Disclosures of Financial Relationships with Relevant Commercial Interests

- Contracted Research - CD Diagnostics, Merck, Hutchison Biofilm Medical Solutions, Accelerate Diagnostics, ContraFect, TenNor Therapeutics Limited and Shionogi
- Consultant - Curetis, Specific Technologies, Next Gen Diagnostics, PathoQuest, Selux Diagnostics, 1928 Diagnostics, PhAST and Qeula
- Patent - Bordetella pertussis/parapertussis PCR; a device/method for sonication; an anti-biofilm substance issued

MALDI ToF Mass Spectrometry

1. Add colony
2. Add matrix (1-2 μl)
   - Dissolved in acetonitrile (50%) & 2.5% trifluoroacetic acid
3. Dry – room air 5 min

Matrix Assisted Laser Desorption Ionization

Laser
Matrix Assisted Laser Desorption Ionization

Time of Flight
Detector

Accelerating potential

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

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 long standing 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

Burkholderia pseudomallei Laboratory Exposure

**Postexposure Antimicrobial Drug Prophylaxis**

<table>
<thead>
<tr>
<th>Antimicrobial Drug</th>
<th>Dosage</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trimethoprim- sulfamethoxazole (TMP-SMX)</td>
<td>2 × 160–320 mg (960 mg) tablets if &gt;60 kg, 3 × 80–400 (480 mg) tablets if 40 kg-60 kg, and 1 × 160–320 mg (960 mg) or 2 × 80–400 (480 mg) tablets if &lt;40 kg, plus folate 5 mg/d</td>
<td>Every 12 h</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>2.5 mg/kg/dose up to 100 mg orally</td>
<td>Every 12 h</td>
</tr>
<tr>
<td>Amoxicillin– clavulanic acid</td>
<td>295 mg/kg/dose. Equates to 3 × 500/125 tabs if &gt;60 kg, and 2 × 500/125 tabs if &lt;60 kg</td>
<td>Every 8 h</td>
</tr>
</tbody>
</table>

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*
  - *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*

---

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

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Verigene EP</th>
<th>Luminex GPP</th>
<th>BioFire GIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter species</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Salmonella species</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Shigella species/Enteroinvasive E. coli</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Vibrio species</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli 0157</td>
<td></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Enterotoxigenic E. coli</td>
<td></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Enteropathogenic E. coli</td>
<td></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Enteroaggregative E. coli</td>
<td></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Plesiomonas shigelloides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shiga toxin-producing E. coli</td>
<td></td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Clostridioides difficile</td>
<td></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Norovirus GI/GII</td>
<td></td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Rotavirus A</td>
<td></td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Astrovirus</td>
<td></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Adenovirus 40/41</td>
<td></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Sapovirus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryptosporidium species</td>
<td></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Entamoeba histolytica</td>
<td></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Giardia lamblia</td>
<td></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Cyclospora cayetanensis</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**TESTING ALGORITHM FOR ACUTE GASTROENTERITIS**

1. **Positive stool culture**
2. **PCR**
3. **Rapid bacterial antigen test**
4. **PCR**
5. **Antibody test**
6. **PCR**
7. **Antibody test**
8. **PCR**

---

**BIOFIRE FILMARRAY MENINGITIS/ENCEPHALITIS PANEL**

<table>
<thead>
<tr>
<th>Viruses</th>
<th>Bacteria</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytomegalovirus</td>
<td><em>Escherichia coli K1</em></td>
<td><em>Cryptococcus neoformans/gattii</em></td>
</tr>
<tr>
<td>Enterovirus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herpes simplex virus 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herpes simplex virus 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human herpes virus 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human parechovirus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Varicella zoster virus</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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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*.

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. lugdunensis* or another *Staphylococcus* species
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.

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 \( \approx 5-8 \) 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 \( \rightarrow \) 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.

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

QUESTION #8

A 65 year old man has multiple blood cultures positive for Pseudomonas aeruginosa resistant to amikacin, gentamicin, tobramycin, aztreonam, 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 \( bla_{KPC} \).
QUESTION #9

The blaKPC gene product would be expected to confer resistance to which of the following?

A. Cefepime
B. Plazomicin
C. Colistin
D. Ceftazidime/avibactam

QUESTION #10

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

a) R 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

- β-lactam ring hydrolyzed by carbapenemase
- pH detected by indicator dye color change red → yellow
- Rapid (2 hours)
CARBAPENEMASE PRODUCTION TEST
MODIFIED CARBAPENEM INACTIVATION

1. Resuspend test organism in TSB
2. Add meropenem disk
3. Incubate 4h @ 35°C
4. Place disk on Mueller-Hinton agar plate inoculated with lawn of Escherichia coli 25922
5. Incubate 18-24 h

Carbapenemase-Production Negative (zone of growth inhibition)
Carbapenemase-Production Positive (no zone of growth inhibition)

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

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**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. casseli/flavus/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.

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

**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.

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
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 ≥ 22 weeks full-dose therapy before testing
  - Accelerated schedule: Poor host factors, extreme viral loads

<table>
<thead>
<tr>
<th>Gene</th>
<th>Drug(s) Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>UL97</td>
<td>Ganciclovir</td>
</tr>
<tr>
<td>UL54</td>
<td>Ganciclovir and cidofovir (if selected for by these agents), foscarnet (if selected for by foscarnet)</td>
</tr>
</tbody>
</table>