



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

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Mayo Clinic

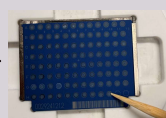
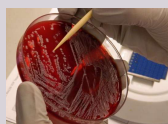
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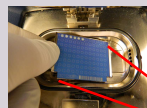
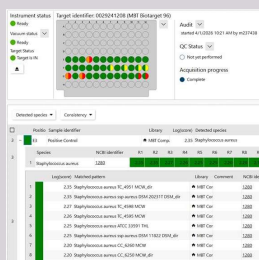
Disclosures of Financial Relationships with Relevant Commercial Interests

- Consulting: PhAST, Iterum Therapeutics, DEEPULL DIAGNOSTICS, Claryx, Inc., Nostics, CARB-X
- Grant Funding: MicuRx Pharmaceuticals, ImmuCell
- Patent: Bordetella pertussis/parapertussis PCR; a device/method for sonication (royalties paid by Samsung to Mayo Clinic); Anti-biofilm substance; PET imaging of bacterial infection with a PET probe filed
- Honoraria: UpToDate

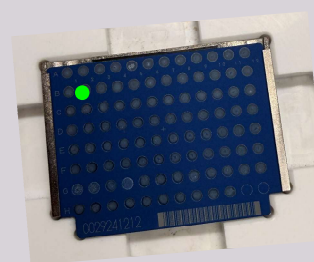
MALDI ToF Mass Spectrometry



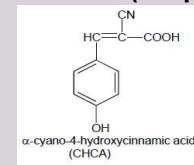
Add Matrix;
Dry



MALDI ToF Mass Spectrometry

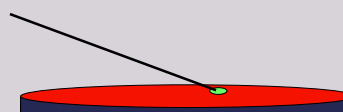


1. Add colony
2. Add matrix (1-2 μ l)

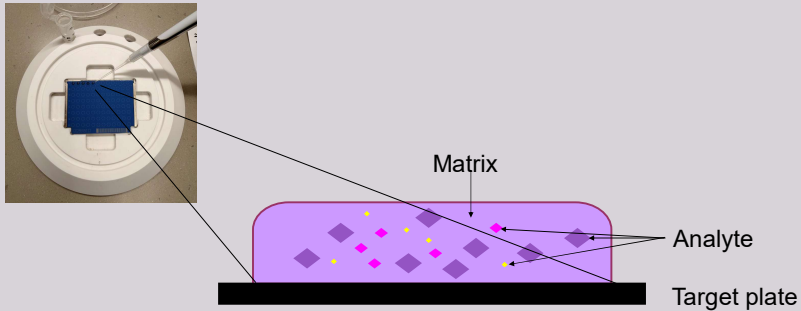


Dissolved in acetonitrile (50%)
& 2.5% trifluoroacetic acid

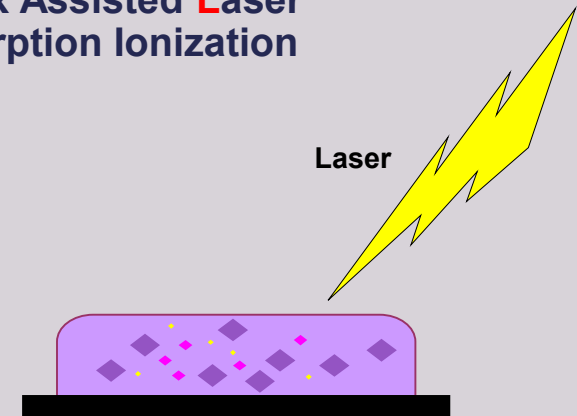
3. Dry – room air 5 min



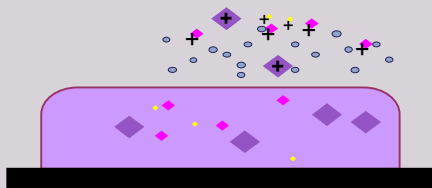
Matrix Assisted Laser Desorption Ionization



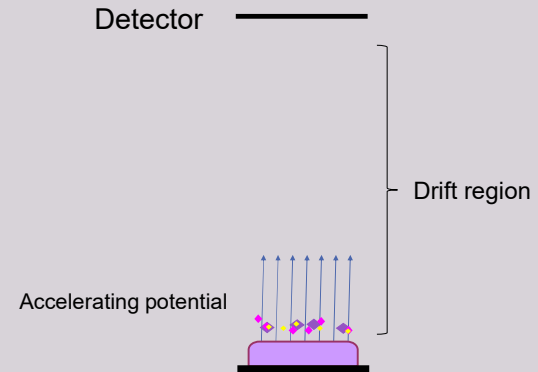
Matrix Assisted Laser Desorption Ionization



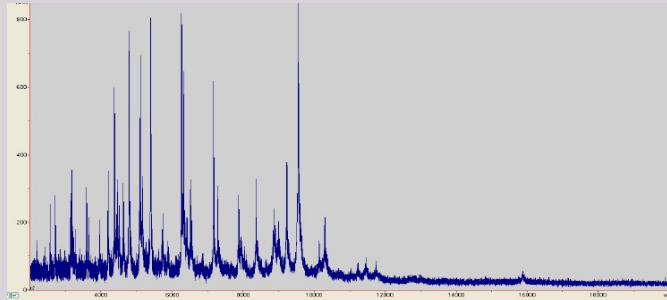
Matrix Assisted Laser Desorption Ionization



Time of Flight



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*

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Bacteria Requiring Specialized Media

- *Bordetella pertussis*
- *Brucella* species (+/-)
- *Burkholderia 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. *Fingoldia* 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. *Fingoldia* 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
- *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.

Which of the following would you recommend?

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

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- B. Amoxicillin
- C. Streptomycin
- D. Cephalexin
- E. None

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/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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- 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*

FDA-Approved/Cleared Multiplex Panels for Gastrointestinal Pathogens in Stool (For Reference)

	Vengene EP	xTAG [®] GPP	BioFire GIP	BioCode [®]	Qiasat-DX
<i>Campylobacter</i> species	✓	✓	✓	✓	✓
<i>Salmonella</i> species	✓	✓	✓	✓	✓
<i>Shigella</i> species/Enteroinvasive <i>E. coli</i>	✓	✓	✓	✓	✓
<i>Vibrio</i> species	✓		✓		✓
<i>Vibrio vulnificus</i>					✓
<i>Vibrio parahaemolyticus</i>				✓	✓
<i>Vibrio cholerae</i>		✓	✓		✓
<i>Yersinia enterocolitica</i>	✓	✓	✓	✓	✓
<i>Escherichia coli</i> O157		✓	✓	✓	✓
Enterotoxigenic <i>E. coli</i>		✓	✓	✓	✓
Enteropathogenic <i>E. coli</i>			✓		✓
Enteraggregative <i>E. coli</i>			✓	✓	✓
<i>Plesiomonas shigelloides</i>			✓		✓
Shiga toxin-producing <i>E. coli</i>	✓	✓	✓	✓	✓
<i>Clostridioides difficile</i>		✓	✓		✓
Norovirus	✓	✓	✓	✓	✓
Rotavirus A	✓	✓	✓	✓	✓
Astrovirus			✓		✓
Adenovirus 40/41		✓	✓	✓	✓
Sapovirus			✓		✓
<i>Cryptosporidium</i> species		✓	✓	✓	✓
<i>Entamoeba histolytica</i>		✓	✓	✓	✓
<i>Giardia lamblia</i>		✓	✓	✓	✓
<i>Cyclospora cayentanensis</i>			✓		✓

SEE ENLARGED SLIDE FOLLOWING ENTIRE PRESENTATION

Gastroenteritis Panel Testing Key Points

- If available, use culture independent method
- Indications: Dysentery, moderate-to-severe disease, symptoms lasting >7 days
 - Not recommended for chronic diarrhea
 - If *C. difficile* main consideration, test for *C. difficile* alone
- What's missing?
 - Some bacteria (e.g., *Aeromonas* species)
 - Fungi (e.g., microsporidia)
 - Some parasites (e.g., *Strongyloides stercoralis*)
 - Not all panels include same parasites (e.g., *Cyclospora cayatanensis*)
 - Some viruses (e.g., cytomegalovirus)

Meningitis/Encephalitis Panels [BIOFIRE FILMARRAY & QIAstat-Dx QIAGEN*] (For Reference)

Viruses	Bacteria	Fungi
Cytomegalovirus	<i>Escherichia coli</i> K1	Cryptococcus neoformans/gattii
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>Mycoplasma pneumoniae</i> *	
Human parechovirus	<i>Streptococcus agalactiae</i>	
Varicella zoster virus	<i>Streptococcus pneumoniae</i>	
	<i>Streptococcus pyogenes</i> *	

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 $\pm$ VZV PCR an alternative
- May be helpful with current/recent antibiotic treatment
- HHV-6 & CMV may not be clinically significant
- What's missing?
 - Some bacteria (e.g., *Capnocytophaga canimorsus*)
 - Some fungi (e.g., *Aspergillus* species)
 - Some parasites (e.g., *Toxoplasma gondii*)
 - Some viruses (e.g., West Nile virus)

Question #5

A 73-year-old man with a history of coronary artery disease, hypercholesterolemia and colon cancer presents to the Emergency Department with a 4-day history of fever, non-productive cough, and shortness of breath.

Physical examination reveals an ill-appearing man in respiratory distress, pulse 120/min, blood pressure 110/70, respiratory rate 24/min, temperature 39°C, O₂ sat on room air 89.

Chest x-ray shows bilateral pulmonary infiltrates.

Blood cultures, urine Legionella and pneumococcal antigen, CBC, basic chemistries are collected. Sputum is submitted for Gram stain and culture but rejected due to poor specimen quality. A pneumonia panel, which includes Legionella pneumophila, is performed on the sputum and returns a negative result for all organisms on the panel. Urine Legionella and pneumococcal antigen return negative.

He is started on intravenous ceftriaxone and nasal cannula O₂ and transferred to the intensive care unit where he is ultimately intubated and mechanically ventilated.

Question #5

Which of the following is the most appropriate empiric antibiotic to add to this patient's treatment?

- A. Gentamicin
- B. Daptomycin
- C. Trimethoprim-sulfamethoxazole
- D. Vancomycin
- E. Azithromycin

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- E. Azithromycin*

Lower Respiratory Tract Panels (For Reference)

	Curetis Unyvero	BioFire		Curetis Unyvero	BioFire
Bacteria			Viruses		
<i>Acinetobacter</i> spp.	✓		Influenza A		✓
<i>Acinetobacter calcoaceticus-baumannii</i> complex		✓	Influenza B		✓
<i>Chlamydia pneumoniae</i>	✓	✓	Respiratory Syncytial Virus		✓
<i>Citrobacter freundii</i>	✓		Human Rhinovirus/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 oxytoca</i>	✓	✓	Fungi		
<i>Klebsiella pneumoniae</i>	✓		<i>Pneumocystis jirovecii</i>	✓	
<i>Klebsiella pneumoniae</i> group	✓	✓	Resistance genes		
<i>Klebsiella varicola</i>	✓		<i>bla_{PEC}</i>	✓	✓
<i>Legionella pneumophila</i>	✓	✓	<i>bla_{CM}</i>	✓	✓
<i>Moraxella catarrhalis</i>	✓	✓	<i>bla_{IMP}</i>	✓	✓
<i>Morganella morganii</i>	✓		<i>bla_{OXA-23}</i>	✓	
<i>Mycoplasma pneumoniae</i>	✓	✓	<i>bla_{OXA-24}</i>	✓	
<i>Proteus</i> spp.	✓	✓	<i>bla_{OXA-48}</i>	✓	
<i>Pseudomonas aeruginosa</i>	✓	✓	<i>bla_{OXA-58}</i>		
<i>Serratia marcescens</i>	✓	✓	<i>bla_{OXA-49-like}</i>		✓
<i>Staphylococcus aureus</i>	✓	✓	<i>bla_{YJM}</i>	✓	✓
<i>Stenotrophomonas maltophilia</i>	✓	✓	<i>bla_{TX-M}</i>	✓	✓
<i>Streptococcus agalactiae</i>	✓	✓	<i>bla_{TEM}</i>	✓	
<i>Streptococcus pneumoniae</i>		✓	<i>mecA</i>	✓	
<i>Streptococcus pyogenes</i>		✓	<i>mecA/C</i> and <i>MREJ</i>		✓

SEE ENLARGED SLIDE FOLLOWING ENTIRE PRESENTATION

Lower Respiratory Tract Panels Key Points

- Perform alongside Gram stain and culture
- Deploy with antimicrobial stewardship guidance
- Detection of members of the usual respiratory microbiota may be clinically insignificant
- What's missing?
 - Fungi ALL (except one includes *Pneumocystis jirovecii*)
 - Some parasites (e.g., *Toxoplasma gondii*)
 - Some viruses (e.g., varicella zoster virus)
 - Some bacteria (e.g., *Burkholderia cepacia* complex, *Streptococcus dysgalactiae*, non-pneumophila *Legionella* species) - one includes *Stenotrophomonas maltophilia*

Question #6

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.

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*

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- 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*

FDA-Approved Multiplex Panels for Detection of Gram-Positive Bacteria in Positive Blood Cultures (For Reference)

	FilmArray MDx-Chex BCID2	VERIGENE® Gram-Positive Blood Culture Test	cobas® eplex BCID-GP Panel	cobas® 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				✓

SEE ENLARGED SLIDE FOLLOWING ENTIRE PRESENTATION

FDA-Approved Multiplex Panels for Detection of Gram-Positive Bacteria in Positive Blood Cultures (For Reference), cont.

	FilmArray MDx-Chex BCID2	VERIGENE® Gram-Negative Blood Culture Test	cobas® eplex BCID-GP Panel	cobas® eplex BCID-GN Panel
<i>Klebsiella oxytoca</i>	✓	✓		✓
<i>Klebsiella pneumoniae</i>		✓		✓
<i>Klebsiella pneumoniae</i> group	✓			✓
<i>Klebsiella aerogenes</i>	✓	*		✓
<i>Salmonella</i> species	✓			✓
<i>Morganella morganii</i>	✓			✓
<i>Stenotrophomonas 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>Cronobacter sakazakii</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			✓	

SEE ENLARGED SLIDE FOLLOWING ENTIRE PRESENTATION

*Detected as *Enterobacter* species

FDA-Approved Multiplex Panels for Detection of Selective Resistance Genes in Positive Blood Cultures (For Reference), cont.

	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 MREJ	✓				
<i>vanA</i>		✓		✓	
<i>vanB</i>		✓		✓	
<i>vanA/B</i>	✓				
<i>bla_{KPC}</i>	✓		✓		✓
<i>bla_{NDM}</i>	✓		✓		✓
<i>bla_{OXA}</i>	✓		✓		✓
<i>bla_{VIM}</i>	✓		✓		✓
<i>bla_{IMP}</i>	✓		✓		✓
<i>bla_{CTX-M}</i>	✓		✓		✓
<i>mcr-1</i>	✓				

FDA-Approved Multiplex Panels for Detection of Fungi in Positive Blood Cultures (For Reference), cont.

	FilmArray MDx-Chex BCID2	cobas®		
		ePlex BCID-GP Panel	eplex BCID-FP 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 species</i>			✓	
<i>Rhodotorula species</i>			✓	
Pan <i>Candida</i>		✓		✓

SEE ENLARGED SLIDE FOLLOWING ENTIRE PRESENTATION

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 β-lactams (except ceftaroline, ceftobiprole)
 - Even if staphylococci that are methicillin-resistant appear susceptible to these other β-lactams, they are not effective
- Oxacillin or ceftazidime tested
- mecA/C* and MREJ specific for *Staphylococcus aureus*
- For serious infections, oxacillin susceptibility confirmed using PBP2a testing or *mecA* (and *mecC*) nucleic acid amplification test (NAAT)

BioFire Joint Infection Panel (Synovial Fluid)

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

SEE ENLARGED SLIDE FOLLOWING ENTIRE PRESENTATION

Joint Infection Panel Key Points

- Only synovial fluid
- Doesn't nullify need for culture, cell count/differential, crystal assessment
- Used for gonococcal septic arthritis
- Gaps for periprosthetic joint infection (e.g., *Staphylococcus epidermidis*)

Periprosthetic Joint Infection Unified Definition

- STANDALONE CRITERIA – CONFIRMED PJI = One criterion
- Clinical features
 - Sinus tract
- Microbiology
 - Two positive cultures - phenotypically indistinguishable organism from periprosthetic tissue
 - One positive synovial or sonicate fluid culture PLUS one positive periprosthetic tissue culture- phenotypically indistinguishable organism
- Inflammatory markers and histology
 - Synovial fluid leukocyte count >3000 cells/ μ L
 - Synovial fluid polymorphonuclear cells >75%
 - Positive histology: 5 or more neutrophils in each of 5 or more high power fields (400x)
- SUPPORTIVE CRITERIA – PROBABLE PJI = One microbiology PLUS one inflammatory or imaging criterion
- Microbiology
 - Single positive synovial fluid, sonicate fluid or periprosthetic tissue culture
 - Positive molecular test for any organism in synovial fluid, periprosthetic tissue or sonicate fluid
- Imaging
 - Positive WBC-scintigraphy
 - Positive [18F]-FDG-PET/CT when performed more than 6 months after index arthroplasty
- Inflammatory markers
 - Synovial fluid leukocyte count 1500 - 2999 cells/ μ L
 - Synovial fluid polymorphonuclear cells 65-74%
 - Any alternative positive synovial fluid biomarker

SEE ENLARGED SLIDE FOLLOWING ENTIRE PRESENTATION

Wouthuysen-Bakker et al. N Engl J Med. In Press.

Question #7

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*_{KPC}.

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

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- C. Colistin
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Typical Susceptibility of a *bla*_{KPC}-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
IDSA Guidance on the Treatment of Antimicrobial-Resistant Gram-Negative Infections (idsociety.org)

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

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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

Which of the following tests for carbapenemase production?

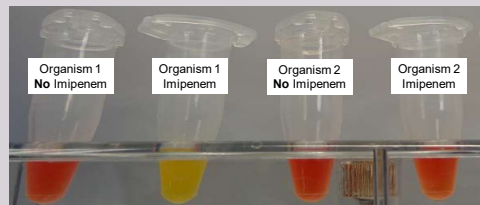
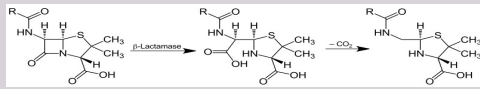
- A. PBP2a test
- B. D-test
- C. Carba NP test*
- D. Polymerase chain reaction assay

Lateral Flow Assay

*bla*_{KPC}, *bla*_{OXA-48-like}, *bla*_{VIM}, *bla*_{IMP}, *bla*_{NDM}



Carbapenemase Production Test Carba NP Test

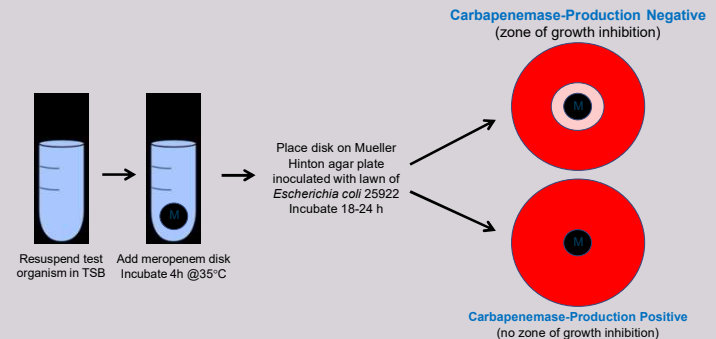


Positive = Carbapenemase Producer
Negative = Carbapenemase Non-Producer

- β -lactam ring hydrolyzed by carbapenemase
- $\downarrow$ pH (detected by indicator dye color change red $\rightarrow$ yellow)
- Rapid (2 hours)

SEE ENLARGED SLIDE FOLLOWING ENTIRE PRESENTATION

Carbapenemase Production Test Modified Carbapenem Inactivation



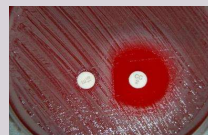
SEE ENLARGED SLIDE FOLLOWING ENTIRE PRESENTATION

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?

- Erythromycin susceptibility, inducible clindamycin resistance
- Erythromycin resistance, constitutive clindamycin resistance
- Erythromycin resistance, inducible clindamycin resistance
- Erythromycin susceptibility, constitutive clindamycin resistance



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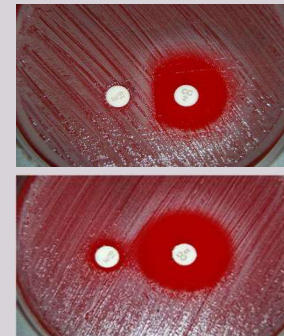


Inducible Clindamycin Resistance (D-Test)

- 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)

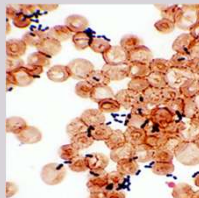
Inducible Clindamycin Resistance (D-Test)

- 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*

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- 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 #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.

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

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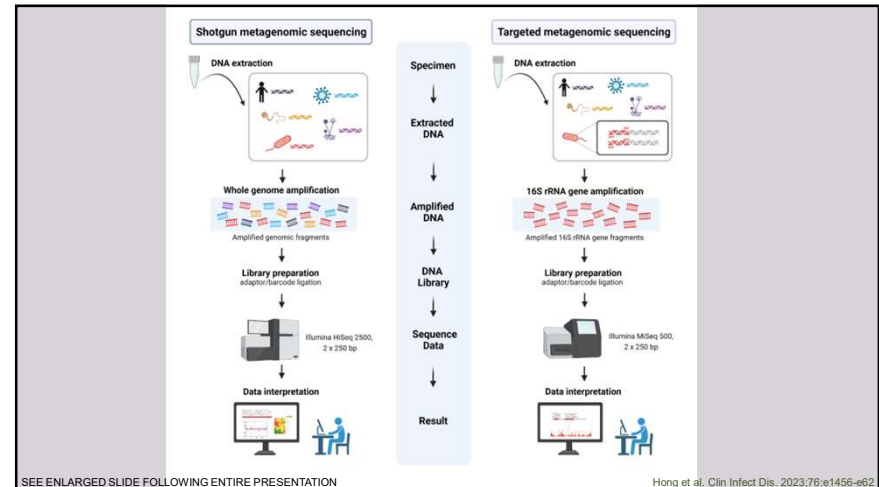
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- A. Cefepime
- B. Ceftriaxone
- C. Trimethoprim-sulfamethoxazole
- D. Azithromycin
- E. *Doxycycline**

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>



Shotgun Metagenomic Sequencing - CSF

- May identify CNS infection not found with conventional diagnostics
 - e.g., hepatitis E virus, St. Louis encephalitis virus, Taenia solium
- Not always positive in CNS infection
 - Infections for which serology preferred (e.g., syphilis, Lyme)
 - Low pathogen titer in CSF
 - Diagnosis from samples other than CSF (tissue)
- May detect microorganisms not causing clinical syndrome
 - e.g., HHV-6, EBV

Plasma Shotgun Metagenomic Sequencing (Cell Free DNA)

- 18,690 reports; 15,165 patients; 22,792 detections
- 701 unique taxa
 - 75% bacteria
 - 15% fungi
 - 7% viruses (herpesviruses, BK virus, adenovirus, torque teno virus)
 - 3% parasites

Park et al. J Clin Microbiol. 2023;61:e01855

Plasma Shotgun Metagenomic Sequencing (Cell Free DNA)

- *Legionella*-like bacteria (80), 40% *Legionella pneumophila*
- *Nocardia* detections (76), 25% *Nocardia cyriacigeorgica*
- *Mycobacterium* species detections (156)
- Zoonotic and vector-borne bacteria detections (247)
 - *Bartonella henselae*, *Bartonella quintana*, *Bartonella clarridgeiae*, *Bartonella vinsonii*
 - *Rickettsia typhi*, *Rickettsia rickettsii*, *Rickettsia felis*
 - *Ehrlichia chaffeensis*, *Ehrlichia muris*, *Anaplasma phagocytophilum*, *Borrelia hermsii*, *Borrelia burgdorferi*
 - *Coxiella burnetii*, *Streptobacillus moniliformis*, *Capnocytophaga canimorsus*
 - *Leptospira kirschneri*, *Leptospira interrogans*, *Leptospira santarosai*, *Leptospira borgpetersenii*, *Leptospira noguchii*
 - *Francisella tularensis*, *Brucella melitensis*
- 632 candidal, 374 Aspergillus species, 196 Mucorales, 78 dimorphic and 33 dematiaceous fungal detections
- 9 microsporidia
- 57 protozoa - 68% *Toxoplasma gondii*, 14% pathogenic amoebae
- 7 helminths - 4 *Strongyloides stercoralis*, 2 *Echinococcus multilocularis*, 1 *Schistosoma mansoni*

SEE ENLARGED SLIDE FOLLOWING ENTIRE PRESENTATION

Park et al. J Clin Microbiol. 2023;61:e01855

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 "typical" pathogens; coagulase negative staphylococci, *Corynebacterium striatum* and *jeikeium*, *Serratia marcescens*, *Pseudomonas aeruginosa*, *Cutibacterium acnes*, nontuberculous mycobacteria (especially *M. chimaerae*), *Candida* spp.
- MAJOR CRITERIA
 - 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

SEE ENLARGED SLIDE FOLLOWING ENTIRE PRESENTATION

Fowler et al. Clin Infect Dis. 2023;77:518-26

Question #13

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 3.6×10^6 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 #13

Which of the following would you recommend?

- Intravenous ganciclovir
- Oral valganciclovir
- Oral acyclovir
- Intravenous foscarnet
- No antiviral therapy is indicated

Question #13

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 log₁₀ 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 #14

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 #14

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

IDSA Guidelines on the Diagnosis of COVID-19

Question #15

A 22-year-old man presents to an urgent care clinic with a cough of 9 day's duration. Prior to the onset of cough, he experienced a runny nose for a couple of days. He has not had fever. The cough is keeping him up at night and has caused him to vomit on two occasions. He has not received any vaccinations since he was a baby. Physical examination is unremarkable, except that he looks tired.

Which of the following is the test of choice for this patient?

- A. Nasopharyngeal swab antigen
- B. Nasopharyngeal swab culture
- C. Nasopharyngeal swab NAAT
- D. Serology

Question #15

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Which of the following is the test of choice for this patient?

- A. Nasopharyngeal swab antigen
- B. Nasopharyngeal swab culture
- C. Nasopharyngeal swab NAAT*
- D. Serology

Question #16

A 60-year-old man presents to an emergency department in Texas with a maculopapular rash that began 7 days prior to presentation. The rash started on his face and subsequently spread to his neck, chest and then arms and legs, and has been accompanied by fever. Before onset of the rash, he had two days of rhinorrhea and red eyes.

Which of the following is recommended?

- A. Throat or nasopharyngeal swab NAAT and serum IgM test
- B. Serum IgM test
- C. Urine NAAT and serum IgG test
- D. Throat or nasopharyngeal swab culture and serum IgM test

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- B. Serum IgM test
- C. Urine NAAT and serum IgG test
- D. Throat or nasopharyngeal swab culture and serum IgM test