Action points for laboratory investigation of suspected foodborne/ADD outbreak and routine laboratory based surveillance

1. Each district laboratory to:
   - Prepare **specimen collection information sheet** for stool specimen including all information needed by those who collect specimens (***Annexure L1: Prototype specimen collection information sheet***). This must be prepared in language understood by all users (English and if required in local language also). This specimen collection information sheet to be made available to all specimen collection sites (blocks, PHCs etc.)
   - Ensure a **specimen referral log book** (register) is available at PHC/CHC ***(Annexure L2: Specimen referral logbook),*** in which the details of the specimens being referred to district laboratory/other laboratories are to be entered.
   - Make a separate **specimen log book** (register) for all stool specimens received and cultured (routine and during suspected ADD/FBD outbreaks) ***(Annexure L3: Specimen logbook).***
   - Prepare Cary Blair medium and make it available at all blocks/blocks from where ADD cases/outbreaks are reported frequently (minimum 15 each), ensure that at all times this many are available at all blocks. (E.g. at the time of receipt of specimens provide more to replenish the stock).

2. While investigating suspected foodborne/ADD outbreak, the laboratory staff to ensure collection and testing of stool specimens for as many case-patients as possible (at least 20 cases, in large outbreaks at least from 10-20% cases). The specimen must be collected as per specimen collection guidelines, see 1a above. Each specimen must be accompanied with the duly filled **specimen referral form** ***(Annexure L4: Specimen referral form)***

3. On receipt of specimen, district laboratory to ensure that duly filled specimen referral form is available for each specimen. Enter all details in the specimen log book.

4. Process the specimen as per the **specimen processing flow charts** provided under the Pilot Project***. (Annexure L5: Specimen processing flow charts)***

5. Maintain a **stool culture worksheet** for each specimen processed in the district laboratory, routine samples and during suspected outbreak samples. ***(Annexure L6: Stool culture worksheet).***

6. Compile results of stool specimens processed weekly in the **weekly reporting format for stool culture** and send to DSU and SSU every week ***(Annexure L7: Weekly reporting format for stool cultures).***

7. Send the clinically significant isolates (significant non lactose fermenters, pure isolates of significant lactose fermenters) to IDSP state referral laboratory for confirmation if required.

8. Maintain a stock of the significant isolates in nutrient agar stabs (refer to IDSP lab manual) at district/state referral labs.

PILOT PROJECT FOR SURVEILLANCE OF ADD/FOODBORNE DISEASE

_____ District

Sample Collection and transport

Laboratory Name: _____ District Laboratory

Nodal officer: _____

Phone:_____

E mail-_____

Working days of the Lab: Monday to Saturday

Working hours of the Lab: 8am to 4pm

List of tests available at the lab:

S No.	Name of the test	Results available by
1	Stool microscopy	Within 2 hrs of receipt of sample in the lab (if received during working hrs); otherwise the next working day
2	Stool Culture sensitivity for common aerobic bacterial pathogens	48 to 72 hrs after receipt of sample in the lab

Sample Collection and Transport Procedure:

- Stool specimens are most useful for microbiological diagnosis if collected soon after onset of diarrhoea (for bacteria < 4 days), and preferably before the initiation of antibiotic therapy. If required, two or three specimens may be collected on separate days, multiple specimens are particularly useful when suspecting parasitic infections.
- Stool is the preferred specimen for bacterial culture and detection of parasitic diarrhoeal pathogens.
- Rectal swabs showing faeces may be collected from infants (where collection of stool sample may not be possible).
- Collect diarrheal stool samples from at least 10 ill persons. Sometimes more specimens may have to be collected to be able to establish the etiology of disease. If the number of ill persons is less than 10 then collect specimens from all ill persons. In large outbreaks, specimens must be collected from at least 10% of cases.

<u>Materials for specimen collection</u>

- Sterile, leak-proof, screw capped container
- Label
- Appropriate bacterial transport media for transport of stool/ rectal swabs from infants
- Parasitology transport pack: 10% formalin in water

<u>Method of collecting a stool specimen</u>

- Label the container (name, unique case Id, specimen type, date and time of collection)

- Collect freshly passed stool, 5 ml liquid or 5 g solid (pea-size), in the container. (You may first ask the patient to pass the stool in a separate clean wide mouthed container and then transfer enough faeces with a spatula to the specimen container)
- Screw cap the container tightly and place in a sealed bag (zip lock bag) and send to laboratory immediately.
- The specimen referral form, to be filled for each specimen, is to be placed in a separate outer zip lock bag.

Method of collecting a rectal swab from infants

- Label the screw capped sterile tube/container containing the Cary Blair medium (transport medium).
- Moisten a swab in sterile saline.
- Insert the swab tip just past the anal sphincter and rotate gently.
- Withdraw the swab and examine to ensure that the cotton tip is stained with faeces.
- Place the swab in the labelled sterile screw capped tube/container containing the transport medium.
- Break off the top part of the stick without touching the tube and tighten the screw cap firmly.
- Place in a sealed bag and send to laboratory immediately.

Handling and transport

- In case a delay of more than two hours in processing of the specimen is expected, the stool specimen is to be transported at 4-8°C. In case a delay of more than 8 hrs is expected before the specimen will be processed, the stool specimen must be transferred to Cary Blair transport medium using two swabs. Cary Blair tubes with stool specimen swabs must be transported to laboratory at 4 - 8°C and must be examined within 48hrs of collection.
- Rectal swabs in Cary Blair medium must be transported at 4-8°C and must be processed within 48 hrs of collection.
- Bacterial yields may fall significantly if specimens are not processed within 48hrs of collection. Shigella are particularly sensitive to elevated temperatures.
- Specimens to be examined for parasites should be mixed with 10% formalin- 3 parts stool to 1 part preservative and transported at ambient temperature in screw capped containers sealed in plastic bags.

How to transfer stool specimen into a tube containing Cary Blair transport medium

- Gloves to be worn at all times when handling the specimen.
- Take a sterile swab. Do not touch the cotton tip of the swab.
- Insert the cotton tip of the swab into the stool specimen.
- Make sure the cotton tip of the swab is completely coated with the specimen.
- Push the swab completely to the bottom of the tube of refrigerated Cary Blair transport medium.
- Break off the top portion of the stick so the cap can be tightly screwed onto the tube.
- After screwing cap tightly onto the Cary Blair tube, seal the tube with tape to prevent leakage.
- Adhere specimen label to the Cary Blair tube.
- Keep the tube at 4–8°C.
- Safely dispose of all contaminated materials.

Specimen Referral Log for Foodborne and Acute Diarrheal Disease Outbreaks

Sl.No	Specimen/Patient's ID	Patient's Name	Age	Sex (Male/Female)	Block, Village & District of Patient	Specimen Source: Outbreak or Routine (O or R)	Date of Onset of Illness	Key Clinical Sign/Symptoms	Specimen Type (Fresh Stool/ Stool Swab in Cary Blair/Rectal Swab)	Date & Time of specimenCollection	Contact Details of Facility to which specimen was sent	Date of Dispatch of specimen	Test Result	Date of Receipt of Test Result

Specimen Log Book for Foodborne and Acute Diarrheal Disease Outbreaks

Sl.No	Laboratory ID	Case ID	Patient Name	Age (Years/Months)	Sex (Male/Female)	Block, Village & District of Patient	Date of Illness Onset	Diarrhea (Tick if YES)	Vomiting (Tick if YES)	Fever (Tick if YES)	Antibiotics received prior to sample submission (Tick if YES)	Specimen type: Outbreak or Routine (O or R)	Specimen collected at District Hospital or Periphery (DH or P)	Type of Sample (Fresh Stool/Stool Swab in Cary Blair/Rectal Swab)	Date Sample Collection	Time Sample Collection	Name & Contact Details of Facility Sending Specimen	Date of Sample Receipt in Lab	Time of Sample Receipt in Lab	Was Specimen received at 4-8 ^{o}C (yes/No)	Test Result	Date of Reporting of Test Results	Name of Person/Facility Receiving the Report	Signature of Person Reporting Results	Comments

CASE ID NUMBER_____

Specimen Referral Form

SPECIMEN INFORMATION

Specimen source/type:
□ Fresh stool □ Stool Swab □Rectal Swab
□ Other (please list)_____

Collection date_____/_____/_____
DD MM YYYY

Time collected_____/_____
HH MN

INFORMATION ABOUT PERSON/FACILITY REQUESTING TEST

Name of Person sending the specimen: _____

Designation:_____ Phone Number_____

Email_____

Type of Facility from where the specimen was sent:

□ District Hospital □ CHC/Block PHC □ PHC □ Other (List) □ N/A, specimen sent directly from field

Facility Name:_____

PATIENT DEMOGRAPHICS

Name_____ Age _____/_____ Sex □ Male □ Female

S/O, D/O,W/O_____ Years Months

Address_____ _____ _____ _____ _____

Street Address Village Block District State

Patient Phone Number: _____

PATIENT CLINCIAL HISTORY

Date of Onset of Illness_____/_____/_____
DD MM YYYY

Time of Onset of Illness _____ □ AM □ PM

Clinical Signs/Symptoms (TICK ALL THAT APPLY):

□ Diarrhea □ Vomiting □ Nausea □ Fever

□ Presence of Mucous/Blood in Stool

□ Other (please specify)_____

Antimicrobial Treatment Received Prior to Specimen Collection: □ Yes □ No □ Unknown

If Yes, Specify which antibiotic:_____

Hospitalized for this illness: □ Yes □ No □ Unknown

Primary Diagnosis:_____

Laboratory Examination Requested

Facility to which specimen is being sent: _____

Type of Examination Requested (Tick all that apply):

□Stool Microscopy and Bacterial Culture □ Microscopic Examination for Parasites

□ Antimicrobial Susceptibility Testing □ Other_____

Stool Culture Worksheet

(to be maintained for each sample at the District Laboratory where culture is performed)

Sample#_____ Date Received_____ Date Inoculated_____

Findings of Macroscopic (naked-eye) examination of Stool:					
Consistency:	□ Liquid	□ Semisolid	□ Solid	□ Presence of blood	□ Presence of Mucous
Findings of Microscopic examination on fresh stool					
□ Pus cells		□ RBCs	□ Any Other_____		

Medium	**Record growth from Direct Plating (after 24 hr incubation at 35-37°C)**	**Record Growth from Selective Enrichment (S/C from Selenite-F and APW) 24 hr incubation at 35-37°C**
MacConkey Agar (MAC)		
Hektoen Enteric Agar (HE)*		
Xylose Lysine Desoxycholate Agar (XLD)*		
Thiosulphate Citrate Bile salt Sucrose (TCBS) agar		

**Either use HE agar or XLD agar, not both*

Results of Biochemical tests and Serotyping:

Substrate/Test	**Colony 1**	**Colony 2**	**Colony 3**
Oxidase			
TSI (slant)			
TSI (butt)			
TSI (H2S)			
TSI (gas)			
LIA			
MIO (Motility)			
MIO (Ornithine)			
MIO (Indol)			
Citrate			
Urea			
String test			
V. cholerae Poly O1 serum			
V. cholerae O139 serum			
V. cholerae Inaba serum			
V. cholerae Ogawa serum			

Final Result:

□ **No *Salmonella* / *Shigella* Isolated**
□ ***Salmonella spp.* Isolated**
□ ***Salmonella* ser. Typhi Isolated**
□ ***Salmonella* ser. Paratyphi A Isolates**
□ ***Shigella spp.* Isolated**
□ ***Vibrio cholerae* 01 isolated (Serotype: Ogawa/Inaba/Hikojima)**
□ ***Vibrio cholerae* 0139 isolated**

Reported by_____ Reviewed by_____ Date____/_____/_____

Weekly reporting format For Reporting Results of Stool Culture performed at District hospital labs (to be sent to the DSU and SSU)

Name of the laboratory:					
District:					
State					
Reporting Week:			**(_____)**	**(_____)**	**(_____)**
			Start Date	**End date**	**Date of Reporting**
Number of stool samples received	**Number of Stool samples rejected**	**Number of Stool samples tested**	**Number of stool samples found positive**	**Name and number of isolates**	**Number of isolates referred to designated referral laboratory**

Line list of positive cases

Sl. No	**Name**	**Age**	**Sex**	**Address (Village/Town)**	**Test done (stool culture)**	**Name of bacteria isolated and AST Result**	**Referred to designated referral lab (Yes/No) & Date of Referral**

**AST: Antimicrobial Susceptibility Testing*