Laboratory Diagnosis of Gastrointestinal Infections

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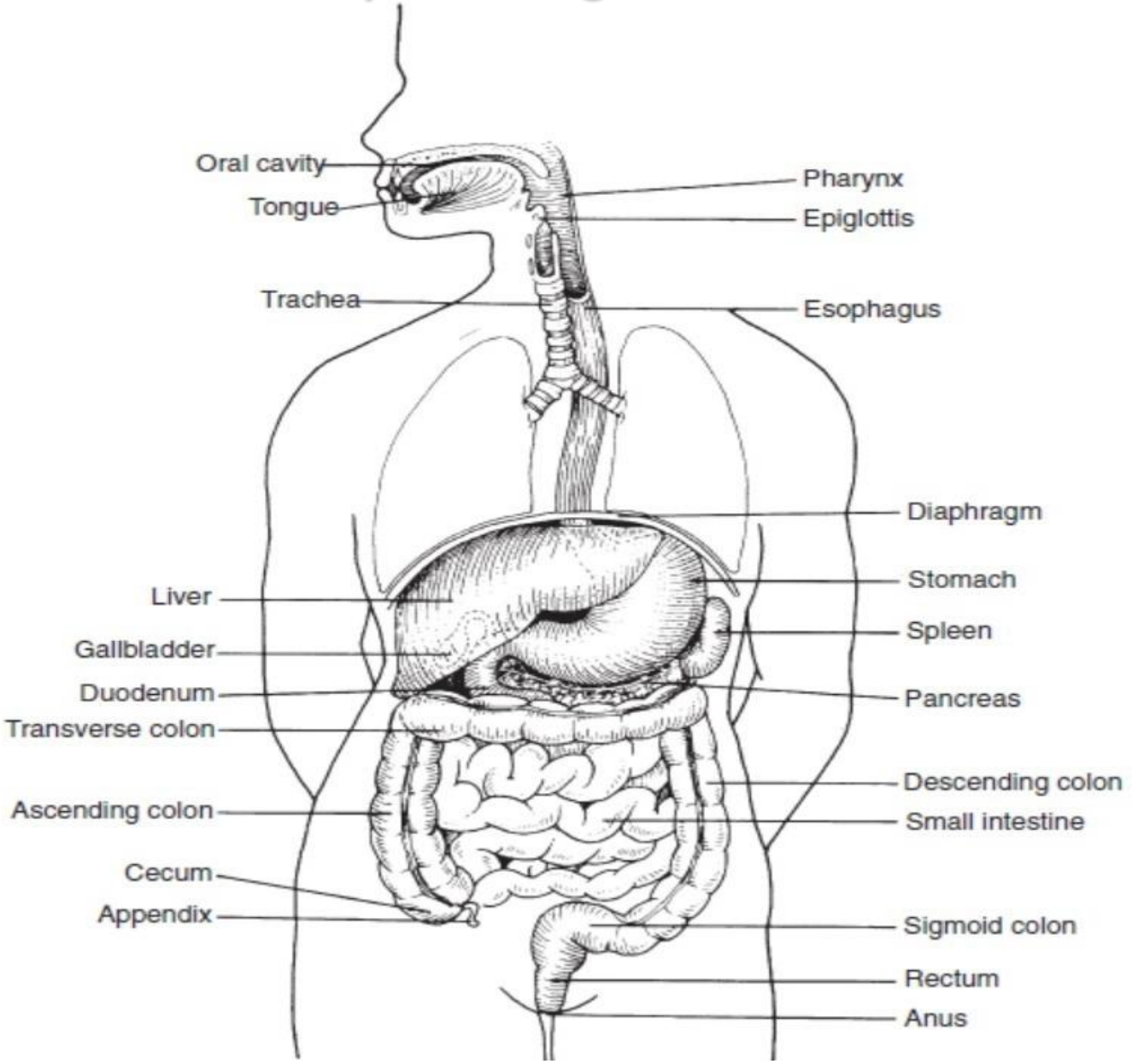
General anatomy of the gastrointestinal tract



General anatomy of the gastrointestinal tract

Components of the Gastrointestinal Tract

Mouth

Oropharynx

Esophagus

Stomach

- *Fundus:* enlarged portion of the stomach to the left and above the opening of the esophagus into the stomach
- *Body:* central part of the stomach
- *Pylorus:* lower portion of the stomach

Small intestine

- *Duodenum:* uppermost division; attached to pyloric end of the stomach
- *Jejunum:* midsection of the small intestine
- *Ileum:* lower portion of the small intestine

Large intestine

- Cecum
- Colon

Ascending colon: lies on the right side of the abdomen and extends up to the lower portion of the liver; the ileum joins the large intestine at the junction of the cecum and the ascending colon

Transverse colon: passes horizontally across the abdomen

Descending colon: lies on the left side of the abdomen in a vertical position

Sigmoid colon: extends downward, subsequently joining the rectum

- Rectum
- Anal canal

General anatomy of the gastrointestinal tract

- Accessory organs and structures include the **salivary glands** , **tongue**, **teeth**, **liver**, **gallbladder**, and **pancreas**.

General physiology of the gastrointestinal tract

- Normal adult GI tract receives up to **8 L** of ingested fluid daily, plus the secretions of the various glands that contribute to digestion (salivary glands, pancreas, gallbladder, stomach)

- Small intestine (Duodenum & Jejunum & Ileum) : more than **90%** of physiologic fluid absorption occurs

Gastrointestinal Infections

Resident Flora / Microbiota

- **Upper small intestine**: 10^1 to 10^3 /mL
- *Streptococci* & *lactobacilli* & yeasts

- **Distal Ileum**: 10^6 to 10^7/mL
- Enterobacteriaceae and Bacteroides

Gastrointestinal Infections

Resident Flora / Microbiota

- Sigmoid colon : **10^{11} to 10^{12}** colony-forming units **(CFU) /g** of stool = **80%** of dry weight of feces
- **Anaerobes** : **Aerobes** ==> **1000 : 1**
- **Anaerobes** : Bacteroides, Clostridium, Peptostreptococcus, Bifidobacterium & Eubacterium
- **Aerobes** : Enterobacteriaceae & Enterococci
- **E.coli** : Other Enterobacteriaceae ==> **10 : 1**

Gastrointestinal Infections

Resident Flora / Microbiota

- The normal flora / resident microbiota **prevents** colonization by potential pathogens

- Normal **peristalsis** helps move organisms toward the rectum, interfering with their ability to adhere to the mucosa.

Treating Gastrointestinal Infections

Resident Flora / Microbiota

- Many episodes of **acute gastroenteritis** are **self-limiting** and require fluid replacement and supportive care and not Antibiotic

- Routine use of **Antidiarrheal agents** is not recommended because many of these agents have potentially serious adverse effects in **infants** and **young children & geriatric / Eldery**

Treating Gastrointestinal Infections

Resident Flora / Microbiota

- **Choice of antimicrobial therapy should be based on:**
- Clinical signs and symptoms
- Organism detected in clinical specimens
- Antimicrobial susceptibility tests

- Some enteric bacterial infections should not be treated / EHEC

Treating Gastrointestinal Infections
Antibiotic-associated Diarrhea

- When normal flora is reduced many Antibiotic resistance microorganism able to multiply:

- Pseudomonas spp
- Candida spp
- Enterococci
- Staphylococci
- various Enterobacteriaceae

Treating Gastrointestinal Infections
Antibiotic-associated Colitis

- Antimicrobial or Antimetabolite treatment / Chemotherapy agents that has altered the normal flora

- When normal flora is reduced, C. difficile is able to multiply and produce its toxins

Gastrointestinal Infections
PATHOGENESIS

- Host Factors - Human Defenses:
- Acidity of stomach
  - Acid-sensitive organisms such as *Salmonella* - 10^5

- Acidity of stomach reduced by
  - Bicarbonate
  - Ranitidine / H2 blockers
  - Milk

Gastrointestinal Infections
PATHOGENESIS

- Host Factors - Human Defenses:
- **Acidity of stomach** -organisms are able to **withstand** exposure to gastric acids and thus require much **smaller** infectious doses

  - ***Shigella*** - 10^2
  - ***E. coli*** O157:H7 - 10^2
  - ***C. difficile*** / spore-forming Clostridium spp

Gastrointestinal Infections

- **Foodborne & Waterborne Illnesses** :
- **Viruses** are considered the most common cause of foodborne illness

- **Bacterial agents**:
  - *Salmonella*
  - *Shigella*
  - *Vibrio & Aeromonas & Plesiomonas*
  - *Yersinia*
  - *E. coli*
  - *Campylobacter*

Etiologic agents of Foodborne & Waterborne Illnesses – 1 / CDC

- Gastroenteritis (vomiting as primary symptom; fever and/or diarrhea also may be present)

- Viral gastroenteritis, most commonly **rotavirus** in an infant or **norovirus** and other in an older child or adult
- Food poisoning due to preformed toxins :
- **Staphylococcus aureus**
- **Bacillus cereus**
- Heavy metals

Etiologic agents of Foodborne & Waterborne Illnesses – 2 / CDC

- Noninflammatory diarrhea (acute watery diarrhea without fever/dysentery; some patients may present with fever)

- Caused by virtually all enteric pathogens (bacterial, viral, parasitic)
- **Enterotoxigenic *Escherichia coli***
- **Giardia**
- ***Vibrio cholerae***
- Enteric viruses (**Noroviruses, enteric Adenovirus, Rotavirus**)
- **Cryptosporidium**
- **Cyclospora**

Etiologic agents of Foodborne & Waterborne Illnesses – 3 / CDC

- Inflammatory diarrhea (invasive gastroenteritis; grossly bloody stool and fever may be present)

- ***Shigella species***
- ***Campylobacter***
- ***Salmonella species***
- ***Enteroinvasive E. coli***
- ***Enterohemorrhagic E. coli***
  - ***E. coli O157:H7***
- ***V. parahaemolyticus***
- ***Yersinia enterocolitica***
- ***Entamoeba histolytica***

Etiologic agents of Foodborne & Waterborne Illnesses – 4 / CDC

- **Persistent diarrhea (lasting >14 days)**

- **Parasites** particularly in travelers to areas where untreated water is consumed.
- Cyclospora **(raspberries)**
- Cryptosporidium
- Entamoeba histolytica
- Giardia lamblia

Etiologic agents of Foodborne & Waterborne Illnesses – 5 / CDC

- Neurologic manifestations (eg, paresthesias, respiratory depression, bronchospasm, cranial nerve palsies)

- **Botulism (*Clostridium botulinum* toxin)**
- Organophosphate pesticides
- Ciguatera fish poisoning
- Neurotoxic shellfish poisoning
- Paralytic shellfish poisoning
- Mushroom poisoning
- **Guillain-Barré syndrome (associated with infectious diarrhea due to Campylobacter jejuni)**

Etiologic agents of Foodborne & Waterborne Illnesses – 6 / CDC

- Systemic illness (eg, fever, weakness, arthritis, jaundice)

- ***Listeria monocytogenes***
- ***Brucella***
- *Trichinella spiralis*
- *Toxoplasma gondii*
- **Vibrio vulnificus**
- Hepatitis A and E viruses
- ***Salmonella* Typhi *and* S. Paratyphi**
- Amebic liver abscess

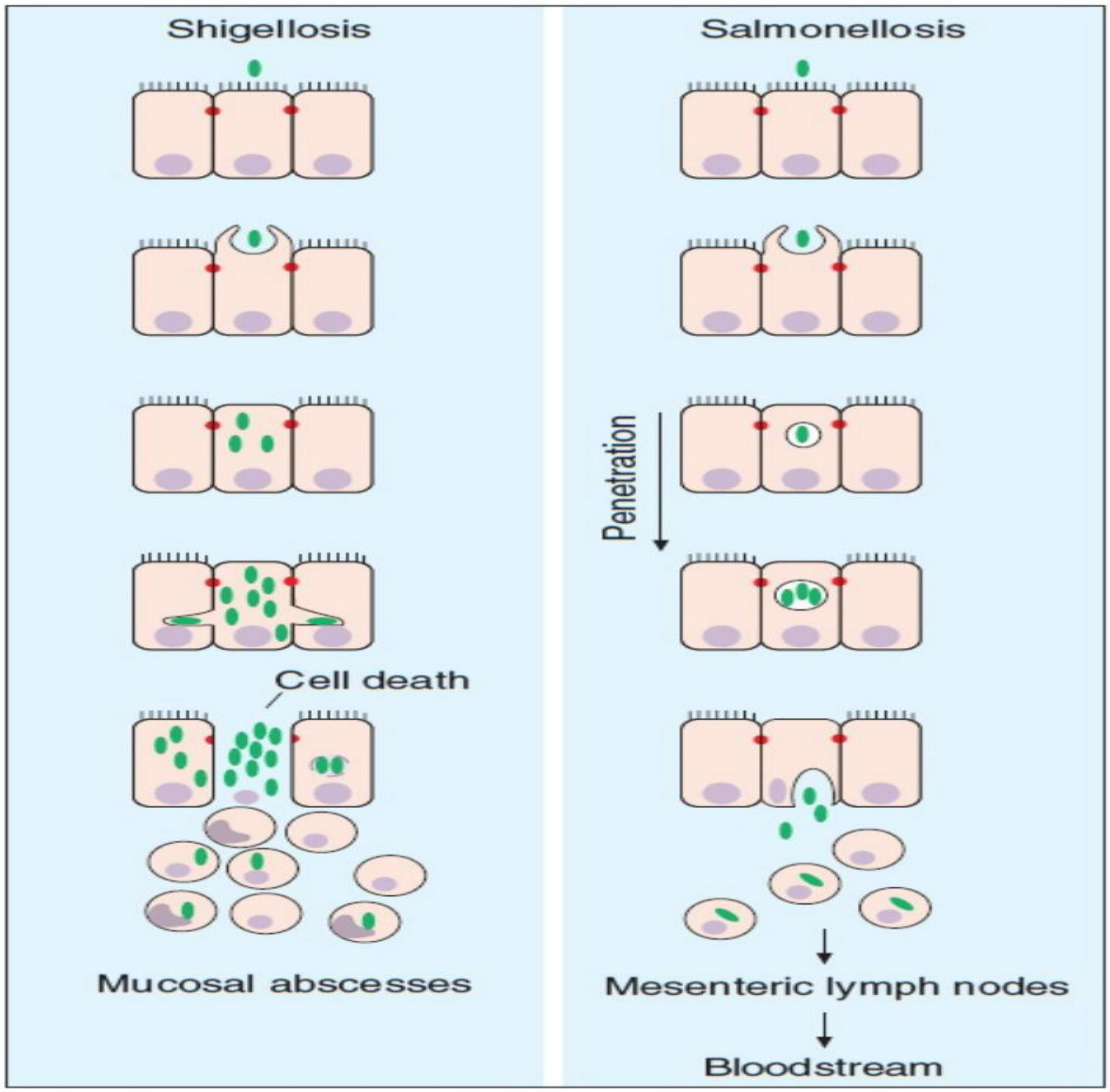
Examples of Microorganisms That Cause GI Infection for Each Primary Pathogenic Mechanism

Mechanism	Examples of Microorganisms
Toxin Production Enterotoxin	*Vibrio cholera* Noncholera vibrios *Shigella dysenteriae* type 1 Enterotoxigenic *Escherichia coli* *Salmonella* spp. *Clostridium difficile* (toxin A) *Aeromonas* *Campylobacter jejuni*
Cytotoxin	*Shigella* spp. *Clostridium difficile* (toxin B) Enterohemorrhagic *Escherichia coli*
Neurotoxin	*Clostridium botulinum* *Staphylococcus aureus* *Bacillus cereus*
Attachment Within or Close to Mucosal Cells/Adherence	Enteropathogenic *Escherichia coli* Enterohemorrhagic *Escherichia coli* *Cryptosporidium parvum* *Isospora belli* Rotavirus Hepatitis A, B, C Norwalk virus
Invasion	*Shigella* spp. Enteroinvasive *Escherichia coli* *Entamoeba histolytica* *Balantidium coli* *Campylobacter jejuni* *Plesiomonas shigelloides* *Yersinia enterocolitica* *Edwardsiella tarda*

Types of Enteric Infections

Pathogenic Mechanism	Major Symptoms	Examples of Etiologic Agents
Upsetting of fluid and electrolyte balance/ noninflammatory	Watery diarrhea No fecal leukocytes No fever	*Vibrio cholerae* Rotavirus Norwalk virus Enterotoxigenic *Escherichia coli* *Giardia lamblia* *Bacillus cereus*
Invasion and possible cytotoxin production/ inflammatory (dysentery)	Dysenteric-like diarrhea (mucus, blood, white cells) Fever Fecal leukocytes	*Shigella* spp. Enteroinvasive *E. coli* *Salmonella enteritidis* *Entamoeba histolytica*
Penetration with subsequent access to the bloodstream (enteric fever)	Signs of systemic infection (headache, malaise, sore throat) Fever	*Salmonella typhi* *Yersinia enterocolitica*

The invasion of Shigella and Salmonella into intestinal epithelial cells



Bacterial Diarrhea

- When bacterial enteropathogens are suspected, a stool culture or toxin assay will help to establish the diagnosis

- Indications for stool culture include the presence of severe diarrhea (passage of six or more unformed stools per day)

Bacterial Diarrhea

- When multiple stool samples are obtained from patients with diarrhea, the increased yield of bacterial pathogens is approximately **20%** **(one in five additional samples**)

Bacterial Dysentery

- *Shigella*
- *Campylobacter*
- Nontyphoid *salmonella*
- Shiga toxin–producing *E. coli*
- *Aeromonas* species
- Noncholeraic vibrios
- *Yersinia enterocolitica*

Traveler's Diarrhea

- Bacterial enteropathogens cause up to 80% of cases
- The diarrhea producing *E. coli* (enterotoxigenic *E. coli*, enteroaggregative *E. coli*, and possibly diffusely adherent *E. coli*) account for more than half of cases

- *Shigella, Salmonella, Campylobacter*, *Aeromonas* species, noncholeraic Vibrios, and *Plesiomonas* also cause this condition

Stool Culture / Specimen collection

- Submit specimen during the acute stage of infection (**usually 5 to 7 days**)
- Submit and culture fresh stool within 30 min of collection to allow for isolation of Shigella spp., which are extremely fragile

Stool Culture / Specimen collection & transportation

- Transfer at least **5** ml of diarrheal stool
- **1** g of stool
- Modified Cary-Blair medium pH : 8.4
- AGAR ; 1.5 G

- Store and transport stool in transport medium at **4'C** and submit within 24h for best recovery of pathogens

Stool Culture / Specimen collection & transportation

- Generally submit **two** stools per patient from different days to diagnose bacterial causes of gastroenteritis

Rejection criteria

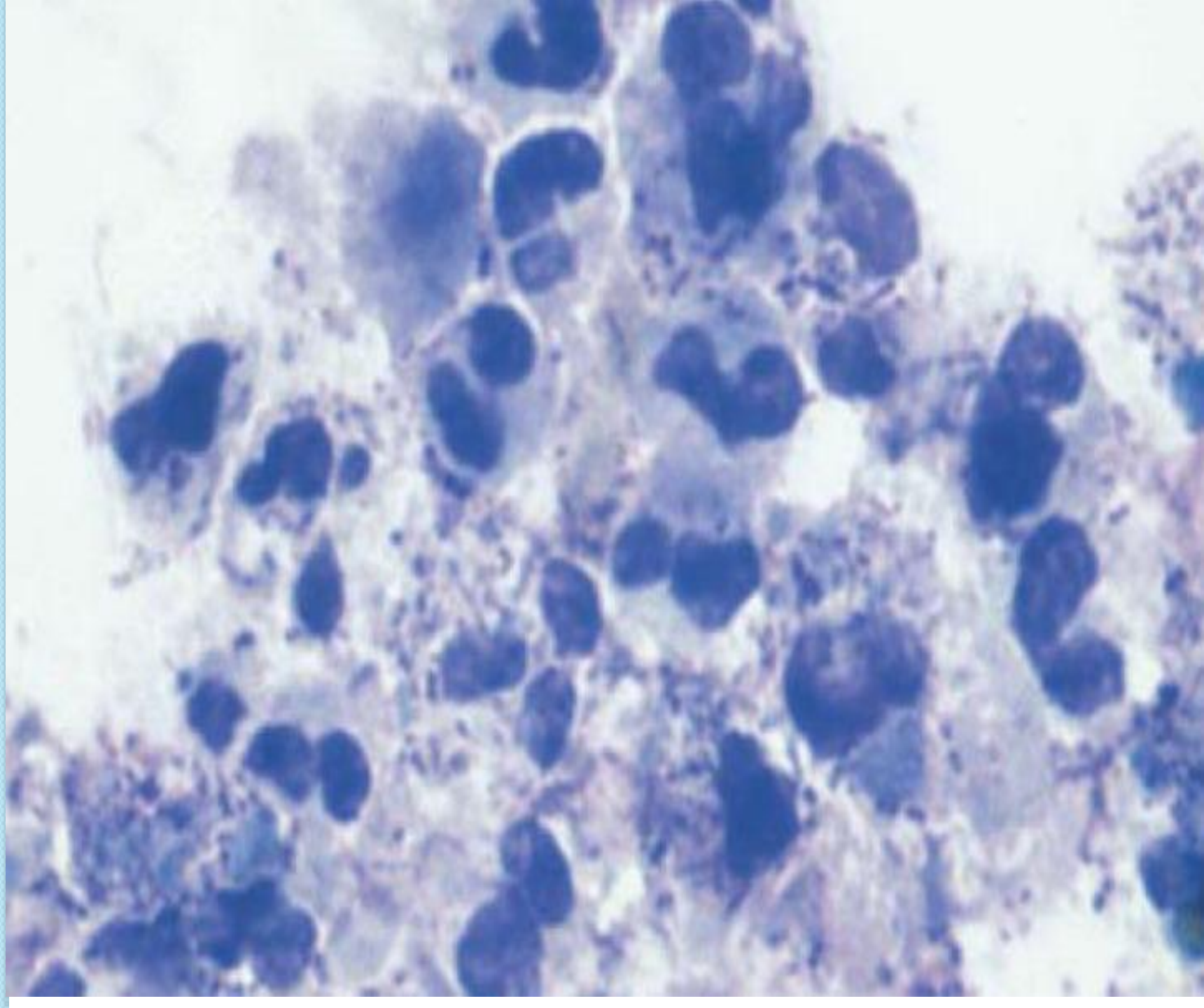
- Reject stools not in transport medium received >2 h after collection as changes occur that are detrimental to most ***Shigella spp***

- If specimen in transport medium is delayed for more than 3 days at 4 C or is delayed for more than 24 h at 25 C

Table 3.8.1–4 Microscopic and gross observations of fecal specimens associated with various infections[a]

Organism or toxin	Other observations	Cells seen in smear	
		PMNs	RBCs
Campylobacter	Darting motile rods	Yes	Yes
Clostridium difficile toxin		Yes	Yes
Escherichia coli O157 H7, enterohemorrhagic	Watery	No	Yes
Escherichia coli, enteroinvasive	Mucous	Yes	Yes
Escherichia coli, enterotoxigenic	Watery	No	No
Salmonella spp.	Motile rods	Few	Yes
Shigella spp.	Lack of motile rods	Yes	
Vibrio cholerae	Rice water	No	No
Staphylococcus toxin		No	No
Viruses		No	No

[a] Data are only a guideline, and in any infection, observations are variable. For example, only 50% of *C. difficile*-associated cases of diarrhea demonstrate the presence of PMNs.



REPORTING RESUTS

- Final reports : No Salmonella, Shigella, or Campylobacter spp. Isolated
- Preliminary reports : to date ,……..

REPORTING RESUTS

Antibiotic-associated Diarrhea

- No normal enteric gram-negative rods isolated
- Identify numerous P.aeruginosa & S. aureus organisms; do not perform AST
- Report yeast, if found in pure or predominating culture, without genus or species identification
- Do not report enterococci in stool

Media

Method of streaking plating medium

Plate 8 or10 cm / Not 6 cm

>30 isolated cfu /plate

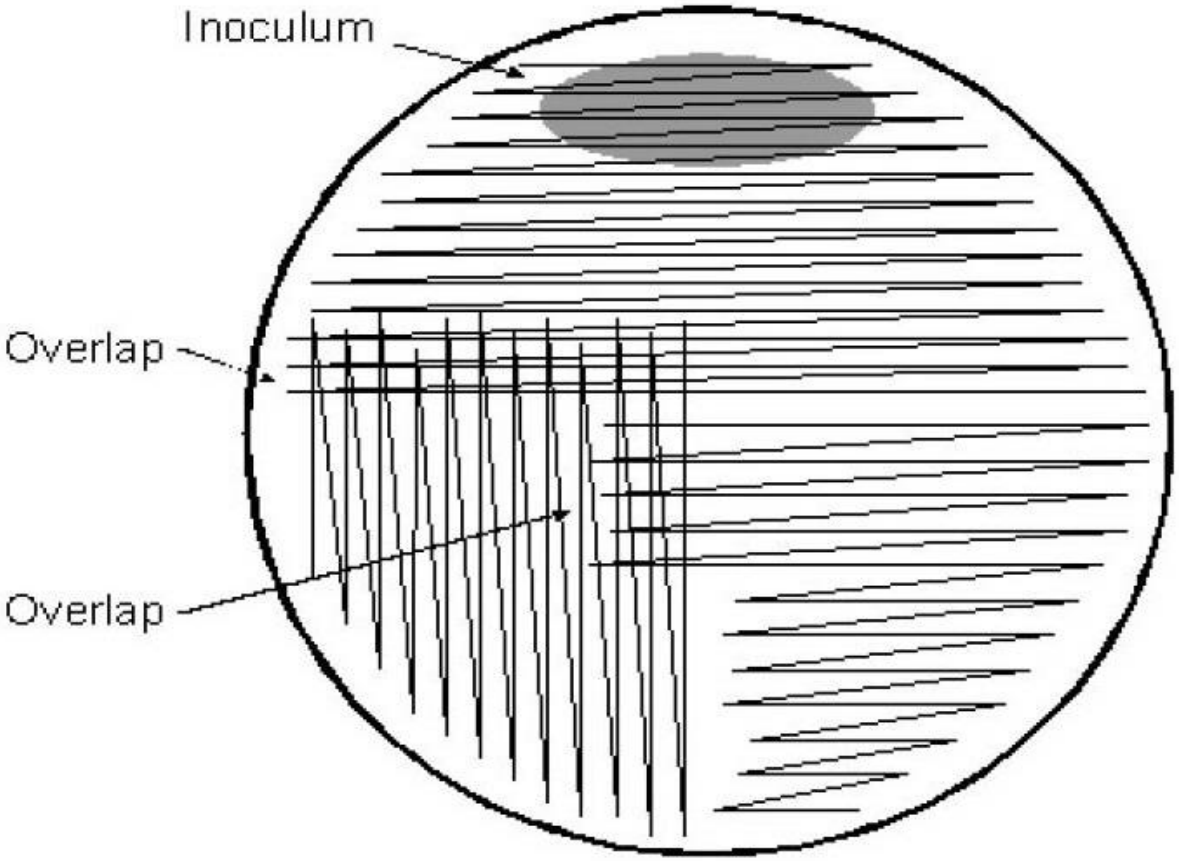


Figure 4-2. Method of streaking plating medium for isolation of *Shigella*

Blood agar (sheep) (SBA,BAP)

- Beta Hemolysis of RBCs
- Screening colonies for the oxidase enzyme

MacConkey agar (MAC)

- Bile salts and crystal violet inhibit most gram-positive organisms and permit growth of gram-negative rods

- Lactose fermenters produce **pink or red** colonies, may be **precipitated bile salts** may surround colonies. **Non–lactose** fermenters appear colorless or transparent

Salmonella-Shigella agar(SS)

- Lactose is the sole carbohydrate
- Select for Salmonella spp. and some strains of Shigella from stool specimens

Xylose-lysine deoxycholate agar (XLD)

Xylose-lysine-deoxycholate agar (XLD)	Sodium deoxycholate inhibits gram-positive cocci and some gram-negative rods. Contains less bile salts than other formulations of enteric media (e.g., SS, HEK) and therefore permits better recovery.	Sucrose and lactose in excess concentrations and xylose in lower amounts. Phenol red is the pH indicator. Lysine is included to detect decarboxylation. Sodium thiosulfate/ferric ammonium citrate allows the production of H_2S. The following types of colonies may be seen: *Yellow:* Fermentation of the excess carbohydrates to produce acid; because of the carbohydrate use, the organisms do not decarboxylate lysine, even though they may have the enzyme. *Colorless or red:* Produced by organisms that do not ferment any of the sugars. *Yellow to red:* Fermentation of xylose (yellow), but because it is in small amounts, it is used up quickly, and the organisms switch to decarboxylation of lysine, turning the medium back to red. Black precipitate is formed from the production of H_2S.		Selective media used to isolate *Salmonella* and *Shigella* spp. from stool and other specimens containing mixed flora

Triple sugar iron agar(TSI)

Media	Selective	Differential	Nutritional	Purpose
Triple sugar iron agar (TSI)		Contains glucose, sucrose, and lactose. Sucrose and lactose are present in 10 times the quantity of the glucose; phenol red is the pH indicator. Turns to yellow when sugars are fermented because of drop in pH. Sodium thiosulfate plus ferric ammonium sulfate as H_2S indicator. Acid/acid (A/A): Glucose and lactose and/or sucrose (or both) fermentation. Gas bubbles: Production of gas. Visible air breaks or pockets in agar. Black precipitate: H_2S. Alkaline/acid (K/A): Glucose fermentation but not lactose or sucrose. Alkaline/alkaline (K/K): No fermentation of dextrose, lactose, or sucrose.		Differentiates glucose fermenters from non–glucose fermenters; also contains tests for sucrose and/or lactose fermentation, as well as gas production during glucose fermentation and H_2S production.

A B C D E F

A: Uninoculated TSI

B: *Salmonella* serovar Typhi K/A^{TR} (Alkaline slant / Acid Butt / Trace H_2S / No Gas)

C: *Salmonella* serovar Newport K/A_g $^{+++}$(Alkaline slant / Copious H_2S / Gas)

D: *Shigella flexneri* K/A (Alkaline slant / Acid Butt / No H_2S / No Gas)

E: *E. coli* A/A (Acid slant / Acid Butt / No H_2S / Copious Gas)

F: *Pseudomonas aeruginosa* (Non-fermenter / No H_2S / No Gas)

Lysine iron agar (LIA)

Lysine iron agar (LIA)		Contains lysine, glucose, and protein, bromocresol purple (pH indicator) and sodium thiosulfate/ferric ammonium citrate. Purple denotes alkaline (K), red color (R), acid (A). K/K: Organism decarboxylates but cannot deaminate, ferments glucose, first butt is yellow. Decarboxylates lysine producing alkaline; changes back to purple. K/A: Organism fermented glucose but was unable to deaminate or decarboxylate lysine. Bordeaux red and yellow butt. R/A: Organism deaminated lysine but could not decarboxylate it. The lysine deamination combines with the ferric ammonium citrate, forming a burgundy color. Blackening of the butt indicates production of H_2S.		Measures three parameters that are useful for identifying Enterobacteriaceae (lysine decarboxylation, lysine deamination, and H_2S production)

A B C D E

A: Uninoculated LIA
B: LDC negative /lysine deaminase negative/ H_2S negative (*Citrobacter freundii*)
C: LDC positive /lysine deaminase negative/ H_2S negative (*Salmonella* ser. Typhi)
D: LDC negative /lysine deaminase positive/ H_2S negative (*Proteus mirabilis*)
E: LDC positive /lysine deaminase negative/ H_2S positive (*Salmonella* ser. Newport)

Stool Culture Screening for Enteric Pathogens Utilizing TSI and LIA in Combination

LIA Reactions	TSI Reactions: K/A H_2S	K/AG H_2S	K/AG	K/A	A/A H_2S	A/AG	A/A	K/K
R/A		*P. vulgaris* *P. mirabilis*	*M. morganii* *Providencia*	*M. morganii* *Providencia*	*P. vulgaris* *P. mirabilis*		*Providencia*	
K/K H_2S	* *Salmonella* *Edwardsiella*	* *Salmonella* *Edwardsiella*	* *Salmonella*	* *Salmonella*	*			
K/K	* *Salmonella*		*Hafnia* *Klebsiella* *Serratia*	* *Salmonella* *Plesiomonas*† *Hafnia* *Serratia*		*Klebsiella* *Enterobacter* *E. coli*	*Serratia*	*Pseudomonas*†
K/A H_2S		*Salmonella* *Citrobacter*			*Citrobacter*			
K/A			* *Salmonella* *Shigella* *Aeromonas*† *E. coli* *Enterobacter* *Citrobacter*	* *Shigella* *Yersinia* *Aeromonas*† *E. coli* *Enterobacter*		* *Aeromonas*† *E. coli* *Citrobacter* *Enterobacter*	* *Aeromonas*† *Yersinia* *Citrobacter* *Enterobacter*	

Data from the Microbiology Laboratory, OSU Hospitals and Maureta Ott, Columbus, Ohio.
LIA, Lysine-iron agar; *TSI*, triple sugar iron; *K*, alkaline; *A*, acid; *G*, gas; *R*, deamination (red slant).
* Results of TSI and LIA reactions in this category indicate a potential pathogen; additional tests must be performed.
† Oxidase positive.

Table 3.8.1–1 Commonly used primary plating and broth media for isolation of *Salmonella* and *Shigella*[a]

Medium (abbreviation)	Type	Expected isolates	Inhibitors or indicators	Reactions of lactose fermenters	Reactions of pathogens	Comments
Hektoen enteric agar (HEK) (17)	D, S plate	*Salmonella* and *Shigella* spp. (especially for *Shigella* spp.)	Bile salts, ferric ammonium citrate, sodium thiosulfate, lactose, sucrose, salicin, bromthymol blue, fuchsin	Yellow-orange or salmon pink; pink precipitate around colonies, may have black centers.	*Shigella* is green. *Salmonella* is blue or green; may have black centers.	Inhibits *Citrobacter* but is small and blue-green, if present. *Proteus* and *Providencia* are yellow or green; may have black centers. Detects H_2S.
MacConkey agar (MAC)	D, S plate	Gram-negative enteric bacilli	Bile salts, crystal violet, lactose, neutral red	Pink	Colorless or transparent	5% Agar will inhibit swarming of *Proteus* spp.
Salmonella-shigella agar (SS)	D, highly S plate	*Salmonella* and *Shigella* spp. (*S. sonnei* inhibited)	Bile salts, lactose, citrate, thiosulfate, ferric citrate, brilliant green, neutral red	Pink, red; may have black centers.	Colorless or transparent; may have black centers.	Detects H_2S.
Xylose, lysine, deoxycholate agar (XLD) (25)	D, S plate	*Salmonella* and *Shigella* spp. (especially for *Shigella* spp.)	Deoxycholate, thiosulfate, ferric ammonium citrate, xylose, lactose, sucrose, lysine, phenol red	Yellow; may have black centers.	*Salmonella* and *Shigella* are red. *Edwardsiella* and *Salmonella* may be red with black centers.	*Providencia rettgeri*, *Morganella morganii*, and *Proteus* spp. are yellow even though they are lactose negative. Detects H_2S.
Gram-negative (GN) broth	E broth	*Shigella* and possibly *Salmonella* spp.	Deoxycholate, citrate, dextrose, mannitol	Initially enhances growth of mannitol-fermenting rods		Subculture at 6–8 h.
Selenite-F	E broth	*Salmonella* and *Shigella* spp. (some shigellae may be inhibited)	Selenite, lactose	Selenite is toxic to *Escherichia coli* and some other enteric bacteria.		Subculture at 18–24 h. Selenite broth with cystine may inhibit some salmonellae.

[a] Either bile salts, deoxycholate, or Selenite is present in each medium to inhibit gram-positive microbiota. Abbreviations: D, differential; E, enriched; S, selective. Ferric ammonium citrate reacts with hydrogen sulfide (H_2S) from organism to produce black color of colony.

A B C

A: Uninoculated Urea Agar
B: Positive (*Proteus mirabilis*)
C: Negative (*E. coli*)

Motility test

- Nonmotile organisms grow clearly only on stab line, and the surrounding medium remains clear
- Shigella are nonmotile
- Yersinia sp. are motile at room temperature

Serogrouping

- Determination of O serogroups associated with the cell wall lipopolysaccharides
- e.g. ;O111 in EPEC & STEC

Serotyping

- Determination of O serogroups associated with the cell wall lipopolysaccharides and H of the flagella
- E. coli are serotyped on the basis of their O (somatic), H (flagellar), and K (capsular) surface antigen profiles
- e.g. O111:H2 in EPEC & O111:H8 in STEC

Serogrouping & Serotyping

- Serogrouping & Serotyping should be performed from a **non–sugar-containing medium**, such as **5% sheep blood agar** or **LIA**.

- Use of sugar-containing media, such as MacConkey or TSI agars, can cause the organisms to **autoagglutinate**.

Staphylococcus aureus

- Vomiting lasting ≤12 hr, with an incubation period of 2–7 hr

- Food may be cultured for staphylococcus or enzyme immunoassay may be performed for enterotoxin in food

Clostridium perfringens

- Potentially very large foodborne outbreaks of watery diarrhea without fever or vomiting;
- Incubation period of 8–14 hr
- Confirmed in foodborne outbreaks by detecting $\geq 10^6$ C. perfringens spores/g of feces in affected persons or $\geq 10^5$ organisms/g in food

Bacillus cereus

- Gastroenteritis
- Syndromes resembling *S. aureus* with vomiting after 2–7 hr or C. perfringens disease with watery diarrhea after 8–14 hr
- Confirmed in foodborne outbreaks by detecting $>10^5$ organisms in food

Clostridium difficile

Antibiotic-associated Colitis

- Antimicrobial or Antimetabolite treatment / Chemotherapy agents that has altered the normal flora
- When normal flora is reduced, C. difficile is able to multiply and produce its toxins

Clostridium difficile Associated Disease (CDAD / CDI)

- **Almost every antimicrobial agent** and several cancer agents have been associated with the development of CDAD

- **Clindamycin, Ampicillin, Amoxicillin or Cephalosporins** were the most often associated with an increased risk of CDAD

SHEA-IDSA GUIDELINE (DIAGNOSIS)

- "Testing for *C. difficile* or its toxins should be performed only on diarrheal (unformed) stool"

- "Testing of stool from asymptomatic patients is not clinically useful, including use as a test of cure. It is not recommended, except for epidemiology studies."

SHEA-IDSA GUIDELINE (DIAGNOSIS)

- "Repeat testing during the same episode of diarrhea is of limited value and should be discouraged."
- "PCR testing appears to be rapid, sensitive, and specific and may ultimately address testing concerns. More data on utility are necessary before this methodology can be recommended for routine testing."

Clostridium difficile DETECTION

Test	Target	Sensitivity (%)	Specificity (%)	Turnaround Time
Enzyme immunoassay	Toxin	70-80	>97	Hours
Glutamate dehydogenase	Common antigen	70-80	<90	Hours
PCR	Toxin	>90	>97	Hours
Toxigenic culture	Toxin	>90	95-97	Days
Cytotoxin tissue culture	Toxin	70-80	>97	Days

PERFORMANCE CHARACTERISTICS

Parameter	Toxin A/B EIA	*tcdB* PCR
Sensitivity (%)	58.5	90.0
Specificity (%)	86.2	99.0
Positive predictive value (%)	45.3	94.7
Negative predictive value (%)	91.4	98.1
Percentage agreement	80.8	

RECENT GUIDELINES

- "Only stools from patients with diarrhea should be tested for *Clostridium difficile*."
- "Repeat testing should be discouraged."
- "Testing for cure should not be done."

RECENT GUIDELINES

- Nucleic acid amplification testing for *C. difficile* toxin genes is superior to toxin A & B enzyme immunoassay as a standard diagnostic test for CDI

- GDH screening tests for *C. difficile* can be utilized in algorithms with subsequent toxin testing, but sensitivity of strategies is lower than nucleic acid amplification testing

Antibiotic-associated hemorrhagic colitis (AAHC)

- Antibiotic-associated hemorrhagic colitis (AAHC) is not linked to C. difficile infection
- AAHC symptoms include a sudden onset of bloody diarrhea and abdominal cramps during antibiotic therapy
- **Toxin-producing *Klebsiella oxytoca* has been identified as a causative agent of AAHC**

E. coli

Type	Primary Mode of Pathogenesis	Other Comments
Enterotoxigenic (ETEC)	Produces heat-labile (LT) or heat stable (ST) enterotoxins; genes of both toxins reside on a plasmid; LTs are closely related in structure and function to cholera toxin; STs result in net intestinal fluid secretion by stimulating guanylate cyclase	Common cause of traveler's diarrhea; infects all ages
Enteroaggregative (EAEC)	Binds to small intestine cells via fimbriae encoded by a large molecular weight plasmid, forming small clumps of bacteria on the cell surface; other plasmid-borne virulence factors include structured pilin, a heat-stable enterotoxin, novel anti-aggregative protein, and a heat-labile enterotoxin, all believed to be the cause of the associated diarrhea	Infects primarily young children
Enteroinvasive (EIEC)	Pathogenesis has yet to be totally elucidated; studies suggest that mechanisms by which diarrhea results are virtually identical to those of *Shigella* spp.	Very difficult to distinguish from *Shigella* spp. and other *E. coli* strains
Enteropathogenic (EPEC)	Initially attaches in the colon and small intestine and then becomes intimately adhered to intestinal epithelial cells, subsequently causing the loss of enterocyte microvilli (effacement); genes for attachment/effacement reside in a cluster on the bacterial chromosome (i.e., pathogenicity island)	Diarrhea in infants, particularly in large urban hospitals
Enterohemorrhagic (EHEC) OR	Attaches to and effaces gut epithelial cells in a similar manner as EPEC; in addition, EHEC elaborates shiga toxins	Although many outbreaks are caused by *E. coli* 0157:H7, other serotypes have been implicated in outbreaks and sporadic cases Gene recombination among strains makes classification difficult
Enterohemorrhagic (EHEC); or serotoxigenic (STEC); verotoxigenic (VTEC) (newest, terminology)	Produce one or more shiga toxins referred to as verocytotoxins. Attaches to and effaces gut epithelial cells in a similar manner as EPEC	0157 STEC serotypes; contains most common serotypes 0157:H7 and nonmotile 0157:NM. There are more than 150 non-0157 serotypes that have been isolated from patients with diarrhea or hemolytic uremic syndrome

Features of Pathogenic *Escherichia coli*

Type	Virulence Factor(s)	Disease	Relevant Serotypes	Relevant Laboratory Tests
Uropathogenic *E. coli*				
UPEC	P pilus/*pap* pili, type 1 fimbriae	UTIs		
DAEC*	Afa/Dr adhesions	UTIs		
Enteric pathogens				
EPEC	Pathogenicity islands	Infantile diarrhea	055:NM 055:H6 0111:NM 0111:H2 0114:NM 0111:H2	
EHEC	Shiga Toxin/Vero toxin	Hemorrhagic diarrhea, colitis, HUS	0157:H7 0157:NM 026:H11 0104:H21	SMAC plates, MUG
EIEC	Invasin	Dysentry	0124:H30 0143:NM 0164:NM	DNA probes, Sereny test
ETEC	LT, ST	Traveler's diarrhea/turista	06:NM 06:H16 08:H9 025:NM	
Enteroadherent *E. coli*				
EAggEC	AAF fimbriae	Persistent pediatric diarrhea		
DAEC*	Afa/Dr adhesions, AIDA-1, pathogenicity islands	Pediatric diarrhea, UTIs		HeLa cell adherence assay, DNA probes
Extra intestinal pathogens				
	Capsule	Septicemia and meningitis	K1	

Summary of Epidemiology, Clinical Features, Pathogenesis, Diagnosis, and Therapy of *E. coli* Pathotypes That Cause Diarrhea

Pathotype[*]	*Epidemiology*	*Clinical Features*	*Pathogenesis*	*Diagnosis*	*Adjunctive Therapy*[†]
ETEC	Contaminated water and food. Major cause of childhood diarrhea in developing countries; leading cause of travelers' diarrhea	Acute watery diarrhea, occasionally severe	Large number of fimbrial adhesins; heat-stable and heat-labile enterotoxins	PCR or DNA probes for enterotoxins	Fluoroquinolones plus loperamide for travelers
EPEC	Person-to-person transmission. Leading cause of infantile diarrhea in developing countries	Severe acute diarrhea and vomiting, may be persistent	Localized adherence via bundle-forming pilus; attaching and effacing via intimin-Tir	PCR or DNA probes for *bfp*[‡] or *eae* genes or tissue culture assay for localized adherence[‡]	Antibiotics guided by susceptibility testing for severe or protracted cases
EHEC and other STEC	Food, water, and person-to-person spread. Major cause of bloody diarrhea in developed countries	Watery and bloody diarrhea, may be complicated by hemolytic uremic syndrome	Shiga toxins; intimin-Tir–mediated attaching and effacing in EHEC strains	Sorbitol-MacConkey agar,[§] PCR or DNA probes for *stx* genes	Supportive care. Antibiotics and antimotility agents contraindicated
EAEC	Mode of transmission unknown. Important cause of chronic diarrhea in developing countries; emerging cause of travelers' diarrhea	Mucoid diarrhea, often persistent	Aggregative adherence via several fimbriae; Pet and other toxins	Tissue culture assay for aggregative adherence or PCR for *aggR* gene	Fluoroquinolones may be of benefit for travelers and HIV patients
EIEC	Contaminated food. Outbreaks in developed countries	Watery diarrhea or dysentery	Cellular invasion, intracellular motility, and cell-to-cell spread	PCR or DNA probes for *inv* genes	Unknown
DAEC	Mode of transmission unknown. Diarrhea in older children in developing countries	Poorly described	Unknown	Tissue culture assay for diffuse adherence	

[*]ETEC, enterotoxigenic *E. coli;* EPEC, enteropathogenic *E. coli;* EHEC, enterohemorrhagic *E. coli;* STEC, Shiga toxin–producing *E. coli;* EAEC, enteroaggregative *E. coli;* EIEC, enteroinvasive *E. coli;* DAEC, diffuse adhering *E. coli.*

[†]The cornerstone of therapy for all diarrheal disease is rehydration, preferably via oral route.

[‡]Detects typical strains only.

Enterohemorrhagic E. coli (EHEC)

- Shiga toxin-producing E. coli (STEC) / Verotoxin & E. coli O157:H7

- The infection is **potentially fatal**, especially in **young children** and **elderly persons** in nursing homes

- **Meats (beef)** , such as undercooked **hamburgers served at fast-food restaurants**, unpasteurized dairy products and apple cider

Shiga toxin–producing E. coli

- Infection by Shiga toxin–producing E. coli is the main cause of renal failure in childhood

- In the hemolytic– uremic syndrome, Shiga toxin released in the gut enters the bloodstream and reaches the renal endothelium

- Two thirds of children with the hemolytic–uremic syndrome require dialysis

Shiga toxin–producing E. coli

- **Infectious dose : 10^2**

- **Inoculum to cause infection with E. coli O157:H7 is low so that person-to-person spread can occur**

Shiga toxin–producing E. coli

- Shiga toxin–producing E. coli strains cause watery diarrhea that becomes bloody in 1 to 5 days in 80% of patients

- Characteristic features of this condition include severe abdominal pain and cramps and passage of five or more unformed stools per 24 hours in the absence of fever

Shiga toxin–producing E. coli

- It appears that Shiga toxin 2 is more important in the pathogenesis of the hemolytic–uremic syndrome than Shiga toxin 1

- As well as examination of the stools for Shiga toxins 1 and 2 by means of commercial enzyme immunoassay

- EIA for detection of STEC (MaC Broth or GN Broth)

Shiga toxin–producing E. coli
E. coli O157:H7

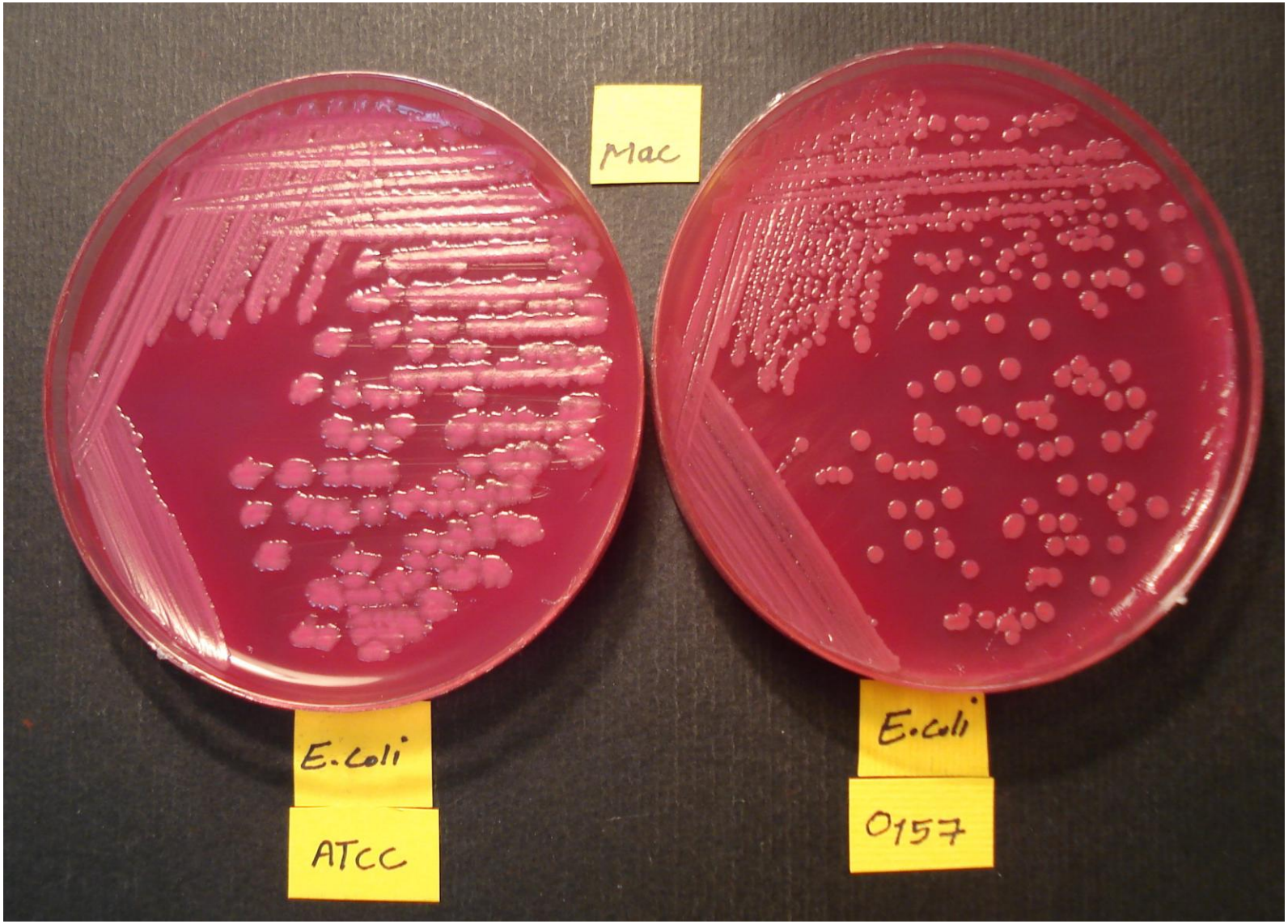
- Stool cultures have a low positivity rate

- Isolation of E.coli O157:H7 is possible only during the acute phase of illness, and the organisms may not be detectable 5-7 days after onset

- Laboratory evaluation of bloody stools should include assays for sorbitol-negative E. coli / O157:H7 strains

Shiga toxin–producing E. coli
E. coli O157:H7

- Only one serotype, namely E. coli O157:H7 can be detected in clinical laboratories
- Sorbitol-MacConkey agar (SMAC)
- E. coli O157:H7 does not ferment sorbitol in 24 (48) hours, a characteristic that differentiates it from most other E. coli
- E. coli O157:H7 appears colorless on Sorbitol MacConky agar (SMAC)


Mac
E.coli
ATCC
E.coli
0157


SmaC
E.coli
E.coli
0157

Confirm by latex agglutination / Antiserum

- Agglutination test for rapid presumptive detection of E. coli 0157 from SMAC / Serogrouping

- Sorbitol-negative colonies are subsequently subculture for Serotyping using E. coli 0157:H7 antiserum

Shiga toxin–producing E. coli
E. coli O157:H7

CHROMagar
Microbiology

CHROMagar Product Range: | **CHROMagar ™ O157**

Browse Products

For isolation and direct differentiation of enterohaemorrhagic *E.coli* O157 by colony color.

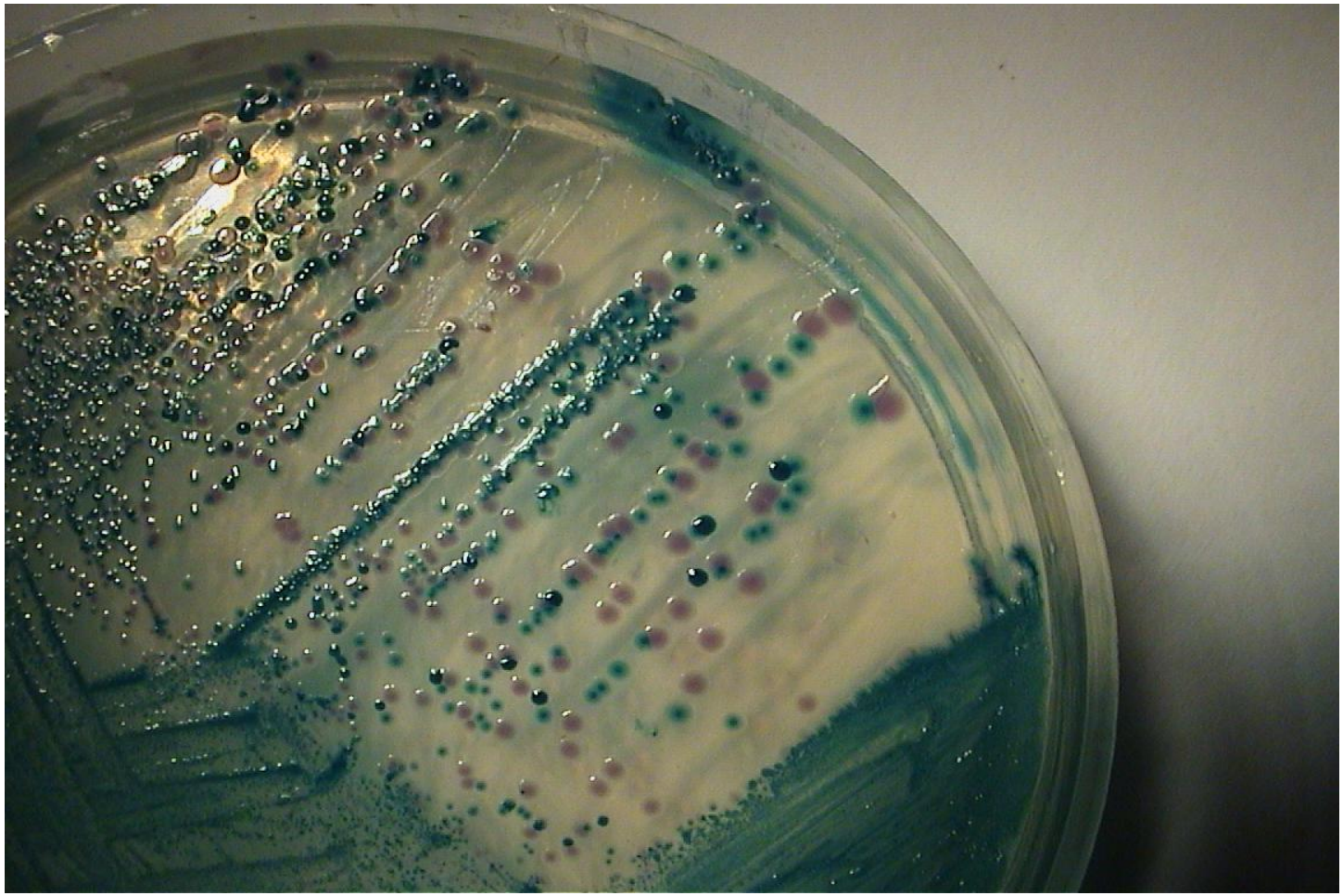
- *E.coli* O157 - mauve
- other bacteria - blue, colourless or inhibited

Ordering information:

5000ml..... EE222
4 x 250ml..... EE220

CHROMagar O157

Shiga toxin–producing E. coli
E. coli O157:H7



Vibrio cholerae

VIBRIONACEAE

- Oxidase –positive
- Glucose-fermenting
- Gram-negative
- Grow on MacConkey agar
- Halotolerant
- **VIBRIO**
- **AEROMONAS**
- **PLESIOMONAS→ Enterobacteriacae**

Vibrio cholerae / CHOLERA

(O1 and non-O1)

- In acute cases as a severe gastroenteritis accompanied by vomiting followed by diarrhea

- The stools produced by cholera patients are described as "rice-water"

- Number of stools,, may be as many as 10 to 30 per day

Vibrio cholerae

Vibrio cholerae

Vibrio cholerae

- Stool / Rectal swab should be collected as early as possible in the course of the illness
- Rectal Swab : Pass tip of sterile swab approximately 2-3 cm
- Cary-Blair Transport

Vibrio cholerae

- **TCBS** (Thiosulfate Citrate Bile Salts Sucrose Agar) is the most widely used selective medium

- Screen TCBS at **24** and **48** h

Vibrio cholerae

- TCBS differentiates sucrose-fermenting (yellow) from the nonsucrose-fermenting (green) vibrios
- Proteus is yellow & Enterococci may grow
- Quality-control : there is great lot to lot variation in performance and not all Vibrio spp.grow on TCBS

Vibrio cholerae

Vibrio cholerae

- Alkaline peptone water : Enrichment procedure enhance isolation of vibrios & Aeromonas
- Alkaline peptone water : 1% NaCl , pH 8.5; Subculture to TCBS at 24 h at 35 C
- Subculture at is not necessary 5-8h (optional)

Vibrio cholerae

- Sheep blood agar plate should be examined for the presence of Hemolysis & Oxidase

- On MacConkey agar, the pathogenic vibrios grow as nonlactose fermenters

- lactose-positive colonies from selective-differential media such as MacConkey may give false positive oxidase reactions

Vibrio cholerae

- Oxidase test must be performed from 5% sheep blood agar or another medium without a fermentable sugar

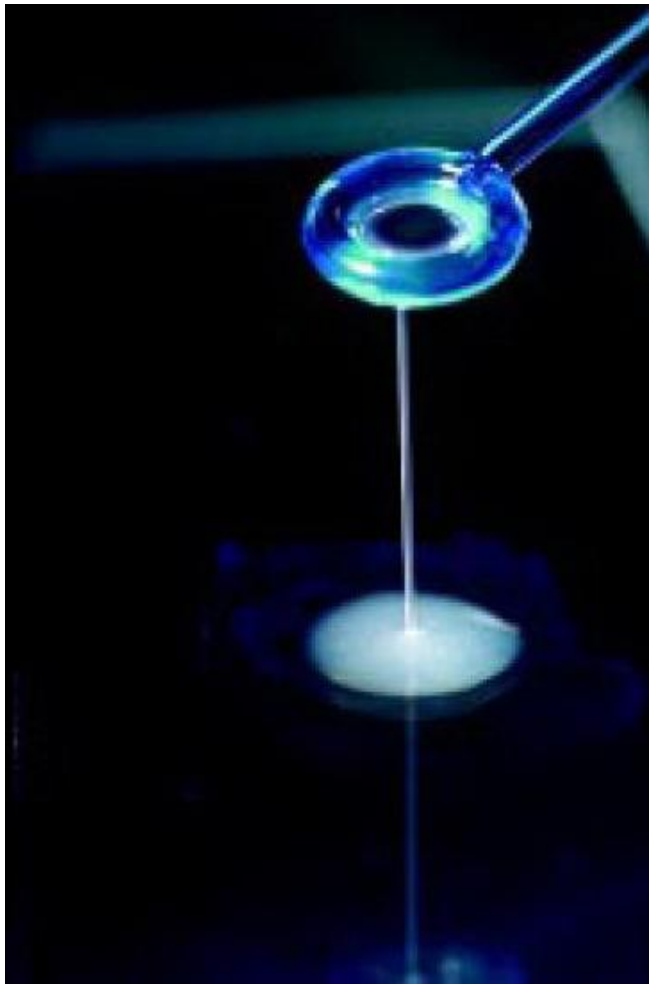
- Acidification of medium if surrounding pH is below 5.1 may result a false-negative Oxidase
- **Oxidase** performed from KIA : YES
- **Oxidase** performed from TSI : NO

Vibrio cholerae

- Oxidase test : **N,N,N,N-Tetramethyl-1,4-phenylendiammonium dichloride for oxidase test**

Vibrio

- **String test** : Most vibrios also exhibit a positive string test observed as a mucoid "stringing" reaction after emulsification of colonies in 0.5% sodium desoxycholate



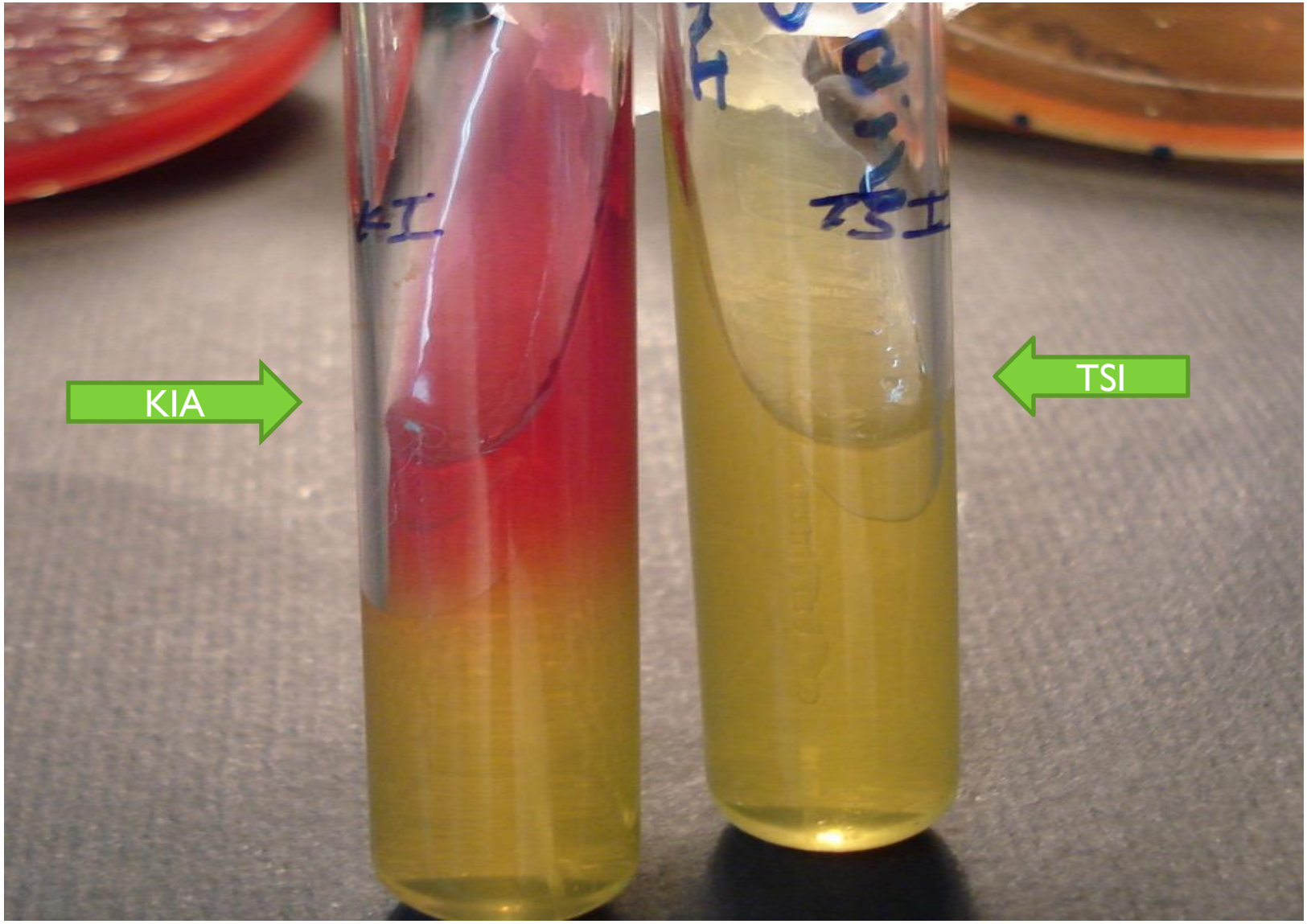
Vibrio

- Most species are generally susceptible to the vibriostatic compound O/129 150-microgram (2,4-diamino-6,7-diisopropylpteridine)
- Exhibiting a zone of inhibition on either Mueller-Hinton or trypticase soy agar
- MHA 4% NaCL if no growth on MHA

Vibrio cholerae

- Negative for Gas from Glucose
- Positive for Growth in 6% NaCl
- Positive for Sucrose
- Negative for Lactose
- Positive for ODC & LDC
- Negative for ADH

Vibrio cholerae



Vibrio cholerae / Antigenic Structure

- Three major subgroups of V. cholerae are

- V. cholerae O1
- V. cholerae O139/ Bangal

- Strains of V.cholerae O1 and V. cholerae O139 are associated with epidemic cholera

Vibrio cholerae / Antigenic Structure

- V.cholerae non-O1 / NAG
- O2 – 138 NOT EPIDEMIC ASSOCIATED
- Strains that phenotypically resemble V. cholerae but fail to agglutinate in 01 antisera are referred to as V. cholerae non-O 1

Vibrio cholerae / Antigenic Structure

- V.cholerae / non-O1/ NAG

- Strains are phenotypically similar to toxigenic V. cholerae O1, but most lack the cholera toxin gene and appear to cause a milder form of gastroenteritis or cholera-like disease.

Vibrio cholerae / Antigenic Structure

- Based on the composition of the O antigen, V.cholerae O1 organisms are divided into the following serotypes:

- Ogawa (A, B) , 1377…
- Inaba (A, C) , 1384 …
- Hikojima(A, B, C)

Vibrio cholerae / Biogroups

- V. cholerae O1 strains occur in two biogroups:
- Classic
- El Tor

- El Tor has been the predominant biogroup in the last two pandemics

Vibrio cholerae / Biogroups

LABORATORY TESTING

TEST	CLASSIC	EL TOR
STRING	+	+
BETA HEME	0	+
VP	0	+
POLYMYXIN B	SUSCEPTIBLE	RESISTANT

Vibrio cholerae / Reporting

- Vibrio cholorae serogroup O1….
  - or O139 or (non-O1 or non-O139)or NAG
- …serotype (Inaba or Ogawa or Hikojima)
- …..biotype (El Tor or Classic)

Aeromonas

Aeromonas

- Aeromonas are currently recognized as human pathogens causing a variety of clinical infections including gastroenteritis
- Most cases are self-limiting
- In the **pediatric** and **geriatric** populations, supportive therapy and antimicrobials are often indicated

Aeromonas/ Intestinal Infections

- An acute, secretory diarrhea often accompanied by vomiting
- An acute, dysenteric form of diarrhea with blood and mucus
- A chronic diarrhea usually lasting more than **10** days
- A cholera-like disease including rice-water stools
- Traveler's diarrhea

Aeromonas/ Intestinal Infections

- Most frequently:
- *Aeromonas veronii* biovars *sobria*
- *Aeromonas hydrophila* complex
- *Aeromonas caviae* :
  - Pediatric diarrhea

Aeromonas/ Intestinal Infections

- ***A. veronii bv. sobria*** has been linked to cholera-like disease characterized by abdominal pain, fever, and nausea

- Complications, usually from ***A. hydrophila*** and ***A. veronii bv. sobria*** include hemolytic uremic syndrome or kidney disease that may require a kidney transplant

Aeromonas/ Laboratory Diagnosis

- ***Aeromonas*** grow readily on most media used for both routine and stool cultures

- After **24-hou**r incubation at 35° C, ***Aeromonas*** appear as large round, raised, opaque colonies with an entire edge and a smooth, often mucoid surface

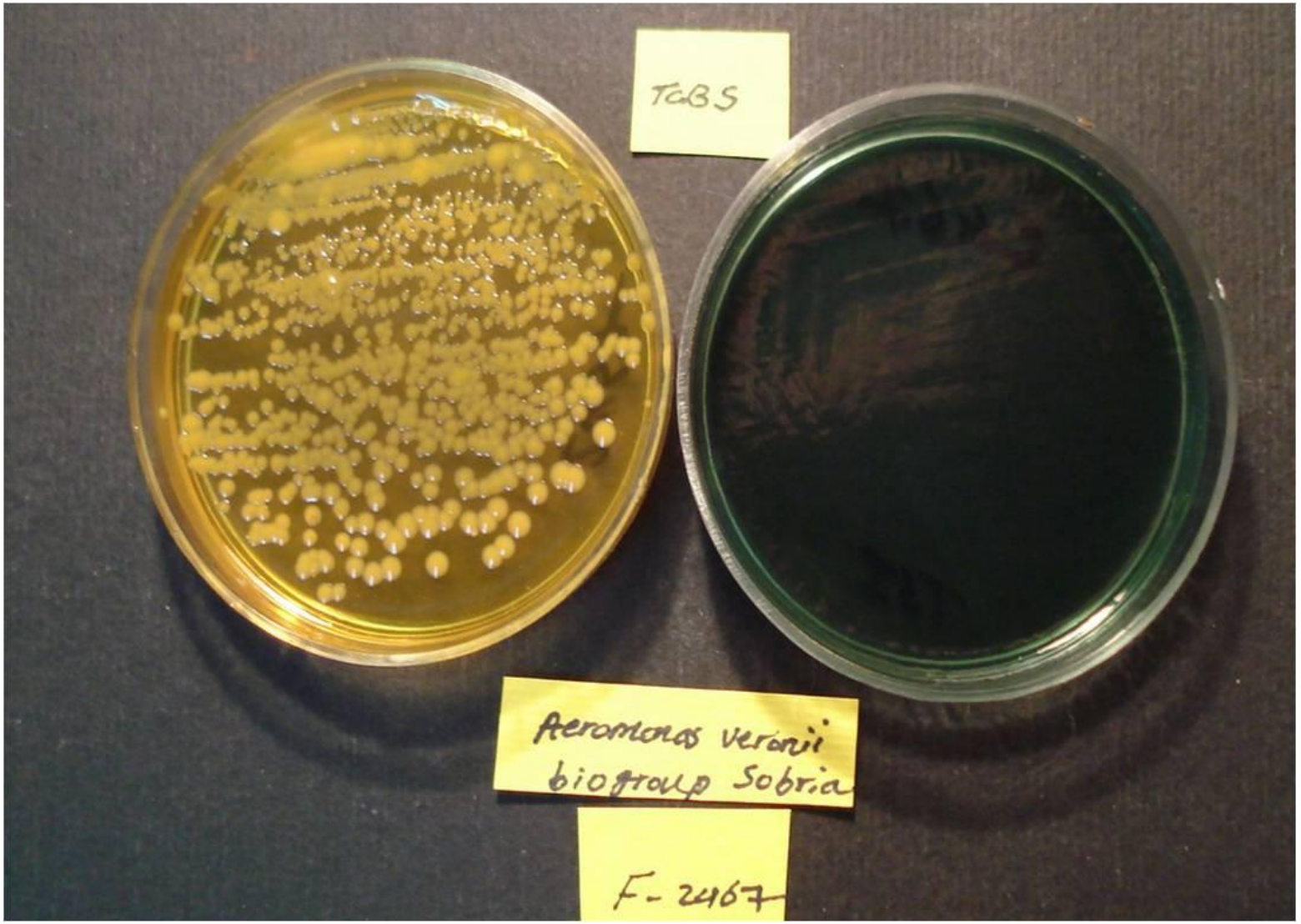
Aeromonas/ Laboratory Diagnosis

- Often an extremely strong odor is present

- Hemolysis is variable on SBA ,with most species displaying B-hemolysis.

Aeromonas/ Laboratory Diagnosis

Aeromonas/ Laboratory Identification



Aeromonas/ Laboratory Identification

- Oxidase positive
- Glucose-fermenting
- Gram-negative rods
- Growth on MacConkey
- Most are INDOLE +
- CATALASE / Dnase / Gelatinase : positive
- TCBS GROWTH - / +

Table 21-5 Differential Characteristics for Mesophilic Clinical *Aeromonas* Species

Characteristic	*Aeromonas hydrophila*	*Aeromonas veronii* biogroup sobria	*Aeromonas veronii* biogroup veronii	*Aeromonas caviae*	*Aeromonas schubertii*	*Aeromonas jandaei*	*Aeromonas trota*
Esculin hydrolysis	+	−	+	+	−	−	−
Voges-Proskauer	+	+	+	−	V+	−	
Pyrazinamidase activity	+	−	−	+	−	−	−
Arginine dihydrolase	+	+	−	V	+	+	+
Fermentation:							
Arabinose	V	−	−	+	−	−	−
Cellobiose	−	−	+	V	−	−	+
Mannitol	+	+	+	+	−	+	+
Sucrose	+	+	+	+	−	−	−
Susceptibility:							
Ampicillin	R	R	R	R	R	R	S
Carbenicillin	R	R	R	R	R	R	S
Cephalothin	R	S	S	R	S	R	R
Colistin*	V	S	S	S	S	R	S
Decarboxylase:							
Lysine	+	+	+	−	+	+	+
Ornithine	−	−	+	−	−	−	−
Indole	+	+	+	+	−	+	+
$H_2S^{\dagger}$	+	+	+	−	−	+	+
Glucose (gas)	+	+	+	−	−	+	+
Hemolysis (5% sheep erthyrocytes)	+	+	+	V	+	+	V

Plesiomonas shigelloides

Plesiomonas shigelloides

- Plesiomonas shigelloides is found in both soil and aquatic environments
- Like the genus Aeromonas, they are widely distributed among both warm- and **cold-blooded animals**
- Potential cause of enteric disease in humans

Plesiomonas shigelloides

- **Three major clinical types of gastroenteritis :**
- More common **watery** or secretory diarrhea
- **Subacute or chronic disease that lasts between 14 days and 2 to 3 months**
- Invasive, dysenteric form that resembles colitis

Plesiomonas shigelloides

- Probably underreported because of the similarity to *Escherichia coli* on most ordinary enteric media

- Recent phylogenetic studies have presented evidence that Plesiomonas is actually closely related to members of the family **Enterobacteriaceae**, particularly the genus **Proteus**

Plesiomonas shigelloides

- Oxidase-positive
- Glucose-fermenting
- Facultatively anaerobic
- Gram-negative rods tend to be pleomorphic gram-negative rods
- Motile

Plesiomonas shigelloides

- *Plesiomonas* and *Shigella* share antigenic features and *Plesiomonas* often cross-agglutinate with
-
- *Shigella sonnei*
- *S. dysenteriae*
- *S. boydii*
- Hence the species name *shigelloides*

P.shigelloides / Laboratory Identification

- *Plesiomanas* grows readily on most media routinely used in the clinical laboratory

- After 18 to 24 hours incubation at 35° C, shiny, opaque, **nonhemolytic** colonies appear, with a slightly raised center and a smooth and entire edge

P.shigelloides / Laboratory Identification

- Ability to ferment **Inositol** separates it from all Aerornanas and nearly all Vibria spp

- Its unique profile of **positive** Ornithine and Lysine decarboxylases and Arginine dihydrolase reactions, combined with the fermentation of inositol

Table 21-2 Salient Features for the Identification of *Vibrio*, *Aeromonas*, and *Plesiomonas*

	Vibrio	*Aeromonas*	*Plesiomonas*
Gram-stain reaction	–	–	–
Oxidase activity	+	+	+
Resistance to O/129*			
10 μg	+/–	+	+/–
150 μg	–	+	–
Growth in nutrient broth with:			
0% NaCl	–/+	+	+
6.5% NaCl	+	–	–
Acid from:			
Glucose	+	+	+
Inositol	–	–	+
Mannitol	+	+/–	–
Sucrose	+/–	+/–	–
Gelatin liquefaction	+	+	–

Shigella

Shigella

- Shigellosis is a global human health problem
- >90% occur in developing countries
- In developing countries 69% of episodes occur in children under five years of age

Shigella

- Humans are the only known reservoir of Shigella organisms
- No animal reservoir has been identified
- Shigella may be isolated **1 to 3** days after the infection develops

Shigella

- ***S. sonnei*** is the predominant isolate **(77%),** followed by ***S. flexneri***

- ***S. sonnei*** is more resistant and survive better in environment (e.g**. 5 days** in feces dried on cloth in cool, damp & dark condition)

- Shigella dysenteriae type 1 produces severe disease

Shigella

- Even the **best technique** with fresh specimens may miss fragile organisms such as shigella

- Fecal cultures failed to yield shigella in **40%** of volunteers with inflammatory diarrhea from experimental shigella infection

- Positive cultures are most often obtained from **blood-tinged plugs of mucus** in freshly passed stool specimens obtained during the acute phase of disease

Shigella

- Gram-negative bacilli
- Nonmotile
- Gas from glucose : Negative
- Urease : Negative
- H_2S : Negative
- Lysine decarboxylase / LDC : Negative
- Oxidase negative
- IMViC = - + - - or (+ + - -)

Shigella

Table 4-2. Reactions of *Shigella* in screening biochemicals

Screening medium	*Shigella* reaction
KIA	K/A, no gas produced (red slant/yellow butt)[a]
TSI	K/A, no gas produced (red slant/yellow butt)[a]
H_2S (on KIA or TSI)	Negative
Motility	Negative
Urea	Negative
Indole	Positive or negative
LIA	K/A (purple slant/yellow butt)[b]

[a] K = alkaline (red); A = acid (yellow); some strains of *S. flexneri* serotype 6 and *S. boydii* produce gas from glucose.

[b] K = alkaline (purple); A = acid (yellow); an alkaline reaction (purple) in the butt of the medium indicates that lysine was decarboxylated. An acid reaction (yellow) in the butt of the medium indicates that lysine was not decarboxylated.

Shigella / Antigenic Structures

- The genus consists of four species
- Shigella spp. are also divided into four major O antigen groups , Serogroup (A,B,C and D)
- These species are subdivided into Serotypes on the basis of O-specific polysaccharide of the LPS
- Several serotypes exist within each species with the exception of S. sonnei, which has only one serotype

Shigella / Antigenic Structures

Subgroups, Serotypes, and Subtypes of Shigella

Subgroup	Serotypes and Subtypes
Group A: *Shigella dysenteriae*	15 serotypes (type 1 produces Shiga toxin)
Group B: *Shigella flexneri*	8 serotypes and 9 subtypes
Group C: *Shigella boydii*	19 serotypes
Group D: *Shigella sonnei*	1 serotype

Shigella sonnei

- IMViC = - + - -
- LDC= -
- Ornithine decarboxylase / ODC : Positive
- ONPG : Positive

Shigella

Differentiation of Species Within the Genus *Shigella*

BIOCHEMICAL TEST	*S. DYSENTERIAE*	*S. FLEXNERI*	*S. BOYDII*	*S. SONNEI*
Serogroup	A	B	C	D
ONPG	−	−	−	+
Ornithine decarboxylase	−	−	−	+
Fermentation of:				
Lactose	−	−	−	−
Mannitol	−	+	+	+
Raffinose	−	D	−	−
Sucrose	−	−	−	−
Xylose	−	−	D	−
Indole production	D	D	D	−

+, 90% or more strains positive; −, 90% or more strains negative; D, different strains positive/negative.

Shigella

- Shigella dysenteriae type 1 : Catalase – Negative
- Touch the center of well-isolated young colony (18-24 hrs.) on SBA or MacConkey with a wooden stick to transfer to a clean ,dry glass slide.
- Dose not test from Muller-Hinton Agar (MHA)
- Place 1 drop of 3% H_2O_2 and observe immediately bubbles

Salmonella

Salmonella / New Classification

- Salmonella enterica DNA group1,2,3,4,6
- Salmonella bongori DNA group 5
- Salmonella enterica subspecies enterica (DNA group1)****** > 90 % of human infections
- e.g;Salmonella enterica subspecies enterica serotype Typhi
- Salmonella serotype Typhi
- Salmonella Typhi
- More than 2400 of Salmonella serotype

TABLE 6 Biochemical reactions useful for differentiating *Salmonella* species and subspecies[a]

Test	Species or subspecies (no. of strains tested)						
	S. enterica						*S. bongori* (formerly V) (16)
	I (650)	II (146)	IIIa (120)	IIIb (155)	IV (120)	VI (9)	
Dulcitol	+	+	−	−	−	d[b]	+
Lactose	−	−	−[c]	+[d]	−	d[e]	−
ONPG	−	−[f]	+	+	−	d[g]	+
Salicin	−	−	−	−	+[h]	−	−
Sorbitol	+	+	+	+	+	−	+
Galacturonate	−	+	−	+	+	+	+
Malonate	−	+	+	+	−	−	−
Mucate	+	+	+	−[i]	−	+	+
Growth in KCN	−	−	−	−	+	−	+
Gelatin (strip)	−	+	+	+	+	+	−
L(+)-Tartrate (*d*-tartrate[j])	+	−	−	−	−	−	−

[a] Reactions after incubation at 37°C. +, 90% or more positive within 1 or 2 days; (+), positive reaction after 3 or more days; −, no reaction (90% or more) in 7 days; d, different reactions [+, (+), −]. Adapted from reference 42.

[b] A total of 67% were positive.

[c] A total of 15% were positive.

[d] A total of 85% were positive.

[e] A total of 22% were positive.

[f] A total of 15% were positive.

[g] A total of 44% were positive.

[h] A total of 60% were positive.

[i] A total of 30% were positive.

[j] Sodium potassium tartrate (42).

Salmonella

- Gram-negative bacilli
- Motile except Gallinarum & Pullorum
- Gas from glucose : Positive except Typhi
- Urease : Negative
- Indole : Negative
- H_2S : Positive except Paratyphi A
- Lysine decarboxylase / LDC : Positive except Paratyphi A
- Oxidase negative

Table 6-12 Differential Characteristics of Salmonella Species and Subspecies [modified from Ewing (1986)][157]

SPECIES	*S. ENTERICA*						*S. BONGORI*
SUBSPECIES	I *enterica*	II *salamae*	IIIA *arizonae*	IIIB *diarizonae*	IV *houtenae*	VI *indica*	
Biochemical test							
Dulcitol	+	+	−	−	−	d	+
ONPG (2 hr)	−	−	+	+	−	d	+
Malonate	−	+	+	+	−	−	−
Gelatinase	−	+	+	+	+	+	−
Sorbitol	+	+	+	+	−/+	−	+
KCN	−	−	−	−	+	−	+
D-tartrate	+	−	−	−	−	−	−
Galacturonate	−	+	−	+	+	+	+
β-glucuronidase (MUG)	D	D	−	+	−	D	−
Mucate	+	+	+	− (70%)	−	+	+
Salicin	−	−	−	−	+	−	−
Lactose	−	−	− (75%)	+ (75%)	−	D	−

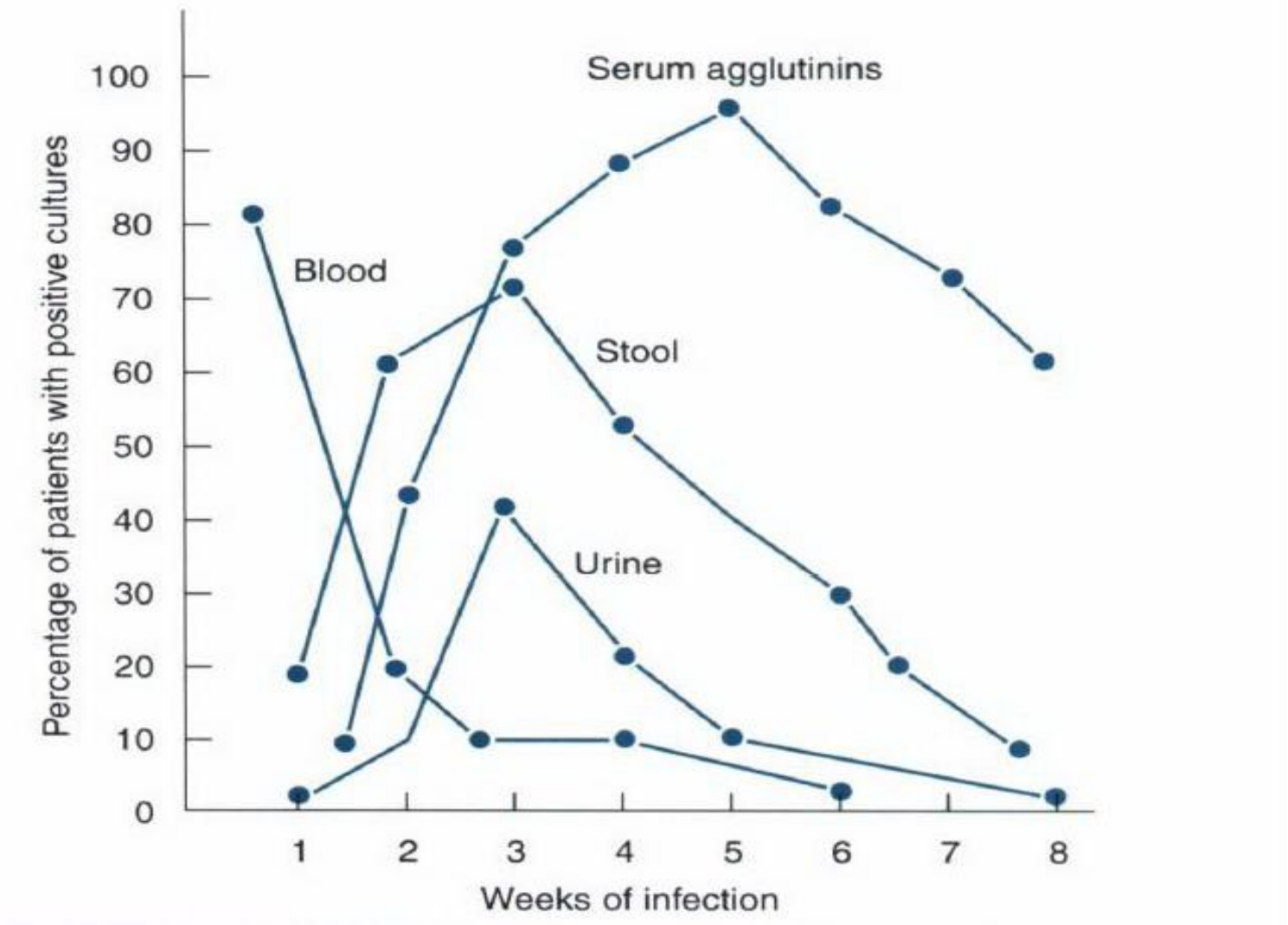
+, 90% or more strains positive; −, 90% or more strains negative; D, different reactions by different serovars.

Typhoid and paratyphoid salmonella

- Organism reservoir is infected humans

- Systemic toxic effects and fever, abdominal symptoms (pain, diarrhea, constipation)

Typhoid and paratyphoid salmonella



Nontyphoid salmonella

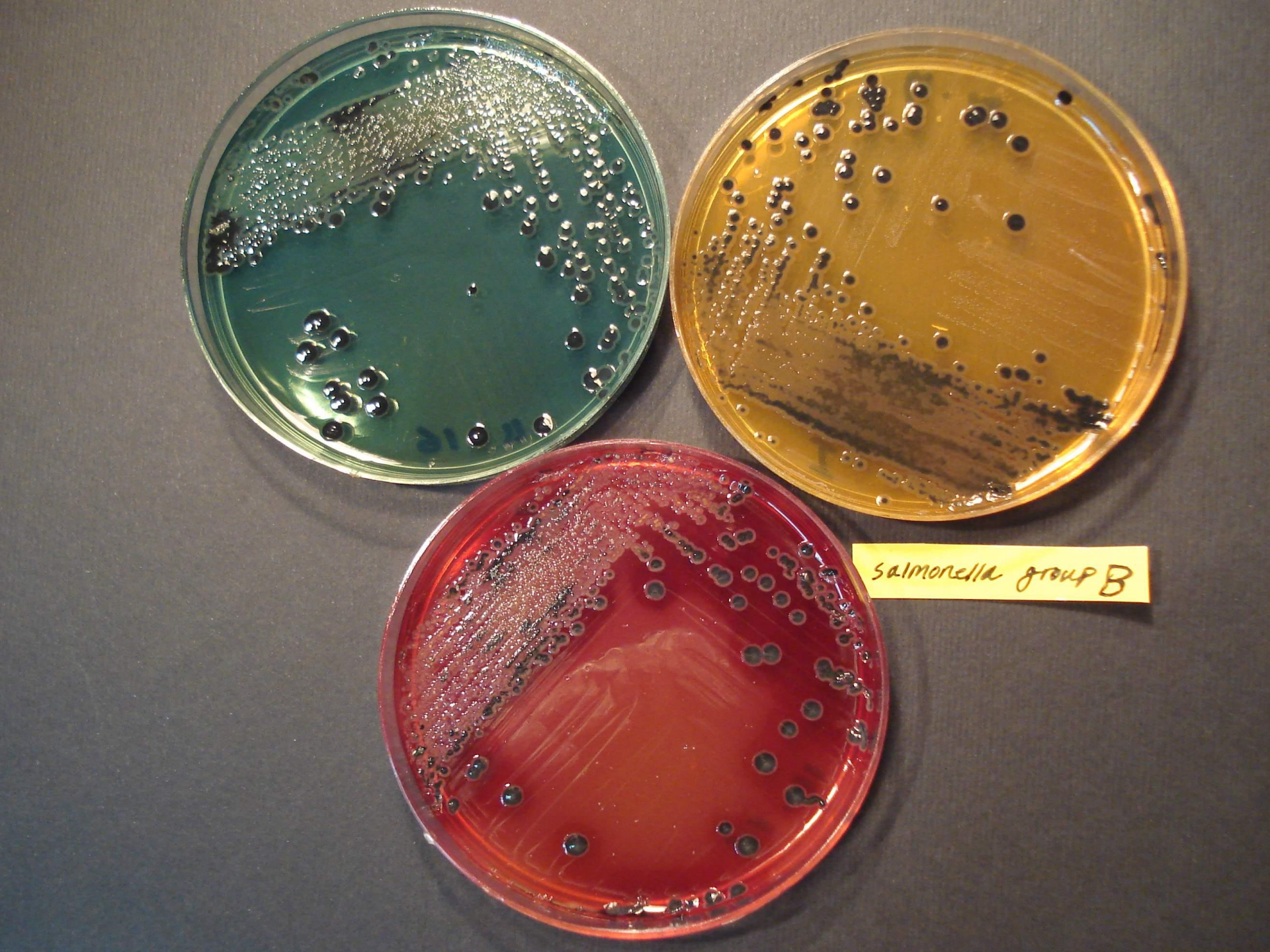
- An acute gastroenteritis or food poisoning characerized by vomiting and watery diarrhea often with fever, occasionally with dysenteric characteristics

- 95% of cases are a result of foodborne transmission (from poultry or hens' eggs);

- Commonly seen in infants

Nontyphoid salmonella

- Many Salmonella serotypes are usually found in cold-blooded animals (e.g. turtles, snakes) as well as in rodents and birds


salmonella group B

Salmonella

- **Salmonella serotype Typhi**
- **IMViC = - + - - , LDC = + , ODC= -**

- **Nontyphoidal Salmonella group I**
- **IMViC = - + - + , LDC=+ , ODC = +**

- **Salmonella serotype Paratyphi A**
- **IMViC = - + - - , LDC= - , ODC = +**

SALMONELLA serovar NEWPORT

(Representative of most non-typhoidal serovars of *S. enterica*)

A B C D E F

A) TSI: Alkaline slant / Acid Butt / H_2S Positive / Gas (K / A_g^{+++})

B) Urea: Negative

C) LIA: Lysine Decarboxylase Positive

D) Citrate: Positive

E) MIO: Motile / Ornithine Positive

F) MIO w/ indol reagent: Indol negative

SALMONELLA serovar TYPHI

A B C D E F

A) TSI: Alkaline slant / Acid Butt / Trace H_2S / No Gas (K / A^{TR})
B) Urea: Negative
C) LIA: Lysine Decarboxylase Positive
D) Citrate: Negative
E) MIO: Motile / Ornithine Negative
F) MIO w/ indol reagent: Indol negative

Salmonella serovar Paratyphi A

A B C D E F

A) TSI: Alkaline slant / Acid Butt / No H_2S / Gas (K / A_g)
B) Urea: Negative
C) LIA: Lysine Decarboxylase Negative
D) Citrate: Negative
E) MIO: Motile / Ornithine Positive
F) MIO w/ indol reagent: Indol negative

Table 3.8.1–5 Biochemical differentiation of selected members of the *Salmonella* group[a]

Test	Serogroup Choleraesuis	Serogroup Paratyphi A	Serogroup Typhi	Other
Salmonella group	C	A	D	A–E
Arabinose fermentation	−	+	−	+
Citrate utilization	V	−	−	+
Glucose gas production	+	+	−	+
Lysine decarboxylase	+	−	+	+
Ornithine decarboxylase	+	+	−	+
Rhamnose fermentation	+	+	−	+
Trehalose fermentation	−	+	+	+

[a] Symbols: −, ≤9% of strains positive; V, 10 to 89% of strains positive; +, ≥90% of strains positive.

Table 21.2 Antigenic formulae of some representative serotypes of Salmonella (Kauffmann–White classification).

Serogroup	O-antigen group[a]	Serotype name[b]	O antigens[c] and Vi	H antigens	
				Phase 1	Phase 2
2	A	Paratyphi A	1,2,12	a	[1,5]
4	B	Paratyphi B	1,4,[5],12	b	1,2
		Stanley	1,4,[5],12,27	d	1,2
		Schwarzengrund	1,4,12,27	d	1,7
		Saintpaul	1,4,[5],12	e,h	1,2
		Derby	1,4,[5],12	f,g	[1,2]
		Agona	1,4,12	f,g,s	–
		Typhimurium	1,4,[5],12	i	1,2
		Bredeney	1,4,12,27	l,v	1,7
		Brandenburg	1,4,12	l,v	e,n,z15
		Heidelberg	1,4,[5],12	r	1,2
7	C1	Choleraesuis	6,7	c	1,5
		Paratyphi C	6,7[Vi]	c	1,5
		Typhisuis	6,7	c	1,5
		Montevideo	6,7,14	g,m,[p],s	[1,2,7]
		Thompson	6,7,14	k	1,5
		Virchow	6,7	r	1,2
		Infantis	6,7,14	r	1,5
		Mbandaka	6,7,14	z10	e,n,z15
8	C2–C3	Muenchen	6,8	d	1,2
		Newport	6,8,20	e,h	1,2
		Hadar	6,8	z10	e,n,x
		Miami	1,9,12	a	1,5
		Sendai	1,9,12	a	1,5
9	D1	Typhi	9,12[Vi]	d	–
		Enteritidis	1,9,12	g,m	[1,7]
		Dublin	1,9,12,[Vi]	g,p	–
		Panama	1,9,12	l,v	1,5
		Gallinarum	1,9,12	–	–
3,10	E1	Anatum	3,10,[15],[15,34]	e,h	1,6
		Weltevreden	3,10,[15]	r	z6
1,3,19	E4	Senftenberg	1,3,19	g,[s],t	–
11	F	Rubislaw	11	r	e,n,x
13	G	Kedougou	1,13,23	i	l,w

[a] Former letter designation.
[b] Serotypes in subsp. I are named; those in subsp. II–VI are presented by antigenic formulae and unnamed (see Old 1992).
[c] Somatic factors associated with phage conversion are underlined.
Antigens in brackets [x] are not always present.

Campylobacter

Campylobacter

- Fecal samples from chicken
- 83% of the samples yielded more than 10^6 colony-forming units Campylobacter, per gram of feces
- Usually transmitted via contaminated food (chicken), milk, or water

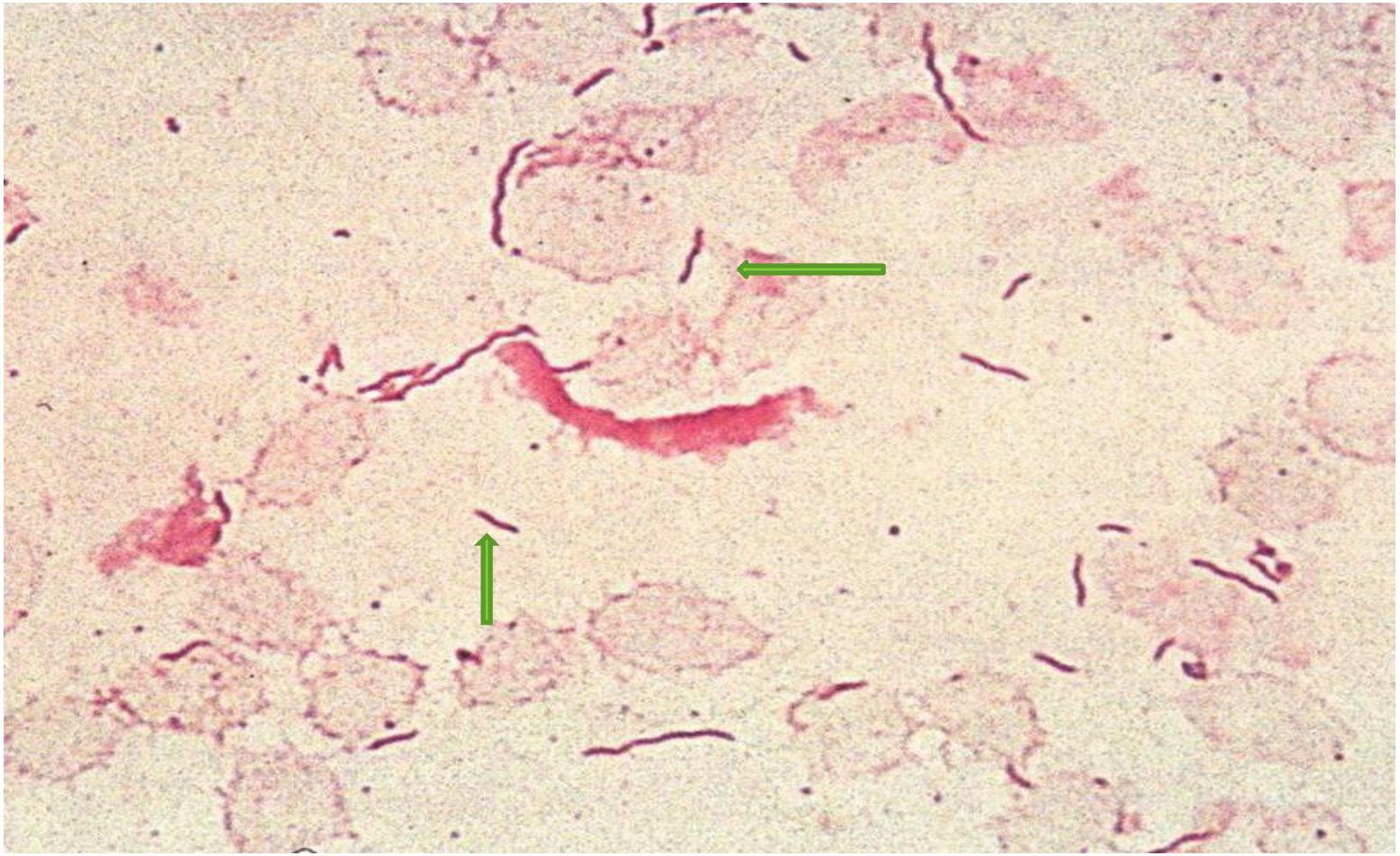
Campylobacter

- Campylobacter jejuni (90)% and C. coli are most often associated with infections in humans
- Motile / Darting Motility across the field in a zigzag fashion in fresh stool (<30 minute) & from colony in broth (e.g..TSB)
- Emulsify a loopful of 24 to 48-h bacterial growth in broth ,not saline or distilled water

Campylobacter

- Gram-negative bacilli, faintly staining (Safranin)
- Gram stain with carbol fuchsin or 0.1% basic fuchsin
- Curved, seagull-winged
- Acute phase of diarrhea ; Sensitivity :66 to 94%
  - Report: Campylobacter-like organism
- Coccoidal forms may be seen in the Gram stain, especially in older cultures

Campylobacter



Campylobacter

- For optimum recovery, the inoculation of two selective agars is recommended
- The use of more than one type of selective medium increases the yield from stools by as much as 15%
- Two sets of selective plates should be incubated, one at 42° C / (40 ° C) for 72 h and one at 37° C for 4-5 days

Selective Media and Incubation Conditions to Recover *Campylobacter* and *Arcobacter* spp. from Stool Specimens

Organism	Primary Plating Media	Incubation Conditions
C. jejuni *C. coli*	Modified Skirrow's media: Columbia blood agar base, 7% horse-lysed blood, and antibiotics (vancomycin, trimethoprim, and polymyxin B) Campy-BAP: *Brucella* agar base with antibiotics (trimethoprim, polymyxin B, cephalothin, vancomycin, and amphotericin B) and 10% sheep blood Blood-free, charcoal-based selective medium: Columbia base with charcoal, hemin, sodium pyruvate, and antibiotics (vancomycin, cefoperazone, and cyclohexamide) Modified charcoal cefoperazone deoxycholate agar (CCDA) Semisolid motility agar: Mueller-Hinton broth II, agar, cefoperazone, and trimethoprim lactate Campy-CVA: *Brucella* agar base with antibiotics (cefoperazone, vancomycin, and amphotericin B) and 5% sheep blood	42° C under microaerophilic conditions* for 72 hr
C. fetus subsp. *fetus*† *C. jejuni* subsp. *doylei* *C. upsaliensis* *C. lari* *C. hyointestinalis*	Modified Skirrow's media Blood-free charcoal-based selective media Campy-CVA CCDA Semisolid motility agar	37° C under microaerophilic conditions for at least 72 hr up to 7 days‡
A. cryaerophilus, A. butzleri	Campy-CVA	37° C under microaerophilic conditions§ for 72 hr

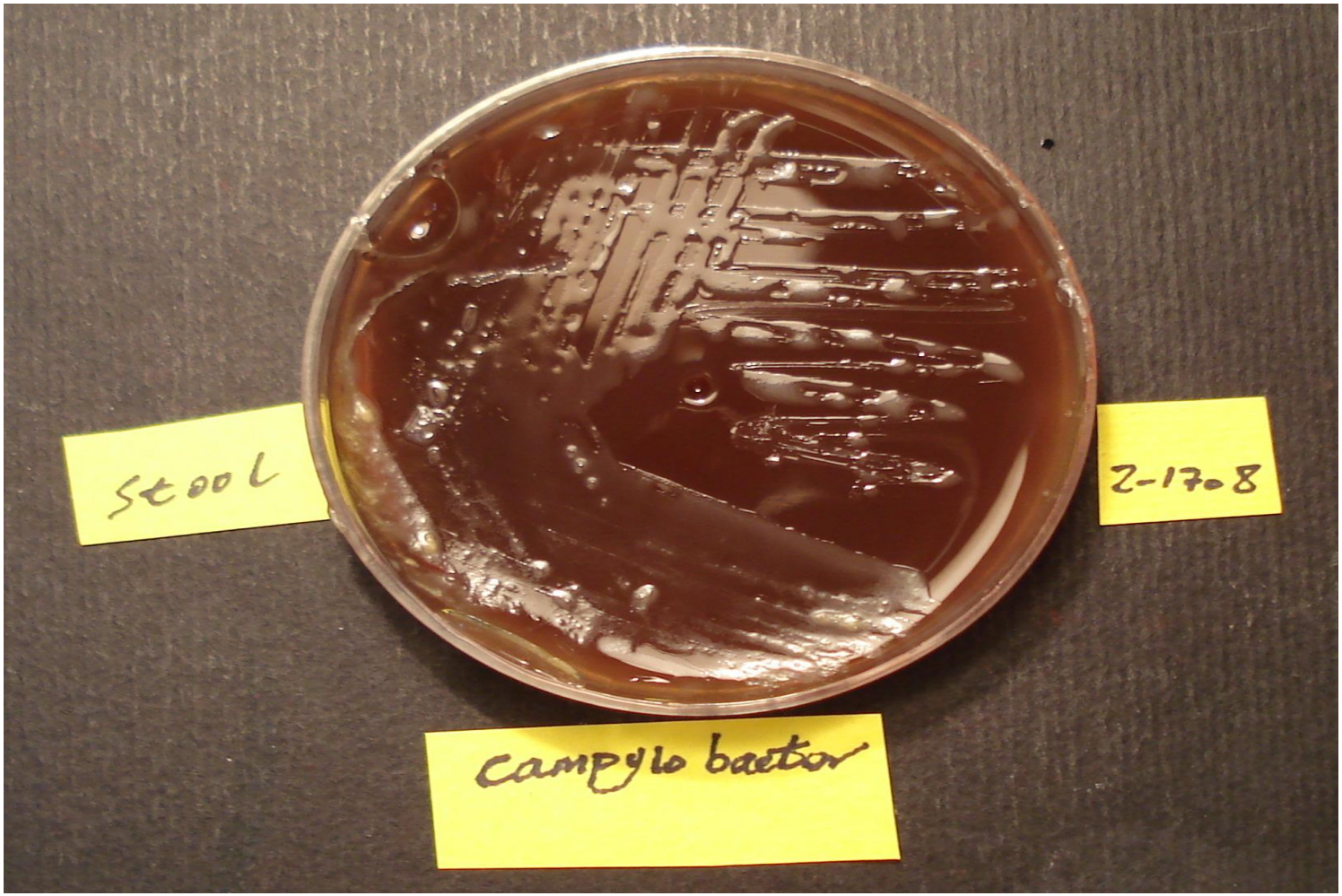
*Atmosphere can be generated in several ways, including commercially produced, gas-generating envelopes to be used with plastic bags or jars. Evacuation and replacement in plastic bags or anaerobic jars with an atmosphere of 10% CO_2, 5% O_2, and the balance of nitrogen (N_2) is the most cost-effective method, although it is labor intensive.

†All these organisms are susceptible to cephalothin.

‡*C. upsaliensis* will grow at 42° C but not on cephalothin-containing selective agar.

§*A. cryaerophilus* does not require microaerophilic conditions.

Campylobacter



Presumptive *Campylobacter*

- Gram Stain
- Growth at 42 C
- Darting Motility
- Oxidase & Catalase positive colonies

- Campylobacter jejuni
- Hippurate hydrolysis : positive

Table 3.8.2–4 Phenotypic reactions of clinically important *Campylobacter* and *Helicobacter* species[a]

Identification test	*C. jejuni*	*C. jejuni* subsp. *doylei*	*C. coli*/ *C. jejuni*, hippurate negative	*C. lari*[b]	*C. fetus* subsp. *fetus*	*C. upsaliensis*	*A. cryaerophilus*/ *A. butzleri*[c]	*C. hyointestinalis*	*H. cinaedi*/ *CLO1B*[d]	*H. fennelliae*[d]	*H. pull*
Oxidase	+	+	+	+	+	+	+	+	+	+	
Catalase	+	V	+	+	+	0/W	V	+	+	+	
Aerobic growth, 35–37°C	0	0	0	0	V[e]	0	+	0	0	0	
Microaerobic growth, 25°C	0	0	0	0	V	0	+	V	0	0	
Microaerobic growth, 35–37°C	+	+	+	+	+	+	+	+	+	+	
Microaerobic growth, 42°C	+	V	+	+	V	+	V	+	V	V	
Hippurate hydrolysis	+	+	0	0	0	0	0	0	0	0	
Indoxyl acetate	+	+	+	0	0	+	+	0	0	+	
NA resistant	0[f]	0	0[f]	+	+	0[f]	V	+	0	0	
CF resistant	+	V	+	+	V	0	V	0	0	V	
Nitrate reduction	+	0	+	+	+	+	V	+	+	0	
H_2S in TSI agar	0	0	V[g]	V	0	0	0	+	0	0	
Urea hydrolysis[h]	0	0	0	0	0	0	0	0	0	0	

[a] +, positive reaction; 0, negative reaction; w, weakly positive; V, variable reaction, NA, not available. See procedure 3.8.4 for *H. pylori* identification.

[b] Urease-positive thermophilic campylobacters or *C. lari*-like strains may be found (11).

[c] Growth at 42°C; catalase negativity suggests *A. butzleri.*

[d] *H. cinaedi*/*CLO1B* can be separated by DNA homology tests. *H. cinaedi*/*CLO1B, H. fennelliae,* and *H. pylori* can be definitively identified by cellular fatty acid analysis (9).

[e] Rare *C. fetus* subsp. *fetus* strains are aerobic.

[f] These species are historically sensitive to NA; however, resistant strains are seen in as high as 35% of isolates due to acquired fluoroquinolone resistance, which may make this assay less use identification.

[g] H_2S in TSI suggests *C. coli.*

[h] There are isolated reports of other *Helicobacter* species that are urease producing other than *H. pylori* (24).