

Culture of Stool Specimens

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Objectives

- List the culture media and incubation conditions used for stool specimens.
- Discuss which organisms are considered to be pathogens vs. normal flora in stool and how to distinguish the pathogens from the normal flora.
- Discuss when susceptibility testing should be performed on a stool specimen isolate.

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SH__ Happens

- History
- Incubation Period
- Antibiotic exposure
- Recent travel
- Clinical presentation
 - Dysentery
 - Bloody diarrhea
 - Rice water stools
 - Diarrhea and vomiting in a young child
 - Hemorrhagic colitis
 - Subacute or chronic diarrhea and flatulence
 - Appendicular syndromes
 - Vomiting

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Incubation Period

- 1 – 6 hours
 - Toxin ingestion
- 24 – 48 hours
 - Most bacterial pathogens
- 3 – 5 days
 - Shiga-toxin producing E. coli
- 3 – 11 days
 - Campylobacter jejuni

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Pathogenesis

- Enterotoxin
- Enteroinvasion
- Cytotoxin
- Attaching and effacing

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Specimen Collection and Transport

- Specimen
 - Diarrheal stool collected during the acute phase of disease
 - Rectal swabs should be restricted to patients with active disease from whom feces may be difficult to obtain, such as infants and children
- Transport to lab within 2 hours or use transport medium
 - Modified Cary-Blair
- Should not need more than two stools from different days

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Rejection Criteria

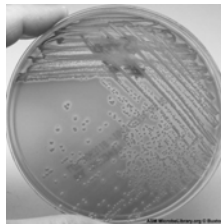
- Not in transport medium and > 2 hours after collection
- In transport medium > 3 days at 4 C or > 1 day at RT
- Transport medium is yellow
- Patients hospitalized > 3 days
- Hard, solid stools that cannot be sampled
- Dry swabs
- Barium stools
- More than three stools from one patient or multiple specimens on the same day

Direct microscopic examination

- Fecal leukocytes
 - Method
 - Stain
 - Lactoferrin
 - Sensitivity less than 90% for the identification of inflammatory diarrhea

Specific Pathogens

- Aeromonas spp.
 - hydrophila, caviae and veronii complex
 - BAP-A or CIN

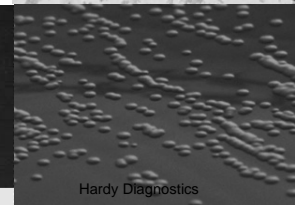
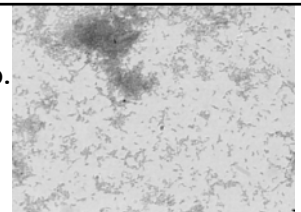


Campylobacter spp.

- jejuni or coli
- Campy CVA or charcoal



Campy-BAP medium in a microaerophilic bag



Hardy Diagnostics

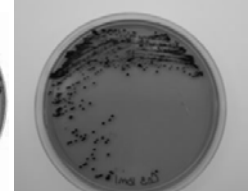
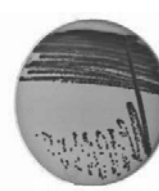
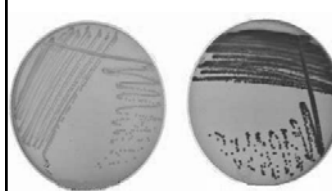
E. coli

- Enteropathogenic E. coli
- Enterotoxigenic E. coli
- Enteroinvasive E. coli
- Enteroadherent E. coli
- Shiga toxin producing E. coli



Salmonella

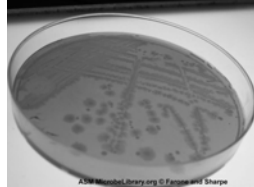
- enterica
- Lactose nonfermenter, H₂S producer
- Biochemical and serological identification



ASM MicrobelLibrary.org © Chamberlain

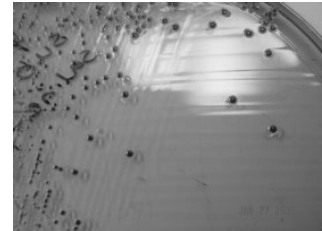
Shigella

- dysenteriae (A), flexneri (B), boydii (C), sonnei (D)
- Lactose nonfermenter



Yersinia enterocolitica

- CIN or MacConkey (RT)



Other less common isolates

- Plesiomonas shigelloides
- Vibrio spp.
- Edwardsiella tarda

Media

- Differential, poorly selective media
 - MacConkey, EMB
- Moderately selective media
 - XLD or Hektoen
- Highly selective media
 - SS
- Enrichment broth
 - GN
 - Selenite F
- Campylobacter media
- GN or MacConkey broth for Shiga Toxin Test
 - sMac agar plates may also be included
- Blood agar plate or BAP-A plate
- Incubate all media (except CIN) at 35 C for 24 hours

Examination of Culture Media

- BAP or BAP-A
 - Sweep oxidase
 - Subculture oxidase positive to BAP and TSI or KIA as screen or perform identification
 - Perform susceptibility testing
 - Report overgrowth with yeast
 - Do not report enterococci

- HE, SS, XLD, Mac
 - Screen plates for lactose negative and/or H₂S positive colonies
 - Subculture to BAP, TSI or KIA, urea or perform identification
 - H₂S positive
 - Discard urea +, oxidase +
 - Urea -, oxidase -, PYR + can be discarded
 - Urea-, oxidase-, PYR -,
 - » Indole + r/o Edwardsiella
 - » Indole - r/o Salmonella
 - Alk/No change - discard
 - H₂S negative, with acid butt
 - Oxidase positive – r/o Aeromonas, Plesiomonas, Vibrio
 - Oxidase negative
 - » Discard acid/acid, PYR positive and motile or gas, PYR negative and Urea positive
 - » Identify all others

- CIN
 - Identify colonies with deep red center with a sharp border surrounded by translucent zone
 - Subculture to BAP and perform identification
 - If oxidase positive, r/o *Aeromonas*
- TCBS
 - Screen or identify
 - yellow colonies for *V. cholerae*, *V. fluvialis* and *V. furnissi*
 - blue colonies for *V. parahaemolyticus*, *V. mimicus* and *V. hollisae*
- sMAC
 - Screen transparent or colorless colonies
 - Perform Mug test
 - Identify
 - Perform serotype or refer to Public Health Laboratory



- Shiga toxin test
 - Perform test for Shiga toxin on GN or MacConkey broth, plate sweep or organism



Susceptibility testing

- Perform and report susceptibility testing on *Shigella*, *Aeromonas*, *Plesiomonas*, *Edwardsiella*, *Vibrio*, *Yersinia* and selected cases of *Salmonella*
 - Report Ampicillin, TMP-SMX and a quinolone. For resistant isolates or isolates from extraintestinal sites, report 3rd generation cephalosporin
 - 1st and 2nd generation cephalosporins and aminoglycosides are not effective. A comment to this effect may be added
 - Antimicrobial treatment is contraindicated for Shiga-toxin producing *E. coli*. A comment to this effect may be added.



Reporting

- Negative
 - Report negative results for each organism
 - "No *Salmonella*, *Shigella*, *Campylobacter* or Shiga-toxin producing *E. coli* detected".
 - Report negative results for specific organisms
 - "No *Vibrio* spp. detected".
- Comments
 - If no growth of gram negative enteric bacilli, report
 - If overgrowth of *Pseudomonas* or *S. aureus*, report
 - If pure or predominant yeast, report



- Positive
 - Report presumptive pathogens as "probable" until both biochemical and serologic identification is performed
 - Notify infection control and/or licensed care provider
 - Report to public health agency



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