis, where eosinophils are present, from non-allergic rhinitis. The clinical differential diagnosis of non-allergic rhinitis and allergic rhinitis is difficult due to the significant overlap of clinical symptomatology. In addition to the nasal smear, skin prick tests, serum IgE levels, and RAST tests may be used in conjunction with the clinical presentation to differentiate allergic and non-allergic rhinitis. Several rod-shaped bacteria which stain deeply basophilic with the Wright-Giemsa stain are also present.



## Clinical Microscopy Miscellaneous Photomicrographs/Photographs

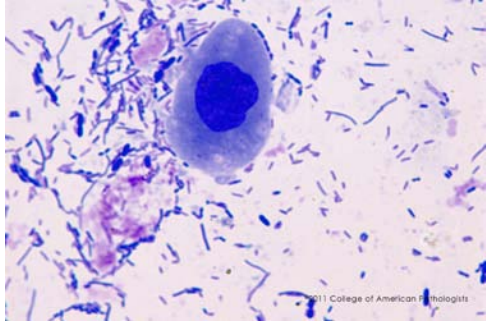


CMMP-39

Identification	Referees		CMMP Participants		Performance Evaluation
	No.	%	No.	%	
Pinworm absent	14	100.0	2096	99.4	Good

This stool specimen is negative for *Enterobius vermicularis* (pinworm) and exhibits only cellular debris. *Enterobius vermicularis* is also called human pinworm. (Adult females: 8 to 13 mm, adult male: 2 to 5 mm.) To make the diagnosis of pinworm in a patient who presents with anal itching, place either a piece of transparent tape or a pinworm paddle on the anal skin. This is ideally done first thing in the morning, when the number of eggs on the skin surface is highest. The tape is then applied to a glass slide. Following additional of toluidine blue, the slide is examined for pinworm. The eggs are elongated, flattened on one side, 50-60  $\mu\text{m}$  long by 20-32  $\mu\text{m}$  wide, with a thick shell. Multiple samples may be required to make the diagnosis.

# Clinical Microscopy Miscellaneous Photomicrographs/Photographs



CMMP-40

Identification	Referees		CMMP Participants		Performance Evaluation
	No.	%	No.	%	

Neutrophils absent	14	93.3	2377	96.9	Good
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This stool specimen is negative for neutrophils. Assessment of stool specimens for neutrophils is a test that can be used in conjunction with a bacterial culture in the evaluation of enteritis/colitis. While the presence of neutrophils is consistent with a bacterial infection, the findings are not specific. Stool cultures are more sensitive and specific for the evaluation of enteric pathogens.

## Clinical Microscopy Miscellaneous Photomicrographs/Photographs



CMMP-41

Identification	Referees		CMMP Participants		Performance Evaluation
	No.	%	No.	%	
Trichomonas absent	19	100.0	3058	99.4	Good
Sperm present	16	100.0	2383	99.8	Good
Clue cells absent	12	66.6	1477	54.1	Non-consensus
Epithelial cells present	15	88.2	2282	92.6	Good

This photomicrograph demonstrates an unstained vaginal wet preparation. The wet preparation is often examined to diagnose causes of vaginal discharge or a postcoital wet preparation can be used to assess for sperm and the interaction between sperm and cervical mucus. A sample of vaginal secretions is taken from the posterior vaginal pool using a cotton or dacron-tipped swab. It is mixed with nonbacteristatic saline on a slide. Spermatozoa are identified in this photo. The sperm head is about 4 to 6 micrometers long while the slender tails are about 40 to 60 micrometers long. The intended responses are sperm present, Trichomonas absent, epithelial cells present and clue cells absent.

Clue cells are vaginal epithelial cells encrusted with the bacterium *Gardnerella vaginalis*. Clue cells have a heavy stippled or granular, very refractile cytoplasm with shaggy or bearded cell borders due to the heavy coating of the coccobacilli. Most of the cell surface should be covered by bacteria for it to be identified as a clue cell. The epithelial cells in this photo do not exhibit this shaggy or bearded appearance, and thus, should not be considered clue cells.

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