What Every Pediatrician needs to know about Microbiology

Pediatric Grand Rounds
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Disclosures

• Steven D. Dallas, PhD, D(ABMM) has no relationships with commercial companies to disclose.

My pediatrics background:


Former micro lab director: Presbyterian/Novant Health in Charlotte, NC, included Hemby Children’s Hospital

Objectives

• By the end of this presentation, participants will be able to:
  – Review classic microbiology tests still in use at UHS
  – Describe new molecular ID and susceptibility methods in use at UHS
  – Examine the importance of appropriate specimen ordering, timing, collection, and transport
  – List susceptibility testing methods and drugs reported
  – Demonstrate use of antibiogram to guide therapy

Culture is still the workhorse

• About 80% of workload is classic culture and susceptibility
• Depends on culture type, but prelim in 18-24 hours, final in 48-72 hours
• Susceptibility testing takes additional 10-24 hours
• Extreme incubation: TB cultures held 42 days!
Minimize use of swabs

- Swabs absorb 100 to 150 microliters
- Mini-tip swab absorbs 10 microliters
- Swabs release only 5-10 microliters of specimen after streaking across 3 plates
- Swab use can be minimized in controlled environments like surgery.

Ask yourself as the physician: Are we getting the right specimen at the right time in sufficient quantity?

Improved swab: E-swabs

- Liquid-based instead of swab based
- Swab tip is “flocked”
- Some degree of standardization
- Can be used for aerobes and anaerobes

Body sites that are not clear to us

- Eye:
  - Is it conjunctiva, vitreous, aqueous, anterior chamber, posterior chamber
- Wound:
  - Is it post-surgical, peri-rectal, intra-abdominal, post trauma?

Urine culture specimen collection

Urine cultures are interpreted in the context of a “significant colony count of a known UTI pathogen.” Boric acid collection tubes stabilize the colony count to reflect what was actually in the bladder.

Gram stain: the first real-time multiplex test

CSF from newborn

CSF is cytocentrifuged to increase test sensitivity
gram stain limitations

- Detects and differentiates bacteria with cell walls. Will also detect yeast, fungi, most human cells, some parasite cysts, but mostly used to detect bacteria.
- Will not detect cell wall deficient bacteria such as Mycoplasma.
- Will not detect Chlamydia or Legionella. Not good for Mycobacterium.
- Not as sensitive as culture, what does this mean?

Fungal hyphae in gram stain

How blood cultures work

Instrument reads bottle every ten minutes, no need to call us!
Positive blood culture gram stains are called on shift too!

Cepheid Xpert MRSA/SA BC

- If blood culture gram stain shows GPC in clusters
- "Allows de-escalation from broad-spectrum therapy to a targeted antimicrobial approach 18-48 hours sooner"—quote from product literature
- Results in one hour

A shotgun approach?
Pediatric blood culture bottles?

- Not in use at UHS.
- A pink bottle won’t make the bugs grow faster.
- Volume is biggest predictor of success
- Open to consideration if compelling case presented

Septic arthritis? Place the aspirated synovial fluid, up to 10 ml, in aerobic blood culture bottle and send to lab ASAP? Can you say Kingella?

Blood culture fill volume report Feb 2015

Blood culture bottles chronically under-filled!

Isolator tubes for fungi

- Lysis-centrifugation
- Identifiable colonies in 18-24 hrs
- Very labor intensive
- Good for Histo and AFB
- Must be requested

Blood culture contamination rates

Contaminated blood cultures

- A monitor of the SPECIFICITY of blood culture (false positives)
- Add estimated $4000.00 per episode*
- Q: At 25,000 cultures per year, if you reduce contamination rate from 3% to 2% you save how much money?


Answer?

A. $10,000
B. $100,000
C. $1,000,000
D. $10,000,000
AFB culture is slow

- PCR to the rescue
- Performed on smear-positive sputum, takes about 2 hours

<table>
<thead>
<tr>
<th>Specimen</th>
<th>AFB Positive</th>
<th>AFB Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>99.8%</td>
<td>99.1%</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>99.7%</td>
<td>99.1%</td>
</tr>
<tr>
<td>Specificity</td>
<td>99.4%</td>
<td>99.5%</td>
</tr>
<tr>
<td>Probability</td>
<td>99.1%</td>
<td>99.1%</td>
</tr>
</tbody>
</table>

Why can’t you run that test on this specimen?

- Some reasons:
  - Regulations regarding “off-label testing”
  - Matrix effects – inhibitors in certain specimens
  - Lack of data to support the test or the interpretation of the results
  - Lack of reimbursement $$
  - Because it makes no sense! No, we won’t test gentamicin on *Bacteroides fragilis* even if you are the doctor!

“Molecular tests”?

- Detect genes rather than bugs
- Generally must amplify before detection (PCR)
- Are not dependent on growth or viable bugs
- Are fast, highly sensitive, and specific
- Are expensive
- *Should not be used as a “test of cure”*

Molecular testing has replaced:

- The PAP smear? (HPV testing)
- Respiratory viral culture (Biofire Filmarray)
- GC and Chlamydia culture (NAAT)
- Herpes culture (Quidel AmpliVue helicase)
  - Detects HSV 1 and HSV 2

Parasites

- *Giardia, Cryptosporidium, E. histolytica* testing is by EIA – sensitive, specific, fast, does not require experienced parasitologist
  - Do not need to order x 3
- Standard Ova and Parasite exam is a send out test due to staffing and expertise issues
- Scabies and pinworm prep and identification of botflies, Ascaris, tapeworms done in house
- Malaria smear: Hematology

Stool cultures

- What organisms do we look for in a routine stool culture?
  a) *Salmonella* and *Shigella*
  b) *Salmonella, Shigella, Campylobacter*
  c) *Salmonella, Shigella, E. coli O157:H7*
  d) *Salmonella, Shigella, Campylobacter, E. coli O157:H7, Vibrio, Plesiomonas, Aeromonas, Yersinia*
Shiga toxin testing

• If you order a stool culture, we only routinely look for Shigella, Salmonella, Campylobacter.
• If you suspect something exotic, let us know!
• If a stool is grossly bloody, we add a SMAC plate to screen for E. coli O157:H7.
• CDC recommends Shiga toxin testing on all community-acquired diarrhea stools for all patient ages so we have added a Shiga toxin EIA, detects SLT1 and SLT2.

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http://www.cdc.gov/ecoli/clinicians.html

C. difficile testing

• PCR
• No formed stools
• Not under age 2, unless....

Asymptomatic colonization

• “Characterization of Human Milk Components Which Block the Binding of Clostridium difficile toxin A to Intestinal Receptors” – S. D. Dallas – Texas Tech University Health Sciences Center

• Populations colonized with no symptoms:
  – Human infants to age two
  – Cystic fibrosis patients
  – 3 to 20% of adults
  Colonized false-positive populations cause confusion!

The future of Poo?

Who decides what is tested and reported?

• Antibiotic subcommittee of P and T
• Some considerations:
  – Match formulary
  – Inpatient versus outpatient
  – Only report drug B if drug A is resistant
  – CLSI guidelines
  – Practical constraints

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"From Doug to Bug to Drug"
Real life involves six Doug-Bug-Drug interactions

Drug

1. Bacteriostatic or bactericidal, time or concentration dependent?
2. Beta lactamase, efflux, altered target or permeability
3. Ototoxic, nephrotic?
4. Antibodies, phagocytosis, fever, cytokines
5. Metabolize, excrete, concentrate, PK/PD, protein binding
6. Unable to reach infection site?

Bug

Doug

*Resemblance to any real life Doug is purely coincidental and unintended.

Four susceptibility methods
- Vitek 2 automated panel MIC
- Disk diffusion, S, I, R only, no MIC
- Etest MIC
- Custom microbroth dilution tray MIC
- Review article: [http://cid.oxfordjournals.org/content/49/11/1749.full](http://cid.oxfordjournals.org/content/49/11/1749.full)

Interpreting susceptibilities
- S, I, R?
- What about S-DD?
- What is an MIC? How is it interpreted?

MIC is like a serial dilution
- Minimum inhibitory concentration
- Standard inoculum of bug with increasing drug
- Measured in micrograms per milliliters

MIC = 4 micrograms/ml

Can I just pick the lowest MIC?
- Must compare apples to apples, i.e., within a class of drugs.
- An Levo MIC of .25 is not better than an SXT MIC of 10, because it is not the same drug class. Both of these MICs are the lowest concentration we test for the respective drug.
  - This is really complicated, it involves PK/PD, and voodoo, see your ID pharmacist.

What if no interpretation available?
- Example: *Propionibacterium acnes* from ventricular shunt fluid?
- You might hear a new term soon: ECV
Resistant Bugs

- MRSA – slight decline in incidence
- VRE
- ESBL – confirmatory tests
- *Acinetobacter baumannii* complex
- KPC (CRE) – modified Hodge test
- *Stenotrophomonas maltophilia*
  - Still SXT susceptible
- *Burkholderia cepacia* complex
  - Still SXT susceptible

Contact Precautions!

How to use the antibiogram?

Antifungal susceptibilities

- UHS micro lab tests *Candida* with fluconazole
- Trek Yeast One MIC tray by request

- True fungi are tested at Fungus Testing Lab at UTHSCSA

Rapid respiratory virus PCR

- UHS Respiratory Virus Activity 2016-2015
- UHS Respiratory Requests vs Positive samples

Reverse syphilis algorithm

- Syphilis serologic screening algorithms
Cystic fibrosis cultures
• Very mixed cultures with resistant organisms
• Mucoid organisms
• Algorithms – guidance from CF foundation
• Longer incubation, lots of subculturing
• Throat as surrogate for sputum
• Two special media for B. cepacia

Summary
• Understanding how the micro lab works helps us all to do a better job together
• Microbiology is advancing toward more molecular testing
• Traditional culture and susceptibility will be around for awhile
• Pediatrics is new to us but we want to help you help the little ones!
• Resources: my email: dallass@uthscsa.edu
  – office 210 4329 cell 704 579 1261
• For virology, immunology
  – Director: Kristin Fiebelkorn, MD, FCAP, FASCP

Come to Wednesday Plate Rounds, 1030 AM, UH micro lab, we have Peds cases!