Gastrointestinal Tract Module

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Objectives

To become familiar with:

- ✓ The selective and differential media used to identify the GIT infections associated bacteria
- ✓ The biochemical tests used to isolate these bacteria

Types of specimen

- ✓ Stool (direct culture)
- ✓ Stool in fecal transport system (no d
  longer than 1 hour)
- ✓ Rectal swab
  - Pass swab beyond anal sphincter, carefully rotate, and withdraw

Criteria of specimen rejection

- ✓ Specimen contaminated with urine, residual soap, or disinfectants.
- ✓ Specimens received in grossly leaking transport containers
- ✓ Dry specimens
- ✓ Specimens submitted in fixative or additives

Patient preparation

- ✓ Instruct the patient on how the specimen should be collected and transferred to the container
- ✓ provide him with sticks and containers

Specimen collection

- ✓ A sample is transferred with the sticks to the container
- ✓ The specimen should contain at least 5 g of feces
- ✓ Those parts that contain blood and/or mucus should be selected
- ✓ Close the lid properly

Who will collect the specimen?

The patient. If stool is unobtainable, nursing staff or physician will collect fecal swab

Time relapse before processing the sample

Stool samples should be examined and cultured as soon as possible after collection.

Indications for stool culture include:

Doctors are most likely to order a stool culture for patients with any of the following characteristics:

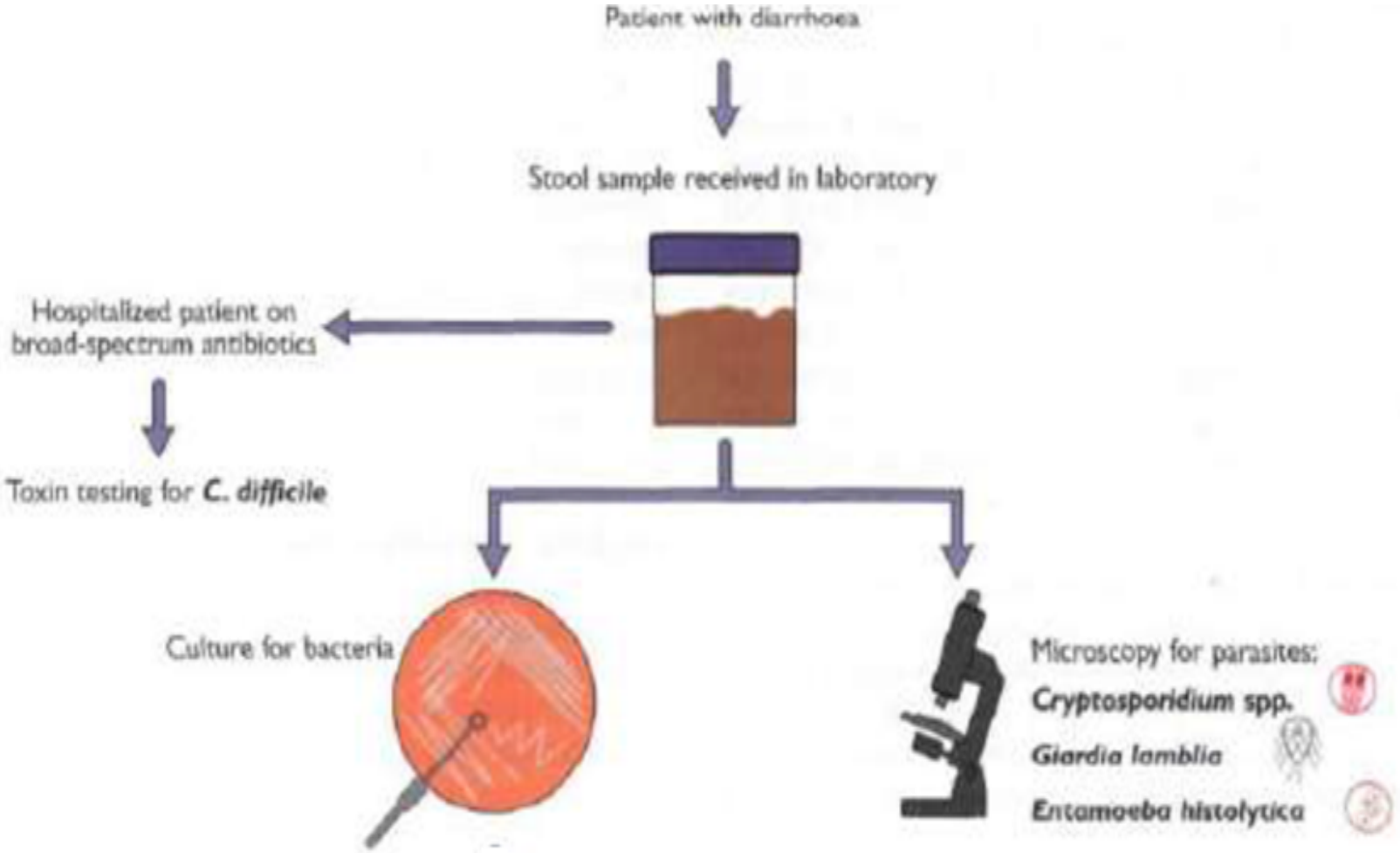
- ✓ Bloody diarrhea and/or fecal leukocytes
- ✓ Tenesmus (is the constant feeling of the need to empty the bowel, accompanied by pain, and cramping)
- ✓ Severe or persistent symptoms
- ✓ Recent travel to a third world country
- ✓ Sever dehydration

Enterobacteriaceae

General Characteristics

- Gram-negative bacilli
- Oxidase –ve
- Catalase +ve
- Ferment glucose with or without gas production
- Reduce nitrate to nitrite (with few exceptions)
- facultative anaerobes

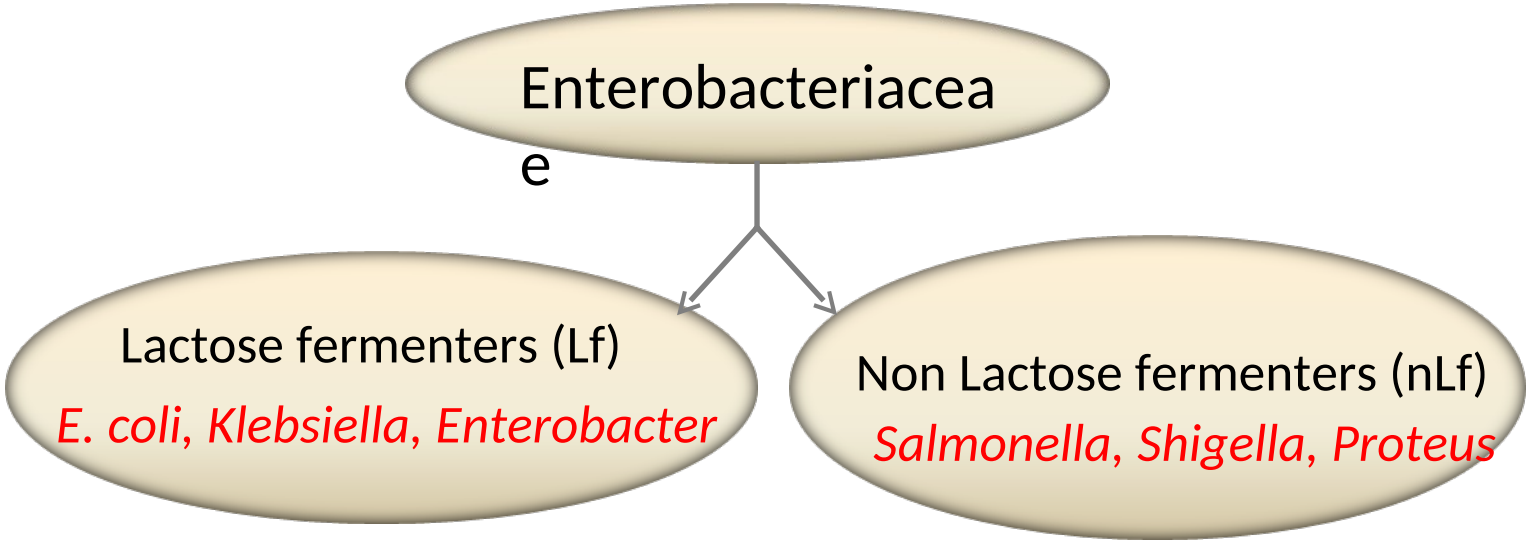
Processing of stool samples



Identification of *Enterobacteriaceae*

1- Using selective and differential media

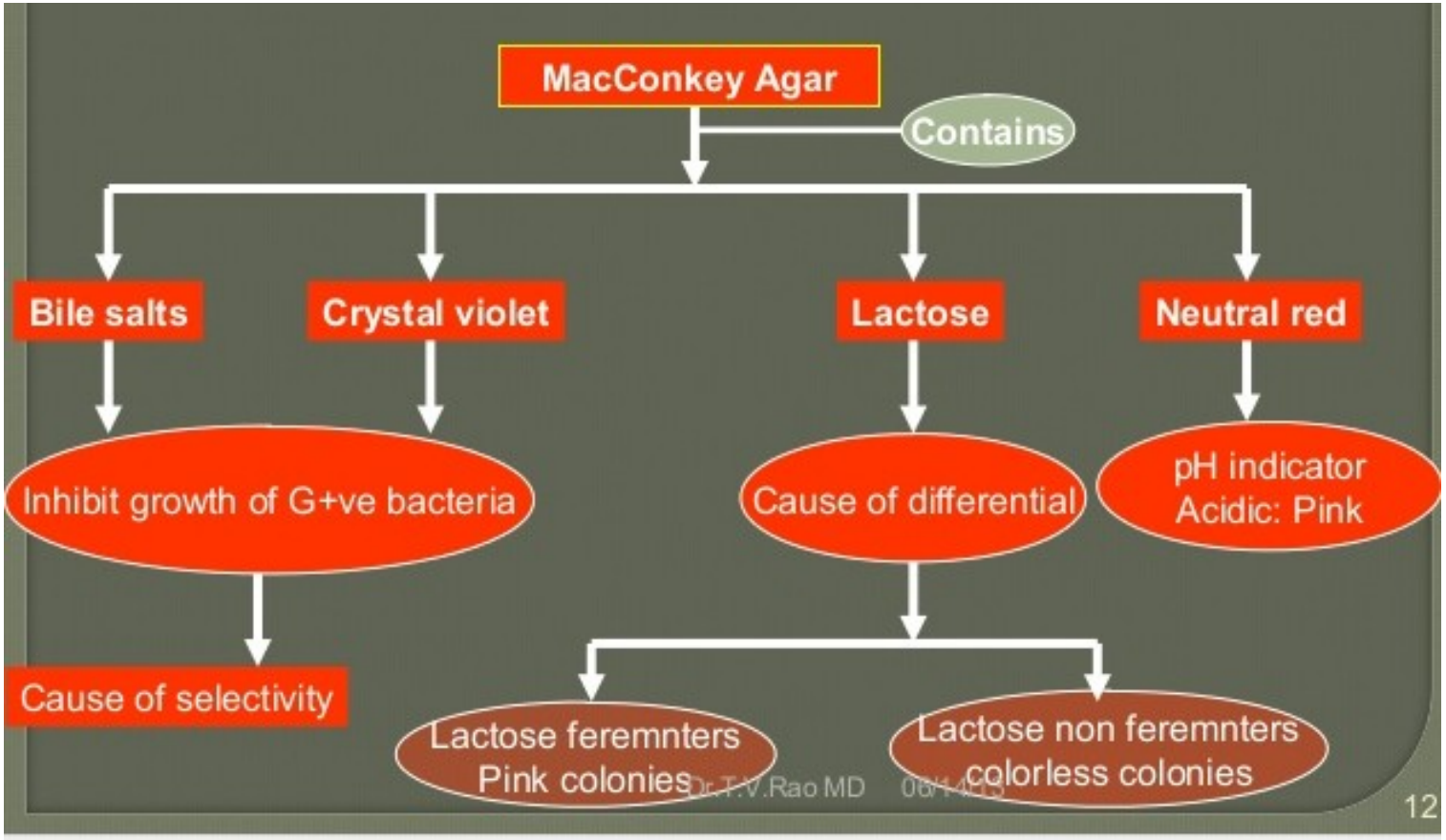
Enterobacteriaceae divided into two main groups according to lactose fermentation



There are several selective and differential media used to isolate and distinguish between Lf & nLf including

- ✓ MacConkey agar
- ✓ *Salmonella Shigella* agar (SS agar)

MacConkey agar is a selective and differential media for Enterobacteriaceae



Escherichia coli
Klebsiella spp
Enterobacter spp
Citrobacter spp

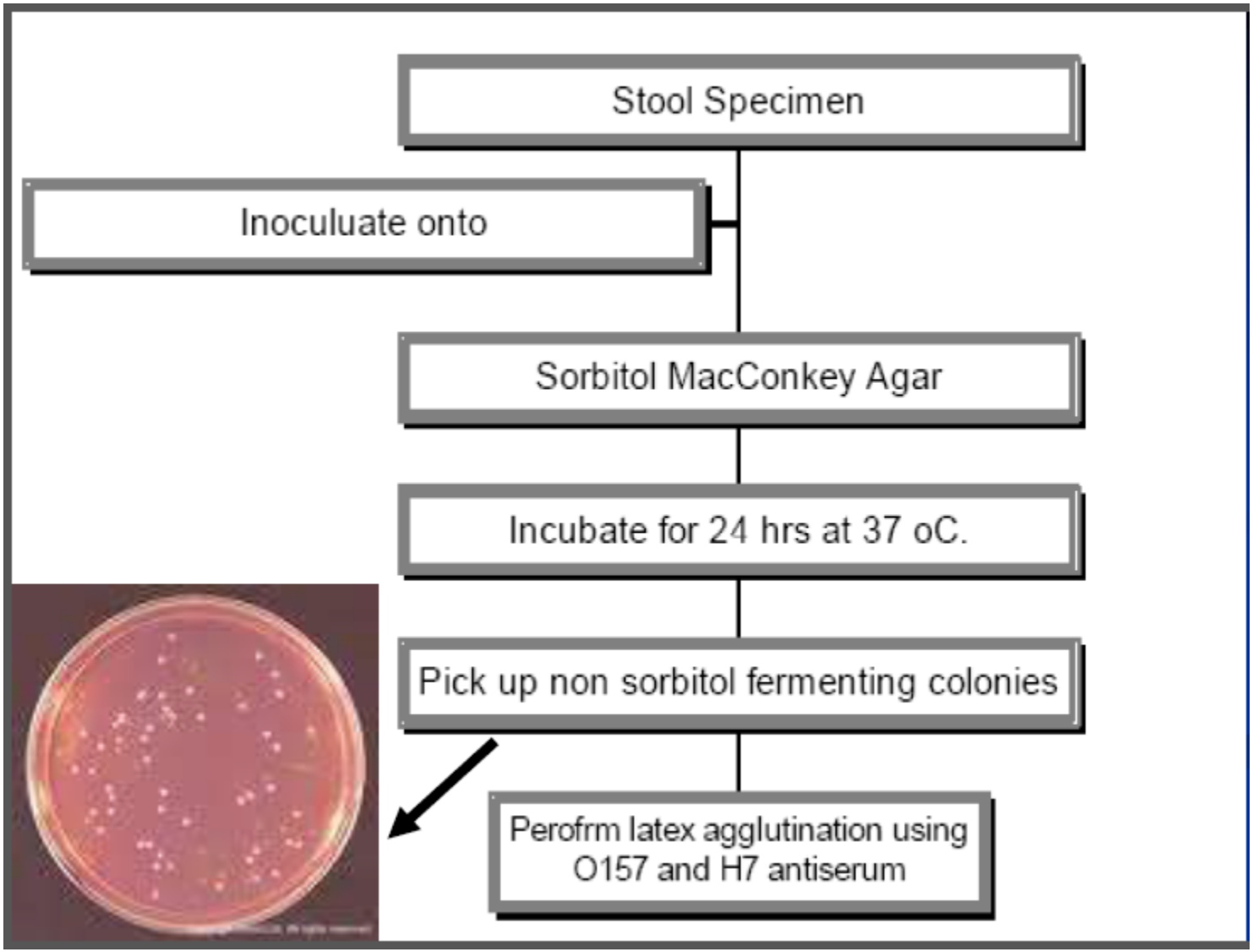
Salmonella spp
Schigella spp
Proteus spp
Yersinina spp

MacConkey agar

Lactose fermenter

Non Lactose fermenter

Suspected EHEC (O157:H7)



Diagnosis of *Salmonella Shigella*

1- *Salmonella Shigella* agar (SS agar)

Purpose

For isolation and differentiation of *Salmonella* & *Shigella*

Components

- ✓ Brilliant green dye & sodium citrate: inhibit the growth of most intestinal flora
- ✓ Lactose
- ✓ Neutral red: pH indicator, red in acidic conditions
- ✓ Sodium thiosulfate ($Na_2S_2O_3$): sulfur source
- ✓ Ferric citrate: H2S indicator

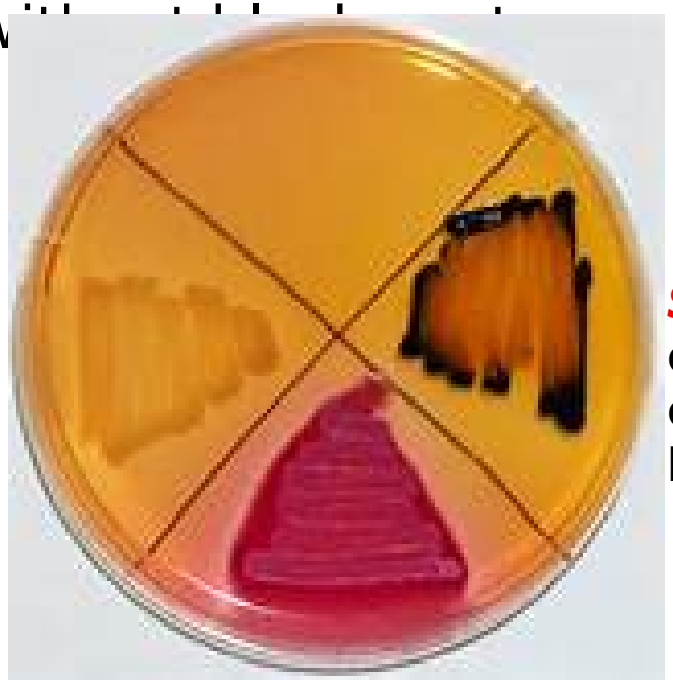
Diagnosis of *Salmonella Shigella*

1- *Salmonella Shigella* agar (SS agar)

Results

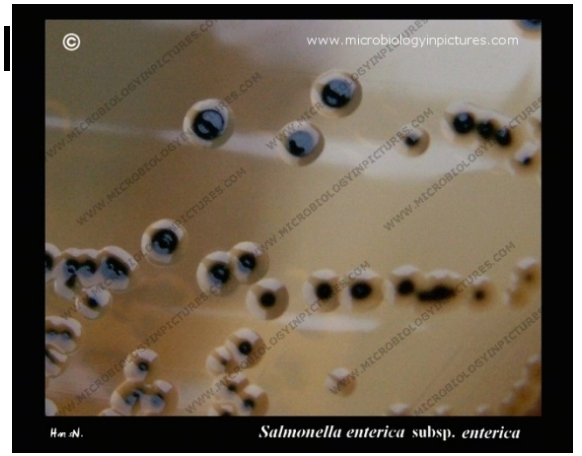
- ✓ Lactose fermenters: pink to red colonies (few can grow)
- ✓ Non lactose fermenters: translucent, colorl
  with or w

***Shigella*:**
colorless colonies without black centers



Lactose fermenter flora:
pink to red

***Salmonella*:**
colorless colonies with black centers



Diagnosis of *Salmonella Shigella*

2- Hektoen enteric agar (cat eye apperance)

Identification of Enterobacteriacea

2- Using special biochemical reactions

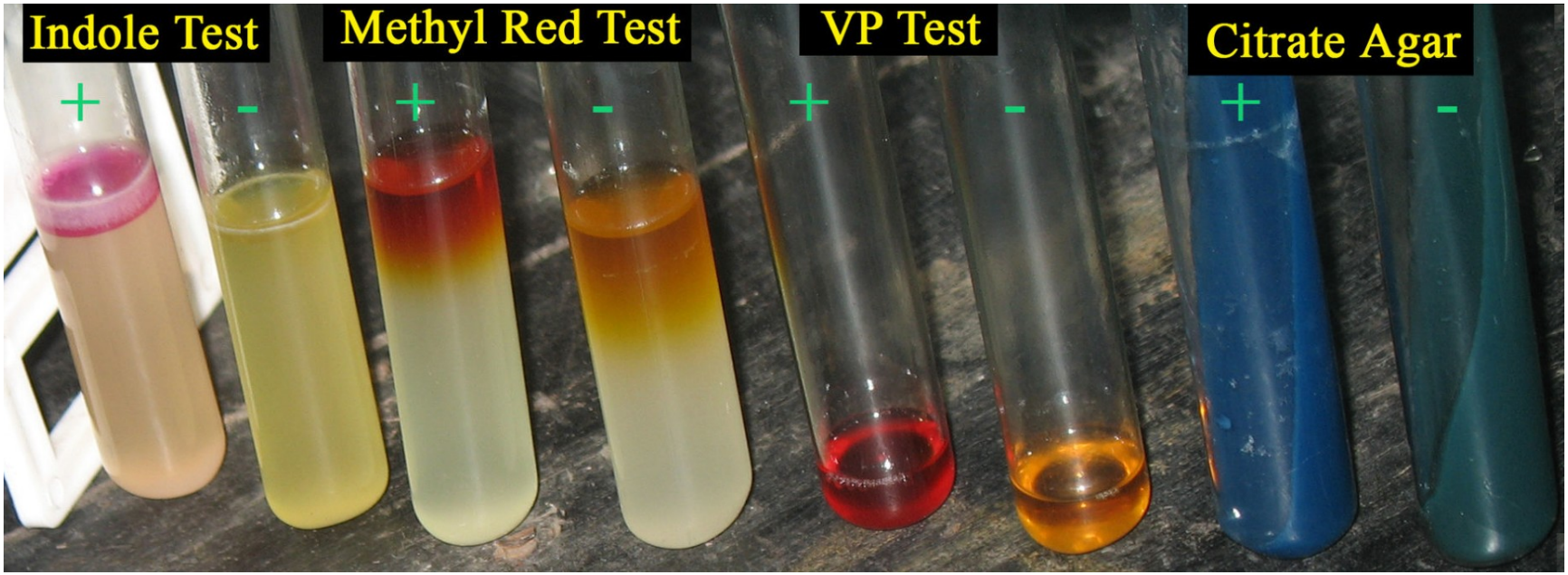
The differentiation of the principle groups of Enterobacteriaceae Can be accomplished on the basis of their biochemical prosperities and enzymatic reactions in the presence of the specific substrate

One important group of biochemical reactions is:

Indoile, **M**ethyle red, **V**ogus proskaur, **C**itrate utilization tests (IMViC)

IMViC Tests

- Indole test, Methyl red test, Voges-Proskauer test, (i; for ease of pronounciation), Citrate test.



IMViC Tests

Species	Indole	Methyl Red	Voges-Proskauer	Citrate
Escherichia coli	Positive	Positive	Negative	Negative
Shigella spp.	Negative	Positive	Negative	Negative
Salmonella spp.	Negative	Positive	Negative	Positive
Klebsiella spp.	Negative	Negative	Positive	Positive
Proteus vulgaris	Positive	Positive	Negative	Negative
Proteus mirabilis	Negative	Positive	Negative	Positive
Citrobacter freundii	Negative	Positive	Negative	Positive
Enterobacter aerogenes	Negative	Negative	Positive	Positive

Citrate Utilization Test

- **Use**: to determine bacterial ability to use citrate as the sole source of carbon.
- **Culture medium**: **Simmons citrate agar**; contains sodium citrate, inorganic ammonium salts (sole source of nitrogen), & pH indicator bromthymol blue (**neutral; green** & **alkaline; blue**).
- **Principle**: citrate use → ammonia production → alkaline pH.
- **Method**:

1- Use a needle to lightly touch tip of single 16- to 24-hour-old colony & inoculate.

2- Incubate at 35°C.

3- Observe for development of blue colour.

Citrate Utilization Test

- **Results**:

1- **Positive**: Growth, with or without colour change. A colour change is due to acid or alkali production during bacterial growth. The usual colour change is from **green (neutral)** to **blue (alkaline).**

2- **Negative**: No growth, colour remains green.

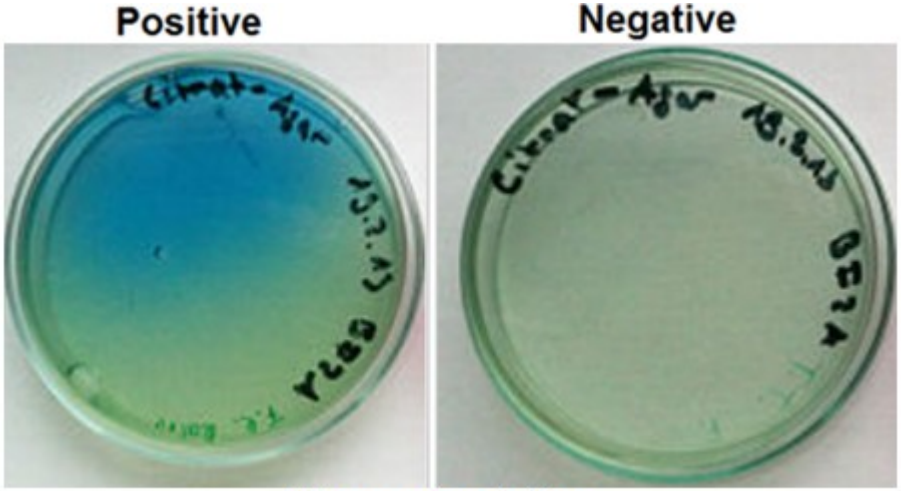
- **Important citrate-positive bacteria**:

1- Klebsiella sp.

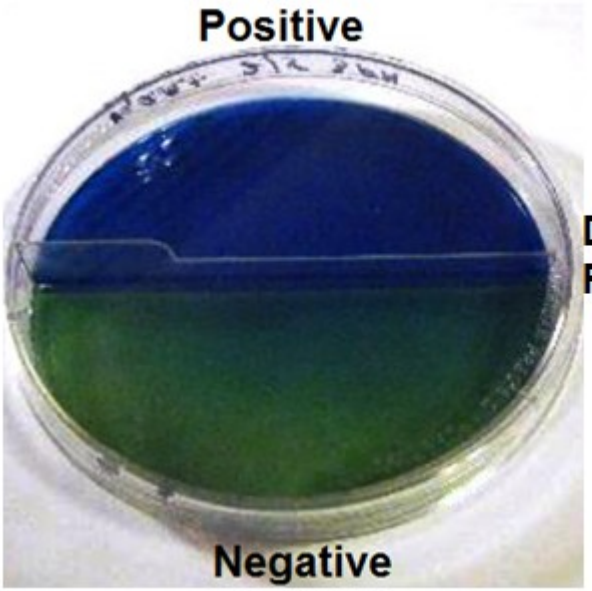
2- Citrobacter sp.

3- Proteus sp.

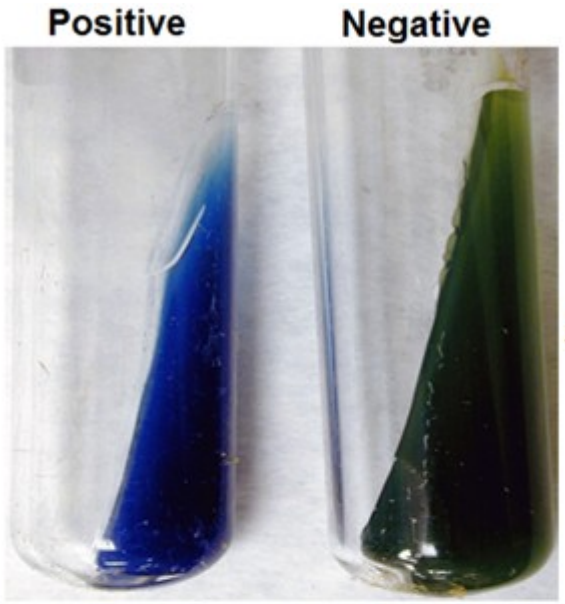
Citrate Utilization Test



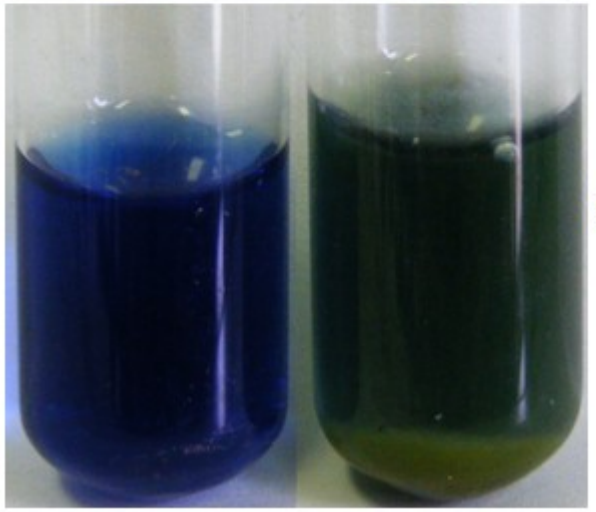
Separate plates



Divided Plate



Slant



Butt

Indole Test

- **Use**: to determine bacterial ability to degrade amino acid tryptophan (by tryptophanase enzyme) into indole (+ Kovac's reagent (yellow) indicator) → red colour.
- **Method**:

1- Inoculate tryptophane broth with 1-2 drops from overnight bacterial enrichment broth.

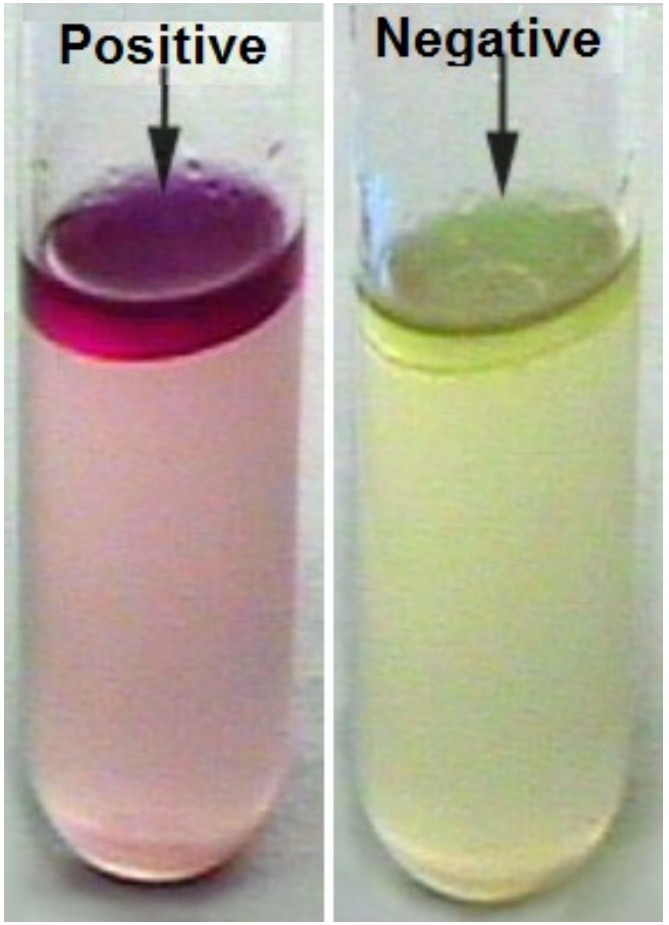
2- Incubate at 35°C.

3- Add 0.5 mL (5-10 drops) of Kovac's reagent.

Indole Test

- **Results**:
- **Positive**: enzyme present, indole produced, red ring on top of broth e.g. **E.coli.**
- **Negative**: enzyme absent, indole NOT produced, NO colour change or clear yellow ring e.g. **Klebsiella sp., Enterobacter sp., Salmonella sp.**

Kovac's Reagent



Methyl Red (MR) & Voges-Proskauer (VP) Tests

- **Use**:

1- **MR tests** for **acids production** from glucose fermentation.

2- **VP tests** for **acetoin production** from glucose fermentation.

•**Culture media**: MRVP Glucose Broth, & **Reagents**:

1- Methyl Red indicator for acids produced using **mixed acid fermentation pathway** using pyruvate as a substrate.

2- VP indicators (5% Alpha-naphthol & potassium hydroxide) for acetoin production using **2,3-butanediol fermentation pathway**.

•**Method**:

1- Inoculate tube aseptically with inoculating loop.

2- Incubate at 35℃ for 48 hours of incubation.

3- Separate bacterial broth into 2 separate tubes.

4- Add few drops of MR to one tube.

5- Add both VP reagents to the other tube, shake vigorously then allow to sit for 5-10 minutes.

Methyl Red (MR) & Voges-Proskauer (VP) Tests Results

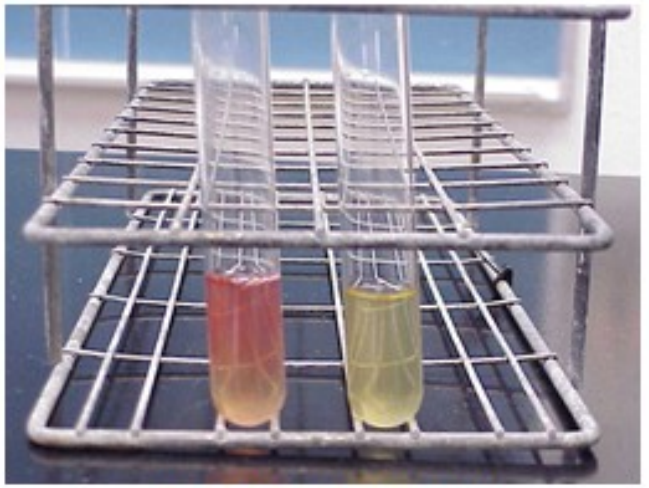
MR

- **Positive**: acids, pH <4.2, red
- **Negative**: NO acids produced, pH >6.2, yellow.

Negative Positive

VP

- **Positive**: acetoin present, red.
- **Negative**: acetoin absent, NO colour change.



Positive Negative

Cholera identification

Identification

- ✓ Thiosulfate citrate bile salt sucrose agar or TCBS agar
- ✓ The medium is alkaline (pH 8.6) which enhances the growth of Vibrio species

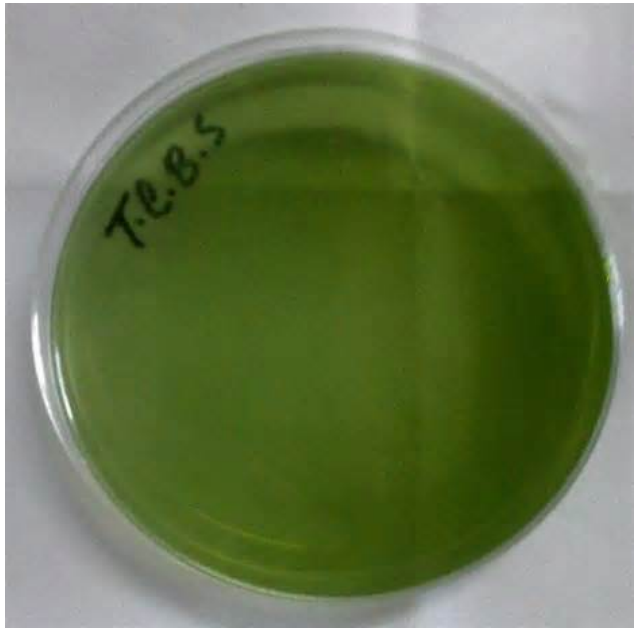
Important components

- ✓ Sucrose: sugar source
- ✓ Bromothymol blue: pH indicator
  - pH<6.0 - yellow
  - pH>7.6 –blue

Cholera identification

Results

- ✓ *Vibrio cholera*: Ferment sucrose and gives smooth yellow colonies
- ✓ *Vibrio parahemolyticus*: non-sucrose fermenter, green colonies

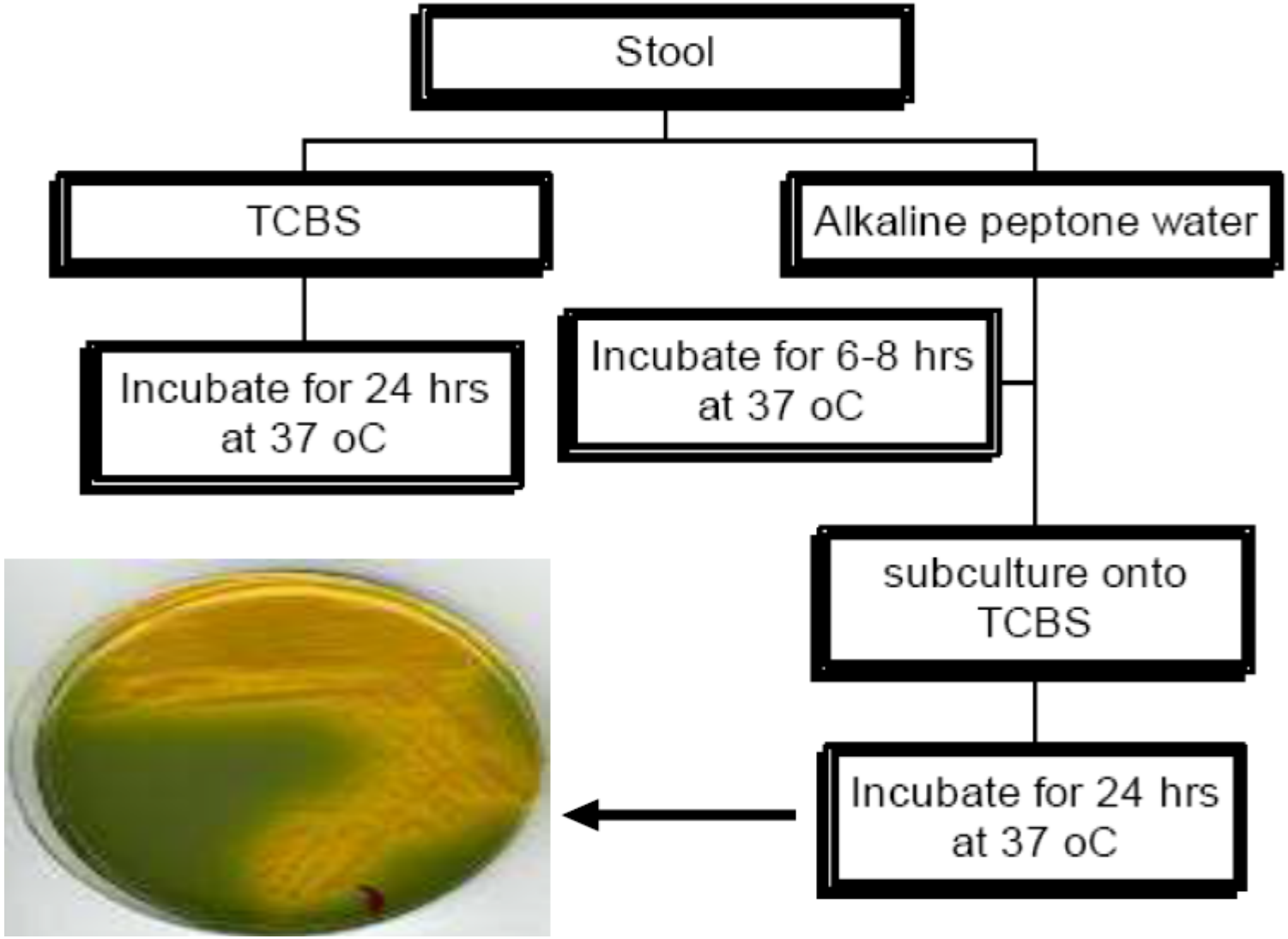


TCBS media

V. cholera

V. parahemolyticus

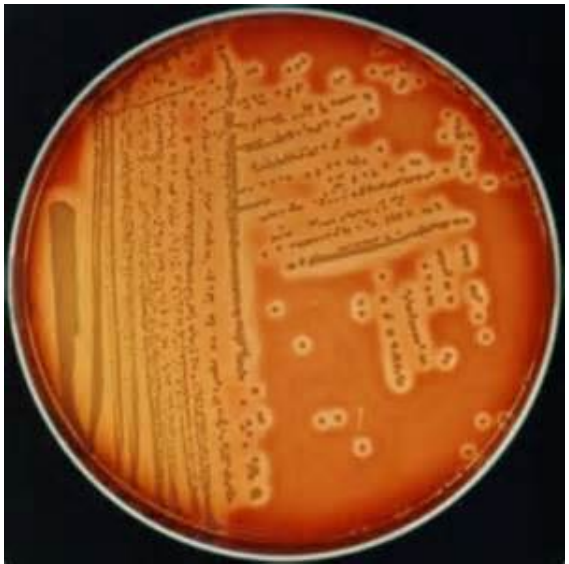
Cholera identification



S. aureus associated food poisoning

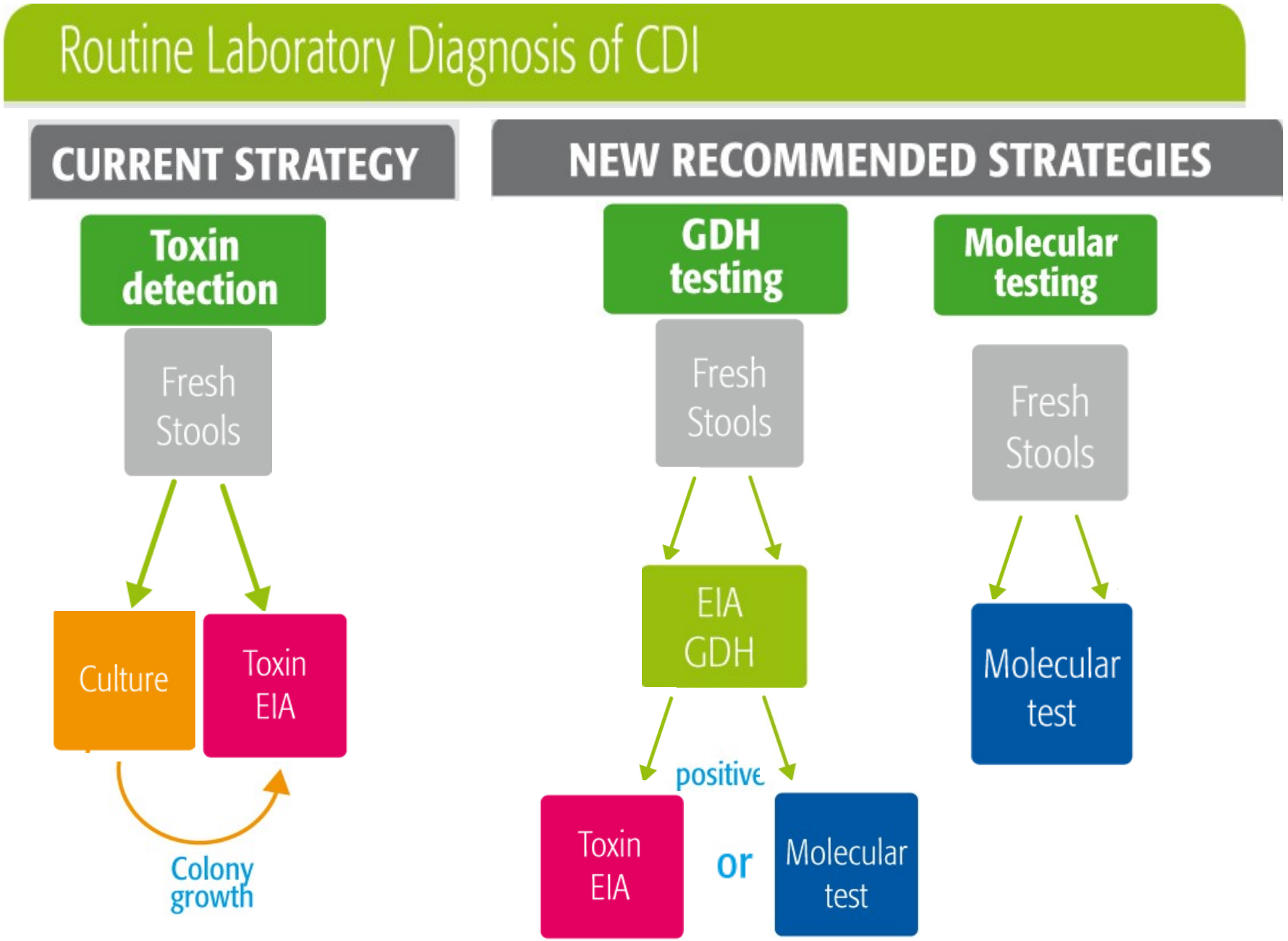
- 25% of healthy people are carriers
- Mainly *S. aureus* food poisoning is diagnosed in case of outbreaks
- Diagnosis based on
  - ✓ gram positive cocci
  - ✓ catalase & coagulase positive
  - ✓ oxidase positive
  - ✓ ß-hemolysis on blood agar
  - ✓ Grow on MSA(Mannitol Salt Agar) with mannitol fermentation

Blood agar



MSA

Diagnosis of *C. difficile* infection (Glutamate Dehydrogenase toxin)



Diagnosis of *C. difficile* infection

Culture

- Clostridium difficile bacteria. Colonies of C. difficile bacteria after 48 hours growth on blood agar.
- Results: Clostridium difficile will appear as flat yellow colonies with a ground glass-like appearance and a slightly filamentous edge.