Clinical Microbiology and Infectious Disease: * * Important Concepts and Vignettes

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Diagnosis and Therapy of Infectious Disease

- Physical exam
 - A. Clinical
 - Patient history
 - Travel
 - Immunologic status
 - Time of year
 - B. Laboratory
 - Supportive of clinical diagnosis (but should not be considered ancillary)
 - Pathways
 - Guidelines
 - Directional studies





Clinical Relevance vs. Cost of Laboratory Testing and Results

- Screening tests should be limited to clinically relevant situations
- The more testing performed the more false-positives will be picked up
- Issues with testing:
 - sensitivity/specificity
 - diagnostic predictive values
 - false-positives usually expands inappropriate testing, and increases inappropriate therapy



Clinical Relevance of Laboratory Results

Diagnostic Sensitivity:

- Ability of test to detect a condition
 - Frequency of abnormal or positive test results in individuals who have a selected disease

Diagnostic Specificity:

- Ability of test to define a true condition
 - Frequency with which a normal or negative test results in individuals free of the disease

Predictive Value of Positive (abnormal) Result

Number of true positives divided by the total number of patients with positive results (both true and false)

Predictive Value of Negative (normal) Result

Number of true negatives divided by the total number of patients with negative results (both true and false)

What does the predictive value of a test depend on?



Clinical Relevance of Laboratory Results

Predictive value of a test is significantly affected by the **prevalence** of the disease in the selected population

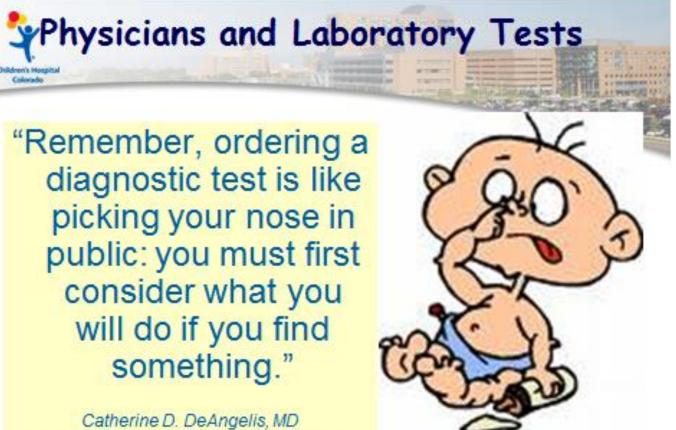
Prevalence of Disease within Population (%)

Predictive Value of Positive Result (%)

1	16.1
2	27.9
5	50.0
10	67.9
25	86.4
50	95.0

Test Sensitivity 95%, Test Specificity 95%





Catherine D. DeAngelis, MD Arch Pediatr Adolesc Med 1994; 148:1277.



Considerations When Ordering Microbiology-related Studies

1. Does initiation of antimicrobial therapy have any effect on recovery of a true pathogen by culture?



Effect of Antibiotic Therapy on Sputum Cultures in Patients with Consolidated Pneumonia

******Collect specimen BEFORE initiation of therapy**

	Prior Rx <u>(52 Pts)</u>	No Prior Rx <u>(24 Pts)</u>
S. pneumoniae	0	15 (63%)
H. influenzae	0	7 (29%)
S. aureus	1 (2%)	0
Gram-negative bacilli	15 (29%)	0
No pathogen	36 (69%)	2 (8%)

Lancet. 1973;2:349.





CASE 1

- A 72-year-old man with benign prostatic hypertrophy admitted for increasing symptoms of hesitation to urinate, reduced force of stream, dribbling after urination, and nocturia. Hx of urinary tract infections twice in the preceding 8 months.
- Day # 2 he underwent transurethral prostatectomy.
- Day # 3 temperature of 38.3° C (100.9° F);
 6 hours later he reported shaking chills and temperature 39.0° C (102.2° F).



What diagnoses should be considered?

What studies should be carried out to reach a diagnosis?



What diagnoses should be considered?

- Urinary tract infection (pyelonephritis) \pm bacteremia
- Bacteremia related to an IV catheter
- ? Drug reaction

What studies should be carried out to reach a diagnosis?



What studies should be carried out to reach a diagnosis?

- WBC, differential: 9.8 (78% polys, 10% bands)
- Urinalysis with microscopy
- Culture of urine, with Gram-stained smear of urine
- Blood cultures
- How are blood cultures performed?



Blood Culture Process

1. Collection of blood cultures (1 aerobic bottle and 1 anaerobic bottle per culture set).

2. Insertion into incubator/reader.

3. Culture bottles rocked and read by reader every 10-15 mins); incubated for up to 5 days (longer incubation not needed – even for HACEK organisms).

- 4. Alarm notifies tech when critical read achieved.
- 5. Bottles pulled for Gram-stain and work-up.
- 6. Floor called with critical results.











- What patients require a blood culture?
- When are blood cultures not warranted?
- What constitutes a blood culture? How much blood in each?
- How many blood cultures are warranted per episode?
- How do blood cultures become contaminated and where does the contamination originate ?
- How does one differentiate between contamination in a culture and a true pathogen?
- Is collection of blood cultures through existing lines acceptable?



Patients Requiring Blood Cultures

Reason to suspect a clinically significant bacteremia with presence of:

- Chills, fever, hypothermia (may be significantly altered in young children, the elderly or the immunocompromised)
- Leukocytosis (especially with shift to immature granulocytes), granulocytopenia
- Signs of hemodynamic compromise (without know source)
- Any patient with indwelling lines, shunts and other long term implanted foreign object is at higher risk of infection and bacteremia.
- Sudden failure to thrive in children and the elderly, since "typical" signs may not be present
- Other factors: low serum albumin, low functional status, renal failure

Examples in which bacteremia may be suspected:

- Endocarditis; Pneumonia (hospitalized); Pyelonephritis; Meningitis
- Septic arthritis; Intraabdominal infection; Presence of abscesses



Patients in whom blood cultures are not warranted:

- Patients who are asymptomatic and without clinical suspicion for infection (surveillance cultures are not usually needed or warranted)
- Patients with community acquired pneumonia who do not require hospital admission (outpatients or seen in ED)

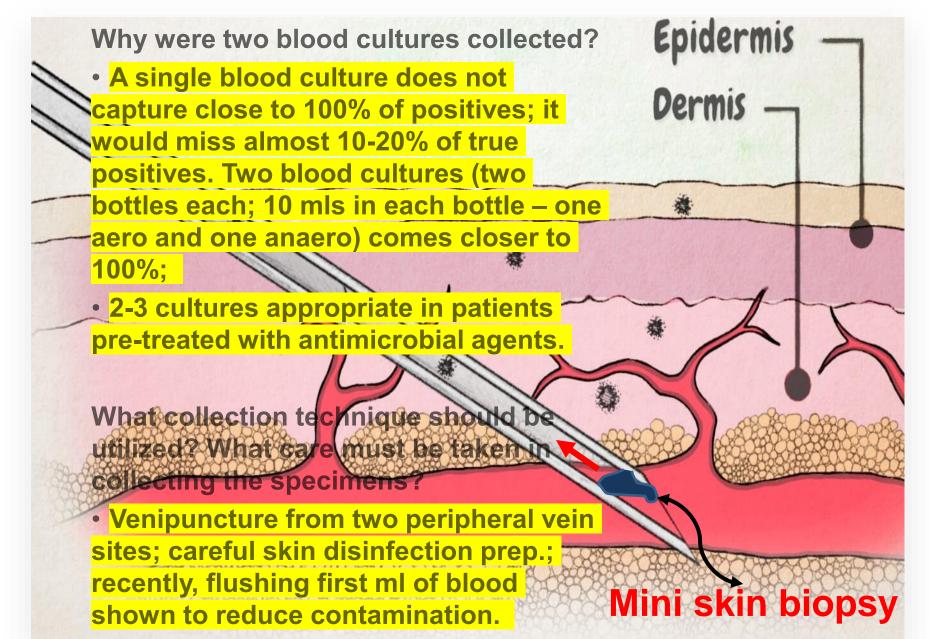


Is Specimen Collection Important?

Two blood cultures were collected in this thent and provided a definitive isolate formis the infectious process. Why? 森 Why were two blood cultures collected? What collection technique utilized? What care must be taken in collecting specimens?

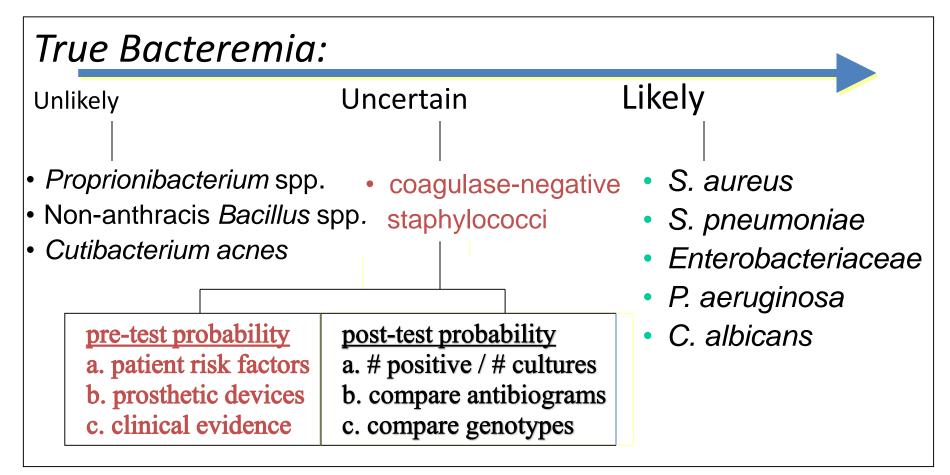


Is Specimen Collection Important?





Interpreting a "Positive" Blood Culture



Source: Kim SD, et al: Infect Control Hosp Epidemiol 2000;21:213-7



Summary of Blood Cultures

Routine (includes the yeast Candida)

- Number (2-3; 2 bottles/culture); 10-15% of significant bacteremias in adults will be missed when less than two blood cultures are performed.
- 20 mls/culture of blood (10 mls per bottle; >8mls/bottle minimum)
- Prior to Rx; peripheral venous draw;
- Contamination
 - skin disinfection (following contamination rates, \$2-6 thousand/case)
 - interpretation: isolate types and number positive cultures
 - Do not collect through indwelling catheter (higher contamination rate)

Fungal (Histo, Crypto, Coccy, do not need for other yeasts) AFB (Rapid growers: MAC, TB, other NTM in IC pts)



How many Blood Cultures?

ADULTS

•A minimal of two blood cultures sets are to be ordered per febrile episode

- Septic, acutely ill: Two blood cultures collected in sequence from two separate percutaneous sites **prior** to initiation of appropriate empiric therapy (dependent on presentation and antimicrobial stewardship recommendations).
- Intermittent fevers: Two blood culture sets (as above) at first, followed by one more at 2-6 hrs; possibly one more at 24 hrs if initial cultures no growth (after 24 hrs).
- Endocarditis: Two cultures the first day (before therapy initiated) followed by two additional blood cultures the second day if initial blood cultures are negative at 24 hrs.



Patients with the suspected organisms listed below shall have blood cultures drawn using the appropriate organism-associated systems:

- Bacterial blood culture systems will be used for Candida species of yeast and the Rapidly Growing Nontuberculous Mycobacteria such as *Mycobacterium chelonae* and *fortuitum*.
- Fungal blood culture systems (Lysis Centrifugation Blood Cultures) will be used for Coccidioidomycosis, Histoplasmosis, Cryptococcosis, and other fungal infections.
- Specialized AFB blood culture systems are reserved for severely immunocompromised patients in combination with routine bacterial blood cultures (call lab)
 - Note: TB is never recovered from blood in a non-immunocompromised patient and other sites have far greater efficacy in recovery.



Follow-up Blood Culture as Test of Clinical Improvement:

A. Patients in whom follow-up cultures **MAY BE WARRANTED**:

• Patients with primary bacteremia (presence in blood stream without an identifiable source), especially if slow in resolving.

B. Patients in whom follow-up cultures are **NECESSARY** include those with suspected:

- **MRSA**: Two follow-up blood cultures are to be performed either on day 2 or 4 after initial cultures and every two days thereafter until clinical improvement.
- Endocarditis: Blood cultures are to be performed 2-4 days after initial cultures and then every two days until clinical improvement (CLSI, 2007).
- **Candida**: Blood cultures are to be performed two days after initial culture and every two days thereafter until clinical improvement (Pappas, 2009).



Patients in whom repeat blood cultures are **NOT** WARRANTED include those with:

Secondary Bacteremia (presence of pathogen in blood which has spread from a known primary source of infection):

 Follow-up cultures not needed (especially with Gramnegative bacilli or pneumococcus) in patients who are NOT immunocompromised, who are doing well clinically and whose primary infection is improving (Baron 2005, CLSI 2007, Mandell 2007, Tabriz 2004).



Question.

Your post-surgical patient in the ICU with peripheral lines in place spikes a fever and has an increase in the blood WBC count. You suspect a possible line sepsis potentially caused by any of the bacteria or yeast usually seen in this setting (Staph., Gram-negative rods, *Candida* species yeast). Which one of the following would provide the correct etiology most efficiently and economically?

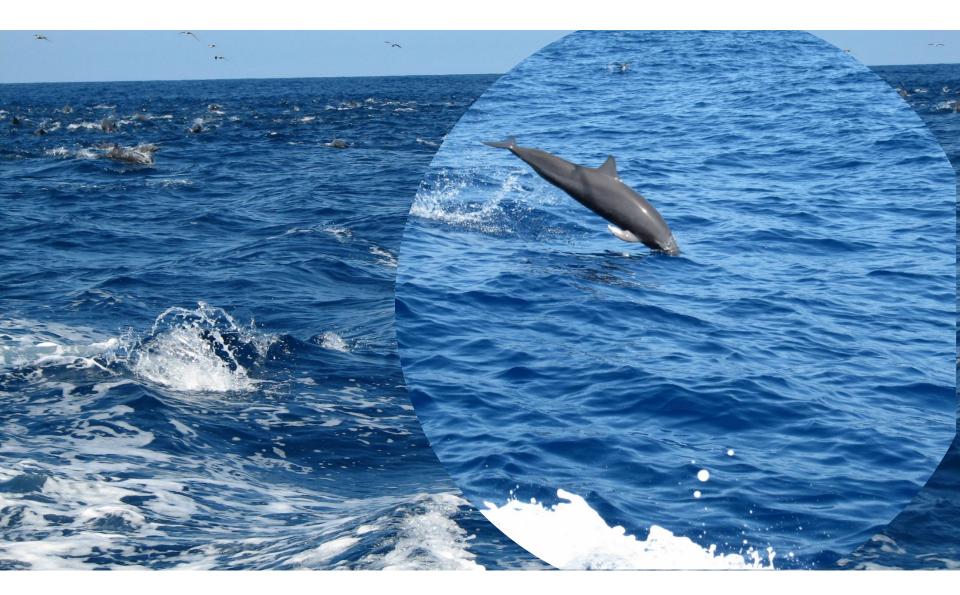
- A. Three routine blood cultures as well as two fungal blood cultures.
- B. Two routine blood cultures collected one after the other
- C. Five routine blood cultures collected over a five-hour period
- D. Fungal blood cultures as well as a buffy coat smear
- E. A single blood culture collected as close to the spiking fever as possible



Question.

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Case 1 continued



What about Urine Culture in our Patient? Is Method of Specimen Collection Important?

- When should urine specimens be collected?
- What is the best way to collect?
- Can urine bags be used?
- Can urine specimens be set at room temp before transport to lab?
- What is the best way to transport?



Urine Culture - Order tests for the right patient

Urine analysis and culture should not be ordered on asymptomatic patients unless they are pregnant, undergoing urologic procedures and +/- kidney transplant in first month

Symptoms may include Dysuria; Urinary frequency; Urinary urgency; Suprapubic pain; CVA (costovertebral angle) pain or tenderness; Fever; Altered mental status in elderly patients (i.e., confusion); Hematuria

Antibiotic therapy in patients with asymptomatic bacteriuria exposes patients to risk of development of side effects, toxicities, antimicrobial resistance, *Clostridioides difficile* colitis, treatment of future UTIs with second- and third-tier antibiotics and increased cost (Avelluto & Bryman, 2018).

The CDC reports that nearly 40% of all antibiotics prescribed for presumed UTI could have been avoided.



C. diff Colonic Lesions



Study in 3 Banner EDs Over 3 Different Time Periods – 2017-2018

ED 1	ED 2	ED 3
 897 Clean Catch Urines Collected in ED and Reflexed to Culture 506 grew organisms (56.4%) 23.6% True Positives 32.8% Contaminated 156 Clean Catch Urines Submitted for Culture 49.4% No Growth 22.4% True Positives 26.9% Contaminated 	 108 Clean Catch Urines submitted for culture over 1 Week 30.6% No Growth 36.1% True Positives 33% Contaminated Pharmacy evaluated 38 asymptomatic patients with mixed cultures 10 (26.3%) were treated without indications of UTI or pregnancy 	 95 Clean Catch Urines submitted for Culture over 1 Week 27% No Growth 29.5% True Positives 41.1% Contaminated

One other study found: 27 urine cultures contaminated; 13 of those received Abx in ED; **ZERO** were DC'd after negative results received (pharmacy review)



Study Summary of Urine Cultures in Banner EDs 2018

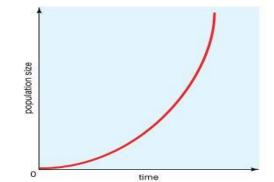
- Most UAs with reflex to culture are not collected correctly
 - Causes specimen contamination with bacteria from the meatus
 - Causes falsely high bacterial counts
 - Triggers automatic Cerner reflex to order a urine culture on the sample
- 35-50% of urine specimens grow mixed and contaminated flora
- High bacteria counts cause misdiagnosis and treatment of asymptomatic bacteriuria
 - Results in potential over-prescription of antibiotics
 - In the study 26-35% of patients with contaminated UAs were treated inappropriately
 - Can negatively impact patients and increase costs



Sending the sample

E. coli can double in number every 20 minutes at room temperature if urine sample not transferred to the proper stasis tube, falsely elevating bacterial counts.

The collected urine should be sent to the microbiology lab immediately, refrigerated for up to 2 hours, or (preferably) transferred to a **Gray-Top urine stasis tube immediately upon collection to be sent ASAP** to the microbiology laboratory.





Collect urine properly: Midstream, clean catch, single catheter or urine bladder tap

Depending upon hospital or ED, **35-50% of urine cultures have been contaminated,** an inordinate and unacceptable number, caused by inappropriate collection and transport methods

"Midstream Clean Catch" Urine Specimen - the preferred type of specimen for culture because of the reduced incidence of cellular and microbial contamination from the urethral meatus Patients should be given both oral and written instruction as to how to collect a urine specimen properly; written instruction by itself is usually inadequate.

NEVER collect urine specimens from urine/toilet pans, urine bags or urine which might have been contaminated by fecal material.



<u>Urinalysis</u>: presence of nitrite and leukocyte esterase <u>Microscopy</u>: WBCs but no casts seen Reflex from UA:

- > moderate + Leukocyte esterase
- + Nitrite
- > 10 hpf WBC
- >= few Bacteria

NOTE: Reflex to culture cannot be ordered on children of 2 yrs and younger. Should be predicated on clinical symptoms and not on results of UA. UTI's may occur despite negative UA results.



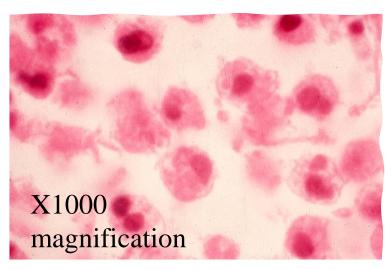
- >10⁵ cfu/ml
- GNR, Lactose Fermenter
- Indole-positive
- Oxidase-negative

= E. coli





Case 2. A Pneumonic Presentation (The AZ Desert might as well be in North Africa)



- A 64-year-old man with a history of smoking and well-controlled diabetes mellitus presents to ED with a 3 –day history of low-grade fevers, mild diarrhea and non-productive cough. He works as a maintenance worker in a local apartment complex and states that many tenants have been hospitalized with a "lung infection". Workup of the case includes a Gram-stain (X1000) of the patient's lower respiratory secretions which shows the following:
- Prominent polymorphonuclear leukocytes, but no organisms were seen.



- X-ray of chest reveals diffuse, patchy bilateral infiltrates.
- Other lab results included:

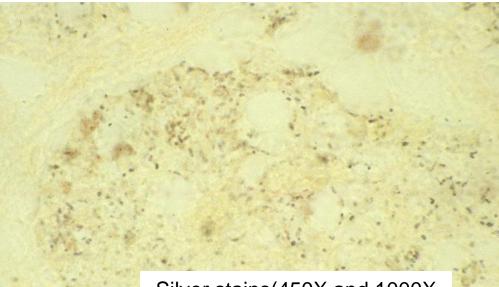
Hemoglobin: 14 g/ml Sodium: 128mEq/L Hematocrit: 40% Platelets: 200,000/mm³ WBC count: 15,000/mm³ Potassium: 4.2 mEq/L Bicarbonate: 17 mEq/L Blood urea nitrogen: 16mg/dL Glucose: 110mg/dL Creatinine: 1.2 mg/dL

Urinalysis: 2+ proteinuria; no glucose, ketones or blood

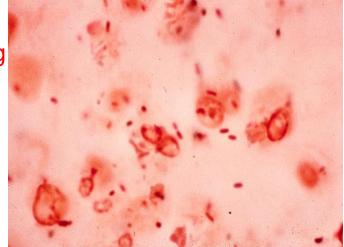
Acid-fast-stain of lower respiratory secretions was negative. Silver stain shows the following (next slide) Sputum culture: light growth normal oral flora



- Q: What lab values and studies stand out in this case?
- Q: What is the clinical and differential diagnosis?
- Q: Based on lab values what additional studies would one ask for in the laboratory?
- Q: What are the risk factors for this patient of having this infection?
- Q: What other tests might be helpful or diagnostic?
- Q: What are the best treatments for this infection?



Silver stains(450X and 1000X (Dieterle stain)





Q: What lab values and studies stand out in this case?

- Diffuse, patchy bilateral infiltrates on chest x-ray
- High WBC count: WBC count: 15,000/mm³
- Low sodium : 128mEq/L
- Negative Gram-stain
- Positive Silver stain
- Sputum culture shows normal oral flora
- Q: Based on lab values what studies would one ask for in the laboratory?



- Based on lab values what studies would one ask for in the laboratory?
 - PCR (viral and other causes of atypical pneumonia, including chlamydia and mycoplasma)
 - Legionella culture and PCR
 - Culture had been "gold standard" but being replaced by PCR
- What other tests might be helpful or diagnostic?
 - Legionella urine antigen test? (NOT in AZ)
 - L. pneumophila, mostly serogroup 1
 - East coast: EIA-sens 70-90%; Latex sens 55-90%, spec 85-99%)
 - Legionella DFA? (NO)
 - *L. pneumophila* abs; sens 20-75%; spec >95%)
 - Legionella serologies? (NO)

Acute/convalescent needed; 1-3 months post; sens 80%)



Legionella Culture using Buffered Charcoal Yeast Extract (BCYE) Agar

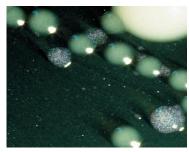
Black light fluorescence*



Young colonies with light shining transversely

viewed legionella agar with dissecting microscope microscope





*Only blue-white *Legionella* species fluoresce. Clinically significant ones include *L. bozemanii* and *L. dumoffii*.

BCYE Agar contains charcoal and yeast extract to enhance the growth of Legionella. Charcoal also serves to absorb toxic metabolic products. Ferric pyrophosphate and L-cysteine are added to satisfy the specific nutritional requirements of Legionella.

- Respiratory secretions may be thin, clear and watery; thus, sputum should not be screened for adequacy when testing for legionella
- Delayed specimens should not be rejected as the legionella may survive for several days

(48hrs at 5°C)

• PCR was positive for Legionella pneumophila





Case 3. A 21-year-old army recruit, who is now in the middle of his boot camp training, is found to have confusion, a stiff neck and a fever of 39.2°C (102.5°F).

- What diagnosis do you suspect?
- What laboratory studies would you initiate?



- What diagnosis do you suspect?
 meningitis
- What laboratory studies would you initiate?
 - Spinal tap (is CT needed before tap)?
 - which tube(s) should be sent to micro?
 - what do you expect PMN values to be?
 - Glucose, protein?
 - Blood cultures
 - What organism or organisms would you suspect if there had been an outbreak of meningitis in the recruits in the boot camp ?



Routine CSF Evaluation for Bacterial Meningitis

Primary bacterial studies:

- Evaluation of the CSF is obligatory (never submit tube #1 to micro; always submit additional tube with CSF fluid, if possible, for additional studies if needed):
 - White count with differential
 - o Glucose
 - o Protein
 - Bacterial culture (automatically includes Gram stain)
- Blood cultures x 2 or 3

Note: Most of the bacterial meningitis cases are characterized by CSF with PMN pleocytosis, low glucose and high protein. However, early meningitis may have normal values and culture is always indicated

Note: Secondary bacterial studies ONLY if culture is negative at 24 hrs or if patient was treated prior to collection of CSF and Gram Stain is negative:

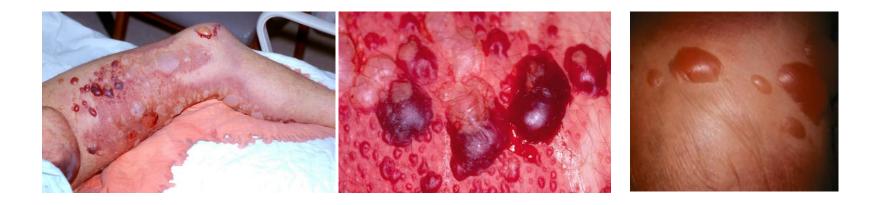
- Antigen testing of CSF for :
 - Streptococcus pneumoniae
 - Neisseria meningitidis







- A 68-yr-old patient arrives at ED in sepsis. His lower extremity has lesions, with bullae and crepitus.
- WBC count was 8,900 cells/mm3 with 47% polys and 38% bands
- X-ray reveals extensive air in soft tissue
- Significantly, his wife tells the ED doctor that the patient has advanced colon cancer



• He is immediately sent to the OR for debridement and started on broad spectrum Rx



What about Wound Cultures

- What do we need to consider?
- Location? Type? Severity? Anything else?
- Is culture necessary? How to culture?
- Are blood cultures needed?



Clinical and Microbiological Relevance

- Wound specimens:
 - Normally sterile sites / tissue, pus
 - Topical : ulcers, diabetic foot, mixed colonization (only after debridement) careful attention to organism types and numbers
 - Pay attention to interpretation of results

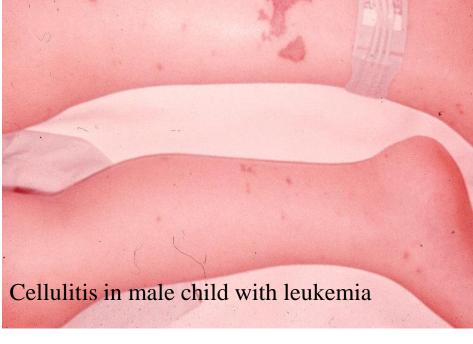
Diagnosis of Wound Infections

- CBC, Blood cultures X 2-3 in severe infections
- Culture and Gram stain of any drainage from lesion (PUS is good)
- Needle aspiration
- Punch biopsy (advancing margin)
- But do not use swabs (even on pus)
 - DO NOT SWAB infected pressure ulcers or diabetic foot wounds

Stevens, et al. CID 2005;41:1373



Cellulitis





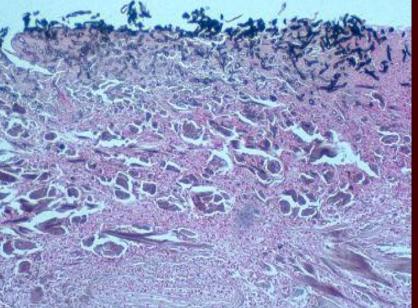


Obtaining Specimens for Culture

- Culture in cellulitis infrequently positive (neg. in 75-80% of cases; including blood cultures, < 5% are positive; except in immunocompromised e.g., Ecthyma gangrenosum)
- Gram stain and culture should be obtained from other SSTIs (except perhaps diabetic feet and infected pressure ulcers)
- Tissue or pus are superior specimens
 - Tissue biopsy or curettage (scraping with a scalpel blade) are better sources for culture
 - Needle aspiration of pus : (do not use swabs)

Granulated tissue and ulcer





Histopathology of DF ulcer and fungal elements (X450)



Ulcers

Diabetic foot ulcer





Soft Tissue Specimen Collection

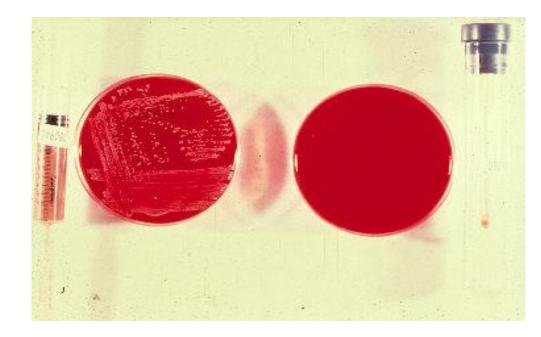
When

- · Culturing clinically uninfected lesions is unnecessary, unless done as part of an infection-control surveillance protocol (C-III).
- Cultures of infected wounds are valuable for directing antibiotic choices, but may be unnecessary in cases of acute mild infection in an antibiotic-naive patient (B-III).
- Blood cultures should be performed for a patient with a severe infection, especially if the patient is systemically ill (C-III).
 How
- · Cleanse and debride the lesion before obtaining specimens for culture.
- In cases involving an open wound, obtain tissue specimens from the debrided base (whenever possible) by means of curettage (scraping with a sterile dermal curette or scalpel blade) or biopsy (bedside or operative) (A-I).
- Avoid swabbing undebrided ulcers or wound drainage. If swabbing the debrided wound base is the only available culture option, use a swab designed for culturing aerobic and anaerobic organisms and rapidly transport it to the laboratory (B-I).
- · Needle aspiration may be useful for obtaining purulent collections or, perhaps, a specimen from an area of cellulitis.
- Clearly identify samples (specimen type and anatomic location), and promptly send them to the laboratory in an appropriate sterile container or transport media for aerobic and anaerobic culture.

From: Lipsky, et al. Clin Infect Dis 2004; 39:885



Specimen Efficacy at Recovery of Microorganisms





Swabs are notoriously poor transport systems for pus, sterile body fluids or tissue

If there is an Issue, Get Some Tissue



- A 68-yr-old patient arrives at ED in sepsis. His lower extremity has lesions, with bullae and crepitus.
- WBC count was 8,900 cells/mm3 with 47% polys and 38% bands
- X-ray reveals extensive air in soft tissue
- Significantly, his wife tells the ED doctor that the patient has advanced colon cancer
- He is immediately sent to the OR for debridement and started on broad spectrum Rx
- So, what grew out in our patient?
 - Motile Gram-positive bacilli in the anaerobic bottles of the two blood cultures submitted.

Based on patient's history, presentation and blood culture data so far, what bacterial etiology do you suspect in this case?



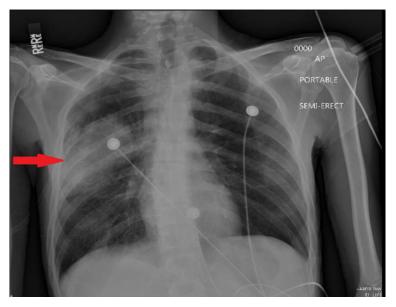
- A second, 68-yr-old patient arrives at ED in sepsis. His lower extremity has lesions, with bullae and crepitus.
- WBC count was 8,900 cells/mm3 with 47% polys and 38% bands
- X-ray reveals extensive air in soft tissue
- Significantly, his wife tells the ED doctor that the patient has advanced colon cancer
- He is immediately sent to the OR for debridement and started on broad spectrum Rx
- So, what grew out in our patient?
 - Motile Gram-positive bacilli in the anaerobic bottles of the two blood cultures submitted.
 - From this information one can highly suspect *Clostridium* spp. not *C. perfringens*
- *C. perfringens* is nonmotile (though other *Clostridium* species are motile)
- C. septicum, C. sordelii other species frequently involved with sepsis and fasciitis (often associated with cancer of the colon or other GI tract issues)





A 65-year-old woman presents with a several day history of coughing, right-sided pleuritic chest discomfort, and fever. WBC count is elevated at 18,000/mm³, with shift to the left. A chest radiograph reveals infiltrate in the right middle lobe, compatible with pneumonia.

Would you perform a blood culture on this patient? What if the patient is sick enough to be admitted? What respiratory specimen(s) would you submit for culture if any?

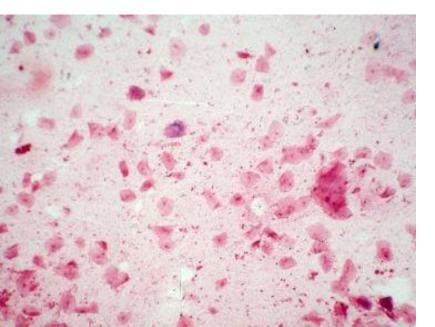


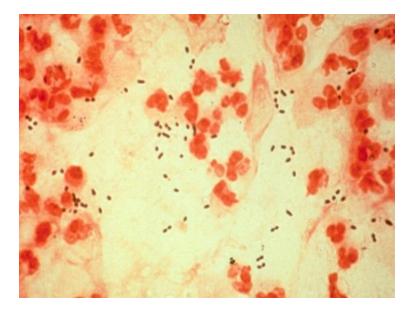


Specimens:

Sputum if patient is productive Blood cultures if patent admitted

How would you interpret this Gram-stain of sputum (X11200)?





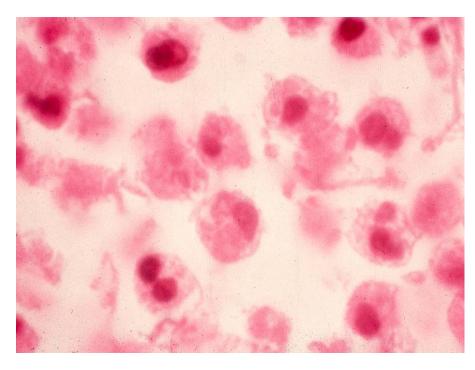
How would you interpret this Gram-stain of sputum (X450)?

Clinical and Microbiological Relevance

- Lower Respiratory Tract
 - sputum: routine culture screen for WBCs/ squamous epithelial cells (expectorated, induced, endotracheal aspirate)
 - Most groups use <10-12 epis, > 25 WBCs per LPF
 - Work up only organism(s) associated with WBCs as indicated by Gram-stain
 - Protected-Brush Bronchoscopy or Bronchoalveolar lavage: quantitative culture and cytospin-centrifuged Gram-stain (intracellular organisms)

What would you think of this Gram-stain of sputum, or other lower respiratory tract secretions?

What other causes would you consider?



It is always better to think in advance and see where the thoughts might take you. Think before you act.



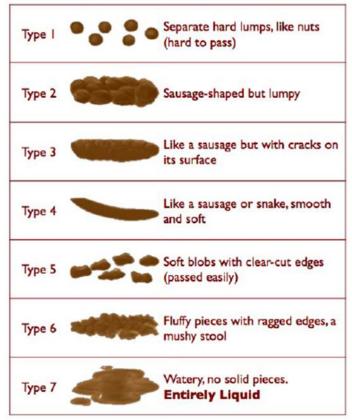
The END

3:04



Appropriate specimen types for C. difficile testing

Bristol Stool Chart



The Brecher Guidelines

- If it ain't loose, it's of no use
- Put a lab stick in the stool:
 If the stick stands, the test is banned; if the stick falls, test them all
- And my favorite so far...
 "If you can't slurp it with a straw it ain't diarrhea"

An anonymous physician from Tampa General Hospital