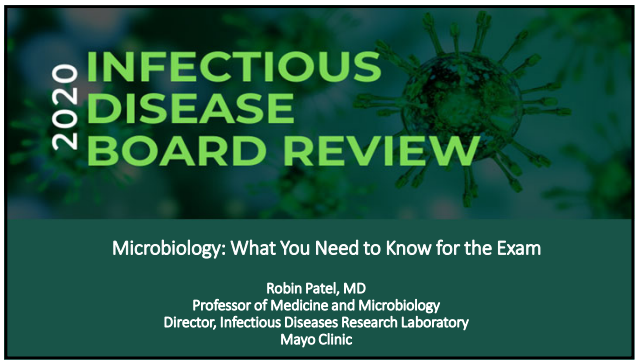
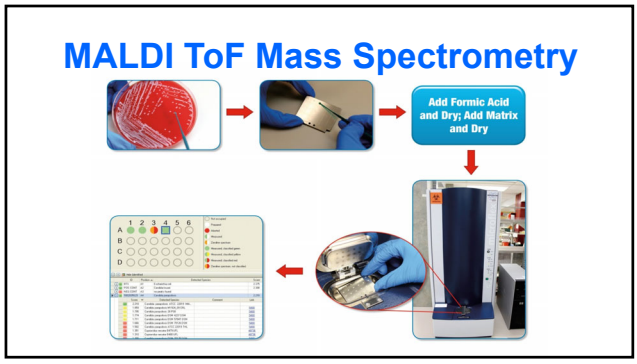
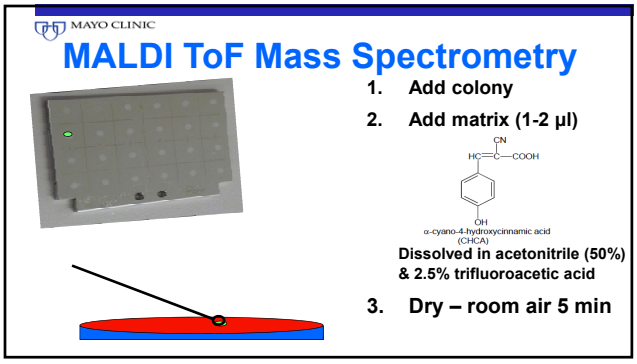
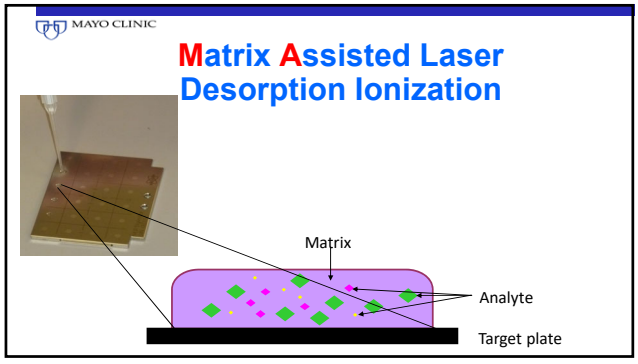
53 - Microbiology: What You Need to Know for The Exam

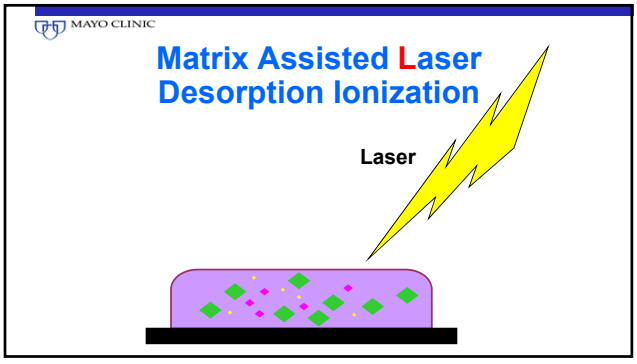
Speaker: Robin Patel, MD











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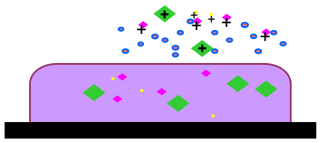
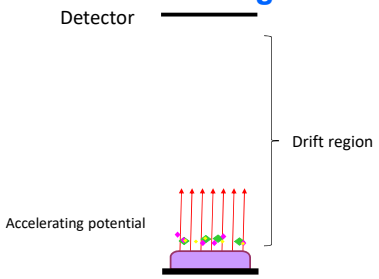
MAYO CLINIC

Matrix Assisted Laser Desorption Ionization



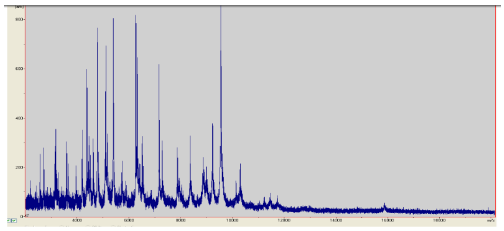
MAYO CLINIC

Time of Flight



MAYO CLINIC

Mass Spectrum Generated Compared with Library (Database)



QUESTION #1

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***

BACTERIA REQUIRING SPECIALIZED MEDIA

- *Bordetella pertussis*
- *Brucella* species (+/-)
- *Burkholdheria pseudomallei* (+/-)
- *Campylobacter* species
- *Francisella tularensis* (+/-)
- *Helicobacter pylori*
- *Legionella* species
- *Mycoplasma* species (+/-)
- *Ureaplasma* 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
    - *Tsukamurella* species
    - *Dietzia* species
- *Tatlockia* (*Legionella*) *micdadei* and some *Corynebacterium* species
  - [But not *Cutibacterium* (or *Propionibacterium*) 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 *Burkholdheria 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

Burkholderia pseudomallei Laboratory Exposure

Low risk Events	Inadvertent opening of the lid of an agar plate growing *B. pseudomallei* outside a biologic safety cabinet
	Inadvertent sniffing of agar plate growing *B. pseudomallei* in the absence of contact between worker and bacterium
	Splash event leading to visible contact of *B. pseudomallei* with gloved hand or protected body, in the absence of any evidence of aerosol
	Spillage of small volume of liquid culture (<1mL) within a functioning biologic safety cabinet
	Contamination of intact skin with culture
High risk Events	The presence of any predisposing condition without proper personal protective equipment (PPE): diabetes mellitus; chronic liver or kidney disease; alcohol abuse; long-term steroid use; hematologic malignancy; neutropenia or neutrophil dysfunction; chronic lung disease (including cystic fibrosis); thalassemia; any other form of immunosuppression
	Needlestick or other penetrating injury with implement contaminated with *B. pseudomallei*
	Bite or scratch by experimental animal infected with *B. pseudomallei*
	Splash event leading to contamination of mouth or eyes
	Generation of aerosol outside biologic safety cabinet (e.g., sonication, centrifuge incident)

Peacock SJ et al. Emerg Infect Dis. 2008 Jul http://wwwnc.cdc.gov/eid/article/14/7/07-1501

Burkholderia pseudomallei Postexposure Antimicrobial Drug Prophylaxis

Antimicrobial Drug	Dosage	Frequency
Trimethoprim-sulfamethoxazole (TMP-SMX)	2 × 160–800 mg (960 mg) tablets if >60 kg, 3 × 80–400 (480 mg) tablets if 40 kg-60 kg, and 1 × 160–800 mg (960 mg) or 2 × 80–400 (480 mg) tablets if adult <40 kg plus folate 5 mg/d	Every 12 h
Doxycycline	2.5 mg/kg/dose up to 100 mg orally	Every 12 h
Amoxicillin–clavulanic acid	20/5 mg/kg/dose. Equates to 3 × 500/125 tabs if >60 kg, and 2 × 500/125 tabs if ≤60kg	Every 8 h

Peacock SJ et al. Emerg Infect Dis. 2008 Jul http://wwwnc.cdc.gov/eid/article/14/7/07-1501

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

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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
- *Burkholdheria pseudomallei*
  - (*Burkholdheria 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*

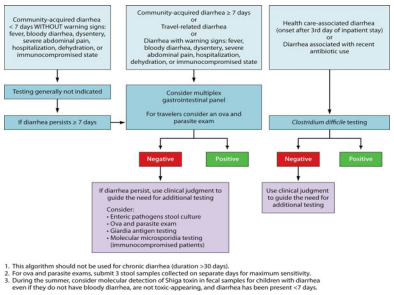
ORGANISMS ABOUT WHICH THE LABORATORY SHOULD BE NOTIFIED IF SUSPECTED

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

FDA-APPROVED/CLEARED MULTIPLEX PANELS FOR GASTROINTESTINAL PATHOGENS IN STOOL

	Verigene EP	Luminex GPP	BioFire GIP
Number of targets	8	14	22
Campylobacter species	✓	✓	✓
Salmonella species	✓	✓	✓
Shigella species/EnteroInvasive *E. coli*	✓	✓	✓
Vibrio species	✓	✓	✓
Yersinia enterocolitica	✓		✓
Escherichia coli 0157		✓	✓
Enterotoxigenic *E. coli*		✓	✓
Enteropathogenic *E. coli*			✓
Enteroaggregative *E. coli*			✓
Plesiomonas shigelloides			✓
Shiga toxin-producing *E. coli*	✓	✓	✓
Clostridioides difficile		✓	✓
Norovirus GI/GII	✓	✓	✓
Rotavirus A	✓	✓	✓
Astrovirus			✓
Adenovirus 40/41		✓	✓
Sapovirus			✓
Cryptosporidium species		✓	✓
Entamoeba histolytica		✓	✓
Giardia lamblia		✓	✓
Cyclospora cayetanensis			✓

TESTING ALGORITHM FOR ACUTE GASTROENTERITIS



1. This algorithm should not be used for chronic diarrhea (duration >30 days).
2. For ova and parasite exams, submit 3 stool samples collected on separate days for maximum sensitivity.
3. During the summer, consider molecular detection of Shiga toxin in fecal samples for children with diarrhea even if they do not have bloody diarrhea, are not toxic-appearing, and diarrhea has been present <7 days.

BIOFIRE FILMARRAY MENINGITIS/ENCEPHALITIS PANEL

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

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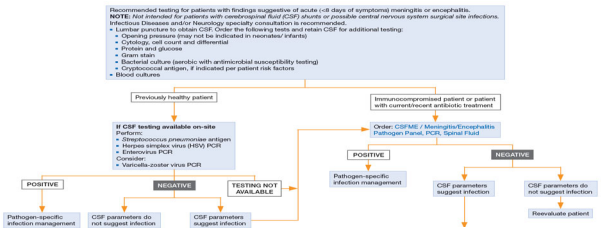
Meningitis/Encephalitis Panel Algorithm



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FDA-Approved Multiplex Respiratory Panels

Parameter	FilmArray	Verigene	x-TAG RVP	x-TAG RVP FAST	NxTAG-RPP	eSensor RVP	ePlex
Analysis platform	FilmArray system or FilmArray/Torch	Verigene system	Luminex 100/200	Luminex 100/200	Luminex MAGPIX	eSensor	ePlex system
Acceptable specimens	NP swab	NP swab	NP swab	NP swab	NP swab	NP swab	NP swab
Number of targets	20	16	12	8	20	14	17
Pathogens							
Viruses							
Adenovirus	✓	✓	✓	✓	✓	✓(differentiates B/E from C)	✓
Coronavirus							✓
Coronavirus HKU1	✓				✓		
Coronavirus NL63	✓				✓		
Coronavirus 229E	✓				✓		
Coronavirus OC43	✓				✓		
SARS-CoV-2	✓						
Human bocavirus					✓		
Human metapneumovirus	✓	✓	✓	✓	✓	✓	✓
Influenza A	✓	✓	✓	✓	✓	✓	✓
Subtype H1	✓	✓	✓	✓	✓	✓	✓
Subtype H3	✓	✓	✓	✓	✓	✓	✓
Subtype 2009 H1N1	✓					✓	✓
Influenza B	✓	✓	✓	✓	✓	✓	✓
Parainfluenza 1	✓	✓	✓		✓	✓	✓
Parainfluenza 2	✓	✓	✓		✓	✓	✓
Parainfluenza 3	✓	✓	✓		✓	✓	✓
Parainfluenza 4	✓	✓			✓		✓
Respiratory syncytial virus	✓			✓			
Respiratory syncytial virus A		✓	✓		✓	✓	✓
Respiratory syncytial virus B		✓	✓		✓	✓	✓
Rhinovirus/enterovirus	✓	✓	✓	✓	✓	✓	✓
Bacteria							
Chlamydophila pneumoniae	✓				✓		✓
Mycoplasma pneumoniae	✓				✓		✓
Bordetella pertussis	✓	✓					
Bordetella parapertussis	✓						
B. parapertussis/Bordetella bronchiseptica		✓					
Bordetella holmesii		✓					
Throughput	Scalable	Low	Medium	Medium	High	Medium	Scalable
Time to result	1 hour	~2.3 hours	~8 hours	~6 hours	~4 hours	~6 hours	~1.5 hours

The acceptable specimen type for all panels is nasopharyngeal swab. RVP, respiratory virus panel; RPP, respiratory pathogen panel. Ramanan et al. Clin Microbiol Rev 2018;31:1-28

MAYO CLINIC

FilmArray® Respiratory Panel EZ (RP EZ)

- VIRUSES:
  - Adenovirus, Coronavirus, Human Metapneumovirus, Human Rhinovirus/Enterovirus, Influenza A, Influenza A/H1, Influenza A/H3, Influenza A/H1-2009, Influenza B, Parainfluenza Virus, Respiratory Syncytial Virus
- BACTERIA:
  - *Bordetella pertussis, Chlamydophila pneumoniae, Mycoplasma pneumoniae*

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Lower Respiratory Tract Panels

	Curetis Unyvero	BioFire
Bacteria		
***Acinetobacter* spp.**	✓	
***Acinetobacter calcoaceticus-baumannii* complex**		✓
Chlamydia pneumoniae	✓	✓
Citrobacter freundii	✓	
Enterobacter aerogenes		✓
Enterobacter cloacae		✓
***Enterobacter cloacae* complex**	✓	
Escherichia coli	✓	✓
Haemophilus influenzae	✓	✓
Klebsiella oxytoca	✓	✓
Klebsiella pneumoniae	✓	
***Klebsiella pneumoniae* group**		✓
Klebsiella variicola	✓	
Legionella pneumophila	✓	✓
Moraxella catarrhalis	✓	✓
Morganella morganii	✓	
Mycoplasma pneumoniae	✓	✓
***Proteus* spp.**	✓	✓
Pseudomonas aeruginosa	✓	✓
Serratia marcescens	✓	✓
Staphylococcus aureus	✓	✓
Stenotrophomonas maltophilia	✓	
Streptococcus agalactiae		✓
Streptococcus pneumoniae	✓	✓
Streptococcus pyogenes		✓

	Curetis Unyvero	BioFire
Viruses		
Influenza A		✓
Influenza B		✓
Respiratory Syncytial Virus		✓
Human Rhinovirus/Enterovirus		✓
Human Metapneumovirus		✓
Parainfluenza virus		✓
Adenovirus		✓
Coronavirus (non-SARS-CoV)		✓
Resistance genes		
bla_{KPC}	✓	✓
bla_{NDM}	✓	✓
bla_{IMP}		✓
bla_{OXA-23}	✓	
bla_{OXA-48}	✓	
bla_{OXA51}	✓	
$bla_{OXA55-like}$		✓
bla_{VIM}	✓	✓
bla_{CTX-M}	✓	
bla_{TEM}	✓	
mecA	✓	
mecA/C* and *MREJ		✓

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* species as well as *mecA*.

QUESTION #5

Which of the following is the interpretation of this finding?

A. The patient has methicillin-resistant *Staphylococcus aureus* bacteremia

B. The patient has methicillin-susceptible *S. aureus* bacteremia

C. The patient has methicillin-resistant *Staphylococcus epidermidis* bacteremia

D. The patient has methicillin-susceptible *Staphylococcus lugdunensis* bacteremia

E. The patient has a methicillin-resistant staphylococcal bacteremia that could be due to *S. aureus*, *S. epidermidis*, *S. lugdenensis* or another *Staphylococcus* species

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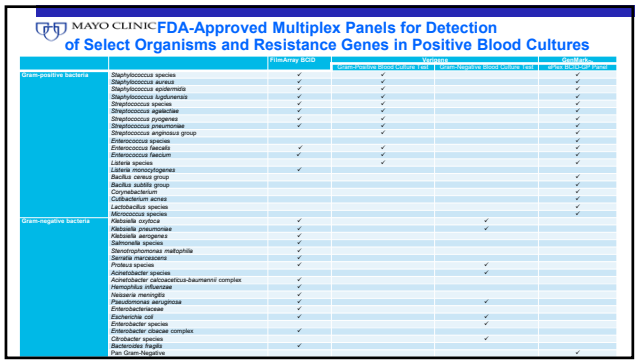
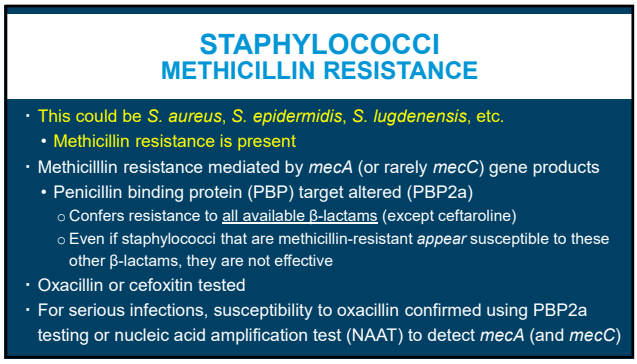


MAYO CLINIC

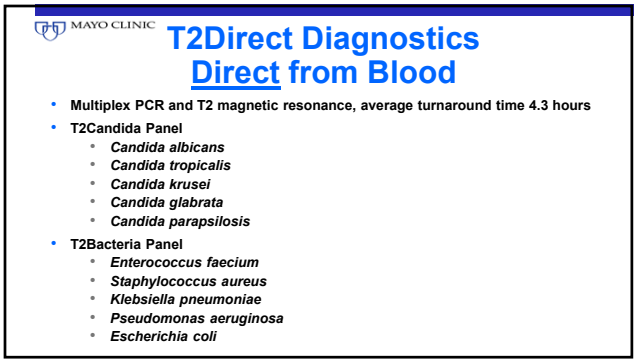
FDA-Approved Multiplex Panels for Detection of Select Organisms and Resistance Genes in Positive Blood Cultures

		FilmArray BCID	Verigene		GenMark
			Gram-Positive Blood Culture Test	Gram-Negative Blood Culture Test	ePlex BCID Gram-Positive Panel
Gram-positive bacteria	*Staphylococcus* species	✓	✓		✓
	Staphylococcus aureus	✓	✓		✓
	Staphylococcus epidermidis	✓	✓		✓
	Staphylococcus lugdunensis	✓	✓		✓
	Streptococcus species	✓	✓		✓
	Streptococcus agalactiae	✓	✓		✓
	Streptococcus pyogenes	✓	✓		✓
	Streptococcus pneumoniae	✓	✓		✓
	Streptococcus anginosus group		✓		✓
	Enterococcus species				✓
	Enterococcus faecalis	✓	✓		✓
	Enterococcus faecium	✓	✓		✓
	Listeria species		✓		✓
	Listeria monocytogenes	✓			
	Bacillus cereus group				✓
	Bacillus subtilis group				✓
	Corynebacterium				✓
	Cutibacterium acnes				✓
	Lactobacillus species				✓
	Micrococcus species				✓
Gram-negative bacteria	*Klebsiella oxytoca*	✓		✓	
	Klebsiella pneumoniae	✓		✓	
	Klebsiella aerogenes	✓			
	Salmonella species	✓			
	Stenotrophomonas maltophilia	✓			
	Serratia marcescens	✓			
	Proteus species	✓		✓	
	Acinetobacter species	✓		✓	
	Acinetobacter calcoaceticus-baumannii complex	✓			
	Haemophilus influenzae	✓			
	Neisseria meningitis	✓			
	Pseudomonas aeruginosa	✓		✓	
	Enterobacteriaceae	✓			
	Escherichia coli	✓		✓	
	Enterobacter species			✓	
	Enterobacter cloacae complex	✓			
	Citrobacter species			✓	
	Bacteroides fragilis	✓			
	Pan Gram-Negative				✓



STAPHYLOCOCCI
METHICILLIN RESISTANCE

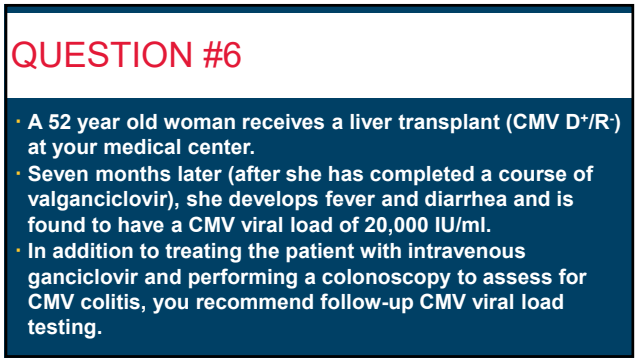
- This could be *S. aureus*, *S. epidermidis*, *S. lugdenensis*, etc.
  - Methicillin resistance is present
- Methicilllin resistance mediated by *mecA* (or rarely *mecC*) gene products
  - Penicillin binding protein (PBP) target altered (PBP2a)
    - Confers resistance to all available β-lactams (except ceftaroline)
    - Even if staphylococci that are methicillin-resistant *appear* susceptible to these other β-lactams, they are not effective
- Oxacillin or cefoxitin tested
- For serious infections, susceptibility to oxacillin confirmed using PBP2a testing or nucleic acid amplification test (NAAT) to detect *mecA* (and *mecC*)



MAYO CLINIC

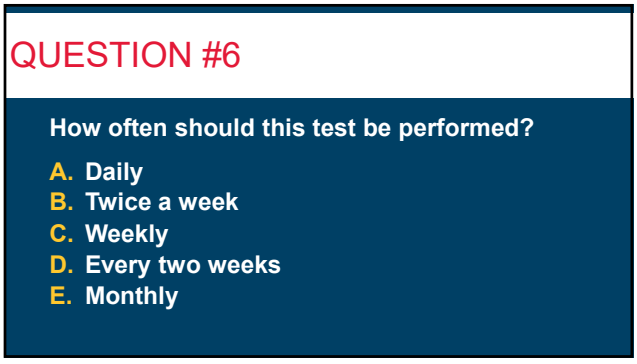
T2Direct Diagnostics
Direct from Blood

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



QUESTION #6

- **A 52 year old woman receives a liver transplant (CMV D^+/R^-) at your medical center.**
- **Seven months later (after she has completed a course of valganciclovir), she develops fever and diarrhea and is found to have a CMV viral load of 20,000 IU/ml.**
- **In addition to treating the patient with intravenous ganciclovir and performing a colonoscopy to assess for CMV colitis, you recommend follow-up CMV viral load testing.**



QUESTION #6

How often should this test be performed?

A. Daily
B. Twice a week
C. Weekly
D. Every two weeks
E. Monthly

OPTIMAL FREQUENCY CMV VIRAL LOAD TESTING

- Weekly viral load testing sufficient to document antiviral response, antiviral resistance emergence
  - $T_{1/2}$ virus ~5-8 days
  - May rise 1st few days on therapy
  - Obtain baseline viral load day therapy started
- Treatment
  - Until viral clearance, symptom resolution and 2 week minimum
- Changes >3-fold (>0.5 log)
  - Biologically important changes in viral replication
- Preemptive treatment → weekly viral load testing

QUESTION #7

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^+/R^+) who has a whole blood HHV-6 viral load of $3.6x10^6$ 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 #7

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

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

QUESTION #8

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?

A. Dalbavancin
B. Tedizolid
C. Ceftolozane/tazobactam
D. Oritavancin

QUESTION #9

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 *Enterobacteriaceae* and bla_{KPC}.

53 - Microbiology: What You Need to Know for The Exam

Speaker: Robin Patel, MD

QUESTION #9

The *bla*$_{KPC}$ gene product would be expected to confer resistance to which of the following?

A. Cefepime

B. Plazomicin

C. Colistin

D. Ceftazidime/avibactam

TYPICAL SUSCEPTIBILITY OF A KPC-PRODUCER

Klebsiella pneumoniae carbapenemase producer

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

– Extended spectrum beta-lactamase producer

Ampicillin	>16 R	Ampicillin/Sulbactam	>16/8 R	Piperacillin/Tazobactam	16/4 S
Cefazolin	>16 R	Oral cephalosporins	R	Cefepime	>16 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

QUESTION #10

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 #11

Which of the following tests for carbapenemase production?

A. PBP2a test

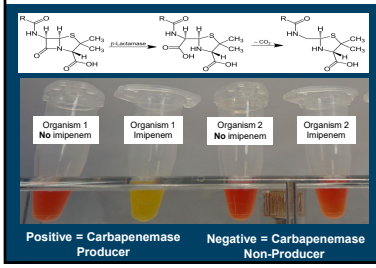
B. D-test

C. Carba NP test

D. Polymerase chain reaction assay

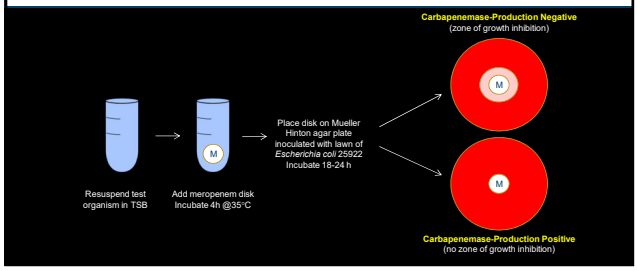
CARBAPENEMASE PRODUCTION TEST

Carba NP TEST



- **β-lactam ring hydrolyzed by carbapenemase**
- **↓pH (detected by indicator dye color change red → yellow)**
- **Rapid (2 hours)**

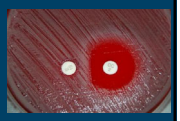
CARBAPENEMASE PRODUCTION TEST MODIFIED CARBAPENEM INACTIVATION



QUESTION #12

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?



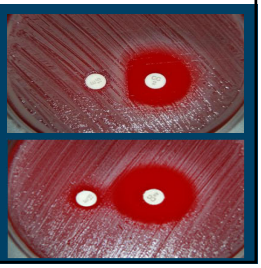
A. **Erythromycin susceptibility, inducible clindamycin resistance**
B. **Erythromycin resistance, constitutive clindamycin resistance**
C. **Erythromycin resistance, inducible clindamycin resistance**
D. **Erythromycin susceptibility, constitutive clindamycin resistance**

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)

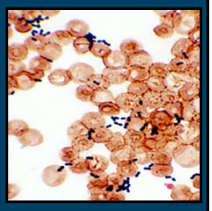
INDUCIBLE CLINDAMYCIN RESISTANCE (D-TEST)

- 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 #13

- **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 #13

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***

ENTEROCOCCI
VANCOMYCIN SUSCEPTIBILITY TESTING

- **Vancomycin MICs ≥32 µg/ml**
  - Typically VanA or VanB mediated resistance
  - Typically *E. faecium*
  - Epidemiologically significant
- **Vancomycin MICs, 8-16 µg/ml (intermediate)**
  - VanC
  - *E. gallinarum* or *E. casseliflavus/flavescens*
  - Not epidemiologically significant

QUESTION #14

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 #14

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

QUESTION #15

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

7/01/16: 26,000 IU/ml (day of diagnosis)
7/11/16: 25,000 IU/ml
7/20/16: 22,000 IU/ml
7/31/16: 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 1st and his diarrhea is unchanged.

QUESTION #15

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

A. UL51
B. UL54
C. UL89
D. UL97
E. Testing is unlikely to be helpful given the patient's viral load

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QUESTION #16

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

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

CYTOMEGALOVIRUS ANTIVIRAL RESISTANCE

- **Risk factors**
  - **Prolonged drug exposure**
  - **D^+R^-, lung transplant recipient**
- **Amplify and sequence directly from plasma**
  - **(viral load ~1,000 IU/ml required)**
- **≥6 weeks antiviral drug exposure**
  - **Should include ≥2 weeks full-dose therapy before testing**
  - **Accelerated schedule: Poor host factors, extreme viral loads**

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

Kotton CN et al. Transplantation 2013;96:333 and Chou S. Curr Opin Infect Dis 2015;28:293