



## WHO Global Foodborne Infections Network

**"A WHO network building capacity to detect, control and prevent foodborne and other enteric infections from farm to table"**

**Laboratory Protocol:**

**"Isolation of *Salmonella* and *Shigella* from Faecal Specimens"**

19 November 2010

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WHO Global Foodborne Infections Network Laboratory Sub-Committee

## LABORATORY SOP TEMPLATE

<b>Title:</b> Isolation of <i>Salmonella</i> and <i>Shigella</i> from Human Faecal Specimens	
Protocol Number: 2010GFNLAB004	Effective Date: <b>19 November 2010</b>
Revision Number:	

### REVISION HISTORY

HISTORY OF CHANGES				
Rev. Level	Sections Changed	Description of Change (From—To)	Date	Approval
0				
1				

## I. PURPOSE

A standardised protocol for the culture and isolation of *Salmonella* species and *Shigella* species from human feces.

## II. TEST PRINCIPLES

Bacterial gastroenteritis; commonly referred to as bacterial diarrhoea or infectious diarrhoea can be caused by a number of different agents including *Salmonella*, *Shigella*, *Vibrio*, *Campylobacter*, *Plesiomonas*, and *E. coli*. In most cases, the aetiology of infectious diarrhoea cannot be accurately determined simply on the basis of clinical symptoms. Laboratory confirmation both verifies the diagnosis and provides critical information for disease surveillance and response programmes.

The following is a procedure for the culture and isolation of *Salmonella* and *Shigella* from faeces. *Salmonella* and *Shigella* are two of the most common etiologic agents of bacterial diarrhoea. Sensitive and specific laboratory methods for the isolation, identification, and serotyping of *Salmonella* and *Shigella* are key to monitoring and control programmes. The ideal diagnostic test for these organisms will be rapid, inexpensive, easily reproducible, sensitive, and specific. Currently however, no single method meets all these criteria. While molecular methodologies have the potential to increase sample throughput, sensitivity, and specificity, while simultaneously reducing turn around time, traditional culture remains the diagnostic standard for identification of *Salmonella* species and *Shigella* species. Theoretically, traditional culture is 100% specific and provides the investigator with an actual isolate. Pure isolates are crucial for antimicrobial susceptibility testing and most subtyping methods (e.g. serotyping and PFGE).

Fecal specimens are the preferred laboratory samples for diagnosis of infectious diarrhea. However, the recovery of enteric pathogens from feces is often complicated by multiple factors including: prior antibiotic treatment, transport stress, intermittent shedding of pathogens in the feces, and low numbers of *Salmonella* or *Shigella* in relation to other enteric flora. These factors necessitate the use of culture algorithms that employ selective enrichment and the use of selective / differential media.

Selective enrichment suppresses fecal-flora while allowing the target pathogen to grow. Selective and/or differential media (described as “selective media” in this manual) suppresses the growth of fecal-flora while allowing the growth of target pathogens; these media also utilise various phenotypic characteristics to preliminarily differentiate potential pathogens from fecal-flora.

## III. RESPONSIBILITIES

### A. Staff Responsibilities

*This section summarizes personnel responsibilities specific to execution of this SOP.*

*Example: “Daytime Microbiology Technician: The Daytime Microbiology Technician will be responsible for verifying that the sample submission form is properly completed and will inoculate the stool culture as specified in the SOP.”*

*Job descriptions and responsibilities vary by institution; as such this section must be individualized to your specific institution. Please refer to applicable manuals within the facility/locality for the complete set of responsibilities to properly conduct this procedure.*

### B. Specific Safety Requirements and Responsibilities

This SOP describes a method for the culture and isolation of *Salmonella* species and *Shigella* species from human feces. Blood-borne pathogen protection (gloves, lab coat, etc) must be utilized when handling human clinical samples.

Biosafety level-2 (BSL-2 / RG-2) practices and procedures must be followed when handling *Salmonella* species, *Shigella* species, or clinical samples suspected to contain these organisms.

#### **IV. SAMPLE COLLECTION/TRANSPORT/STORAGE**

Samples may consist of fecal or rectal swabs submitted in transport media or unpreserved feces. The preferred sample is 2 fecal swabs submitted in transport media (e.g. Cary-Blair). Rectal swabs are not a preferred sample and should only be utilized when the patient cannot produce a fecal sample. The rectal swab should be examined after collection; fecal matter should be visible on the swab. If fecal matter is not visible on the swab, the swab should not be submitted for culture,

Samples in transport media should be kept cold and arrive at the laboratory within 48 hours. If appropriate transport media is not available, unpreserved feces may be submitted. The unpreserved fecal sample must be submitted in a clean, container with no soap or disinfectant residue. The sample must be kept cold and transported to the lab within 8 hours of collection. If the sample cannot reach the laboratory within specified time (48 hours in transport media / 8 hours without media); the sample should be frozen at < 70°C or stored on dry ice.

#### **Rejection Criteria:**

Samples which have been exposed to excessive temperatures for prolonged periods of time, rectal swabs which do not contain visible fecal matter, and samples which are submitted with inappropriate preservatives (e.g formalin or PVA) will not be tested.

#### **V. MATERIALS / SUPPLIES**

##### **A. Media**

- 1) 5% Sheep Blood agar (5% SBA)  
Note: 5% SBA may be substituted with another non-selective media such as Mueller-Hinton agar
- 2) MacConkey agar
- 3) Hektoen Enteric (HE) agar or Xylose-Lysine-Desoxycholate (XLD) agar  
Note: HE or XLD are recommended. Choice between HE and XLD should be based on the quality and availability of HE and XLD in your region.
- 4) Selenite F Broth

##### **B. Other supplies and equipment**

- 1) Inoculating loops
  - 2) Applicator swabs
- 

#### **VI. EQUIPMENT**

Incubator 36°C (non-CO<sub>2</sub>)

#### **VII. QUALITY ASSURANCE**

Each lot and shipment of media utilized in this SOP will be quality control tested prior to use. The results of QC testing (performance characteristics and sterility) will be recorded on the QC Log Sheet. Only media which has passed QC will be used for testing.

#### **VIII. PROCEDURE**

Note: All incubations are performed at 36°C (+/- 1°C).

## **A. Media Inoculation and Media Incubation**

**Label all culture media with the laboratory accession number before inoculating media.**

### **Inoculation:**

If possible, perform all sample manipulation in a biological safety cabinet.

- 1) **Unpreserved feces**
  - a) Using an applicator swab, collect a small amount of feces. Collect from areas with visible blood or mucous, if present.
  - b) Roll swab over the first quadrant of the MacConkey & Hektoen plate (or MacConkey & XLD plate).
  - c) Using a sterile 1uL inoculating loop or inoculating needle, streak for isolation.
  - d) Insert a new, sterile swab into the sample and then drop the swab into the tube of selenite broth. Loosely cap the tube.

**Note: It is critical that the swab is lightly coated with sample. Do not place excessive amounts of sample into the selenite broth. Excessive amounts of sample will cause overgrowth of enteric flora.**

- 2) **Rectal swab** – inoculate media as for raw stool
- 3) **Fecal swab in Cary-Blair**
  - a) Gently agitate sample to mix contents.
  - b) Roll swab over the first quadrant of the MacConkey & Hektoen Agar plate (or MacConkey & XLD plate).
  - c) Using a sterile 1uL inoculating loop or inoculating needle, streak for isolation.
  - d) Insert a new, sterile swab into the sample and then drop the swab into the tube of selenite broth. Loosely cap the tube.

**Note: It is critical that the swab is lightly coated with sample. Do not place excessive amounts of sample into the selenite broth. Excessive amounts of sample will cause overgrowth of enteric flora.**

### **Incubation:**

Incubate all media at 36C (+/- 1°C) in a non-CO2 incubator for 18-24 hours (overnight).

## **B. Examination of Cultures**

### **Examination:**

Following overnight (18-24 hours) incubation, plates are examined for *Salmonella*-like or *Shigella*-like colonies:

### 1) MacConkey Agar (MAC)

Both *Salmonella* and *Shigella* produce colourless (lactose negative) colonies (2-4 mm) on MAC.

Most coliforms produce red colonies.

- a) Examine plate and identify any *Salmonella*-like or *Shigella*-like (clear/colourless) colonies for additional testing.
- b) If no *Salmonella*-like or *Shigella*-like colonies are present, no additional testing is performed on this plate.
- c) Record results on "Stool Culture Worksheet"

### 2) Hektoen Enteric Agar (HE)

*Salmonella* typically produce clear colonies with distinct black centres (hydrogen sulphide: H<sub>2</sub>S +) on HE. Colonies of *Salmonella* ser. Typhi are typically clear with pinpoint black centres and colonies of *Salmonella* ser. Paratyphi A are typically clear (no H<sub>2</sub>S).

*Shigella* spp. typically produce colonies on HE which range in colour from clear to white / pale-green.

- a) Examine plate and identify any *Salmonella*-like or *Shigella*-like colonies (clear or pale-green colonies and colonies with black centres) for additional testing.
- b) If all colonies are orange without black centers, no additional testing is performed on this plate.
- c) Record results on "Stool Culture Worksheet"

### 3) Xylose Lysine Desoxycholate Agar (XLD)

*Salmonella* typically produce clear to light pink colonies with distinct black centres on XLD. Colonies of *Salmonella* ser. Typhi are typically clear with pinpoint black centres and colonies of *Salmonella* ser. Paratyphi A are typically clear (no H<sub>2</sub>S).

*Shigella* spp. typically produce colonies on XLD which range in colour from clear to white / pale-red.

- a) Examine plate and identify any *Salmonella*-like or *Shigella*-like colonies (clear or pale-green colonies and colonies with black centres) for additional testing.
- d) If all colonies are orange without black centers, no additional testing is performed on this plate.
- e) Record results on "Stool Culture Worksheet"

The type or types of colonies present are recorded on the "Stool Culture Worksheet". The amount of growth present (1+, 2+, 3+, or 4+) is estimated and recorded.

For example, a MAC plate may be recorded as:  
MAC: 3+ NLF / 1+ LF

NLF: non lactose fermenting colonies – clear colonies / LF: lactose fermenters – red colonies

### **C. Broth Enrichment:**

Following 18-24 hours of incubation, the selenite broth is plated to HE (or XLD) using same technique as above. The plates are incubated for 18-24 hours at 36°C in a non-CO2 incubator.

Following overnight incubation, the plate is examined as described above.

#### Salmonella-like or Shigella-like Colonies (Suspect Colonies):

Suspect colonies must be biochemically confirmed.

Many different organisms can produce *Salmonella*-like or *Shigella*-like colony morphology on selective media. Therefore, when possible, three suspect colonies should be tested from each sample. If suspect colonies are present on both the MAC and HE plate, then colonies from both plates should be selected for biochemical testing.

Suspect colonies are harvested with a sterile inoculating loop and transferred to 5% sheep blood agar (or another non-selective agar) and incubated 18-24 hours (overnight) at 36°C (+/- 1°C). It is important to select individual, well isolated colonies. Following incubation isolates are biochemically confirmed using the protocol "Identification of *Salmonella* and *Shigella* Using an Abbreviated Panel of Tests"

## **IX. INTERPRETATION OF RESULTS**

- A. Positive culture: Regardless of quantity, the isolation of *Salmonella* species or *Shigella* species is a significant finding indicating infection with the organism.
- B. Negative culture: The isolation of normal fecal flora only.

## **X. LIMITATIONS OF PROCEDURE**

Typical morphology on selective agar is not a definitive identification. All suspect isolates must be confirmed biochemically.

Results must be interpreted in the context of available clinical data.

This procedure is not all inclusive, additional media and/or procedures must be utilised for the isolation or detection of other enteropathogens including (but not limited to): *Vibrio*, *Yersinia*, diarrhoeagenic / toxigenic *E. coli* and *Campylobacter*.

Fecal-shedding of *Salmonella* by chronic carriers may be intermittent. Three cultures, performed on three stool samples, collected 24 hours apart, may increase the likelihood of recovering *Salmonella* from a suspected carrier.

## **XI. REPORTING**

If no suspect colonies are present, the sample may be reported as:

"No *Salmonella* spp. or *Shigella* spp. Isolated"

If suspect colonies are present, the colonies must be subjected to biochemical testing as described in the SOP: "Identification of *Salmonella* and *Shigella* Using an Abbreviated Panel of Tests".

If the colonies are tested biochemically and *Salmonella* and *Shigella* are biochemically excluded, the sample may be reported as:

"No *Salmonella* spp. or *Shigella* spp. Isolated"

If the colonies are tested biochemically and a *Salmonella* or *Shigella* is biochemically confirmed, the sample may be reported according to the reporting guidelines specified in the SOP: "Identification of *Salmonella* and *Shigella* Using an Abbreviated Panel of Tests".

## **XII. REFERENCES**

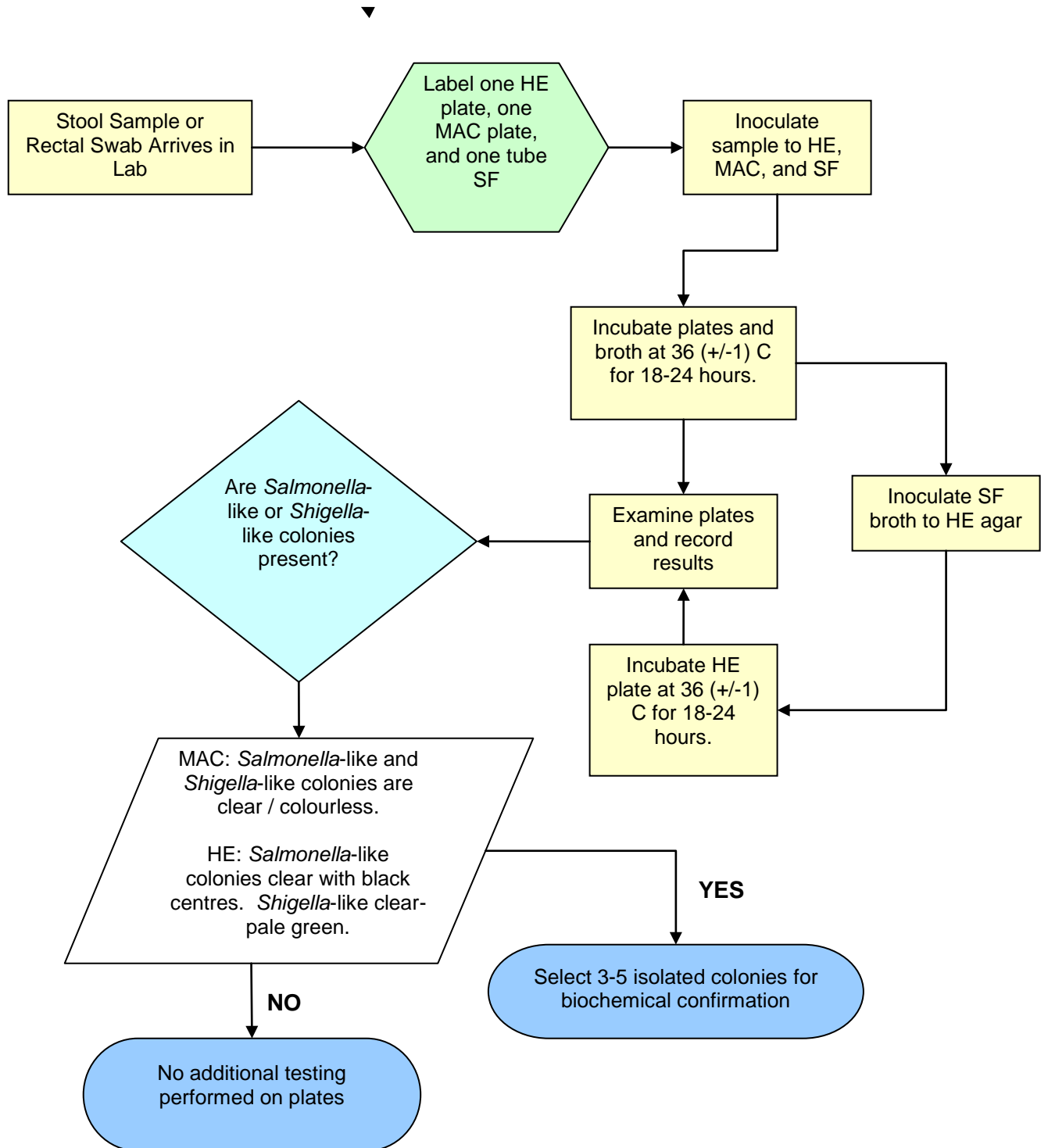
- A. Centers for Disease Control and Prevention. Laboratory Methods for the Diagnosis of Epidemic Dysentery and Cholera. Atlanta, GA. CDC. 2002.
- B. Isenberg, Henry D., editor. 2004. *Clinical Microbiology Procedures Handbook*, American Society for Microbiology, Washington DC. p.3.8.1.1-21.
- C. WHO Global Salm-Surv. Laboratory Protocol: "Isolation of *Salmonella*, *Shigella*, and *V. cholerae*" 1<sup>st</sup> Edition. 2009.

## **XIII. ATTACHMENTS:**



## Appendix I

### Flow Diagram for the Culture of *Salmonella* spp. and *Shigella* spp. From Fecal Samples



**Appendix 2**

**Morphology of *Salmonella* spp. , *Salmonella* serovar Typhi, and *Shigella* spp. on Various Selective and Differential Media after 18-24 incubation at 36 °C**

	<i>Salmonella</i> (majority)	<i>Salmonella</i> Typhi	<i>Shigella</i> spp.
MacConkey Agar (MAC)	Smooth, colourless colonies. 2-4 mm	Smooth, colourless colonies. 1-3 mm	Smooth, colourless colonies. 2-3 mm
Hektoen Enteric Agar (HE)	Clear colonies with black centres. 2-4 mm	Clear colonies. Some may produce pinpoint black centres. 1-3 mm	Clear / green colonies 2-3 mm
Xylose Lysine Desoxycholate Agar (XLD)	Colonies may range in colour from clear to pink /red. Most colonies 2-4 mm with black centres.	May be inhibited. 1-3 mm clear colonies. Some with pinpoint black centres.	Red colonies 1-2 mm

**Appendix 3**  
**Morphology of *Salmonella* spp., *Shigella* spp., and *E. coli* on Various Selective and Differential Media after 18-24 incubation at 36 °C**



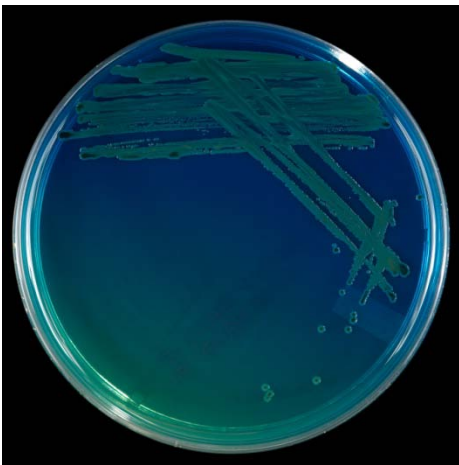
MacConkey agar with *Salmonella* spp.: Lactose negative colonies. Similar morphology will be observed with *Shigella* spp.



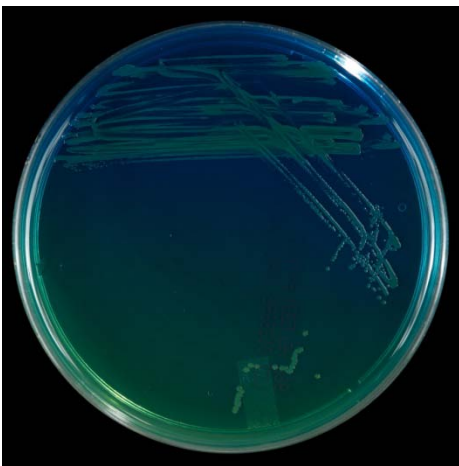
MacConkey agar with *E. coli*: Lactose positive colonies.



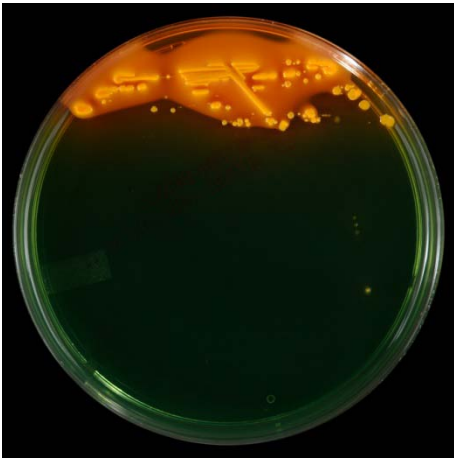
MacConkey agar with mixture of lactose negative colonies (*Shigella* spp.) and lactose positive colonies (*E. coli*).



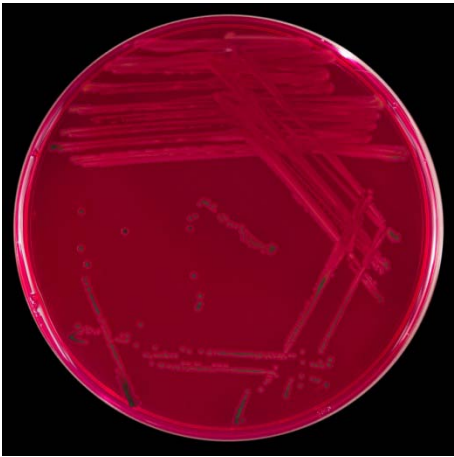
Hektoen Enteric Agar with *Salmonella* Typhi: clear colonies with small black centres: trace hydrogen sulphide production. Most other *Salmonella* serovars will produce colonies with larger black centres (similar to XLD plate below).



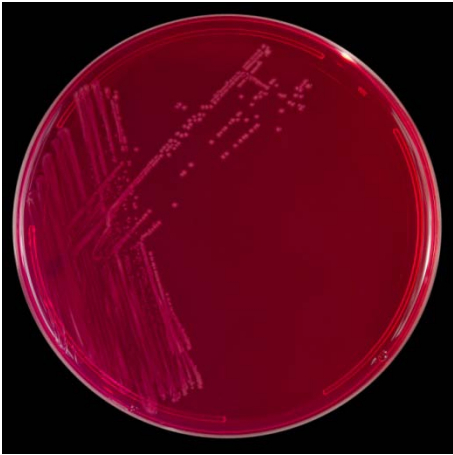
Hektoen Enteric Agar with *Shigella* spp.: small, clear (lactose, sucrose, salicin, H<sub>2</sub>S negative) colonies.



Hektoen Enteric Agar with *E. coli*: yellow colonies



XLD agar with *Salmonella* spp.: clear colonies with black centres.



XLD agar with *Shigella* spp.: clear colonies



XLD agar with *E. coli*: yellow colonies

**Appendix 4**  
**STOOL CULTURE WORKSHEET**

Sample # \_\_\_\_\_ Date Received: \_\_\_/\_\_\_/\_\_\_ Date Inoculated: \_\_\_/\_\_\_/\_\_\_

Medium	Direct Plating 24 Hour Incubation at 36C	Selenite Enrichment 24 Hour Incubation at 36C
MacConkey Agar (MAC)		
Hektoen Enteric Agar (HE)		
Xylose Lysine Desoxycholate Agar (XLD)		

Substrate	Colony 1	Colony 2	Colony 3
TSI (slant)			
TSI (butt)			
TSI (H <sub>2</sub> S)			
TSI (gas)			
LIA			
MIO (Motility)			
MIO (Ornithine)			
MIO (Indol)			
Citrate			
ONPG			

**Final Result:**

**No *Salmonella* / *Shigella* Isolated**

***Salmonella* spp. Isolated**

***Salmonella* ser. Typhi Isolated**

***Salmonella* ser. Paratyphi A Isolated**

***Shigella* spp. Isolated**

Reported by: \_\_\_\_\_ Reviewed by: \_\_\_\_\_ Date: \_\_\_/\_\_\_/\_\_\_