GIT Module
Bacterial infections
Lab 1
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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 (in delay 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:

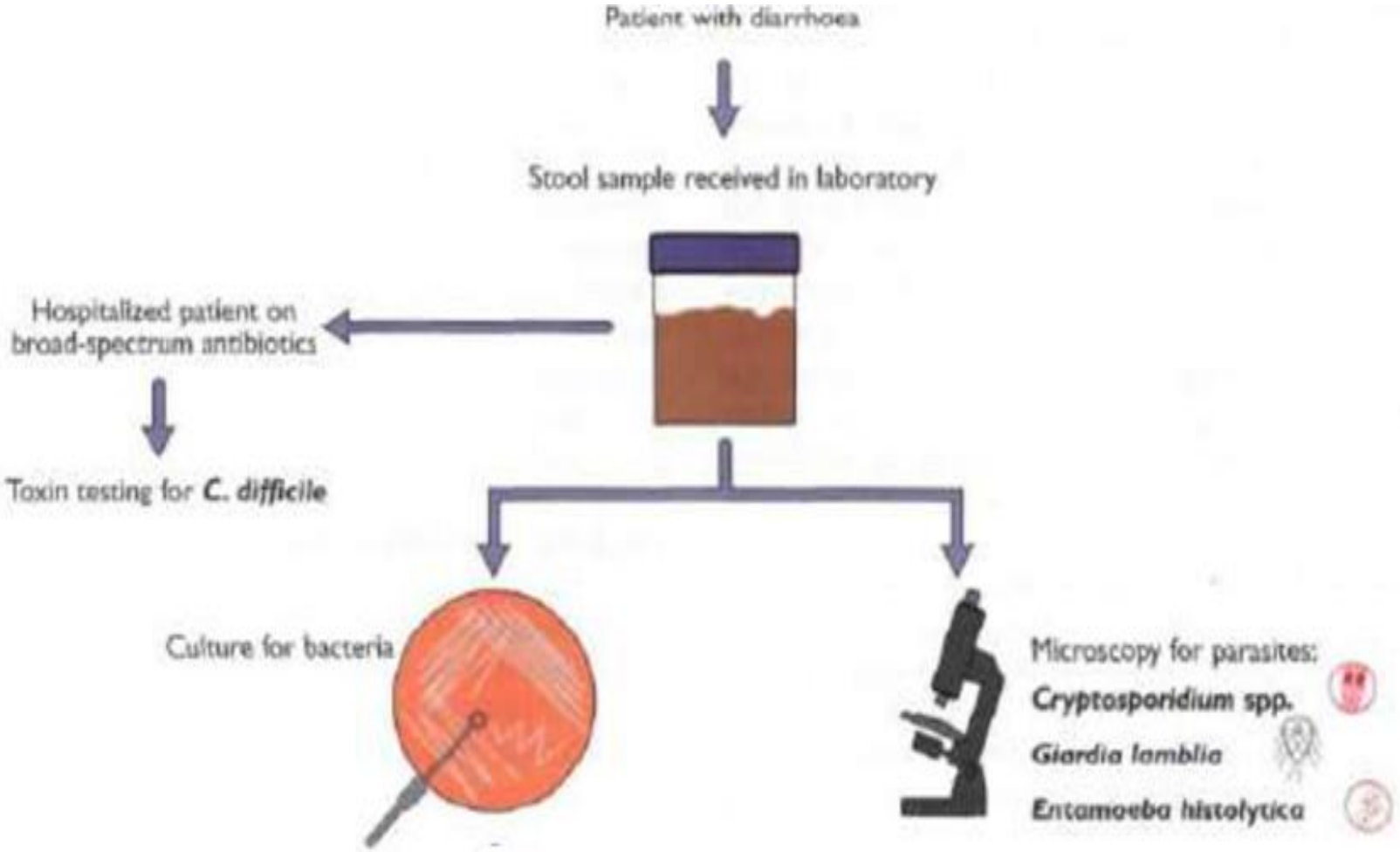
- ✓ Bloody diarrhea and/or fecal leukocytes
- ✓ Tenesmus (is the constant feeling of the need to empty the bowel, accompanied by pain, and cramping)
- ✓ Severe or persistent symptoms
- ✓ Recent travel to a third world country
- ✓ Sever dehydration

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

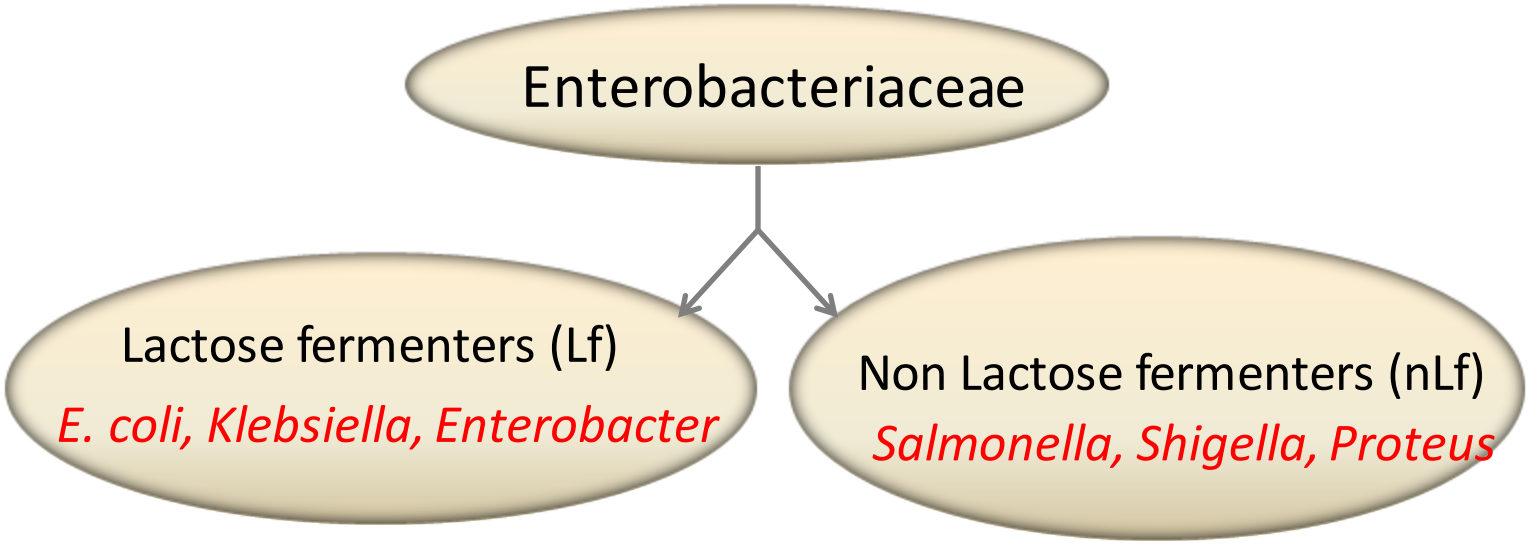
Processing of stool samples



Identification of *Enterobacteriaceae*

1- Using selective and differential media

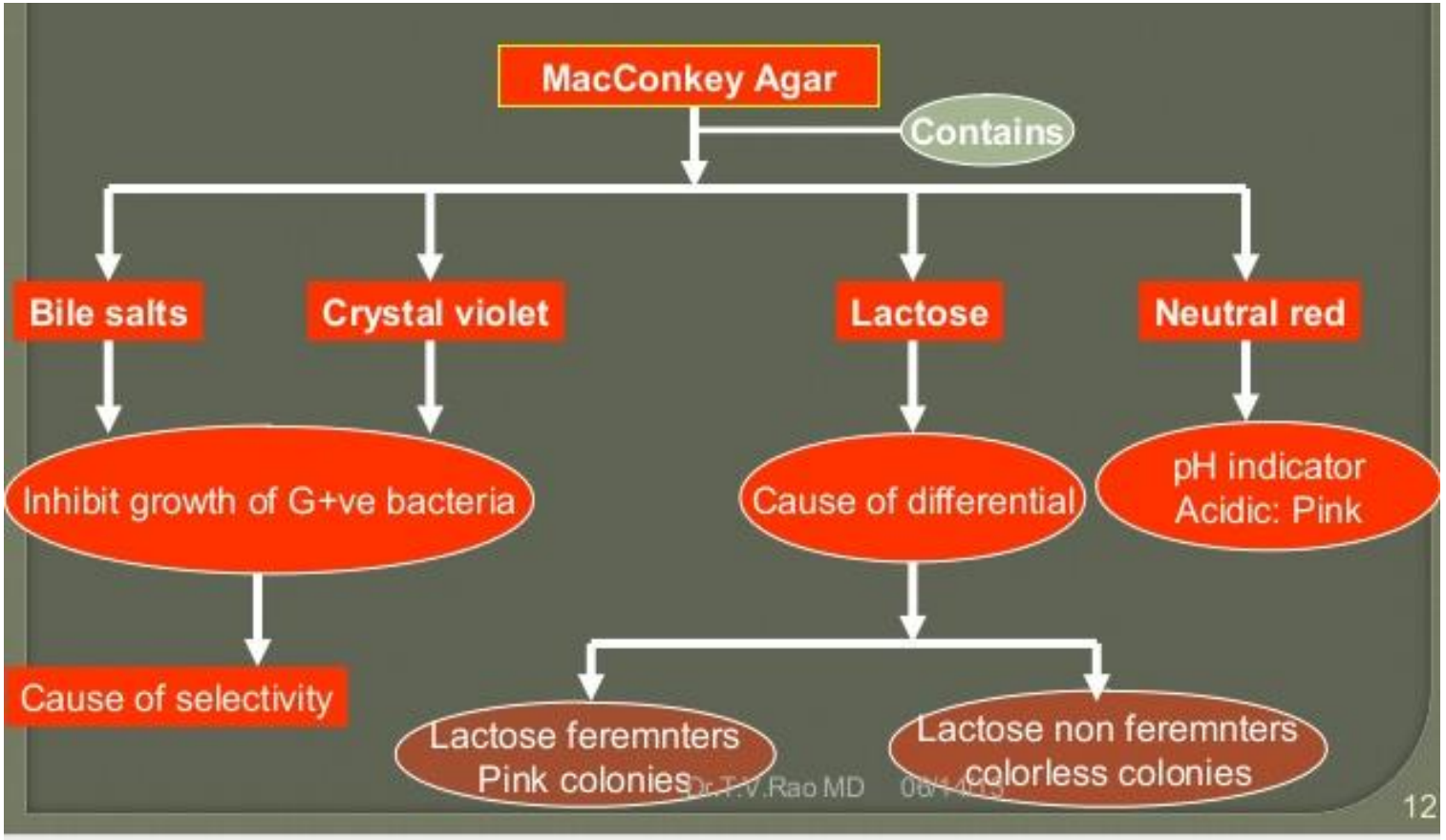
Enterobacteriaceae divided into two main groups according to lactose fermentation



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

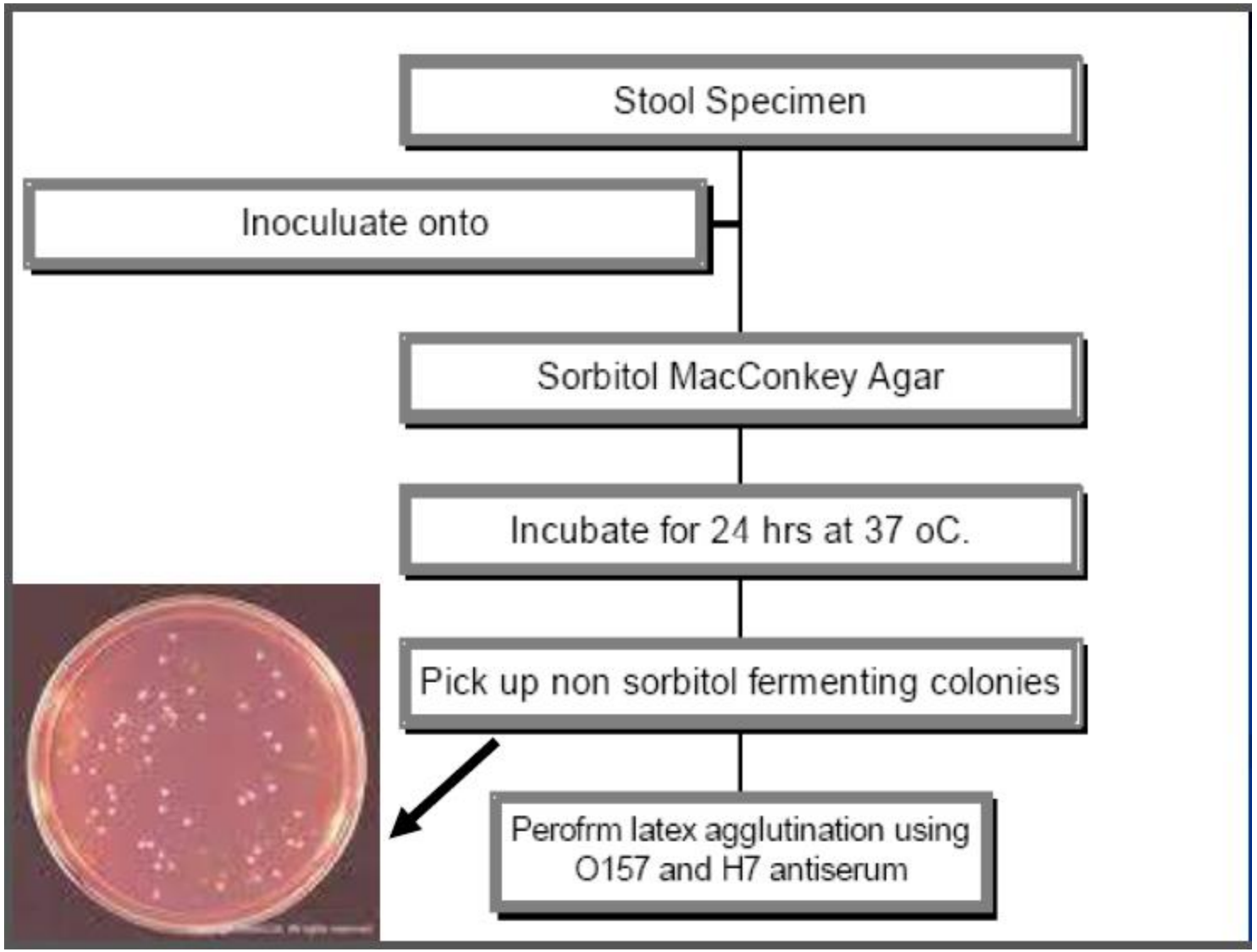


MacConkey agar

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, colorless colonies with or without black centers

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

Lactose fermenter flora: pink to red colonies

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

Diagnosis of *Salmonella Shigella*

2- Hektoen enteric agar (cat eye apperance)

Identification of Enterobacteriaceae

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)

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

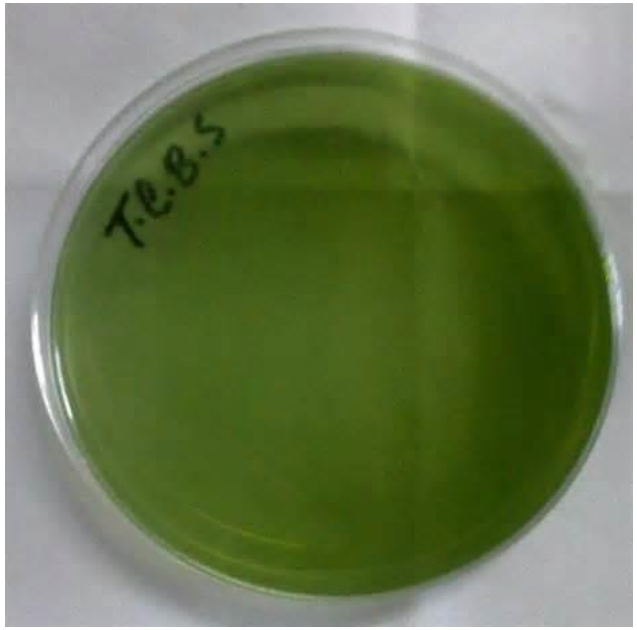
Important components

- ✓ 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

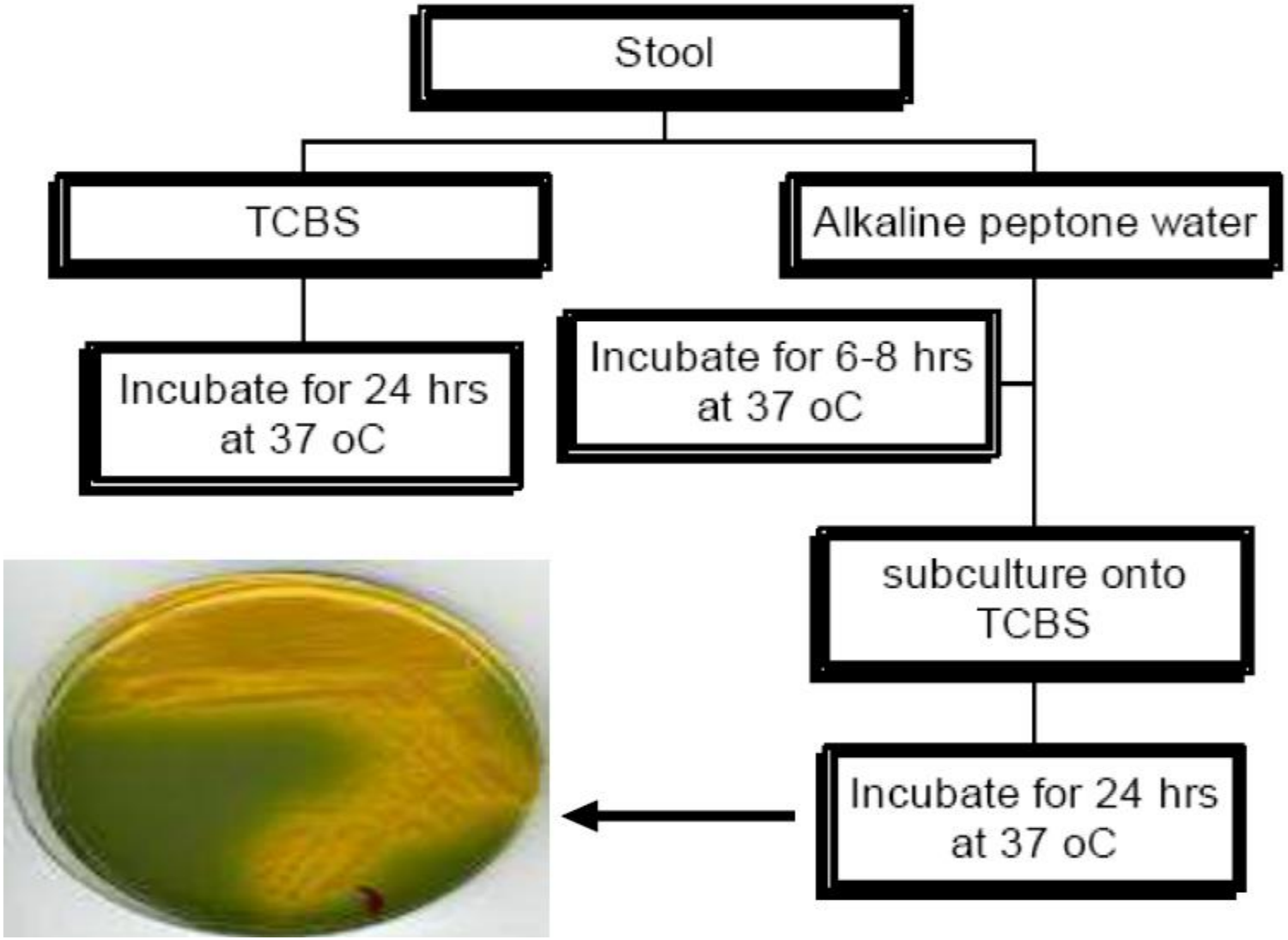


TCBS media

V. cholera

V. parahemolyticus

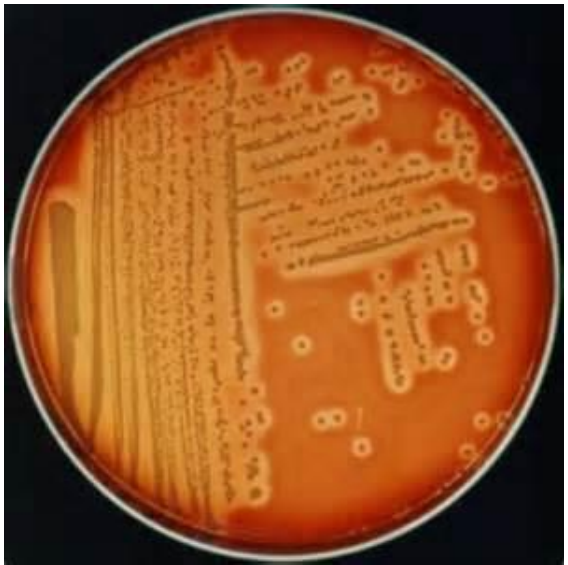
Cholera identification



S. aureus associated food poisoning

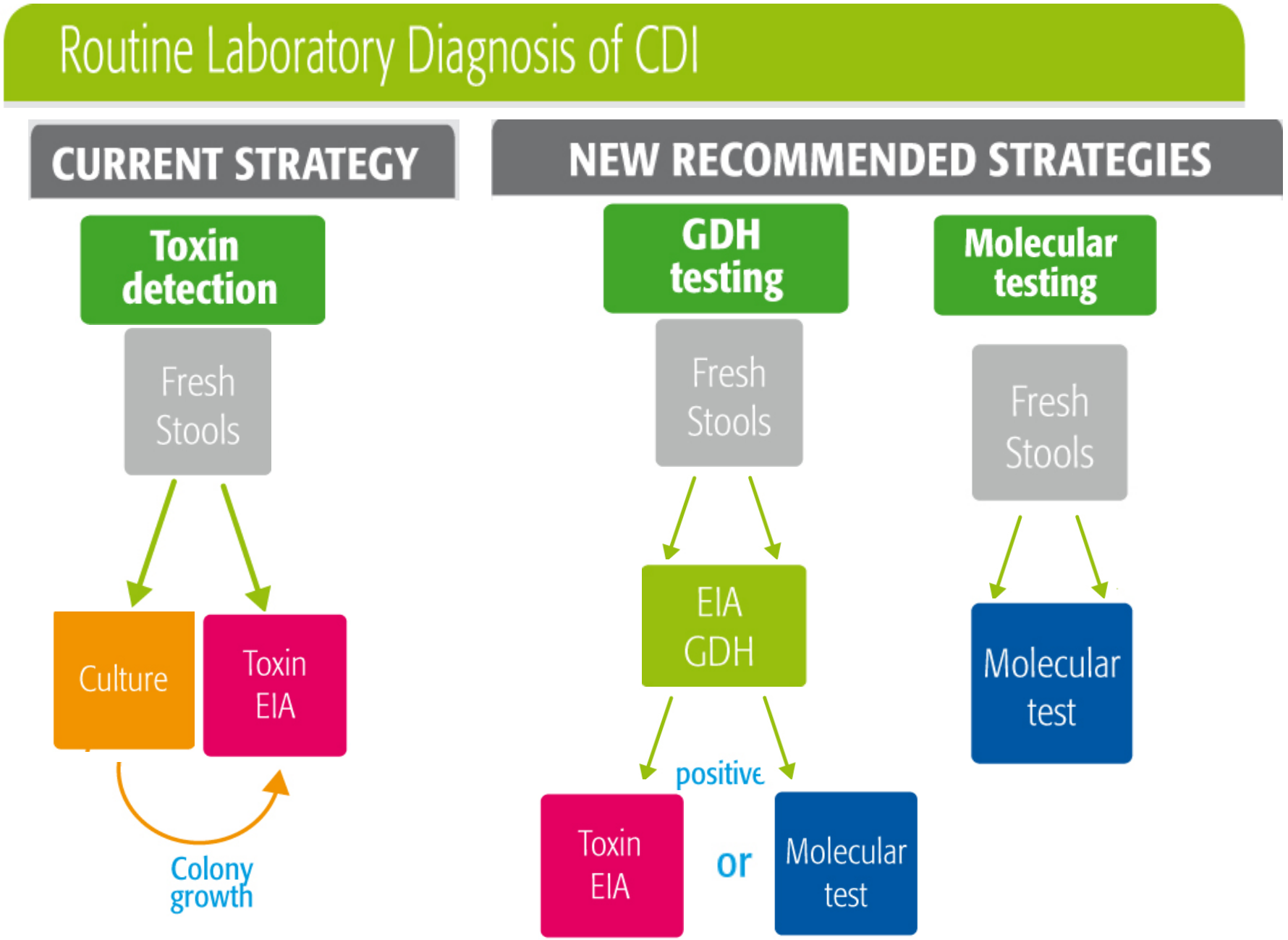
- 25% of healthy people are carriers
- Mainly *S. aureus* food poisoning is diagnosed in case of outbreaks
- Diagnosis based on
  - ✓ gram positive cocci
  - ✓ catalase & coagulase positive
  - ✓ oxidase negative
  - ✓ ß-hemolysis on blood agar
  - ✓ Grow on MSA with mannitol fermentation

Blood agar



MSA

Diagnosis of *C. difficile* infection



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 to low umbonate, yellow colonies with a ground glass-like appearance and a slightly filamentous edge.