POLICY STATEMENT	The intestinal tract is home for a large variety of organisms. In cases of intestinal infection, therefore, our efforts are guided toward the isolation of a specific pathogen or for an imbalance in the normal flora. The recovery of enteric pathogens is dependent on how quickly the stool specimen is received and processed after collection. EHEC Shiga toxin testing for Shiga toxin 1 and Shiga toxin 2 is performed on all stool specimens submitted for stool culture, along with *Salmonella, Shigella, Vibrio, E. coli 0157:H7* and *Campylobacter*. *Campylobacter* testing is performed via an Immunochromatography methodology with all positive *Campylobacter* antigen tests being reflexed to a *Campylobacter* culture for confirmation. Yersinia cultures with the incubation of selective differential plates at room temperature which enhances the recovery of Yersinia are performed upon request.
PURPOSE	This procedure provides technical instruction for the performance of the Enteric Cultures.
SCOPE	This procedure applies to testing personnel authorized to perform testing. This group includes, but is not limited to Laboratory Technologists as well as leads and supervisory personnel.
RESPONSIBILITY	All the above personnel are responsible for following the Enteric Cultures procedure without exception. In addition, testing personnel are also responsible for evaluating the results and taking proper remedial action.
RELATED DOCUMENTS	MICR 6140 R Specimen Processing MICR 6305 R Bacterial Cultures MICR 6915 R ImmunoCard STAT! CAMPY MICR 6920 R ImmunoCard STAT! EHEC

SPECIMEN HANDLING

See *MICR 6140 R Specimen Processing*

- Stool specimens should be collected in Cary-Blair based media and stored at 2-8°C for up to 48 hours.
- Do not collect more than 2 to 3 specimens per patient with one specimen per day for diagnostic testing. Reject multiple specimens from the same day.
- Do not collect specimens on inpatients after 4 days of admission (5 days for pediatric patients) for diagnostic testing. Consider testing for Clostridium difficile.
- Fungal cultures on stool are rejected. Add statement: "Fungal cultures of stool have not been shown to be clinically useful."

CULTURE WORKUP

1. Examine all plates for macroscopic growth at 24 and 48 hours
2. If Gram-positive flora and Gram-negative flora are present, report as **"**normal flora**"**
    - Normal fecal flora
        - Staphylococcus
        - Streptococcus viridians
        - Enterococcus
        - diphtheroids
        - Lactobacilli
        - Enterobacteriaceae
3. Subculture non lactose fermenting colonies to TSI, LIA and urea slants and BAP to screen for pathogens. Lactose fermenting colonies need not be screened.
4. Set up a Rapid ID panel on organisms that conform to biochemical types for enteric pathogens (See Chart below). If the identification is confirmed as a pathogen, report the identification and perform antimicrobial susceptibility testing (AST).
    - Enteric Pathogens
        - Salmonella
        - Shigella

- o E. coli 0157:H7
- o Aeromonas
- o Plesiomonas
- o Edwardsiella
- o Vibrio
- o Yersinia
- o Campylobacter
- o Bacillus cereus

	TSI REACTIONS						
LIA REACTIONS	K/A H2S+	K/AG H2S+	K/AG	K/A	A/AG H2S+	A/AG	A/A
R/A		P.vulgaris (rare) P. mirabilis	P.morganii (rare) Providencia	P.morganii (rare) P.rettgeri Providencia	P.vulgaris P.mirabilis (rare)		P.rettgeri (rare)
K/K or N H2S+	S.typhi Salmonella (rare) Arizona (rare) Edwardsiella	Salmonella Arizona Edwardsiella	Salmonella (rare) Arizona (rare)	S. typhi (Rare)	Arizona Salmonella (rare)		
K/K or N	Salmonella (rare)		Enterobacter hafniae Klebsiella Serratia (occ.)	Serratia S.typhi (rare) Klebsiella (rare) Enterobacter hafniae (rare		Klebsiella Ent. aerogenes Serratia liquefaciens E.coli	Serratia
K/A H2S+		Citrobacter			Citrobacter		
K/A			E.agglomerans E.coli P.morganii Paratyphi A S.flexneri (some biotypes) (uncommon) C.diversus	E.coli (A.D.) Shigella P.morganii E.agglomerans Y.pseudo-tuberculosis Y.pestis A.acitinmycet-emcomitans C.violaceum		E.coli (rare) Citrobacter (rare) E.cloacae E.agglomerans H.aphrophilus	E.coli E.agglomerans C.diversus H.aphro-philus Y. entero-colitica C.violaceum Y.Pseudo tuberculosis (occ)

Key: R = red, oxidative deamination of lysine; K= Alkaline slant; A= acid slant; /K= alkaline butt; /A =acid butt; g = gas in butt; H_2S = Hydrogen sulfide production. N = neutral.
For TSI Acid reaction = yellow. Alkaline reaction = Red.
For LIA Acid reaction = Yellow. Alkaline reaction = Purple.
For H_2S positive reaction = Blacking of media.

5. Examine the Macconkey Sorbitol agar plate at 24 and 48 hours for clear colonies (non-sorbitol fermenter) suspicious of E. coli 0157:H7. Perform identification and AST. Send sorbitol negative E.coli isolates to reference laboratory for serologic identification. Pink colonies (sorbitol fermenter) need not be screened.
6. Examine the TCBS agar plate at 24 and 48 hours for large yellow colonies and/or colonies with blue-green centers
   - If colonies are present, perform a Gram stain. *Vibrio* is a curved comma shaped Gram-negative rod.
   - Isolate colonies to a BAP and TSI
   - Perform oxidase, if positive and the TSI is a glucose fermenter, *Vibrio* is suspected. Set up MicroScan using sterile 0.85% saline. See MICR 6540 R Microscan Panels for Saline Procedure.
7. Identify if heavy growth **and** predominate **and** in quantities greater than the normal flora. Lesser amounts are reported as normal flora.
   - Pseudomonas aeruginosa – Do not perform an AST
   - Staphylococcus aureus – perform PBP2. Do not perform an AST
   - Yeast – perform Germ tube and report as Candida albicans or Candida sp.not albicans.
   - Bacillus – send to reference lab to screen for Bacillus cereus (β-hemolytic) or Bacillus anthracis (non β-hemolytic)

Yersina Culture

To detect the presence or absence of *Yersina*

1. Examine the room temperature CIN agar plate at 24 and 48 hours for colonies that are dark pink with a translucent border. There may be zone-precipitated bile surrounding the colonies. Mucoid colonies need not be screened.
2. If colonies are present, inoculate a urea slant.
3. If urea positive, set up a Microscan panel.

Campylobacter Culture

To detect the presence or absence of *Campylobacter*

4. All positive Campylobacter antigen tests will reflex to a Campylobacter culture.
5. Subculture all positive antigen specimens to a Campy BAP and incubate in the appropriate environment for 72 hours.
6. Examine the Campy BAP plate at 72 hours for flat, gray to yellow or pink, smooth non-hemolytic colonies with irregular edges; sometimes colonies maybe mucoid or a thin film.
7. If colonies are present, perform a Gram stain. *Campylobacter* is a small curved Gram-negative rod (Seagull shaped). Kinyoun's carbol fuchsin in a 1 to 4 dilution counter stain may be used instead of Safranin.
8. Perform an oxidase and catalase test after exposing the plate to air for ½ hour before performing tests. *Campylobacter* is strongly oxidase and catalase positive.
9. Perform a rapid hippurate hydrolysis test
    - Positive = *Campylobacter jejuni*
    - Negative = send to reference lab for identification

REPORTING

- Enter preliminary reports daily until final at 48 hours.
- Enter preliminary report of "No growth after <24 hours" or "Culture in Progress" if the culture was processed after 4pm.
- If only normal fecal flora is present report as normal flora with the quantitation and the following comments:
    - o NEP = No Salmonella Isolated
    - o NEP2 = No Shigella Isolated
    - o NEP3 = No E.coli 0157:H7 isolated
    - o NEP5 = No Vibrio isolated

- If only Gram positive fecal flora is present report as “Normal Fecal Flora Altered” with the quantitation and add the FFA comment “the normal fecal flora is altered due to the absence of Gram negative flora” and the following comments:
  - o NEP = No Salmonella Isolated
  - o NEP2 = No Shigella Isolated
  - o NEP3 = No E.coli 0157:H7 isolated
  - o NEP5 = No Vibrio isolated
- Report enteric pathogens with quantitation and AST along with normal flora if present or no normal flora if not present and the appropriate following comments:
  - o NEP = No Salmonella Isolated
  - o NEP2 = No Shigella Isolated
  - o NEP3 = No E.coli 0157:H7 isolated
  - o NEP5 = No Vibrio isolated
- Enter all results in the appropriate spot in the Meditech workcard.

Campylobacter Culture

- If negative, report as “No Campylobacter Isolated”
- If positive, report as *Campylobacter jejuni*
- Enter all results in the appropriate spot in the Meditech workcard.

Yersina Culture

- If negative, report as “No Yersina Isolated”
- If positive, report as *Yersina* with speciation
- Enter all results in the appropriate spot in the Meditech workcard.

REFERENCES:

Garcia, Lynne, *Clinical Microbiology Procedure Handbook,* 3rd Edition, 2010, Volume 1, Section 3.8 Fecal and Other Gastrointestinal Cultures and Toxin Assays.