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# Biochemical test for *Enterobacteriaceae*

## IMVC tests

Each of the letters in "IMVC" stands for one of these tests. "I" is for indole; "M" is for methyl red; "V" is for Voges-Proskauer, and "C" is for citrate. IMVC is an acronym that stands for **four different tests.**

### 1- Indole test

Principle

Media used: tryptophan water broth

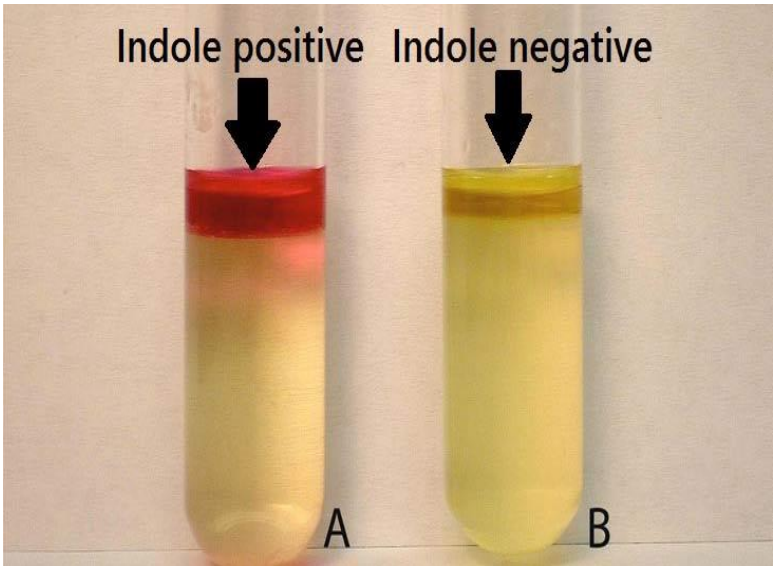
Certain microorganisms can metabolize tryptophan by tryptophanase. The enzymatic degradation leads to the formation of pyruvic acid, indole and ammonia. The presence of indole is detected by addition of Kovac's reagent.

**Method:**

- Inoculate tryptone water with the tested microorganism
- Incubate at 37°C for 24 hours
- After incubation interval, add 1 ml Kovacs reagent, shake the tube gently and read immediately

**Result:**

- A red ring color in the top layer indicates the presence of indole (indole positive)
- The absence of color means that indole was not produced i.e. indole is negative




## 2- Methyl red- vogues Proskauer

These tests both use the same broth for bacterial growth. The broth is called MR-VP broth or glucose broth. After growth, the broth is separated into two different tubes, one for the methyl red (MR) test and one for the Voges-Proskauer (VP) test.

- The methyl red test detects production of **acids** formed during metabolism of glucose. **Reagent is methyl red**

Result: red color appears at pH's lower than 4.2, indicating a positive test

The solution remaining yellow (pH = 6.2 or above) indicates a negative test

- The VP test detects production of **(acetoin)** formed during metabolism of glucose. **Reagent** is alpha-naphthol and 40% potassium hydroxide **(Barrett's reagent)**

**Result:** after 5-10 minutes. A pinkish-red color indicates a positive test

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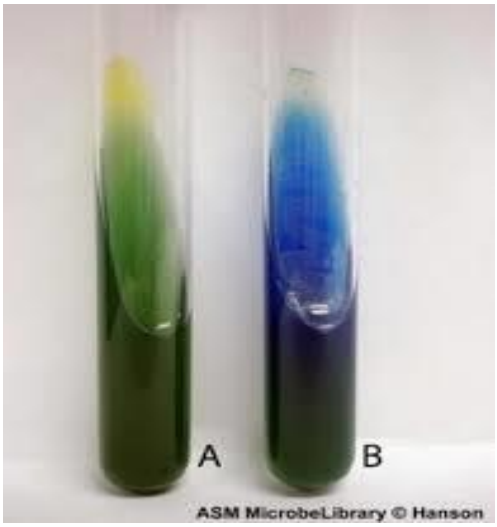
### 3- Citrate test

This test uses Simmon's citrate agar to determine the ability of a microorganism to use citrate as its sole carbon source. The agar contains citrate and ammonium ions (nitrogen source) and **bromothymol blue** as an indicator. The citrate agar is green before inoculation, and turns blue as a positive test indicator, meaning citrate is utilized.




| Species | Indole | Methyl Red | Voges-Proskauer | Citrate |
|---|---|---|---|---|
| *Escherichia coli* | Positive | Positive | Negative | Negative |
| *Klebsiella spp.* | Negative | Negative | Positive | Positive |
| *Proteus vulgaris*[3] | Positive | Positive | Negative | Negative |
| *Salmonella spp.* | Negative | Positive | Negative | Positive |

| | | | | |
|---|---|---|---|---|
| *Proteus mirabilis* | Negative | Positive | Negative | Positive |
| *Citrobacter freundii* | Negative | Positive | Negative | Positive |
| *Enterobacter aerogenes* | Negative | Negative | Positive | Positive |

- **Kliger's Iron Agar (KIA)**

This is a differential medium.

**Uses**: 1- It tests for organisms' abilities to ferment glucose and lactose to acid and acid plus gas end products. 2- It also allows for identification of sulfur reducers (H2S production).

Organisms capable of fermenting glucose will use it up within the first few hours of incubation. Glucose fermentation will create acidic byproducts that will turn the **phenol red** indicator in the media **yellow**. At this point, when the glucose has been all used up, the organism must choose another food source. If the organism can ferment lactose, this is the sugar it will choose. Lactose fermentation will continue to produce acidic byproducts and the media will remain yellow . If gas is produced as a result of glucose or lactose fermentation, then fissures will appear in the agar or the agar will be lifted off the bottom of the tube.

If an organism cannot use lactose as a food source it will be forced to use the amino acids / proteins in the media. The deamination of the amino acids creates $NH_3$, a weak base, which causes the medium to become alkaline. The alkaline pH

causes the phenol red indicator to begin to turn red. Since the incubation time is short (18-24 h), only the slant has a chance to turn red and not the entire tube. Thus, an organism that can ferment glucose but not lactose, will produce a red slant and a yellow butt in a KIA.

If an organism is capable of using neither glucose nor lactose, the organism will use solely amino acids / proteins. The slant of the tube will be red and the color of the butt will remain unchanged. *Pseudomonas aeruginosa* is an example of a nonfermented.

KIA tubes are also capable of detecting the production of $H_2S$. It is seen as a black precipitate. Sometimes the black precipitate obscures the butt of the tube. In such cases, the organisms should be considered positive for glucose fermentation (yellow butt).

Figure 5-68

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# - Urease test

Medium used for urease test: **urea medium, agar or broth**

**Indicator used in urease test:** Phenol red

**Color change:**

- Original: orange yellow color
- Final color (in positive test): Bright pink

**Urease test principle**

Urea hydrolyzed with the release of ammonia and carbon dioxide.

Many organisms especially those that infect the urinary tract, have a urease enzyme which is able to split urea in the presence of water to release ammonia and carbon dioxide. The ammonia combines with carbon dioxide and water to form ammonium carbonate which turns the medium alkaline, turning the indicator phenol red from its original orange yellow color to bright pink.

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## - Oxidase test

The oxidase test is used to identify bacteria that produce cytochrome c oxidase, an enzyme of the bacterial electron transport chain. When present, the **cytochrome c oxidase** oxidizes the reagent **(tetramethyl-p-phenylenediamine)** to **(indophenols)** purple color end product. When the enzyme is not present, the reagent remains reduced and is colorless. **Enterobacteriaceae** family are characterized as **oxidase negative.**