34th Annual Meeting
Southwestern Association of Clinical Microbiology

Stool Culture Work Up
Someone's got to do it... at least for now

Yvette S. McCarter, PhD, D(ABMM)
Director, Clinical Microbiology Laboratory
UF Health Jacksonville
Professor of Pathology
University of Florida College of Medicine-Jacksonville
Disclosures

• No financial disclosures
• No discussion of off label uses
• Cat and parrot mommy
Objectives

- List the organisms most commonly associated with bacterial diarrhea in the US
- Describe emerging bacterial pathogens associated with diarrheal disease
- Discuss appropriate specimen collection, transport and processing for stool culture
- Discuss methods that can be used to streamline stool culture work up
- Discuss appropriate antimicrobial susceptibility testing and culture reporting
- Explore the impact of nucleic acid testing on performance of stool cultures
Let’s talk stool cultures...
Laboratory Diagnosis of Bacterial Gastroenteritis

Romney M. Humphries, a Andrea J. Linscott b
Pathology and Laboratory Medicine, University of California, Los Angeles, California, USA a; Department of Pathology, Ochsner Health System, New Orleans, Louisiana, USA b

Impact of Bacterial Gastroenteritis

Global
• > 1.7 billion cases of diarrheal disease reported annually
  ▫ 22 million deaths
• Second leading cause of death in children <5 years of age

United States
• ~211-375 million episodes of diarrheal illness annually
  ▫ 1.8 million hospitalizations
  ▫ 3100 deaths
• 48 million cases – foodborne disease
  ▫ 128,000 hospitalizations
Inquiring minds want to know...

Which bacteria has the highest incidence of foodborne disease?

- Aeromonas
- Campylobacter
- Salmonella
- Shigella
- STEC
- Vibrio
- Yersinia

Which bacteria has the highest association with outbreaks?

- Aeromonas
- Campylobacter
- Salmonella
- Shigella
- STEC
- Vibrio
- Yersinia
Incidence of Foodborne Infection

FoodNet

Incidence/100,000 population
% infections associated with outbreaks

MMWR. 2015. 64:495-499
When is stool culture indicated?

**From the patient perspective**

- American College of Gastroenterology
  - Severe or persistent diarrhea or bloody diarrhea
  - Temperature > 38.5°C
  - Presence of fecal WBC/lactoferrin or occult blood
- Infectious Diseases Society of America
  - Diarrhea > 1 day
  - Fever or dehydration or systemic illness
  - Bloody stool

**From the Public Health perspective**

- Identify and track outbreaks of bacterial gastroenteritis

* Clin Infect Dis. 2001. 32:331-51
Specimen Collection and Transport

• Collect specimen in acute stage (5-7 days)
  ▫ Clean, dry container
  ▫ Rectal swabs less sensitive

• Transport
  ▫ Fresh specimens
    • Clean, leakproof container
    • Transport and process within 2 hrs collection

Transport medium – Cary Blair
  • Buffered – prevent pH shifts
  • Low nutrient content – inhibit growth of other species
  • NaCl (Vibrio) and sodium thioglycollate (Campylobacter)
  • Transport and process within 48 hrs
Optimizing Stool Culture

- Fecal leukocyte testing
  - Screen for evidence of inflammation
  - Poor sensitivity – differentiating infectious and non-infectious diarrhea in inpatients

Methods
- Microscopy (Methylene blue/Gram stain)
- Fecal lactoferrin
  - Detects a glycoprotein component of neutrophilic granules
  - More stable (does not rely on detection of intact PMN); rapid

Optimizing Stool Culture

The Dos...

- **Apply the 3 Day Rule**
  - Low yield of stool culture for patients developing diarrhea while hospitalized >3 days
  - Think *C. difficile*-associated disease

- **The Don'ts...**
  - Don’t process...
    - Fresh specimens not received within 2 hrs of collection
    - Specimens in Cary Blair after 48 hours
    - Specimens in Cary Blair if the indicator has turned yellow
    - Multiple specimens collected on the same day
Which of the following organisms are included in your routine stool culture?

- *Salmonella, Shigella, Campylobacter*
- *Salmonella, Shigella, Campylobacter* + *Aeromonas* and/or *Plesiomonas*
- *Salmonella, Shigella, Campylobacter* + *Vibrio* and/or *Yersinia*
- Everything!
What should I look for in a stool culture?

- **Always**
  - *Salmonella, Shigella, Campylobacter, STEC*

- **Sometimes**
  - *Vibrio, Yersinia, Aeromonas and Plesiomonas*
    - Geography/patient population dependent; seasonal
    - Selective media used for optimal detection

- **Never - infrequently diagnosed by clinical laboratory**
  - *Bacillus cereus*
  - *Clostridium perfringens*
  - *Listeria monocytogenes*
  - *Staphylococcus aureus*
Do you routinely test for STEC in your laboratory?

- Yes - routinely test for both O157 and non-O157 STEC
- Yes - perform culture for O157 STEC only
- Yes - perform shiga toxin testing only
- No - do not routinely perform STEC testing
Why test all stools for STEC?

- Selective testing strategies will miss many STEC infections
  - Blood
    - Not reliably present
    - Other pathogens can cause bloody stools
  - Seasonality
    - More common during summer months but infections and outbreaks occur year-round
  - Age
    - More frequent in children but almost half of all isolates are obtained from persons >12 years old
Why Culture and STEC-EIA?

- More effective for identifying STEC than either technique alone
- Early detection of O157 STEC
  - High predictive positive value for severe disease
    - Almost all strains contain Stx2
  - Prompt treatment with parental volume expansion decreases renal injury and improves outcomes
  - Antibiotics should not be given for STEC
  - Early recognition of public health problem
- Non-O157 STEC are important cause of infection
    - 5 yr study – detected additional 66 cases 47% non-O157
Should all stools be screened for STEC?

• Current CDC recommendation – Simultaneous culture for O157 STEC and toxin assay for STEC
• Selective testing approach
  ▫ Screen all stools received for culture for a 12-month period to determine STEC prevalence in the population
  ▫ Low incidence
    • Consider testing by request only
    • Apply combination of screening criteria

*MMWR.* 2009. 58 (RR-12):1-14  
What about emerging enteropathogens?

- Other less common bacteria can cause gastroenteritis
- Enterotoxigenic *Bacteroides fragilis*
- *Edwardsiella tarda*
- *Escherichia albertii*
- *Klebsiella oxytoca*
- *Providencia alcalifaciens*
Emerging Enteropathogens

• Enterotoxigenic *Bacteroides fragilis*
  ▫ Implicated as a cause of diarrhea in children < 5 years of age and inflammatory diarrhea in children/adults
  ▫ Conflicting information in the literature about pathogenicity – additional factors likely play a role in infective process
  ▫ No easy method of detection
    • Culture on BBE and test for enterotoxigenicity with PCR for *B. fragilis* toxin gene
    • CPE produced by toxin in human colon cell lines
Emerging Enteropathogens

- *Edwardsiella tarda*
  - Associated with < 1% of cases of gastroenteritis
  - Asymptomatic carriage → watery diarrhea → dysentery
  - Most susceptible < 5 and > 50 years of age

- *Escherichia albertii*
  - Involved in at least one outbreak of gastroenteritis; isolated from patients with gastroenteritis
  - Harbors known enteropathogenic virulence factors
  - Frequently misidentified using phenotypic ID systems
  - Can be identified using 16S rRNA sequencing and MALDI-TOF
Emerging Enteropathogens

- *Klebsiella oxytoca*
  - Linked to antibiotic-associated hemorrhagic enterocolitis in *C. difficile* negative patients
    - Confirmation requires detection of *K. oxytoca* cytotoxin
  - Also suggested to cause mild-moderate diarrhea

- *Providencia alcalifaciens*
  - Studies link diarrheal disease and outbreaks to foreign travel or consuming contaminated food
  - Most isolates recovered in pure culture, as predominant flora or in absence of other enteropathogens
Emerging Enteropathogens

• Should I be looking for these organisms routinely?
  • **NO!**
    • Some can be found in the absence of symptoms
    • Difficult to differentiate from other resident fecal flora
• Culture only after discussion with clinicians to determine which patients are unique enough to look for these potential pathogens
What media should I use?

• Dependent on organisms you want to recover
  ▫ Patient population
  ▫ Organisms routinely isolated

• Suggested media
  ▫ MacConkey
  ▫ Selective/differential for *Salmonella/Shigella*
  ▫ Selective media for *Campylobacter*
  ▫ Selective media for STEC O157 and/or enrichment broth for shiga toxin testing

What about enrichment broth?
Do you include enrichment broth (specifically for *Salmonella* and *Shigella*) in your routine culture set up?

- Yes - include enrichment broth on all routine stool culture
- No - do not include enrichment broth
- Enrichment broth added selectively to certain cultures
Enrichment Broth
Do we need it?

• Yes
    • 35% of *Salmonella* only recovered in Selenite
    • 41% of newly identified *Salmonella* only recovered in Selenite

• No
  ▫ Lue (*Clin Microbiol NewsL*. 1986. 8:5-6)
    • Appropriate subculture important (GN – 6-8 hr; Selenite – 18-24 hr)
    • Yield does not justify the cost

Review historical data to determine if enrichment broth provides additional recovery – if not, discontinue
What media should I use?

**Campylobacter**

- Options
  - Blood-free – Charcoal cefoperazone-desoxycholate agar (CCDA), charcoal based selective agar
  - Blood-containing – Campy CVA, Skirrow
- Avoid media with cephalothin, colistin, and polymyxin B – inhibitory to some strains of *C. jejuni* and *C. coli*, and are inhibitory to *C. fetus*
- Use of a combination of media, including one that is charcoal-based, increases yield 10-15%

*J Clin Microbiol. 1991. 29:1007-10*
What media should I use?

**Aeromonas, Yersinia, Vibrio**

- **Aeromonas**
  - Blood agar
  - Cefsulodin-irgasan-novobiocin (CIN) agar (35°C)
- **Yersinia enterocolitica**
  - Cefsulodin-irgasan-novobiocin (CIN) agar
    - 22-25°C – produces colonies with a more distinct "bull's-eye"
- **Vibrio**
  - Thiosulfate-citrate-bile salts-sucrose (TCBS) agar
    - *Grimontia hollisae* and *Vibrio metschnikovii* - inhibited
  - Blood agar
Stool Culture Work Up Algorithm

Screen plates for colorless or H$_2$S positive colonies

Screen suspicious colonies using biochemical tests
- Classic – TSI + LIA + urea
- Alternatives – MIO, MIL, MILS

Perform confirmatory biochemical and/or antigen testing

- Poor specificity
- False positive colonies
Optimizing Stool Culture Work up

CHROMagar *Salmonella*

- Inhibits gram positives, yeast, *Proteus* spp., Non-glucose fermenters
- *Salmonella* – mauve/rose
  - *Salmonella enterica* subspecies *arizonae* (lactose +) = blue-violet to purple
- Coliforms – blue-green
- Others – colorless (white)

Incubate 24 hrs; if negative, reincubate additional 24 hr

Biochemical/serological confirmation
Optimizing Stool Culture Work up

**CHROMagar *Salmonella***


- CHROMagar *Salmonella* vs. Hektoen ± enrichment
  - CHROMagar – higher specificity; reduced confirmatory testing = more economical than Hektoen


- SS – XLD – HEK – GN vs. CHROMagar Sal ± enrichment
  - CHROMagar + XLD sensitivity = 100% ; 27% reduction in annual stool culture cost; 78% reduction in false positive results
Optimizing Stool Culture Work up

CHROMagar *Salmonella*

*Church et al. 2010. DMID 68:13-19*

- **Stool**
  - Selenite
  - CHROMagar
  - HEK
  - MAC + CHROMagar

- **n=2999; 51 (1.7%) *Salmonella***

<table>
<thead>
<tr>
<th></th>
<th>CHROMagar</th>
<th>CHROM + Sel</th>
<th>HEK + Sel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>94.1</td>
<td>98.0</td>
<td>84.3</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>99.9</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>“Colony Picks” (CP)</td>
<td>114</td>
<td><strong>156 (+82%)</strong></td>
<td>880</td>
</tr>
<tr>
<td>CP not <em>Salmonella</em></td>
<td>66 (58%)</td>
<td>105 (67%)</td>
<td>841 (96%)</td>
</tr>
</tbody>
</table>

:. CHROMagar – higher sensitivity than HEK-Sel; 52% reduction in annual stool culture cost
Optimizing Stool Culture Work up
HardyCHROM *Salmonella Shigella*

- Facilitates detection of *Salmonella* and *Shigella*
  - *Salmonella* – teal blue colored colonies with/without black centers
  - *Shigella* – teal blue colored colonies
- Coliforms – pink colonies, with or without purple centers; dark blue; pink
Optimizing Stool Culture Work up

CHROMagar O157

• Facilitates detection of \textit{E. coli} O157
  ▫ Potassium tellurite
  ▫ Antimicrobials (cefixime, ceftazidime)
• \textit{E. coli} O157 – mauve
• Non \textit{E. coli} O157 – blue/blue-green, colorless (white)

CHROMagar STEC (RUO)
Detects shiga toxin-producing \textit{E. coli}
Optimizing Stool Culture Work up

CHROMagar O157

Church et al. 2007. *J Clin Microbiol* 45:3098-3100

- CHROMagar O157 vs. sorbitol MAC
- 27 (0.9%) positive for *E. coli* O157
  - 26/27 (96.3%) on CHROMagar
  - 23/27 (85.2%) on sorbitol MAC
- Costs with CHROMagar
  - Labor – decreased 21%
  - Materials – decreased 64%
    - Less indole testing and O157 serotyping

∴ CHROMagar = improved diagnostic efficiency compared to sorbitol MAC
Optimizing Stool Culture Work up

MALDI-TOF

- Cost-effective alternative to screening of colonies and biochemical testing
  - Accurate identification of *Aeromonas, Campylobacter, Plesiomonas, Salmonella, Vibrio spp.* (including *V. cholerae*), *Yersinia enterocolitica*
- Caveats
  - Cannot differentiate *Shigella* and *E. coli*
  -Cannot differentiate *E. coli* from STEC
  - Media type may effect identification
    - Blood = MAC = XLD >HEK >SS

*J Clin Microbiol.* 2015. 53:329-31  
*J Thorac Dis.* 2014. 6:539-44  
*J Clin Microbiol.* 2012. 50:1008-13
Antimicrobial Susceptibility Testing

- Antimicrobials not routinely indicated in healthy patients with bacterial gastroenteritis
- Routine susceptibility testing of stool culture isolates not indicated
  - Exceptions
    - Infants ≤ 6 mo of age
    - Elderly or immunocompromised
    - Prolonged disease
    - Isolation of *Salmonella* Typhi/Paratyphi A
Result Reporting

• Positive Cultures
  ▫ Pathogen with susceptibility testing, if appropriate

• Negative Cultures
  ▫ Include each organism routinely included in screening
  ▫ No *Salmonella, Shigella or Campylobacter* isolated
  ▫ No enteric pathogens isolated

• Verbal reporting/automated electronic alerts to healthcare providers – especially for STEC

• Rapid reporting to Public Health
  ▫ Forward isolates/broths to Public Health Lab as required
Do you perform/plan to perform a multiplex molecular panel instead of stool culture?

- Yes - we have switched to a multiplex molecular panel and have discontinued stool culture totally
- Yes - we have switched to a multiplex molecular panel but continue to perform stool culture for some organisms
- No - we have not switched to a multiplex molecular panel, but plan to do so in the next 6 months
- No - we do not plan to switch to a multiplex molecular panel
The Future is now…
Nucleic Acid Amplification Testing

• Currently 5 FDA approved assays

<table>
<thead>
<tr>
<th>Assay</th>
<th>Manufacturer</th>
<th>Ease of Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>xTAG Gastrointestinal Pathogen Panel (GPP)</td>
<td>Luminex</td>
<td>☠</td>
</tr>
<tr>
<td>Prodesse ProGastro SSCS</td>
<td>Hologic-GenProbe</td>
<td>☠</td>
</tr>
<tr>
<td>BD MAX Enteric Bacterial Panel</td>
<td>BD Diagnostics</td>
<td>☀</td>
</tr>
<tr>
<td>Verigene Enteric Pathogens Test</td>
<td>Nanosphere</td>
<td>☀</td>
</tr>
<tr>
<td>FilmArray Gastrointestinal Panel</td>
<td>BioFire Diagnostics</td>
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</tbody>
</table>
# Nucleic Acid Amplification

## What’s available...

## What’s included....

<table>
<thead>
<tr>
<th>Test</th>
<th>Analytes</th>
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</thead>
<tbody>
<tr>
<td>xTAG Gastrointestinal Pathogen Panel</td>
<td><em>Salmonella, Shigella, Campylobacter, Shiga toxin producing E. coli (stx1/2), E. coli O157, ETEC LT/ST, C. difficile, Norovirus, Rotavirus, Giardia, Cryptosporidium</em></td>
</tr>
<tr>
<td>Prodesse ProGastro SSCS</td>
<td><em>Salmonella, Shigella, Campylobacter, Shiga toxin producing E. coli (stx1/2)</em></td>
</tr>
<tr>
<td>BD MAX Enteric Bacterial Panel</td>
<td><em>Salmonella, Campylobacter, stx1/2, Shigella/EIEC</em></td>
</tr>
<tr>
<td>Verigene Enteric Pathogens Test</td>
<td><em>Salmonella, Shigella, Campylobacter, stx1/2, Vibrio spp., Y. enterocolitica, Norovirus, Rotavirus</em></td>
</tr>
<tr>
<td>FilmArray Gastrointestinal Panel</td>
<td><em>Salmonella, Campylobacter, C. difficile, Plesiomonas shigelloides, Y. enterocolitica, Vibrio spp., Vibrio cholerae, Shiga toxin producing E. coli (stx1/2), ETEC LT/ST, EAEC, EPEC, Shigella/EIEC, diarrheagenic E. coli/Shigella, Norovirus, Rotavirus, Adenovirus 40/41, Sapovirus, Astrovirus, Giardia, Cryptosporidium, Cyclospora, E. histolytica</em></td>
</tr>
</tbody>
</table>
Nucleic Acid Amplification

• Will multiplex NAA assays replace culture and antigen/toxin testing?
  ▫ High sensitivity
  ▫ Rapid
  ▫ Multiplex capability for parasites and viruses

• What is the impact of culture-independent testing on public health surveillance?
  ▫ Lack of isolate for susceptibility testing or subtyping
Stool Culture Work up

Conclusions

• Number and types of agents cultured should be driven by geographic location and patient history
• Chromogenic media available to help make culture work up easier
• MALDI-TOF is a useful alternative to traditional work up algorithms
• Simultaneous culture for *E. coli* O157 and toxin assay for STEC EIA represent the best practice for detection of shiga toxin-producing *E. coli*
Stool Culture Work up

Conclusions

• Effective communication with physicians regarding need for AST and culture for “emerging pathogens” a must
  ▫ Includes physician understanding of organisms included in routine stool culture
• Still to be determined – the role of stool culture in the era of NAAT multiplex testing for detection of common pathogens
  ▫ Decisions on which method to choose
    • Cost
    • Expertise required/level of automation
    • Extent of testing required
    • Availability of organism isolate for additional testing
Questions??
yvette.mccarter@jax.ufl.edu