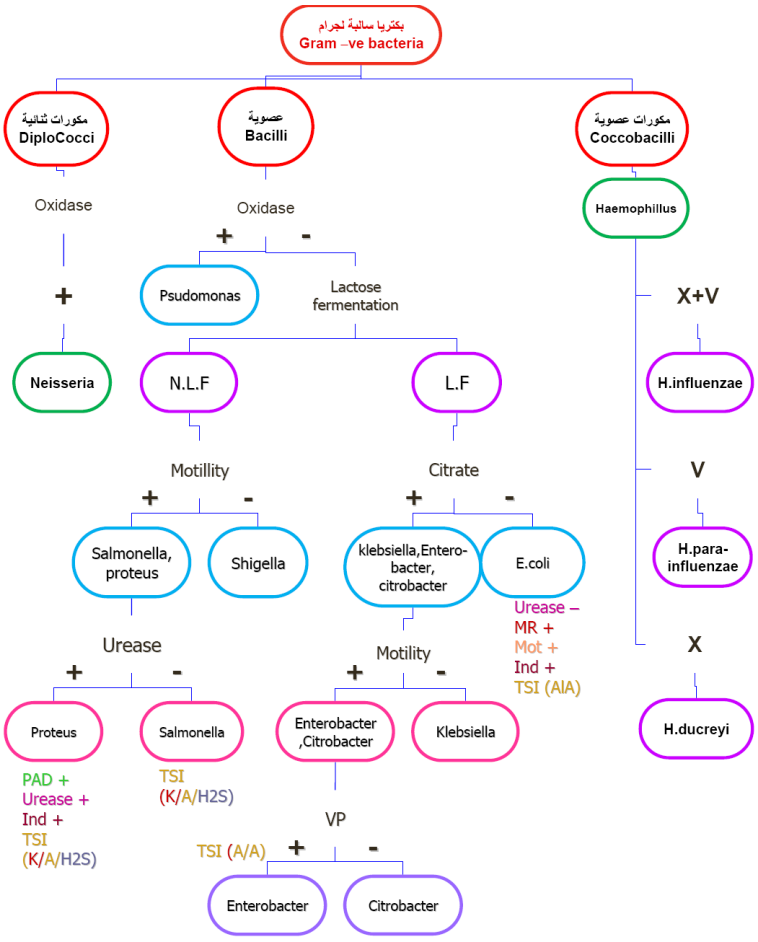
Lab diagnosis of Enterobacteriaceae and Other Gram negative Rods



	Klebsiella	E.coli	Salmonella	Shigella	Proteus
Indole	--	++	--	variable	P. valgaris ++ P. mirabilis --
VP	++	--	--	--	--
MR	--	++	variable	variable	++
Citrate	++	--	Most are ++ Except: S.typhi, S.paratyphi	--	++
Urease	++	--	--	--	++
TSI (Butt)	Yellow	Yellow	Yellow	Yellow	Yellow
TSI (Slant)	Yellow	Yellow	Red	Red	Red
TSI (H2S)	--	--	S.typhi ++ S.paratyphi --	--	++
TSI (Gas)	++	Mostly ++	S.typhi -- S.paratyphi ++	--	++
Motility	--	++	++	--	++
Lysine Decarboxylase	++	++	Typhi ++ Paratyphi --	--	--
ONPG	++	++	--	--	--

1. Oxidase test:

This test is used to differentiate those bacteria that produce the enzyme Oxidase from non-oxidase producer bacteria.

Method of the test:

1. Place a piece of filter paper in a clean Petri- dish.

2. Add 2 or 3 drops of oxidase reagent on the filter paper.

3. Using a wooden stick, smear a colony of the test organism across the reagent on the filter paper.

4. Observe the color change to deep blue-purple within 10 seconds.

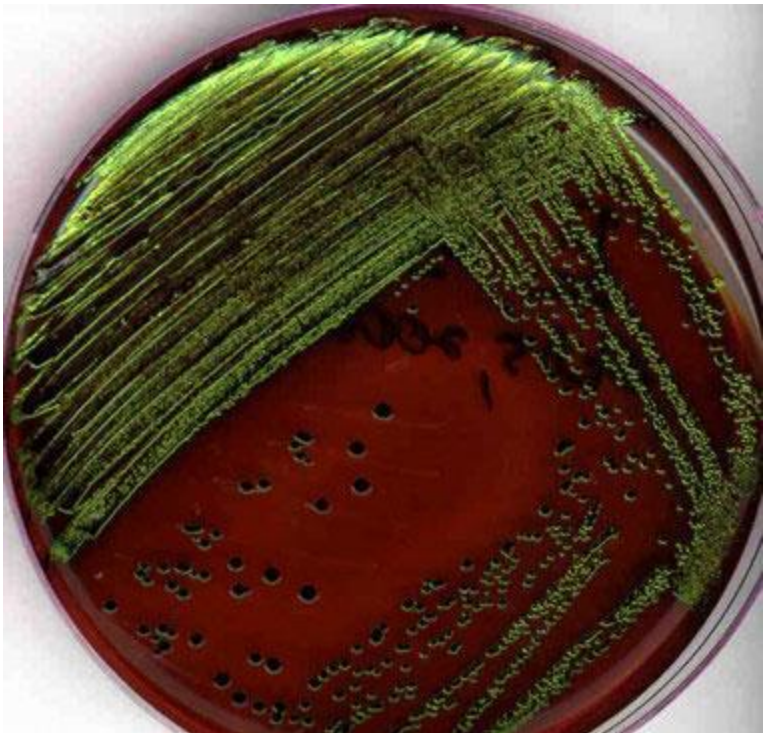
Results:

- ✓ If the filter paper shows deep blue-purple within 10 seconds: **positive (ignore any blue-purple color that develops after 10 seconds).**
- ✓ If no color appears within 10 seconds: Negative.
- ✓ Pseudomonas, Neisseria, Vibrio and Brucella species: Oxidase positive.
- ✓ Escherichia coli and other **Enterobacteriaceae**: Oxidase **negative**.

2. Differential media:

a. Eosin Methylene blue (EMB) agar:

- Contains the dyes eosin and methylene as well as the carbohydrates lactose-and sucrose. The dyes inhibite gram+ve bacteria and act as indicators of those bacteria capable of fermenting lactose (appear as dark-colored), lactose non-fermenter (translucent or colorless colonies).
- EMB agar is particularly valuable in identifying Escherichia coli (green metallic sheen).



E.coli on EMB medium showing a green metallic sheen

b. MacConkey's Agar.

- Distinguishes between lactose fermenters (red or pink) and lactose nonfermenters (translucent or colorless).
- pH indicator, neutral red, which gives a red color under acid conditions.
- The growth of gram-positive bacteria is inhibited on MacConkey's agar because of the presence of bile salts and the dye crystal violet.

c. Deoxycholate Agar. very similar to MacConkey's agar (neutral red and distinguishes between lactose fermenters and lactose nonfermenters).

Selective media: Salmonella-Shigella (SS) agar

- Contains the pH indicator neutral red and the carbohydrate lactose.
- Contain salts (ferric citrate and sodium thiosulfate). The presence of these salts provides the medium with an indicator of hydrogen sulfide production. The colonies of bacteria producing hydrogen sulfide may have blackened centers, which is the result of the precipitation of ferric sulfide.

3.IMViC test: **INDOLE TEST:** Principle:

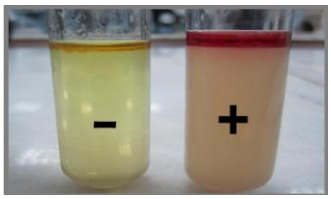
The test organism is cultured in a medium which contains tryptophan. This amino acid (Tryptophan) is broken down and indole is released. Indole production is detected by addition of Kovac's reagent which contains 4 (p)-dimethylaminobenzaldehyde which reacts with the indole to produce colored compound.

Method:

1. Prepare peptone water.
2. Inoculate the test colony to the tube containing peptone water.
3. Incubate overnight in 37 ˚C.
4. After incubation period, add drops of Kovac's reagent to the tube.
5. Shake gently and then examine for a red color (red ring) in the surface layer within 10 minutes.

Results:

- ✓ After adding kavoc's reagent: red ring appears positive result for indole production (e.g. E.coli).
- ✓ If the reagent remain as yellow ring: it is negative and no indole produced.

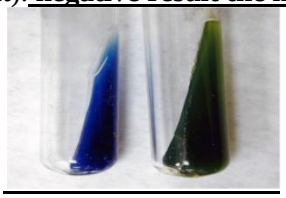


Methyl red-Voges proskauer test:

- **How to Perform Tests**: Inoculate 2 glucose broths with inoculating loop. After 48 hours of incubation, add a few drops of MR to one tube, and VP reagents to the other tube.
- **Properties they test for**: Both tests are used to differentiate species of the family Enterobacteriaceae.
  - MR—tests for acid end products from glucose fermentation.
  - VP—tests for acetoin production from glucose fermentation.
- **Media and Reagents Used**:
  - Glucose Broth
  - Methyl Red indicator for acid
  - Voges Proskauer reagents—A: 5% Alpha-Naphthol, & ethanol, B: Potassium Hydroxide, & Deionized Water.

Citrate test:

- **How to Perform Test:** Inoculate slant with inoculating loop.
- **Property of test:** This test is used to help differentiate species of the family *Enterobacteriaceae.* It is selective for bacteria that has the ability to consume citrate as its sole source of carbon and ammonium as sole nitrogen source.
- **Media and Reagents Used:** Simmon's Citrate Agar contains sodium citrate (carbon source), ammonium ion (nitrogen source), & pH indicator—bromthymol blue.
- **Reading Results:**
  - **positive result is blue** (meaning the bacteria metabolize citrate and produced an acid end product). **negative result the media remains green.**



4. **TSI: triple sugar iron test**

This agar contains three sugars are: lactose (10 parts), sucrose (10 parts), and glucose (1 part). The medium also contains a pH indicator (phenol red) and ferrous sulfate to detect hydrogen sulfide (H2S) production. TSI tube determine the following:

- ✓ can the organism ferment glucose, and if so, can it also ferment lactose and sucrose?
- ✓ Is gas produced as a byproduct of fermentation?
- ✓ Does the organism produce H2S?

TSI tube has a slant portion and butt (deep) portion, the uninoculated medium is red, meaning it is alkaline. The slant portion is aerobic and the butt portion is relatively unaerobic.

Method:

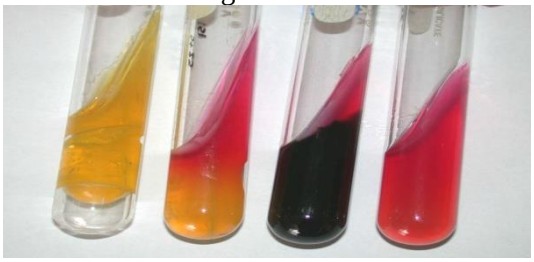
Inoculate the butt portion by stabbing the needle through the center of the medium until reaching the butt.

Inoculate the surface of the slant using zigzag motion.

Inocubate the tube at 35c for 18-24hr.

Result:

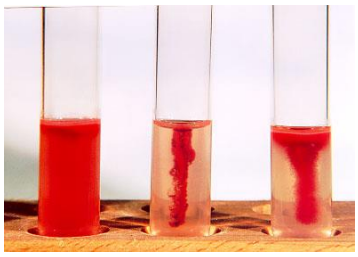
Yellowing the butt indicates glucose fermentation. Yellowing the slant indicate that the organism ferment sucrose and/or lactose. Bubbles and /or cracks in the agar or separation of the agar indicate gas production. Blackening the medium indicate the H2S production.



5. Motility test:

- **How to Perform Test:** Stab motility media with inoculating needle.
- **Property of the test:** This test is done to help differentiate species of bacteria that are motile.
- **Media and Reagents Used:** Motility media contains tryptose, sodium chloride, agar, and a color indicator.

➲ **Reading Results:** If bacteria is motile, there will be growth going out away from the stab line, and test is positive. If bacteria is not motile, there will only be growth along the stab line. A colored indicator can be used to make the results easier to see.



6. **UREASE TEST:**

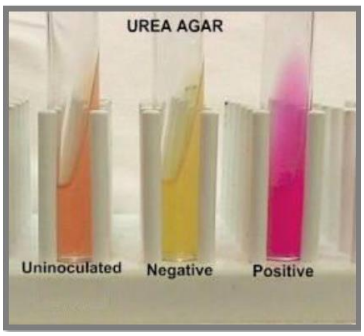
Testing for urease activity is important in differentiating enterobacteria that produce the enzyme urease from non-urease producers.

Principle:

The test organism is cultured in a medium which contains urea and the indicator phenol red. When the strain is urease-producing, the enzyme will break down the urea (by hydrolysis) to give ammonia and carbon dioxide.

Results:

- ✓ Urease positive organisms yield a bright pink or bright red color to the agar (e.g. Proteus species are strong urease producers).
- ✓ Yellow color indicates negative reaction and no urease enzyme in the test organism (e.g. E. coli).



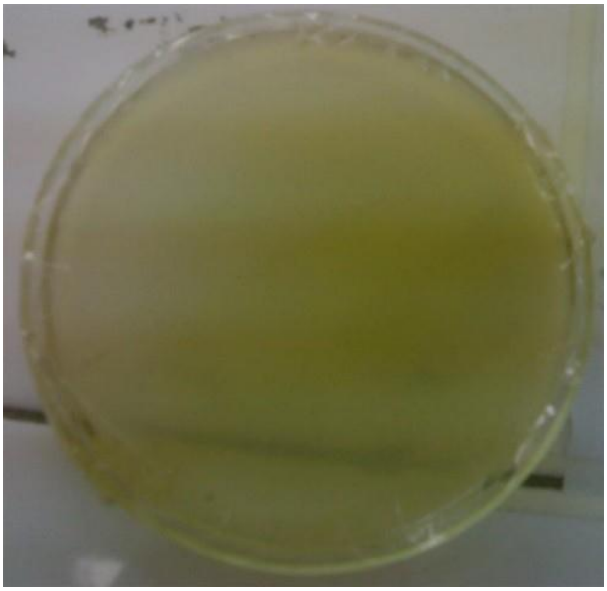
Note:

Swarming of proteus can be demonstrated on either nutrient or blood agar. It is done by performing a spot inoculation of the organism on a N.A petri-dish and incubating the plate at 37 C for 24 hours.

Swarming of Proteus on nutrient

Pseudomonas

Pseudomonas is a gram negative pathogenic bacterium that doesn't belong to the Enterobacteriaceae family. It is oxidase positive and doesn't ferment lactose or glucose.
Culture: Strict aerobe, Produces green pigments on nutrient agar and dark colonies on blood agar due to pigmentation.

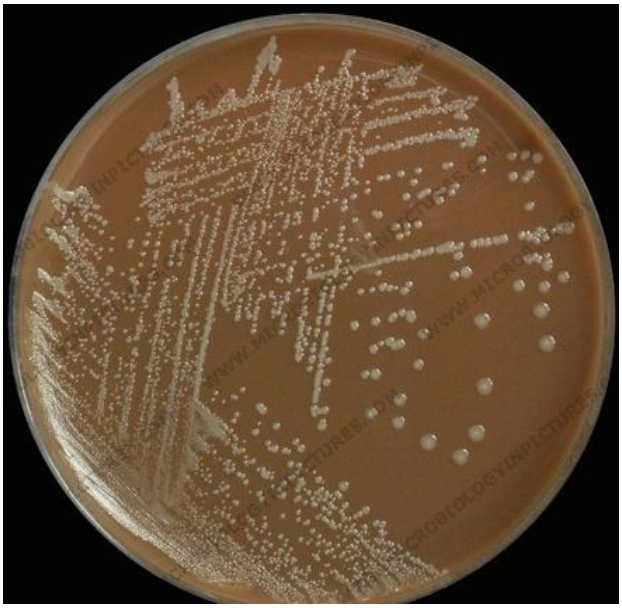


picture showing *Pseudomonas* pigment production on nutrient agar

Hemophilus Influenzae

- Gram reaction: gram negative coccobacilli, pleomorphic in shape
- Culture: fastidious organisms that need enrichment media (chocolate agar- provide (X) and (V) factor)

Colonial Morphology:
Hemophlius Sp. give grey moist colonies on chocolate agar.



Picture showing *H.influenzae* on chocolate agar. The colonies are grey and moist.

Satellitism

Principle:
Satellitism is the growth of one bacteria in culture contains colonies of another bacteria supplies needed micronutrients or growth factors. The Hemophilus

influenzae satellitism test is performed on blood agar and it uses Staphylococcus aureus as a source of NAD (V factor). The X factor (heamin) is obtained from the agar itself since it's naturally one of the constituents of the agar. This test is used to differentiate between *H.influenzae* and *H.parainfluenzae*.

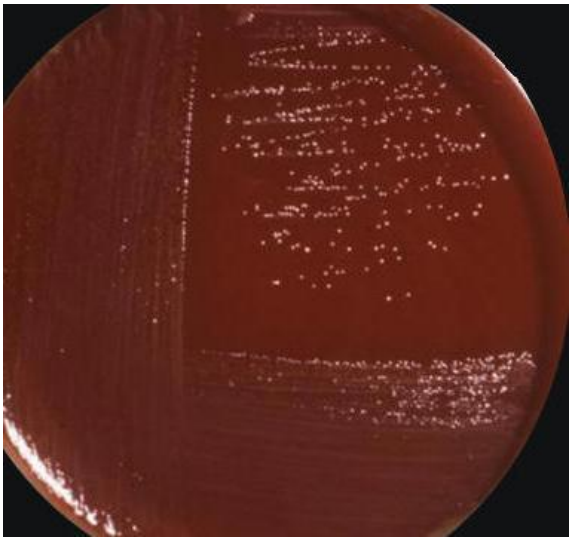
Procedure:
- Using a cotton swab, pick up 2-3 colonies of *H.influenzae* and wipe the swab on the surface of the blood agar plate.
- Make a straight line streak of *S.aureus* across the middle of the plate.
- *Hemophilus influenza* colonies will grow all over the plate, but they will increase in size and number as they get closer to the colonies of *S.aureus*.

H.influenzae satellitism on blood agar.

Campylobacter

Campylobacter bacteria are a common cause of diarrhea and enteritis which makes them clinically significant. They are characterized as being microaerophilic (need CO2 for growth), oxidase positive, catalase positive and thermophilic (require high temp. for growth 42°-43° C).

Gram stain: gram negative curved seagull-shaped bacilli.
Culture medium: Campylobacter is grown on a selective medium that prevents the growth of fecal normal flora. The medium is called Campy agar which is composed of blood agar incorporated with an antibiotic.
Incubation should be at a temperature of 42° – 43° C because the organism is thermophillic.

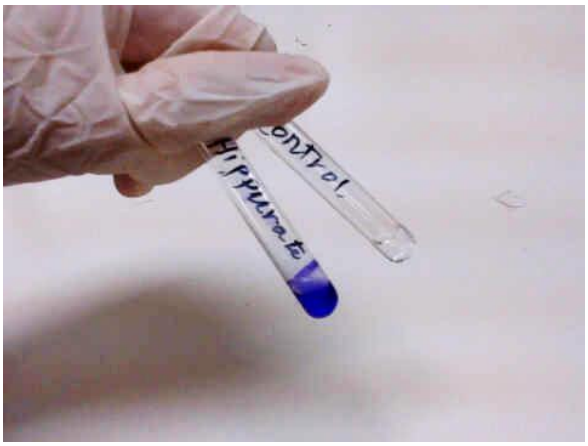


Campylobacter colonies on campy agar. Colonies are flat, droplet like and glistening.

Hippurate Test

The Hippurate test is used to differentiate between two different species of Camplylobacter: Campylobacter jeujeni (hippurate +ve) and Campylobacter coli (hippurate –ve).

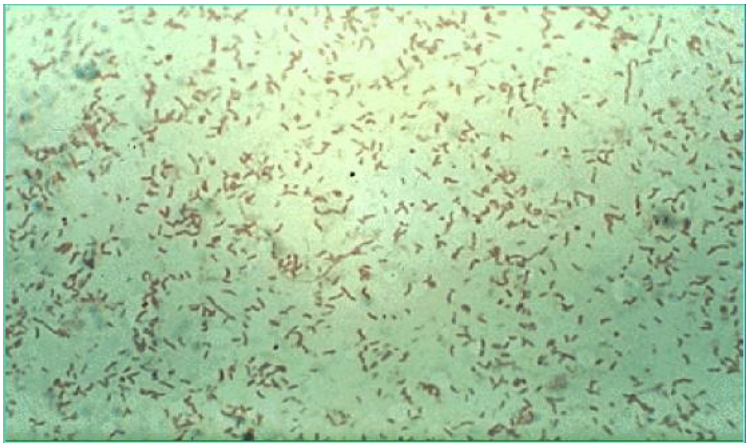
Procedure:
Hippurate broth is inoculated with the organism and then incubated. After incubation ninhydrin is added to check if glycine is present. A purple color means that glycine is present and this indicates that hippurate has been hydrolyzed and that the organism is a hippuricase producer.



Hippurate test. A purple color indicates a positive hippurate result.

Vibrio Species

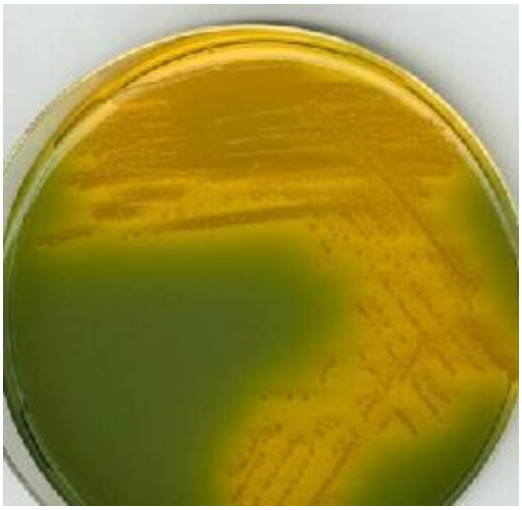
Gram stain: gram negative comma shaped bacilli
Culture: the medium Thiosulphate citrate bile sucrose (T.C.B.S) is used for culturing Vibrio. It is a selective medium which inhibits the growth of normal fecal flora.



gram stain of Vibrio sp. showing gram negative comma-shaped bacilli

Thiosulphate Citrate Bile Sucrose Agar (T.C.B.S)

Vibrio cholera grows at 8.2 pH and ferments sucrose which causes the colonies to turn yellow in color. Vibrio parahemolyticus does not ferment lactose and produces blue-green colonies on T.C.B.S. Both species are non-lactose fermenting and they both give moist colonies.



Vibrio cholera on T.C.B.S medium. The colonies are yellow due to sucrose fermentation.