Understanding the Basics of Clinical Microbiology.

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Disclosures:

- None
Objectives:

1. Describe the utility of laboratory testing/chemistries in the workup of infection.
2. Describe general microbiologic culture and susceptibility methods and their associated time courses.
3. Describe some forms of rapid diagnostic testing (RDTs)
Learning Assessment:

1. T/F – Elevations in inflammatory biomarkers including (ESR, CRP, PCT, and WBC) indicate the presence of an infectious condition.

2. Which of the following is a catalase positive, coagulase positive, latex positive GPC?
   - Staphylococcus aureus
   - Streptococcus pyogenes
   - Staphylococcus epidermidis
   - Streptococcus pneumoniae

3. Which susceptibility testing method provides a formal MIC? (circle all that apply)
   - Broth microdilution (BMD)
   - Epsilometer test (E-test)
   - Kirby-Bauer disk diffusion
70 y/o female found down in her assisted living facility (ALF). Nurse at ALF notes patient complaining vaguely of “malaise” and appeared to be slightly more confused for 1-2 days prior to being found down.

She is intubated in the ED for hypoxia and inability to protect airway.

PMH: Diabetes, dementia, CVA, hypertension, hyperlipidemia, COPD, CHF, and history pneumonia (5 months prior).

Physical exam:

- Gen: intubated obese white female
- Neuro: Not responsive to voice or touch, passive movement of all extremities
- ENMT: NG, Endotracheal tube in place
- Resp: Lungs with crackles bilaterally
- CV: Hypotensive (starting norepi), tachycardic, no murmers
- GI: Distended with present bowel sounds
- GU: Foley in place
- Musculo: No edema
- Skin: open wound to left calf (chronic appearing – venous stasis ulcer) with surrounding erythema
Vitals from ED: Temp 101.8, BP 90/55 (non-responsive to 2L NS), Pulse 113, RR 32, O₂ Saturation = 82% on 10 L NC

Labs: WBC 27 (34% bands), SCr 2.3 (baseline 1.15), H/H 9.5/28.1, plts 225, lactate 5.5, CRP 225, ESR 12, and Procalcitonin 9.1.

- UA: 4+ Leukocyte esterase, 2+ bacteria, >150 wbc, nitrite positive, 0 RBC, and hazy brown

Imaging:
- CXR: Bibasilar infiltrates vs. atelectasis (R>L), cannot exclude pneumonia

Cultures:
- Blood (peripheral): Pending
- Urine (foley): Pending
- Respiratory (Endotracheal aspirate): Pending
Why do we think she is infected?

Clinical Presentation!

1. Constellation of symptoms → Septic Shock
   - Hypotension (not responsive to fluids → vasopressor dependent)
   - Tachycardia
   - Fever
   - Leukocytosis
   - End organ damage: Altered mental status and elevated SCr

2. Imaging:
   - CXR with ? Pneumonia

3. Non-specific labs:
   - WBC (bands)
   - Lactate
   - Pro-inflammatory markers: CRP, ESR, Procalcitonin
   - Positive UA?
Non-specific Lab Tests: \(^1,2\)

- There is NO single definitive test for identification of infection!
  - i.e. All have limitations
  - Always should be paired with clinical presentation.

- Examples:
  - White Blood Cell (WBC) Count:
    - Elevate in response to infection
    - Also elevate in response to: Drugs (i.e. steroids), stress, inflammation, etc.
    - Bands = immature neutrophils
      - “Left Shift”: >9% bands
  - Lactate:
    - Demonstrates shift to anaerobic metabolism / illustrates tissue hypoperfusion
    - Elevates in response to shock, tissue ischemia, severe liver disease, and some medication (metformin)
Non-specific Lab Tests:¹⁻³

- CRP and ESR:
  - C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR):
    - Non-specific acute phase reactant (i.e. non-specific inflammatory marker)
    - Elevate vaguely in response to inflammation
    - Generally CRP reacts faster than ESR
    - Better for trending chronic infections vs. determining if infection present.
  - Erythrocyte sedimentation rate (ESR)
    - Non-specific inflammatory marker
    - Generally reacts slower than CRP

- Procalcitonin:
  - 116 amino acid precursor of calcitonin
  - More sensitive than CRP at detecting bacterial infection
  - Detectable in 2-4 hours / Peak = 8-24 hours / Half-life = 24 hours
  - Rises not impaired by neutropenia or immunosuppression
  - Most useful in community-acquired lower respiratory tract and sepsis
Non-specific Lab Tests:4,5

- **Urinalysis:**
  - Color and Clarity: Non-specific
  - Nitrites:
    - Reductase: Nitrates → Nitrites
    - Weakly sensitive/highly specific for the PRESENCE of bacteria.
  - Leukocyte Esterase:
    - Produced by neutrophils
    - Indicates pyuria
  - WBC: Grades the pyuria
  - Bacteria: May signal contamination, ASB, or infection
  - Squamous Epithelial Cells: May help determine quality of specimen.

<table>
<thead>
<tr>
<th>COLOR</th>
<th>Amber</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLARITY</td>
<td>Cloudy</td>
</tr>
<tr>
<td>Specific Gravity</td>
<td>1.017</td>
</tr>
<tr>
<td>PH UA</td>
<td>5.0</td>
</tr>
<tr>
<td>GLUCOSE UA</td>
<td>1+</td>
</tr>
<tr>
<td>KETONES UA</td>
<td>Trace</td>
</tr>
<tr>
<td>PROTEIN UA</td>
<td>2+</td>
</tr>
<tr>
<td>BLOOD UA</td>
<td>3+</td>
</tr>
<tr>
<td>BILIRUBIN UA</td>
<td>Negative</td>
</tr>
<tr>
<td>NITRITE UA</td>
<td>Negative</td>
</tr>
<tr>
<td>UROBILINOGEN UA</td>
<td>Normal</td>
</tr>
<tr>
<td>ASCORBIC ACID UA</td>
<td>Negative</td>
</tr>
<tr>
<td>LEUKOCYTES ESTERASE UA</td>
<td>3+</td>
</tr>
<tr>
<td>WBC UA</td>
<td>20 - 50</td>
</tr>
<tr>
<td>RBC UA</td>
<td>50 - 100</td>
</tr>
<tr>
<td>SQUAMOUS EPITHELIAL UA</td>
<td>0 - 1</td>
</tr>
<tr>
<td>BACTERIA UA</td>
<td>1+</td>
</tr>
<tr>
<td>HYALINE CASTS UA</td>
<td>&gt;20</td>
</tr>
<tr>
<td>GRANULAR CASTS UA</td>
<td>2 - 4</td>
</tr>
<tr>
<td>MUCUS UA</td>
<td>Present</td>
</tr>
</tbody>
</table>
Asymptomatic Bacteriuria (ASB):\textsuperscript{5}

- No matter what Bear Grylls tells you... urine is not a sterile body fluid.

- A “dirty” UA or “pyuria” in the absence of symptoms is NOT an indication for antimicrobial therapy.

- Caveats:
  - Pregnancy
  - ASB during procedures which will compromise of urinary mucosa.

### Table 2. Prevalence of asymptomatic bacteriuria in selected populations.

<table>
<thead>
<tr>
<th>Population</th>
<th>Prevalence, %</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy, premenopausal women</td>
<td>1.0–5.0</td>
<td>[31]</td>
</tr>
<tr>
<td>Pregnant women</td>
<td>1.9–9.5</td>
<td>[31]</td>
</tr>
<tr>
<td>Postmenopausal women aged 50–70 years</td>
<td>2.8–8.6</td>
<td>[31]</td>
</tr>
<tr>
<td>Diabetic patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Women</td>
<td>9.0–27</td>
<td>[32]</td>
</tr>
<tr>
<td>- Men</td>
<td>0.7–11</td>
<td>[32]</td>
</tr>
<tr>
<td>Elderly persons in the community\textsuperscript{a}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Women</td>
<td>10.8–18</td>
<td>[31]</td>
</tr>
<tr>
<td>- Men</td>
<td>3.6–19</td>
<td>[31]</td>
</tr>
<tr>
<td>Elderly persons in a long-term care facility</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Women</td>
<td>25–50</td>
<td>[27]</td>
</tr>
<tr>
<td>- Men</td>
<td>15–40</td>
<td>[27]</td>
</tr>
<tr>
<td>Patients with spinal cord injuries</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Intermittent catheter use</td>
<td>23–89</td>
<td>[33]</td>
</tr>
<tr>
<td>- Sphincterotomy and condom catheter in place</td>
<td>57</td>
<td>[34]</td>
</tr>
<tr>
<td>Patients undergoing hemodialysis</td>
<td>28</td>
<td>[28]</td>
</tr>
<tr>
<td>Patients with indwelling catheter use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Short-term</td>
<td>9–23</td>
<td>[35]</td>
</tr>
<tr>
<td>- Long-term</td>
<td>100</td>
<td>[22]</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Age, \(\geq\)70 years.
Cultures...the basics: 1,6-9

1. Only culture something...if you plan to use the culture to guide therapy.
   - May NOT always be necessary (i.e. uncomplicated CAP or perforated appendicitis)

2. Always culture PRIOR TO administration of antibiotics if possible.
   - Sepsis is the obvious exception
     - 7.6% increase in mortality for every hour antimicrobial therapy is delayed.

3. Take care to avoid contamination with patient’s usual flora.

4. Interpret cultures with a critical eye:
   - What source/type of culture was obtained?
   - What was the culture method?
   - What did the gram-stain/grouping show?
   - What grew?
   - Does what grew match the clinical suspicions?
   - What did susceptibilities show?
Culture Source/Type:

- **Source:**
  - Blood
  - Wound, bone, tissue, abscess
  - CSF
  - Respiratory
  - Stool
  - Urine
  - Body fluid (pleural, ascites, etc.)

- **Type:**
  - Aerobic
  - Anaerobic
  - Fungal
  - Acid fast bacilli
Culture Source/Type:¹

- Some variance in microbiology processing:
  - BACTEC alert device
    - Blood
    - Gram-stain negative Body Fluids
  - Required additional processing:
    - Tissue
    - Bone
  - Straight to plate
    - Wound
    - Respiratory
    - Urine
    - Gram-stain positive
    - CSF
Culture Source/Type: 1,8-11

- Anticipate pathogen based on location of infection.
  - Skin and Soft Tissue Source:
    - Skin flora (Staphylococcus/Streptococcus)
  - Respiratory Source: *S. pneumoniae*, *M. catarrhalis*, *L. pneumophila*, *M. Pneumoniae*, *C. pneumoniae*, *H. influenzae*.
    - Hospital-acquired: MDRO gram-negative rods (including *P. aeruginosa*) and *S. aureus*
  - GI Source: *E. coli*, *Klebsiella spp.*, *B. fragilis*, *S. anginosus*, *Enterococcus spp.*
Culture Method: 10-14

- How was it collected?
- Is it a good specimen?
- Could it be contaminated?

General features:
- Look for presence of WBC
- Look for absence of squamous epithelial cells

Respiratory:
- Sputum (expectorated) vs. sputum (suction)
- Endotracheal aspirate vs. Bronchoalveolar lavage (BAL) vs. mini-BAL

Wounds:
- Purulent vs. Non-purulent
- Superficial swab vs. tissue/biopsy
- Chronic ulcer vs. acute wound

Blood:
- ? Contaminant
  - How many bottles of how many draws?
  - How long did it take to grow?
Culture Method:

- **Quantitative:**
  - Provides more robust estimate of number of organisms in a given culture.
  - Requires fluid specimen.
  - One mL of specimen is plated in plated and depending on growth on streak estimate made.
  - i.e. >100,000 cfu/mL

- **Semi-quantitative:**
  - Attempts to estimate the quantity of organisms in a given culture.
  - Cultures plated in one quadrant on plate.
  - Growth in primary quadrant = 1+
  - Extension characterized as 2+, 3+, and 4+

[Link to intranet.tdmu.edu.ua/data/cd/disk2/ch010.htm]
Gram Stains:¹

1. Specimen applied to slide
2. Crystal violet stain applied followed by iodine.
3. Alcohol decolorizing solution applied
4. Counterstain with safranin
5. Gram-negative: Red/Pink
6. Gram-positive: Purple in appearance
Gram Stains: \(^{16}\)

- **Grouping:**
- More relevant in gram positive cocci (GPC):
  - Staphylococcus spp. → GPC pairs, tetrads, and clusters
  - Streptococcus spp. / Enterococcus spp. :
    - Generally: GPC in chains
    - LONG chains → Beta-hemolytic strep or *S. viridans*
    - Diplococci and chains → *S. pneumoniae*

- What about... GPC pairs?
Gram Stains/Grouping:

[Diagram showing the classification of bacteria based on their staining properties and size.]

- **Gram-positive**
  - **Cocci**
    - **Clusters**
      - Staphylococcus
    - **Coagulase-positive**
      - Staphylococcus aureus
    - **Coagulase-negative**
      - Staphylococcus epidermidis
    - **Pairs (diplococci)**
      - Pneumococcus
    - **Streptococcus pneumoniae**
  - **Chains**
    - Streptococcus
      - **α-hemolytic**
        - Streptococcus pyogenes
      - **β-hemolytic**
        - Viridans Streptococcus
        - Strptococcus pneumoniae
    - **γ-hemolytic**

- **Gram-negative**
  - **Cocci**
    - Neisseria meningitidis
    - Neisseria gonorrhoeae
  - **Bacilli**
    - Lactose fermenter
      - Oxidase-positive
        - Achromobacter
      - Oxidase-negative
        - Pseudomonas aeruginosa
        - Serratia marcescens
        - Moraxella spp.
    - Non-lactose fermenter
      - Oxidase-positive
        - Pseudomonas aeruginosa
        - Rhodobacter spp.
        - Achromobacter spp.
      - Oxidase-negative
        - Pseudomonas aeruginosa
        - Pseudomonas putida
        - Pseudomonas aeruginosa

[Diagram continues with more detailed classifications and examples.]
Gram Stains:

16
Gram Stains x 2:

• Specimen received
• Initial gram stain (1-6 hours)
• Plated and grown (24-48 hours)
• Growth gram stain
• Organism identification (0-24 hours)
• Susceptibilities (18-24 hours)
Gram Stains ➔ Plate Growth:

Culture, Respiratory, Lower, Smear

Order Status: Completed
Specimen Information: Respiratory from Endotracheal Aspirate

Culture

Lab Status: Final result

Gram Stain Result

Result:
>25 per low power field: White blood cells, polymorphonuclear

Narrative:
Tracheostomy or Endotracheal tubes are followed by colonization within 24 hours of insertion, and results may not correlate with disease. Culture only if clinical pneumonia is present.

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Staphylococcus aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>VITEK</td>
<td></td>
</tr>
<tr>
<td>Clindamycin</td>
<td>&lt;=0.25 ug/mL</td>
</tr>
<tr>
<td></td>
<td>Sensitive</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>&lt;=0.25 ug/mL</td>
</tr>
<tr>
<td></td>
<td>Sensitive</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>0.5 ug/mL</td>
</tr>
<tr>
<td></td>
<td>Sensitive</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>&gt;0.5 ug/mL</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
</tr>
<tr>
<td>Trimethoprim+Sulfamethoxazole</td>
<td>&lt;=10 ug/mL</td>
</tr>
<tr>
<td></td>
<td>Sensitive</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>1 ug/mL</td>
</tr>
<tr>
<td></td>
<td>Sensitive</td>
</tr>
</tbody>
</table>

1 Oxacillin susceptible strains are susceptible to other anti-staphylococcal B-lactams (except amoxicillin, ampicillin, penicillin, piperacillin and ticarcillin).
Plate Growth:

- Identification...
  - ...what is taking so long?
- Pure plates vs. mixed plates
  - “re-isolating for more information”
- Poor vs. no plate growth plates
- Oddly behaving organisms
Plate Growth (GNR):
Plate Growth (GNR):¹⁷

- **Gram Negatives:**
  - **Lactose fermentation:**
    - Helps to distinguish between GNRs prior to formal ID.
      - Pseudomonas vs. other
  - **MacConkey agar:**
    - Inhibits gram-positive growth
    - Lactose Fermenting:
      - Lowers pH → red agar
    - Non-lactose fermenting:
      - Ammonia production raises pH → Clear/opaque agar

¹⁷ Local Reference 17
Plate Growth (GNR):\textsuperscript{17}

\begin{itemize}
\item \textbf{Oxidase:}
\item Assesses for presence of cytochrome oxidase
\item Not produced by Enterobacteriaceae
  \begin{itemize}
  \item Produced by pseudomonas
  \end{itemize}
\item Positive test = Purple stain
  \begin{itemize}
  \item i.e. agent is oxidized
  \end{itemize}
\item Negative test = Colorless
  \begin{itemize}
  \item i.e. agent remains reduced
  \end{itemize}
\end{itemize}

http://www.medical-labs.net/oxidase-test-1291/
Plate Growth (GPC):¹
Plate Growth (GPC):

- **Catalase:**
  - \(2\text{H}_2\text{O}_2 \rightarrow \text{O}_2 + \text{H}_2\text{O}\)
    - \(\text{O}_2\) released as gas = bubbles
  - Differentiates staphylococcus from streptococcus
    - Staphylococcus = catalase positive
    - Streptococcus = catalase negative

[Image: http://4.bp.blogspot.com/-pGWy_YzoaD4/UZXnPopWsUI/AAAAAAAAAH0/nrnpu-kKufg/s1600/slide+catalase+test+results.jpg]
Plate Growth (GPC): 17

- **Catalase positive:**
  - **Latex agglutination:**
    - Antibody for *S. aureus* on latex beads
      - Latex positive = *S. aureus*
      - Latex negative = CoNS
  - **Coagulase:**
    - Converts fibrinogen to fibrin clot with help of plasma factors
      - *S. aureus* = positive
      - *S. epidermidis* and other CoNS = negative

- **Catalase negative:**
  - **Hemolysis:**
    - Does it growth cause hemolysis of blood agar
      - Alpha = green = partial hemolysis
        - *S. viridans*
        - *S. pneumonia*
        - Maybe *S. anginosus*
      - Beta = clear = full hemolysis
        - “Typeable” streptococcus
          - Group A, B, C, G
      - Gamma = red = no hemolysis
        - Enterococcus spp. (PYR)
        - Maybe *S. anginosus*
Organism Identification:

- Specimen received
- Initial gram stain (1-6 hours)
- Plated and grown (24-48 hours)
- Growth gram stain
- Organism identification (0-24 hours)
- Susceptibilities (18-24 hours)
Organism Identification:

- VITEK vs. Microscan vs. Phoenix

- PAMC = VITEK2
  - Performs both:
    - Organism identification
    - Susceptibilities
      - Automated broth microdilution (BMD)
    - We will come back to this!
Organism ID vs. Clinical Suspicion:

- Culture Source
- Method of Collection
- Suspicion for Contamination
  - Gram stains

- Growth
- Organism ID

- Presence of Usual flora?
- Odd organism for culture source?
- Growth that doesn’t match the initial gram stain?
- Initial gram stain that doesn’t match the growth?
Susceptibilities:

• Specimen received
• Initial gram stain (1-6 hours)
• Plated and grown (24-48 hours)
• Growth gram stain
• Organism identification (0-24 hours)
• Susceptibilities (18-24 hours)
Susceptibilities:¹

### Quantitative Results:
- Susceptible
- Intermediate
- Resistant

### Qualitative Results:
- Driven by MIC
- Actual determinant behind quantitative results
- Multiple methods (BMD vs. KB vs. E-test)

Valuable...but sometimes hard to interpret.

User friendly...but perhaps oversimplified.
“Semi” -Qualitative Susceptibilities: $^1$

- Disk diffusion test (i.e. Kirby Bauer)
  - Grow bug $\rightarrow$ Drop Disk $\rightarrow$ Measure zone of inhibition
  - Susceptibility of organism is determined by “zone of inhibition”
    - Determined by CLSI standards
    - Varies depending on organism
    - Varies depending on drug
  - Generally:
    - Bigger zone of inhibition = more susceptible bug
    - Can perform multiple tests (up to 12) on same plate
    - Able to choose specific agents to test
  - Pros: Reliable, flexible, cheap, and simple
  - Cons: May be impacted by incubation temp or bacterial inoculum
Qualitative Susceptibilities:¹

- **Minimum Inhibitory Concentration:**
  - “The lowest antimicrobial concentration that prevents visible growth of an organism after ~24 hours of incubation in a specified growth medium”
  - Susceptibility breakpoints determined by CLSI
  - Traditionally
    - Macrotube dilution method vs. Solid agar
    - Labor intensive!
  - Present day:
    - Automated
      - VITEK2 vs. Microscan (turbidity) vs. Phoenix
      - Epsilometer Test (i.e. E-test)
  - Tells us the level of susceptibility of an organism rather than just the interpretation of that level.
    - E. coli: Piperacillin/tazobactam \(\leq 4\) vs. 32 mcg/mL
    - MRSA: Vancomycin <0.5 vs. 2 mcg/mL
Qualitative Susceptibilities:¹

- **Automated Broth Microdilution (BMD):**
  - Inoculate card ➔ Put in machine ➔ Wait
  - Tests organism to multiple concentrations of multiple drugs
    - Drugs in card determined by manufacturer or card selected.
  - Determines organism MIC to multiple agents in single test
    - Run time = 18-24 hours
  - Pros: Easy, reliable, provides formal MIC
  - Cons: Requires machine ($$$) and lacks flexibility in agent selection.
Qualitative Susceptibilities: \textsuperscript{1,18}

- Epsilometer test (i.e. E-test)
  - Grow bug $\rightarrow$ Drop strip $\rightarrow$ look for ellipse/strip intersection.
- E-Strip:
  - Single agent
  - Increasing concentrations
- One strip per plate.
  - Has been at times to be more accurate than automated broth microdilution
- Pros: Easy to perform, \textsuperscript{?} Easy to read, cheap
- Cons: One per plate, \textsuperscript{?} Easy to read
Susceptibilities:

Summary:

1. Do you have qualitative, quantitative, or both?
2. If qualitative, was it performed via BMD, E-test, or Kirby-Bauer?
3. If BMD or E-test, just how susceptible was the organism (i.e. what was the MIC)?

Select a therapy!

1. What is the narrowest spectrum agent that treats all presently identified organisms?
Rapid Diagnostic Testing: Sensitivity vs. Specificity

Varies depending on testing method and specific test

**Sensitivity:**
- If a person HAS the disease how often will the test be positive?
  - I.e.
    - 10 influenza patients present and are swabbed for EIA
    - Rapid flu swab (EIA) detects 5/10.
    - Sensitivity = 50%
- Rate of true positive vs. false negative.

**Specificity:**
- If a person does NOT HAVE the disease how often will the test be negative?
  - I.e.
    - 10 patients WITHOUT influenza present and are swabbed for EIA
    - Rapid flu swab (EIA) detects 2/10
    - Specificity = 80%
- Rate of true negative vs. false positive.
Rapid Diagnostic Testing (RDT):  

- **Antibody testing:**
  - **Agglutination testing:**
    - Antibodies (polyclonal or monoclonal) attached to latex beads and specimen introduced.
      - If lattice structure forms then antigen is present (antibody-antigen complexes)
    - Typically tested from growth (not generally from direct specimen)
    - Ex: *S. aureus* from plate growth
  - **Enzyme immunoassay (EIA)/Enzyme-linked immunosorbent assay (ELISA):**
    - Antibody coated wells/trays → Specimen (antigen) introduced → Well washed out → Second antibody introduced → well washed out → coloring agent added.
      - Wells that change color = positive for antigen.
    - Typically tested direct from specimen
    - Ex: Influenza A and B, Ag EIA (i.e. rapid flu swab)
Rapid Diagnostic Testing (RDT): \(^1,\text{17}\)

- **Polymerase Chain Reaction (PCR / NAAT):**
  - Testing done directly from collection specimen
  - Target amplification system
    - Amplifies SMALL sections of DNA using DNA polymerase and short oligonucleotide primers for detection.
      - If more than one primer used = improved sensitivity (multiplex PCR)
  - Ex: Respiratory viral pathogen panel (Biofire\textsuperscript{TM})
    - Blood Culture Identification Panel (BCID)
    - GI Panel
    - Meningitis/Encephalitis Panel
  - C. diff, NAAT

- **16S rRNA:**
  - Looks for specific section of ribosomal RNA that helps to identify specific organisms in a specimen.
    - Draws on LARGE bank of known sequencing vs. specific testing on specific platform
    - Testing of direct specimen
Rapid Diagnostic Testing (RDT):\textsuperscript{17}

- Mass Spectrometry:
  - Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF)
    - Thin smear on metallic slide
    - Hit with pulses of laser
    - Desorbed and deionized particles then accelerated through electrostatic field and drifted through vacuum tube
    - Contact mass spectrometers detector
    - Different particles fly at different speeds which indicates the presence of components of specific organisms
    - Typically run off of organism growth
Rapid Diagnostic Testing (RDT): \(^{19}\)

- **Accelerate Diagnostics – Pheno\(^{TM}\)**
  - **Gel electro-filtration (GEF)**
    - Sample loaded into gel well that contains pores smaller than bacterial cells
    - Electric current applied which removes cellular debris to isolate/concentrate bacterial cells
  - **Electro-kinetic concentration (EKC)**
    - Cells are drawn to surface where analysis will take place by exposure to mild electric charge.
  - **FISH (Fluorescence in-site hybridization)**
    - Cells exposed to probes with fluorescent tags looking for specific nucleic acid sequences.
  - **Fast phenotypic susceptibility testing**
    - Cell exposed to single concentration of agent and time lapse imaging correlates growth patterns to MICs.
Patient Case: (HD1)

- **Blood:**
  - Drawn vial peripheral draw
  - Aerobic and anaerobic bottles drawn from 2 separate sites
  - In BACTEC and pending

- **Urine:**
  - Collected from foley
  - No gram-stain ordered
  - Plated and pending

- **Respiratory:**
  - Collected as endotracheal aspirate
  - Gram stain: 1+ usual respiratory flora (<10 WBC / 0 epithelial)
  - Plated and pending
Patient Case: (later on HD1)

- **Blood:**
  - 1/2 draws positive for gram-negative rods at 8 hours → plated
  - Collected from peripheral draw x 2 (aerobic and anaerobic on each)

- **Urine:**
  - Cultures currently pending
  - Collected from foley catheter

- **Respiratory:**
  - Cultures currently pending
  - Collected from ETA with initial gram stain (1+ URF, <10 WBC, 0 epithelial)
Patient Case: (HD2)

- **Blood:**
  - Growth of GNR on plates at 24 hours (lactose fermenter / oxidase negative)
  - Collected from peripheral draw x 2 (aerobic and anaerobic on each)

- **Urine:**
  - 40,000 cfu gram negative rods on plates at 24 hrs (lactose fermenter / oxidase negative)
  - Collected from foley (initial plate growth = >100,000 cfu GNR)

- **Respiratory:**
  - Plates growing 1+ usual respiratory flora (at 24 hours)
  - Collected from ETA with initial gram stain (1+ URF, <10 WBC, 0 epithelial)
Patient Case: (HD3)

- Blood:
  - E. coli identified as organism
- Urine:
  - E. coli identified as organism
- Respiratory:
  - Cultures “finaled” as 1+ “usual respiratory flora”
  - Collected from ETA with initial gram stain (1+ URF, <10 WBC, 0 epithelial)
Patient Case: (HD4)

Blood:
- E. coli identified as organism

Urine:
- E. coli identified as organism
Learning Assessment:

1. T/F – Elevations in inflammatory biomarkers including (ESR, CRP, PCT, and WBC) indicate the presence of an infectious condition.

2. Which of the following is a catalase positive, coagulase positive, latex positive GPC?
   - Staphylococcus aureus
   - Streptococcus pyogenes
   - Staphylococcus epidermidis
   - Streptococcus pneumoniae

3. Which susceptibility testing method provides a formal MIC? (circle all that apply)
   - Broth microdilution (BMD)
   - Epsilometer test (E-test)
   - Kirby-Bauer disk diffusion
References:


