


# 02 – Core Concepts: Microbiology: What You Need to Know for the Exam


Speaker: Robin Patel, MD



## Core Concepts: Microbiology: What You Need to Know for the Exam

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### Disclosures of Financial Relationships with Relevant Commercial Interests

- Grants: MicuRx Pharmaceuticals, BioFire
- Consultant: PhAST, Day Zero Diagnostics, Abbott Laboratories, Sysmex, DEEPULL DIAGNOSTICS, S.L., Netflix, Oxford Nanopore Technologies, HealthTrackRx, CARB-X
- Patent: *Bordetella pertussis/parapertussis* PCR issued; Device/method for sonication with royalties paid by Samsung to Mayo Clinic; Anti-biofilm substance issued
- Honoraria: Up-to-Date

## MALDI ToF Mass Spectrometry




1. Add colony

2. Add Formic Acid and Dry; Add Matrix and Dry

3. Analyze

4. View mass spectrum



## MALDI ToF Mass Spectrometry


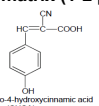
1. Add colony
2. Add matrix (1-2 µl)

NC(=O)C(O)C1=CC=C(O)C=C1

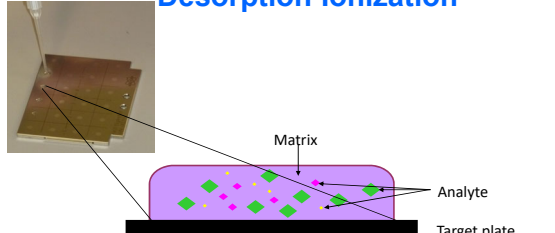
o-cyano-4-hydroxybenzoic acid (CHCA)

Dissolved in acetonitrile (50%) & 2.5% trifluoroacetic acid

3. Dry – room air 5 min




## Matrix Assisted Laser Desorption Ionization



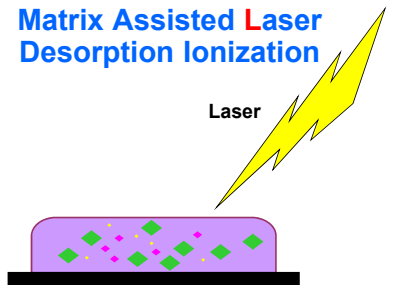
Matrix

Analyte

Target plate



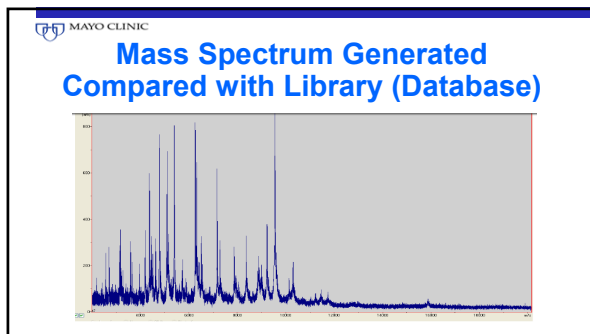
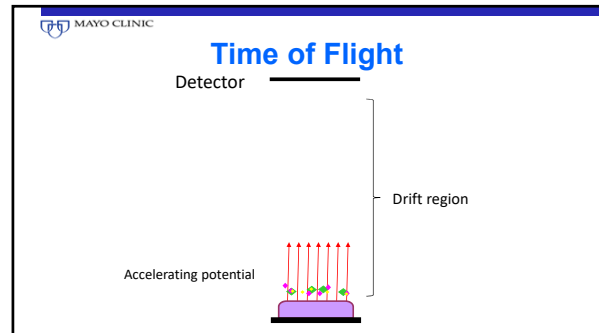
## Matrix Assisted Laser Desorption Ionization




Laser

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**QUESTION #1**  **PREVIEW QUESTION**

Which of the following will not grow on sheep blood, chocolate and/or MacConkey agar?

- A. *Granulicatella adiacens*
- B. *Bordetella pertussis*
- C. *Brucella melitensis*
- D. *Vibrio cholerae*
- E. *Abiotrophia defectiva*

**QUESTION #1**  **PREVIEW QUESTION**

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- C. *Brucella melitensis*
- D. *Vibrio cholerae*
- E. *Abiotrophia defectiva*

**BACTERIA REQUIRING SPECIALIZED MEDIA**

- *Bordetella pertussis*
- *Brucella* species (+/-)
- *Burkholderia pseudomallei* (+/-)
- *Campylobacter* species
- *Francisella tularensis* (+/-)
- *Helicobacter pylori*
- *Legionella* species
- *Mycoplasma* species (+/-)
- *Ureaplasma* species

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## QUESTION #2

Which of the following bacteria may stain acid-fast positive?

- A. *Rhodococcus* species
- B. *Cutibacterium* species
- C. *Finegoldia* species
- D. *Microbacterium* species
- E. *Wolbachia* species

## QUESTION #2

Which of the following bacteria may stain acid-fast positive?

- A. *Rhodococcus* species \*
- B. *Cutibacterium* species
- C. *Finegoldia* species
- D. *Microbacterium* species
- E. *Wolbachia* species

## ACID-FAST BACTERIA (MYCOLIC ACIDS)

- *Mycobacterium* species
- "Modified" acid fast stain positive
  - Weaker decolorizing agent (0.5-1% sulfuric acid in place of 3% acid-alcohol); do not stain well with Ziehl-Neelsen or Kinyoun stain
    - *Nocardia* species
    - *Rhodococcus* species
    - *Gordonia* species
    - *Tsakamurella* species
    - *Dietzia* species
- *Legionella micdadei* and some *Corynebacterium* species
  - [But not *Cutibacterium* species]

## QUESTION #3

A laboratory technologist who has a longstanding history of diabetes mellitus inadvertently opens the lid of an agar plate growing an organism which is subsequently determined to be *Burkholderia pseudomallei*.

You are asked to make a recommendation regarding postexposure prophylaxis.

## QUESTION #3

Which of the following would you recommend?

- A. Trimethoprim-sulfamethoxazole
- B. Amoxicillin
- C. Streptomycin
- D. Cephalexin
- E. None

## QUESTION #3

Which of the following would you recommend?

- A. Trimethoprim-sulfamethoxazole \*
- B. Amoxicillin
- C. Streptomycin
- D. Cephalexin
- E. None

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## *Burkholderia pseudomallei*

- Postexposure antimicrobial prophylaxis
- Trimethoprim-sulfamethoxazole
- Doxycycline
- Amoxicillin–clavulanic acid

Peacock SJ et al. Emerg Infect Dis. 2008 Jul <http://wwwnc.cdc.gov/eid/article14/7/07-1501>

## QUESTION #4

Which of the following, if present in a clinical specimen, poses a hazard for laboratory personnel?

- A. *Entamoeba histolytica*
- B. *Trichuris trichiura*
- C. *Enterobius vermicularis*
- D. *Strongyloides stercoralis*
- E. *Babesia microti*

## QUESTION #4

Which of the following, if present in a clinical specimen, poses a hazard for laboratory personnel?

- A. *Entamoeba histolytica*
- B. *Trichuris trichiura*
- C. *Enterobius vermicularis*
- D. *Strongyloides stercoralis* \*
- E. *Babesia microti*

## *Strongyloides stercoralis*

- Larvae - two forms
  1. Rhabditiform (in stool)
  2. Filariform

Infectious stage that develops in soil and occasionally in patient (leads to autoinfection and is hazardous to laboratory personnel)

- Larvae detected
  - Microscopically (top) or
  - By placing feces on plate and detecting migrating larvae where they leave a trail of bacterial colonies (bottom)



## LABORATORY- ACQUIRED BACTERIAL, FUNGAL AND PARASITIC INFECTIONS (SELECTED)

- *Bacillus anthracis*
- *Brucella* species
- *Burkholderia pseudomallei* \* (*Burkholderia mallei*)
- *Coxiella burnetii*
- *Coccidioides immitis/posadasii* (*Blastomyces dermatitidis*, *Histoplasma capsulatum*)
- Dermatophytes
- Enteric pathogens
- *Francisella tularensis*
- *Mycobacterium tuberculosis*
- *Neisseria meningitidis*
- *Salmonella enterica* subsp. *enterica* serovar Typhi
- *Staphylococcus aureus*
- *Strongyloides stercoralis*
- *Yersinia pestis*

## ORGANISMS ABOUT WHICH THE LABORATORY SHOULD BE NOTIFIED IF SUSPECTED

- Avian influenza
- *Bacillus anthracis*
- *Brucella* species
- *Burkholderia pseudomallei*
- *Burkholderia mallei*
- *Clostridium botulinum*
- *Coxiella burnetii*
- *Coccidioides immitis/posadasii*
- Hemorrhagic fever viruses (e.g. Ebola, Marburg, Chapare, Crimean-Congo, Guanarito, Hanta, Junin, Kayasur Forest Disease, Lassa fever, Lujo, Machupo, Omsk Hemorrhagic Fever, Sabia)
- *Francisella tularensis*
- Measles
- MERS, SARS-CoV
- Nipah virus, Hendra virus
- Smallpox
- *Yersinia pestis*

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**FDA-APPROVED/CLEARED MULTIPLEX PANELS FOR GASTROINTESTINAL PATHOGENS IN STOOL (for reference)**

	Verigene EP	xTAG <sup>®</sup> GPP	BioFire GI <sup>®</sup>	BioCode <sup>®</sup>	Qiasoft-DX
Enterobacter species	✓	✓	✓	✓	✓
Salmonella species	✓	✓	✓	✓	✓
Shigella species/Enteroinvasive E. coli	✓	✓	✓	✓	✓
Vibrio species	✓	✓	✓	✓	✓
Vibrio vulnificus	✓	✓	✓	✓	✓
Vibrio parahaemolyticus	✓	✓	✓	✓	✓
Vibrio cholerae	✓	✓	✓	✓	✓
Yersinia enterocolitica	✓	✓	✓	✓	✓
Escherichia coli O157	✓	✓	✓	✓	✓
Enterotoxigenic E. coli	✓	✓	✓	✓	✓
Enteropathogenic E. coli	✓	✓	✓	✓	✓
Enterococci	✓	✓	✓	✓	✓
Shiga toxin-producing E. coli	✓	✓	✓	✓	✓
Campylobacter	✓	✓	✓	✓	✓
Neisseria	✓	✓	✓	✓	✓
Rotavirus A	✓	✓	✓	✓	✓
Adenovirus	✓	✓	✓	✓	✓
Adenovirus 40/41	✓	✓	✓	✓	✓
Staphylococcus	✓	✓	✓	✓	✓
Cryptosporidium species	✓	✓	✓	✓	✓
Enterococcus faecium	✓	✓	✓	✓	✓
Cyclospora cayentensis	✓	✓	✓	✓	✓

## GASTROENTERITIS PANEL TESTING KEY POINTS

- If available, culture independent methods of diagnosis recommended
- Indications: Dysentery, moderate-to-severe disease, and symptoms lasting >7 days (define etiology, inform potential treatment)
- Not recommended for chronic diarrhea
- If *C. difficile* main consideration, test for *C. difficile* alone
- *Aerococcus* species not included

Riddle et al. Am J Gastroenterol 2016;111:602-622

**BIOFIRE FILMARRAY MENINGITIS/ENCEPHALITIS PANEL (for reference)**

Viruses	Bacteria	Fungi
Cytomegalovirus	<i>Escherichia coli</i> K1	<i>Cryptococcus neoformans/gattii</i>
Enterovirus	<i>Haemophilus influenzae</i>	
Herpes simplex virus 1	<i>Listeria monocytogenes</i>	
Herpes simplex virus 2	<i>Neisseria meningitidis</i>	
Human herpes virus 6	<i>Streptococcus agalactiae</i>	
Human parechovirus	<i>Streptococcus pneumoniae</i>	
Varicella zoster virus		

## MENINGITIS/ENCEPHALITIS PANEL KEY POINTS

- Doesn't nullify need for cell count, differential, protein, glucose, Gram stain, culture
- Cryptococcal antigen more sensitive than PCR
- *Streptococcus pneumoniae* antigen plus HSV, enterovirus and possibly VZV PCR an alternative
- May be helpful with current/recent antibiotic treatment
- HHV-6 & CMV may not be clinically significant

**Lower Respiratory Tract Panels (for reference)**

	Curetis Unyvero	BioFire		Curetis Unyvero	BioFire
<b>Bacteria</b>			<b>Viruses</b>		
<i>Acinetobacter</i> spp.	✓	✓	Influenza A	✓	✓
<i>Acinetobacter baumannii</i> complex	✓	✓	Influenza B	✓	✓
<i>Chlamydia pneumoniae</i>	✓	✓	Respiratory Syncytial Virus	✓	✓
<i>Citrobacter freundii</i>	✓	✓	Human Rotavirus/Enterovirus	✓	✓
<i>Klebsiella aerogenes</i>	✓	✓	Human Metapneumovirus	✓	✓
<i>Enterobacter cloacae</i> complex	✓	✓	Parainfluenza virus	✓	✓
<i>Escherichia coli</i>	✓	✓	Adenovirus	✓	✓
<i>Haemophilus influenzae</i>	✓	✓	Coronavirus (non-SARS-CoV)	✓	✓
<i>Klebsiella pneumoniae</i>	✓	✓	Egag	✓	✓
<i>Klebsiella pneumoniae</i> group	✓	✓	<i>Pneumocystis jirovecii</i>	✓	✓
<i>Klebsiella variicola</i>	✓	✓	<i>Rhizopus</i> genus	✓	✓
<i>Legionella pneumophila</i>	✓	✓	<i>Staphylococcus aureus</i>	✓	✓
<i>Moraxella catarrhalis</i>	✓	✓	<i>Staphylococcus epidermidis</i>	✓	✓
<i>Morganella morganii</i>	✓	✓	<i>Staphylococcus saprophyticus</i>	✓	✓
<i>Mycoplasma pneumoniae</i>	✓	✓	<i>Streptococcus pneumoniae</i>	✓	✓
<i>Proteus</i> spp.	✓	✓	<i>Streptococcus pyogenes</i>	✓	✓
<i>Pseudomonas aeruginosa</i>	✓	✓	<i>Streptococcus pneumoniae</i>	✓	✓
<i>Serratia marcescens</i>	✓	✓	<i>Streptococcus pneumoniae</i>	✓	✓
<i>Staphylococcus aureus</i>	✓	✓	<i>Streptococcus pneumoniae</i>	✓	✓
<i>Stenotrophomonas maltophilia</i>	✓	✓	<i>Streptococcus pneumoniae</i>	✓	✓
<i>Streptococcus agalactiae</i>	✓	✓	<i>Streptococcus pneumoniae</i>	✓	✓
<i>Streptococcus pneumoniae</i>	✓	✓	<i>Streptococcus pneumoniae</i>	✓	✓
<i>Streptococcus pneumoniae</i>	✓	✓	<i>Streptococcus pneumoniae</i>	✓	✓
<i>Streptococcus pyogenes</i>	✓	✓	<i>Streptococcus pneumoniae</i>	✓	✓

## QUESTION #5

- You are asked to see a 62-year-old man with a positive blood culture to advise on management.
- Gram stain of the positive blood culture bottle shows Gram positive cocci in clusters.
- A rapid PCR panel performed on the positive blood culture bottle contents detects *Staphylococcus aureus*, *Staphylococcus epidermidis* as well as *mecA/C* but not *mecA/C* and *MREJ*.

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## QUESTION #5

Which of the following is the interpretation of this finding?

- A. Methicillin-susceptible *S. aureus* and methicillin-resistant *S. epidermidis*
- B. Methicillin-susceptible *S. aureus* and methicillin-susceptible *S. epidermidis*
- C. Methicillin-resistant *S. aureus* and methicillin-resistant *S. epidermidis*
- D. Methicillin-resistant *S. aureus* and methicillin-susceptible *S. epidermidis*

## QUESTION #5

Which of the following is the interpretation of this finding?

- A. Methicillin-susceptible *S. aureus* and methicillin-resistant *S. epidermidis* \*
- B. Methicillin-susceptible *S. aureus* and methicillin-susceptible *S. epidermidis*
- C. Methicillin-resistant *S. aureus* and methicillin-resistant *S. epidermidis*
- D. Methicillin-resistant *S. aureus* and methicillin-susceptible *S. epidermidis*

MAYO CLINIC  
FDA-Approved Multiplex Panels for Detection of Gram-Positive Bacteria in Positive Blood Cultures (for reference)

	FilmArray MDx-Chex BCID2	VERIGENE®		cobas®	
		Gram-Positive Blood Culture Test	Gram-Negative Blood Culture Test	eplex BCID-GP Panel	eplex BCID-GN Panel
<i>Staphylococcus</i> species	✓	✓		✓	
<i>Staphylococcus aureus</i>	✓	✓		✓	
<i>Staphylococcus epidermidis</i>	✓	✓		✓	
<i>Staphylococcus lugdunensis</i>	✓	✓		✓	
<i>Streptococcus</i> species	✓	✓		✓	
<i>Streptococcus agalactiae</i>	✓	✓		✓	
<i>Streptococcus pyogenes</i>	✓	✓		✓	
<i>Streptococcus pneumoniae</i>	✓	✓		✓	
<i>Streptococcus anginosus</i> group		✓		✓	
<i>Enterococcus</i> species		✓		✓	
<i>Enterococcus faecalis</i>	✓	✓		✓	
<i>Enterococcus faecium</i>		✓		✓	
<i>Listeria</i> species		✓		✓	
<i>Listeria monocytogenes</i>	✓	✓		✓	
<i>Bacillus cereus</i> group		✓		✓	
<i>Bacillus subtilis</i> group		✓		✓	
<i>Corynebacterium</i> species		✓		✓	
<i>Cutibacterium acnes</i>		✓		✓	
<i>Lactobacillus</i> species		✓		✓	
<i>Micrococcus</i> species		✓		✓	
Pan Gram-Positive					✓

MAYO CLINIC  
FDA-Approved Multiplex Panels for Detection of Gram-Negative Bacteria in Positive Blood Cultures (for reference), continued

	FilmArray MDx-Chex BCID2	VERIGENE®		cobas®	
		Gram-Negative Blood Culture Test	Gram-Negative Blood Culture Test	eplex BCID-GP Panel	eplex BCID-GN Panel
<i>Klebsiella oxytoca</i>		✓	✓		✓
<i>Klebsiella pneumoniae</i>		✓	✓		✓
<i>Klebsiella pneumoniae</i> group		✓	✓		✓
<i>Klebsiella aerogenes</i>		✓	✓		✓
<i>Serratia</i> species		✓	✓		✓
<i>Morganella morganii</i>		✓	✓		✓
<i>Streptophomonas maltophilia</i>		✓	✓		✓
<i>Serratia</i> species		✓	✓		✓
<i>Serratia marcescens</i>		✓	✓		✓
<i>Proteus</i> species		✓	✓		✓
<i>Proteus mirabilis</i>		✓	✓		✓
<i>Acinetobacter</i> species		✓	✓		✓
<i>Acinetobacter baumannii</i>		✓	✓		✓
<i>Acinetobacter calcoaceticus-baumannii</i> complex		✓	✓		✓
<i>Hemophilus influenzae</i>		✓	✓		✓
<i>Campylobacter jejuni</i>		✓	✓		✓
<i>Neisseria meningitidis</i>		✓	✓		✓
<i>Pseudomonas aeruginosa</i>		✓	✓		✓
<i>Enterobacter</i> species		✓	✓		✓
<i>Escherichia coli</i>		✓	✓		✓
<i>Enterobacter</i> species		✓	✓		✓
<i>Enterobacter cloacae</i> complex		✓	✓		✓
<i>Citrobacter</i> species		✓	✓		✓
<i>Bacteroides fragilis</i>		✓	✓		✓
<i>Fusobacterium necrophorum</i>		✓	✓		✓
<i>Fusobacterium nucleatum</i>		✓	✓		✓
Pan Gram-Negative					✓

MAYO CLINIC  
FDA-Approved Multiplex Panels for Detection of Select Resistance Genes in Positive Blood Cultures (for reference), continued

	FilmArray MDx-Chex BCID2	VERIGENE®		cobas®	
		Gram-Positive Blood Culture Test	Gram-Negative Blood Culture Test	eplex BCID-GP Panel	eplex BCID-GN Panel
<i>mecA</i>		✓		✓	
<i>mecC</i>		✓		✓	
<i>mecA/C</i>	✓				
<i>mecA/C</i> and <i>MREJ</i>	✓				
<i>vanA</i>		✓		✓	
<i>vanB</i>		✓		✓	
<i>vanA/B</i>	✓				
<i>bla<sub>oxp</sub></i>	✓		✓		✓
<i>bla<sub>NDM</sub></i>	✓		✓		✓
<i>bla<sub>OXA</sub></i>	✓		✓		✓
<i>bla<sub>IMP</sub></i>	✓		✓		✓
<i>bla<sub>CTX-M</sub></i>	✓		✓		✓
<i>mcr-1</i>	✓				

MAYO CLINIC  
FDA-Approved Multiplex Panels for Detection of Fungi in Positive Blood Cultures (for reference), continued

	FilmArray MDx-Chex BCID2	cobas®	
		ePlex BCID-GP Panel	ePlex BCID-GN Panel
<i>Candida albicans</i>	✓		✓
<i>Candida auris</i>	✓		✓
<i>Candida dubliniensis</i>			✓
<i>Candida famata</i>			✓
<i>Nakaseomyces glabrata</i>	✓		✓
<i>Candida guilliermondii</i>			✓
<i>Candida kefyr</i>			✓
<i>Pichia kudriavzevii</i>	✓		✓
<i>Candida lusitanae</i>			✓
<i>Candida parapsilosis</i>	✓		✓
<i>Candida tropicalis</i>	✓		✓
<i>Cryptococcus gattii</i>			✓
<i>Cryptococcus neoformans</i>			✓
<i>C. neoformans/gattii</i>	✓		✓
<i>Fusarium</i> species			✓
<i>Rhodotorula</i> species			✓
Pan <i>Candida</i>		✓	✓

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## STAPHYLOCOCCI METHICILLIN RESISTANCE

- Methicillin resistance mediated by *mecA* (or rarely *mecC*) gene products
- Penicillin binding protein (PBP) target altered (PBP2a)
  - Confers resistance to all available  $\beta$ -lactams (except ceftaroline)
  - Even if staphylococci that are methicillin-resistant *appear* susceptible to these other  $\beta$ -lactams, they are not effective
- Oxacillin or ceftioxin tested
- *mecA/C* and MREJ specific for *Staphylococcus aureus*
- For serious infections, susceptibility to oxacillin confirmed using PBP2a testing or nucleic acid amplification test (NAAT) to detect *mecA* (and *mecC*)

## FDA-APPROVED RAPID PHENOTYPIC SUSCEPTIBILITY TESTS - POSITIVE BLOOD CULTURE BOTTLES

- Accelerate Diagnostics
  - Gram-negative and –positive bacteria
- Selux Dx
  - Gram-negative bacteria



## T2Direct Diagnostics Direct from Blood

- Multiplex PCR and T2 magnetic resonance, average turnaround time 4.3 hours
- T2Candida Panel
  - *Candida albicans*
  - *Candida tropicalis*
  - *Pichia kudriavzevii*
  - *Nakaseomyces glabrata*
  - *Candida parapsilosis*
- T2Bacteria Panel
  - *Enterococcus faecium*
  - *Staphylococcus aureus*
  - *Klebsiella pneumoniae*
  - *Pseudomonas aeruginosa*
  - *Escherichia coli*



## BioFire Joint Infection Panel (Synovial Fluid)

<i>Anaerococcus prevotii/vaginalis</i>	<i>Escherichia coli</i>
<i>Clostridium perfringens</i>	<i>Haemophilus influenzae</i>
<i>Cutibacterium avidum/granulosum</i>	<i>Kingella kingae</i>
<i>Enterococcus faecalis</i>	<i>Klebsiella aerogenes</i>
<i>Enterococcus faecium</i>	<i>Klebsiella pneumoniae</i> group
<i>Finexidia magna</i>	<i>Morganella morganii</i>
<i>Parvimonas micra</i>	<i>Neisseria gonorrhoeae</i>
<i>Peptoniphilus</i>	<i>Proteus</i> spp.
<i>Peptostreptococcus anaerobius</i>	<i>Pseudomonas aeruginosa</i>
<i>Staphylococcus aureus</i>	<i>Salmonella</i> spp.
<i>Staphylococcus lugdunensis</i>	<i>Serratia marcescens</i>
<i>Streptococcus species</i>	<i>Candida</i> spp.
<i>Streptococcus agalactiae</i>	<i>Candida albicans</i>
<i>Streptococcus pneumoniae</i>	<i>bla</i> <sub>MR</sub> , <i>bla</i> <sub>SPC</sub> , <i>bla</i> <sub>NDM</sub> , <i>bla</i> <sub>OXA-48-like</sub>
<i>Streptococcus pyogenes</i>	<i>bla</i> <sub>IMP</sub> , <i>bla</i> <sub>CTX-M</sub>
<i>Bacteroides fragilis</i>	<i>mecA/C</i> and MREJ
<i>Citrobacter</i>	<i>vanA/B</i>
<i>Enterobacter cloacae</i> complex	

## QUESTION #6

A 65-year-old man has multiple blood cultures positive for *Pseudomonas aeruginosa* resistant to amikacin, gentamicin, tobramycin, aztreonam, cefepime, ceftazidime, meropenem, piperacillin-tazobactam, ciprofloxacin, and levofloxacin. You call the clinical microbiology laboratory to request susceptibility testing of an additional antimicrobial.

Which of the following is most appropriate?

- Dalbavancin
- Tedizolid
- Ceftolozane/tazobactam
- Oritavancin

## QUESTION #6


A 65-year-old man has multiple blood cultures positive for *Pseudomonas aeruginosa* resistant to amikacin, gentamicin, tobramycin, aztreonam, cefepime, ceftazidime, meropenem, piperacillin-tazobactam, ciprofloxacin, and levofloxacin. You call the clinical microbiology laboratory to request susceptibility testing of an additional antimicrobial.

Which of the following is most appropriate?

- Dalbavancin
- Tedizolid
- Ceftolozane/tazobactam \*
- Oritavancin


# 02 – Core Concepts: Microbiology: What You Need to Know for the Exam

Speaker: Robin Patel, MD

**QUESTION #7**  **PREVIEW QUESTION**


You are asked to see a 43-year-old woman to advise on management of a positive blood culture.

- Gram stain of her blood culture bottle shows Gram-negative bacilli.
- A rapid PCR panel performed on the positive blood culture bottle contents detects *Klebsiella pneumoniae* and *bla<sub>KPC</sub>*.

**QUESTION #7**  **PREVIEW QUESTION**

The *bla<sub>KPC</sub>* gene product would be expected to confer resistance to which of the following?

- Cefepime
- Plazomicin
- Colistin
- Ceftazidime/avibactam

**QUESTION #7**  **PREVIEW QUESTION**

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- Cefepime \*
- Plazomicin
- Colistin
- Ceftazidime/avibactam

**TYPICAL SUSCEPTIBILITY OF A *bla<sub>KPC</sub>*-PRODUCER**

*Klebsiella pneumoniae*

Ampicillin	>16 R	Ampicillin/Sulbactam	>16/8 R	Piperacillin/Tazobactam	64/4 R
Cefazolin	>16 R	Oral cephalosporins	R	Cefepime	>16 R
Ceftazidime	>16 R	Ceftriaxone	>32 R	Ertapenem	>1 R
Meropenem	>8 R	Aztreonam	>16 R	Ciprofloxacin	>2 R
Levofloxacin	4 I	Amikacin	>32 R	Gentamicin	>8 R
Tobramycin	4 S	Tigecycline	2 S	TMP/SMX	>2/38 R

**TYPICAL SUSCEPTIBILITY OF AN ESBL-PRODUCER**

*Escherichia coli*

Ampicillin	>16 R	Ampicillin/Sulbactam	>16/8 R	Piperacillin/Tazobactam	S/R*
Cefazolin	>16 R	Oral cephalosporins	R	Cefepime	S/SDD/R
Ceftazidime	>16 R	Ceftriaxone	>32 R	Ertapenem	≤0.5 S
Meropenem	≤1 S	Aztreonam	>16 R	Ciprofloxacin	≤1 S
Levofloxacin	≤2 S	Amikacin	≤8 S	Gentamicin	≤1 S
Tobramycin	4 S	Tigecycline	2 S	TMP/SMX	>2/38 R

\*Not currently recommended for infection outside of urinary tract

**TYPICAL SUSCEPTIBILITY OF INDUCIBLE, CHROMOSOMALLY-ENCODED AmpC β-LACTAMASE PRODUCER**

*Enterobacter cloacae*\*

Ampicillin	>16 R	Ampicillin/Sulbactam	>16/8 R	Piperacillin/Tazobactam	S/R*
Cefazolin	>16 R	Oral cephalosporins	R	Cefepime	S/SDD
Ceftazidime	>16 R	Ceftriaxone	>32 R**	Ertapenem	≤0.5 S
Meropenem	≤1 S	Aztreonam	S/R	Ciprofloxacin	≤1 S
Levofloxacin	≤2 S	Amikacin	≤8 S	Gentamicin	≤1 S
Tobramycin	4 S	Tigecycline	2 S	TMP/SMX	>2/38 R

\**Enterobacter cloacae*, *Klebsiella aerogenes*, *Citrobacter freundii*  
 \*\*Avoid ceftriaxone or ceftazidime even if test susceptible; cefepime an acceptable choice  
 [ISA Guidance on the Treatment of Antimicrobial-Resistant Gram-Negative Infections. (idsociety.org)]



# 02 – Core Concepts: Microbiology: What You Need to Know for the Exam

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## QUESTION #8

Which of the following susceptibility patterns would be typical for an *Escherichia coli* isolate carrying a New Delhi metallo-β-lactamase (NDM)?

	Cefazolin	Cefotaxime	Ceftazidime	Piperacillin/ tazobactam	Imipenem	Aztreonam
A.	R	S	S	S	S	S
B.	R	R	R	S	S	R
C.	R	R	R	R	S	R
C.	R	R	R	R	R	R

## QUESTION #8

Which of the following susceptibility patterns would be typical for an *Escherichia coli* isolate carrying a New Delhi metallo-β-lactamase (NDM)?

	Cefazolin	Cefotaxime	Ceftazidime	Piperacillin/ tazobactam	Imipenem	Aztreonam
A.	R	S	S	S	S	S
B.	R	R	R	S	S	R
C.	R	R	R	R	S	R
D*.	R	R	R	R	R	R

## QUESTION #9

Which of the following tests for carbapenemase production?

- A. PBP2a test
- B. D-test
- C. Carba NP test
- D. Polymerase chain reaction assay

## QUESTION #9

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### CARBAPENEMASE PRODUCTION TEST

Carba NP TEST

β-lactam ring hydrolyzed by carbapenemase

pH (detected by indicator dye color change red → yellow)

Rapid (2 hours)

Positive = Carbapenemase Producer      Negative = Carbapenemase Non-Producer

### CARBAPENEMASE PRODUCTION TEST

MODIFIED CARBAPENEM INACTIVATION

Re-suspend test organism in TSB

Add meropenem disk  
Incubate 4h @35°C

Place disk on Mueller Hinton agar plate  
Inoculated with lawn of *Escherichia coli* 25922  
Incubate 18-24 h

Carbapenemase-Production Negative  
(zone of growth inhibition)

Carbapenemase-Production Positive  
(no zone of growth inhibition)

# 02 – Core Concepts: Microbiology: What You Need to Know for the Exam

Speaker: Robin Patel, MD

### QUESTION #10

The image shows *Staphylococcus aureus* grown with an erythromycin disc (left) and a clindamycin disc (right).

Which of the following is the correct interpretation of these results?

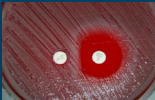


- A. Erythromycin susceptibility, inducible clindamycin resistance
- B. Erythromycin resistance, constitutive clindamycin resistance
- C. Erythromycin resistance, inducible clindamycin resistance
- D. Erythromycin susceptibility, constitutive clindamycin resistance

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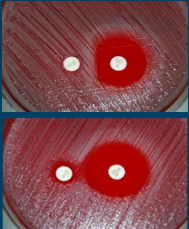
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- B. Erythromycin resistance, constitutive clindamycin resistance
- C. Erythromycin resistance, inducible clindamycin resistance \*
- D. Erythromycin susceptibility, constitutive clindamycin resistance

### INDUCIBLE CLINDAMYCIN RESISTANCE (D-TEST)

- Macrolide resistance from alteration in ribosomal target → co-resistance to clindamycin; constitutive or inducible
- Constitutive, erythromycin & clindamycin test resistant
- Inducible, erythromycin tests resistant but clindamycin tests falsely susceptible
- (Macrolide resistance due to efflux → no effect on clindamycin)

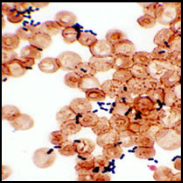
### INDUCIBLE CLINDAMYCIN RESISTANCE (D-TEST)

- Erythromycin & clindamycin disks incubated on plate
- Flattening of zone of inhibited growth between disks = inducible clindamycin resistance (top)
- If erythromycin does not influence zone around clindamycin disk, clindamycin susceptible (bottom)



### QUESTION #11

- You are asked to see a 95-year-old woman who is a resident of a long-term care facility to advise on therapy for bacteremia associated with a urinary tract infection.
- She has had two sets of blood cultures collected, both of which signaled positive after 17 hours of incubation.
- Gram stain of the bottles is shown.
- A rapid PCR panel performed on the positive blood culture bottle detects *Enterococcus* species as well as *vanA/vanB*.



### QUESTION #11

Which of the following is the most likely identity of the blood culture isolate?

- A. *Enterococcus gallinarum*
- B. *Enterococcus faecium*
- C. *Enterococcus faecalis*
- D. *Enterococcus casseliflavus*
- E. *Enterococcus avium*

# 02 – Core Concepts: Microbiology: What You Need to Know for the Exam

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## QUESTION #11

Which of the following is the most likely identity of the blood culture isolate?

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- B. *Enterococcus faecium* \*
- C. *Enterococcus faecalis*
- D. *Enterococcus casseliflavus*
- E. *Enterococcus avium*

## ENTEROCOCCI VANCOMYCIN SUSCEPTIBILITY TESTING

- Vancomycin MICs  $\geq 32$   $\mu\text{g/ml}$ 
  - Typically VanA or VanB mediated resistance
  - Typically *E. faecium*
  - Epidemiologically significant
- Vancomycin MICs, 8-16  $\mu\text{g/ml}$  (intermediate)
  - VanC
  - *E. gallinarum* or *E. casseliflavus/flavescens*
  - Not epidemiologically significant

## QUESTION #12

A 44-year-old man who underwent bilateral lung transplantation for pulmonary hypertension develops a sternal wound infection with sternal dehiscence 15 days post-transplant.

Blood cultures are negative. He undergoes sternal debridement with the finding of purulence and negative Gram and KOH stains.

After three days of incubation, pinpoint, clear colonies are visualized on cultures on sheep blood agar, however Gram stain of these colonies is negative.

## QUESTION #12

Which of the following is the most appropriate empiric antibiotic to treat this patient?

- A. Cefepime
- B. Ceftriaxone
- C. Trimethoprim-sulfamethoxazole
- D. Azithromycin
- E. Doxycycline

## QUESTION #12

Which of the following is the most appropriate empiric antibiotic to treat this patient?

- A. Cefepime
- B. Ceftriaxone
- C. Trimethoprim-sulfamethoxazole
- D. Azithromycin
- E. **Doxycycline** \*

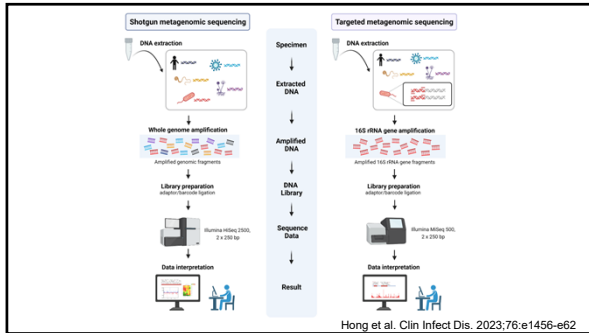
## *Mycoplasma hominis*

- Post-cardiothoracic transplant
- Pleuritis, surgical site infection and/or mediastinitis
- Treatment
  - Inactive
    - Cell wall active antibiotics
    - Trimethoprim/sulfamethoxazole
    - Aminoglycosides
    - Erythromycin and azithromycin
  - Active
    - Tetracyclines (doxycycline preferred)
    - Fluoroquinolones
    - Clindamycin

Sampath, R., et al. EBioMedicine (2017), <http://dx.doi.org/10.1016/j.ebiom.2017.04.026>

# 02 – Core Concepts: Microbiology: What You Need to Know for the Exam

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### 2023 DUKE-INTERNATIONAL SOCIETY FOR CARDIOVASCULAR INFECTIOUS DISEASES CRITERIA FOR INFECTIVE ENDOCARDITIS (IE)

- Pathologic Criteria - Microorganisms detected (appropriate sample) - PCR, amplicon/metagenomic sequencing, *in situ* hybridization
- Blood cultures - Removed required timing (and separation) venipunctures for blood cultures
- MAJOR CRITERIA
  - Positive blood cultures - Microorganisms that commonly cause IE ≥2 blood culture sets (typical) or that occasionally or rarely cause IE ≥3 blood culture sets (nontypical)
    - Typical: *Staphylococcus aureus*; HACEK group; *Staphylococcus lugdunensis*; *Enterococcus faecalis*; all streptococcal species (except for *Streptococcus pneumoniae* and pyogenes); *Granulicatella* and *Abiotrophia* spp.; *Gemella* spp. In setting of intracardiac prosthetic material, include these as "atypical" pathogens: coagulase negative staphylococci, *Geopilobacterium striatum* and *jelkeium*, *Serratia marcescens*, *Pseudomonas aeruginosa*, *Cultibacterium acnes*, non-*Berckelous* mycobacteria (especially *M. chimaerae*), *Candida* spp.
  - Blood PCR or amplicon/metagenomic sequencing detection of *Coxiella burnetii*, *Bartonella* spp., *Tropheryma whipplei*
  - IFA ≥1:800 for IgG antibodies *Bartonella henselae* or *B. quintana*
- MINOR CRITERIA - Positive culture, PCR, or other nucleic acid-based test (amplicon/metagenomic sequencing, *in situ* hybridization) organism consistent with IE from sterile body site other than cardiac tissue, cardiac prosthesis, or arterial embolus; or single finding of skin bacterium by PCR on valve or wire without additional clinical or microbiological supporting evidence

Fowler et al. Clin Infect Dis. 2023;77:519-20

### QUESTION #13

A transplant hepatologist calls to inquire about ganciclovir resistance testing on a liver transplant patient with CMV colitis and the following CMV viral loads:

7/04/23: 26,000 IU/ml (day of diagnosis)  
 7/14/23: 25,000 IU/ml  
 7/23/23: 22,000 IU/ml  
 8/13/23: 27,000 IU/ml

- The patient is CMV D\*/R-, received 3 months of valganciclovir prophylaxis, and now has CMV disease after discontinuing valganciclovir.
- He has been receiving full dose intravenous ganciclovir since July 4th and his diarrhea is unchanged.

### QUESTION #13

A plasma test for mutations in which of the following genes is most appropriate?

- UL51
- UL54
- UL89
- UL97
- Testing is unlikely to be helpful given the patient's viral load

### QUESTION #13

A plasma test for mutations in which of the following genes is most appropriate?

- UL51
- UL54
- UL89
- UL97 \*
- Testing is unlikely to be helpful given the patient's viral load

### QUESTION #14

Results of testing show a M460V UL97 mutation. This mutation would be expected to confer resistance to:

- Cidofovir
- Foscarnet
- Ganciclovir
- Ganciclovir and foscarnet
- Ganciclovir and cidofovir

# 02 – Core Concepts: Microbiology: What You Need to Know for the Exam

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## QUESTION #14

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- A. Cidofovir
- B. Foscarnet
- C. **Ganciclovir \***
- D. Ganciclovir and foscarnet
- E. Ganciclovir and cidofovir

## CYTOMEGALOVIRUS ANTIVIRAL RESISTANCE

- Risk factors
  - Prolonged drug exposure
  - D<sup>+</sup>R<sup>+</sup>, lung transplant recipient
- Amplify and sequence directly from plasma
  - (viral load ~1,000 IU/ml required)
- ≥2 weeks full-dose therapy before testing

Gene	Drug(s) affected
UL97	Ganciclovir, marabavir
UL54	Ganciclovir and cidofovir (if selected for by these agents); foscarnet (if selected for by foscarnet)
UL56	Letemovir

Kitter DA et al. Transplantation 2013;96:333 and Chou S. Curr Opin Infect Dis 2015;28:203

## QUESTION #15

You are consulted to advise on the course of action for a 57-year-old female liver transplant recipient (transplant for alcoholic steatohepatitis; CMV D<sup>+</sup>R<sup>+</sup>) who has a whole blood HHV-6 viral load of  $3.6 \times 10^6$  copies/ml at three months post-transplant. The test was performed because of a report of subjective fever of four days' duration. She has no other new symptoms. The patient received one month of acyclovir prophylaxis post-transplant and is currently receiving mycophenolate mofetil, prednisone and trimethoprim-sulfamethoxazole. Her post-transplant course was complicated by one episode of treated rejection on day 30 post transplant. Physical examination is unremarkable and she is afebrile.

## QUESTION #15

Which of the following would you recommend?

- A. Intravenous ganciclovir
- B. Oral valganciclovir
- C. Oral acyclovir
- D. Intravenous foscarnet
- E. **No antiviral therapy is indicated**

## QUESTION #15

Which of the following would you recommend?

- A. Intravenous ganciclovir
- B. Oral valganciclovir
- C. Oral acyclovir
- D. Intravenous foscarnet
- E. **No antiviral therapy is indicated \***

## CHROMOSOMALLY INTEGRATED HUMAN HERPESVIRUS-6

- High HHV-6 levels in whole blood
  - ( $>5.5 \log_{10}$  copies/ml)
- Suggest chromosomally integrated HHV-6
  
- **1:1 ratio of viral to human genomes**

Pelletti et al. Rev Med Virol 2012;22:144-55

## 02 – Core Concepts: Microbiology: What You Need to Know for the Exam

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### QUESTION #16

A 76-year-old woman presents with three days of cough, difficulty breathing and fever. She has never received a COVID-19 vaccine and has never been diagnosed with COVID-19. Which of the following COVID-19 tests is recommended?

- A. Antigen
- B. Serology
- C. NAAT

### QUESTION #16

A 76-year-old woman presents with three days of cough, difficulty breathing and fever. She has never received a COVID-19 vaccine and has never been diagnosed with COVID-19. Which of the following COVID-19 tests is recommended?

- A. Antigen
- B. Serology
- C. NAAT \*

### COVID-19 DIAGNOSTICS

- NAAT generally preferred over antigen testing
  - Symptomatic individuals suspected of having COVID-19
  - Asymptomatic individuals exposed to SARS-CoV-2 infection
  - Interpret Ct values with caution
- Healthcare provider or patient collected specimens acceptable
- Swabs from nasopharynx, anterior nares, oropharynx, or mid-turbinate regions; saliva or mouth gargle acceptable
  - Compared to nasopharyngeal swabs, anterior nares or oropharynx swabs alone yield more false-negative results than combined anterior nares/oropharynx swabs, mid-turbinate swabs, saliva, or mouth gargle
- Suspected lower respiratory infection → upper respiratory sample; if negative, lower respiratory sample

USA Guidelines on the Diagnosis of COVID-19