Blood cultures: past, present and future

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Faculty/ Presenter Disclosure

* Faculty: Dr Natalia Solomon
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  * Speakers Bureau/Honoraria: None
  * Consulting Fees: None
  * Other: Consultant for DynaLIFE_{Dx} Laboratories
Disclosure of Commercial Support

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• Potential for conflict(s) of interest:
  * Dr Natalia Solomon has received no honorarium and provides consulting services to \( \text{DynaLIFE}_{\text{Dx}} \).
  * \( \text{DynaLIFE}_{\text{Dx}} \) provides laboratory services which will be discussed in this program.
Mitigating Potential Bias

* DynaLIFE$_{Dx}$ operates in accordance with Alberta Health Services, testing and solutions are a direct result of provincial standards.
Case

* 36 y.o. previously healthy female, developed nasal stuffiness, mild sore throat, and a cough 3 days ago.
* Today she presented with fever up to 40°C, chills, progressively worsening cough with purulent sputum, and fatigue.
* Based on a history and physical examination you are suspecting bacterial pneumonia and in addition to other investigations ordering blood cultures...
What is the **recommended** total volume of blood per culture?

a) 5-10 ml  
b) 20-30 ml  
c) 40 ml  
d) 50 ml  
e) More is better
Percentage increase for all pathogens recovered related to the volume of blood cultured

<table>
<thead>
<tr>
<th>Patient group</th>
<th>20 mL vs. 10 mL</th>
<th>30 mL vs. 10 mL</th>
<th>30 mL vs. 20 mL</th>
<th>40 mL vs. 10 mL</th>
<th>40 mL vs. 20 mL</th>
<th>40 mL vs. 30 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>No endocarditis</td>
<td><strong>29.8</strong></td>
<td><strong>47.2</strong></td>
<td>13.4</td>
<td><strong>57.9</strong></td>
<td>21.6</td>
<td><strong>7.2</strong></td>
</tr>
<tr>
<td>Endocarditis</td>
<td>7.7</td>
<td>7.7</td>
<td>0</td>
<td>7.7</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**NOTE.** A total of 40 mL of blood was obtained within a 30-min period; 20 mL was obtained separately from each of 2 phlebotomies and distributed equally between 1 aerobic (BACTEC Plus Aerobic/F resin; Becton Dickinson) and 1 anaerobic (BACTEC Lytic/10 Anaerobic/F; Becton Dickinson) bottle.

Blood cultures: Volume

* The **volume of blood** that is obtained for each blood culture **is the single most important variable** in recovering microorganisms from patients with blood stream infections.

* Recommended volume of blood to obtain from adults per culture is **20-30 ml**

* Recommended volume of blood per bottle for adult patient is **8-10 ml**
Checklist for blood cultures collection

- **Timing** of blood cultures and the **optimum interval** between successive blood cultures
- **Volume** of blood (total and per bottle)
- **Number of sets** (2-3 sets per episode)
- **Separate** blood cultures
- **Site**
- **Clinical information**
Case: You received a phone call from the lab...

**Scenario 1**
- Gram positive cocci in chains
- 1/3 vials
- After 26 hours of incubation
- Culture: Streptococcus sanguinis (VGS)

**Scenario 2**
- Gram positive cocci in chains
- 3/3 vials
- After 10 hours of incubation
- Culture: Streptococcus pneumoniae
Check list for blood culture results interpretation

- Organism
- Time to detection
- Total number of bottles vs number of positives
- **Aerobic vs anaerobic**
- Clinical diagnosis
- Previous/following/other cultures available
Blood cultures: Limitations

* **Mycobacteria, filamentous and dimorphic fungi** will *not* grow using **routine** BC method—will require special media (MycoF/lytic vials, lysis-centrifugation vials) and extended incubation
* **Coxiella burnetii, Chlamydia, Rickettsia, Tropheryma whippelii** - uniformly uncultivable, use serology or molecular methods
* **Bartonella, Legionella**- can be isolated using lysis-centrifugation vials and special culture protocols but more optimally diagnosed by serology or molecular methods
* **Blood volume** and diagnostic yield
* **Time** from sampling to incubation
Blood cultures: Instrument
Identification of the organisms-past, present, and future

* Gram stain
Identification of the organisms-past and present

* Morphologic characteristics
Identification of the organisms-past

* Manual biochemical tests

* Culture-based automated systems
  * Longer turnaround time
  * Expensive consumables
  * Priori knowledge of organism type
Identification of the organisms-present

* MALDI-TOF MS (Matrix Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry)
MALDI-TOF MS: How it works

- The target slide is prepared and introduced to a high-vacuum environment
- A precise laser burst desorbs and ionizes the sample
- A “cloud” of proteins is released and accelerated by an electric charge
- The ionized analytes (proteins) enter a flight tube and are separated by their individual mass
- A mass spectrum is generated and represents the number of ions hitting the detector at the end of the flight tube at a specific time (time of flight)
- Generated mass spectrum compared against a database and software scores the relatedness of the test isolate’s spectrum to the known spectra stored in the system’s library
MALDI-TOF MS: Advantages

- **Volume** - single or multiple isolates may be tested at a time, capable of analyzing thousands of samples per day
- Decreased **Turnaround Time**, superior speed (the analytical turnaround time is $\leq 3$ min per isolate)
- **Cost-effectiveness**
- **Accuracy** - identifies organisms to the genus and species level. No interpretation required by technologist.
- **Database** - comprehensive collection of clinically relevant bacteria and fungi that can be customized and consistently updated
MALDI-TOF MS: Limitations

- **Does not provide antimicrobial susceptibility** results
- Can not be **routinely** applied directly to clinical specimens - organism must first be cultivated
- **Uncultivable** organisms cannot be identified
- Database may be less comprehensive for esoteric organisms
MALDI-TOF: Future Developments

* Organism identification **directly from clinical specimens**
  * Specimen with relatively little human protein (blood, urine)
  * Requires high bacterial counts
  * Identification of organisms in mixed cultures
  * Requires extensive preparatory specimen processing
  * Would **not** provide antimicrobial susceptibility results
Identification of the organisms: Molecular Methods

Molecular methods for the diagnosis of sepsis

- Hybridization techniques
- Amplification techniques
- Non-nucleic acid based techniques
Identification of the organisms: Molecular Methods

* Hybridization
  * FISH

* Amplification
  * PCR
  * Multiplex PCR
  * Broad-range PCR

* Non-nucleic acid based
  * Spectrometry
  * Phage assays
Diagnosis of sepsis independent of blood culture: Molecular Methods

- Applied directly on whole blood samples
- No time-consuming culture required
- Include broad-range and multiplex PCR
- Most of them still used as a research tool
- Clinical utility and benefits to be determined
Faster than conventional blood cultures

<table>
<thead>
<tr>
<th>Method</th>
<th>TAT after positive BC</th>
<th>TAT after whole blood sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional</td>
<td>18-24h-few days</td>
<td></td>
</tr>
<tr>
<td>Culture growth needed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Hybridization</td>
<td>1.5-3h</td>
<td>12-24+h</td>
</tr>
<tr>
<td>• Amplification</td>
<td>1-8h</td>
<td>12-24+h</td>
</tr>
<tr>
<td>• Mass spectrometry</td>
<td>4-6h</td>
<td>12-24+h</td>
</tr>
<tr>
<td>Directly from blood sample</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Whole blood amplification methods</td>
<td></td>
<td>6h</td>
</tr>
</tbody>
</table>
Molecular methods: Advantages (continue)

- May yield **better sensitivity**
- Better detection in antimicrobial **pre-treated** patients
- May detect specific resistance profiles (e.g. meca gene)
Critical hours after diagnosis are still lost
Pathogen panels may not include all relevant organisms
Detect DNAemia, not bacteremia, therefore, interpretation of positive result may be challenging (viable? contamination? infection?)
A limited number of bacterial resistance genes can be routinely detected, however, those methods do not provide full antimicrobial susceptibility results for the detected pathogen
* Microbial nucleic acid extraction required
* **Expensive** (equipment, reagents, labor)
* **Resources** (dedicated space, trained personnel)
* Data indicate that more rapid microbiological diagnosis **does not** ensure more rapid adequate antimicrobial therapy
* Antibiogram-cumulative antimicrobial susceptibility test data summary
* Generated by analysis of results on isolates from a particular institution(s) in a defined period of time (annually)
* Reflects the percentage of first isolates (per patient) of a given species that is susceptible to each of the antimicrobial agents routinely tested
* Should be used to guide empirical therapy decisions
Antibiogram

* [www.dynalifedx.com](http://www.dynalifedx.com)
Rapid Antigen Detection Test (RADT) for Group A Streptococcal Pharyngitis: an update
Group A Streptococcal Pharyngitis: RADT

* Developed in early 1980’s to provide rapid diagnosis of acute Streptococcal pharyngitis
* Test identifies the presence of the carbohydrate antigen unique to Group A Streptococcus by an immunologic reaction
* Sensitivity 70-95%, specificity 95%
# Group A Streptococcal Pharyngitis: RADT

<table>
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<tr>
<th>RADTs</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Comments</th>
</tr>
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</table>
| Latex agglutination       | 70-90%      | 95%         | • Point of care test  
• Sensitivity increased with the newer generation assays (the most sensitive test being OIA)  
• Takes about 15 min to complete  
• Very simple to perform |
| Enzyme immunoassay (EIA)  | 70-90%      | 95%         |                                                                          |
| Optical immunoassay (OIA) | 86-94.8%    | 95-100%     | • Employ molecular biology methods  
• Some can be a point of care tests  
• Involve multiple steps, require special equipment and batching  
• Takes 1.5-2 h to complete |
| DNA probe                 | 86-94.8%    | 95-100%     |                                                                          |
| Real-time PCR             | 93%         | 98%         |                                                                          |
**RADT**: Principles

* RADT

![Image of RADT principles diagram]

- **Add all the extracted solution**
- **Strep A**
  - **C**
  - **T**
  - **ID**

- **POSITIVE**
- **NEGATIVE**
- **INVALID**
RADT: what is available today
RADT : Advantages

* **Rapid Diagnosis and Treatment**
  * Results in approximately 5-10 minutes
  * Patients can be tested and treated in the same office visit
  * Helps effectively manage antibiotic use

* **Easy to use**
* **Convenient**
* **Reasonable** performance
* **Room temperature storage** and **long shelf life**
Qualitative

Unable to differentiate asymptomatic carriers from infections

**False positive** results can be due to cross reacting with other Streptococcus species such as Streptococcus anginosus group, heavy colonization with S.aureus

**False negative** may occur in patients with acute pharyngitis due to Group C and Group G Streptococci, with low bacterial load in the specimen (carriers, collection technic, prior antibiotics, etc.)
Group A Streptococcal Pharyngitis: RADT

* **In children and adolescents**, negative test requires throat culture
* **In adults**, routine back-up cultures for negative results generally not recommended
* Sensitivity to be determined by each practice/group
* Sensitivity can be improved by applying clinical score
RADT: Clinical Score

Criteria:
1. Tonsillar exudate or erythema
2. Anterior cervical adenopathy
3. Cough absent
4. Fever present
5. Age:
   * Age 3 to 14 years: +1 point
   * Age 15 to 45 years: 0 points
   * Age over 45 years: -1 points

Approach:
1. Strep Score 4 to 5:
   * Treat with antibiotics
2. Strep Score 2 to 3:
   * **Perform rapid antigen test**
     * Antigen test positive: Treat with antibiotics
     * Antigen test negative: Throat Culture
3. Strep Score 0 to 1:
   * Provide Pharyngitis Symptomatic Treatment
Thank you!