

Enteric Pathogens

Susan Harrington PhD D(ABMM)
MLS(ASCP)CM
2022

Today is all about...



Enteric pathogens

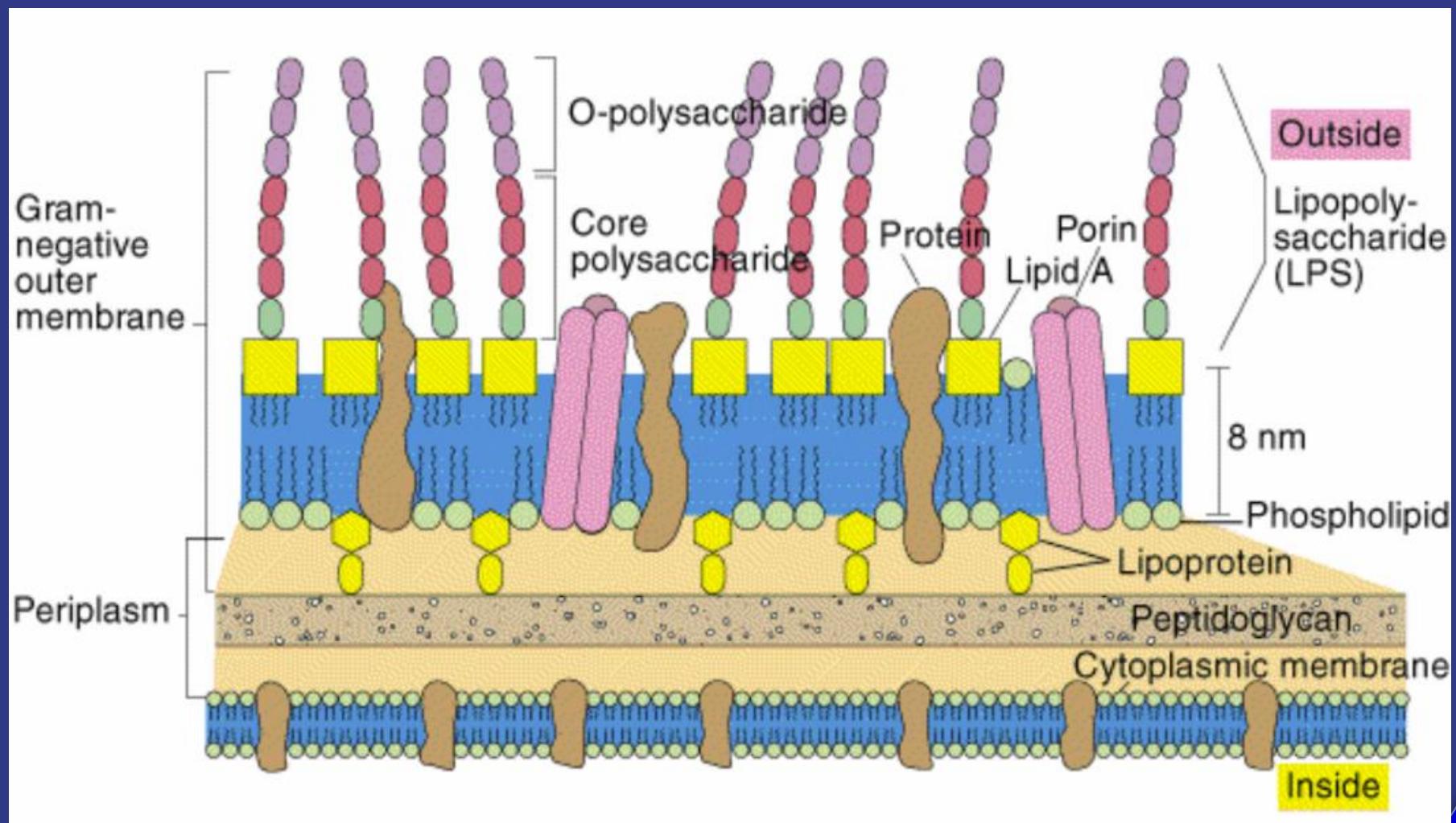
Enterobacteriales

Oxidase-positive, gram-negative
rods

Enterobacteriales

- Gram negative rod ($0.3\text{-}1.0 \times 1\text{-}6 \mu\text{m}$)
- Non-spore forming
- Facultative anaerobes
- If motile, peritrichous flagella present
- Good growth on MacConkey (MAC) agar*
- Ferment glucose
- Catalase positive
- Oxidase negative*
- Reduction of nitrate to nitrite

Gram-negative cell wall



Antigens - Serologic Grouping

O = somatic

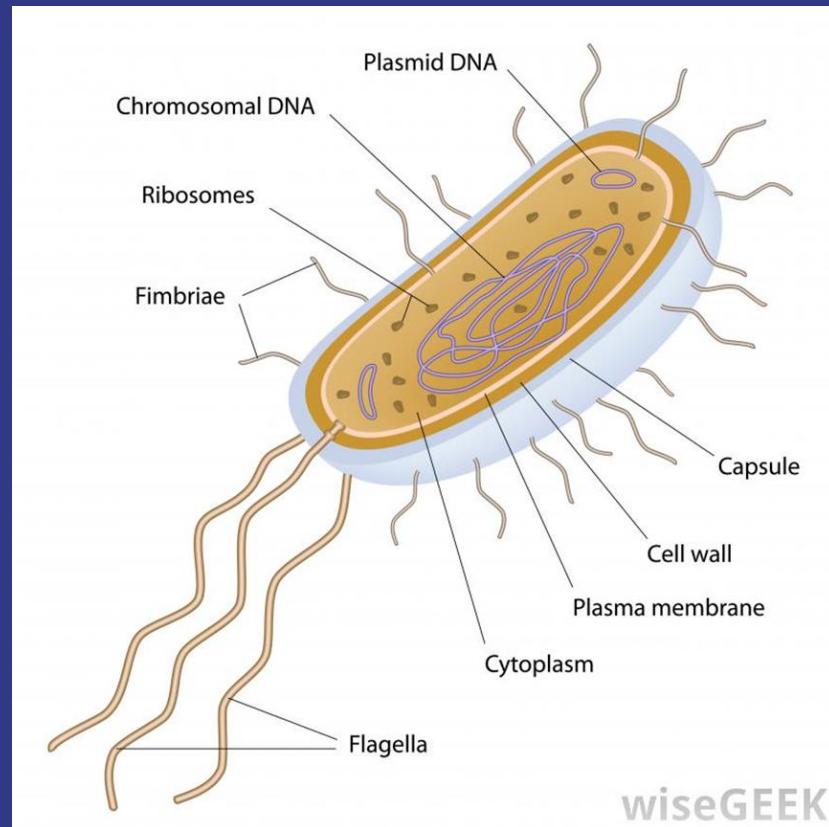
- heat-stable
- cell wall

H = flagellar

- heat-labile
- flagellar surface

K = capsular

- heat-labile polysaccharide
- certain encapsulated species



wiseGEEK

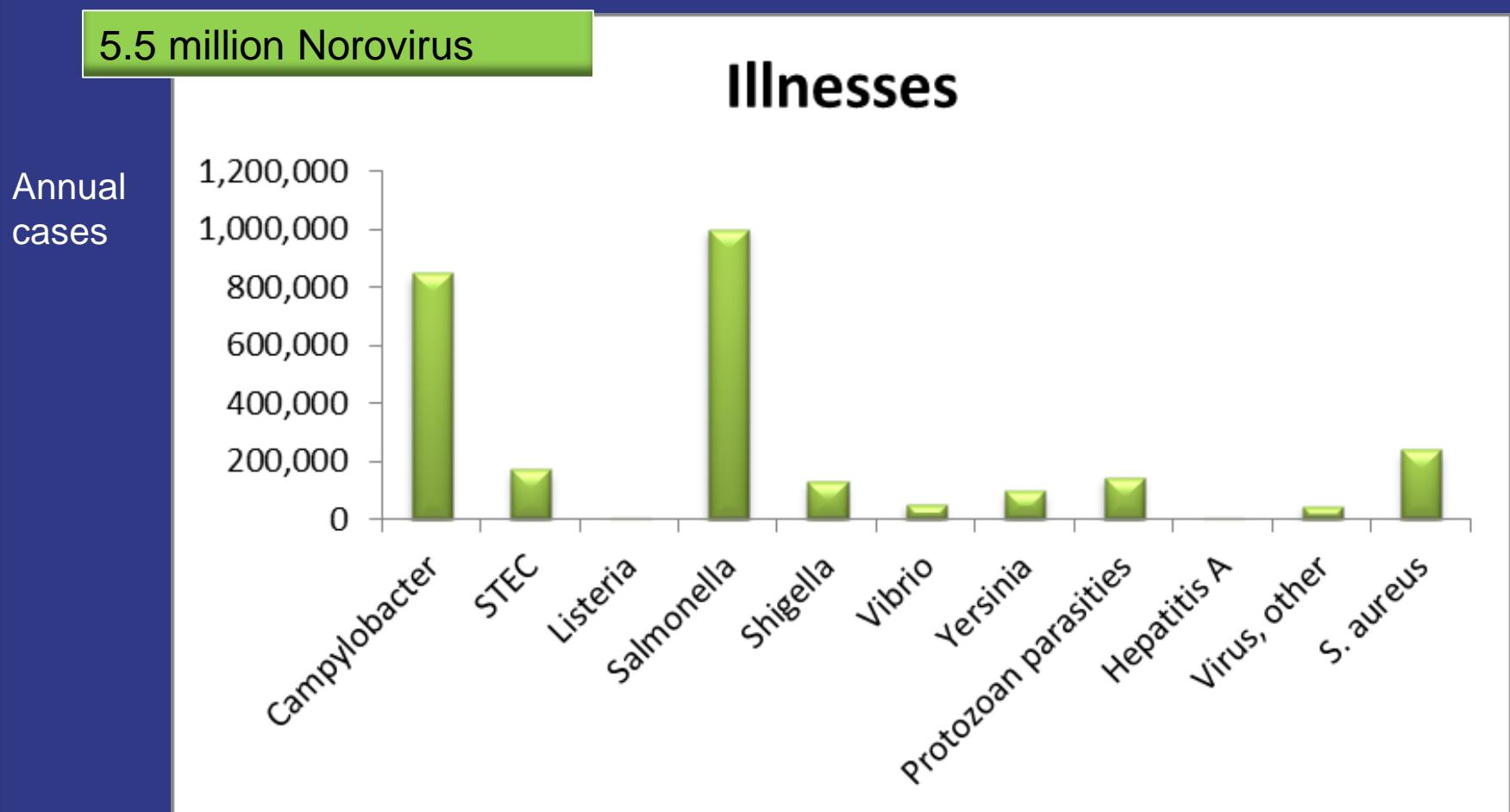
Clinical Significance

Intestinal infections

- *Salmonella*
- *Shigella*
- Pathogenic *E. coli*
- *Yersinia*
- *Plesiomonas*
- *Aeromonas*
- *Vibrio*
- *Campylobacter*

[*Clostridium difficile* – antibiotic associated colitis]

Burden of Foodborne Illness United States, 2000-2008



Clinical Significance

Extraintestinal Infections - examples

<i>Salmonella</i> ser. Typhi	Typhoid fever
<i>Yersinia pestis</i>	Regional lymph node buboes
	Pneumonic fever
	septicemia
<i>Aeromonas</i> spp.	Wound infections
<i>Campylobacter</i> spp.	bloodstream infection

Specimen Collection & Processing

- Extra-intestinal Infections
 - utilize normal protocols for collecting blood, respiratory, wound and urine specimens.

Specimen Collection & Transport

Gastroenteritis

- Obtain specimen early in the illness.
- Don't culture pts in hospital >3d.
- Stool. Not swab.
- Select bloody and mucoid portions.
- Culture within 2 hrs or use transport medium (Cary-Blair). Keep refrigerated.



Evaluation of Inflammation

- Gram stain & methylene blue stains – limited value
 - WBCs degrade in stool



- Fecal lactoferrin immunoassays
 - Iron-binding protein in granules of PMNs
 - Antibacterial & antifungal activity
 - Not sensitive/specific enough to rule-in or rule-out infectious diarrhea

Media for Isolation

- Non-selective, enriched media
 - Trypticase soy blood agar
- Selective media – antibiotics, salts, dyes, etc.
 - Campylobacter agars, CHROMagars
- Selective and Enrichment broth
 - Selenite or GN; subculture to selective agar
- Selective & Differential Media
 - MacConkey, XLD & HEK agar (*Salmonella*, *Shigella*)
 - CIN agar (*Yersinia*)
 - MacConkey Sorbitol (*E. coli* O157)
 - TCBS (*Vibrio*)

MacConkey Agar

- Bile salts
- Crystal violet
- Neutral red
- Lactose



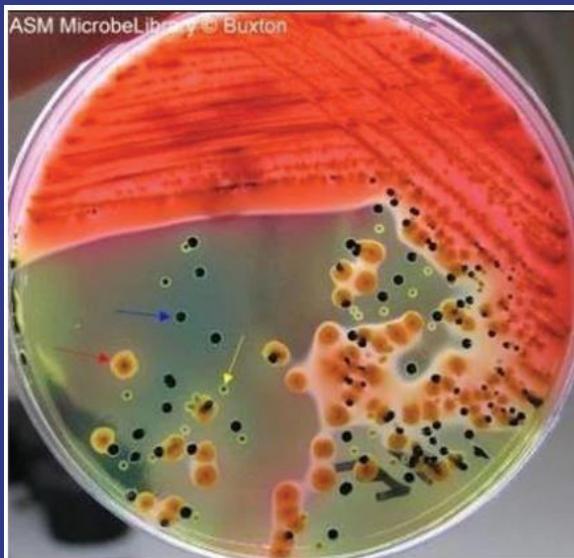
Lactose fermenters: *E. coli, Klebsiella, Enterobacter.*

Non-lactose fermenters: *Proteus, Providencia, Morganella, Salmonella, Shigella.*

Sometimes *E. coli* and *Enterobacter*.

Citrobacter species +/-

HE (Hektoen enteric) agar



- *Salmonella* – green, black center due to H_2S prodn
- *Shigella* – green
- Lactose-fermenters – yellow-orange

Ingredients:

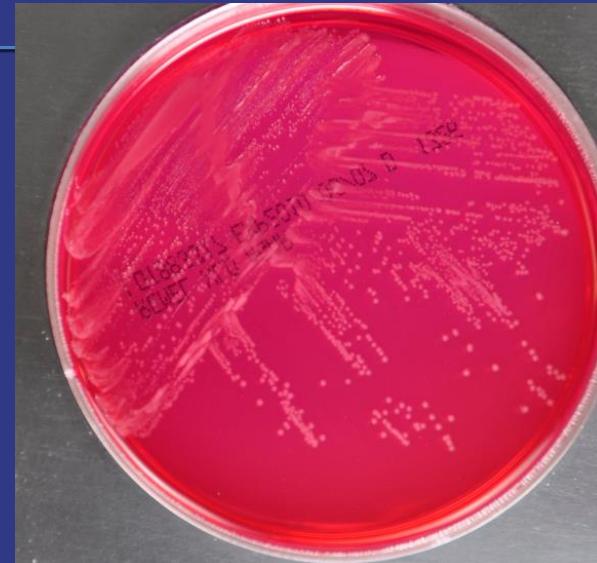
- yeast extract
- bile salts (deoxycholate)
- lactose, sucrose, salicin
- bromthymol blue – yellow if acid; blue if base
- ferric ammonium citrate & sodium thiosulfate

Xylose Lysine Deoxycholate

Salmonella – red w/black center

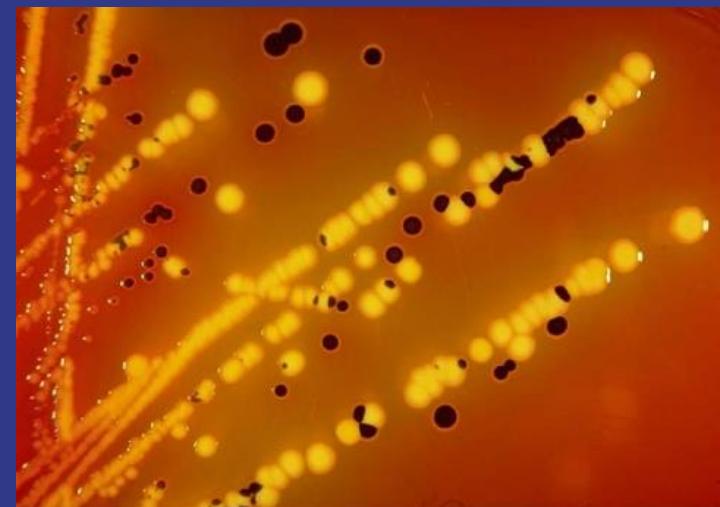
Shigella – colorless (red of media)

LFs - yellow



Ingredients:

- yeast extract
- xylose, lactose, sucrose
- lysine
- bile salts (deoxycholate)
- ferric ammonium citrate & sodium thiosulfate
- phenol red - yellow if acid/red if base

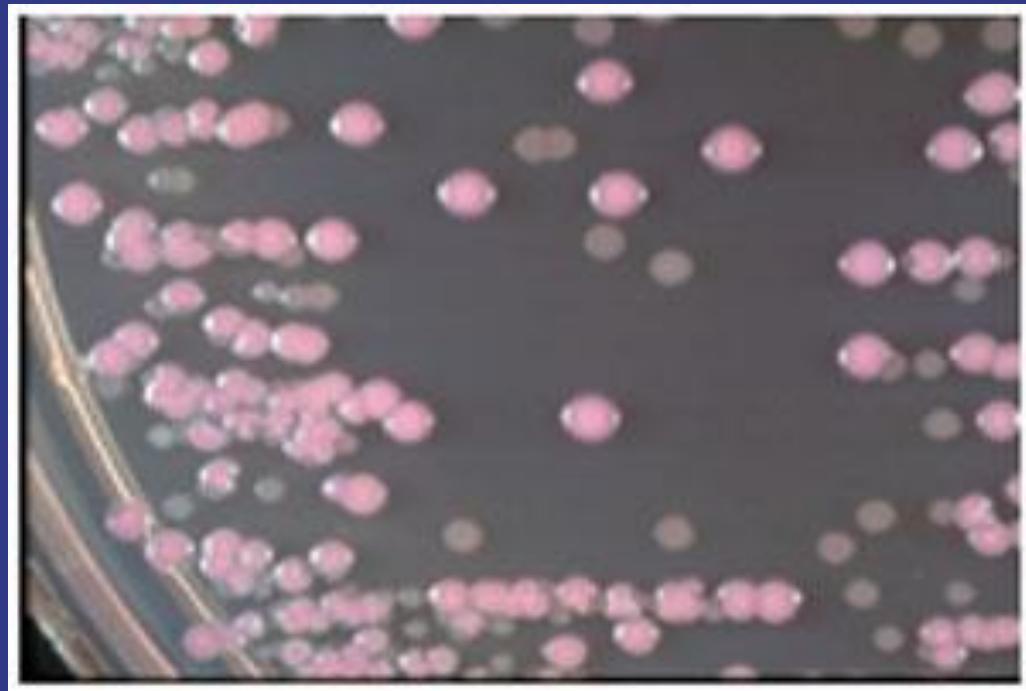


Sorbitol - MacConkey

- *E. coli* O157 does not ferment sorbitol

Ingredients:

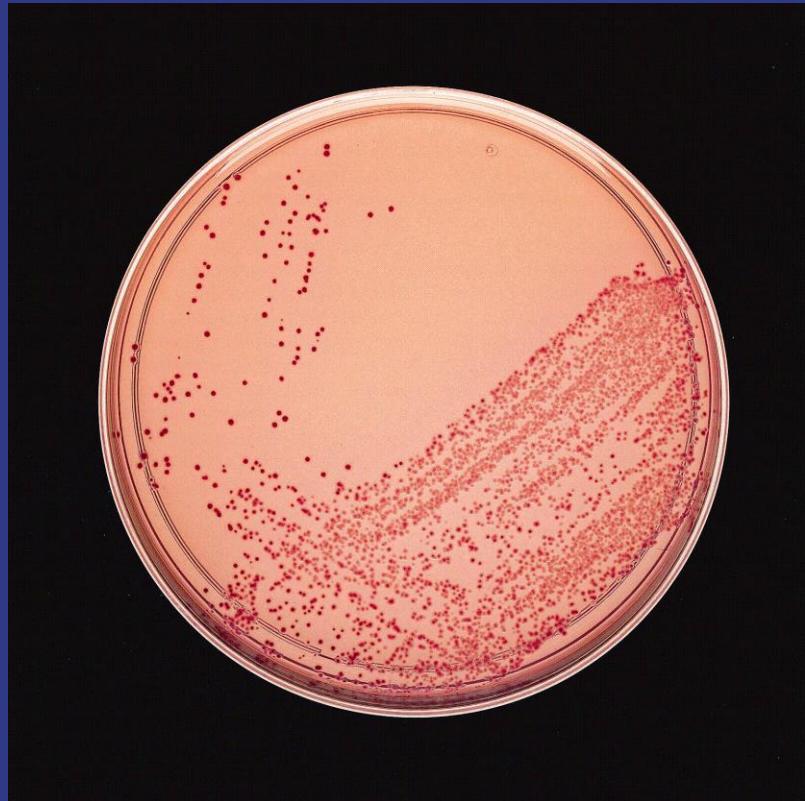
- bile salts
- crystal violet
- neutral red
- sorbitol



CIN Agar

Y. enterocolitica & *Y. pseudotuberculosis*

- Incubate at 25 - 30°C
- CIN - selective/differential
- Ingredients:
mannitol, cefsulodin,
irgasin, novobiocin, Na
deoxycholate, crystal
violet
- Also supports *Aeromonas*
- Pale colony w/red center
due to mannitol ferm



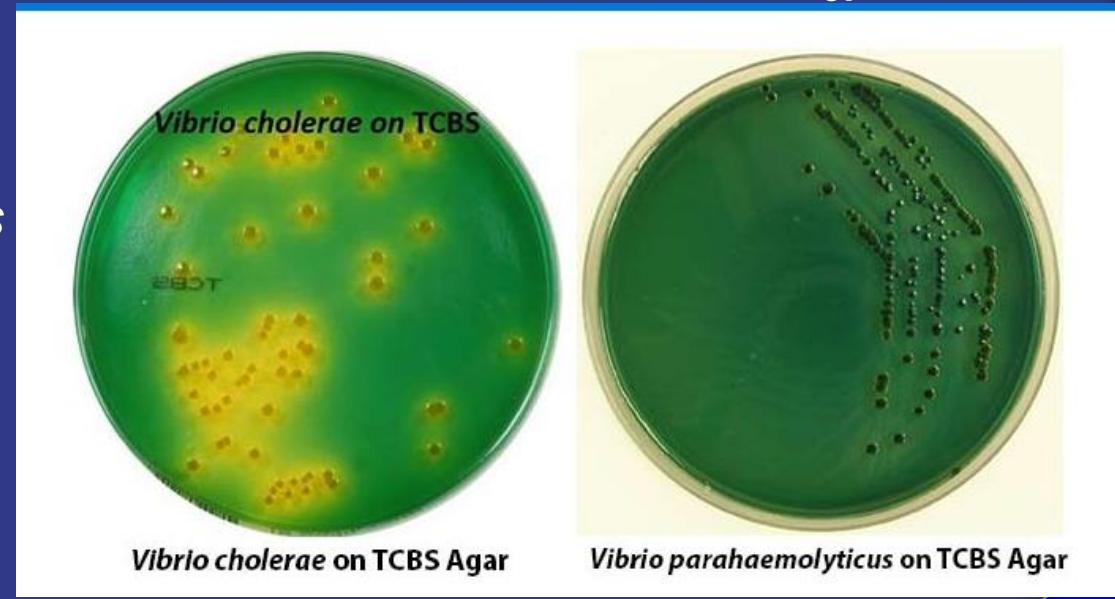
TCBS Agar

thiosulfate citrate bile salts sucrose
(bromthymol blue indicator)

Appearance of Colonies:

- Flat, 2-3 mm in diameter, yellow
V. cholerae
- Small, blue-green center
V. parahaemolyticus, *V. vulnificus*
- Large, yellow
V. alginolyticus
- Blue
Pseudomonas, *Aeromonas*
- Very small, translucent
Enterobacterales

Microbiology Info.com



Broth Media Options

- Selenite F broth

- Recommended for *Salmonella*
- Digest of casein, lactose, sodium selenite

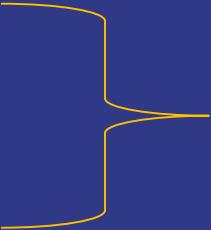
- GN broth

- Enriches for *Salmonella* & *Shigella*
- Mannitol – enhances for S & S
- Digest of casein, dextrose
- Sodium citrate, sodium deoxycholate

- Subculture to selective media after incubation

Culture

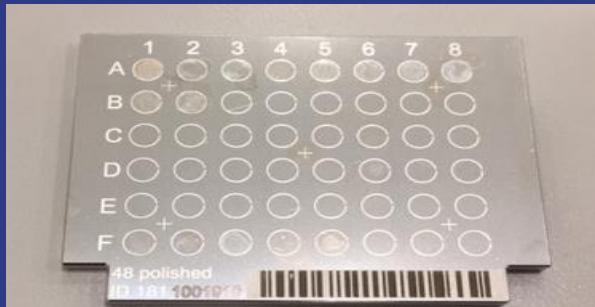
Routine: *Salmonella*, *Shigella*, Shiga toxin-producing *E. coli*, *Campylobacter*.

- MAC
 - HE or XLD
 - SMAC
 - Campy Selective agar – Incubate 72 hrs, 42°C, miroaerophilic
- 
- Incubate up to 48 hrs at 35–37°C

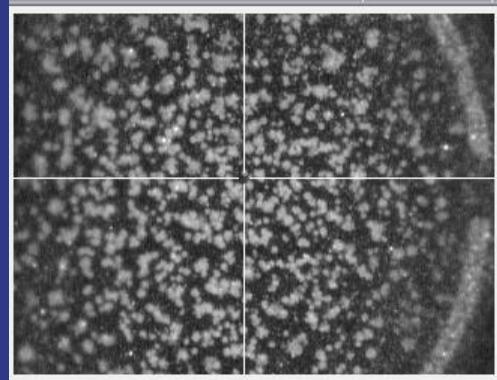
Additional media based on exposures or history:

- BAP
- CIN – incubate RT
- TCBS

Pick “suspicious” colonies for MALDI TOF MS

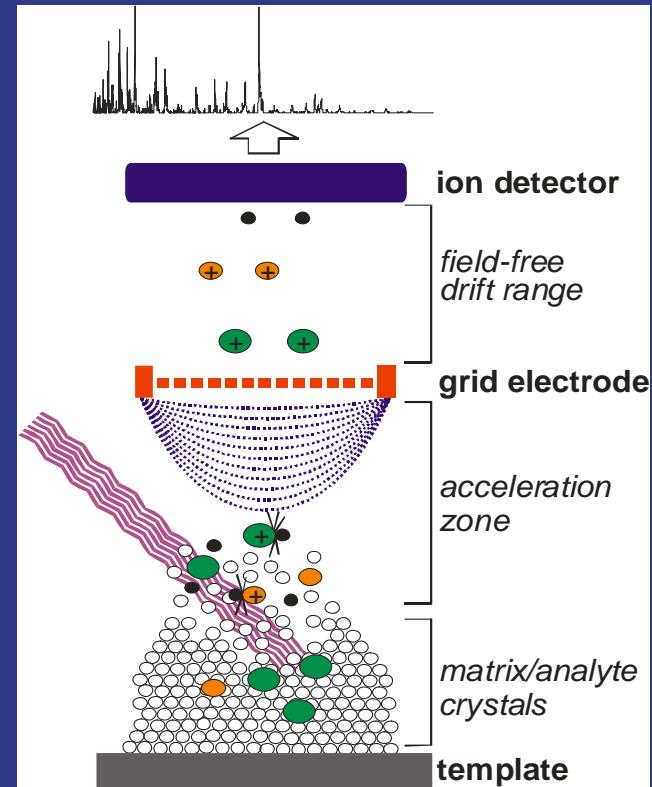


MALDI TOF
Target Plate



Camera Image of spot

detection
separation
acceleration
ionization
desorption



Courtesy bioMerieux

The Enteric Pathogens

- Four genera of *Enterobacterales* are common enteric pathogens, isolated from the intestines of humans and animals.
 - *Salmonella*
 - *Shigella*
 - *Escherichia*; several pathotypes
 - *Yersinia*

Shigella: Clinical Significance

- Causes both bloody and non-bloody diarrhea
 - Shigellosis begins with watery diarrhea, fever, and cramps, and may progress to scant stools with blood, mucous and pus.
 - *Shigella dysenteriae* is associated with dysentary, due to Shiga toxin.
- *Shigella* rarely causes extra-intestinal infections.
- Complications include:
 - HUS (*S. dysenteriae*)
 - Reiter's chronic arthritis syndrome (*S. flexneri*)

Shigella Epidemiology

- Humans and other large primates are the only natural reservoir for *Shigella*.
- Transmission occurs by person-to-person spread or ingestion of contaminated food or water.
- Infection dose is ~10-100 organisms

Shigella

- Same 16S as *Escherichia coli*
 - *Shigella* is less fermentative; lysine decarboxylase -; non-motile; no gas produced.
- 4 serogroups:
 - “A” - *S. dysenteriae*
 - “B” - *S. flexneri*
 - “C” - *S. boydii*
 - “D” - *S. sonnei*

Shigella Epidemiology

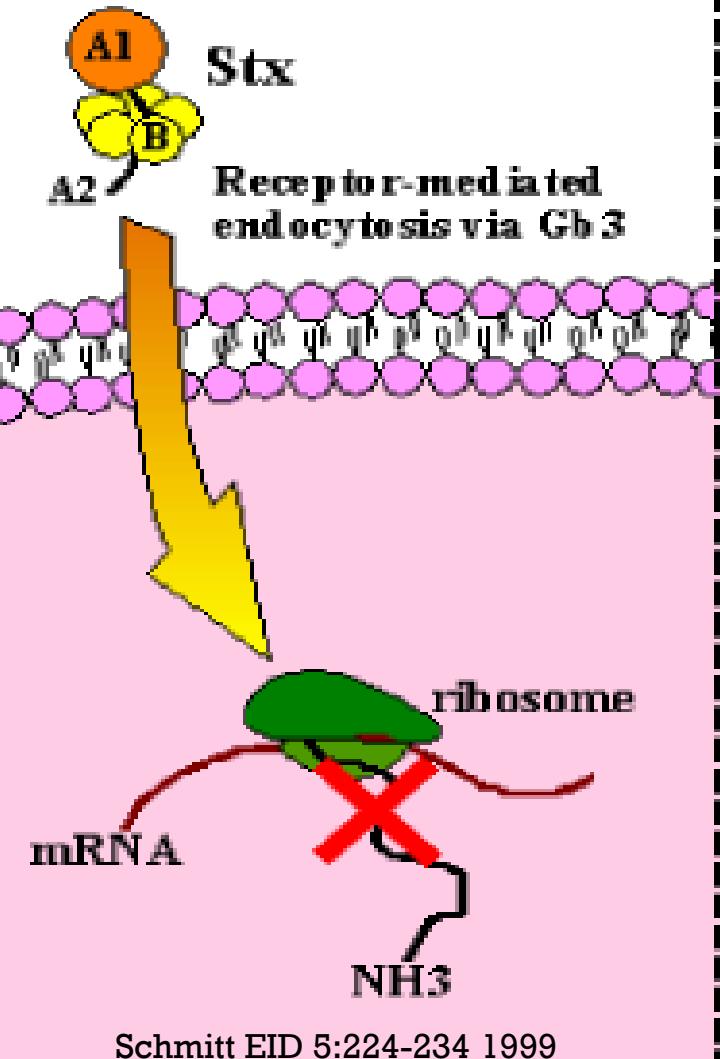
- *S. sonnei* - developed countries
- *S. flexneri* - developing countries

- *S. dysenteriae* - developing world;
 - can get disease from contaminated food/water; only infects humans

Shiga Toxin

- Secreted by *S. dysenteriae*, EHEC
- A₁B₅ toxin encoded in bacteriophage
- N-glycosidase cleaves 28S rRNA, causing decreased protein synthesis, leading to cell death
- Colon, kidney (HUS), CNS damage

B. INHIBIT PROTEIN SYNTHESIS



Salmonella – Clinical Significance

Non-typoidal *Salmonella*:

- Watery diarrhea, nausea, vomiting, fever, abdominal cramps.
- 8-36 hrs post exposure; May last >1 week.
- Extra-intestinal infections uncommon



Transmission:

- Foods of animal origin
- Direct animal contact (pet turtles, reptiles)
- Water, rarely human contact

Salmonella Clinical Significance

Typhoid fever (*Salmonella* serotype Typhi)

- Common in developing world, rare in the U.S.
 - Humans are only known reservoir.
 - High fever, headache, abdominal tenderness & inflammation.
 - Usually without diarrhea.
 - Septicemia – disseminates to liver, spleen, bone marrow
 - Low infectious dose (<10³) and 1-6 weeks incubation
- *Salmonella* ser. Paratyphi & Choleraesuis cause similar, less severe syndrome.

Salmonella Identification

- Two species are currently recognized:
 - *S. enterica* (6 subspecies)
 - *S. enterica* subspecies *enterica* (subspecies I) - humans and warm-blooded animals
 - Subspecies II, IIIa, IIIb, IV, VI - cold-blooded animals and the environment
 - *S. bongori* (subspecies V)
- Biochemicals/MALDI used to identify *Salmonella*
Citrobacter: urease (+); lysine (-)
Salmonella: urease (-); lysine (+)
Salmonella produce H₂S; except *Salmonella* ser Paratyphi

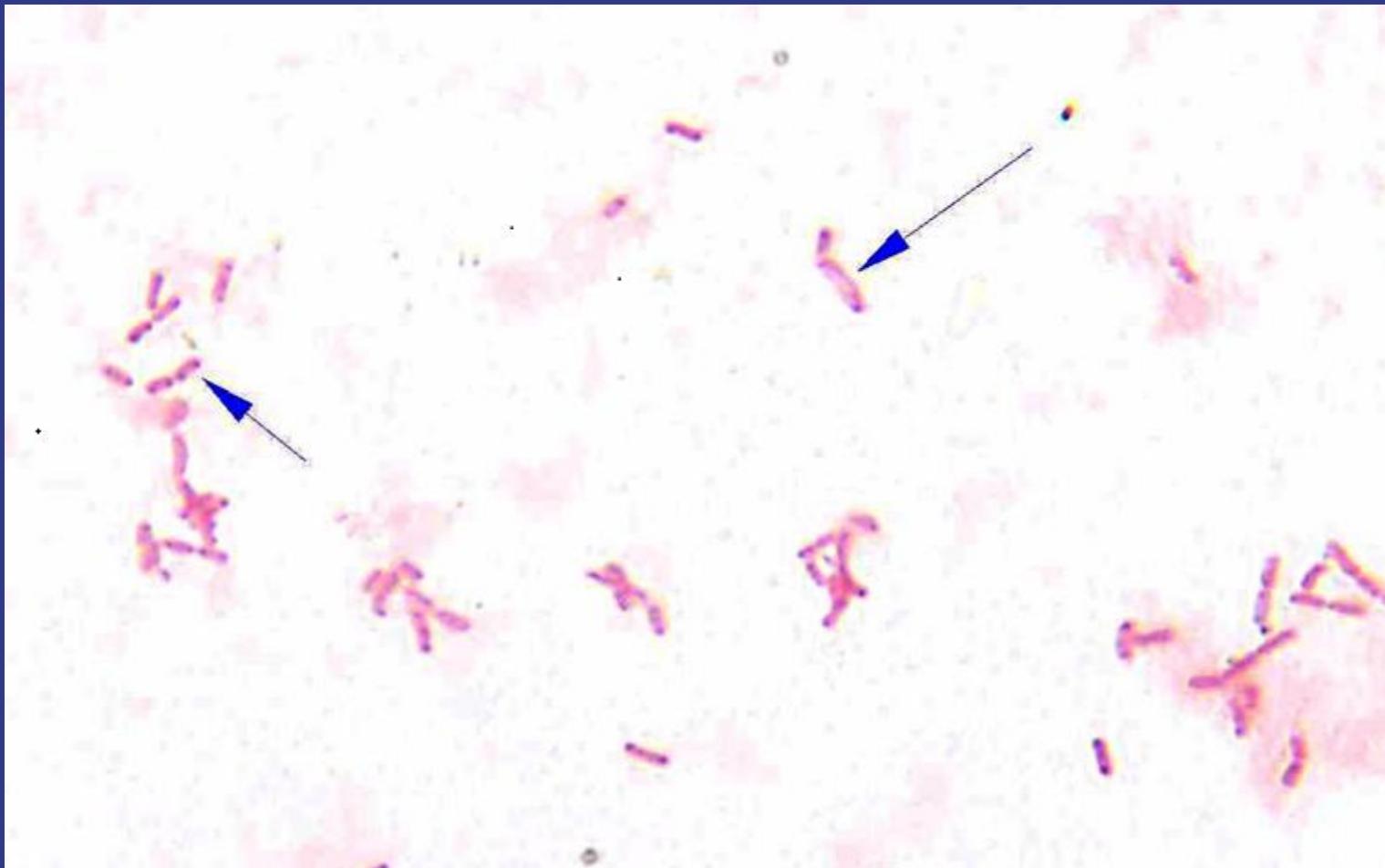
Referral to Public Health

- “O” group polyvalent antisera to confirm *Salmonella* species; 2,501 “O” serotypes
 - Most are “O” groups A through E.
- Public Health does serotyping with 3 antigens:
 - O - LPS
 - Vi - capsule
 - H - flagella
- *Salmonella ser. Typhi* is O Ag group “D” and Vi positive

Yersinia

- Currently, 18 species.
 - *Yersinia pestis*, *Y. pseudotuberculosis*, and some strains of *Y. enterocolitica* are pathogenic to humans.
- Non-spore forming rods or coccoid cells
- Motile at 22-30°C, but not at 37°C.
- Optimally grow at 25-28°C; cold enrichment
- Urease positive
- *Y. pestis* is a bioterrorism organism:
 - non-motile; urease negative; catalase pos; oxidase neg

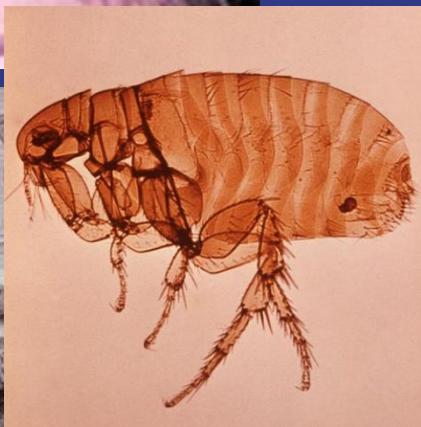
Yersinia Gram stain – pronounced bipolar staining



Yersinia - Natural reservoirs

- Pathogens require a host, but can survive extended periods externally.
 - *Y. pestis*
 - Rodents, domestic cats, prairie dogs, etc.
 - Transmission is mainly through the bites of infected fleas, cat scratches.
 - Desert southwest. California. Oregon.
 - *Y. enterocolitica*
 - Humans and all warm-blooded animals, esp. pigs
 - Sometimes in reptiles, fish and shellfish

Clinical Significance - Plague



- Three syndromes:

- Bubonic – bacteria spread to regional lymph node, developing “bubo”.
- Pneumonic – fever, malaise. Then cough, chest pain, hemoptysis. Highly infectious.
- Septicemia – CFR = 90% if untreated. Death in 1 day.
 - High fever, delirium, seizures, shock, DIC, black hemorrhagic splotches = “the Black Death”

Clinical Significance – *Y. enterocolitica*

- Acquired by ingestion of contaminated food and water.

- Incubation period 4-7 days.
- Adheres to & invades M cells of terminal ileum
- Symptoms are diarrhea, sometimes bloody, fever, vomiting, and abdominal pain.

(like appendicitis because mesenteric lymph nodes are involved and enlarged).

- Associated with

- Transfusion reactions w/rbcs
- Iron overload & hemolytic anemias – pts get septicemia
- Preparation of chitterlings

Antimicrobial Therapy

● *Shigella*

- Ciprofloxacin or azithromycin
- Perform susceptibility testing including: ampicillin, quinolone, trimethoprim/sulfa; report ceftriaxone if blood

● *Salmonella*

- Not usually treated if intestinal infection
- If typhoid fever or invasive disease, report abx as for *Shigella*
- Some highly resistant strains reported worldwide.

● *Yersinia*

- Not usually treated if intestinal infection
- *Y. pestis*: Streptomycin, doxycycline & ciprofloxacin if BT attack

Plesiomonas shigelloides

- Found in soil & fresh water
- Undercooked seafood & contaminated water
- Clinical: watery diarrhea; may be persistent or prolonged; can become invasive dysentery-like
- Abdominal pain common; fever, vomiting occur
- Usually self-limiting

Identification:

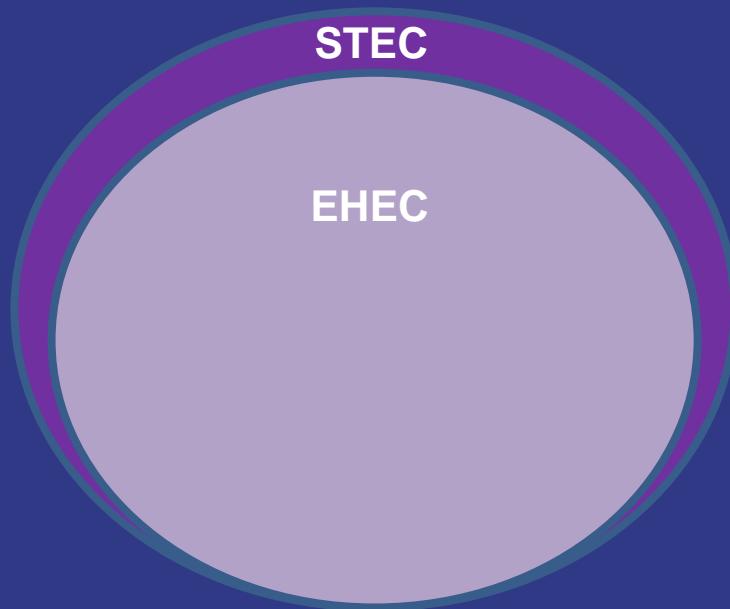
- Only oxidase + *Enterobacterales*
- Inositol +; distinguishes from *Aeromonas*
- Identifies w/biochems or MALDI TOF
- May cross-rx w/*Shigella* antisera

Escherichia

- There are 6 categories of diarrheagenic *E. coli*:
 - Shiga-toxin producing *E. coli* (STEC) aka enterohemorrhagic *E. coli*. (EHEC)
 - Enterotoxigenic *E. coli* (ETEC)
 - Enteropathogenic *E. coli* (EPEC)
 - Enteroinvasive *E. coli* (EIEC)
 - Enteroaggregative *E. coli* (EAEC)
 - Diffusely adherent *E. coli* (DAEC)

EHEC vs. STEC

- EHEC – Pathogenic *E. coli* w/specific virulence factors: Shiga toxin & LEE pathogenicity island
- STEC – Shiga-toxin producing *E. coli*.



STEC

- STEC cause a broad spectrum of illnesses:
 - Mild, non-bloody diarrhea
 - Bloody diarrhea (hemorrhagic colitis)
 - Hemolytic uremic syndrome (HUS)– approximately 6% of O157 STEC infections
- HUS is a condition characterized by:
 - Microangiopathic hemolytic anemia
 - Thrombocytopenia
 - Acute renal failure
- STEC causes about 80% of HUS in North America

STEC

- Ground beef is imp vehicle of transmission
- Others:
 - Raw milk
 - Sausage
 - Unchlorinated municipal water
 - Apple cider
 - Raw vegetables
 - Salads - spinach
 - Mayonnaise
 - Petting Zoos
- The infectious dose is low: 10-100 CFU



STEC

- Two Shiga toxins (verotoxins): Stx1 and Stx2
 - Stx1 = Stx from *Shigella dysenteriae*
- Additional virulence factors contribute to pathogenicity.
- *E. coli* O157:H7 is common serotype
- BUT: about 40-50% of STEC disease in U.S. is caused by other serotypes.
- Best to test for presence of toxin.

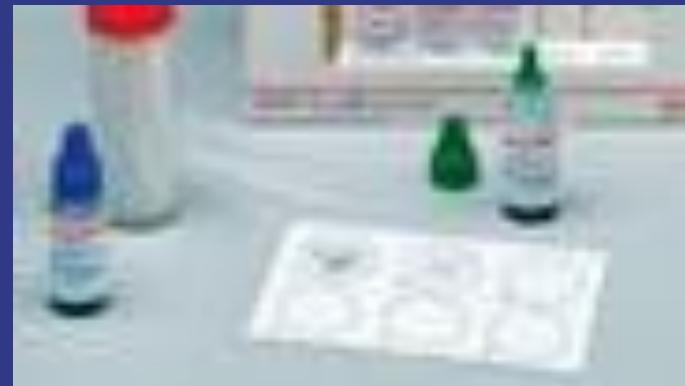
Lab Methods for STEC

- Culture on SMAC = MacConkey Sorbitol Agar or CHROMagar
- *E. coli* O157 does not ferment sorbitol

- Confirm identification biochemically/MUG neg



Latex agglutination for O157



Toxin Assays

- Culture stool O/N in MacConkey or GN broth
- Perform an EIA or PCR for Shiga-toxins
- Send broth (or isolate) to State Health Dept for strain typing

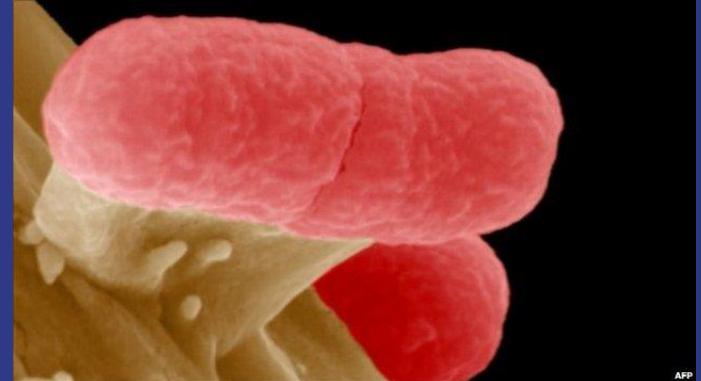


Other Pathogenic *E. coli*

- watery diarrhea in developing world

- Enteropathogenic *E. coli* (EPEC)

- acute, may persist, childhood
- LEE Pathogenicity island



- Enterotoxigenic *E. coli* (ETEC)

- abrupt onset, childhood & travelers diarrhea
- Labile and stable toxins (LT & ST)

- Enteroinvasive *E. coli* (EIEC)

- sometimes dysentery, invasive like *Shigella*; food and water-borne

Other Pathogenic *E. coli*

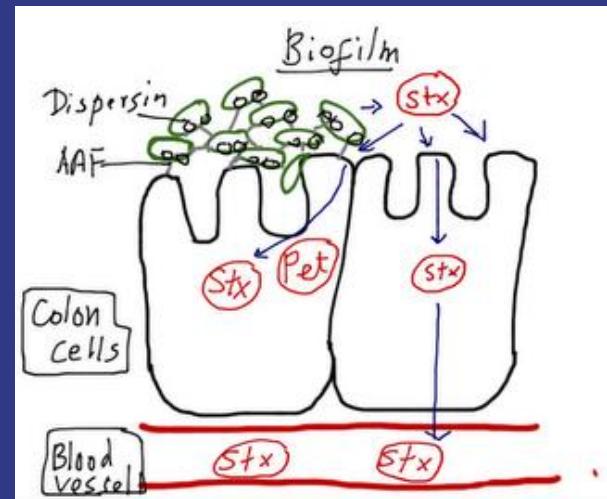
-watery diarrhea in developing world

- Enteroaggregative *E. coli* (EAEC)

- mucoid, persistent;
- infant, food-borne, travelers, AIDS
- Virulence factors cause adherence in aggregates

- ◎ Diffusely adherent *E. coli* (DAEC)

- young children, associated with diarrheal disease & UTI



Non-Enterobacteriales – Agents of Gastrointestinal Illness

Vibrio species

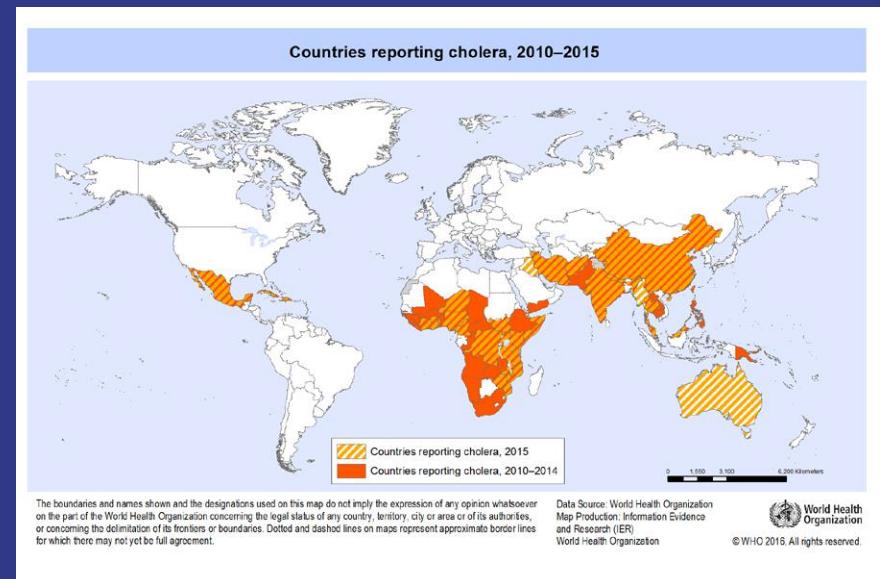
Oxidase positive
Small, curved
Polar flagella

- Habitat: salt water, marine animals, shellfish
- 10 species - diarrhea & extra-intestinal infections

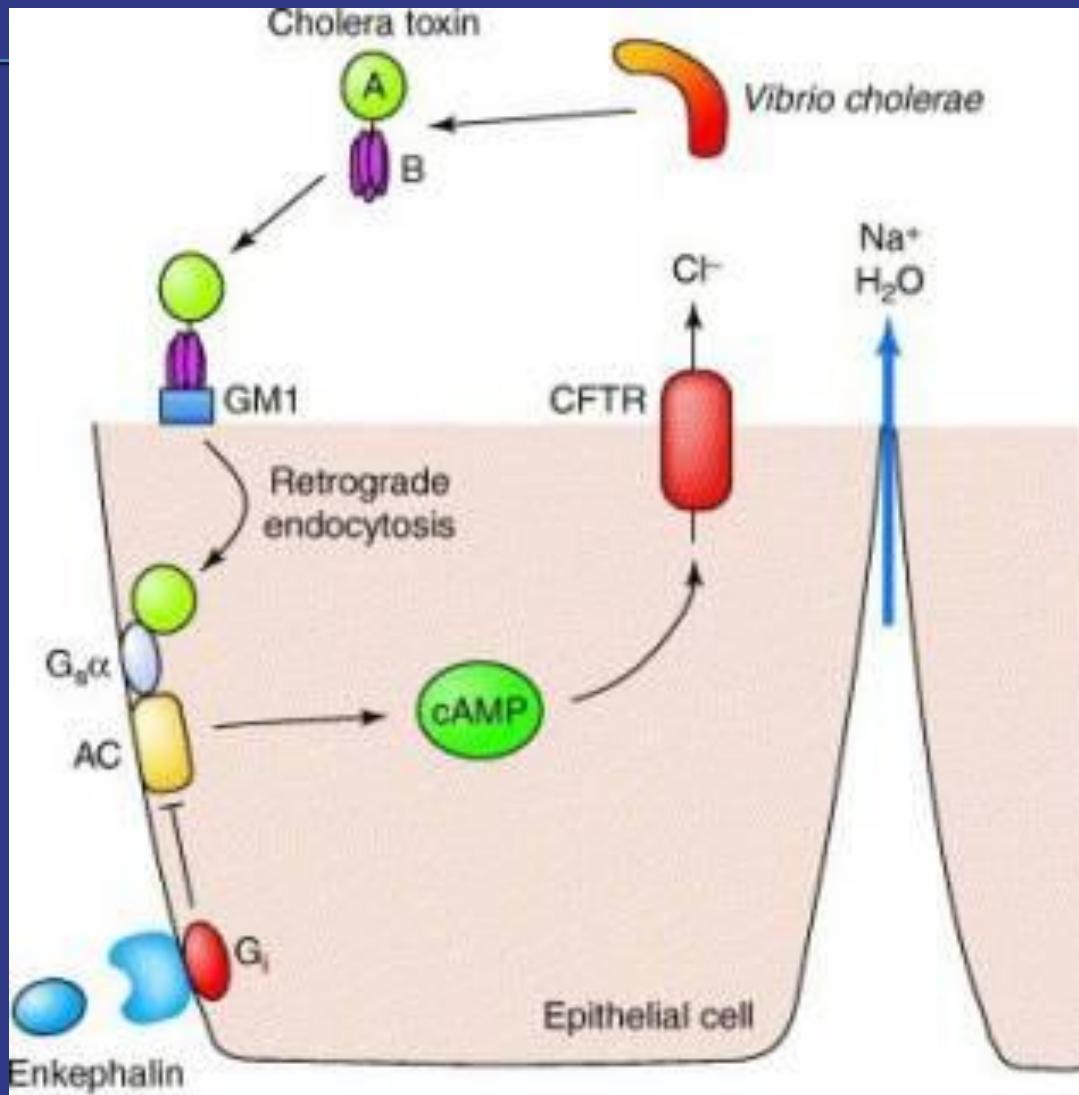


Vibrio cholerae

- *V. cholerae*: massive watery diarrhea, dehydration, vomiting. (rice water stools)
- Treat mainly with ORT
- Mediated by cholera toxin
- 3 Major subgroups causing pandemics:
O1, O139 and non-O1.



Cholera toxin



TCBS Agar

thiosulfate citrate bile salts sucrose

Appearance of Colonies:

- Flat, 2-3 mm in diameter, yellow
V. cholerae
- Small, blue-green center
V. parahaemolyticus, *V. vulnificus*
- Large, yellow
V. alginolyticus
- Blue
Pseudomonas, *Aeromonas*
- Very small, translucent
Enterobacteriaceae



V. cholerae

Clinical Presentation – *Vibrio* spp.

- *V. parahemolyticus*

- most common Vibrio in U.S.
- Raw fish, shellfish.
- Causes gastroenteritis, nausea, vomiting, diarrhea, cramps, low grade fever, chills.

- *V. alginolyticus*

- ear and eye infections and wound infections following exposure to sea water.

- *V. vulnificus*

- septicemia and severe wound infections
- oyster consumption & exposure to sea/brackish water.

Confirmatory Identification

Presumptive *Vibrio* spp.:

- Curved GNB
 - Growth on TCBS
 - positive oxidase
-
- *V. cholera* does not require salt
 - Na⁺ enhances or stimulates growth – distinguishes from *Aeromonas*.
 - Commercial ID systems poor, but MALDI TOF identifies.

Aeromonas – clinical significance

- Extraintestinal infections: septicemia, wounds
 - Septicemia usually in immunosuppressed
- Gastroenteritis: may be watery, dysentery-like, chronic
 - Abdominal pain, fever, vomiting may occur.
 - Usually self-limiting

Aeromonas Epidemiology

- Found in aquatic environments
- Fresh produce, meat, dairy products.
- *A. veronii* bv. *sobria* – gut of medicinal leeches

Aeromonas Isolation & Identification

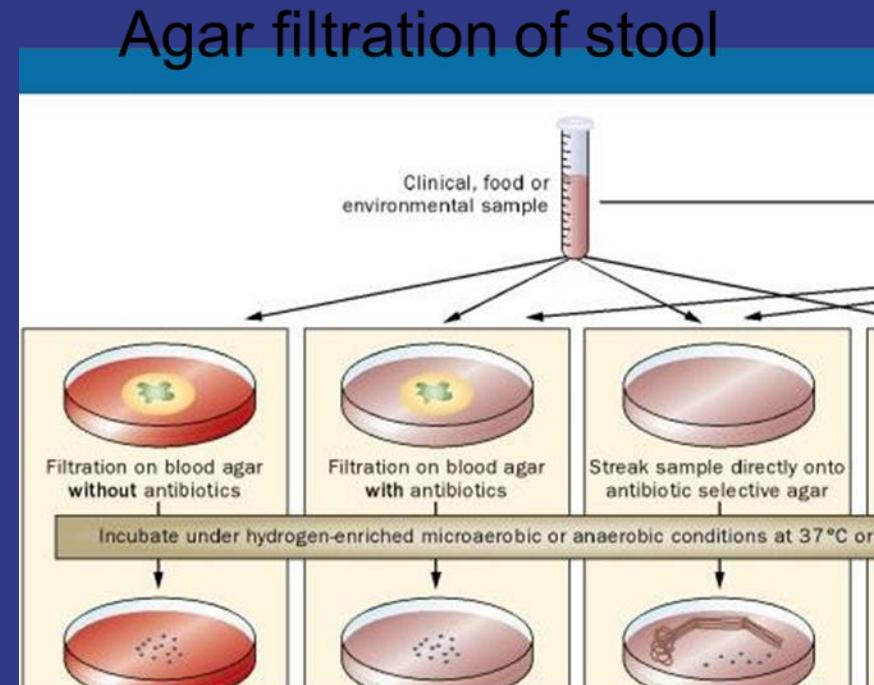
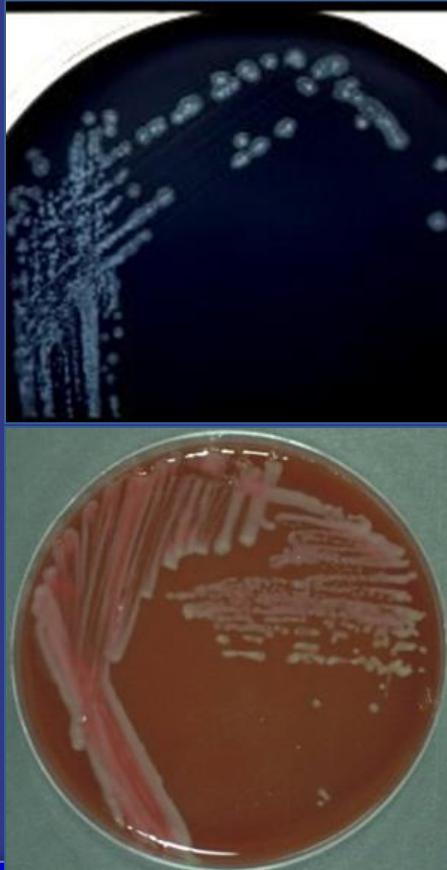
- Oxidase & catalase positive
- Glucose fermenter
- Maybe beta-hemolytic
- Lactose fermenter or non-fermenter
- Many grow well at room temperature;
 - BAP or CIN (pink w/clear edge)
- Straight rods or coccoid
- Not salt tolerant

Campylobacter disease

- Mostly causes gastroenteritis;
 - watery to bloody diarrhea.
- Infectious dose is 10^2 !
- Colonizes the intestines of poultry, cattle, pigs, cats, dogs. Eggs, water, unpasteurized foods may be contaminated.
- Therapy: Rehydration is primary goal.
 - Ciprofloxacin or macrolide if severe, prolonged, bloody, immunocompromised.

Campylobacter culture

- Colonies grow in 48 hrs on selective medium w/abx
- Incubation = 42°C
- Micro-aerophilic atmosphere: 10% CO₂, 5% O₂; 85% N₂.



Curved, small gram-neg bacillus



- 0.2-0.9 x 0.5-5.0 μm
- Spirals; gull wing shapes
- Single, polar flagellum;
darting motility
- Presumptive Campy:
 - Curved GNB
 - oxidase pos
 - darting motility

Campylobacter (kampylos – curved)

Major Species Pathogenic for Humans:

- *C. jejuni* – most common (95%)
- *C. coli*, *C. lari*, *C. upsaliensis*, *C. hyoilectinalis*
- *C. fetus* – bacteremia

- All oxidase (+).
- If hippurate (+) identification is *C. jejuni*.
 - If hippurate (-), need molecular test

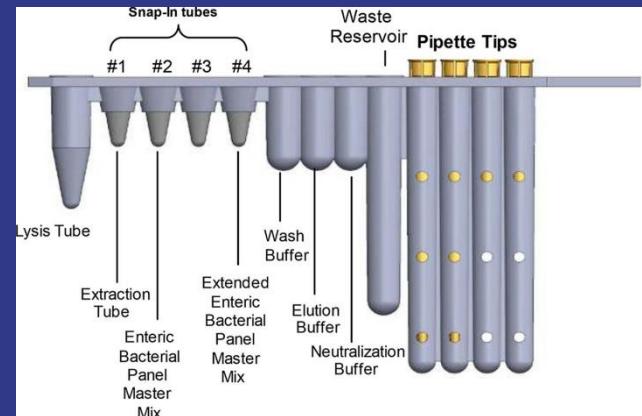
Molecular Tests vs. Culture Stool Pathogens

- Culture takes 3 days for neg result
- Molecular test improves sensitivity
- Molecular test usually less technical time
- Culture materials cheap
- Multiplexed molecular test can include more pathogens

BD MAX - PCR



PCR chamber n=12



Reagents and pipette assembly – 1/sample

BD MAX Target Panels

Bacteria in 2 panels:

- Campylobacter
- Salmonella
- Shigella
- Shiga toxins

- Y. enterocolitica
- ETEC
- P. shigelloides
- Vibrio spp

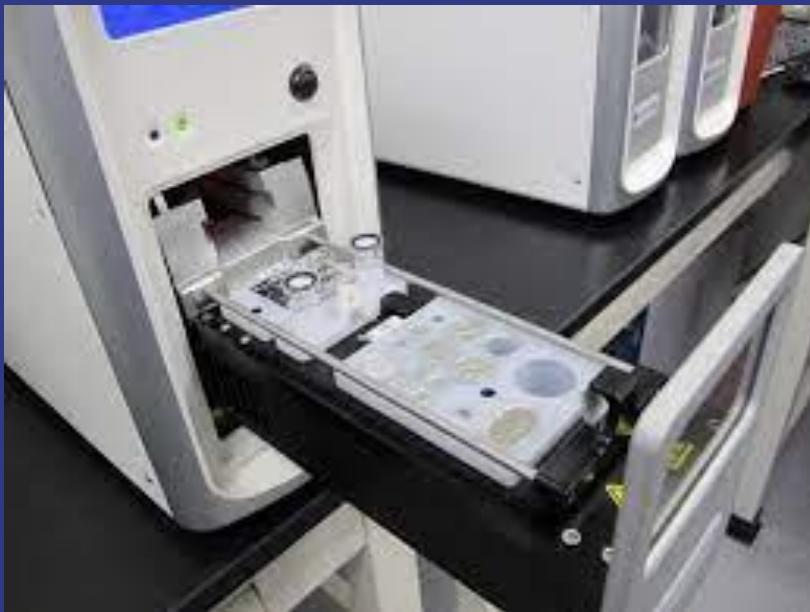
Parasites:

- Giardia
- Cryptosporidium
- E. histolytica

Viruses:

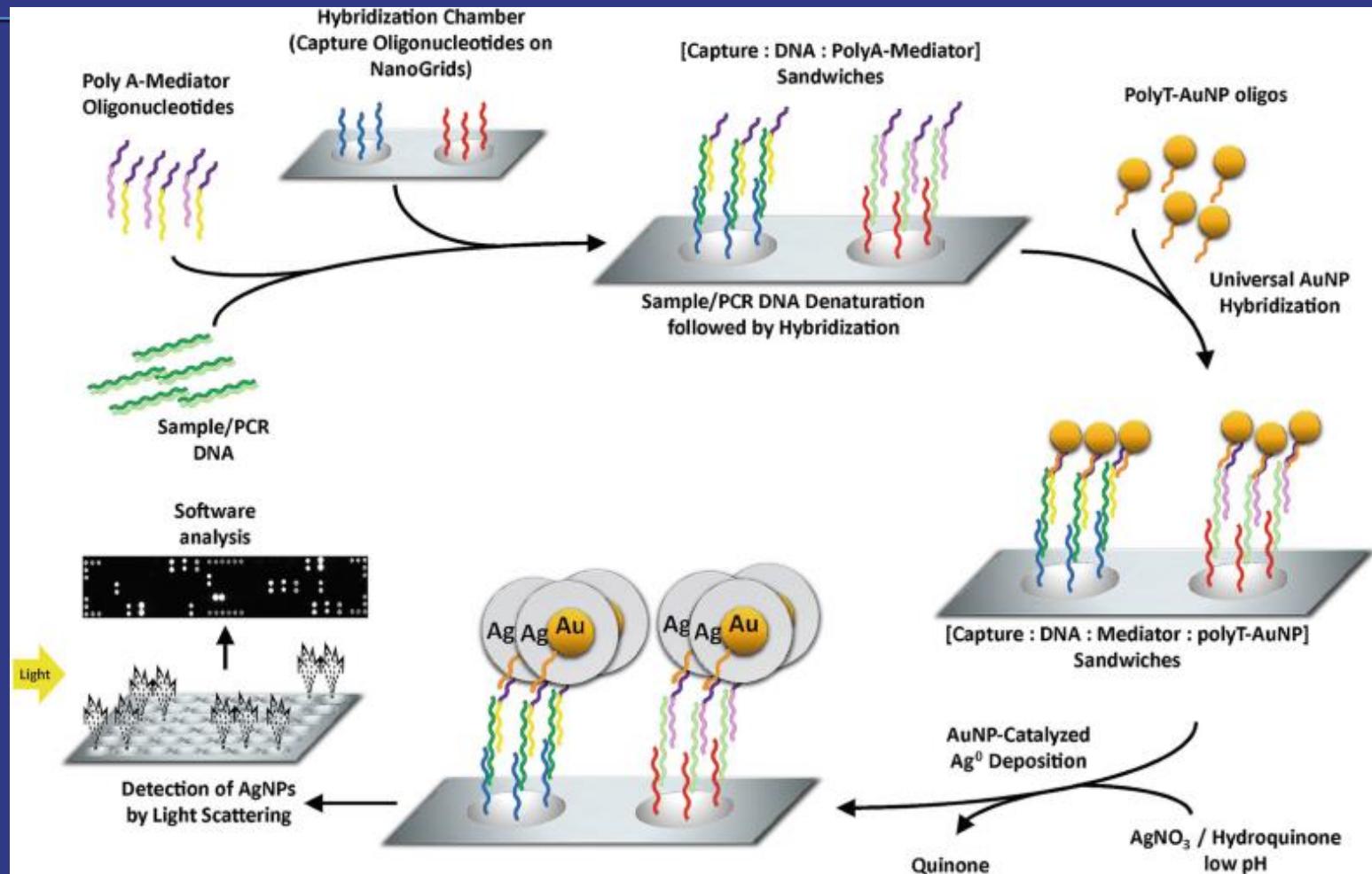
- Norovirus
- Rotavirus
- Adenovirus
- Sapovirus
- Astrovirus

Luminex Verigene - Microarray

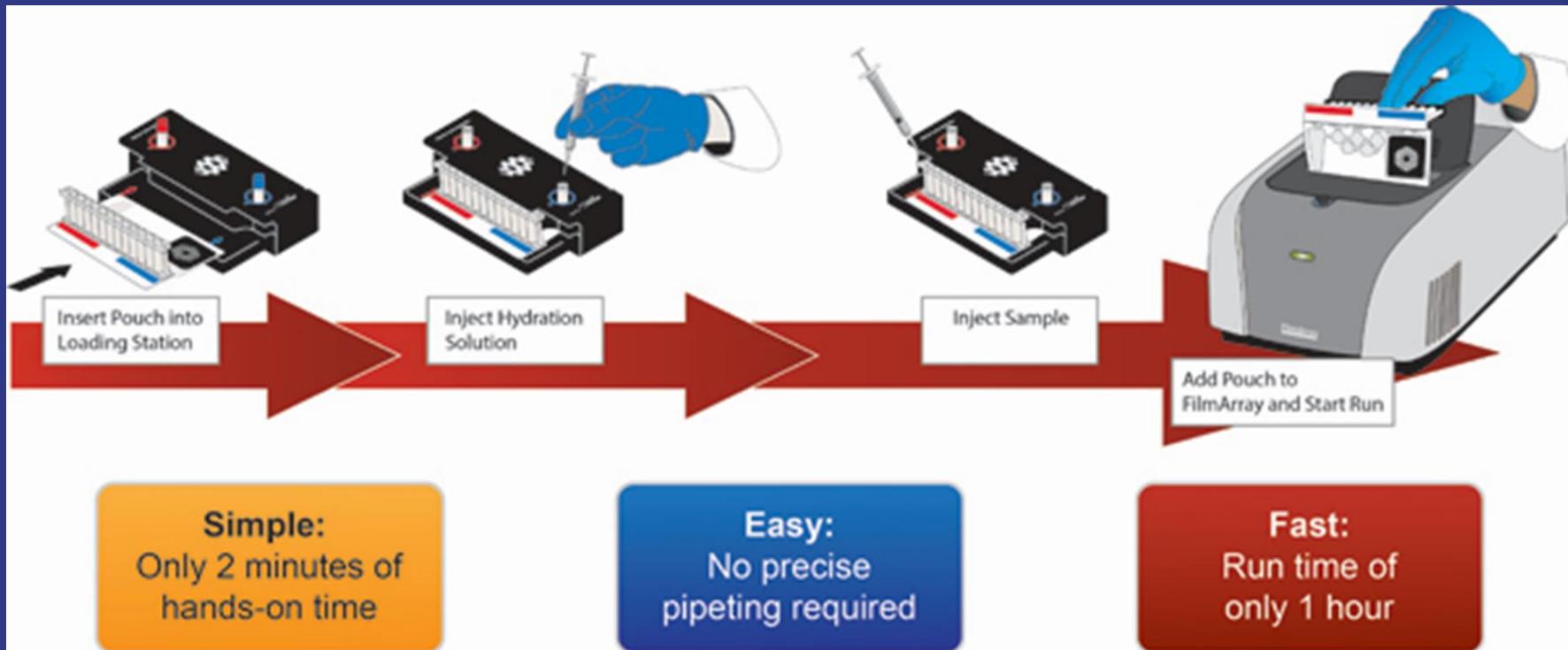


- Campylobacter
- Salmonella
- Shigella
- Shiga toxins
- Vibrio
- Y. enterocolita
- Norovirus
- Rotavirus

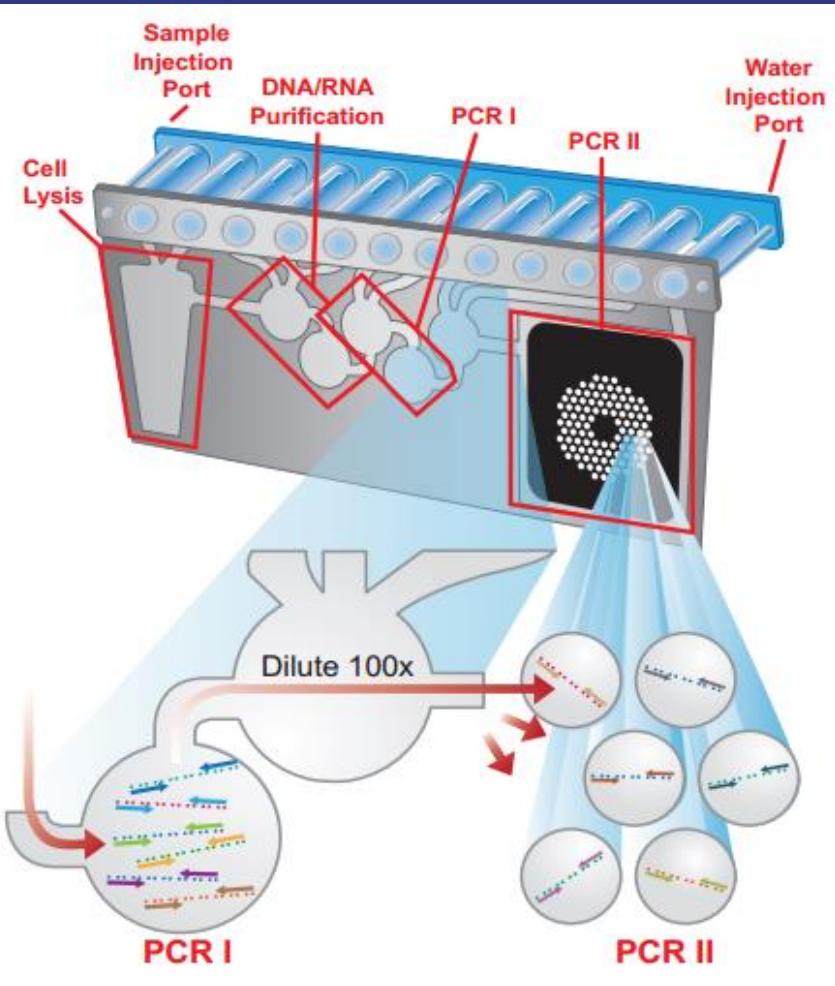
Luminex Verigene - Microarray



Biofire – Nested PCR



Biofire – Nested PCR



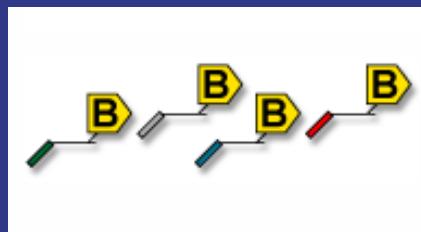
- Campylobacter
- Giardia
- Salmonella
- Cryptosporidium
- Shigella
- E. histolytica
- Shiga toxins
- Cyclospora
- E. coli O157
- Norovirus
- LT/ST (ETEC)
- Rotavirus A
- EPEC
- Adenovirus 40/41
- EAEC
- Astrovirus
- Aeromonas
- Sapovirus
- Yers. enterocolitica
- Plesiomonas
- Vibrio spp.

Luminex xTAG™ GPP

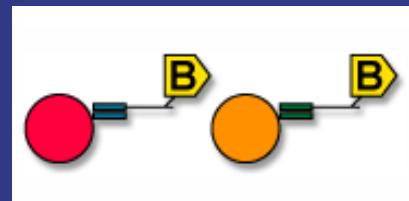
96 well throughput



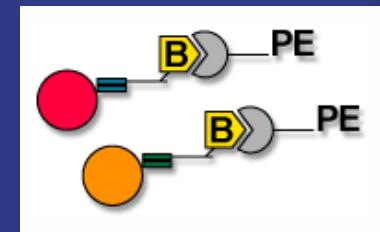
- Extraction (45 min)
- Multiplex PCR, cleanup (2.5 hr)



Target specific
Primer Extension



Hybridization to
anti-Tag sequence
coupled to xMAP
bead set



Wash. Detect
hybridized
beads in
Luminex xMAP
system

(1 hr)

Luminex XTAG GPP (U.S. IVD Targets)

- Targets:

- *Campylobacter*
- *Clostridium difficile*
- *E. coli* O157
- *stx1/stx2*
- LT/ST (ETEC)
- *Salmonella*
- *Shigella*
- *Norovirus* GI/GII
- Rotavirus A
- *Giardia*
- *Cryptosporidium*
- *E. histolytica*



Comparison of Molecular Assays

	BD MAX	Verigene	Luminex GP Panel	Biofire
Time to results	3 hrs	2 hrs	~4.5 hrs	1 hr
Hands-on time per sample	1.25 min	5 min	depends on batch size	2 min
Specimens per batch	$12 \times 2 = 24$	1	96	1
Targets	4	8	13	23

Considerations for implementation

- Test volume to manage
- Costs and savings
- Turn-around time
- Test menu
- Reporting
- Need for supplemental testing

Questions?



I'm really tired!