# **Enterobacteriaceae (Family Overview – Oxidase-Negative Fermenting Gram-Negative Rods)**

**Characteristics:**

* The Enterobacteriaceae are **Gram-negative rods**
* **facultative anaerobes**.
* They ferment **glucose** with acid production
* **oxidase negative** (with rare exceptions like Plesiomonas)
* usually **reduce nitrate to nitrite**.
* Most are **motile by peritrichous flagella,** exceptions: *Klebsiella, Shigella, Yersinia pestis* are non-motile.
* They are common inhabitants of the GI tract (coliforms) or environment and include primary pathogens as well.

**General Identification Approach:**

* **Colony on MacConkey Agar:**
  + Enterics grow on MacConkey (which inhibits Gram-positives)
  + Divided by **lactose fermentation**:
    1. **Lactose-fermenters (LF)** form **pink/red colonies** on MacConkey (due to acid from lactose). Examples: *Escherichia coli*, *Klebsiella*, *Enterobacter, Citrobacter (some)*, *Serratia (slow LF)*.
    2. **Non-lactose fermenters (NLF)** remain **colorless** on MacConkey. Examples: *Salmonella, Shigella, Proteus, Yersinia, Morganella, Providencia*.
    3. **late or slow lactose fermenters** that may appear weakly pink after 48h (e.g. *Serratia marcescens*, *Shigella sonnei*, some *Citrobacter*).
* **TSI (Triple Sugar Iron) or KIA (Kligler Iron Agar) Reactions:** Useful to assess fermentation of glucose (butt reaction), lactose/sucrose (slant), gas, and H₂S production:
  + 1. Typical **coliforms (E. coli, Klebsiella, Enterobacter)**: **A/A** (Acid slant/Acid butt = yellow/yellow) with gas, no H₂S (they ferment lactose/sucrose as well as glucose).
    2. **Non-lactose fermenters (like Salmonella, Shigella, Proteus)**: **K/A** (Alkaline slant/red, Acid butt/yellow) – only glucose fermented (butt yellow), slant reverts to red from peptone usage). H₂S production turns the butt black for H₂S-positive ones (e.g. Salmonella, Proteus). Example: *Salmonella* = K/A + H₂S + gas (often), *Shigella* = K/A no gas, no H₂S, *Proteus mirabilis* = K/A + H₂S + **swarming on blood** (and often rapid urease).
* **IMViC Tests:** Indole, Methyl Red, Voges-Proskauer, Citrate – a classic suite for coliforms:
  + 1. *E. coli*: **I+ MR+ VP– C–** (indole positive, methyl red positive, VP negative, citrate negative).
    2. *Klebsiella pneumoniae*: **I– MR– VP+ C+**.
    3. *Enterobacter cloacae*: **I– MR– VP+ C+** (similar to Kleb, but motile and ornithine decarb +).
    4. *Citrobacter freundii*: **I– MR+ VP– C+** (and often H₂S +).
    5. *Proteus mirabilis*: **I– MR+ VP– C+** (and strongly urease +).
    6. *Proteus vulgaris*: **I+ MR+ VP– C–**. - (These patterns help differentiate major groups; remembering a couple key ones like E. coli vs Kleb helps).
* **Urease Test:** 
  + 1. *Protea (Proteus, Morganella, Providencia)* are **rapid urease positive** (bright pink in urea broth within hours).
    2. *Klebsiella* and *Yersinia* are slower urease positive.
    3. Most others (E. coli, Salmonella, Shigella) are urease negative.
* **Motility:** Most enterics are motile (exhibiting hazy growth in motility agar). Notable **non-motile** genera/species: *Klebsiella*, *Shigella*, and *Yersinia pestis* (at 37°C).
* **Phenylalanine Deaminase (PAD) Test:** Positive for the **Proteae** tribe (Proteus, Morganella, Providencia turn green on adding ferric chloride to PAD agar). Negative for most other Enterobacteriaceae.
* **H₂S Production:** Common in *Salmonella* (except *S. Paratyphi A*), *Proteus, Citrobacter freundii*, *Edwardsiella*. Absent in *Escherichia, Klebsiella, Shigella, Yersinia* (and many others).
* **Special Media:**
  + *Eosin Methylene Blue (EMB) agar*: E. coli gives distinctive **green metallic sheen** colonies due to strong lactose fermentation.
  + *XLD (Xylose Lysine Deoxycholate) agar*: Salmonella appear **red with black centers** (red = lysine decarboxylation, black H₂S), Shigella appear red (no Xylose fermentation), coliforms yellow.
  + *HE (Hektoen Enteric) agar*: Salmonella = green with black centers; Shigella = green; coliforms = orange/salmon colonies.

**Examples of Common Genera and Differentiating Features:**

* **Escherichia coli:** 
  + **Lactose fermenter** (strong LF on MacConkey)
  + indole **positive** (spot indole from colony often positive)
  + motile (peritrichous flagella)
  + gas from glucose
  + usually β-hemolytic on blood agar (many strains).
  + paradigm MR+ VP– organism.
  + also **ornithine decarboxylase positive** (most strains) which helps differentiate it from some Shigella.
  + **Serology**: certain serotypes (O157:H7, etc.) identified for pathogenic strains (see STEC section).
* **Klebsiella pneumoniae/oxytoca:** 
  + **Lactose fermenters** (often extremely mucoid colonies due to polysaccharide capsule).
  + Non-motile.
  + **Urease weakly positive**.
  + Indole: *K. pneumoniae* is indole negative, *K. oxytoca* is indole positive.
  + Klebsiella are typically **VP positive and citrate positive**.
* **Citrobacter:**
  + Some are LF or slow LF.
  + *Citrobacter freundii* often **produces H₂S** (and can be mistaken for Salmonella on initial screens).
  + However, Citrobacter are **urease positive** (weak) and most **utilize citrate** and **do not decarboxylate lysine** (LDC negative), whereas Salmonella (most serovars) are urease negative, citrate variable (many pos), and LDC positive.
* **Enterobacter & Serratia:**
  + Generally LF or slow LF.
  + Motile.
  + *Enterobacter* (e.g. *E. cloacae*) is ornithine positive
  + *Serratia marcescens* produces a red pigment at room temp (not always)
  + Serratia is **DNase positive** and gelatinase positive.
  + Both are usually urease negative or very weak.
* **Proteus:**
  + NLF.
  + **Swarming motility** on non-inhibitory agar (waves of growth over blood agar).
  + Strong **urease positive** (can rapidly alkalinize urea media).
  + *P. mirabilis* is indole negative, *P. vulgaris* indole positive;
  + both produce H₂S (especially mirabilis).
  + PAD positive.
  + Burned chocolate or fishy odor is common.
* **Providencia & Morganella:**
  + NLF
  + motile
  + **PAD positive** like Proteus
  + *Providencia* (e.g. *P. rettgeri, P. stuartii*) are **urease variable** (rettgeri strongly urease +, stuartii weak), **citrate positive**,*Morganella morganii* is **urease positive**, **ornithine positive**, **citrate negative** (distinguishing it from Providencia), and can produce a fishy odor.
* **Salmonella:**
  + NLF
  + most produce H₂S
  + **Motile**
  + Lysine decarboxylase **positive** (most serovars) – this helps differentiate from *Citrobacter* which can look similar but many Citrobacter are LDC negative.
  + Urease negative
  + indole negative.
  + **Does not ferment lactose or sucrose**, hence K/A on TSI with black if H₂S.
  + Produces gas from glucose (except *S. Typhi* which typically does not).
  + **Serotyping** by O and H antigens is used for identification to serovar. -
* **Shigella:**
  + NLF.
  + **Non-motile**.
  + Biochemically rather inert: they do not decarboxylate lysine (LDC negative), do not produce gas from glucose (except some *S. flexneri* strains weakly), do not produce H₂S.
  + **Urease negative, Indole variable** (e.g. *S. flexneri* often indole positive; *S. sonnei* indole negative).
  + *Shigella sonnei* is unique in being **ONPG positive** (ferments lactose slowly) and **ornithine decarboxylase positive**; these tests separate *S. sonnei* (Group D) from other Shigella groups.
* **Yersinia:**
  + Typically NLF (some *Y. enterocolitica* are slow lactose fermenters on Mac after 48h).
  + **Motility is temperature-dependent**: *Y. enterocolitica* and *Y. pseudotuberculosis* are motile at 25–30°C (peritrichous flagella) but **non-motile at 37°C**. *Y. pestis* is non-motile at all temperatures.
  + Most strains of *Y. enterocolitica* and *Y. pseudotuberculosis* rapidly urease positive; *Y. pestis* is usually urease negative or very weak
  + *Y. enterocolitica* ferments **sucrose** and is **ornithine decarboxylase positive** (differentiating it from *Y. pseudotuberculosis* which is ornithine neg and does not ferment sucrose). Special media: Yersinia can be isolated on **CIN agar** (Cefsulodin-Irgasan-Novobiocin) where *Y. enterocolitica* produces bull’s-eye red colonies.

# **Salmonella Species**

# **About:**

* *Salmonella* are motile **Gram-negative rods** (except *S. Gallinarum/Pullorum* which are non-motile) in the Enterobacteriaceae.
* Pathogens causing gastroenteritis (e.g. *S. Enteritidis, S. Typhimurium*), enteric fever (typhoid fever caused by *S. Typhi* or paratyphoid by *S. Paratyphi*), and other infections.
* They are **non-lactose fermenters**
* many produce **H₂S** on media (XLD).

**Key Identification Tests:**

* **Culture Characteristics:**
  + On MacConkey or DCA agar, Salmonella form **nonlactose fermenting colonies**.
  + On XLD agar, colonies are **red with black centers** (red indicates lysine decarboxylation and no xylose left, black from H₂S).
  + On Hektoen agar, they are **green with black centers**.
  + **TSI Agar:** Salmonella typically give **K/A, H₂S positive, gas positive**. That is a red slant (no lactose/sucrose fermentation), yellow butt (glucose fermented), often with a black precipitate in butt (H₂S) and bubbles/cracks (gas from glucose).
  + *S. Typhi* is an outlier: it usually produces only slight H₂S (at the interface), and typically no gas.
* **Urease: Negative** (distinguishes from Proteus which is positive).
* **Indole: Negative** (distinguishes from some indole-positive Proteus or E. coli).
* **Motility: Motile** (peritrichous flagella) at 20°C + 37°C
* **Lysine Decarboxylase:** 
  + **Positive** for *Salmonella enterica* subspecies enterica (which includes the human pathogens) – they decarboxylate lysine, hence the red slant on LIA (Lysine Iron Agar) and the red colonies on XLD.
  + *Salmonella Paratyphi A* is a notable exception (LDC negative).
* **Citrate:** Most Salmonella are **citrate positive**, except *S. Typhi* which is citrate negative.
* **Serological Identification:**
  + **agglutination with Salmonella antisera**.
  + **O (somatic) antigen grouping:** Polyvalent O antisera (A–G groupings) then specific group factor antisera can identify which group (e.g. Group D for *S. Typhi and S. Enteritidis*, Group B for *S. Typhimurium*, etc.).
  + **H (flagellar) antigen typing:** Salmonella typically have phase 1 and phase 2 flagellar antigens. Slide agglutination can determine these. If only one phase is expressed, labs may use techniques to induce the switch to the alternate phase (e.g. Craigie tube method).
  + **Vi antigen:** *S. Typhi* and *S. Paratyphi C* have a capsular Vi antigen that may cause weak or no agglutination in O antisera until it’s removed (by boiling or specific anti-Vi serum). Detection of Vi antigen (e.g., with anti-Vi serum) is specifically important for identifying *S. Typhi*.

**Identification Algorithm Simplified:**

1. **Screen** colony from stool or sample on differential media (e.g. MacConkey: pick NLF colonies).
2. Perform **oxidase test** to rule out oxidase-positive NLF (like Aeromonas). Salmonella will be oxidase negative.
3. Inoculate **TSI (or KIA)** and **urease** and perhaps **LIA**: - If TSI = K/A + H₂S, urease negative, LIA = purple (LDC positive), the organism is highly suggestive of Salmonella.
   1. If urease was positive, think Proteus/Citrobacter, not Salmonella.
   2. If no H₂S, consider Shigella or others (some Salmonella can rarely be H₂S negative, like *S. Paratyphi A*).
4. Perform **agglutination tests**: First with a polyvalent anti-O serum that covers A–E groups. If positive, proceed to specific group antisera (A, B, C1, C2, D, etc.). A positive O agglutination confirms a Salmonella *somatic* group.
5. Optionally, perform **flagellar (H) antigen typing** to determine serovar, or send to reference lab for full serotyping if required. For example, an isolate agglutinating in Group D O antisera and in H phase 1 “Vi” and “d” might be *Salmonella Typhi* (which has O group D1 9,12; Vi; H:d). 6
6. Biochemical **confirmation kits** or automated systems will also identify Salmonella genus/species, but serotyping is needed to name the serovar (like *Typhimurium, Enteritidis*).

**Differentiating from Citrobacter:** *Citrobacter freundii* can mimic Salmonella (NLF, H₂S+). Key differences: Citrobacter is often **urease positive** (weak) and **ONPG positive** (has β-galactosidase, meaning it can slowly ferment lactose, Salmonella cannot). Also Citrobacter typically does not have the Salmonella antigenic profile. If an “H₂S-positive, citrate-positive, urease-variable NLF” is isolated, labs will do a **Salmonella latex agglutination** screen; Citrobacter will not agglutinate with Salmonella polyvalent antisera.

**Special Notes:**

* *Salmonella enterica* has many serovars (>2600). The SMIs describe them using the Kauffmann-White scheme.
* *Salmonella Typhi*: Biochemically, Typhi is a bit unique: It often produces only a small amount of H₂S (must look carefully at the butt of TSI for a ring of black at the interface), is citrate negative, and ornithine decarboxylase negative (this combination differentiates it from most other Salmonella). It also has the Vi capsular antigen which can be tested.

# **Shigella Species**

**About:** *Shigella* are Gram-negative rods, very closely related to E. coli genetically, but are distinguished by a non-motile, biochemically limited profile.

**Clinical:**

* high transmissibility person-to-person and low infectious dose.
* *S. dysenteriae* type 1 is particularly severe (causes epidemic dysentery and can lead to HUS due to Shiga toxin).
* *S. sonnei* is the most common in many regions, causing usually milder illness.
* Antibiotic sensitivity is important as resistance is common (and treatment shortens illness and carriage).
* Labs report Shigella isolations to public health for surveillance.

There are four serogroups corresponding to species:

1. **Group A – *S. dysenteriae*** (most severe, produces Shiga toxin, and crucially only one to NOT ferment mannitol),
2. **Group B – *S. flexneri*** (common in developing world),
3. **Group C – *S. boydii***,
4. **Group D – *S. sonnei*** (most common in developed countries).

**Key Identification Tests:**

* **Non lactose Fermentation:** *Shigella* are typically **non-lactose fermenters** (clear colonies on MacConkey).
* **TSI:** *Shigella* usually gives **K/A with no gas, no H₂S**. They ferment glucose (acid butt) but not lactose/sucrose (alkaline slant). They typically do **not produce gas** from glucose (a differentiator from many Salmonella, although *S. flexneri* can sometimes produce slight gas).
* **Motility:** *Shigella* are **non-motile** (no spreading in motility medium, no flagella observed).
* **Urease: Negative**.
* **Indole:** Variable by species
  + *S. flexneri* is often **indole positive**;
  + *S. sonnei* is indole negative;
  + *S. dysenteriae* often indole negative.
* **Ornithine Decarboxylase (ODC):** *S. sonnei* is **ODC positive** (which means it can decarboxylate ornithine, turning Moeller’s broth purple). All other Shigella (Groups A, B, C) are ODC negative. This is a very useful test: an NLF, non-motile, ODC-positive organism from stool is highly suggestive of *Shigella sonnei*.
* **ONPG (β-galactosidase test):** *S. sonnei* is **ONPG positive** (it has βgalactosidase, allowing it to slowly ferment lactose or at least cleave ONPG to a yellow product). *S. sonnei* is often called a “late lactose fermenter” because of this slow fermentation. Other Shigella are ONPG negative.
* **Lysine Decarboxylase:** All Shigella are **LDC negative** (they do not decarboxylate lysine), which differentiates them from Salmonella (which are mostly LDC positive).
* **Serological confirmation:** Presumptive Shigella (NLF, non-motile, urease neg) can be confirmed by **slide agglutination with Shigella antisera**. Typically, polyvalent antisera for each group (A, B, C, D) are used. Note: cross-reactions can happen; *E. coli* with certain O antigens might agglutinate, so biochemical compatibility with Shigella profile must be confirmed.
* **Differentiation from E. coli:**
  + Some E. coli strains (especially enteroinvasive E. coli – EIEC) can be biochemically very similar to Shigella (non-motile, NLF).
  + But most E. coli are motile or lactose-fermenting or indole positive
  + The ODC/ONPG pattern helps; also E. coli are typically **DNase negative** (as are Shigella) and **oxidase negative** (same as Shigella). If an organism is non-motile, NLF, ODC-neg, ONPG-neg, and indole-neg, it’s likely Shigella or EIEC. Serology is the tiebreaker: Shigella have specific O antigens – if it agglutinates with Shigella antisera, it’s confirmed; if not, it could be an odd E. coli
  + **Special notes:**
  + For exam recall: *S. sonnei* = ODC+, ONPG+; *S. flexneri* = mannitol +, indole often +, ODC–; *S. dysenteriae* type 1 = mannitol –, most biochemically inert of all.

**Identification Workflow:**

1. Pick NLF colonies from Mac/XLD.
2. Oxidase test negative likely Enterobacteriaceae.
3. TSI: K/A, no H₂S, no gas;
4. Urease: negative; Motility: negative > suspect Shigella (or possibly Yersinia which is motile at 25°C, or E. coli variant).
5. Do ODC and ONPG tests: if **ODC+ and ONPG+** strongly suggests *Shigella sonnei* (Group D). If **ODC– and ONPG–** (and other biochems inert) suggests *S. flexneri* or *S. dysenteriae*.
6. Confirm with **Shigella antisera agglutination** (slide test). No agglutination might mean it’s an E. coli or requires boiling to remove any capsule if present (though Shigella don’t really have a prominent capsule like Salmonella Typhi does).

# **Yersinia Species (Identification of *Yersinia*)**

* *Yersinia* are Gram-negative rods
* bipolar staining (“safety pin”) by Wayson stain
* Three main species in human pathology:
  1. **Y. enterocolitica:** enterocolitis, pseudoappendicitis, reactive arthritis - contaminated pork or water – CIP/SXT
  2. **Y. pseudotuberculosis:** mesenteric adenitis, sepsis, often in rodents and birds
  3. **Y. pestis:** plague – Strep/GEN or DOX

**Key Identification Tests:**

* GI bugs: motile, urease positive NLF and grows on CIN. Y. enterocoliticia is ODC+.
* Y pestis non-motile, urease negative (also catalase +, oxidase -, indole -). Fried eff colonies. Does not grow on CIN.
* **Temperature-Dependent Motility:**

*Y. enterocolitica* and *Y. pseudotuberculosis: m***otile at 25°C** (room temperature, swirling motility) and **non-motile at 37°C.** *Y. pestis* is **non-motile at any temperature** (no flagella).

* **Lactose:** *Yersinia* are **non-lactose fermenters** (clear on MacConkey), though *Y. enterocolitica* can sometimes appear as a pinpoint LF after 48h (due to slow fermentation of lactose by some strains). They are usually taken as NLF.
* **Urease:** *Y. enterocolitica* and *Y. pseudotuberculosis* are **urease positive** (often rapidly within minutes for *Y. pseudotuberculosis*, within a few hours for *Y. enterocolitica*). *Y. pestis* is urease negative (useful to note in lab if working with a culture under special conditions, though plague is typically identified by reference methods).
* **TSI:** 
  + *Yersinia enterocolitica* – usually **A/A or A/A with a slight alkali slant** (often described as weak acid slant, acid butt) *with no H₂S*. This is because *Y. enterocolitica* can ferment **sucrose** (TSI has lactose and sucrose in the slant). It may or may not produce gas.
  + *Y. pseudotuberculosis* might appear K/A (no sucrose fermentation). Both do not produce H₂S.
* **Ornithine Decarboxylase (ODC):** *Y. enterocolitica* is **ODC positive** (this is an important trait; it decarboxylates ornithine, which differentiates it from *Y. pseudotuberculosis* which is ODC negative). *Y. pestis* is also ODC negative.
* **Fermentation of certain sugars:** *Y. enterocolitica* typically ferments **sucrose** and **mannitol** (acid from mannitol is a trait, and it ferments sucrose so TSI slant can be yellow). *Y. pseudotuberculosis* does **not ferment sucrose** or mannitol.
* **Selective isolation:** Labs often use **CIN agar** (Cefsulodin-Irgasan-Novobiocin) for Yersinia from stool. *Y. enterocolitica* on CIN agar shows **bull’s-eye colonies**: a deep red center with a translucent edge, due to mannitol fermentation and neutral red indicator uptake in the center. These colonies at 48 hours at room temp are highly indicative of *Y. enterocolitica*. I.E. IS RESISTANT TO cefsulodin
* **Serotyping**: *Y. enterocolitica* has multiple serogroups (O:3, O:8, O:9 common in human infection). *Y. pseudotuberculosis* has its own O serotypes. *Y. pestis* is usually identified by other means (DFA for capsular F1 antigen, phage lysis test, PCR).

**Y. pestis special notes:**

* If there’s suspicion of plague (usually by epidemiology, e.g. patient from endemic area with buboes or pneumonia), the lab should be alerted.
* *Y. pestis* forms rough, fried-egg colonies at 48h and shows bipolar “safety-pin” on Wayson or Wright stain.
* catalase positive, oxidase negative, urease negative indole negative, and non-motile.
* It will not grow on CIN like enterocolitica does.

Identification is confirmed by reference labs (DFA for F1 antigen or bacteriophage lysis test). **Summary of Differentiation:**

Trait Y. enterocolitica Y. pseudotuberculosis Y. pestis

|  |  |  |  |
| --- | --- | --- | --- |
| Motility at 25°C | Yes (motile) | Yes (motile) | No (non-motile) |
| Motility at 37°C | No (non-motile) | No (non-motile) | No |
| Urease | Positive (often rapid) | Positive (rapid) | Negative |
| Ornithine decarboxylase | Positive | Negative | Negative |
| Sucrose fermentation | Positive (A/A on TSI) | Negative (K/A on TSI) | Negative (K/A) |
| Key infections | Gastroenteritis (can mimic appendicitis, mesenteric lymphadenitis) | Mesenteric lymphadenitis, septicemia (far-east scarletlike fever) | Plague (bubonic, pneumonic, septicemic) |

# **Shiga Toxin-Producing *E. coli* (STEC) and other Pathogenic *E. coli***

**About:**

* Shiga toxin-producing *E. coli* (STEC), also known as **enterohemorrhagic E. coli (EHEC)**, > cause hemorrhagic colitis and hemolyticuremic syndrome (HUS), especially in children. Antibiotics contraindicated.
* e.g. **E. coli O157:H7**, but other serotypes (called non-O157 STEC, like O26, O111, O103, etc.) also cause similar disease.

**Identification of E. coli O157:H7:**

* **Biochemical profile:** O157:H7 is otherwise like a typical E. coli: Gram-negative rod, oxidase negative, catalase positive, indole positive, lactose fermenting (on regular MacConkey it *can* ferment lactose, although some O157 strains are slow lactose fermenters), usually motile
* **Sorbitol-MacConkey Agar (SMAC):** *E. coli O157:H7* is unique among most E. coli in that it does **not ferment sorbitol rapidly,** forms **colorless colonies** (sorbitol-negative), whereas most normal fecal E. coli and other STEC serotypes ferment sorbitol and form pink/red colonies.
* **Indole positive** likely typical E. Coli
* **MUG negative:** Most E. coli produce β-glucuronidase (which cleaves MUG to a fluorescent product), but **E. coli O157:H7 is typically MUG-negative** (no fluorescence with 4-methylumbelliferyl-β-Dglucuronide).
* **Agglutination:** A positive agglutination confirms the isolate as belonging to the O157 serogroup.
* **Flagellum** Often a second test for H7 flagellar antigen is done at reference lab
* **Toxin detection:** To confirm as STEC, detection of **Shiga toxins (Stx1, Stx2)** is performed. This can be done by immunoassays (like an EIA on stool broth) or PCR for the toxin genes. Many labs screen stools with a PCR or EIA for Shiga toxin genes and then culture for O157 if positive.

**Other Pathogenic E. coli Groups:**

* Enterotoxigenic E. coli (ETEC) – traveler's diarrhea (identified by toxin assays, not routine labs).
* Enteropathogenic E. coli (EPEC) – infant diarrhea (identified by serotype historically).
* Enteroinvasive E. coli (EIEC) – dysentery-like illness (biochemically can look like Shigella, as mentioned).
* Enteroaggregative E. coli (EAEC) – persistent diarrhea (identified by HEp-2 cell adherence assay historically, now by PCR).

**Laboratory workflow for STEC:**

1. Culture stool on SMAC – look for **sorbitol-negative colonies**.
2. Test those colonies with **oxidase** (should be neg) and **indole** (most likely pos if E. coli). Also ensure they are Gram-negative rods.
3. Perform **O157 slide agglutination**. If positive, presumptive E. coli O157. 4
4. Parallel approach: Do a **Shiga toxin assay** on stool broth (many labs do this first). If toxin positive and O157 culture negative, alert for possible non-O157 – these might be further tested with a PCR panel that identifies the serogroup or just reported as “Shiga toxin positive E. coli” and sent out. 5
5. Confirm O157 by reference lab for H7 antigen and perhaps PFGE or sequencing for epidemiology, especially if part of an outbreak.