

به نام خدا

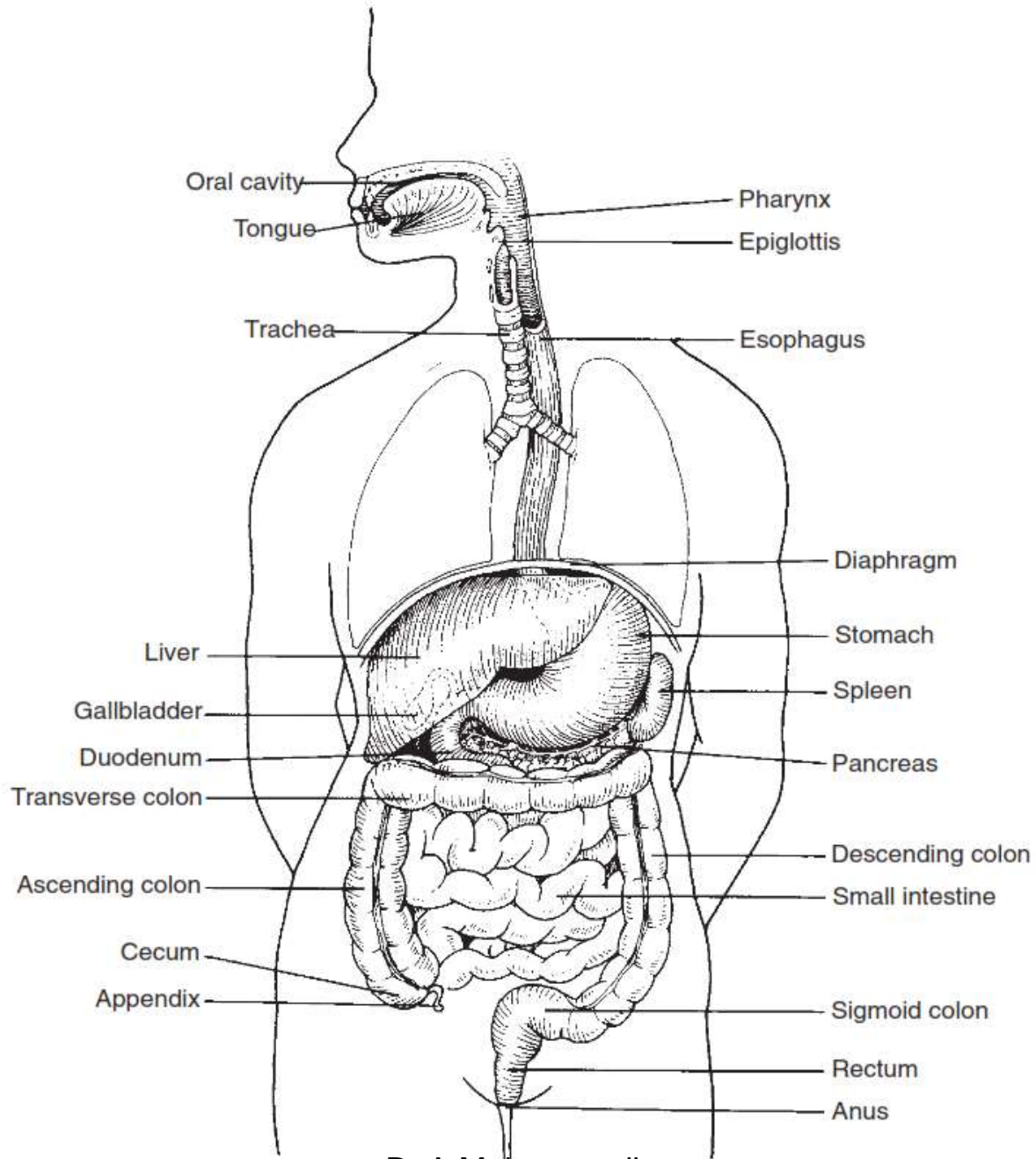


Bacteriology Lab 2

By: **Dr. A. Mohammadi**

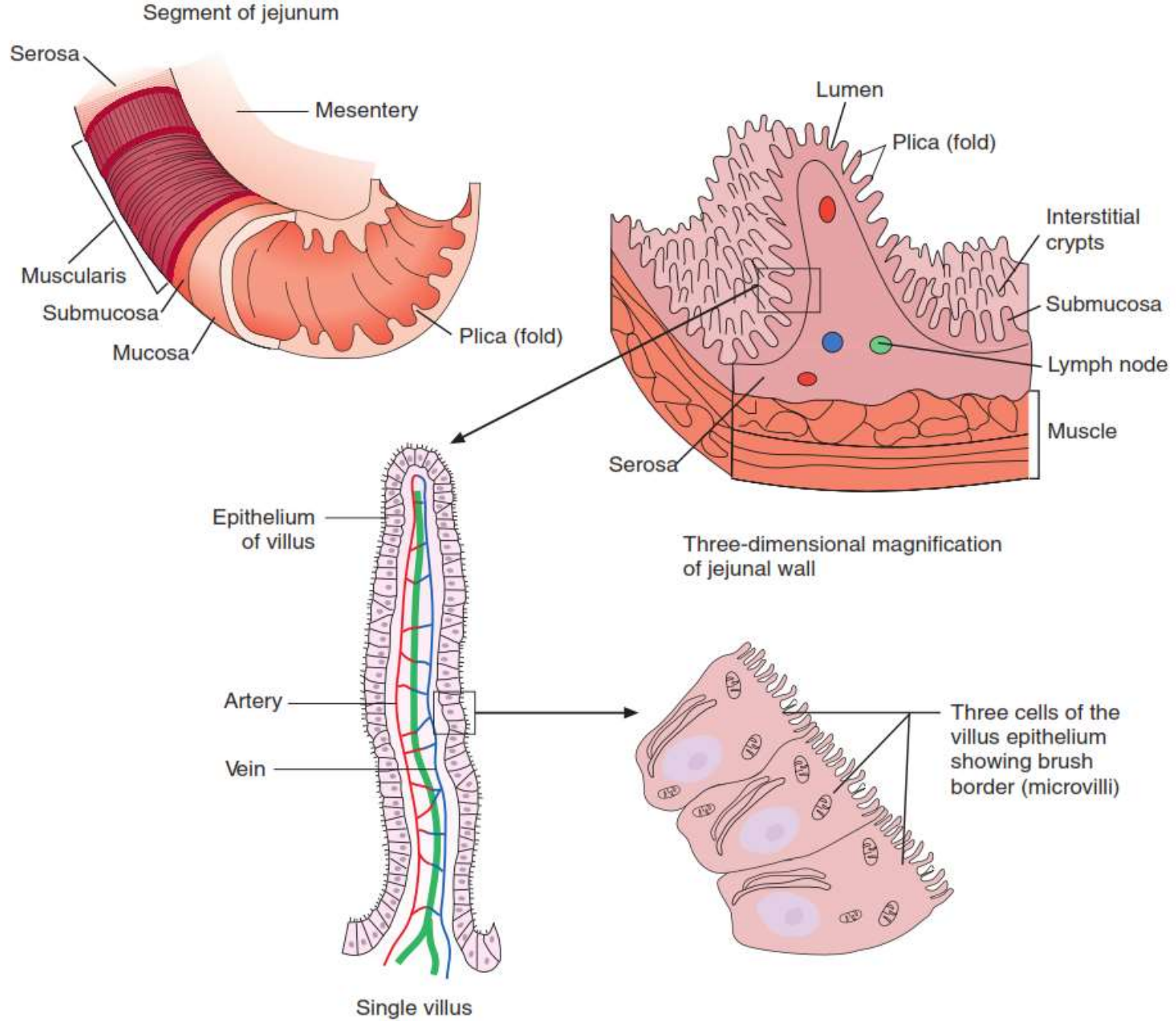
Department of Biology,
Faculty of science,
University of Alzahra

میکروارگانیزم های دستگاه گوارش



Dr A.Mohammadi

• **Figure 74-1** General anatomy of the gastrointestinal tract. (From Broadwell DC, Jackson BS: *Principles of ostomy care*, 1982, St Louis, Mosby.)



• **Figure 74-2** Wall of the small intestine. Villi cover the folds of the mucosal layer; in turn, each villus is covered with epithelial cells.

TABLE 34-1 Microbiota Found in the Large Intestine

Bacterial Species*	Incidence (%)
Strict Anaerobes	
Gram-Negative	
<i>Bacteroides fragilis</i>	100
<i>Bacteroides</i> spp.	100
<i>Fusobacterium</i> spp.	100
Gram-Positive	
Lactobacilli	20-60
<i>Clostridium perfringens</i>	25-35
<i>Clostridium</i> spp.	1-35
<i>Peptostreptococcus</i> spp.	Common
<i>Peptococcus</i> spp.	Common
Facultative Anaerobes	
Gram-Positive Cocci	
<i>Staphylococcus aureus</i>	30-50
<i>Enterococcus</i> spp.	100
β -Hemolytic streptococci, groups B, C, F, and G	0-16
Gram-Negative Bacilli (Enterobacteriaceae)	
<i>Escherichia coli</i>	100
<i>Klebsiella</i> spp.	40-80
<i>Enterobacter</i> spp.	5-55
<i>Proteus</i> spp.	3-11
<i>Salmonella enteritidis</i> (1400 serotypes)	3-7
<i>Shigella</i> , groups A-D	0-1
<i>Pseudomonas aeruginosa</i>	3-11
<i>Candida albicans</i>	15-30

Modified from Sommers HM: The indigenous microbiota of the human host. In Youmans GP, Paterson PY, Sommers HM, editors: *The biologic and clinical basis of infectious diseases*, ed 2, Philadelphia, 1980, WB Saunders, p. 83.

*Strict anaerobes are present in ratio of 1000:1 with facultative aerobes.

**TABLE
74-1**

**Examples of Microorganisms That Cause
Gastrointestinal Infection for Each Primary
Pathogenic Mechanism**

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

Dr A.Mohammadi

TABLE 34-2 Common Pathogens Involved in Diarrhea

Pathogen	Fever	Nausea, Vomiting	Bloody Stool	Fecal Inflammation
<i>Campylobacter</i> spp.	Common	Occurs	Occurs	Common
<i>Salmonella</i> spp.	Common	Occurs	Occurs	Common
<i>Shigella</i> spp.	Common	Common	Occurs	Common
Enterohemorrhagic <i>Escherichia coli</i>	Atypical	Occurs	Common	Often not found
<i>Clostridium difficile</i>	Occurs	Not characteristic	Occurs	Common
<i>Yersinia enterocolitica</i>	Common	Occurs	Occurs	Occurs
<i>Entamoeba histolytica</i>	Occurs	Variable	Variable	Variable
<i>Cryptosporidium</i> spp.	Variable	Occurs	Not characteristic	None to mild
Cyclospora	Variable	Occurs	Not characteristic	Not characteristic
<i>Giardia lamblia</i>	Not characteristic	Occurs	Not characteristic	Not characteristic
Viruses	Variable	Common	Not characteristic	Not characteristic

Modified from Thielman NM, Guerrant RL: Clinical practice. Acute infectious diarrhea, *N Engl J Med* 350:38, 2004.

TABLE 34-3 Common Food Vehicles for Specific Pathogens or Toxins

Vehicle	Pathogen or Toxin
Undercooked chicken	<i>Salmonella</i> spp., <i>Campylobacter</i> spp.
Eggs	<i>Salmonella</i> spp. (especially <i>S. enteritidis</i>)
Unpasteurized milk	<i>Salmonella</i> , <i>Campylobacter</i> spp., <i>Yersinia</i> spp.
Water	<i>Giardia lamblia</i> , noroviruses, <i>Campylobacter</i> spp., <i>Cryptosporidium</i> spp., <i>Cyclospora</i>
Fried rice	<i>Bacillus cereus</i>
Fish	
Shellfish	<i>Vibrio cholerae</i> , <i>V. parahaemolyticus</i> , <i>V. vulnificus</i> , other <i>Vibrio</i> spp., neurotoxic shellfish poisoning, paralytic shellfish poisoning, Norwalk virus
Tuna, mackerel, mahi-mahi	Scombroid poisoning
Grouper, amberjack, snapper	Ciguatera
Sushi	<i>Anisakis</i> spp.
Beef, gravy	<i>Salmonella</i> spp., <i>Campylobacter</i> spp., <i>Clostridium perfringens</i>

Modified from Goodman LJ: Diagnosis, management, and prevention of diarrheal diseases, *Curr Opin Infect Dis* 6:88, 1993.

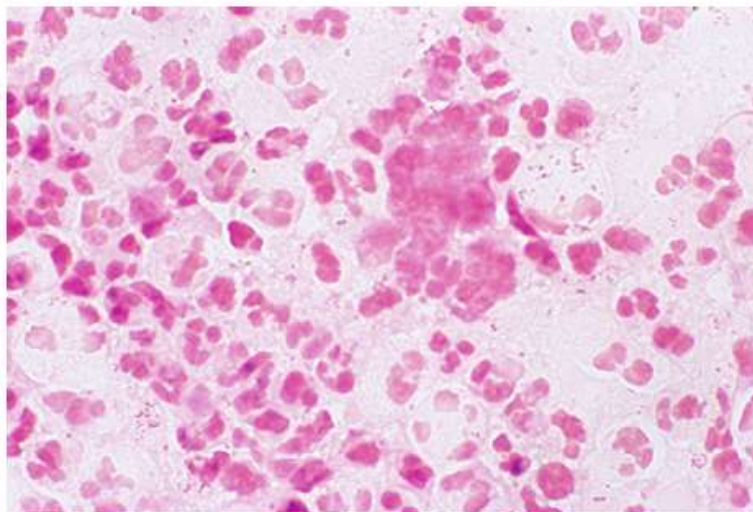


FIGURE 34-2 Gram stain of a direct fecal smear to show the presence of white blood cells, indicative of an invasive process and not an enterotoxin.

**TABLE
74-2**

Overview of the Primary Groups of *Escherichia coli* That Cause Diarrhea in Humans

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 antiaggregative 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 <i>Shigella</i> spp.	Very difficult to distinguish from <i>Shigella</i> spp. and other <i>E. coli</i> 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 (STEC)	Attaches to and effaces gut epithelial cells in a similar manner as EPEC; in addition, STEC elaborates shiga toxins.	Although many outbreaks are caused by <i>E. coli</i> O157:H7, other serotypes have been implicated in outbreaks and sporadic cases. Gene recombination among strains makes classification difficult.
	Produce one or more shiga toxins referred to as verocytotoxins. Attaches to and effaces gut epithelial cells in a similar manner as EPEC.	O157 STEC serotypes; contains most common serotypes O157:H7 and non-motile O157:NM. There are more than 150 non-O157 serotypes that have been isolated from patients with diarrhea or hemolytic-uremic syndrome.

Dr A.Mohammadi

TABLE A-8. Important Groups of *Escherichia Coli*

Organism	Major Clinical Disease
Enterotoxigenic ETEC	Watery diarrhea
Enterohemorrhagic EHEC (O157:H7 and non-O157:H7 strains)	Hemorrhagic colitis Hemolytic uremic syndrome
Enteroinvasive EIEC	Begins with diarrhea, which then develops into dysentery
Enteropathogenic EPEC	Infant watery diarrhea
Enteraggregative EAEC	Infant watery diarrhea

TABLE 34-4 Diarrheagenic *Escherichia coli*

Group	Major Virulence Factors	Reported Food Sources
ETEC	Adhesins LT and ST toxins	Fresh fruits and vegetables, scallops, tuna paste, soft cheeses
EIEC	Invasion proteins	Cheese, guacamole
EHEC	Intimin (adherence to intestinal mucosa) Shiga toxins	Undercooked beef, sausage, chicken, lunch meats, deer jerky, lettuce, radishes, alfalfa sprouts, potatoes, milk, apple juice, cider, cheese curds
EPEC	Intimin (adherence to intestinal mucosa) Bundle-forming pili Surface-associated filaments Translocated intimin receptor	Fresh fruits and vegetables, likely infant formula
EAEC	Aggregative adherence fimbriae Dispersin Plasmid-encoded toxin	Likely foodborne, possibly fruits and vegetables, other food sources uncertain

EAEC, Enteraggregative *E. coli*; EHEC, enterohemorrhagic *E. coli*; EIEC, enteroinvasive *E. coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; ST, heat-stable; LT, heat-labile.

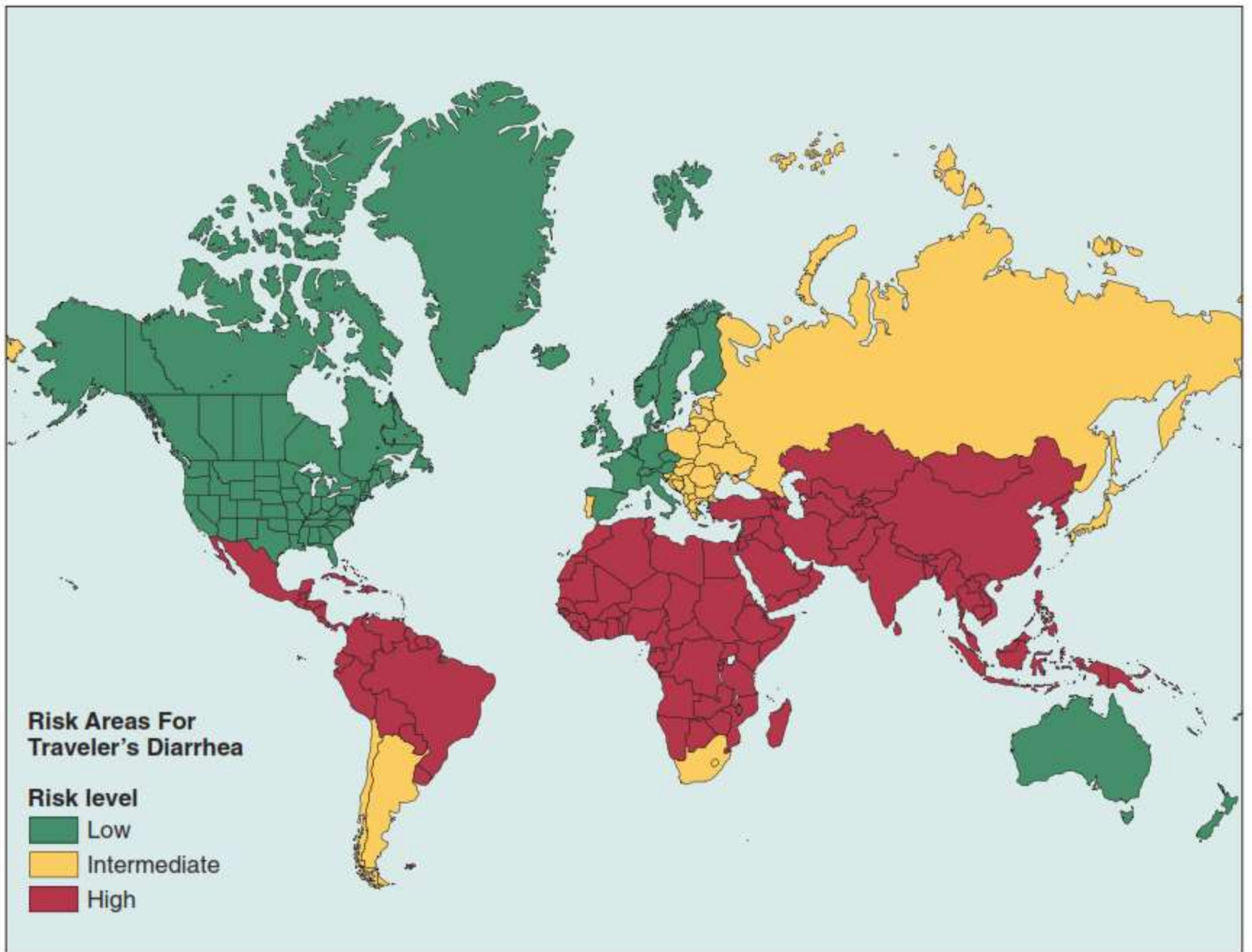
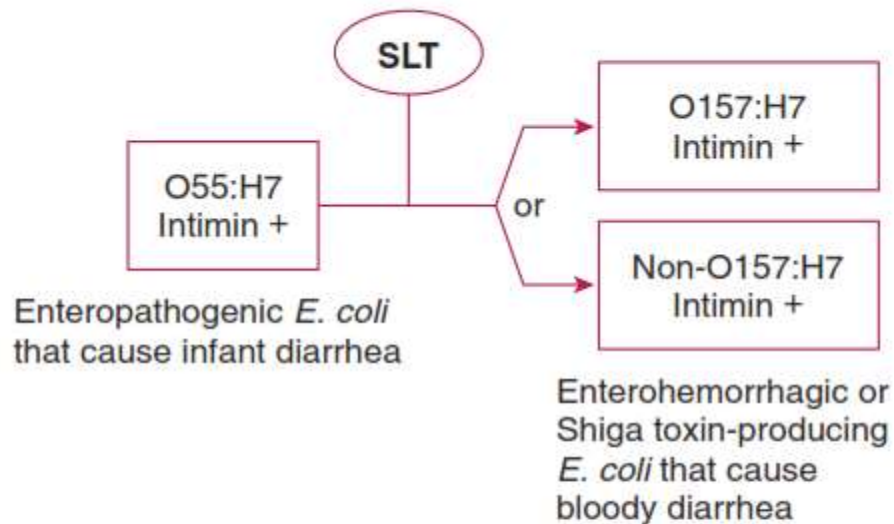


FIGURE 34-6 Geographic distribution of risk for traveler's diarrhea.

Origins of STEC



- **Figure 74-4** It appears that the presence of enterohemorrhagic *Escherichia coli* (STEC) strain O157:H7 was not simply overlooked before 1982. *E. coli* O157:H7 strains are closely related to a Shiga toxin-negative enteropathogenic *E. coli* (EPEC) strain O55:H7. It is proposed that this EPEC strain O55:H7 became infected by a bacteriophage that encoded Shiga toxin (SLT); it is now recognized that at least 25 different virulence-associated genes may be associated with STEC and are classified into five “seropathotypes” A through E.

• **BOX 74-2** Foodborne and Waterborne
Gastrointestinal Syndromes

General Symptoms	Incubation/ Occurrence	Etiology
Nausea and vomiting	1-8 hours	<i>Staphylococcus aureus</i> <i>Bacillus cereus</i>
Abdominal cramps and diarrhea	8-16 hours	<i>Clostridium perfringens</i> <i>B. cereus</i>
Fever, abdominal cramps, and diarrhea	6-48 hours	<i>Salmonella</i> <i>Shigella</i> <i>Vibrio</i> <i>Campylobacter</i> <i>Escherichia coli</i> (STEC) <i>Yersinia enterocolitica</i>
Abdominal cramps and watery diarrhea	16-72 hours	<i>Listeria monocytogenes</i> <i>E. coli</i> (ETEC) <i>Vibrio</i>
Vomiting and non-blood diarrhea	10-51 hours	Noroviruses
Fever, abdominal cramps, with or without diarrhea	1-11 days	<i>Y. enterocolitica</i>
Bloody diarrhea with low fever	3-8 days	STEC
Paralysis	18-36 hours	<i>Clostridium botulinum</i> *
Persistent diarrhea	1-3 weeks	Parasitic <i>Cryptosporidium</i> <i>Giardia</i> <i>Cyclospora</i>
Systemic illness		<i>Vibrio vulnificus</i> <i>Vibrio</i> spp. <i>Toxoplasma gondii</i> <i>Trichinella</i> spp.

*Approximately 50% of patients will present with nausea and vomiting, and 20% with diarrhea; others may demonstrate constipation.

**TABLE
74-3****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	<i>Vibrio cholerae</i> Rotavirus Noroviruses Enteric adenoviruses Enterotoxigenic <i>Escherichia coli</i> <i>Giardia duodenalis</i> <i>Bacillus cereus</i>
Invasion and possible cytotoxin production/ inflammatory (dysentery)	Dysenteric-like diarrhea (mucus, blood, white cells) Fever Fecal leukocytes	<i>Shigella</i> spp. Enteroinvasive <i>E. coli</i> <i>Salmonella</i> spp. <i>Entamoeba histolytica/dispar</i>
Penetration with subsequent access to the bloodstream (enteric fever)	Signs of systemic infection (headache, malaise, sore throat) Fever	<i>Salmonella enterica</i> serotype Typhi <i>Yersinia enterocolitica</i>

Dr A.Mohammadi

**TABLE
74-4**

General Characteristics of Agents of Enteric Infections

Organism	Common Sources or Predisposing Condition	Distribution	Clinical Presentation	Predominant Pathogenic Mechanism	Fecal Leukocytes
<i>Arcobacter</i> spp.	Foodborne.	Unknown	Watery diarrhea or chronic	Unknown	Unknown
<i>Bacillus cereus</i>	Meats, vegetables, rice.	Worldwide	Intoxication: vomiting or watery diarrhea	Ingestion of preformed toxin (food poisoning)	—
<i>Clostridium botulinum</i>	Improperly preserved vegetables, meat, fish.	Worldwide	Neuromuscular paralysis	Ingestion of preformed toxin (food poisoning)	—
<i>Staphylococcus aureus</i>	Meats, salads, dairy products.	Worldwide	Intoxication: vomiting	Ingestion of preformed toxin (food poisoning)	—
<i>Clostridium perfringens</i>	Meats, poultry.	Worldwide	Watery diarrhea	Ingestion of organism followed by toxin production	—
<i>Aeromonas</i>	Water.	Worldwide	Watery diarrhea, dysentery, and chronic diarrhea	Heat labile cytotoxic enterotoxin (<i>alt</i> gene) and/or heat-stable cytotoxic enterotoxin (<i>ast</i> gene)	—
<i>Campylobacter</i> spp.	Water, poultry, milk.	Worldwide	Dysentery	Invasion	+
<i>Clostridium difficile</i>	Antimicrobial therapy; immunosuppression, underlying gastrointestinal disease, health care-associated exposure.	Worldwide	Dysentery	Enterotoxin and cytotoxin	+/-

Diarrheagenic <i>Escherichia coli</i>					
Enteropathogenic (EPEC)		Worldwide	Watery diarrhea	Adherence/ invasion without multiplication	–
Enterotoxigenic (ETEC)	Food, water.	Worldwide— more preva- lent in developing countries	Watery diarrhea	Enterotoxin	–
Enteroinvasive (EIEC)	Food.	Worldwide	Dysentery	Invasion, enterotoxin	+
Enterohemorrhagic (STEC)	Meats.	Worldwide	Watery, often bloody diarrhea	Cytotoxin	–/+
<i>Plesiomonas shigelloides</i>	Fresh water, shellfish.	Worldwide	Watery, invasive dysentery-like, and subacute/ chronic	Enterotoxins: <i>in vitro</i> Demonstration of cholera-like toxin, thermostable and thermolabile toxins, beta-hemolysins and cytotoxins	+/-
<i>Salmonella</i> spp. (nontyphoidal)	Food, water.	Worldwide	Dysentery	Invasion	+
<i>Salmonella enterica</i> serotype Typhi	Food, water.	Tropical, developing countries	Enteric fever	Penetration	+ (monocytes, not PMNs)

Organism	Common Sources or Predisposing Condition	Distribution	Clinical Presentation	Predominant Pathogenic Mechanism	Fecal Leukocytes
<i>Shigella</i> spp.	Food, water.	Worldwide	Dysentery	Invasion	+
<i>Shigella dysenteriae</i>	Water.	Tropical, developing countries	Dysentery	Invasion, cytotoxin	+
<i>Vibrio cholerae</i>	Water, shellfish.	Asia, Africa, Middle East, South and North American (along coastal areas)	Watery diarrhea	Enterotoxin, cytotoxin	-/+
<i>Yersinia enterocolitica</i>	Milk, pork, water.	Worldwide	Watery diarrhea and/or enteric fever	Invasion, penetration	-
<i>Giardia duodenalis</i>	Food, water.	Worldwide	Watery diarrhea	Unknown—impaired absorption	-
<i>Cryptosporidium parvum</i>	Animals, water.	Worldwide	Watery diarrhea	Adherence	-
<i>Entamoeba histolytica/dispar</i>	Food, water.	Worldwide (more common in developing countries)	Dysentery	Invasion, cytotoxin	-/+ (amoebae destroy the white cells)

Rotavirus	Person-to-person; viral shedding often occurs during subclinical presentation and after cessation of diarrhea.	Worldwide	Watery diarrhea	Mucosal damage leading to impaired absorption in small intestine; can be life-threatening	—
Enteric adenoviruses	Day care settings and health care-associated settings (hospitals); immunocompromised patients, particularly hematopoietic stem cell transplant recipients.	Worldwide	Sporadic cases; chronic watery or subclinical presentation	Unknown	—
Astroviruses	Unknown.	Worldwide	Pediatric gastroenteritis or asymptomatic in all ages; watery diarrhea	Unknown	—
Noroviruses (Caliciviruses)	Shellfish, salads. Immunohematology ABH secretors and Lewis blood groups serve as viral receptors.	Worldwide	Watery diarrhea	Mucosal damage leading to impaired absorption in small intestine	—

ABH, A, B, and H, blood group antigens; PMN, Polymorphonuclear cells.

ORGANISMS TRANSMITTED BY RAW MILK

TABLE A-10. Diseases Contracted from Consumption of Raw Milk

Disease	Organism	Symptoms and Complications
Campylobacteriosis	<i>Campylobacter</i>	Bloody diarrhea
Salmonellosis	<i>Salmonella</i>	Bloody diarrhea
Hemolytic uremic syndrome	<i>Escherichia coli</i> O157:H7	Diarrhea; kidney failure; death
Yersiniosis	<i>Yersinia enterocolitica</i>	Diarrhea
Listeriosis	<i>Listeria monocytogenes</i>	Meningitis; blood infections
Tuberculosis	<i>Mycobacterium tuberculosis</i>	Tuberculosis; pneumonia
Brucellosis	<i>Brucella</i>	Blood infections; heart infections
Cryptosporidiosis	<i>Cryptosporidium parvum</i>	Diarrhea
Staphylococcal enterotoxin poisoning	<i>Staphylococcus aureus</i>	Vomiting
Q fever	<i>Coxiella burnetii</i>	High fever, severe headache, muscle aches; can infect the liver and/or heart

TABLE 19-3 Bacterial Species and Infections They Commonly Produce

Bacterial Species	Diseases
<i>Escherichia coli</i>	Bacteriuria, septicemia, neonatal sepsis, meningitis, diarrheal syndrome
<i>Shigella</i> spp.	Diarrhea, dysentery
<i>Edwardsiella</i> spp.	Diarrhea, wound infection, septicemia, meningitis, enteric fever
<i>Salmonella</i> spp.	Septicemia, enteric fever, diarrhea
<i>Citrobacter</i> spp.	Opportunistic and hospital-acquired infections (wound, urinary)
<i>Klebsiella</i> spp.	Bacteriuria, pneumonia, septicemia
<i>Enterobacter</i> spp.	Opportunistic and hospital-acquired infection, wound infections, septicemia, bacteriuria
<i>Serratia</i> spp.	Opportunistic and hospital-acquired infection, wound infections, septicemia, bacteriuria
<i>Proteus</i> spp.	Bacteriuria, wound infection, septicemia
<i>Providencia</i> spp.	Opportunistic and hospital-acquired infection, wound infections, septicemia, bacteriuria
<i>Morganella</i> spp.	Opportunistic and hospital-acquired infections
Yersinia	
<i>Y. pestis</i>	Plague
<i>Y. pseudotuberculosis</i>	Mesenteric adenitis, diarrhea
<i>Y. enterocolitica</i>	Mesenteric adenitis, diarrhea
<i>Erwinia</i> spp.	Wounds contaminated with soil or vegetation
<i>Pectobacterium</i> spp.	Wounds contaminated with soil or vegetation

Modified from Washington J: *Laboratory procedures in clinical microbiology*, ed 2, New York, 1981, Springer-Verlag.

TABLE 34-5 Compendium of Common Foodborne Diseases

Average Incubation Period	Organism	Average Duration	Implicated Foods	Typical Symptoms	Comments
2-16 h	<i>Bacillus cereus</i>	1 day	Boiled and fried rice, meats, vegetables	Nausea, vomiting, (emetic) abdominal cramping, watery diarrhea	Produces two toxins, one emetic form that causes nausea and vomiting within hours, and one diarrhetic form; common year-round; isolation of large numbers from implicated foods and patient stool
6-72 h	<i>Vibrio parahaemolyticus</i>	3 days	Shellfish	Pain, vomiting, fever, watery diarrhea	Blood sometimes in stool; common in spring, summer, fall in coastal states; stool culture using TCBS media recommended
6-72 h	<i>Vibrio cholerae</i>	3-7 days	Seafood, water	Rice water stools, severe diarrhea, no fever	No blood or mucus in stool; mechanism of action, in vivo enterotoxin production; no tissue invasion; stool culture using TCBS media recommended
<8 h	<i>Staphylococcus aureus</i>	<1 day	Egg salad, meat, poultry, pastries	Abrupt onset of nausea, pain and projectile vomiting, infrequent diarrhea	Mechanism of action is preformed enterotoxin in foods; common in summer; ELISA or reverse passive latex agglutination enterotoxin test; gel electrophoresis in lieu of phage typing

8-22 h	<i>Clostridium perfringens</i>	1 day	Beef, poultry, gravy, fish	Abdominal cramping, watery diarrhea; vomiting and fever uncommon	In vivo enterotoxin production; unlike <i>Staphylococcus aureus</i> , viable organisms must be ingested for disease to occur; common in fall, winter, spring
12-48 h	<i>Salmonella</i> spp.	3 days	Eggs, dairy products, fowl, beef	Fever, abdominal cramping, diarrhea, mild vomiting	WBCs in stool; common in summer; culture and serologic identification
16-48 h	<i>Yersinia enterocolitica</i>	1 day to 4 wk	Milk, pork	Fever, severe abdominal pain, diarrhea	WBCs and RBCs in stool; common in winter
18-36 h	<i>Clostridium botulinum</i>	Weeks-months	Vegetables, fruits (canned foods), fish, honey (infants)	Nausea, vomiting, diarrhea, paralysis	Mechanism of action is preformed neurotoxin; common in summer and fall
24-72 h	<i>Shigella</i> spp.	3 days	Egg and tuna salads, lettuce, milk	Fever, abdominal cramping, diarrhea, occasional vomiting	WBCs, RBCs, and mucus in stools; tissue invasion common mechanism of action; common in summer; culture and serologic identification
24-72 h	Enterotoxigenic <i>Escherichia coli</i> (ETEC)	3 days	Fruits, meats, pastries, salads	Abdominal cramping, watery diarrhea, no vomiting or fever	In vivo enterotoxin; major cause of traveler's diarrhea; year-round distribution; patient history includes travel to Mexico and other developing countries
24-72 h	Enterohemorrhagic <i>E. coli</i> (EHEC)	3 days	Undercooked ground beef, cider	Watery diarrhea progressing to bloody diarrhea, abdominal cramping, no fever or vomiting	Implicated shiga-toxin producing <i>E. coli</i> ; organisms disappear rapidly from stool; culture of sorbitol-negative <i>E. coli</i> from stool using SMAC plate recommended

ELISA, Enzyme-linked immunosorbent assay; RBCs, red blood cells; SMAC, sorbitol-MacConkey; TCBS, thiosulfate–citrate–bile salts–sucrose; WBCs, white blood cells.

TABLE A-16. Common Causes of Infectious Diseases of the Gastrointestinal Tract*

Disease	Common Cause(s)
Teeth: dental caries	<i>Streptococcus mutans</i>
Gums: gingivitis and periodontal disease	Polymicrobial infection involving many anaerobic bacteria found in the oropharynx
Sublingual and submaxillary space: Ludwig angina	<i>Streptococcus</i> , <i>Bacteroides</i> , <i>Fusobacterium</i> and/or <i>Staphylococcus aureus</i>
Mouth and tongue: gingivostomatitis or oral herpes	Herpes simplex virus
Mouth: stomatitis or oral candidiasis (thrush)	<i>Candida albicans</i>
Salivary glands: parotitis	Mumps virus: benign viral parotitis <i>Staphylococcus aureus</i> : acute bacterial parotitis
Esophagus: esophagitis	<i>Candida albicans</i>
Stomach and upper duodenum: gastritis and peptic ulcer disease	<i>Helicobacter pylori</i>
Intestine: food poisoning	<i>Staphylococcus aureus</i> <i>Bacillus cereus</i> <i>Clostridium perfringens</i>
Intestine: viral gastroenteritis	Rotavirus (winter infant diarrhea) Noroviruses (winter vomiting disease) Norwalk virus (summer diarrhea)
Intestine: noninflammatory bacterial infections	<i>Escherichia coli</i> (EPEC), <i>E coli</i> (ETEC), and <i>E coli</i> (EAEC) <i>Vibrio cholerae</i> <i>Clostridium difficile</i>
Intestine: inflammatory bacterial infections	<i>Campylobacter jejuni</i> <i>Escherichia coli</i> (EIEC) and <i>E coli</i> (EHEC) <i>Salmonella typhimurium</i> <i>Shigella dysenteriae</i> , <i>S sonnei</i> , and <i>S flexneri</i> <i>Yersinia enterocolitica</i> <i>Clostridium difficile</i>
Intestine: parasitic infections	<i>Giardia lamblia</i> <i>Entamoeba histolytica</i> <i>Cryptosporidium parvum</i> <i>Enterobius vermicularis</i> <i>Ascaris lumbricoides</i>
Liver: viral hepatitis	Hepatitis A, B, and C virus

*Note that this is not an exhaustive list and does not include all possible etiologies for a particular disease.

تشخیص آزمایشگاهی

Dr A.Mohammadi

TABLE 34-6 Selective Media Commonly Used to Recover Diarrheal Agents

Culture Medium	Purpose	Characteristic Morphology	
		Pathogens	Colon Flora
MacConkey agar	To recover Enterobacteriaceae and other nonfastidious, gram-negative bacilli; inhibits gram-positive organisms and some fastidious gram-negative bacilli	<i>Salmonella</i> , <i>Shigella</i> (with few exceptions) organisms; <i>Edwardsiella</i> organisms appear clear and colorless	Lactose fermenters, such as <i>Escherichia coli</i> , <i>Klebsiella</i> spp., <i>Enterobacter</i> spp., and certain <i>Citrobacter</i> spp., appear dark pink to red. Late or slow lactose fermenters, such as <i>Citrobacter</i> spp., <i>Serratia</i> spp., and <i>Hafnia</i> spp., appear colorless in 24 h and slightly pink after 24-48 h; nonlactose fermenters, such as <i>Citrobacter</i> spp., <i>Proteus</i> spp., <i>Providencia</i> spp., and <i>Morganella</i> spp. appear clear and colorless.
Hektoen enteric (HE) agar	Highly selective medium to recover primarily <i>Salmonella</i> and <i>Shigella</i> spp.; inhibits common colon flora; contains indicators to detect hydrogen sulfide (H ₂ S) production	<i>Salmonella</i> spp. appear green to blue-green with black centers because of H ₂ S production; <i>Shigella</i> spp. appear green without black centers because they do not produce H ₂ S	Lactose fermenters, such as <i>E. coli</i> , are slightly inhibited and appear orange to salmon pink; <i>Proteus</i> spp. are slightly inhibited; small, clear colonies with black centers may appear.
Xylose lysine deoxycholate (XLD) agar	Differential and selective medium to isolate <i>Salmonella</i> and <i>Shigella</i> spp. from stool; inhibits most colon flora and most gram-positive bacteria; certain <i>Shigella</i> spp. (<i>S. dysenteriae</i> and <i>S. flexneri</i>) may be slightly inhibited.	<i>Salmonella</i> spp. appears red with black centers because of production of H ₂ S; <i>Salmonella</i> does not ferment lactose or sucrose but does ferment xylose, which is essential in decarboxylating lysine to revert the acid pH (yellow from sucrose fermentation) to an alkaline pH (red from lysine decarboxylation) <i>Shigella</i> spp. do not ferment any of these carbohydrates and appear red or clear	Enterobacteriaceae that may not be completely inhibited, such as <i>Proteus vulgaris</i> , appear yellow (from sucrose) with black centers; <i>Citrobacter freundii</i> , which produces H ₂ S, appears yellow with black centers because of inability to decarboxylate lysine; other intestinal flora that may grow ferment one or all of the carbohydrates in this medium, resulting in yellow colonies.

<i>Campylobacter</i> blood agar (CAMPY-BA)	Enrichment-selective medium primarily to isolate and cultivate <i>Campylobacter</i> spp. from stool	<i>Campylobacter jejuni</i> appears pinkish gray, moist, and runny when incubated at 42°C	
Cefsulodin-irgasan-novobiocin (CIN)	Selective medium primarily to isolate and recover <i>Yersinia enterocolitica</i> and <i>Aeromonas</i> spp.; <i>Plesiomonas shigelloides</i> may also be recovered; inhibits most gram-positive cocci, except for enterococci, and most gram-negative bacilli, particularly the Enterobacteriaceae	<i>Yersinia enterocolitica</i> produce colonies that look like bull's eyes; center is red and periphery appears colorless; <i>Aeromonas</i> species also ferment mannitol present in the medium, like <i>Yersinia</i> ; <i>P. shigelloides</i> does not	Except for <i>Pseudomonas aeruginosa</i> , <i>Citrobacter</i> , and <i>Serratia</i> , most colon flora are inhibited.
Thiosulfate–citrate–bile salts–sucrose agar (TCBS)	Highly selective medium to recover <i>Vibrio</i> spp., including <i>Vibrio cholerae</i> , from stool and food; inhibits most colon flora because of the high pH (preferred by vibrios) and high bile salts content; <i>Aeromonas</i> spp. may be recovered from this medium	TCBS contains sucrose, so sucrose-fermenting <i>Vibrio</i> spp. such as <i>V. cholerae</i> and <i>V. alginolyticus</i> produce yellow colonies; nonsucrose fermenters, such as <i>V. parahaemolyticus</i> and <i>V. vulnificus</i> produce blue-green colonies	Inhibitory to most colon flora, except for occasional <i>Pseudomonas</i> isolates, which may also appear blue-green
Cycloserine–cefoxitin–fructose agar (CCFA), anaerobic incubation required	Selective medium to isolate primarily <i>Clostridium difficile</i> from stool of patients suspected of antibiotic-associated diarrhea or pseudomembranous colitis; inhibits most colon flora, both gram-positive and gram-negative bacteria	<i>C. difficile</i> appears yellow because of fructose fermentation	Colon flora are inhibited.
Sorbitol-MacConkey (SMAC) agar	Differential medium to detect sorbitol-negative <i>Escherichia coli</i> ; contains sorbitol instead of lactose	<i>E. coli</i> O157:H7 appears colorless; does not ferment sorbitol	Most appear pink.

IDENTIFICATION OF COMMON GRAM-NEGATIVE BACTERIAL PATHOGENS (FIGURE A-2)

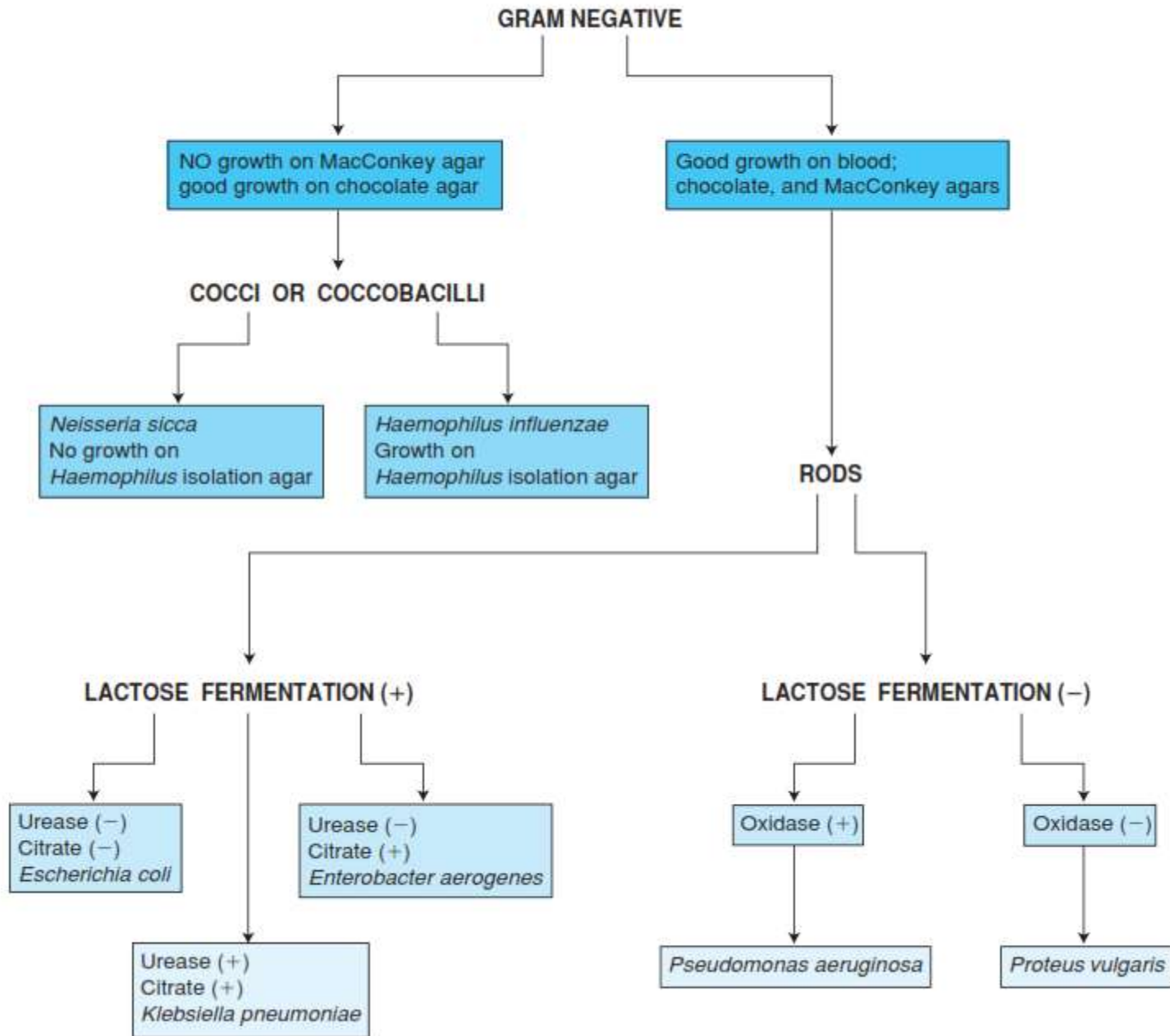


TABLE A-7. Physical Properties of Enteric Gram-Negative Rods

Genera	Pathogenic Species	Shape	Motile	Glucose Fermentation	Oxidase	Lactose Fermentation	H ₂ S Gas	Special
<i>Escherichia</i>	<i>E coli</i>	Rod	Yes	Yes	No	Yes	No	
<i>Salmonella</i>	<i>S enterica, S typhi</i>	Rod	Yes	Yes	No	No	Yes	
<i>Shigella</i>	<i>S flexneri, S dysenteriae, S boydii, S sonnei</i>	Rod	No	Yes	No	No	No	
<i>Yersinia</i>	<i>Y enterocolitica,</i>	Rod	No	Yes	No	No	No	
<i>Campylobacter</i>	<i>C jejuni, C coli, C fetus</i>	Comma-shaped rod, or S-shaped rod, or corkscrew	Yes	No	Yes	No	No	Grows best at 42°C, microaerophile
<i>Helicobacter</i>	<i>H pylori</i>	Spiral-shaped rod	Yes	No	Yes	No	No	Urease producer
<i>Vibrio</i>	<i>V parahaemolyticus, V vulnificus, V cholerae</i>	Comma-shaped rod	Yes	Yes	Yes	No, Yes, No	No	Grows best in high salt
<i>Bacteroides</i>	<i>B fragilis</i>	Rod	No	Not applicable	No	Not applicable	No	Obligate anaerobe

TABLE 19-2 Biochemical Characteristics of Tribes of Enterobacteriaceae

Tests or Substrate	Escherichieae	Edwardsielleae	Citrobacteriaceae	Salmonelleae*	Klebsiellae	Proteeae [†]	Yersiniae
H ₂ S (TSI agar)	–	+	+ or –	+	–	+ or –	–
Urease	–	–	(+ ^w) or –	–	– or (+)	+ or –	+
Indole	+ or –	+	– or +	–	–	+ or –	+ or –
Methyl red	+	+	+	+	–	+	+
Voges-Proskauer	–	–	–	–	+	–	–
Citrate (Simmons)	–	–	+	+	+	d	–
KCN	–	–	+ or –	–	+	+	–
Phenylalanine deaminase	–	–	–	–	–	+	–
Mucate	d	–		d	+ or –	–	
Mannitol	+ or –	–	+	+	+	– or +	+

Modified from Ewing WH: *Edwards and Ewing's identification of Enterobacteriaceae*, ed 4, East Norwalk, CT, 1986, Appleton & Lange.

H₂S, Hydrogen sulfide; KCN, potassium cyanide; TSI, triple sugar iron; +, ≥90% positive within 1 or 2 days; (+), positive reaction after ≥3 days (decarboxylase tests: 3 or 4 days); –, ≥90% no reaction in 30 days; + or –, most cultures positive, some strains negative; – or +, most strains negative, some cultures positive; d, different reactions, +, (+), –, +^w, weakly positive reaction.

**Salmonella* serovars Typhi and Paratyphi and some rare serovars fail to use citrate in Simmons medium. Cultures of serovar Paratyphi and some rare serotypes may fail to produce H₂S.

[†]Some cultures of *Proteus mirabilis* may yield positive Voges-Proskauer tests.

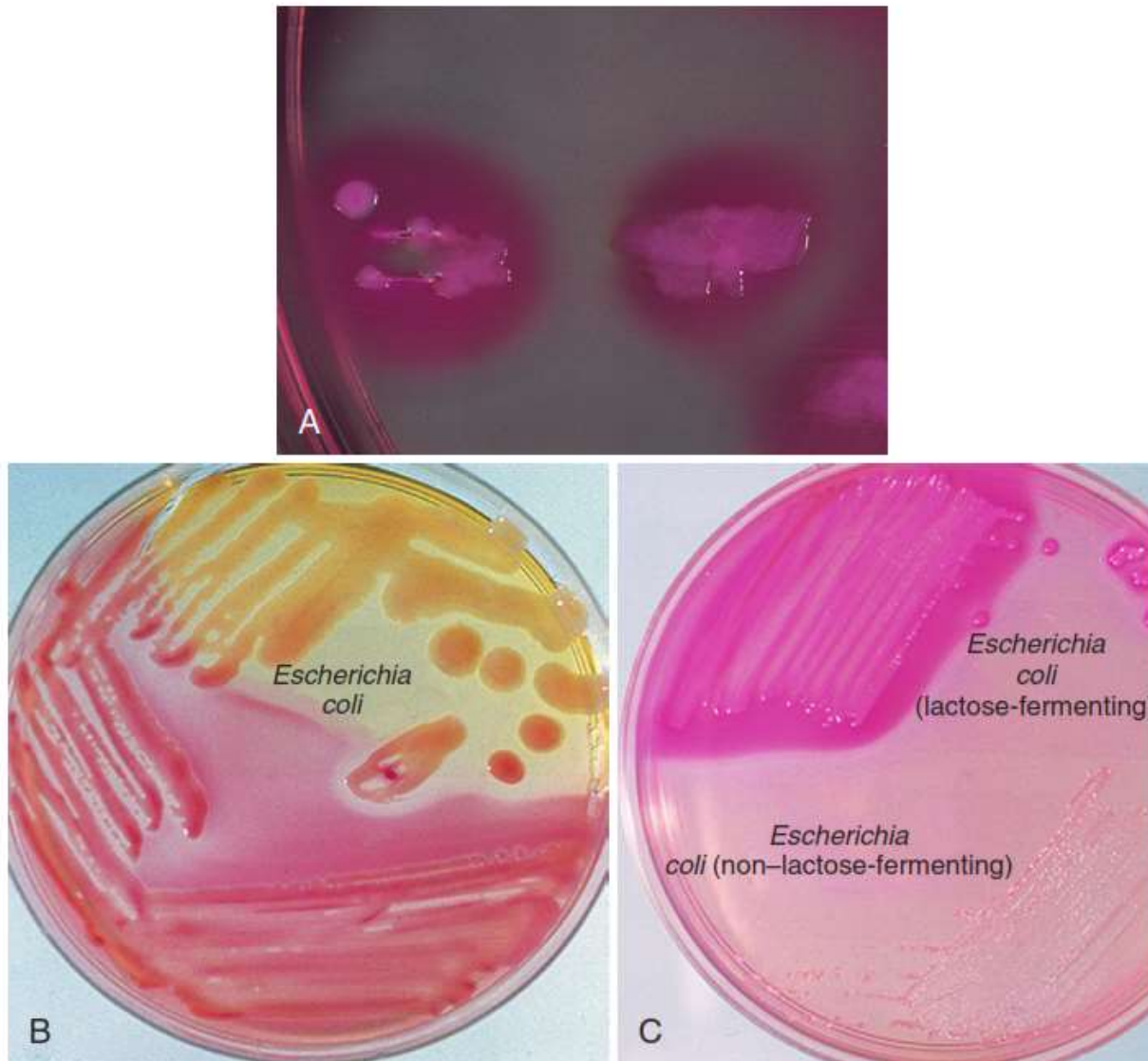


FIGURE 19-1 A, Typical dry, lactose-positive *Escherichia coli* growing on MacConkey (MAC) agar. Note the pink precipitate surrounding the individual colonies. B, Muroid colonies of *E. coli* growing on MAC agar. C, Non-lactose-fermenting *E. coli* compared with typical lactose-fermenting *E. coli* on MAC agar. (B and C, Courtesy Jean Barnishan.)



• **Figure 74-7** Colonies of a lactose-positive organism growing on xylose-lysine deoxycholate (XLD) agar (A) and Hektoen enteric (HE) agar (B). Colonies of *Salmonella enteritidis* (lactose-negative) growing on XLD (C) and HE agar (D). (Note how both agars detect H₂S production.) Colonies of *Shigella* (lactose-negative) growing on XLD (E) and HE agar (F).

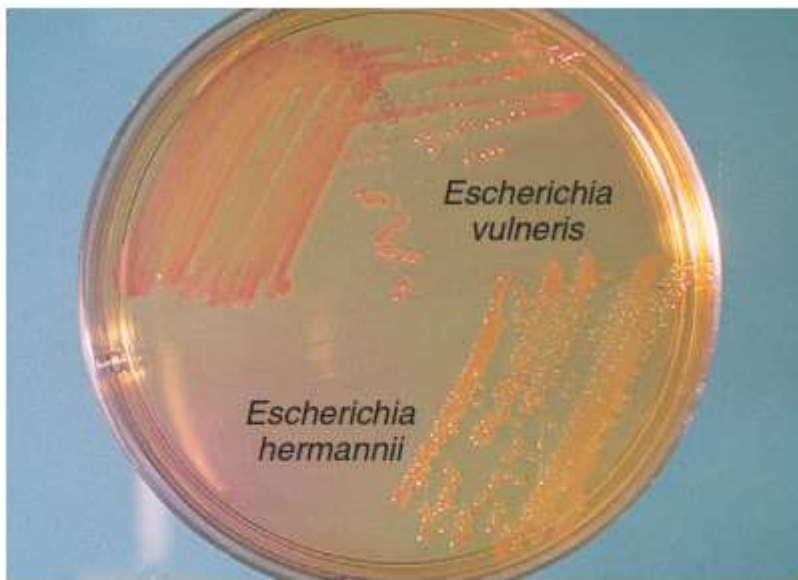


FIGURE 19-2 Comparison of the colony morphology of *Escherichia vulneris* and a yellow-pigmented *Escherichia hermannii* on MacConkey (MAC) agar. *Escherichia vulneris* may also produce a yellow-pigmented colony, but the yellow is more prevalent in *E. hermannii*. (Courtesy Jean Barnishan.)

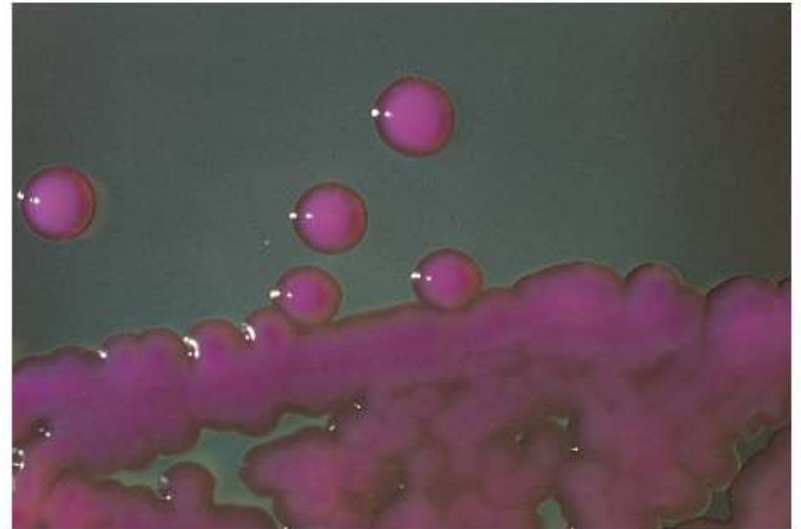


FIGURE 19-3 Mucoid appearance of *Klebsiella pneumoniae* on MacConkey (MAC) agar.

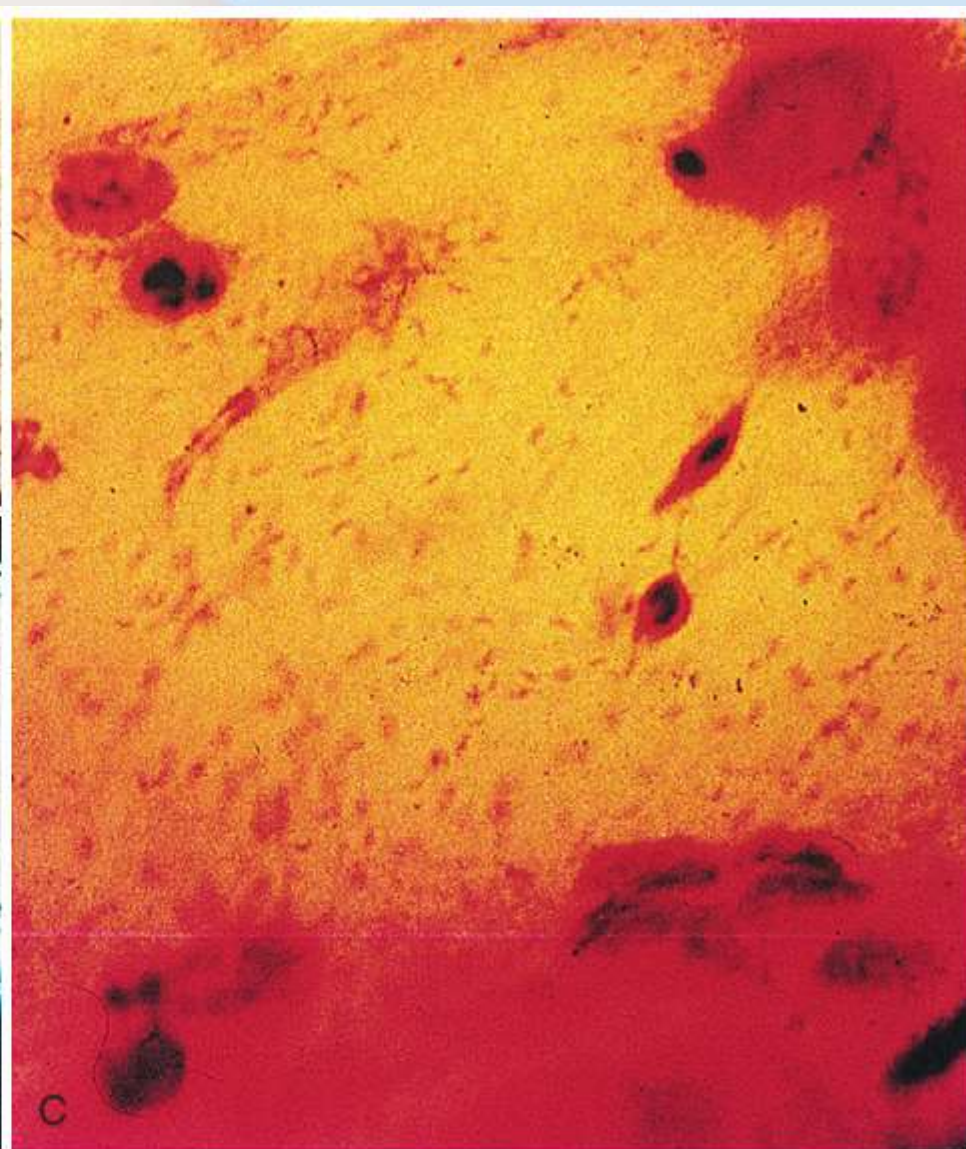
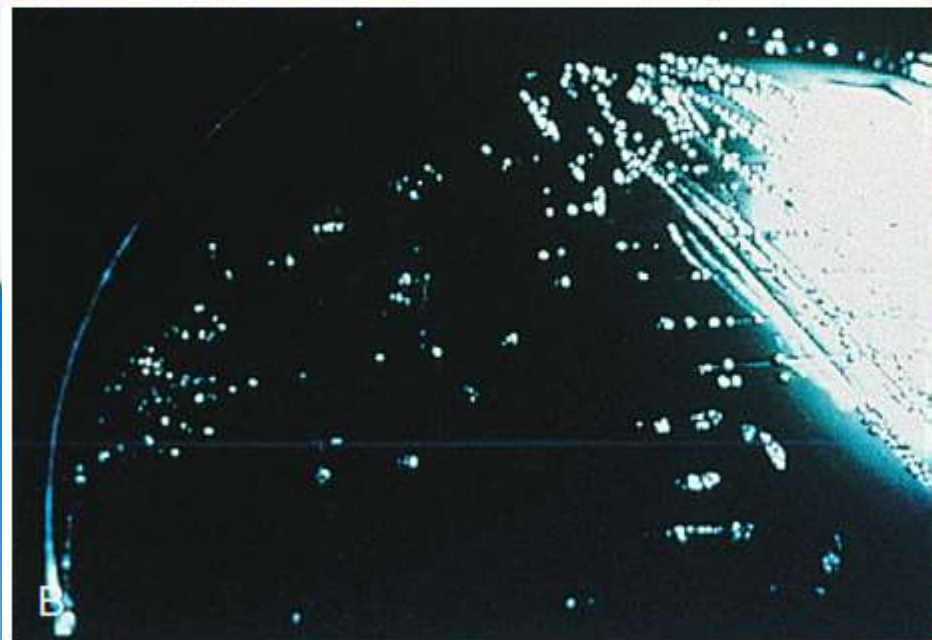
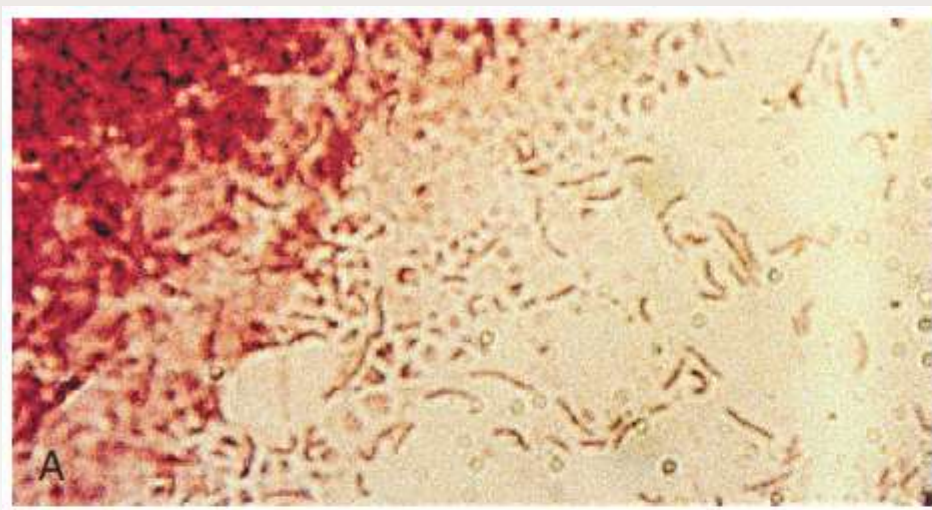


FIGURE 34-3 A, Microscopic morphology of *Helicobacter pylori* Gram stained from a colony. B, Gray translucent *H. pylori* colonies grown on agar culture medium. C, Gram stain on gastric mucus. (Courtesy American College of Gastroenterology and DiaSorin, Stillwater, MN.)

Dr A.Mohammadi

TABLE 19-11 Stool Culture Screening for Enteric Pathogens Using Triple Sugar Iron and Lysine-Iron Agar in Combination

LIA Reactions	TSI Reactions							
	K/A H ₂ S	K/AG H ₂ S	K/AG	K/A	A/A H ₂ S	A/AG	A/A	K/K
R/A		<i>P. vulgaris</i> <i>P. mirabilis</i>	<i>M. morganii</i> <i>Providencia</i>	<i>M. morganii</i> <i>Providencia</i>	<i>P. vulgaris</i> <i>P. mirabilis</i>	—	<i>Providencia</i>	—
K/K H ₂ S	<i>Salmonella</i> [*] <i>Edwardsiella</i>	<i>Salmonella</i> [*] <i>Edwardsiella</i> [*]	<i>Salmonella</i> [*]	<i>Salmonella</i> [*]	—	—	—	—
K/K	<i>Salmonella</i>	—	<i>Hafnia</i> <i>Klebsiella</i> <i>Serratia</i>	<i>Salmonella</i> [*] <i>Plesiomonas</i> [†] <i>Hafnia</i>	—	<i>Klebsiella</i> <i>Enterobacter</i> <i>E. coli</i>	<i>Serratia</i>	<i>Pseudomonas</i> [†]
K/A H ₂ S	—	<i>Salmonella</i> [*]	—	<i>Serratia</i>	—	—	—	—
K/A	—	<i>Citrobacter</i>	<i>Salmonella</i> [*] <i>Shigella</i> <i>Aeromonas</i> [†] <i>E. coli</i> <i>Enterobacter</i> <i>Citrobacter</i>	<i>Shigella</i> [*] <i>Yersinia</i> <i>Aeromonas</i> [†] <i>E. coli</i> <i>Enterobacter</i>	<i>Citrobacter</i>	<i>Aeromonas</i> ^{*†} <i>E. coli</i> <i>Citrobacter</i> <i>Enterobacter</i>	<i>Aeromonas</i> ^{*†} <i>Yersinia</i> <i>Citrobacter</i> <i>Enterobacter</i>	—

Data from Microbiology Laboratory, The Ohio State University Hospitals and Maureta Ott, Columbus, OH.

A, Acid; G, gas; H₂S, hydrogen sulfide; K, alkaline; LIA, Lysine-iron agar; R, deamination (red slant); TSI, triple sugar iron.

*Results of TSI and LIA reactions in this category indicate a potential pathogen; additional tests must be performed.

[†]Oxidase-positive.



FIGURE 19-12 Clear, green colonies of *Shigella* growing on Hektoen enteric (HE) agar. (Courtesy R. Abe Baalness.)

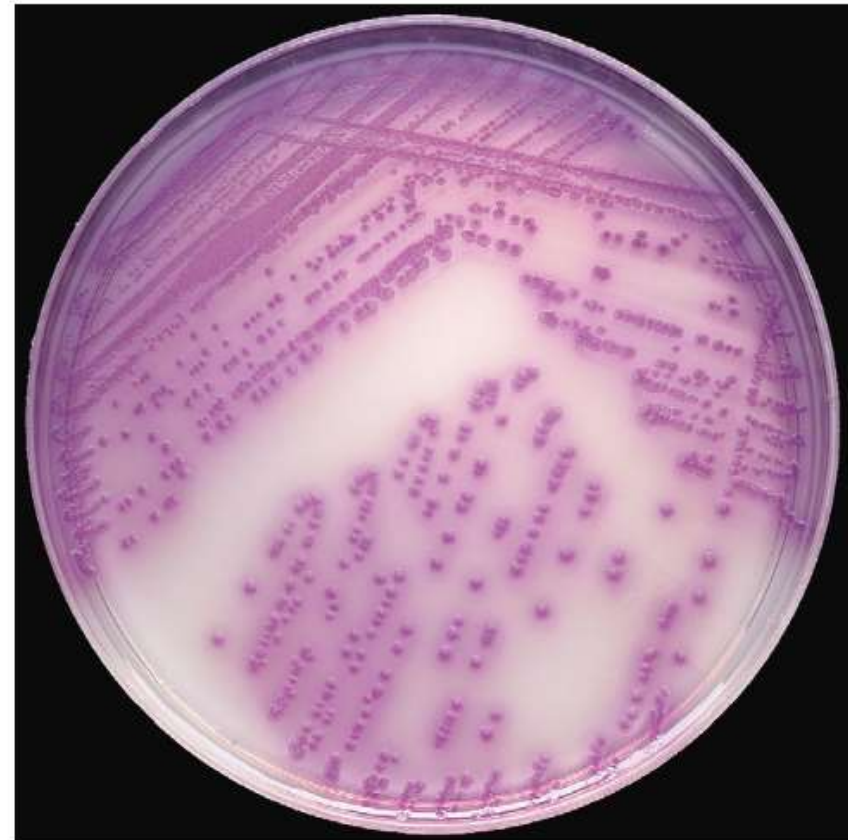


FIGURE 19-14 *Salmonella* growing on CHROMagar Salmonella differential agar. (Courtesy BD Diagnostic Systems, Sparks, MD.)

Dr A.Mohammadi

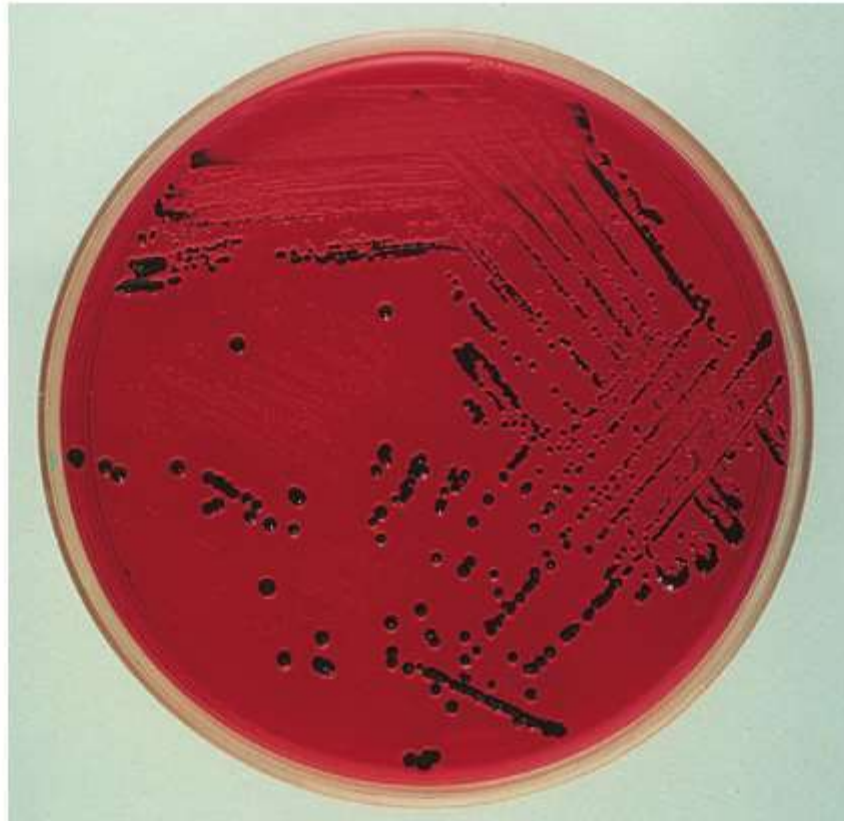


FIGURE 19-13 H₂S-producing colonies of salmonellae growing on xylose-lysine-desoxycholate (XLD) agar. (Courtesy American Society for Clinical Laboratory Science, Education and Research Fund, Inc, 1982.)

Laboratory Identification of Significant Isolates

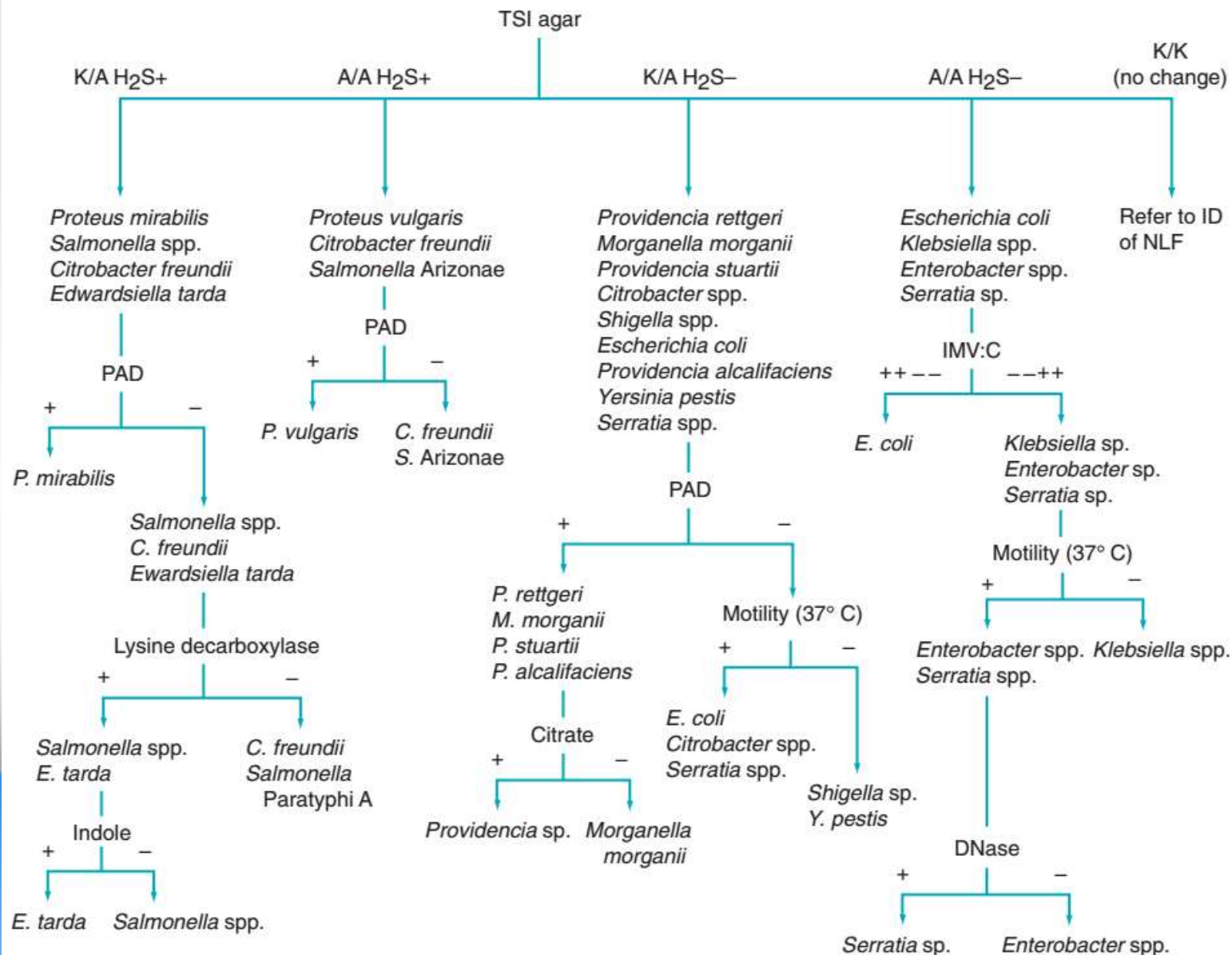


FIGURE 19-15 Flow chart for the presumptive identification of commonly encountered Enterobacteriaceae on triple sugar iron (TSI) agar. A, acid; IMV:C, indole, methyl red, Voges-Proskauer, citrate; K, alkaline; NLF, nonlactose fermenter; PAD, phenylalanine deaminase. (Data from Koneman E, et al: *Color atlas in diagnostic microbiology*, ed 5, Philadelphia, 1997, Lippincott-Raven.)

	<i>Escherichia coli</i>	<i>Ewingella americana</i>	<i>H. alvei</i>	<i>Plesiomonas shigelloides</i> *oxidase +	<i>Shigella sonnei</i>	Other <i>Shigella</i>	<i>S. enteritidis</i>	<i>S. typhi</i>	<i>Edwardsiella tarda</i>	<i>Citrobacter</i>			<i>Klebsiella</i>		<i>Enterobacter</i>		<i>Cronobacter sakazakii</i>	<i>Pantoea agglomerans</i> (was <i>Enterobacter</i>)	<i>Serratia</i>		<i>Proteus</i>		<i>Morganella morganii</i>	<i>Providencia</i>		<i>Yersinia enterocolitica</i>
										<i>C. freundii</i>	<i>C. braakii</i>	<i>C. koseri</i> (formerly <i>diversus</i>)	<i>K. pneumoniae</i>	<i>K. oxytoca</i>	<i>E. cloacae</i>	<i>E. aerogenes</i>			<i>S. marcescens</i>	<i>S. odorifera</i> biotype 2	<i>P. vulgaris</i>	<i>P. mirabilis</i>		<i>P. rettgeri</i>	<i>P. stuartii</i>	
Indole	+	-	-	+	-	V	-	-	+	-	-(v)	+	-	+	-	-	-	-(v)	-	V	+	-	+	+	+	V
Methyl red	+	+	-(v)	V	+	+	+	+	+	+	+	+	V	-(v)	-	-	-	V	V	+(v)	+	+	+	+	+	+
Voges Proskauer	-	+	+(v)	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+(v)	+	+	-	V	-	-	-	-
Simmons citrate	-	+	+	-	-	-	+	-	-	+	+(v)	+	+	+	+	+	+	V	+	+	-(v)	+(v)	-	+	+	-
Hydrogen Sulfide (TSI)	-	-	-	-	-	-	+(v)	+w	+	+	+(v)	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
Urea	-	-	-	-	-	-	-	-	-	-(v)	-(v)	+(v)	+	+	+(v)	-	-	-(v)	-(v)	-	+	+	+	+	-(v)	+
Motility	V	+(v)	+	+	-	-	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	V	+	+(v)	-
Lysine decarboxylase	+(v)	-	+	+	-	-	+	+	+	-	-	-	+	+	-	+	-	+	+	-	-	-	-	-	-	-
Arginine dihydrolase	-(v)	-	-	+	-	V	+(v)	-	-	+(v)	+	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-
Ornithine decarboxylase	+(v)	-	+	+	+	-	+	-	+	-	+	+	-	-	+	+	+	-	+	-	-	+	+	-	-	+
Phenylalanine deaminase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+(v)	-(v)	-	-	+	+	+	+	+	-
Gas from D-glucose	+	-	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+	-(v)	-	-	+	+	+	-	-	-
Lactose	+	+(v)	-	V	-	-	-	-	-	+(v)	+	V	+	+	+	+	+	-(v)	-	+	-	-	-	-	-	-
Sucrose	V	-	-	-	-	-	-	-	-	+(v)	-	-(v)	+	+	+	+	+	+(v)	+	-	+	-	-	-	-	V
D-Mannitol	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-(v)	+
Adonitol	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-(v)	+	-	-	-(v)	+(v)	-	-	-	+	-	-
Inositol	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-(v)	+	+	-(v)	V	+	-	-	-	+	+	-
D-Sorbitol	+(v)	-	-	-	-	V	+	+	-	+	+	+	+	+	+	+	-	-(v)	+	+	-	-	-	-	-	+
L-Arabinose	+	-	+	-	+	V	+	-	-	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	-	+
Raffinose	V	-	-	-	-	V	-	-	-	-(v)	-	-	+	+	+	+	+	-(v)	-	-	-	-	-	-	-	-
L-Rhamnose	-	-(v)	+	-	+(v)	-(v)	+	-	-	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+(v)	-	-
KCN, growth in	-	-	+	-	-	-	-	-	-	+	+	-	+	+	+	+	+	-(v)	+	-	+	+	+	+	+	-
Gelatin (22°C)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-(v)	+	+	+	+	-	-	-	-
DNase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-

• **Figure 19-4** Biochemical differentiation of representative *Enterobacteriaceae*. +(v), greater probability for positive reaction >50%; -(v), greater probability for negative reaction >50%; (+), positive >80%; (-), negative >80%; V, variability can be equally either positive or negative. The pink squares indicate a pattern useful for preliminary recognition. The green squares indicate a key characteristic for biochemical identification.

Lactose Fermenters: <i>E. coli</i> , <i>K. pneumoniae</i> , * <i>C. freundii</i> (LF), * <i>C. koseri</i> (LF), Other * <i>Citrobacter</i> species (LF), <i>E. aerogenes</i> , <i>E. cloacae</i>		Non-lactose Fermenters: * <i>C. freundii</i> , * <i>C. koseri</i> , *Other <i>Citrobacter</i> species, <i>E. tarda</i> , <i>H. alvei</i> , <i>E. coli</i> (inactive), <i>M. morgani</i> , <i>P. mirabilis</i> , <i>P. penneri</i> , <i>P. vulgaris</i> , <i>P. rettgeri</i> , <i>P. stuartii</i> , <i>Salmonella</i> spp., <i>Shigella</i> spp., <i>Serratia marcescens</i>					
Indole Positive	Indole Negative	PPA Positive		PPA Negative			
<i>E. coli</i> , <i>Citrobacter</i> species other than <i>C. freundii</i> , <i>K. oxytoxa</i>	<i>C. freundii</i> , <i>K. pneumoniae</i> , <i>Enterobacter</i> spp.	<i>Proteus</i> spp., <i>Providencia</i> spp., <i>Morganella morgani</i>		<i>Citrobacter</i> spp., <i>Edwardsiella tarda</i> , <i>E. coli</i> [inactive], <i>Hafnia</i> spp., <i>Salmonella</i> spp., <i>Shigella</i> spp., <i>S. marcescens</i>			
Biochemical Division <i>E. coli</i> I+U- C- <i>C. koseri</i> I+U+/-C+ (motile) MR positive <i>K. oxytoxa</i> I+U+C+ (nonmotile) MR negative	<i>C. freundii</i> I-/+U-/+C+/- H ₂ S+ (KI) <i>E. aerogenes</i> I-U-C+ A/A with gas (KI); OD positive <i>E. cloacae</i> I-U+/-C+ A/A with gas (KI); AD positive <i>K. pneumoniae</i> I-U+C+ A/A with gas (KI); gas (KI)	H₂S Positive	H₂S Negative	Mannitol Positive	Mannitol Negative		
		<i>P. mirabilis</i> <i>P. vulgaris</i> <i>P. penneri</i> (30%) <i>M. morgani</i> (20%)	<i>P. rettgeri</i> <i>P. stuartii</i> <i>P. penneri</i> (70%) <i>M. morgani</i> (80%)			<i>Citrobacter</i> spp., <i>E. coli</i> [inactive], <i>Hafnia</i> spp., <i>Salmonella</i> spp., <i>Shigella</i> spp. [other than <i>S. dysenteriae</i>], <i>S. marcescens</i>	<i>Edwardsiella tarda</i> isolated from a variety of body sites I+H ₂ S+ (KI) <i>S. dysenteriae</i> usually isolated from stool I-H ₂ S- (KI)
		Biochemical	Biochemical				
		<i>P. mirabilis</i> I-U+C+/- <i>P. vulgaris</i> I+U+C-/+ <i>P. penneri</i> I-U+C- <i>M. morgani</i> I+U+C-	<i>P. rettgeri</i> M+ no gas I+C+C+ K/A(KI) <i>P. stuartii</i> M- no gas I+C+U-/+ K/A (KI) <i>P. penneri</i> M- with gas I-C-U+ K/A (KI) <i>M. morgani</i> M- with gas I+C-U+ K/A (KI)				
H₂S Positive		<i>C. freundii</i>					
<i>C. freundii</i>							

ONPG +
S. serotype Typhi
C-
S. serotype Paratyphi B
C+
S. serotype Typhimurium
C+
LD positive

H₂S Negative

Citrobacter spp. [other than *C. freundii*]
I-U+C+
K/A with gas (KI)
Motile
C. koseri I+U-C+
K/A with gas (KI)
Motile
E. coli [inactive]
I+/-U-C-
K/A no gas (KI)
Hafnia spp. I-U-C-
K/A with gas (KI)
Motile
AD positive
S. paratyphi A I-U-C-
K/A with gas (KI)
Motile
S. marcescens I-U-C+
K/A 55% no gas (KI)
Motile
Shigella spp. I-U-C-
K/A no gas (KI)
Non Motile

• **Figure 19-5** Algorithm for the identification of *Enterobacteriaceae*. *Denotes variability in lactose fermentation reactions. +, positive >90%; -, negative ≤10%; +/-, >50%; +, -/+, less than 5% positive; AD, arginine decarboxylase positive; C, indicates growth on Simmons citrate agar; I, indicates indole reaction; KI, Kligler iron agar; LD, lysine decarboxylase positive; LF, late fermenter; M, mannitol fermentation; MR, methyl red; OD, ornithine decarboxylase positive; ONPG, ortho-nitrophenyl-beta-galactoside test; PPA, phenylalanine deamination to phenylpyruvic acid; U, indicates urease reaction. Modified from Gould LH, Bopp C, Strockbine N, et al: Recommendations for diagnosis of Shiga toxin-producing *Escherichia coli* in clinical laboratories, *MMR* 58:1, 2009.)

محیط های اختصاصی برای باکتری های آلوده کننده دستگاه گوارش

هکتون انتریک آگار ، گزیلوز لایزین داکسی کولات ، سالمونلا شیگلا آگار، بیسموت سولفیت آگار	سالمونلا و شیگلا (محیط های انتخابی)
آبگوشت سلنیت، آبگوشت گرم منفی، آبگوشت تتراتیونات	سالمونلا و شیگلا (محیط های غنی کننده ی انتخابی)
سفسولودین - ایرگاسون- نووبیوسین آگار، مک کانکی و سالمونلا شیگلا حرارت گذاری شده در دمای اتاق برای ۴۸ ساعت	یرسینیا انتروکولیتیکا
کامپی بلاد آگار	کمپیلوباکتر
تیوسولفات - بایل سالتز سوکروز آگار	ویبریو

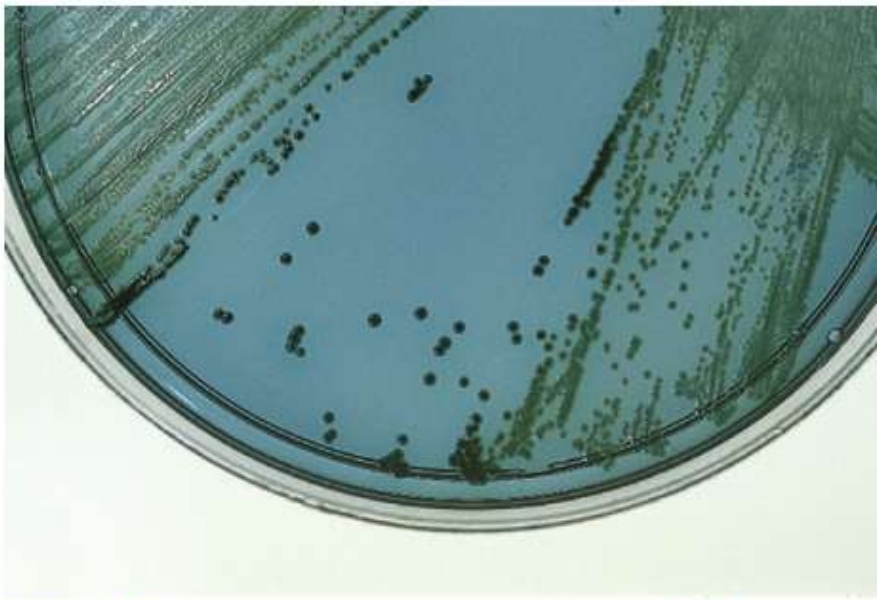


FIGURE 34-8 *Salmonella* colonies growing on Hektoen enteric agar showing black centers resulting from the production of hydrogen sulfide.



FIGURE 34-9 *Shigella* colonies growing on Hektoen enteric agar showing clear green colonies.

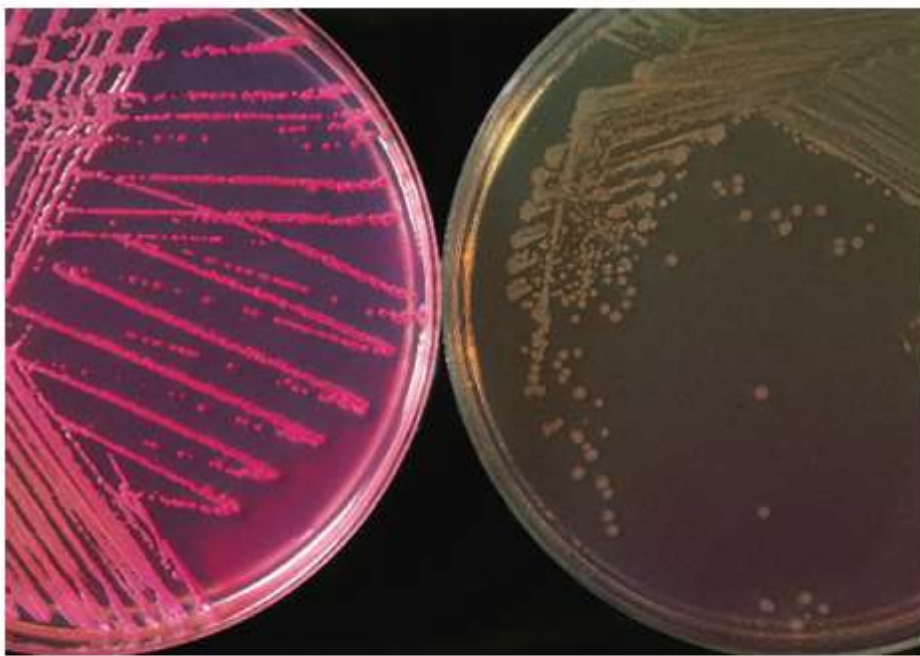


FIGURE 34-10 Left, *Escherichia coli* O157:H7 growing on MacConkey agar. Right, *E. coli* O157:H7 on sorbitol MacConkey agar. *E. coli* O157:H7 does not ferment sorbitol, whereas most other *E. coli* serotypes do ferment sorbitol.



FIGURE 34-11 *Vibrio vulnificus* growing on TCBS (thiosulfate-citrate-bile salts-sucrose). *Vibrio vulnificus* is a non-sucrose-fermenting vibrio.

TABLE 19-5 Differentiation of Common Species within the Genus *Klebsiella*

Test or Substrate	<i>K. pneumoniae</i> subsp. <i>pneumoniae</i>			<i>K. oxytoca</i>			<i>K. pneumoniae</i> subsp. <i>ozaenae</i>		
	Sign	% +	(% +)	Sign	% +	(% +)	Sign	% +	(% +)
Urease	+	95.4	(0.1)	+	90		d	0	(14.8)
Indole	–	0		+	99		–	0	
Methyl red	– or +	10		–	20		+	97.7	
Voges-Proskauer	+	98		+	96		–	0	
Citrate (Simmons)	+	98	(0.6)	+	95		d	30	(32.4)
Gelatin (22°C)	–	0	(0.2)	–	0		–	0	
Lysine decarboxylase	+	98	(0.1)	+	99		– or +	40	(6.3)
Malonate	+	92.5		+	98		–	6	
Mucate	+	90		+	93		– or +	25	
Sodium alginate (utilization)	+ or (+)	88.5	(9.2)	nd			– or (+)	0	(11)
Gas from glucose	+	96		+	97		d	50	(9.4)
Lactose	+	98.7	(1)	+	100		d	30	(61.3)
Dulcitol	– or +	30		+or–	55		–	0	
Organic acid media									
Citrate	+ or –	64.4		nd			– or +	18	
D-Tartrate	+ or –	67.1		nd			– or +	39	

Modified from Ewing WH: *Edwards and Ewing's identification of Enterobacteriaceae*, ed 4, East Norwalk, CT, 1986, Appleton and Lange.
 +, ≥90% positive within 1 or 2 days; (+), positive reaction after ≥3 days (decarboxylase tests: 3 or 4 days); –, ≥90% no reaction in 30 days; + or –, most cultures positive, some strains negative; – or +, most strains negative, some cultures positive; d, different reactions, +, (+), –, nd, no data.

TABLE 19-8 Biochemical Differentiation of Selected Members of the Genus *Salmonella*

Test	<i>S. serotype Choleraesuis</i>	<i>S. serotype Paratyphi</i>	<i>S. serotype Typhi</i>	Other*
Arabinose fermentation	-	+	-	+
Citrate utilization	V	-	-	+
Glucose gas production	+	+	-	+
H ₂ S (TSI)	V	-	+	+
Lysine decarboxylase	+	-	+	+
Ornithine decarboxylase	+	+	-	+
Rhamnose fermentation	+	+	-	+
Trehalose fermentation	-	+	+	+

Data from Farmer JJ, et al: Enterobacteriaceae: introduction and identification. In Murray PR, et al, editors: *Manual of clinical microbiology*, ed 9, Washington, DC, 2007, ASM Press.

H₂S, Hydrogen sulfide; TSI, triple sugar iron agar; -, ≤9% of strains positive; +, ≥90% of strains positive; V, 10% to 89% of strains positive.

*Typical strains in serogroups A through E.

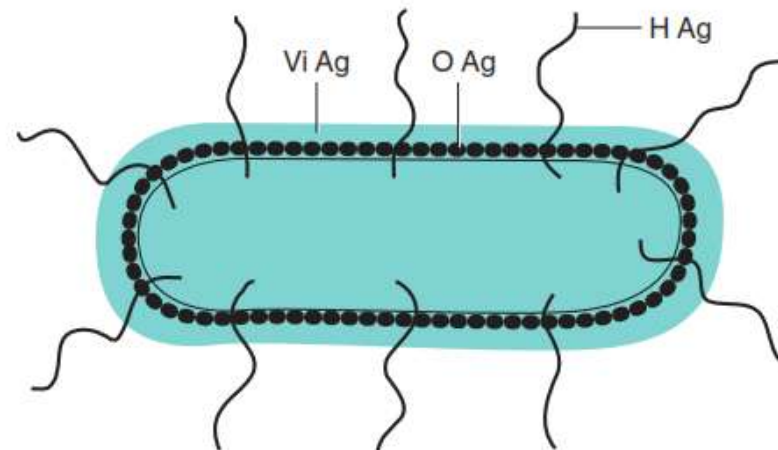


FIGURE 19-8 Antigenic structures of salmonellae used in serologic typing.

جدول ۱ - تستهای بیوشیمیایی کاربردی در افتراق سالمونلا از سایر انتروباکتریاسه ها و تشخیص سالمونلا تایفی و سالمونلا

پاراتایفی A⁽¹⁾

Test	Nontyphoidal <i>Salmonella</i> subsp. I reaction	<i>Salmonella</i> serotype Typhi reaction	<i>Salmonella</i> Paratyphi A reaction
TSI	K/A_{gas}	K/A	K/A_{gas}
Glucose	+ / gas	+	+ / gas
Lactose	-	-	-
Sucrose	-	-	-
H ₂ S(TSI)	+	+^{weak}	- or +^{weak}
Indole	-	-	-
MR	+	+	+
VP	-	-	-
Citrate(simmons)	+	-	-
Urea(Agar)	-	-	-
Lysine decarboxylase	+	+	-
Ornithine decarboxylase	+	-	+
Motility	+	+	+
ONPG	-	-	-

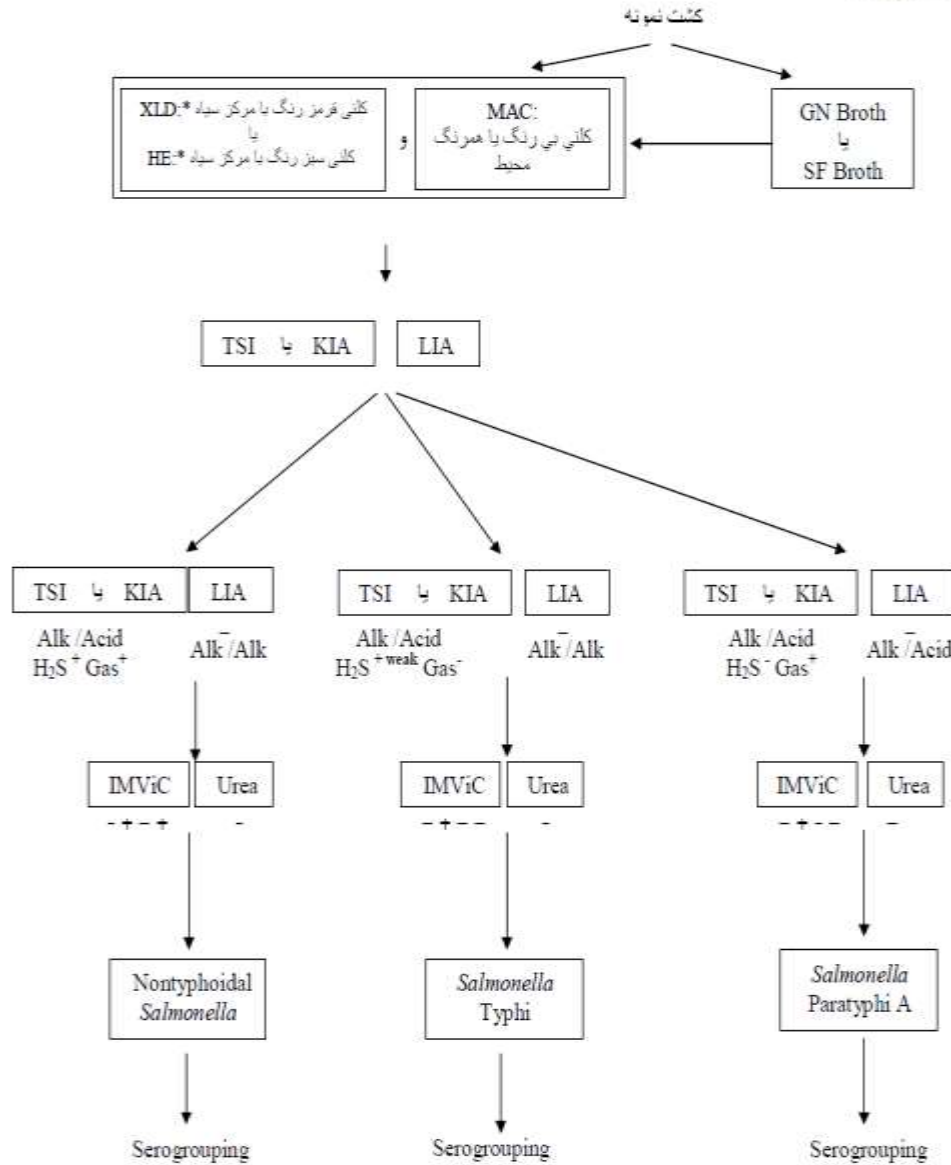
توضیحات:

۱-واکنش مثبت در ۹۰٪ موارد بعد از ۱-۲ روز ایجاد می شود.

۲- H₂S از روی محیط TSI گزارش می شود (نه SIM).

۳- تولید گاز را بر روی TSI یا KIA می توان بررسی نمود.

۴- سالمونلاها اندول منفی و اوره منفی(اوره آگار) هستند و در صورت مثبت شدن جنس سالمونلا رد می شود.



توضیحات:

• رنگ سیاه در مرکز کلنی های سالمونلا بر روی محیطهای XLD و HE ممکن است بعد از ۲۴ ساعت اول انکوباسیون ایجاد شود.
 اگر سوبه مورد آزمون از نظر واکنشهای بیوشیمیایی به طور مشخص شبیه سالمونلا است، اما با آنتی سرمهای سالمونلا آگلوتینه نمی دهد، سوبه باکتریایی مورد آزمون باید به آزمایشگاه مرکز بهداشت استان و از آن جا به آزمایشگاه همکار دانشکده بهداشت دانشگاه تهران یا انستیتو پاستور (مرجع کشوری E.coli) ارسال شود.

TABLE 19-9 Biochemical and Serologic Differentiation of *Shigella* Species

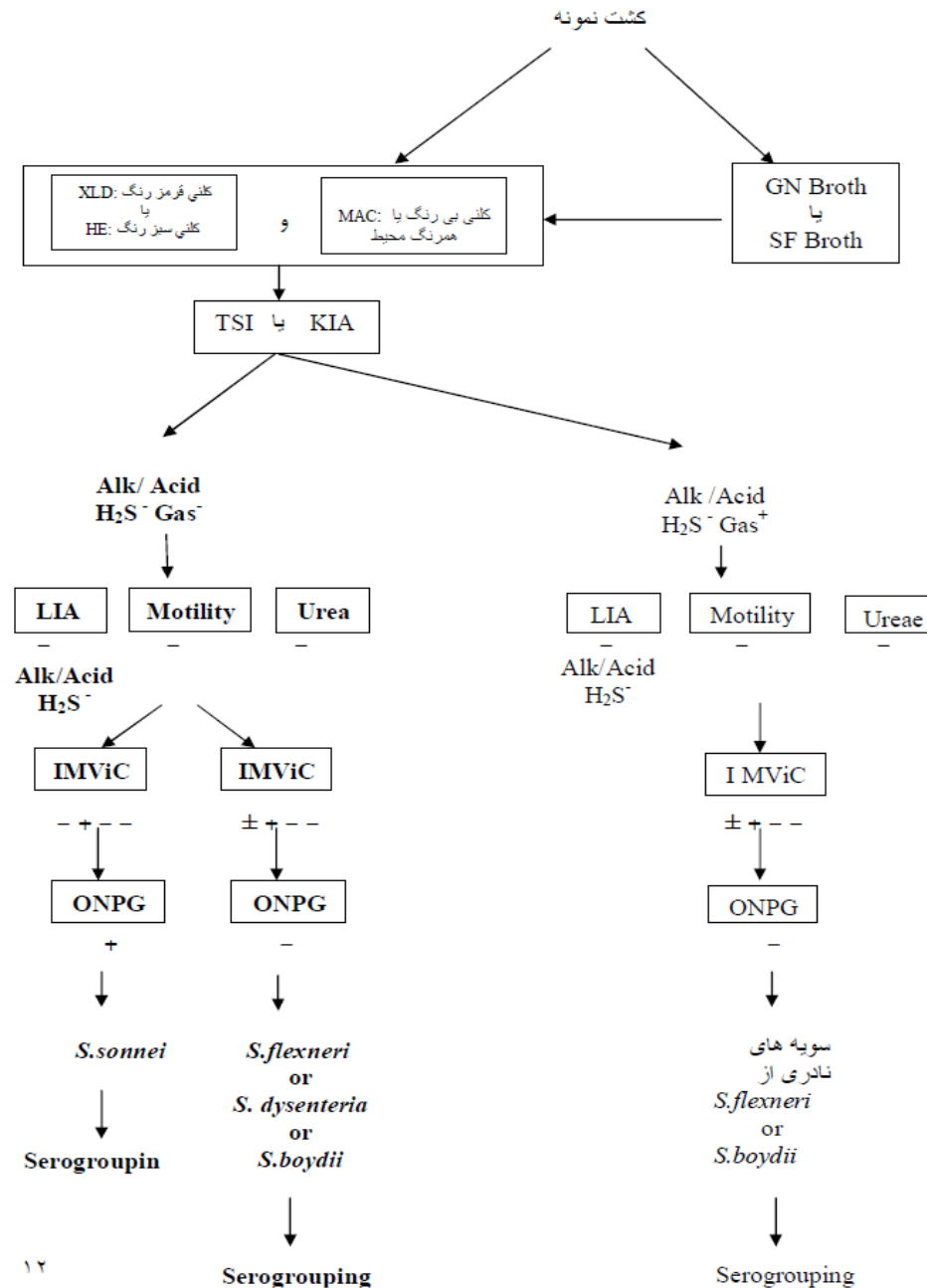
Test	<i>S. dysenteriae</i>	<i>S. flexneri</i>	<i>S. boydii</i>	<i>S. sonnei</i>
Mannitol fermentation	–	+	+	+
ONPG	V	–	V	+
Ornithine decarboxylase	–	–	–	+
Serogroup	A	B	C	D

From Farmer JJ, et al: Enterobacteriaceae: introduction and identification. In Murray PR, et al, editors: *Manual of clinical microbiology*, ed 9, Washington, DC, 2007, ASM Press.

ONPG, *O*-nitrophenyl- β -D-galactopyranoside; –, $\leq 9\%$ of strains positive; +, $\geq 90\%$ of strains positive; V, 10% to 89% of strains positive.



FIGURE 19-10 Left, Lactose-negative appearance of *Shigella sonnei* growing on MacConkey (MAC) agar at 18 to 24 hours of incubation. Right, Lactose-positive appearance of *S. sonnei* growing on MAC agar after 48 hours of incubation.



مواد و روش ها

۱	لوپ و آنس	۸	محیط MRVP و معرف متیل رد، آلفانفتل و پتاس
۲	محیط کشت NA	۹	محیط TSI
۳	محیط کشت Mac	۱۰	محیط LIA
۴	محیط کشت EMB	۱۱	محیط SS agar
۵	کیت رنگ امیزی گرم	۱۲	میکروسکوپ و روغن امرسیون
۶	محیط SIM و معرف کواکس	۱۳	انکوباتور
۷	محیط سیمون سیترات		

کار عملی

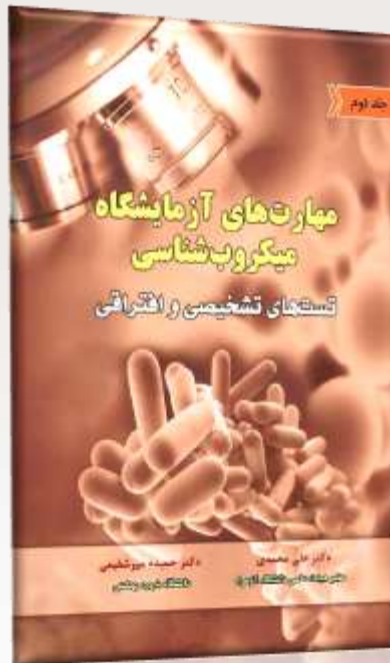
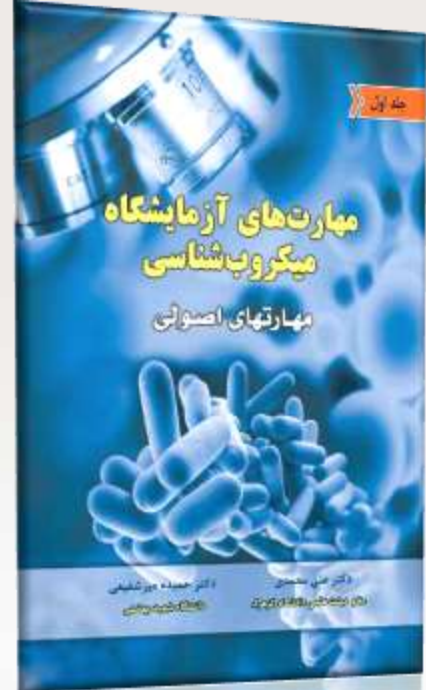
ادامه.

- (۱) نمونه مجهول را در سه محیط کشت Mac، EMB و NA کشت ۴ منطقه ای دهید.
- (۲) پس از رشد از کلنی های مختلف رنگ امیزی گرم تهیه نمایید.
- (۳) برای هر تعداد کلنی که ملاحظه می نمایید استفاده از محیط های انتخابی را آغاز نمایید.
- (۴) از محیط های انتخابی که در قسمت روش ها ذکر شد استفاده نمایید.
- (۵) باکتری ها را پس از کشت در انکوباتور ۳۷ درجه قرار داده و پس از ۱۸-۲۴ ساعت نتیجه را بخوانید.

نام باکتری را با توجه به جدول کمکی زیر ذکر نمائید. شماره ی کد نمونه را در گزارش کار بیان نمائید. تفسیر خود را از نتیجه ذکر کنید.

تفسیر	نتایج
مشکوک به شیگلا	K/K: TSI بدون گاز یا H2 اوره منفی اکسیداز منفی
مشکوک به سالمونلا	K/A: TSI بدون گاز اوره منفی اکسیداز منفی
مشکوک به ویبریو ، آئرو موناس، پلزیوموناس	A/A یا A /K: TSI با یا بدون گاز اوره منفی اکسیداز منفی
مشکوک به یرسینیا	A/A یا K /A: TSI بدون گاز یا H2S اوره منفی اکسیداز منفی
مشکوک به شیگلا	K/A : TSI بدون گاز یا H2S اوره منفی
مشکوک به سالمونلا	K/A : TSI مثبت H2S اوره منفی

Dr A.Mohammadi



منابع:

- **مهارت های آزمایشگاه میکروبی شناسی** ، جلد ۱- ۳
- دکتر علی محمدی-عضو هیئت علمی دانشگاه الزهرا (س).
- دکتر حمیده میرشفیعی - دانشگاه شهید بهشتی
- Mahon, C. R., Lehman, D. C., & Manuselis, G. (2018). *Textbook of Diagnostic Microbiology-E-Book*. Elsevier Health Sciences.
- Tille, P. (2016). *Bailey & Scott's Diagnostic Microbiology-E-Book*. Elsevier Health Sciences.
- Chamberlain, N. (2008). *Medical microbiology: The big picture*. McGraw Hill Professional.
- Carroll, K. C., Butel, J., & Morse, S. (2016). *Jawetz Melnick & Adelbergs Medical Microbiology 27 E*. McGraw Hill Professional.