Chart1: Laboratory Approach to Etiological diagnosis of ADD

Fresh stool or Rectal swab/ Fecal swab in Cary Blair

Macroscopic Examination (of Stool)

Examine and record consistency (example: mucous, blood, liquid)

Microscopic examination

Chart 2

Culture

APW S/c after 4- 8hrs
SF S/c after 16-24 hrs

Direct Plating
MAC, XLD or HE, and TCBS

Enrichment (APW, SF broth) At 36+/-1°C

16-18hrs at 36°C +/-1°C

TCBS

Yellow, Shiny colonies (1-4 mm)

Chart 3

MAC

Colorless (NLF), smooth colonies (1-4 mm)

Chart 4

XLD/HE

XLD: Red colonies with or without black centre (1-3) mm
HE: Colourless colonies with or wihout black centre (1-3)mm

MAC= MacConkey agar, XLD= Xylose Lysine Deoxychlate agar, TCBS= Thiosulphate Citrate Bile salt Sucrose agar, APW= Alkaline Peptone Water, SF= Selenite F broth

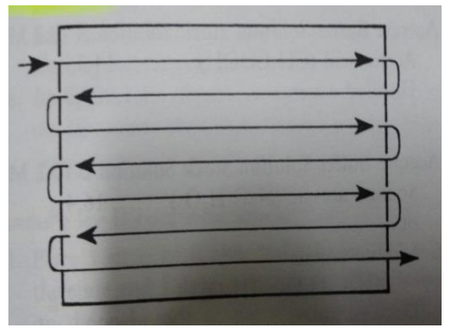
Chart 2:Microscopic Examination of Stool/Rectal Swab/Fecal Swab

Preparation of Saline and Iodine mounts:

- Place 1 drop of 0.85% NaCl on left side of a slide (1- x 3-in) and 1 drop of iodine (5 times diluted Lugol's iodine) on right side of slide
- Take small amount of fecal specimen (using a swab/loop) and thoroughly mix
- Place a 22-mm coverslip on each suspension

Method of examining direct wet film preparation with a 10x objective:

- Scan both the suspensions with 10x objective.
- The entire coverslip area should be examined for motile parasites under low power
- If something suspicious seen-- 40x objective for more detailed study
- At least 1/3 of coverslip should be examined under 40x even if nothing suspicious seen



NaCl: Sodium Chloride; Lugols Iodine: I_2: KI: H_2O= 5 gm:10 gm:100ml

Chart3: Identification of Vibrio cholerae

Specimen

Crystal Vc
(if potential outbreak)

APW
enrichment

S/c after 4-8 hrs
enrichment

Culture on TCBS

16-18hrs at 36°C +/-1°C

Yellow, Shiny colonies (2-4mm)

String
Test

16-18hrs at 36°C +/-1°C

Subculture on Nonselective agar*
(MHA)

Report: *Vibrio cholerae*

Oxidase +ve

String test, Biochemicals

Vibrio cholerae

Serotyping

Antimicrobial Susceptibility Testing*

O1 antisera

Ogawa & Inaba
serology

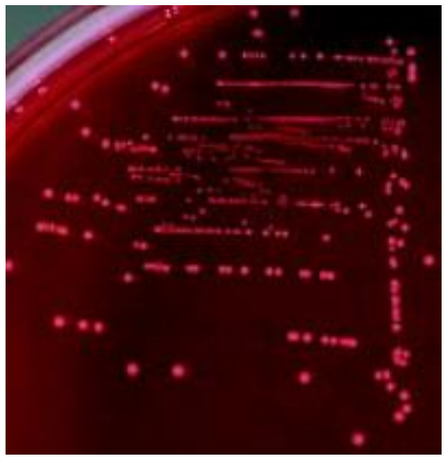
+ve

-ve

Vibrio cholerae
O139 serology

*Antibiotic discs required: Ampicillin (10µg), Tetracycline (30µg), Co-trimoxazole (1.25/23.75µg),

Chart 4: Identification of Salmonella & Shigella



XLD: Pink/Red colonies ± black center (2-4 mm)
HE: Clear colonies ± black center (1-4mm)

Subculture on Nonselective agar (MHA or NA)

Oxidase test

TSI, LIA, MIO, Citrate, Urea

Antimicrobial Susceptibility Testing*

TSI:K/A, g++ LIA: LDC + MIO: +/-/+ Citrate:+ Urea: -	TSI: K/A tr LIA: LDC + MIO: +/-/- Citrate:- Urea: -	TSI: K/Ag LIA: LDC – MIO: +/-/+ Citrate:- Urea: -	TSI: K/A LIA: LDC – MIO: -/v/- Citrate:- Urea: -	TSI: K/A LIA: LDC – MIO: -/-/+ Citrate:- Urea: -
Consistent with *Salmonella species*	Consistent with *Salmonella* serov. Typhi	Consistent with *Salmonella* ser. Paratyphi A	Consistent with *Shigella species*	Consistent with *Shigella sonnei*

*Antibiotic discs required: Ampicillin (10µg), Co-trimoxazole(1.25/23.75µg), Chloramphenicol (30µg), Ceftriaxone (30µg),Pefloxacin (5µg),Ciprofloxacin (5µg)

Chart5 : Disc Diffusion Method for Susceptibility Testing

Isolated colonies

↓

Saline Suspension

↓

Adjust Turbidity to 0.5 Mc Farland

↓

Inoculate Mueller Hinton Agar
Inoculate a quality control plate in the similar manner using
E. Coli 25922

↓

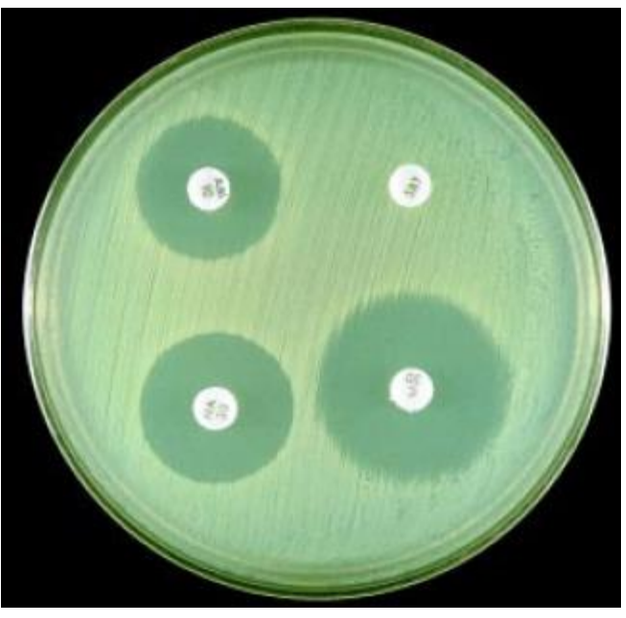
Place discs on Plate

↓

Incubate 16-18 hr, 36±1°C

↓

Measure zone sizes, Interpret



Antibiotic discs required:

Salmonella & *Shigella:* Ampicillin (10µg), Co-trimoxazole(1.25/23.75µg), Chloramphenicol (30µg), Ceftriaxone (30µg),Pefloxacin (5µg),Ciprofloxacin (5µg)

Vibrio cholerae: Ampicillin (10µg), Tetracycline (30µg), Co-trimoxazole (1.25/23.75µg), Ciprofloxacin (5µg)

Antibiotic disc placement:

Discs must be placed so that unacceptable overlapping of zones is avoided.

The maximum number of discs will vary based on the antimicrobial and organism (some agents consistently give larger zones with susceptible isolates).

No more than six (6) disks should be placed on a 90mm plate and no more than twelve (12) disks should be placed onto a 150 mm plate

Triple Sugar Iron (TSI) agar

- Principle:

  To determine following characteristics in a GNB

  – Fermentation of Glucose, Sucrose & Lactose

  – Gas production during glucose fermentation

  – H_2S production

- Medium:
  - Triple Sugar Iron agar containing Glucose, Sucrose, Lactose (1:10:10), Phenol red indicator, Iron salt, Sodium thiosulfate (H_2S source)
- Procedure:
  – Inoculate the agar heavily (Stab and Streak)

  – Incubate at 37°C, examine after 8-12 hrs, & 18-24 hrs

  – If screw capped tubes are used, the caps must be loose.

Triple Sugar Iron (TSI) agar

A B C D E F

Reactions: After at 18-24 hours:

- A: Uninoculated tube
- B: K/A Tr(ace) H_2S: glucose fermented, lactose sucrose not fermented. *Salmonella* Typhi
- C: K/Ag++: Glucose fermentation but no sucrose or lactose fermentation. Non-typhoidal *Salmonella* spp.
- D: K/A: Glucose fermented, no lactose or sucrose fermented, no gas produced (*Shigella flexneri*).
- E: A/A: Glucose and lactose and/or sucrose fermented gas produced. *E. coli*.
- F: No fermention, no gas production. *Pseudomonas aeruginosa.*

Citrate Utilization test

Principle:

- Test the ability to utilize citrate as a sole carbon source

Medium:

- Simmon's citrate medium (solid medium with Bromothymol blue indicator)

Procedure:

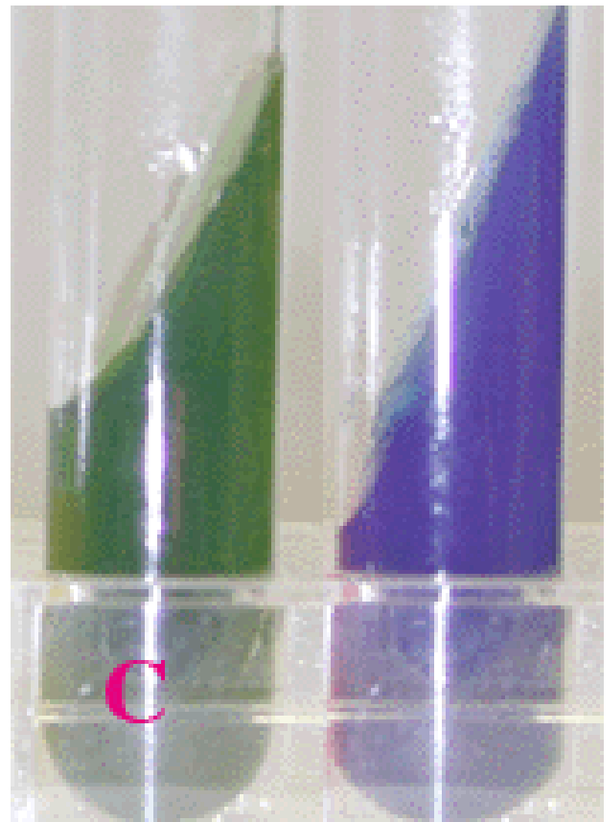
- Light inoculum (Streak)
- Incubation at 37°C, up to 48 hrs

Result Interpretation:

- Positive: Growth & blue color
- Negative: No growth, green color (no change)

Controls:

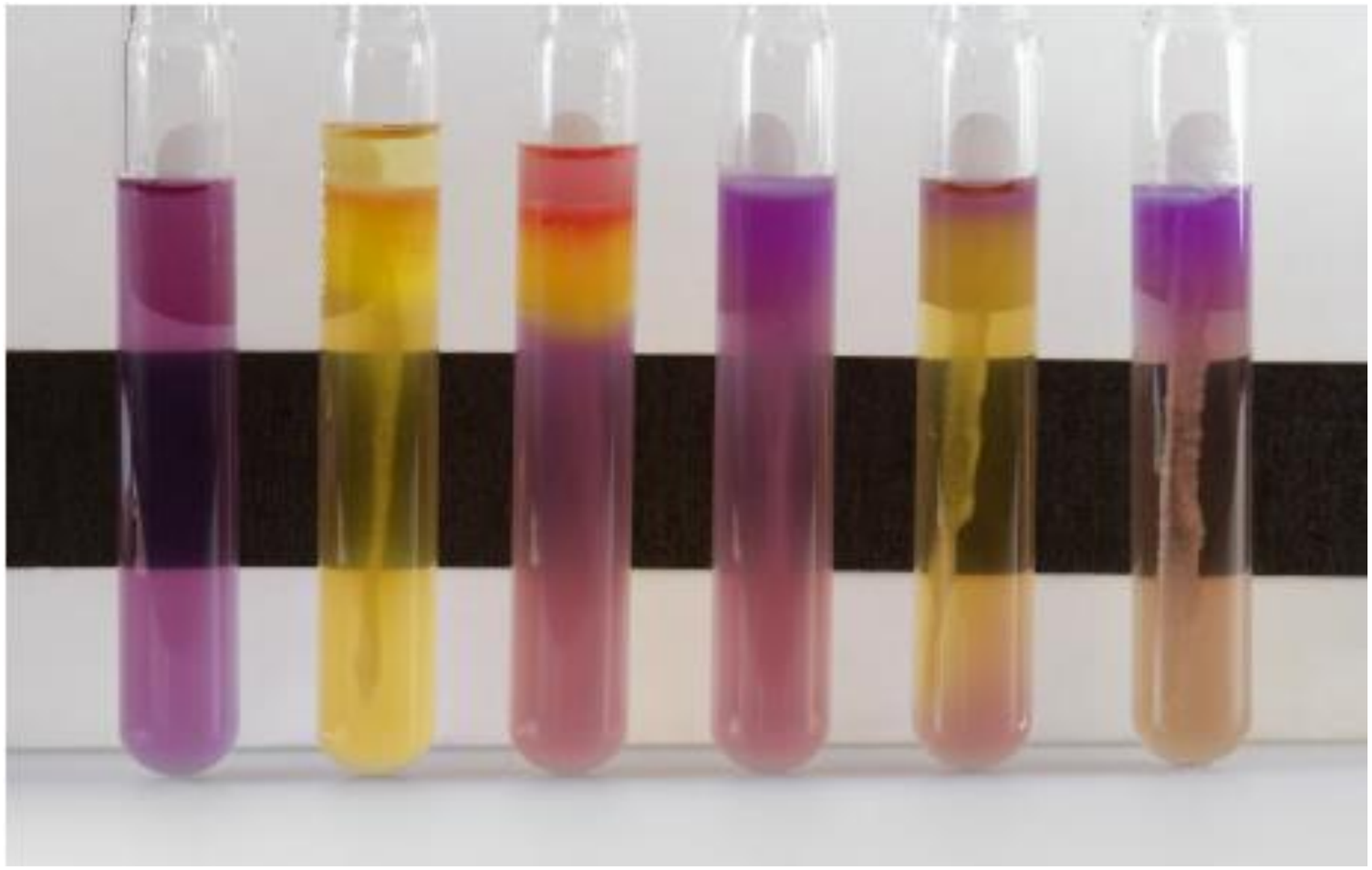
- Positive: Citrobacter spp
- Negative: E. coli



Urease Test

- Principle:
  - Test for the presence of enzyme Urease that breaks urea into NH_3, thus increasing the pH
- Medium:
  - Cristensen's urea agar, Phenol red indicator
- Procedure:
  - Inoculate the agar heavily
  - Incubate at 36°+/- 1°C, examine after 4 hours, after overnight incubation up to 4 days
- Result Interpretation:
  - Pink colour -------Positive
  - No coloration-----Negative
- Controls:
  - Positive: *Proteus spp.*
  - Negative: *E. coli*

Motility Indole Ornithine (MIO) agar



A B C D E F

Reactions

A: Uninoculated MIO tube
B: Indole negative (*Shigella flexneri*)
C: Indole positive (*E. coli*)
D: Motile/Ornithine Positive (*Salmonella* serovar Newport)
E: Nonmotile/Ornithine Negative (*Shigella flexneri*)
F: Nonmotile/Ornithine Positive (*Shigella sonnei*)

Motility Indole Ornithine (MIO) agar

Principle:

- To demonstrate the motility, ornithine decarboxylase activity and Indole production.

Procedure:

- Inoculate the agar with a single stab into agar and stop approx.1cm from the bottom of the tube.
- Incubate at 37°C, examine after overnight (18-24 hrs) incubation .

Result Interpretation::

Motility

- Positive: Turbid.
- Negative: Clear

Motility Indole Ornithine (MIO) agar Cont…..

Orinithine Decarboxylase

- Positive: middle of tube turns light purple colour
- Negative: Yellow

Iodole: (Kovac's reagent)

- Positive: Pink Red
- Negative: No Color Change

Controls:

- Positive: *E.coli spp.*
- Negative: *Shigella flexneri*

Lysine Iron Agar (LIA)

Reactions

A: Uninoculated LIA
B: LDC negative /lysine deaminase negative/ H_2S negative (*Citrobacter freundii*)
C: LDC positive /lysine deaminase negative/ H_2S negative (*Salmonella* ser. Typhi)
D: LDC negative /lysine deaminase positive/ H_2S negative (*Proteus mirabilis*)
E: LDC positive /lysine deaminase negative/ H_2S positive (*Salmonella* ser. Newport)

Lysine Iron Agar (LIA)

Principle:

To demonstrate the Lysine decarboxylation, deamination and H_2S production by enteric organisms.

Procedure:

- Inoculate the agar with a single stab into agar and stop approx.1cm from the bottom of the tube.
- Incubate at 37°C, examine after overnight (18-24 hrs)incubation .

Controls:

- Proteus mirabilis: Slant Red; Butt Yellow; H_2S neg
- *Salmonella* Typhimurium: Slant Purple; Butt Purple; H_2S pos
- *Shigella flexneri*: Slant Purple; Butt Yellow; H_2S neg

Lysine Iron Agar (LIA) Cont....

Result Interpretation:

H2S Production

- Positive: black colour along the streak or throughout medium
- Negative: No Colour change

Lysine Decarboxylation (detected in butt of the tube)

- Positive: Purple
- Negative: Yellow

Lysine Deamination (detected on agar slant)

Positive: Red

Negative: Purple

Salmonella serovar Typhi

A B C D E F

A) TSI: Alkaline slant / Acid Butt / Trace H2S / No Gas (K / A TR)
B) Urea: Negative
C) LIA: Lysine Decarboxylase Positive
D) Citrate: Negative
E) MIO: Motile / Ornithine Negative
F) MIO w/ indol reagent: Indol negative

Salmonella serovar Newport (typical of most nontyphoidal *Salmonellae*

A B C D E F

A) TSI: (K/Ag++) Alkaline slant / Acid Butt / H2S Positive / Gas
B) Urea: Negative
C) LIA: Lysine Decarboxylase Positive
D) Citrate: Positive
E) MIO: Motile / Ornithine Negative
F) MIO w/ indole reagent: Indole negative

Salmonella serovar Paratyphi A

A **B** **C** **D** **E** **F**

A) TSI: Alkaline slant / Acid Butt / No H2S / Gas (K / A g)
B) Urea: Negative
C) LIA: Lysine Decarboxylase Negative
D) Citrate: Negative
E) MIO: Motile / Ornithine Positive
F) MIO w/ indole reagent: Indole negative

Shigella sonnei

A **B** **C** **D** **E** **F**

A) TSI: Alkaline slant / Acid Butt / No H2S / No Gas (K / A)
B) Urea: Negative
C) LIA: Lysine Decarboxylase Negative
D) Citrate: Negative
E) MIO: Nonmotile / Ornithine Positive
F) MIO w/ indole reagent: Indole negative

Shigella flexneri

A **B** **C** **D** **E** **F**

A) TSI: Alkaline slant / Acid Butt / No H2S / No Gas (K / A)
B) Urea: Negative
C) LIA: Lysine Decarboxylase Negative
D) Citrate: Negative
E) MIO: Nonmotile / Ornithine Negative
F) MIO w/ indol reagent: Indol negative

Escherichia coli

A **B** **C** **D** **E** **F**

A) TSI: Acid slant / Acid Butt / No H2S / Copious Gas (A / A g)
B) Urea: Negative
C) LIA: Lysine Decarboxylase Positive
D) Citrate: Negative
E) MIO: Motile / Ornithine Positive
F) MIO w/ indol reagent: Indol positive

References

- Laboratory Protocol: “Isolation of Salmonella and Shigella from Faecal Specimens” http://www.antimicrobialresistance.dk/data/images/protocols/gfn%20stool%20culture-nov2010.pdf
- Laboratory Protocol: “Biochemical Identification of Salmonella and Shigella Using an Abbreviated Panel of Tests” http://www.antimicrobialresistance.dk/data/images/protocols/gfn_biochem_final.pdf
- Laboratory Protocol: “Susceptibility testing of Enterobacteriaceae using disk diffusion” http://www.antimicrobialresistance.dk/data/images/protocols/sop_dd_2010gfnlab002_v2.pdf