

PathMD™: Board Review Letter

Author: Jennifer Linder, DO & Lynda Bradshaw, MT (ASCP) Microbiology – Part 1

Volume 2, Number 6

1. You receive plates from a respiratory specimen that exhibit no growth on a blood agar plate and growth of small gray colonies on a chocolate plate. You perform a gram stain, which reveals small, pleomorphic gram-negative rods. Based on these results you conclude that this organism is a *Haemophilus* species. To further subtype the organism, you test for factor requirements and hemolysis. The next day, you examine the plate and based on the growth, you determine the organism is *H. influenzae*. What growth pattern most closely resembles the expected findings for this organism?

- a. Growth and hemolysis, does not require factor X, does not require factor V
- b. Growth and hemolysis, requires both factors X and V
- c. Growth and hemolysis, does not require factor X, requires factor V
- d. Growth but no hemolysis, requires both factors X and V
- e. Growth but no hemolysis, does not require factor X, requires factor V

Answer: D. *Haemophilus influenzae* type b is one of the leading causes of bacterial meningitis worldwide, especially in young unvaccinated children. Infection with *Haemophilus* can also cause cellulitis, pericarditis and pneumonia. Pneumonia is typically caused by non-type b strains, most commonly serotype f. *Haemophilus* is a gram negative rod. Differentiating species can be accomplished by testing for factor requirements and observing hemolysis. All *Haemophilus* species isolated from humans require factor V. *H. influenzae* does not show hemolysis on blood agar and requires factor X. An alternate method for testing for factor X requirement is the porphyrin test. In addition, *Haemophilus* is one of the few organisms that show satellite growth around a streak of *Staphylococcus aureus*. (Murray, Patrick R, et al. Manual of Clinical Microbiology 9th Edition, Volume 2. Washington DC: ASM Press, 2007, pp.636-648)

2. You receive a proficiency test unknown in your microbiology lab. You are told the specimen is from a wound, so you plate the specimen onto your four standard agars. Pictured are the colonies on MacConkey agar. Based on these findings, you perform an indole test and find that it is positive. What is the MOST LIKELY organism?

- a. *Citrobacter freundii*
- b. *Klebsiella pneumoniae*
- c. *Pseudomonas aeruginosa*
- d. *Klebsiella oxytoca*
- e. *Proteus*

Answer: D. The colonies pictured are growing on MacConkey agar and are pink, which indicates that they are lactose positive. Of the choices, only the *Klebsiella* and *C. freundii* are lactose positive. The colonies are mucoid, which is most characteristic of *Klebsiella* species due to their capsule. Differentiation between *K. pneumoniae* and *K. oxytoca* can be made by testing for indole. *K. pneumoniae* is negative for indole while *K. oxytoca* is positive. *Klebsiella* are gram-negative rods carried in the nasopharynx and the bowel and are a frequent cause of urinary tract infections. (Murray, Patrick R, et al. Manual of Clinical Microbiology 9th Edition, Volume 2. Washington DC: ASM Press, 2007, pp.698-715)

3. In preparation for an upcoming laboratory inspection, you are reviewing your quality control procedures in the microbiology department. You have a list of atypical bacteria-antibiotic resistance profiles that are posted and should alert the technicians to perform confirmatory testing. Which of the following bacteria-antibiotic resistance combinations is INCORRECT and should prompt retesting by another method to confirm resistance?

- a. *Proteus* resistant to cefazolin
- b. *Klebsiella* resistant to ampicillin
- c. *Enterobacter* resistant to ampicillin and cefazolin
- d. *E. coli* resistant to no antibiotics
- e. *Serratia* resistant to ampicillin, cefazolin and cefuroxime

Answer: A. Strains of *Proteus* are resistant to nitrofurantoin but are susceptible to the remainder of the drugs that are commonly tested. The other microorganism-drug combinations are expected patterns of resistance. (Murray, Patrick R, et al. Manual of Clinical Microbiology 8th Edition, Volume 2. Washington DC: ASM Press, 2007, pp.693-796)

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4. Which spiral bacterium has hooks at both ends?
- Leptospira*
 - Borrelia*
 - Treponema*
 - Brachyspira*
 - Helicobacter*

Answer: A. All of the choices are spiral bacteria, but only *Leptospira* has hooked ends. *Leptospira* is a tightly coiled, right-hand spiraled bacteria. Infection of humans occurs most commonly via direct contact with infected animals through cuts on the skin or via the conjunctiva. Most infections have a mild clinical course and resolve while recognized infections present with sudden onset of fever, chills and myalgia. The minority of cases present with jaundice, acute renal failure, pulmonary hemorrhage and cardiac arrhythmias, known as Weil's disease. (Murray, Patrick R, et al. Manual of Clinical Microbiology 8th Edition, Volume 1. Washington DC: ASM Press, 2007, pp.589-599)

5. You finalize a report from a skin wound as no growth after 5 days. The clinician calls and he is concerned that the specimen was not cultured correctly, as he was certain there was adequate material upon collection. He states that the patient had an abscess on the thigh and upon drainage, there was 25ml of purulent fluid expressed from the lesion. Following the drainage, a swab culture was taken for microbiology. The clinician sent the swab for gram stain, aerobic and anaerobic cultures. Your BEST response to address the clinician's concern is:
- Occasionally organisms just do not grow and we cannot explain why. Ask the clinician to send another swab and the lab will repeat the cultures.
 - A swab should only be used if no other type of specimen can be submitted and in that case, one swab should be submitted for each type of culture
 - Swabs never contain adequate material and should not be used for culturing specimens in the microbiology laboratory
 - Occasionally organisms just do not culture and we cannot explain why. Tell the clinician you will re-culture the specimen from the original swab
 - Tell the clinician you will allow the plates to incubate for another 3 days to see if there will be delayed growth and then issue an amended report at that time

Answer: B. Preferred specimens for microbiologic culture include fresh tissue, fluid, exudates and instrumentation (i.e. screws, plates, catheter tips). A specimen should be submitted to microbiology on a swab only when there is no availability of other types of specimens. Material submitted on a swab often yields an inadequate amount of material for culture. To increase the likelihood of having enough material for culture on a swab, one swab should be submitted for each type of culture the clinician desires. For example, one swab should be submitted for each of the following: Gram stain, aerobic culture and anaerobic culture, for a total of three swabs. (Murray, Patrick R, et al. Manual of Clinical Microbiology 8th Edition, Volume 1. Washington DC: ASM Press, 2007, pp.55-66)

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6. You receive a respiratory specimen in the laboratory that is labeled bronchial washing and has been obtained by bronchoscopy. As standard procedure in your lab, you culture the specimen to a blood and buffered charcoal yeast extract plate. On the third day, you examine the plates and notice colonies growing that you would describe as having a ground-glass appearance. You perform a Gram stain and see tiny gram-negative rods. There is no growth on the blood plate. What is the MOST LIKELY organism growing on the BCYE plate?
- Neisseria gonorrhoeae*
 - Mycoplasma pneumoniae*
 - Moraxella catarrhalis*
 - Burkholderia cepacia*
 - Legionella pneumophila*

Answer: E. *Legionella* is a fastidious, slow growing organism. Buffered charcoal yeast extract should be used to allow for the growth of the *Legionella* organism. The cultures must be observed for 7 days due to the slow rate of growth of this organism. *L. pneumophila* is the most common organism and colonies have a ground-glass appearance with iridescent edges. Gram stain of the organism reveals tiny gram-negative rods. Hospital acquired *Legionella* most often originates in the water distribution system and environmental studies should be performed in these cases. (Murray, Patrick R, et al. Manual of Clinical Microbiology 8th Edition, Volume 1. Washington DC: ASM Press, 2007, pp.809-816)

7. You isolate a *Staphylococcus aureus* on a specimen from a wound. You test for Erythromycin susceptibility and find that the organism is resistant. You perform a D-test disk diffusion test to evaluate clindamycin inducible resistance. Based on the results pictured, can you treat this patient's infection with clindamycin?
- No. The discs are too close together and therefore the test is not valid
 - No. The zone of resistance around the clindamycin disc indicates that the bacteria is resistant to clindamycin
 - No. Because there is no zone of inhibition around the erythromycin disc, the test is not applicable
 - No. The flattening of the clindimycin zone of inhibition near the erythromycin disc indicates the organism is resistant to clindamycin
 - Yes. The size of the zone of inhibition surrounding the clindamycin disc indicates that the bacteria is susceptible to clindamycin

Answer: D. D Zone Test for Inducible Clindamycin Resistance in Methicillin Resistant *Staphylococcus Aureus* (MRSA) <http://www.medal.org/visitor/www%5CActive%5Cch25%5Cch25.01%5Cch25.01.08.aspx>

Clindamycin is an excellent antibiotic for the treatment of methicillin sensitive *Staphylococcal* infections. For the test to be valid, there must be a zone of inhibition around the clindamycin and no zone around the erythromycin. For the test to be positive, inferring that the organism is resistant to clindamycin, there must be flattening of the zone of inhibition around the clindamycin, nearest the erythromycin disc. (Murray, Patrick R, et al. Manual of Clinical Microbiology 8th Edition, Volume 1. Washington DC: ASM Press, 2007, pp.1052)

8. You isolate an *Enterococcus faecium* from a urine specimen. Standard susceptibility testing is performed. What is the MOST IMPORTANT antibiotic pattern to look for initially?
- Vancomycin resistance
 - Clindamycin inducible susceptibility
 - Aztreonam susceptibility
 - Trimethoprim-sulfamethoxazole susceptibility
 - Methacillin resistance

Answer: A. Vancomycin or penicillin is typically combined with an aminoglycoside for the treatment of an enterococcal infection. Recently, there is an increased incidence of organisms with acquired antimicrobial resistance to vancomycin. In the lab, it is important to test Enterococcal species for resistance to vancomycin. (Murray, Patrick R, et al. Manual of Clinical Microbiology 8th Edition, Volume 1. Washington DC: ASM Press, 2007, pp.427-428)

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9. You receive a cultured specimen on a MacConkey plate. Based on the growth pictured, you perform an oxidase test, which is positive. What is the BEST organism identification for this specimen?
- Proteus mirabilis*
 - Enterobacter cloacae*
 - Pseudomonas aeruginosa*
 - Klebsiella pneumoniae*
 - Escherichia coli*

Answer: C. Based on the picture, the organism growing on the plate is lactose negative. Of the choices, the only organisms that fall into this category are *Pseudomonas* and *Proteus*. Differentiation can be made by an oxidase test, which is positive with *Pseudomonas* and negative with *Proteus*. *Pseudomonas* is a Gram-negative rod that is slightly curved and has one or more flagella. The organisms are lactose non-fermenters on MacConkey agar, oxidase positive and catalase positive. *Pseudomonas aeruginosa* is known to cause folliculitis in association with swimming pools and whirlpools, “swimmers ear”, as well as infections in patients with cystic fibrosis. (Murray, Patrick R, et al. Manual of Clinical Microbiology 8th Edition, Volume 1. Washington DC: ASM Press, 2007, pp719-722)

10. A urine specimen from a middle aged female is received in the lab and grows small white colonies on sheep blood agar that are coagulase negative and positive for catalase. The Gram stain reveals Gram-positive cocci in clusters. The organism is plated onto a Mueller-Hinton agar and a Novobiocin disc is placed onto the plate. The next morning, the plate is examined and shows no zone of inhibition. What is the MOST LIKELY organism growing on this plate?
- Staphylococcus epidermidis*
 - Staphylococcus aureus*
 - Staphylococcus saprophyticus*
 - Staphylococcus lugdunensis*
 - Staphylococcus haemolyticus*

Answer: C. Many of the different species of *Staphylococcus* can be differentiated by simple testing. The coagulase negative *Staphylococcus* organisms include *S. epidermidis* and *S. saprophyticus*. While both organisms are catalase positive, differentiation can be made using Novobiocin. *S. saprophyticus* is resistant while *S. epidermidis* is sensitive. (Murray, Patrick R, et al. Manual of Clinical Microbiology 9th Edition, Volume 2. Washington DC: ASM Press, 2007, pp.384-394)

Notes for question set:¹

¹ PathMD strives for the highest quality and accuracy. However, the *PathMD: Board Review Letter* is for review purposes and not meant for clinical decision making. It should not be used in place of review of primary reference texts and the current medical literature. If inaccuracies are identified, please notify us so that a correction may be published. (info@PathMD.com)