



STANDARD OPERATING PROCEDURE OF STOOL AND RECTAL SWABS FOR CULTURE AND SENSITIVITY

A. PRINCIPLE

Acute infectious diarrhea may be caused by a number of different agents including bacteria, viruses, and protozoa.

At present there are over 30 subgroups, species and genera of bacteria with proven or reputed enteropathogenicity (Table 1). **Proven enteropathogens** have a definite association with disease production. They exhibit pathogenicity in animal models, they express defined pathogenic mechanisms, and they are associated with disease outbreaks. **Reputed enteropathogens** do not fulfil all of the above criteria of proven enteropathogens, but they are strongly linked to diarrheal disease. There is a growing list of **opportunistic enteropathogens** that have an opportunistic capability but no established virulence mechanism(s) or pathogenicity.

Table 1 Gastrointestinal diseases caused by microorganisms

DISEASE	PATHOGENS / PATHOGENICITY LEVEL	
Viral diarrhea	Proven	Rotavirus (severe diarrhea in children), Norwalk viruses, Adenovirus, Astrovirus, Coronavirus
Parasitic diarrhea	Proven	<i>Entamoeba histolytica</i> , <i>Giardia lamblia</i> , <i>Cryptosporidium parvum</i>
Bacterial diarrhea	Proven	AMR*: <i>Vibrio cholerae</i> , <i>Salmonella</i> spp and <i>Shigella</i> spp
		<i>Campylobacter jejuni</i>, <i>Campylobacter coli</i>, <i>Yersinia enterocolitica</i> and Enterohemorrhagic <i>E. coli</i> O157:H7
		<i>Vibrio mimicus</i> , <i>Vibrio parahaemolyticus</i> , <i>Vibrio fluvialis</i>
		<i>Escherichia coli</i> : enterotoxigenic, enteroinvasive, enterohemorrhagic (O157:H7), enteroaggregative, enteropathogenic
		<i>Clostridium difficile</i> , <i>Clostridium perfringens</i>
		<i>Bacillus cereus</i> , <i>Staphylococcus aureus</i>
	Reputed	<i>Aeromonas</i> spp, <i>Plesiomonas shigelloides</i> , <i>Edwardsiella tarda</i> , <i>Campylobacter lari</i> (formerly <i>laridis</i>), other <i>Campylobacter</i> sp
Opportunistic	<i>Bacteroides fragilis</i> , <i>Citrobacter freundii</i> , <i>Hafnia alvei</i> , <i>Klebsiella oxytoca</i> , <i>Klebsiella pneumoniae</i> , <i>Providencia alcalifaciens</i>	
Gastritis & Duodenal ulcers	Proven	<i>Helicobacter pylori</i>

* AMR Surveillance involves the investigation of the organisms in the shaded box in Table 1.

B. SPECIMEN

Stool and rectal-swab samples sent to the laboratory will be screened for *Salmonella* spp, *Shigella* spp, and *Vibrio cholerae* (see shaded area in Table 1); as required by the AMR Surveillance program.

Stool is the preferred sample for investigation of common bacterial enteropathogens. Refer to the Laboratory Specimen Collection Guide for instructions pertaining to collection. A good stool sample for culture and susceptibility consists of / involves:

- a) Approximately 1 cubic inch of stool
- b) If the stool sample cannot be processed within 2 hours post-collection, it should be placed in Cary-Blair transport medium; awaiting processing at a later time
- c) Stool mixed well in enteric transport medium



- d) Storing the sample in the fridge pending processing
- e) Processing the sample within 24 hours of collection

B. SPECIMEN (Continued)

Stool samples for C&S are considered “repeatable” according to the Laboratory Acceptability / Utilization criteria (SECTION A, Management of Microbiology Specimens. If these specimens are found to be “unsuitable”, the laboratory should ensure that the requesting physician (or designate) is contacted and asked for a repeat specimen. If the physician (or designate) requests that processing of the compromised sample continues, the appropriate reporting modifiers must be appended to the final report; as described in Table 2.

Rectal swab:

Generally, rectal swabs are inferior to well-collected stool samples. They are indicated when dealing with infants, for patients unable to pass a stool sample, or patients with acute diarrheal disease. Feces must be visible on the swab or false-negative cultures may occur. Samples may be held at room temperature and must be processed within 24 hours of collection.

Before processing, confirm or define the nature of the investigation (a call to the requesting doctor or nursing floor may be warranted). Sometimes, rectal swabs are submitted for other investigations (e.g., culture for GC). Rectal swabs for enteric C&S are considered “repeatable” according to the Laboratory Acceptability / Utilization criteria (SECTION A, Management of Microbiology Specimens.



B. SPECIMEN (Continued)

Table 2. Corrective actions for common quality problems specific to enteric samples

CONCERN	CORRECTIVE ACTION	REPORTING *
<p>Not enough specimen was collected:</p> <ol style="list-style-type: none"> 1. Stool: no clear evidence that stool is in Cary-Blair medium 2. Rectal swab: no faecal staining of swab tip 	REJECT: and ask for repeat	Append to report: "QISPNSQ": "Specimen quantity insufficient for test(s) ordered."
Sample not contained in recommended enteric transport medium (e.g., Cary-Blair)	<ol style="list-style-type: none"> 1. If sample > 2 hours old → reject 2. If sample is ≤ 2 hours old → process for C&S; attach comments (see * adjacent) 	Append to report: "QISPSTTRAN": This stool specimen was not submitted in an approved enteric transport medium. Because recovery of enteropathogens cannot be guaranteed, a repeat specimen (collected into an enteric transport medium) is suggested if clinically indicated.
Delay in processing – beyond recommended maximum holding time of ≤ 48 hours post collection	REJECT: ask for repeat samples; if physician (or designate) requests culture, attach qualifying comments	Append to report: "QISP48OLD": "Specimen is > 48 hours old due to a delay in transit" "QISPPEAT": "Please submit a repeat specimen if clinically indicated" "QISPSETUP": "Specimen has been processed according to requesting physician's instructions" "QISPCAUT1": "Interpret culture results with caution"
Stool for C&S in enteric pathogen transport medium – medium is yellow coloured rather than pink	REJECT: and ask for repeat	Append to report: "QISPSTFAIL": Yellow-coloured enteric transport medium indicates build-up of acidity. Culture results of acidic transport samples are not reliable. Please discard other transport containers if medium is yellow. Re-submit another sample if clinically indicated "
Only ONE stool specimen per patient day and a maximum of TWO stools per patient visit should be accepted for culture.	Check requisition for special request. If no special order, select the sample with the most blood and mucous for work-up. Save the other stool sample(s). Add specimen modifiers (see reporting).	Append to report: "QISPMULTST": "Multiple stool samples collected on the same day. Only one sample has been processed (IEDCR Utilization Protocol)."
Stools from in-patients after 3 days of hospitalization should not be cultured for enteric pathogens (routine C&S).	Contact requesting physician or nursing ward to clarify. Discuss the value of a <i>C. difficile</i> toxin assay.	Append to report: "QISPST3D": "After 3 days of hospitalization, stools are not processed for routine C&S. If patient develops diarrhoea during hospital stay, a <i>C. difficile</i> toxin assay should be considered."

Note: All rejected specimens will be kept for 1 week in the fridge.



C. MATERIALS

Refer to Table 8, Set-up of microbiology specimens in MICROBIOLOGY PROCEDURE: MANAGEMENT OF MICROBIOLOGY SPECIMENS [\[Para-Link\]](#) for the list of processing equipment, culture media, and processing techniques used in the processing of stool and rectal swabs for enteropathogens.

Supplies / Reagents needed for processing and culture work-up:

Primary media

MacConkey agar
XLD agar
TCBS agar

Identification tests

Acetate utilization
API 20E
Motility (Wet-Mount or Semisolid medium)
O/129 susceptibility test
Oxidase
PYR
Spot indole
String test
Triple Sugar Iron slant (TSI)
Urease

Serology reagents:

Shigella antisera: for *S. flexneri*, *S. sonnei*, *S. boydii*, and *S. dysenteriae*
Salmonella antisera: Polyvalent O, A-S & Monovalent Vi
V. cholerae antisera: Polyvalent O1 and O139; Inaba, Ogawa and Hikojima monovalent antisera

D. PROCEDURE

1. Processing:

Refer to the Specimen Processing section for faecal samples in MICROBIOLOGY PROCEDURE: MANAGEMENT OF MICROBIOLOGY SPECIMENS.

The AMR enteric screen for stool specimens involves culturing for: *Salmonella* spp, *Shigella* spp, and *Vibrio cholerae*.



D. PROCEDURE (Continued)

3. Routine Examination / Workup:

Refer to Figure 1 for full details pertaining to screening and working up enteric culture plates.

Refer to the corresponding procedure in Section B for instructions as to how to perform each identification test referred to in Figure 1.

MacConkey Agar: Work-up one representative colony of every colonial morphotype that is **colourless** (i.e., a non-lactose fermenter). With a sterile inoculating wire, inoculate the UREA slant and make a blood agar (BA) and MacConkey (MAC) purity plate (1/2 plates are suitable). The purity plates should be examined after overnight incubation. If the growth on both purity plates looks “pure”, the investigation of that particular morphotype can continue; as shown in Figure 1. If the purity plates looked mixed, the original morphotype should be re-selected for testing.

The UREA slant should be read at 4 to 6 hours of incubation. If urease is produced (red tube), stop the investigation of this morphotype. If urease is negative, setup a TSI; as described in the Procedure “Triple Sugar Iron (TSI)” in Section B.

Xylose Lysine Deoxycholate (XLD) Agar: *Shigellae* form pink-red colonies because they do not ferment xylose, lactose, or sucrose (except some *S. sonnei* strains). *Salmonellae* form pink-red colonies even though they ferment xylose with acid production. Hydrogen sulphide (H₂S) producing salmonellae form red colonies with black centres. Target one representative colony (of every colonial morphotype) that is **red to red-orange and any colony with a black centre**. A portion of a colony, representing each colonial morphotype, is transferred to a UREA slant; then streaked to ½ of a MacConkey agar and ½ of a blood agar for purity (for reasons described above). *Vibrio cholerae* grow poorly or not all on XLD (Cheesbrough). When they do grow on this medium, they present as a yellow colony; making this organism indistinguishable from many other sucrose-fermenters that are normal faecal flora.

Thiosulfate-citrate-bile salts-sucrose agar (TCBS): This medium is used only for the recovery of *Vibrio* species. **Yellow** colonies are targeted for investigation of *V. cholerae*. This plate must be read after overnight incubation. Longer incubation may result in the yellow colonies of *V. cholerae* changing to a green colour. Likewise, this colour-change may occur if an overnight-incubated TCBS plate is refrigerated (Manual of Clinical Microbiology, 10th edition). Work-up one representative colony of every colonial morphotype that is yellow; as described in Figure 1. Setup BA and MAC purity plates as described above.

4. Susceptibility Testing:

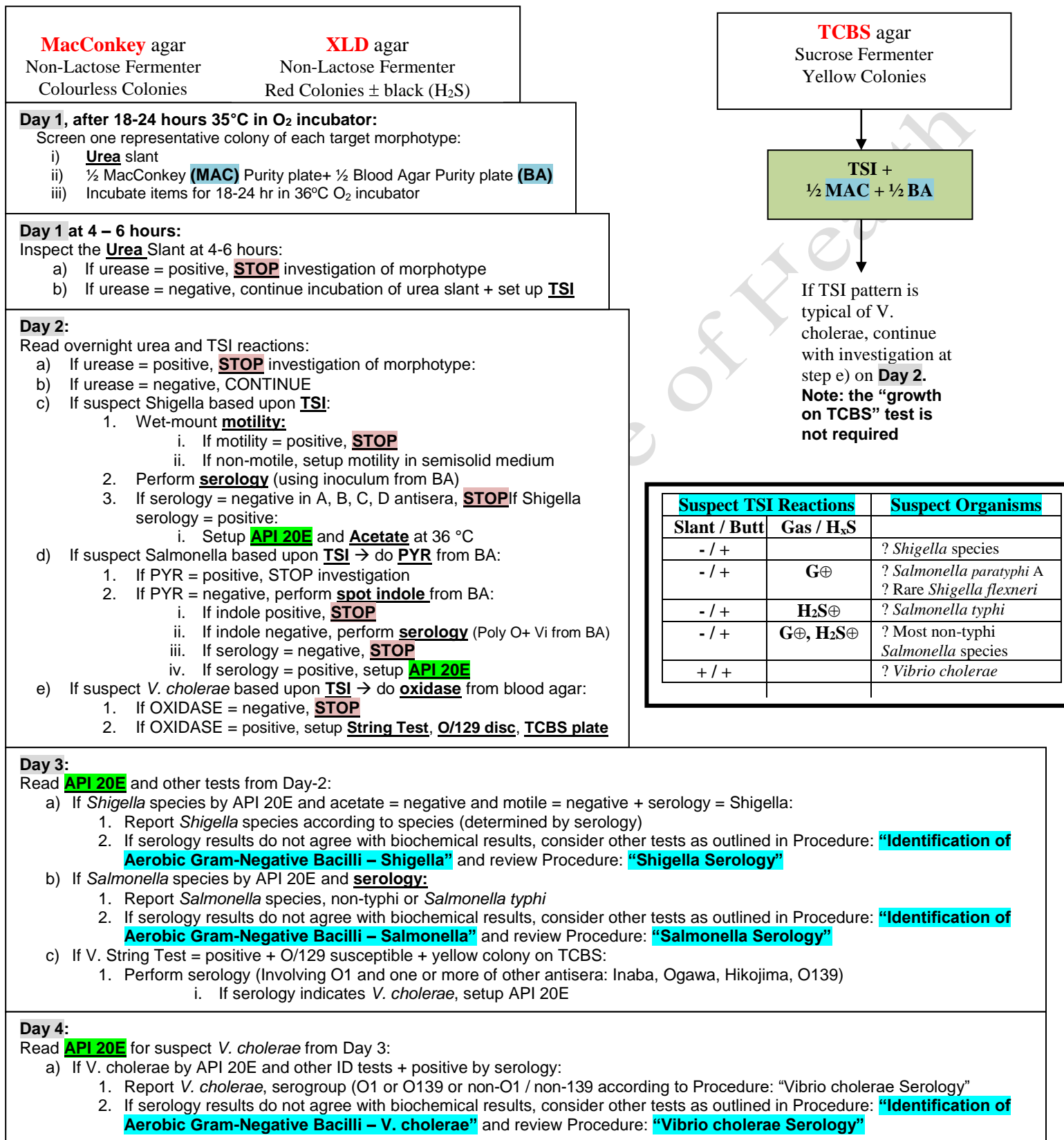
Perform disc-diffusion susceptibility testing on all fully-identified enteropathogens. Refer to Procedure: “**Disc Diffusion Method**” for susceptibility testing of *Salmonella* and *Shigella*. Refer to Procedure: “**Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria**” for susceptibility testing of *Vibrio cholerae*. All three enteropathogens are tested on unsupplemented Mueller Hinton agar.

Susceptibility results are reported to the submitting physician (for patient-care management) according Table 3.



D. PROCEDURE (Continued)

Figure 1. Routine workup of significant growth on enteric culture media





D. PROCEDURE (Continued)

4. Susceptibility Testing (continued):

Table 3. Reporting of susceptibilities for patient-care management [Excerpt from IQMH Stool Guidelines, 2008]

Pathogen	Susceptibility Testing Recommendations
<i>Salmonella</i> spp. (other than <i>S. typhi</i> and <i>S. paratyphi</i>)	Susceptibility testing should not be performed routinely. Most cases resolve without treatment. In cases where antibiotic therapy may be indicated, susceptibility testing should be performed if requested and therapy is required.
<i>Shigella</i> spp., <i>Salmonella typhi</i> , <i>Salmonella paratyphi</i>	Test and report as per current CLSI Standard
<i>Vibrio cholerae</i> <i>Vibrio parahaemolyticus</i>	Test and report as per current CLSI Standard

E. REPORTING

BOTH serology and biochemical / organism characteristics must be positive before *Shigella*, *Salmonella*, and *Vibrio cholerae* are final-reported. Do not issue a final report based upon only one of the two investigations.

If none of the AMR enteropathogens are grown in culture, report:

**** Negative ****

No Salmonella, Shigella, or Vibrio cholerae isolated.

All AMR enteropathogens must be reported to:

- WHO, via the AMR Program Portal
- Most responsible physician: fax or broadcast copy of patient report and call physician (or designate) to ensure transmission



F. SUPPLEMENTARY INFORMATION

1. MacConkey is a differential / selective medium. Fermentation of **lactose** results in acid production and a colour change in the pH indicator – neutral red. Addition of crystal violet and bile salts inhibit gram-positive bacteria.
2. XLD is a differential / selective medium. The selective agent in XLD Agar is sodium deoxycholate, which inhibits the growth of gram-positive organisms. The carbohydrate source is **xylose** which is fermented by most enterics except for *Shigella* species, and *Shigella* colonies appear red on this medium as a result. A second differential mechanism for *Salmonella* is employed by the addition of **lysine**. Lysine decarboxylation reverts the pH of the medium to an alkaline condition. To avoid this reversal to a *Shigella* reaction, lactose and sucrose are added in excess. The addition of sodium thiosulfate and ferric ammonium citrate as a sulfur source and indicator, respectively, allows hydrogen sulfide forming organisms to produce colonies with black centers, under alkaline conditions. Organisms which ferment xylose, are lysine decarboxylase-negative, and do not ferment lactose or sucrose cause an acid pH in the medium, and form yellow colonies. Examples of such organisms are *Citrobacter* spp., *Proteus* spp., and *Escherichia coli*. Addition of crystal violet and bile salts inhibit gram-positive bacteria.
3. Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) Agar is a differential / selective medium. TCBS Agar is used for the selective isolation of *Vibrio cholerae* and other enteropathogenic vibrios. **Thiosulfate** and **sodium citrate**, as well as the **alkalinity** of the medium, inhibit the growth of *Enterobacteriaceae*. **Bile salts** inhibit the development of Gram-positive bacteria. The fermentation of **sucrose** by *Vibrio cholerae* leads to acid production. Acid-producing colonies turn yellow when the pH indicators, bromthymol blue and thymol blue, shift to a yellow colour. The production of hydrogen sulfide is visualized in the presence of **ferric citrate** as a black precipitate. **Yeast extract** and **peptone** provides the nitrogen, vitamins, and amino acids for optimal bacterial growth. **Sodium chloride** provides optimum conditions for growth of halophilic (i.e., salt-loving) *Vibrio* spp.

G. REFERENCES

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