

PathMD™: Board Review Letter

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Microbiology – Part 2

Volume 2, Number 9

1. Small white colonies are growing on a blood agar plate. You are suspicious of *Staphylococcus aureus* and attempt to confirm the diagnosis with a catalase and coagulase. The catalase is positive but the coagulase is negative. Despite the result of the coagulase, you are still highly suspicious that the organism is *S. aureus*. You set up a tube coagulase test to investigate. After 4 hours, you note a firm clot in the tube. You continue incubation and check the tube after 24 hours and notice the clot has dissolved. Your control tubes have appropriate reactions. What is the BEST interpretation of this tube coagulase test?
 - a. The specimen is not *S. aureus*, as the clot should not form before 4 hours
 - b. The specimen is not *S. aureus*, as a formed clot should not dissolve before 24 hours
 - c. The specimen is *S. aureus*, as any clot in the tube within the 24 hours is a positive test
 - d. The specimen is *S. aureus*, as the clot must form before 4 hours and dissolve before 24 hours
 - e. This test is invalid because a clot formed before 4 hours

Answer: C. The tube coagulase test is the definitive test for coagulation. The tube test detects both free and bound coagulase. The tube coagulase test is positive if any clot forms in the tube at any time during the incubation period. Some clots may develop quickly, especially within the first 4 hours. With time, the clot can lyse if the organism produces fibrolysin. This is important to remember, as tubes should not only be checked after 24 hours. The tubes should be checked at 4 hours and if no clot has yet formed, the tubes should incubate to 24 hours and be rechecked. A positive and negative control should be run with the test. The slide coagulase test detects only cell-associated clumping factor, also known as bound coagulase.

W.H. Sperber and S.R. Tatini. Interpretation of the Tube Coagulase Test for Identification of *Staphylococcus aureus*. Appl Microbiol 1975 April; 29(4): 502–505.

Murray, Patrick R, et al. Manual of Clinical Microbiology 8th Edition, Volume 1. Washington DC: ASM Press, 2007, pp.492.

2. A clinician calls for a preliminary report on a wound specimen. As the resident on microbiology, you are asked to review the Gram stain made from the specimen. Based on the image, what is the MOST APPROPRIATE interpretation of this stain?
 - a. Gram positive rods in chains are present, issue a preliminary report
 - b. The specimen is under-decolorized and should be repeated before giving the clinician an interpretation
 - c. The specimen was inappropriately collected, ask the clinician to recollect the specimen
 - d. The Gram stain was over-decolorized and should be repeated before giving the clinician any information
 - e. In preparation of the Gram stain, saffarin was never placed onto the slide

Answer: B. In evaluating a Gram stain, epithelial cells and/or white blood cells are built in controls that will help you discern that the process was performed appropriately. In a Gram stain, epithelial cells/ WBC's should stain pink. If the stain is under-decolorized, as in this case, the epithelial cells are blue. These cells are not as useful in interpreting over-decolorization, as they will be pink in both appropriately decolorized and over-decolorized specimens. If saffarin was not used, there would be no evidence of pink coloration and the epithelial/ WBC's would be colorless.

A detailed description of the Gram stain procedure can be found at <http://www.bd.com/ds/productCenter/212539.asp>

Murray, Patrick R, et al. Manual of Clinical Microbiology 8th Edition, Volume 1. Washington DC: ASM Press, 2007, pp.363.

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3. A patient is admitted to the hospital and the clinician orders a nasal swab to screen for methicillin resistant *Staphylococcus aureus* (MRSA). The swab is received in the microbiology laboratory and is plated onto a CHROMagar MRSA plate. Based on the growth you see in the image, you perform a latex agglutination test, which is positive. The BEST interpretation of this specimen is:
- The growth indicates that the organism is a methicillin resistant *Staphylococcus aureus*
 - The growth, not the color, indicates that the organism is *Staphylococcus aureus* and that it is sensitive to methicillin
 - The color of the organisms indicate that the organisms growing on the plate are methicillin resistant *Staphylococcus aureus*
 - The color of the organisms indicate that the organisms growing on the plate are methicillin sensitive *Staphylococcus aureus*
 - The color of the organism growing on the plate is suggestive of growth of a red pigmented *Serratia* species

Answer: C. CHROMagar MRSA is a qualitative method to detect nasal colonization by methicillin resistant *S. aureus*. This test is only a screening test that is performed on anterior nares swab specimens. CHROMagar is a selective and differential medium that incorporates cefoxitin and specific chromogenic substances to detect MRSA. Growth alone does not confirm that the organism is methicillin resistant *S. aureus*. The mauve-colored colonies confirm methicillin resistant *S. aureus*. If the organisms are not pink, the growth is not methicillin resistant *S. aureus*. Organisms other than MRSA can grow and produce a blue or green color, white or no color at all. Coagulase positivity can then be confirmed. *Serratia* should not grow on this selective media.

Murray, Patrick R, et al. Manual of Clinical Microbiology 8th Edition, Volume 1. Washington DC: ASM Press, 2007, pp.370.

BD BBL CHROMagar MRSA Package Insert: www.bd.com/ds/productcenter/215084.asp

4. You identify a *Klebsiella pneumoniae* on a plate from a respiratory specimen. To test the sensitivities for the organism you use an automated minimum inhibitory concentration (MIC) device. The sensitivity results are ready the next morning and you review the report before sending out the final results. The report shows that the organism is sensitive to all antibiotics. What is your MOST APPROPRIATE next step?
- Finalize the report with the given sensitivities and send the report to the clinician
 - Leave the sample on the automated MIC device for another 24 hours to give the machine more time to evaluate the sensitivities
 - Finalize the report, stating that the organism is resistant to Ampicillin, despite the MIC results, since you know that all *Klebsiella* are intrinsically resistant
 - Replate, incubate and repeat you initial tests that were used to identify the organism as *Klebsiella pneumoniae*
 - Repeat sensitivities with an alternate method, such as Kirby Bauer disc diffusion

Answer: E. Ampicillin is a β -lactam antibiotic, for which *Klebsiella* species are intrinsically resistant. *Klebsiella* organisms are typically sensitive to all other antibiotics. This result is most likely inaccurate, and should not be reported without rechecking the results. Leaving the specimen on the automated MIC device is not effective and will not yield correct results. The best approach for an automated MIC result of a *Klebsiella* organism that does not show resistance to Ampicillin is to manually check the sensitivity, by a method such as Kirby Bauer disc diffusion. Some labs will also repeat the sensitivities on the automated MIC device.

Murray, Patrick R, et al. Manual of Clinical Microbiology 8th Edition, Volume 1. Washington DC: ASM Press, 2007, pp 693.

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5. While examining the plates from a wound specimen that came from the operating room, you identify tiny, white creamy colonies that appear to be yeast, mixed with some larger white colonies. You notice that the plates were cultured late the previous evening, but you do not want to delay your identification and susceptibility another day. You decide to carefully isolate only the larger colonies and perform disc diffusion susceptibility testing on a Mueller-Hinton plate. Based on the image, what is the BEST interpretation of this test?
- Based on the growth of small colonies within the zone of inhibition, the organism is susceptible to TE
 - Based on the growth of small colonies within the zone of inhibition, the organism is resistant to TE
 - The growth of small colonies within the zone of inhibition indicates that there is mixed growth
 - The growth of small colonies within the zone of inhibition indicates that the organism has a delayed resistance to TE
 - The growth of small colonies within the zone of inhibition indicates that the organism has delayed susceptibility to TE

Answer: C. See answer to question 6

Murray, Patrick R, et al. Manual of Clinical Microbiology 8th Edition, Volume 1. Washington DC: ASM Press, 2007, pp. 1251

6. You receive a call from the clinician regarding the previous case. She wants to know if the organism is sensitive to TE. You tell her the zone of inhibition is within the sensitive range but there is mixed growth on the plate. She asks if the sensitivity is valid, since there is mixed growth. What is your BEST response?
- The mixed growth invalidates the sensitivity result for TE
 - The organism is sensitive to TE and can be reported as such, despite the mixed growth
 - The mixed growth can cause a false increase in the zone of inhibition for TE and should not be reported as sensitive
 - The mixed growth can cause a false decrease in the zone of inhibition for TE and the result can confidently be reported as sensitive
 - The organism is most likely sensitive to TE despite the mixed growth but the sensitivity should be performed again on a pure culture

Answer: E. This picture is an example of mixed growth. A report should not be finalized when mixed growth is seen. Growth of colonies within the clear zone typically indicates mixed growth, and colonies showing mixed growth should be subcultured, reidentified and retested. Occasionally, this picture can indicate mutant organisms growing in the clear zone, which will eventually become resistant to the antibiotic. For staphylococci and enterococci only, growth within the zone of inhibition around an oxacillin or vancomycin disc is indicative of resistance.

Murray, Patrick R, et al. Manual of Clinical Microbiology 8th Edition, Volume 1. Washington DC: ASM Press, 2007, pp. 1251

7. A urine specimen from a middle aged female in received in the microbiology laboratory. You notice on the patient's history that her chief complaint is urinary tract infection symptoms with alkaline urine. You examine the blood agar plate and see >100 k/ml of pinpoint smooth whitish-gray colonies growing on the aerobic blood agar plate. There is no growth on the MacConkey plate. You decide to make a Gram stain and notice that as you try to pick a colony from the plate, the colony is very sticky and difficult to sample. While you wait for the Gram stain to dry, you perform a catalase, which is positive. Examination of the Gram stain reveals small slightly curved Gram positive rods. Based on the Gram stain and other findings, what is the MOST LIKELY diagnosis?
- Staphylococcus saprophyticus*
 - Corynebacterium urealyticum*
 - Escherichia coli*
 - Enterococcus fecalis*
 - Stenotrophomonas maltophilia*

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Based on the image and the diagnosis, what type of crystals are commonly found in the urine of these patients?

- a. Calcium oxalate
- b. Tyrosine
- c. Urate
- d. Struvite (triple phosphate)
- e. Cystine

Answer 7a: B.

Answer 7b: D. *C. urealyticum* is one of the more frequently isolated clinically significant *Corynebacterium* species in clinical specimens, often responsible for urinary tract infections. Gram stain reveals Gram positive, slightly curved rods. *C. urealyticum* is catalase positive and strongly urease positive. *C. urealyticum* urine splitting capability results in alkaline urine and the formation of struvite crystals, which have the easily identifiable “coffin-lid” appearance. Colonies tend to be sticky when sampling from agar and are therefore difficult to remove.

Cystine crystals are hexagonal. Tyrosine crystals are needle like. Calcium oxalate crystals are square with a three dimensional point and resemble envelopes. Urate crystals are small, clear, rounded circular objects often with striations radiating from the center.

Murray, Patrick R, et al. Manual of Clinical Microbiology 8th Edition, Volume 1. Washington DC: ASM Press, 2007, pp. 477,492.

8. You receive a blood agar plate and notice small white colonies surrounded by a zone of hemolysis. You suspect the organism growing on the plate is a *Streptococcus* and you confirm your suspicion with Gram stain and negative catalase and negative coagulase tests. You type the *Streptococcus* and find that it is a Lancefield group B *Streptococcus*. What are the identified organism and the typical response to penicillin?
- a. *Streptococcus agalactiae*, resistant to penicillin
 - b. *Streptococcus pyogenes*, resistant to penicillin
 - c. *Streptococcus agalactiae*, sensitive to penicillin
 - d. *Streptococcus pyogenes*, sensitive to penicillin
 - e. *Streptococcus bovis*, resistant to penicillin

Answer: C. *Streptococcus agalactiae* is Lancefield group B while *S. pyogenes* is Lancefield group A and *S. bovis* is Lancefield group D. Of the two answer choices for *S. agalactiae*, C is the best choice. *Streptococcus* organisms are universally sensitive to penicillin, which is the drug of choice in these infections. Susceptibility testing is typically not performed, unless the patient has a penicillin allergy. *S. agalactiae* can be acquired by a newborn at birth, from vaginal colonization, which can result in an early onset of pneumonia and sepsis or a late onset of meningitis (associated with serotype III). Adults infected with *S. agalactiae* can present with meningitis (serotype II). Other manifestations include endocarditis, arthritis and urinary tract infection.

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9. Culture plates are made from the blood culture specimen of a 35-year-old man who clinically appears septic. The following day you examine the plates and notice small translucent glistening colonies growing on the Chocolate agar. You perform a Gram stain and see abundant gram-negative cocci in pairs and are suspicious of *Neisseria*. To identify the organism, you decide to culture the colonies to another media and/or perform additional tests. Which of the following results is LEAST LIKELY to be helpful?
- Indole positivity
 - Growth on Modified Thayer Martin media
 - Butyrate esterase disk negativity
 - Oxidase positivity
 - Catalase positivity

Answer: A. The major differentials in this question include *Neisseria*, *Moraxella* and *Acinetobacter*. Thayer Martin is a useful way to selectively grow all three of these organisms and eliminate other less common diplococci organisms. *Acinetobacter* can be eliminated from the differential by performing the oxidase and catalase, which would both be negative. Both *Moraxella* and *Neisseria* are positive for oxidase and catalase. *Moraxella* can be differentiated from *Neisseria* by performing a butyrate esterase disk test (Catarrhalis test disk), which has a positive blue color change for *M. catarrhalis* and no color change for *Neisseria*.

Murray, Patrick R, et al. Manual of Clinical Microbiology 8th Edition, Volume 1. Washington DC: ASM Press, 2007, pp.589-599.

Catarrhalis Test Disk Package Insert (IFU 21121). Remel, Lenexa, KS USA. Revised September 13, 2006.

10. After confirming that the organism is *Neisseria*, you are asked to determine if the species is *gonorrhoeae* or *meningitidis*. You decide the best method for differentiating is by testing for fermentation of different sugars. Which of the following patterns would be INDICATIVE of *N. meningitidis*?
- Glucose negative, lactose negative, maltose positive, sucrose negative
 - Glucose positive, lactose negative, maltose negative, sucrose negative
 - Glucose positive, lactose negative, maltose positive, sucrose positive
 - Glucose positive, lactose negative, maltose positive, sucrose negative
 - Glucose negative, lactose negative, maltose negative, sucrose positive

Answer: D. Once you determine that the organism is *Neisseria* species, differentiation between *N. gonorrhoeae* and *N. meningitidis* can be made by examining acidification properties with different carbohydrates. *N. gonorrhoeae* acidifies only glucose (**G**, *gonorrhoeae* and **g**lucose) while *N. meningitidis* acidifies both glucose and maltose (**M** and **G**, in **m**altose and **g**lucose and *meningitidis*). *N. meningitidis* most commonly is present in the CSF and the blood, causing meningitis and sepsis.

Murray, Patrick R, et al. Manual of Clinical Microbiology 8th Edition, Volume 1. Washington DC: ASM Press, 2007, pp. 588,593-4.

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)