PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

Stool Culture Procedure

Department of Microbiology

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1.0 Clinical Significance

Gastroenteritis is most commonly caused by viral etiologies in both children and adults. However, bacterial enteric pathogens represent a significant portion of gastrointestinal infections. Enteric pathogens are often associated with food-borne outbreaks and pose a risk to public health. Illness associated with bacterial gastroenteritis can range from mild to severe and usually manifests with symptoms of vomiting, diarrhea, and abdominal discomfort. The most common complication is dehydration. Infections are usually self-limited, but improper management may lead to prolonged symptoms or may even be associated with serious complications.

2.0 Principle

Stool cultures screen for the presence of specific enteric pathogens. Routinely, cultures are screened for the presence of *Salmonella, Shigella, Campylobacter*, and Shiga toxin-producing *Escherichia coli* (STEC), including *E. coli* O157. Other pathogens, such as *Yersinia enterocolitica, Aeromonas* spp*., Plesiomonas shigelloides,* or *Vibrio* spp., may be detected in routine culture, but there is better chance of detection, if the clinician specifies the suspected organism. Specific selective media are used for the isolation and detection of each enteric pathogen.

BBL™ CHROMagar™ Salmonella is a selective and differential medium for the isolation and differentiation of *Salmonella* species. Selective agents in the medium suppress the growth of most fecal flora while the addition of chromogenic substrates in the medium facilitates differentiation of *Salmonella* species. Colonies of *Salmonella* species appear mauve (rose to purple) in color. Fecal flora that is not inhibited by selective agents in the medium will produce blue-green or colorless colonies. CHROMagar™ Salmonella provides increased detection with decreased time to identification by reducing the need for subculture, as compared to conventional, non-chromogenic media. CHROMagar™ Salmonella also reduces unnecessary testing associated with H_2S-producing organisms such as *Proteus* and *Citrobacter* spp.

BBL™ CHROMagar™ O157 is intended for the isolation, differentiation, and presumptive identification of *E. coli* O157. The addition of potassium tellurite, cefixime, and cefsulodin reduces the number of bacteria other than *E. coli* O157:H7 that grow on this medium. The medium contains a mix of artificial substrates, which release an insoluble colored compound when hydrolyzed by a specific enzyme. *E. coli* O157 utilizes one of the chromogenic substrates producing “mauve” colonies. The growth of mauve colonies is considered presumptive for *E. coli* O157 on CHROMagar™ O157. Non-*E. coli* O157 bacteria may utilize other chromogenic substrates resulting in blue to blue-green colored colonies. If none of the chromogenic substrates are utilized, colonies may appear as their natural color. This facilitates the detection and differentiation of *E. coli* O157 from other organisms.

Campy CVA Agar is a selective medium for the primary isolation of *Campylobacter jejuni* from stool specimens. This medium consists of Brucella Agar with sheep blood to support the growth of *Campylobacter* species. Antimicrobial agents are incorporated to suppress the growth of normal fecal flora that could mask the presence of *C. jejuni*. Cefoperazone suppresses the growth of gram-negative enteric bacilli and some gram-positive species, while vancomycin inhibits many species of gram-positive bacteria and amphotericin B serves as an antifungal agent. Inoculated plates are incubated in a reduced oxygen atmosphere using gas jars to support the growth of *Campylobacter*. Jars are incubated at 42 °C to inhibit growth of normal fecal flora while supporting the growth of most *Campylobacter* found in clinical specimens.

CIN (cefsulodin-Irgasan-novobiocin) Agar is used for the selective isolation of *Yersinia enterocolitica*. Fermentation of mannitol in the presence of neutral red results in a characteristic “bull’s-eye” colony (colorless with red centers). Selective inhibition of gram-negative and gram-positive organisms is obtained by means of crystal violet, sodium desoxycholate and the antimicrobial agents, cefsulodin, Irgasan (triclosan) and novobiocin.

Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS) is highly selective for the isolation of *Vibrio* species*.* Inhibition of gram-positive bacteria is achieved by the incorporation of bile salts. Thymol

blue and bromthymol blue are included as indicators of pH changes. Sucrose is included as a fermentable carbohydrate and allows for preliminary differentiation of *Vibrio* species, with *V. cholerae, V. fluvialis,* and *V. alginolyticus* producing yellow colonies while *V. parahaemolyticus*, *V. mimicus,* and most strains of *V. vulnificus* produce green colonies.

Immuno*Card* STAT! EHEC is an immunochromatographic rapid test for the qualitative detection of Shiga toxins 1 and 2 (ST1 and ST2) produced by *E. coli* in GN broth cultures derived from clinical stool specimens. The test utilizes monoclonal antibodies labeled with red-colored gold particles. The device has a circular sample port and an oval-shaped test and control window. The sample is applied to the chromatography paper via the circular sample port. The sample is absorbed through the pad to the reaction zone containing colloidal, gold-labeled antibodies specific to Shiga Toxins. Any Shiga toxin antigen present complexes with the gold-labeled antibody and migrates through the pad until it encounters the binding zones in the test (Toxin 1, Toxin 2) area. The binding zones contain another anti-ST1 or -ST2 antibody, which immobilizes any Shiga toxin-antibody complex present. Due to the gold labeling, a distinct red line is then formed. The remainder of the sample continues to migrate to another binding reagent zone within the control zone, and also forms a further distinct red line (positive control). Regardless of whether any Shiga toxin is present or not, a distinct red line should always be formed in the control zone and confirms that the test is working correctly.

3.0 Scope

This procedure is classified under CLIA as Highly Complex. It should be carried out by technical personnel familiarized and trained on all aspects of testing. Testing includes but is not limited to: evaluation of colony and microscopic morphologies, performance and interpretation of biochemical, serological and immunoassays, instrument use and maintenance, basic troubleshooting, QC checks, and technical proficiency. Records are kept to document employee competence and proficiency. Performance reviews of technical personnel are to be carried out annually.

4.0 Safety - Personal Protective Equipment

Performance of this procedure will expose testing personnel to biohazardous material. All specimens must be handled as potentially infectious material as outlined in the Providence Sacred Heart Microbiology Safety Guidelines.

The reagents and/or chemicals that are used in this procedure may be hazardous to your health if handled incorrectly. Some reagents may be slightly corrosive to skin, slightly toxic, may be weak oxidizers, and may combust if heated. More extensive information concerning the safe handling of the reagents and/or chemicals used in this procedure, as well as other important safety information may be obtained by consulting the Safety Data Sheet (SDS). Before performing any part of this procedure, the technologist must take any and all precautions and adhere to all prescribed policies.

This procedure may expose you to:

- Enteric pathogens
- Bloodborne pathogens
- Airborne pathogens
- Hazardous reagents

To perform this procedure, you must use:

- Gloves – must be worn when handling specimens and concentrates.
- Laboratory Coat – must be worn when handling specimens and reagents.
- Biological Safety Cabinet – must be used when processing specimens.

Disinfectant following procedure:

- Bleach dilution sprayers can be used for on demand disinfectant.

Reference for spill/decontamination:

- SDS
- Chemical hygiene plan

5.0 Specimen Collection, Handling and Storage

5.1 Collection

1. Stool
   Patients should be instructed to pass stool into a clean, dry container. Ideally, up to 5 mL of diarrheal stool or 1 g (a walnut-sized portion of stool) should be transferred to a specimen container with a tight-fitting lid. If specimen transport to the laboratory is expected to exceed 2 h from time of collection, specimen should be refrigerated or placed in enteric transport medium (Modified Cary-Blair). Specimen should be added to liquid transport medium until the fluid reaches the indicator line. Overfilling vials with a transport medium results in improper specimen preservation.
2. Rectal Swabs
   Rectal swabs may be collected by passing the tip of a sterile swab approximately 1 in. beyond the anal sphincter. The swab should be carefully rotated to sample anal crypts and withdrawn. Swabs should be placed in bacterial transport medium to prevent drying of specimen.
3. Duodenal, Colostomy, or Ileostomy Contents
   Specimens should be submitted in leak proof cup.

5.2 Transport & Storage

1. Fresh specimens may be stored and transported at room temperature for up to 2 h or refrigerated for up to 24 h.
2. Specimens may be placed in bacterial transport medium and transported at room temperature up to 24 h or up to 72 h refrigerated.

5.3 Timing & Frequency

1. Specimens should be submitted during the acute stage of infection (usually 5 to 7 days). Pathogens decrease in number with time.
2. Culturing multiple specimens is of questionable value. If the initial culture is negative, then up to 2 more fecal samples may be submitted for testing provided the patient collects them on successive days.
3. Cultures are not routinely recommended from patients who have been hospitalized for 3 or more days.

6.0 Materials

6.1 Equipment and/or Testing System

- Ambient incubator set at 35 ± 2°C
- Ambient incubator set at 30 ± 2°C
- Ambient Incubator set at 42 ± 2°C
- Jars and system for generating microaerophilic environment
- Rotator
- BD Phoenix™ system

6.2 Culture Media

On receipt, store media in the dark at 2-8 °C in original sleeve wrapping. Chromogenic media should be stored in original cardboard box until time of inoculation. Minimize exposure of the medium to light both before and during incubation, as light may destroy the chromogens. Avoid freezing and overheating. Do not open until ready to use. Allow the medium to warm to room temperature before inoculation. Prepared plates may be inoculated up to the expiration date and incubated for the recommended incubation times.

- BBL™ CHROMagar™ O157 (MacConkey with Sorbitol may be used as a substitute)
- BBL™ CHROMagar™ Salmonella

- Campy CVA Agar
- MacConkey II Agar
- GN (Gram-Negative) Broth, 8 mL
- CIN Agar (*Yersinia* selective)
- TCBS Agar (for *Vibrio* isolation)
- Trypticase™ Soy Agar with 5% Sheep Blood (TSA II™)

6.3 Consumables

- Sterile swabs
- Inoculating loops and needles
- Wooden applicators
- Glass slides
- Filter paper

6.4 Reagents & Tests

- TSI slants
- Urea agar slants
- PYR tests
- Spot indole reagent
- Oxidase reagent
- Gram stain reagents
- *E. coli* O157 Latex Kit
- *Salmonella* Wellcolex
- *Shigella* Wellcolex Kits
- Immuno*Card* STAT! EHEC
- Phoenix NID and NMIC/ID panels

7.0 Specimen Processing

Verify that the appropriate test has been ordered for the specific pathogens requested. Label and inoculate the appropriate media and incubate in specified atmospheres.

1. Routine Stool Culture with Shiga Toxin Test (CSTLST)
    - BBL™ CHROMagar™ O157 (or MacConkey with Sorbitol) – ambient air at 35 ± 2 °C
    - BBL™ CHROMagar™ Salmonella – ambient air at 35 ± 2 °C
    - MacConkey II Agar – ambient air at 35 ± 2 °C
    - GN Broth – ambient air at 35 ± 2 °C (lid loose)
    - Campy CVA Agar – microaerophilic jar at 42 ± 2 °C
    - If *Vibrio* requested, add TCBS agar – ambient air at 35 ± 2 °C
    - If *Aeromonas* requested, add BAP & CIN – ambient air at 35 ± 2 °C (if *Yersinia* and *Aeromonas* are both requested, CIN plate may be incubated at 30 ± 2 °C
    - If *Plesiomonas* requested, add BAP – ambient air at 35 ± 2 °C
2. Stool Culture with *Yersinia* and Shiga Toxin Test (CSTLYS)
    - All of the above media for routine stool culture
    - CIN Agar – ambient air at 30 ± 2 °C
3. Stool Culture for *E. coli* O157 with Shiga Toxin Test (CECST)
    - BBL™ CHROMagar™ O157 (or MacConkey with Sorbitol) – ambient air at 35 ± 2 °C
    - GN Broth – ambient air at 35 ± 2 °C (lid loose)

Culture, *Campylobacter* Screen (CCAMS)

- Campy CVA Agar – microaerophilic jar at 42 ± 2 °C

4. Requests for “all pathogens” should be clarified with client. Set up routine stool culture and add additional media if necessary.

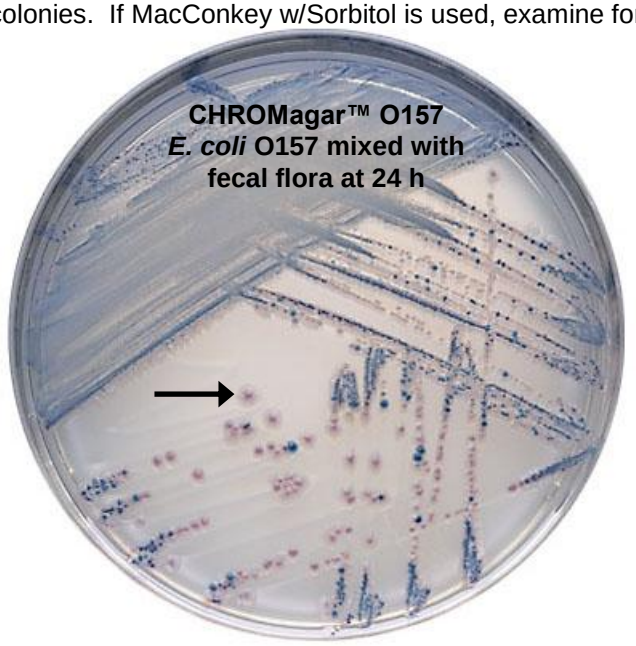
8.0 Culture Examination

8.1 Length of Culture Incubation & Timing of Examination

1. Examine CHROMagar™ media at 18-24 h. Discard negative CHROMagar™ O157 at 24 h. Reincubate and examine CHROMagar™ Salmonella again at 48 h.
2. Examine MacConkey agar plate at 18-24 h and again at 48 h.
3. Examine Campy CVA agar plate at 48 h.
4. Perform Shiga toxin test after 16-24 h incubation of the GN broth.
5. Examine CIN agar plate at 18-24 h and again at 48 h.
6. Examine BAP at 18-24 h and again at 48 h.
7. Examine TCBS agar plate at 18-24 h and again at 48 h.

8.2 CHROMagar™ O157 Work-up

1. Examine medium for suspect "mauve" colonies at 18-24 h. Gram-negative organisms, other than *E. coli* O157, will either be inhibited or produce colorless, blue, green, blue-green or natural color colonies. If MacConkey w/Sorbitol is used, examine for any clear colonies.



2. Select several suspect colonies and test using the latex agglutination test for *E. coli* O157. It is important to test latex-positive colonies with the latex control reagent to rule out non-specific reactions. Refer to the *E. coli* O157 Latex Test Procedure for complete instructions.
3. If the O157 latex is positive, perform biochemical identification by Phoenix™ NID.
4. Do not perform antimicrobial susceptibility testing on *E. coli* O157 isolates.
5. Subculture the isolate to two BHI slants. Send one slant to the public health laboratory for H7 typing and hold the other in the refrigerator with send out stocks.
6. Notify director and/or supervisor during Rounds if *E. coli* O157 is isolated.

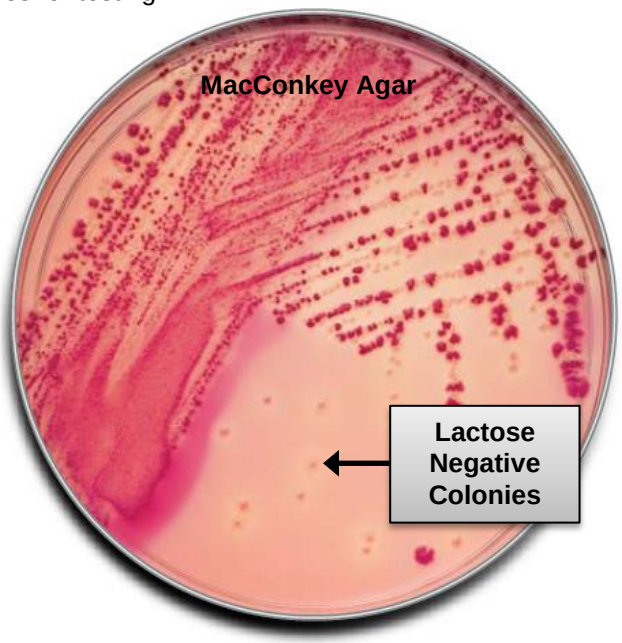
8.3 CHROMagar™ Salmonella Work-up

1. Refer to the CHROMagar Salmonella Procedure for complete instructions for use.
2. Examine plate for "mauve" colonies at 18-24 h and again at 48h. *S. typhimurium* and other *Salmonella* species will appear mauve-colored colonies, with the exception of *Salmonella enterica* subspecies *arizonae* and other *Salmonella* species positive for lactose and beta-glucosidase. Those isolates will appear as blue-violet or purple colonies. *Citrobacter* and other coliforms will appear as blue-green colored colonies. Some organisms that do not hydrolyze any of the chromogenic compounds may appear as colorless colonies.
3. Perform biochemical identification with a Phoenix™ NID panel.
4. If biochemical identification is *Salmonella*, perform Wellcolex latex agglutination test to confirm. Refer to the Salmonella Wellcolex Test Procedure for complete instructions for use.

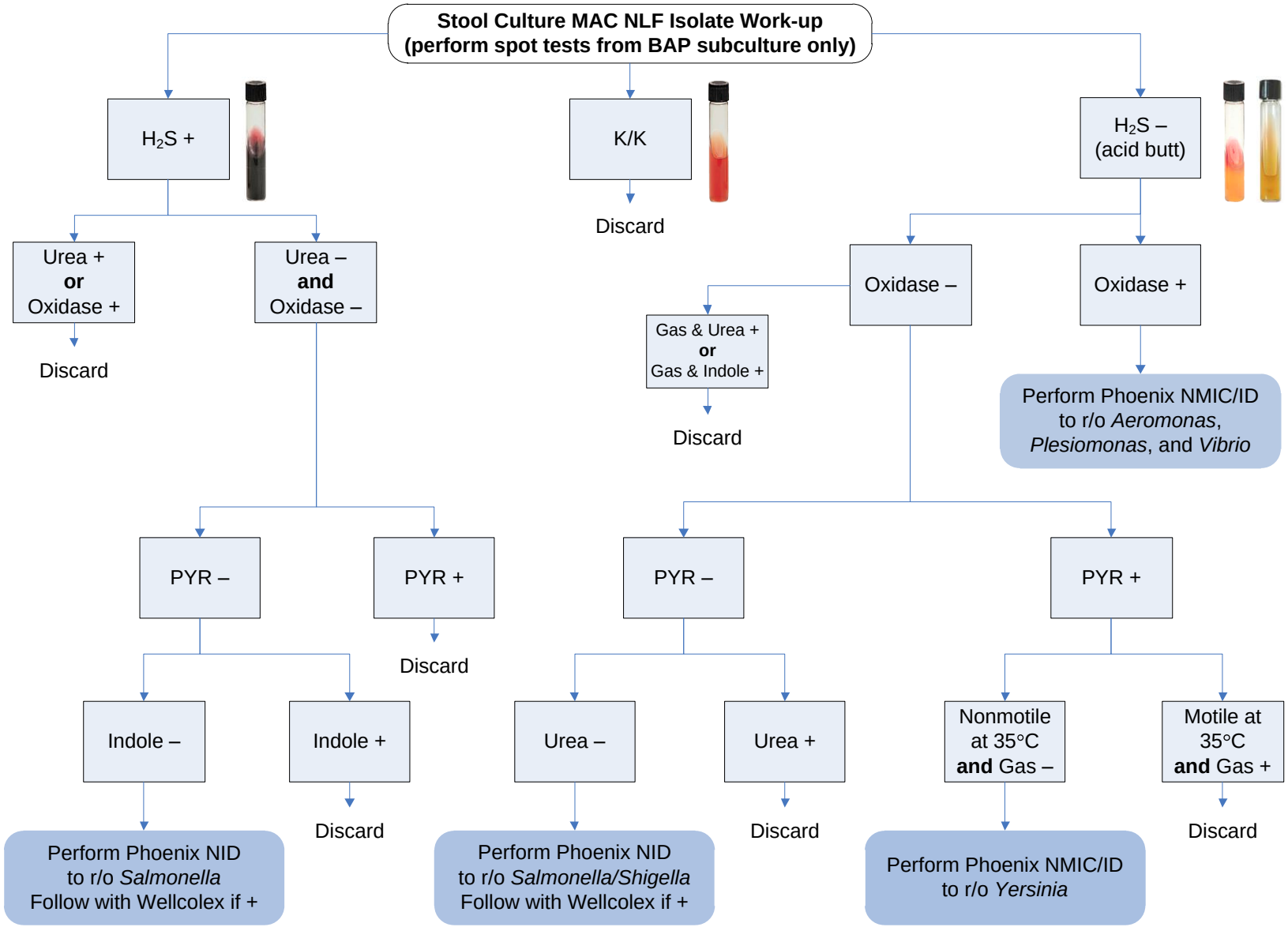
5. Subculture the isolate to two BHI slants. Send one slant to the public health laboratory for typing and hold the other in the refrigerator with send out stocks.
6. Notify director and/or supervisor during Rounds if *Salmonella* is isolated.

8.4 MacConkey Agar Work-up

1. Examine the MacConkey agar plate at 18-24 h and again at 48 h. Any colorless, lactose-negative colonies require further testing. Subcultures may be required to obtain enough isolated colonies for testing.

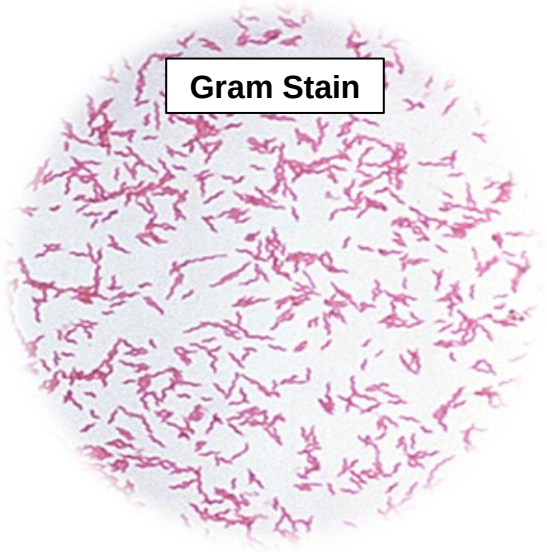


2. Using isolated colonies of the same morphology, inoculate a BAP, TSI, and urea agar slant. Incubate in ambient air at 35 ± 2 °C for 18-24 h.
3. On the following day read biochemical reactions in TSI and urea slants. Refer to TSI Agar and Urea Agar procedures for instructions. Perform spot tests from BAP and follow flowchart below.
4. Notify director and/or supervisor during Rounds if any enteric pathogens are isolated.



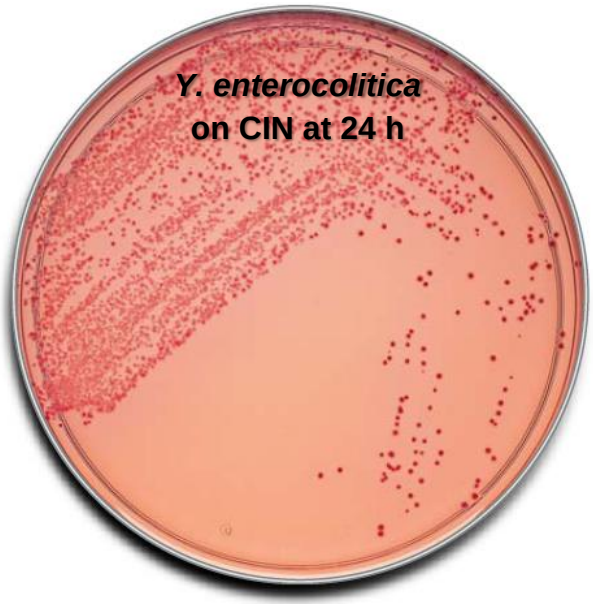
8.5 Campy CVA Work-up

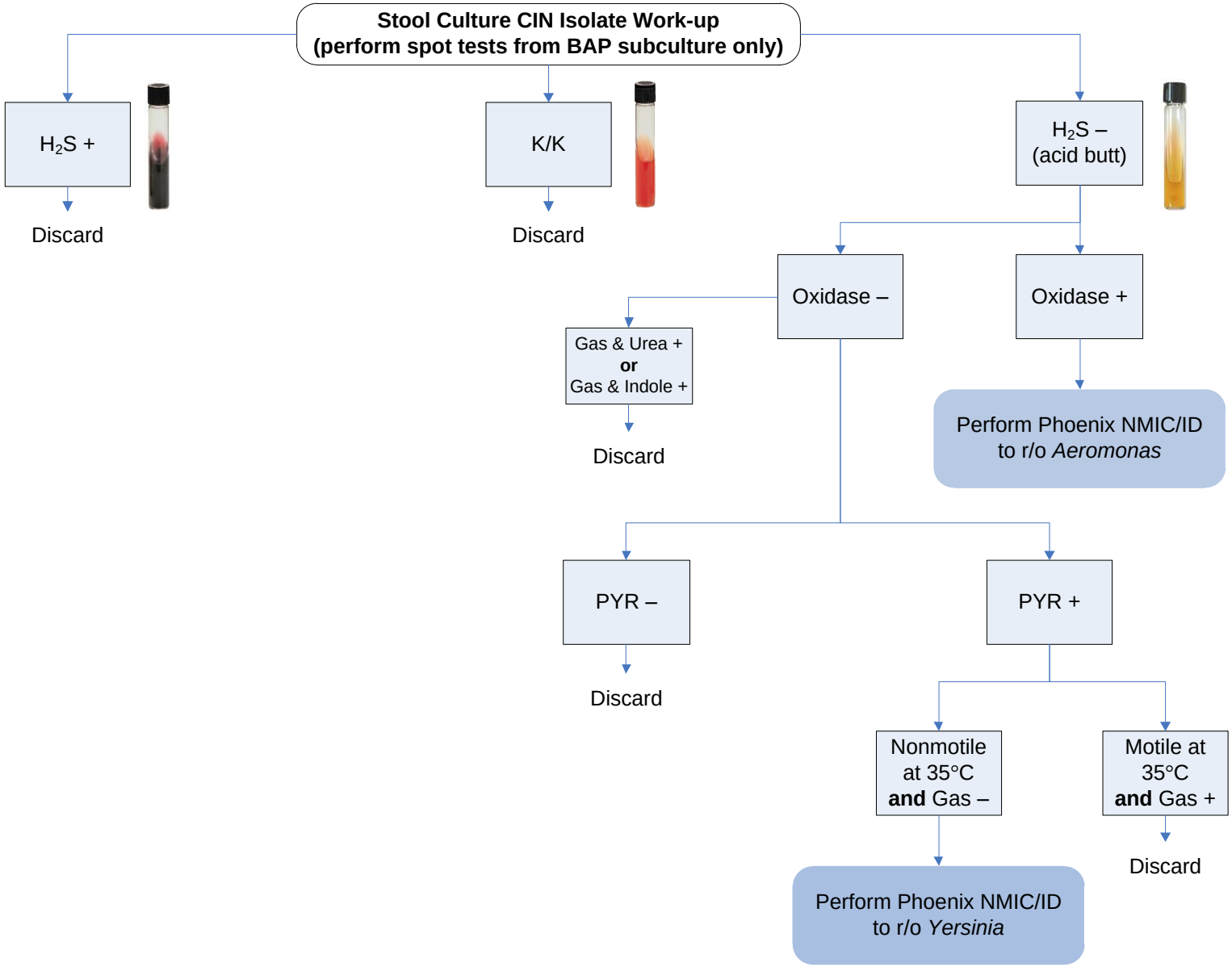
1. After 48 h incubation, open the microaerophilic jars containing the *Campylobacter* cultures and examine the plates. *Campylobacter* colonies are yellowish to gray or pinkish and non-hemolytic. They may appear round or irregular, smooth, raised, convex, and glossy. Colonies may appear to spread along the streak line.
2. Perform an oxidase test on any suspect colonies. *Campylobacter* are oxidase-positive.
3. Prepare a gram stain of suspect colonies. Allow the safranin counter stain to remain on the smear for at least 2 min to improve the visibility of the individual cells. On gram stain, cells are thin, gram-negative rods with marked curved and sometimes spiral shapes. Dividing cells can resemble the wings of a seagull.
4. Confirm identification by performing catalase and motility. *Campylobacter* is strongly catalase positive and, in a wet preparation, has a distinctive motility, darting rapidly across the field of view.
5. Notify director and/or supervisor during Rounds if *Campylobacter* is isolated.



8.6 CIN Agar Work-up

1. Screen at 18-24 h and at 48 h.
2. Look for any colonies with a deep red center with a transparent margin, or “bull’s-eye” appearance.
3. If necessary, subculture suspected colonies for isolation.
4. Using isolated colonies of the same morphology, inoculate a BAP, TSI, and urea agar slant. Incubate in ambient air at 30 ± 2°C for 18-24 h.
5. On the following day read biochemical reactions in TSI and urea slants. Refer to TSI Agar and Urea Agar procedures for instructions. Perform spot tests from BAP and follow flowchart below.
6. Notify director and/or supervisor during Rounds if *Yersinia* or *Aeromonas* are isolated.





8.7 BAP Agar Work-up

1. If *Aeromonas* or *Plesiomonas* were requested, a BAP should be used for screening. Examine BAP at 18-24 h and again at 48 h.
2. Sweep through colonies to test for oxidase production. Pursue all oxidase-positive colonies unless culture smells like *P. aeruginosa*. *Aeromonas* spp. typically produces beta-hemolytic colonies while *Plesiomonas shigelloides* produce opaque, non-hemolytic colonies.
3. If necessary, subculture suspected colonies for isolation.
4. Perform biochemical identification and susceptibility testing with a Phoenix™ NMIC/ID panel.

8.8 TCBS Agar Work-up

1. Examine plate for growth at 18-24 h and again at 48 h. Depending on the species, *Vibrio* may appear as yellow colonies (*V. cholerae* and *V. fluvialis,* & *V. alginolyticus*) or green (*V. parahaemolyticus*, *V. mimicus,* and most strains of *V. vulnificus*).
2. Gram stain any suspicious colonies to rule out gram-positive organisms.
3. Perform Phoenix™ NMIC/ID panel to identify possible *Vibrio* spp.
4. If *Vibrio* is isolated, subculture the isolate to two BHI slants. Send one slant to the public health laboratory for typing and hold the other in the refrigerator with send out stocks
5. Notify director and/or supervisor during Rounds if *Vibrio* is isolated.

9.0 Shiga Toxin Test

1. After 16-24 h of incubation, examine the GN broth for growth.

   DO NOT PROCEED with testing if the broth tube does not exhibit growth after incubation, as testing may produce false negative results.

   - If the MacConkey agar plate in the stool culture set-up has growth, retrieve the original specimen and reset the GN broth.
   - If the MacConkey agar and GN broth are both no growth, report the Shiga toxin results as negative. The lack of growth on the MacConkey agar plate indicates that there are no viable STEC present. Credit the Shiga toxin test portion of the culture with the code, SHIGCR.

2. If growth is evident in the GN broth, perform the Shiga toxin assay. Refer to the Shiga Toxin Test Procedure for complete instructions.

10.0 Reporting Results

10.1 Positive Cultures

1. Positive culture results or Shiga toxin results should be phoned to the clinician as soon as testing is completed.
2. Report the name of the pathogen isolated or the positive Shiga toxin result(s).

3. The following comment should be appended when reporting *Campylobacter* spp., *Salmonella* species, *Shigella* species, Shiga toxin-producing *E. coli, Vibrio* spp., or *Yersinia enterocolitica.* **This is a REPORTABLE DISEASE. Please contact your County/State Health Department.**
4. Always enter significant findings, such as a positive Shiga toxin result or a pathogen, directly under the header. Alternatively, the report order may be changed in LIS so that significant findings will appear in the report before negative, preliminary results that were entered previously.
5. Report antimicrobial susceptibility test results. Susceptibility results for *Salmonella* spp. are reported only by request. Susceptibility results should not be reported for *E. coli* O157. Add the following comment when reporting *E. coli* O157 or a positive Shiga toxin result: **Antimicrobial therapy in patients infected with Shiga Toxin-producing *E. coli* is not recommended as it may increase risk of serious complications such as hemolytic-uremic syndrome.**
6. Report all other specific pathogens that were not isolated on a single line. A routine culture (CSTLST) includes *Salmonella, Shigella, E. coli* O157, and *Campylobacter*. A stool culture with *Yersinia* (CSTLYS) includes the four pathogens in the routine culture plus *Yersinia enterocolitica.*
7. For *Salmonella, Shigella,* Shiga toxin-producing *E. coli, or Vibrio* spp., add the comment: **Sent to state public health laboratory for additional testing.**
8. When the results are received back from the state lab, replace the above comment with, **State lab results:** followed by the exact information provided by the state lab.

10.1.1 *Campylobacter* spp. Reporting Examples

#	Day 1 Report: Shiga toxin results & preliminary culture results
1.	=====SHIGA TOXIN RESULTS=====
2.	Negative for Shiga Toxin 1 and 2
3.	=====CULTURE RESULTS=====
4.	No enteric pathogens isolated to date.

#	Day 2 Final Report: Update culture results for *Campylobacter*
4.	Campylobacter species isolated. This is a REPORTABLE DISEASE. Please contact your County/State Health Department.
5.	Results called to: Lisa R on 9/25/13 at 1310 (inpatient example)
6.	No E. coli O157 Isolated, No Salmonella Isolated, No Shigella Isolated (include Yersinia if that it was ordered)

10.1.2 *Salmonella* spp. Reporting Examples

#	Day 1 Report: Shiga toxin results & preliminary culture results
1.	=====SHIGA TOXIN RESULTS=====
2.	Negative for Shiga Toxin 1 and 2
3.	=====CULTURE RESULTS=====
4.	Results pending further incubation.

#	Day 2 Report: Update culture results for *Salmonella*
4.	Salmonella species isolated. This is a REPORTABLE DISEASE. Please contact your County/State Health Department.
5.	Results called to: Lisa R on 9/25/13 at 1310 (inpatient example)
6.	Sent to state public health laboratory for additional testing.
7.	No Campylobacter Isolated, No E. coli O157 Isolated, No Shigella Isolated (include Yersinia if that it was ordered)

#	Final Report: Add state lab *Salmonella* speciation
6.	State lab results: Salmonella enteritidis

10.1.3 *Shigella* spp. Reporting Examples

#	Day 1 Report: Shiga toxin results & preliminary culture results
1.	=====SHIGA TOXIN RESULTS=====
2.	Negative for Shiga Toxin 1 and 2
3.	=====CULTURE RESULTS=====
4.	Results pending further incubation.

#	Day 2 or 3 Report: Update culture results for *Shigella*
4.	Shigella species isolated. This is a REPORTABLE DISEASE. Please contact your County/State Health Department.
5.	Results called to: Lisa R on 9/25/13 at 1310 (inpatient example)
6.	Sent to state public health laboratory for additional testing.
7.	No Campylobacter Isolated, No E. coli O157 Isolated, No Shigella Isolated (include Yersinia if that it was ordered)

#	Final Report: Add state lab *Shigella* speciation
6.	State lab results: Shigella flexneri

10.1.4 Positive Shiga Toxin & *E. coli* O157 Reporting Examples

#	Day 1 Report: Shiga toxin results & preliminary culture results
1.	=====SHIGA TOXIN RESULTS=====
2.	Positive for Shiga Toxin 2 and Negative for Shiga Toxin 1
3.	Antimicrobial therapy in patients infected with Shiga Toxin-producing E. coli is not recommended as it may increase the risk of serious complications such as hemolytic-uremic syndrome.
4.	Results called to: Lisa R on 9/25/13 at 1310 (inpatient example)
5.	=====CULTURE RESULTS=====
6.	Results pending further incubation.

#	Day 2 Report: Update culture for *E. coli* O157
6.	Escherichia coli O157 isolated. This is a REPORTABLE DISEASE. Please contact your County/State Health Department.
7.	Sent to state public health laboratory for additional testing.
8.	No Campylobacter Isolated, No Salmonella Isolated, No Shigella Isolated (include Yersinia if that it was ordered)

#	Final Report: Add state lab H7 typing
7.	State lab results: Escherichia coli O157/H7. Serotyping results were obtained with research procedures and reagents for epidemiologic purposes. Not to be used for diagnosis, treatment or assessment of patient's health (as per Washington State Laboratory).

10.1.5 Positive Shiga Toxin with No *E. coli* O157 Reporting Examples

#	Day 1 Report: Shiga toxin results & preliminary culture results
1.	=====SHIGA TOXIN RESULTS=====
2.	Positive for Shiga Toxin 1 and Negative for Shiga Toxin 2
3.	Antimicrobial therapy in patients infected with Shiga Toxin-producing E. coli is not recommended as it may increase the risk of serious complications such as hemolytic-uremic syndrome.
4.	This is a REPORTABLE DISEASE. Please contact your County/State Health Department.
5.	Results called to: Lisa R on 9/25/13 at 1310 (inpatient example)
6.	=====CULTURE RESULTS=====
7.	Results pending further incubation.

#	Day 2 Report: Update culture results
7.	Sent to state public health laboratory for additional testing.
8.	No Salmonella, Shigella, Campylobacter, E coli O157 isolated (include Yersinia if that it was ordered)

#	Final Report: Add state lab *E. coli* typing
7.	State lab results: Escherichia coli serotype O111. Serotyping results were obtained with research procedures and reagents for epidemiologic purposes. Not to be used for diagnosis, treatment or assessment of patient's health (as per Washington State Laboratory).

10.1.6 *Yersinia enterocolitica* Reporting Examples

#	Day 1 Report: Shiga toxin results & preliminary culture results
1.	=====SHIGA TOXIN RESULTS=====
2.	Negative for Shiga Toxin 1 and 2
3.	=====CULTURE RESULTS=====
4.	Results pending further incubation.

#	Day 2 or 3 Final Report: Update culture results for *Yersinia enterocolitica*
4.	Yersinia enterocolitica isolated. This is a REPORTABLE DISEASE. Please contact your County/State Health Department.
5.	Results called to: Lisa R on 9/25/13 at 1310 (inpatient example)
6.	No Salmonella, Shigella, Campylobacter, E coli O157 isolated

10.1.7 *Aeromonas* spp. or *Plesiomonas shigelloides* Reporting Examples

#	Day 1 Report: Shiga toxin results & preliminary culture results
1.	=====SHIGA TOXIN RESULTS=====
2.	Negative for Shiga Toxin 1 and 2
3.	=====CULTURE RESULTS=====
4.	Results pending further incubation.

#	Day 2 or 3 Final Report: Update culture results for *Aeromonas* or *Plesiomonas*
4.	Aeromonas/Plesiomonas isolated.
5.	No Salmonella, Shigella, Campylobacter, E coli O157 isolated (include Yersinia if that it was ordered)

10.2 Negative Cultures

#	Day 1 Report: Shiga toxin results & preliminary culture results
1.	=====SHIGA TOXIN RESULTS=====
2.	Negative for Shiga Toxin 1 and 2
3.	=====CULTURE RESULTS=====
4.	No enteric pathogens isolated to date.

#	Day 2 Report: Update culture results
1.	=====SHIGA TOXIN RESULTS=====
2.	Negative for Shiga Toxin 1 and 2
3.	=====CULTURE RESULTS=====
4.	No Salmonella, Shigella, Campylobacter, E coli O157 isolated (include Yersinia if that it was ordered)

11.0 Quality Control & Quality Assurance

11.1 Culture Media

1. Exempt Media – user quality control testing not required.
   - CIN Agar
   - GN Broth
   - MacConkey II Agar
   - TCBS Agar
   - Trypticase™ Soy Agar with 5% Sheep Blood (TSA II™)
2. Nonexempt – user quality control required.
   - BBL™ CHROMagar™ O157
   - BBL™ CHROMagar™ Salmonella
   - Campy CVA Agar

   Each new lot or shipment of media should be examined for product deterioration and tested with the following control strains. Prepare a 0.5 McFarland suspension of each test strain and dilute as indicated. Use a 0.01 mL calibrated loop to inoculate the media. Incubate CHROMagar™ plates at 35 ± 2°C in an aerobic atmosphere for 24 h. Incubate Campy CVA plate at 42 ± 2°C in a microaerophilic jar for 48 h.

Control strain	Dilution	Expected Results
CHROMagar O157		
E. coli ATCC 700728	1 : 100	Growth of mauve colonies
E. coli ATCC 25922	1 : 10	Inhibition (partial to complete)
E. cloacae ATCC 13047	1 : 10	Growth of blue-green to blue colonies
CHROMagar Salmonella		
S. enterica ATCC 14028	1 : 100	Growth of mauve colonies
E. coli ATCC 25922	1 : 10	Inhibition (partial to complete)
S. aureus ATCC 25923	1 : 10	Inhibition (partial to complete)
C. freundii ATCC 8090	1 : 10	Growth of blue-green to blue colonies
Campy CVA		
C. jejuni ATCC 33291	1 : 100	Growth
E. coli ATCC 25922	1 : 10	Inhibition (partial to complete)

11.2 Shiga Toxin Test Kit

1. Internal controls

   Internal controls are contained within the test strip and therefore are evaluated with each test. A pink-red band appearing at the Control line serves as a procedural control and indicates the test has been performed correctly, proper flow occurred and the test reagents were active at the time of use. A clean background around the Control or Test lines also serves as a procedural control. Control or test lines that are obscured by heavy background color may invalidate the test and may be an indication of reagent deterioration, use of an inappropriate sample or improper test performance.

2. External controls

   External control reagents should be tested with each new lot or shipment. The external controls are used to monitor reagent reactivity and test performance. Failure of the controls to produce the expected results can mean that one of the reagents or components is no longer reactive at the time of use, the test was not performed correctly or that reagents or samples were not added. Refer to the Shiga Toxin Test Procedure for instruction on performing QC testing with external controls. The kit should not be used if control tests do not produce the correct results.

12.0 Limitations

12.1 General Limitations

1. Because the media used for culture are highly selective, some pathogens that should grow may be inhibited.
2. Without a specific request for *Aeromonas, Plesiomonas, Vibrio,* or *Yersinia*, appropriate media will not be inoculated, and these pathogens may or may not be detected in culture.
3. Pathogens present in small numbers may not be detected.
4. For identification and susceptibility testing, isolates must be in pure culture. Subcultures may be necessary prior to testing suspect colonies.

12.2 CHROMagar™ O157 Limitations

1. BBL CHROMagar O157 does not detect enterohemorrhagic or enteropathogenic serotypes of *E. coli* other than O157:H7, since they may differ biochemically.
2. β-glucuronidase-positive strains of *E. coli* O157:H7 will not be detected on BBL CHROMagar O157; however, such strains are rare.
3. BBL CHROMagar O157 does not differentiate between toxin-producing and non-toxin-producing strains of *E. coli* O157:H7.
4. Organisms other than *E. coli* O157:H7, such as *Proteus* spp., may grow on this medium. However, they generally produce a different color. If non-isolated mauve colonies are observed, isolation can be achieved by subculturing to another BBL CHROMagar O157 plate.
5. Confirmatory tests are necessary for definitive identification.
   - Rare strains of *E. coli* (biochemically similar to *Shigella*) have been found that produce false positive results on BBL CHROMagar O157.
   - *Salmonella* serotype Heidelberg exhibits mauve colonies when plated on BBL CHROMagar O157 medium.
6. Incubation at lower than recommended temperatures may delay detection of positive reactions.
7. Plates should not be incubated beyond the 24 h time period prior to reading.

12.3 CHROMagar™ Salmonella Limitations

1. Occasionally strains of *Aeromonas hydrophila*, *Hafnia alvei*, *Pseudomonas aeruginosa*, *P. putida*, *Acinetobacter* species, or *Candida* species may not be completely inhibited, and colonies may exhibit light mauve to mauve pigmentation.
2. Rare strains of the following organisms may fail to grow or have reduced growth on this medium: *S. typhi*, *S. paratyphi* A, *S. typhimurium*, *S*. *choleraesuis*, *S. minnesota*, *S. enterica* subsp. *arizonae*, and *S. pullorum*. This is strain specific, and the majority of the strains tested by the manufacturer of each of these serovars were recovered.
3. When testing some samples, a purple discoloration of the medium, without detectable colony growth, may be observed. This should be considered a negative result.

12.4 Campy CVA Agar Limitations

1. The methods described here are intended to recover and identify only the most common pathogenic strains of *Campylobacter*, with isolation and identification of *C. jejuni*, the most common species. Recovery procedures for other *Campylobacter* species are not clearly definied.

12.5 Immuno*Card* STAT! EHEC Limitations

1. The test is qualitative, and no quantitative interpretation should be made with respect to the intensity of the positive line when reporting the result.
2. Test results are to be used in conjunction with information available from the patient clinical evaluation and other diagnostic procedures.
3. Failure to add 150 µL of broth culture to the 5 drops of Sample Diluent will lead to false negative results.

4. The addition of more than 5 drops of Sample Diluent can also lead to false negative test results.
5. Over incubation of tests may lead to false-positive test results. Incubating tests at reduced temperatures or times may lead to false negative results.
6. The performance of Immuno*Card* STAT! EHEC has not been evaluated with direct stool samples. The manufacturer has only evaluated its performance with SMAC plate culture or GN and MacConkey liquid cultures. The GN broth method is the only method validated for use in the PSHMC Microbiology lab.
7. Shiga toxin 1 produced by *E*. *coli* and the toxin produced by *Shigella dysenteriae* type 1 strains are nearly identical. Therefore, Immuno*Card* STAT! EHEC may give a positive result with toxins from *S. dysenteriae* type 1 strains.

13.0 Verification Information

13.1 CHROMagar™ O157 Verification

In the first verification phase, a total of 13 *E. coli* O157 isolates were tested on CHROMagar™ O157 by seeding into a stool matrix. This included 12 isolates from stock cultures of clinical isolates and one ATCC strain, 700728. A suspension equivalent to a 0.5 McFarland turbidity standard was made for each of these 13 isolates. Each of these suspensions was diluted 1:100 to achieve an approximate concentration of 10^6 CFU/mL. Each of these stock suspensions was diluted into a pooled stool matrix to achieve a final concentration of 10^5 CFU/mL. The pooled stool matrix was previously plated to CHROMagar™ O157 and MacConkey with Sorbitol (SMAC) to verify that no colonies resembling O157 would be present. A separate sterile swab was dipped into each of the seeded stool suspensions and plated onto CHROMagar™ O157 and SMAC plates. These plates were incubated overnight in a dark, aerobic incubator at 35 ± 2°C. The plates were examined for colonies consistent with *E. coli* O157. All of the SMAC plates yielded growth of sorbitol fermenting flora that obscured any clear colonies that were present. However, the CHROMagar™ plates inhibited nearly all of the fecal flora. Twelve of the CHROMagar™ plates produced “mauve” colonies consistent with *E. coli* O157. One culture yielded atypical, dark blue colonies that tested positive with the O157 latex reagent (Pro-Lab Diagnostics). This isolate was further characterized by the WA PHL as serotype H7. The table below summarizes the data from this portion of the study.

Recovery of *E. coli* O157 isolates seeded into stool matrix at 10^5 conc.

	n (%) recovered
SMAC	0 (0)
CHROM	12 (92)

The other five isolates included in this evaluation came directly from stool specimens that were submitted for testing. Two samples were submitted in Modified Cary-Blair transport media, and three were submitted as fresh samples. The specimens were inoculated directly onto the CHROMagar™ O157 in the same manner as the other routine stool culture media. The plates were incubated overnight in a dark, aerobic incubator at 35 ± 2°C before they were examined. One (20%) of the isolates was recovered only on CHROMagar™. One (20%) other isolate was recovered on both SMAC and CHROMagar™, but the SMAC culture required subculture to obtain enough isolated colonies for testing while the CHROMagar™ plate had sufficient isolation for a full identification on day 1. The other three (60%) isolates were recovered on both media on day 1. The table below summarizes the data from this portion of the study.

Recovery of *E. coli* O157 isolates from original stool specimens. n (%)

	Recovery Day 1	Total recovery Day 2
SMAC	3 (60)	4 (80)
CHROM	5 (100)	5 (100)

This stool specimen containing the *E. coli* O157 that was only recovered by CHROMagar™ was also subjected to Shiga Toxin testing by three different assays using GN broth culture. None of the Shiga Toxin assays were positive for this specimen, presumably due to the low number of *E. coli* O157 present. The isolate only tested positive for Shiga Toxin when it was subcultured in pure inoculum to GN broth.

Of the 18 total isolates tested in this study, 15 (83%) were serotype H7 and 3 (17%) were the non-motile O157 strain, NM.

13.2 CHROMagar™ Salmonella Verification

The evaluation of BBL™ CHROMagar™ Salmonella was performed with clinical samples and seeded samples created by diluting *Salmonella* isolates into a stool matrix. A total of 50 *Salmonella* isolates from clinical and seeded specimens were evaluated on CHROMagar™ and XLD in parallel.

Clinical specimens included 18 specimens that previously tested positive for *Salmonella* spp. on XLD agar and were frozen at -70°C prior to this evaluation. The specimens were thawed, planted on CHROMagar™ and XLD agar, and incubated overnight at 35 ± 2°C in an aerobic incubator. After incubation, the plates were examined for colonies consistent with *Salmonella*. A total of 17 (94%) of the samples produced characteristic colonies on CHROMagar™. All 18 (100%) of the samples produced characteristic colonies on XLD agar. One of the CHROMagar™ cultures did not yield characteristic *Salmonella* colonies due to overgrowth of competing flora.

An additional 30 clinical samples were tested with both media. These specimens produced black colonies on XLD agar during routine culture but were reported as negative for *Salmonella*. These samples were retrieved from the refrigerator and planted to CHROMagar™ and XLD agar and incubated overnight at 35 ± 2°C in an aerobic incubator. After incubation, the plates were examined for colonies consistent with *Salmonella*. Three (10%) of the specimens produced colonies on CHROMagar™ that were consistent with *Salmonella* spp. Additional testing by Phoenix and Wellcolex confirmed that these colonies were *Salmonella* spp. The companion XLD cultures for two of the three specimens produced black colonies in very low numbers that were overgrown by competing flora. These colonies were either overlooked in the original culture by the bench technologist or they were unable to get the colonies isolated for testing. The third culture produced isolated black colonies on XLD that were tested by the bench technologist to rule out *Salmonella*. However, the black colonies proved to be a mixture of *Proteus mirabilis* and *Salmonella* spp. Selection of the *Proteus* colonies for testing prevented the identification of the *Salmonella*. The CHROMagar™ medium proved to be more sensitive and specific in detecting *Salmonella* in these specimens from patients that would have otherwise been undiagnosed.

The 20 *Salmonella* isolates recovered on CHROMagar™ from clinical specimens included the following strains: 6 *Salmonella enteritidis*, 1 *Salmonella newport*, 2 *Salmonella stanley*, 2 *Salmonella typhimurium*, 1 *Salmonella javiana*, 1 *Salmonella* subspecies I 4,5,12 I, 1 *Salmonella hvittingfoss*, 1 *Salmonella* serogroup B, and 5 *Salmonella* species (no typing available).

To broaden the number and variety of *Salmonella* isolates in the evaluation, 32 additional specimens were created seeding a stool matrix with diluted suspensions of previously isolated *Salmonella*. This included the following strains: 7 *Salmonella enteritidis*, 3 *Salmonella hadar*, 1 *Salmonella heidelberg*, 1 *Salmonella marina* IV, 2 *Salmonella montevideo*, 1 *Salmonella muenchen*, 2 *Salmonella newport*, 1 *Salmonella* paratyphi, 1 *Salmonella rissen*, 1 *Salmonella saintpaul*, 1 *Salmonella thompson*, 1 *Salmonella typhi,* 7 *Salmonella typhimurium*, 1 *Salmonella virchow*, 1 *Salmonella weltevreden*, and 1 *Salmonella* species (no type information available). A suspension equivalent to a 0.5 McFarland turbidity standard was made for each of the isolates. Each of these suspensions was diluted 1:100 to achieve an approximate concentration of 10^6 CFU/mL. Each of these stock suspensions was diluted into a pooled stool matrix to achieve a final concentration of 10^5 CFU/mL. The pooled stool matrix was previously plated to CHROMagar™ and XLD agar to verify that no colonies resembling *Salmonella* would be present. A separate sterile swab was dipped into each of the seeded stool suspensions and plated onto CHROMagar™ and XLD plates. These plates were incubated overnight in a dark, aerobic

incubator at 35 ± 2°C. The plates were examined for colonies resembling *Salmonella*. All 32 (100%) of the CHROMagar™ cultures produced isolated *Salmonella* colonies at 24 h. A total of 29 (91%) of the XLD cultures produced isolated *Salmonella* colonies at 24 h. Three (7%) of the XLD cultures did not demonstrate characteristic colonies until 48 h and were not well isolated.

Even though CHROMagar™ Salmonella costs more than XLD agar, the cost is offset by the reduction in subculturing for isolation and additional screening biochemical tests. Organisms such as *Proteus* and *Citrobacter* spp. produce colonies similar to *Salmonella* spp. on XLD but are inhibited, or easily differentiated, on CHROMagar™. Testing with CHROMagar™ Salmonella reduces the time to identify *Salmonella* isolates. In this evaluation, CHROMagar™ Salmonella demonstrated a higher sensitivity by detecting an additional 3 *Salmonella* isolates that were missed by routine culture with XLD.

13.3 Shiga Toxin Test Verification

In this study, three different assays were evaluated for use. This included two EIA-based products, the Premier EHEC (Meridian Bioscience, Inc.) and ProSpecT Shiga Toxin (Remel). The EIA test results were interpreted visually. The third product is an immunochromatographic product, the ImmunoCard STAT! EHEC (Meridian Bioscience, Inc.).

In the first phase of the evaluation, 17 stool specimens that were submitted for routine culture were tested using all three Shiga Toxin assays. This included 7 fresh samples and 10 that were submitted in modified Cary-Blair (CB) transport medium. Each stool specimen was sampled using a sterile swab which was then used to inoculate a GN broth tube. The GN broths were incubated with loose caps overnight in an aerobic incubator at 35 ± 2°C. The GN broths were then used to perform the Shiga toxin assays. A total of 4 (24%) of the stool samples yielded *E. coli* O157 by culture with BD BBL™ CHROMagar™ O157. A total of 3 (18%) stool samples yielded positive results by all three toxin assays. One (6%) stool sample that was culture positive yielded false-negative results on the Shiga toxin assays. A MacConkey-Sorbitol culture of this stool specimen grew abundant fecal flora that completely obscured the clear colonies. The CHROMagar culture for this sample yielded only a few colonies of *E. coli* O157. Subcultures of the GN broth revealed that the *E. coli* O157 was still in proportionally low numbers with an abundance of enteric flora. To further evaluate this isolate, a colony was picked from the CHROMagar plate and inoculated into another GN broth. Testing of this broth yielded positive toxin results with all three assays. This proved that the isolate was a toxigenic strain. Presumably, it was not detected in the original GN broth cultures because the other enteric flora were able to outgrow the low numbers of the O157. The results from this phase of the study are summarized in Table 1 below.

Table 1: Evaluation of Clinical Specimens

	O157 Culture		Meridian Card		Meridian EIA		Remel EIA	
	n	%	n	%	n	%	n	%
Fresh Positive	3	18	2	12	2	12	2	12
CB Positive	1	6	1	6	1	6	1	6
Total Positive	**4**	**24**	**3**	**18**	**3**	**18**	**3**	**18**
Fresh Negative	6	35	7	41	7	41	7	41
CB Negative	7	41	7	41	7	41	7	41
Total Negative	**13**	**76**	**14**	**82**	**14**	**82**	**14**	**82**

Two of these O157 isolates were identified by the Washington State Public Health Laboratory as type H7, and the other two were identified as the non-motile strain, NM.

In the second phase of the study, 34 additional clinical STEC isolates were tested by seeding each isolate into a STEC-negative, pooled stool matrix. This included 14 *E. coli* O157 isolates from stock cultures and 20 non-O157 isolates that were obtained from the Washington State Public Health Laboratory. The O157 strains included one non-motile strain and 13 that were type H7. The non-O157 strains included a variety of serotypes, including three O26, two O111, one O103, and 14 undefined strains.

A 0.5 McFarland (approximately 10^8 CFU/mL) suspension was prepared from 24-h BAP growth of each test strain. Each suspension was then diluted 1:10 with saline to yield a concentration of approximately 10^7 CFU/mL. 100 µL of each suspension was then added to separate tubes containing 900 µL of the stool matrix to yield a final concentration of 10^6 CFU/mL of STEC. Each test suspension was vortexed and then sampled using a sterile swab which was used to inoculate individual GN broth tubes. The GN broth tubes were incubated with loose caps overnight in an aerobic incubator at 35 ± 2°C. All 14 (100%) of the *E. coli* O157 suspension cultures yielded positive results with the Shiga Toxin assays. A total of 19 (95%) of the non-O157 suspension cultures yielded positive Shiga Toxin results with the ImmunoCard STAT! EHEC assay. All 20 (100%) of the non-O157 suspension cultures yielded positive toxin results by both EIA products. However, 1 of these cultures was very weakly positive with both EIA tests. This was the same culture that yielded a negative result by the ImmunoCard assay. A new seeded specimen was prepared with this isolate and cultured in GN broth as before for repeat testing. The second cultured suspension yielded weak results by all three toxin assays. The results from this phase of the study are detailed in Table 2 below.

Table 2: Evaluation of STEC Seeded Stool Specimens (% Positive)

Serotype	# of isolates	Meridian Card	Meridian EIA	Remel EIA
O157	14	100	100	100
non-O157	20	100*	100	100

*1 non-O157 isolate yielded a negative initial result and a weak positive after repeat testing

For the ImmunoCard STAT! EHEC assay, 9 of the O157 strains were positive for both ST1 and ST2 and 5 strains were positive for ST2 only. A total of 18 of the non-O157 strains were positive for ST1 only, 1 strain was positive for ST2 only, and 1 strain was positive for both ST1 and ST2.

Table 3 below summarizes the data from the first two phases of the evaluation study. One of the clinical samples yielded a false-negative Shiga toxin result with all 3 assays. One of the STEC-seeded samples containing a non-O157 strain was initially negative by the ImmunoCard STAT! EHEC assay and weakly positive by both EIA assays. Repeat testing with the non-O157 strain yielded weak Shiga toxin results with all 3 assays.

Table 3: Data summary for clinical specimens and STEC-seeded stools.

	Negative ST	Positive ST	Total
Negative Cx	13	1	14
Positive Cx	0	37	37
Total	13	38	51

% Agreement = 98.0% (89.7 to 99.7%)

Positive Agreement = 97.4%

Negative Agreement = 100%

The third phase of the evaluation study examined specimen stability. Three specimens were available in pairs of fresh and modified Cary-Blair specimens. Initial colony counts were performed on the specimens. The culture plates were refrigerated to serve as reference comparisons later. The paired specimens were left at room temperature for 7 days after which colony count testing was repeated. The culture plates from the 7-day-old specimens were compared to the initial colony count plates. No difference in the number of colonies recovered was observed when comparing fresh vs. modified Cary-Blair specimens. All 3 fresh and 3 Cary-Blair specimens yielded positive toxin results when cultured in GN broth and when subsequently tested with the three Shiga Toxin assays.

As all 3 Shiga toxin assays performed essentially equally well, the assay selection decision was based on a balance of product and labor costs, along with the feasibility of incorporating the testing into the existing operational work flow in the Microbiology department. While the ImmunoCard STAT! EHEC was the most expensive product of the three, it also required the least amount of technical hands-on time. Each ImmunoCard STAT! EHEC test requires approximately 1 min of hands-on time to perform the test. The EIA products require several wash and incubation steps. The hands-on time is about 30 – 45 min, but the actual time that a technologist is committed to the assay is about 2 h.

14.0 References

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10. Package insert: BD BBL™ CHROMagar™ Salmonella, 8010557, Rev. 01, 12/2008.
11. Package insert: BD BBL™ TCBS Agar, L007414, Rev. 07, 10/2006.
12. Package insert: BBL™ Campy CVA Agar, L007355, Rev. 05, 1/2006.

15.0 Document Control History

Medical Director Approval: Reviewed by Dr. Schappert 3/10/2010. CHROMagar™ O157 Procedure reviewed 03/10/2010. CHROMagar™ Salmonella Procedure reviewed 03/08/2012.

Microbiology Director Approval: Dr. Ann Robinson Stool Culture Procedure 04/01/2006, Updates reviewed 03/12/2012. CHROMagar™ O157 Procedure 11/11/2009. CHROMagar™ Salmonella Procedure 03/14/2012.

Microbiology Supervisor Reviews: Jerry Claridge 04/04/2006, 01/2007, 09/2007, 09/2008, 09/2009, 11/11/2009, 03/2011, 03/14/2012, 03/2013, 03/2015

Revisions & Updates: 11/2009 Changed from MacConkey-Sorbitol to CHROMagar™ O157. 03/08/2012 Changed XLD to CHROMagar™ Salmonella (effective 04/02/2012). Added flowchart for MAC NLF and CIN isolate work-up. Updated reporting instructions, media, and limitations. 08/01/2013 Updated to PPM format and consolidated separate CHROMagar™ procedures into this procedure. 02/24/2014 Changed NMIC/ID to NID Phoenix panel for testing suspected *Salmonella* isolates. 04/15/2014 Added reporting examples. 02/19/2015 Added instructions for MacConkey with Sorbitol as a substitute for CHROMagar O157.