Body Fluid Microbiology
(Pericardium, Peritoneum, Pleura, Synovium)

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Pericardial Space

- The area between the epicardium, which is the membrane surrounding the heart muscle, and the pericardium is called the pericardial space.
- Normally contains 15 to 20 mL of clear fluid.
- Effusion
- Tamponade
The peritoneum is a large, moist, continuous sheet of serous membrane that lines the walls of the abdominal pelvic cavity and the outer coat of the organs contained within the cavity.

Small amount of fluid that maintains moistness of the surface of the peritoneum.

Contain 300 WBC/ml but the protein content and specific gravity of the fluid are low.

Ascites
Pleural Space

- The pleura, a serous membrane of the thoracic cavity lines the entire thoracic cavity
- The fluid usually contains few or no cells and has a consistency similar to that of serum
- Effusion
- Empyema
Body Fluid Cultures

- Infection of normally sterile body fluids often results in severe morbidity and mortality
- Rapid and accurate microbiological assessment of these samples is important to successful patient management
Collection & Transport

- A volume of 1–5 mL is adequate for isolating most bacteria

- 10–15 mL is optimal for recovery of mycobacteria and fungi, which are generally present in low numbers (at least 5 mL for each of these two cultures)
Collection & Transport

- Ten milliliters of fluid is recommended for the diagnosis of peritonitis.

- To diagnose peritonitis associated with chronic ambulatory peritoneal dialysis, collection of at least 50 ml of fluid may improve recovery of the responsible pathogen.
Collection & Transport

- Most specimens (pleural, peritoneal, pericardial, and synovial fluids) are collected by aspiration with a needle and syringe.

- Body fluids from sterile sites should be transported to the laboratory in a sterile screw-capped tubes or vial.
Collection & Transport

- Fluid **should never be transported in a syringe with the needle still attached**

- Syringes that have been capped prior to transport may be accepted
Collection & Transport

- The laboratory may reject specimens that have clotted in a capped syringe.
- Swabs afford the least desirable sample for culture of body fluids and should be discouraged as devices for transport.
Collection & Transport

- Percutaneous catheters are placed during many surgical procedures to prevent the accumulation of exudate.
Collection & Transport

- Laboratory may receives drainage fluids from these catheters for culture when signs and symptoms suggest infection.

- Culture of such fluid is potentially misleading when the fluid becomes contaminated within the catheter or collection device.
Invasively collected specimens in leaky containers must be processed, but alert the physician of the possibility of contamination.
Collection & Transport

- Numerous studies have shown that culture of large volumes of fluids in blood culture bottles, rather than concentration by centrifugation, will result in a higher yield.

- One must always send some of the specimen to the laboratory in a container other than a blood culture bottle.
Collection & Transport

- In general, if sufficient specimen is available, cultures should be inoculated with the same volumes specified by the manufacturer for blood specimen.

- If the volume is insufficient to follow the manufacturer's instructions, as little as 0.1 ml can be inoculated.
Collection & Transport

- Fluid from CAPD patients can be submitted to the laboratory in a sterile tube, urine cup, or in the original bag.

- The bag is entered only once with a sterile needle and syringe to withdraw fluid for culture.
Enteroviruses, primarily Coxsackie viruses A and B, are among the most common causes of infectious pericarditis

Collection of throat washings and stool (which are more likely to yield the virus), in addition to pericardial fluid, is strongly recommended for virus isolation from persons with suspected enteroviral pericarditis
SPECIMEN PROCESSING

- Techniques for laboratory processing of all sterile body fluids are similar except for those previously discussed body fluids that are directly inoculated into blood culture bottles
SPECIMEN PROCESSING

- Clear fluids may be concentrated by centrifugation or filtration, whereas purulent material can be inoculated directly to media.

- Any body fluid received in the laboratory that is already clotted must be homogenized to release trapped bacteria and minced or cut to release fungal cells.
Important Notice

Grinding is not recommended with specimens processed for fungi

Small amounts of whole material from a clot should be aseptically cut with a scalpel and placed directly onto media for isolation of fungi
SPECIMEN PROCESSING

- All fluids should be processed for direct microscopic examination.
- For microscopic examination, cytocentrifugation is better to be used to prepare Gram-stained smears because organisms can be further concentrated up to a 1000-fold (about 0.1 ml of fluid may be removed before centrifugation).
SPECIMEN PROCESSING

- Body fluids for culture should be concentrated by either filtration or high-speed centrifugation
- The fluid is centrifuged at 1500-2500×g for 20–30 minutes
- Once the sample is concentrated, the supernatant is aseptically decanted or aspirated with a sterile pipette, leaving approximately 0.5-1 mL liquid
SPECIMEN PROCESSING

- Vigorous vortexing or drawing the sediment up and down into a pipette several times adequately resuspends the sediment

- SAFETY WARNING:
  This procedure should be done in a biological safety cabinet
SPECIMEN PROCESSING

- If fluids have been concentrated by centrifugation, the resulting sediment should be inoculated to an enrichment broth and blood and chocolate agars.
SPECIMEN PROCESSING

- If only blood culture bottles were received, subculture immediately to CHOC
- If little specimen is received, inoculate only CHOC and omit Gram stain
- For peritoneal fluid contaminated with bowel content add other selective media and omit broth cultures
SPECIMEN PROCESSING

- Examine all plated and for evidence of growth at 24 h
- Read plates daily for 4 days for invasively collected specimen and 2 days for drainage cultures
- Incubate blood culture bottles for 5 to 7 days
Advances in medicine have altered the etiologic spectrum of pericarditis over the course of the 20th century.

Idiopathic and viral pericarditis now predominate and usually result in a benign, self-limited disease.

Purulent bacterial pericarditis and tuberculous pericarditis are now less common, but they still cause significant morbidity and mortality.
In some series, idiopathic pericarditis accounted for 40% to 86% of patients hospitalized with acute pericarditis.

- It is likely that viral infections are responsible for many, if not most, cases of acute pericarditis presently classified as idiopathic.
Of the many viruses associated with heart disease, the enteroviruses, especially the coxsackieviruses, are most frequently implicated in pericarditis.
A wide variety of bacteria can cause pericarditis

*S. pneumoniae* and *S. aureus* accounted for more than half of the cases

With the advent of antibiotics, the incidence of purulent pericarditis decreased markedly
Although staphylococci and streptococci are still etiologic in a substantial number of cases, the incidence of pneumococcal pericarditis has declined substantially, and gram-negative bacilli have assumed a much more important role.

Pericardiocentesis alone establishes a specific etiologic diagnosis in 20% to 25% of cases; the availability of both fluid and tissue improves this yield to 54%.
PERITONITIS
✓ Primary Peritonitis
The peritoneal infection is not related directly to other intra-abdominal abnormalities

✓ Secondary Peritonitis
An intra-abdominal process, such as a ruptured appendix or a perforated peptic ulcer, is evident

✓ Peritonitis complicating peritoneal dialysis (CAPD)
Primary Peritonitis

- Sometimes referred to as *spontaneous bacterial peritonitis*

- Primary peritonitis occurs at all ages

- The prevalence of primary peritonitis in children apparently has been decreasing

- In adults, primary peritonitis usually has been reported in patients with cirrhosis and ascites
The prevalence of primary peritonitis in hospitalized patients with cirrhosis and ascites has been estimated at 10% to 30%.
Primary Peritonitis pathogens

- In cirrhotic patients, microorganisms presumably of enteric origin account for 69% of the pathogens.

- *Escherichia coli* is the most frequently recovered pathogen, followed by *Klebsiella pneumoniae, S. pneumoniae*.
Laboratory Finding

- Ascitic fluid with positive cultures but few leukocytes, termed *bacterascites*, in patients without clinical findings of peritonitis has been noted. This condition may represent early colonization before a host response.

- Conversely, several series have identified cases of primary peritonitis with negative ascitic fluid cultures.

- Sterile cultures occurred in 35% of patients.
Laboratory Finding

- A polymorphonuclear leukocyte count in peritoneal fluid greater than 250 cells/mm³ is considered diagnostic.

- Gram staining of the sediment, when positive, is diagnostic, but it is negative in 60% to 80% of patients with primary peritonitis.

- The ascitic fluid protein concentration may be low.
Laboratory Finding

Other parameters of ascitic fluid that might help in diagnosing primary bacterial peritonitis are lactate concentration greater than 25 mg/dL and pH less than 7.35.

Ascitic fluid in tuberculous peritonitis may have an elevated protein concentration (>3 g/dL) and a lymphocytic pleocytosis.
Secondary Peritonitis

Secondary intra-abdominal infection usually is caused by spillage of gastrointestinal or genitourinary microorganisms into the peritoneal cavity secondary to loss of the integrity of the mucosal barrier.
Secondary Peritonitis

Most cases of secondary peritonitis are endogenous in origin, however, and are caused by the large number and variety of microorganisms that normally colonize mucous membranes lining certain viscera within the abdominal cavity.
Secondary Peritonitis

Secondary peritonitis typically is polymicrobial, and the pathogens in most cases are derived from the gastrointestinal tract, even in patients with a primary gynecologic process.

- Typically the facultative microorganisms are *E. coli*, *Klebsiella/Enterobacter* spp., *Proteus* spp., and enterococci, and the obligate anaerobes are *B. fragilis*, *P. melaninogenica*, *Peptococcus*, *Peptostreptococcus*, *Fusobacterium*, *Eubacterium lentum*, and *Clostridium*

- Other, less commonly isolated pathogens include *S. aureus*, *P. aeruginosa*, and *Candida*
Continuous Ambulatory Peritoneal Dialysis

- For patients with end-stage renal disease, peritoneal dialysis has been shown to be a practical, safe, effective, and cost-effective alternative to chronic hemodialysis.

- Usually 1 to 2 liters of dialysate is infused for a dwell time of 4 to 8 h, and cycles are repeated every 6 h. When empty, bags are reused for recovery of effluent drainage by gravity at the end of a cycle.
Principles of peritoneal dialysis
Continuous Ambulatory Peritoneal Dialysis

The average incidence of peritonitis in these patients is up to two episodes per year per patient.

Peritonitis is best diagnosed clinically by the presence of cloudy dialysate with or without abdominal pain.
Continuous Ambulatory Peritoneal Dialysis

- Cultures of fluids from patients with CAPD peritonitis have been reported as negative in 4 to 48% of all episodes.
- Most series have found coagulase-negative staphylococci to be the most frequently encountered agents (40 to 60% of all positive cultures), followed by *S. aureus* and streptococci (10 to 20% each), members of the family *Enterobacteriaceae* (5 to 20%), nonfermentative gram-negative rods (3 to 15%), and gram positive rods (2 to 4%).
CULTIVATION OF MICROORGANISMS

- Only cloudy fluids should be sent to the microbiology laboratory.
- Total WBC and PMN counts before culture are desirable.
- If the sample cannot be worked up right away, it should be briefly (<6 h) stored at 4°C.
CULTIVATION OF MICROORGANISMS

- The amount of dialysate required should be such that maximum sensitivity and specificity can be expected.
- A minimum of 10 ml should be cultured, using enrichment broth with antiphagocytic and lytic properties.
Subcultures of the enrichment should be done at least on aerobic chocolate agar and anaerobic blood agar plates.

Media should be incubated for up to 7 days.

If peritonitis is suspected but the culture remains negative, mycobacterial and fungal stains and cultures should be initiated.
Pleural Effusion
Pleural Effusion

- Infections of the pleural space remain an important cause of morbidity and mortality around the world.
- Delay in diagnosis, failure to institute appropriate antimicrobial therapy, and inadequate drainage contribute to increased morbidity and mortality.
Causes of Pleural Space Infection

- Most commonly follow pneumonia (40% to 60%)
- Thoracotomy (20%)
- Trauma (4% to 10%)
- Less commonly, empyema can develop as a result of esophageal rupture and subdiaphragmatic spread.
- Other uncommon causes include hematogenous seeding of an existing pleural effusion and direct extension from head and neck infections.
Predisposing factors are most important in predicting the most likely pathogens

- Pneumonia continues to be the most frequent predisposing factor in the development of empyemas

- In otherwise healthy adults with pneumonia, the most common bacteria causing pleural empyema are *S. aureus, S. pneumoniae, or S. pyogenes*
Factors predisposing to aspiration, such as altered mental status, alcoholism, and periodontal disease, are common in patients with anaerobic infections of the pleura. Many of these cases tend to be polymicrobial.
Empyema complicating hemothorax is often staphylococcal, whereas that associated with pneumothorax or hematogenous seeding of a serous effusion is often caused by aerobic gram-negative bacilli.

Immunocompromised patients have a higher frequency of empyema caused by fungi and gram-negative bacilli.
■ Fluid should be sent to the microbiology laboratory for a Gram stain and for both aerobic and anaerobic cultures

■ Only 61% of patients with established empyemas have a positive Gram stain

■ Although most patients with empyemas have a positive culture, the absence of growth does not mean that a pleural effusion does not require drainage
Pleural tuberculosis

- Can be diagnosed by stains of pleural fluid in only 18% to 23% of patients
- Cultures of pleural fluid and histologic examination of pleural biopsy specimens permit the diagnosis in up to 95% of patients
- Tests for adenosine deaminase (ADA) and interferon-γ, and the polymerase chain reaction (PCR) are now available to help establish the diagnosis of tuberculous pleural disease
THANK YOU FOR YOUR ATTENTION