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College of Sciences
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Bacterial Taxonomy

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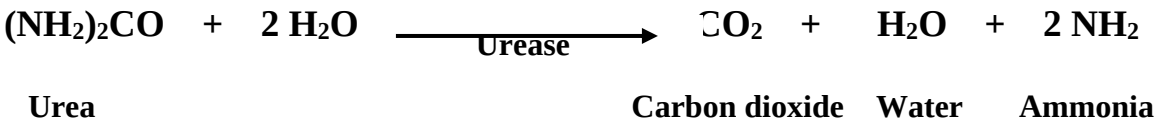
## Lab. 4: Biochemical tests

## 4. Urease test

Many organisms especially those that infect the urinary tract, have an 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 from ammonium carbonate which turns the medium alkaline, turning the indicator phenol red from its original orange yellow color to bright pink.




**► Procedure for urease test**

1. The broth medium is inoculated with a loopful of a pure culture of the test organism; the surface of the agar slant is streaked with the test organism.

2. Incubate the test tube at 35 °C for 18 to 24 hours.

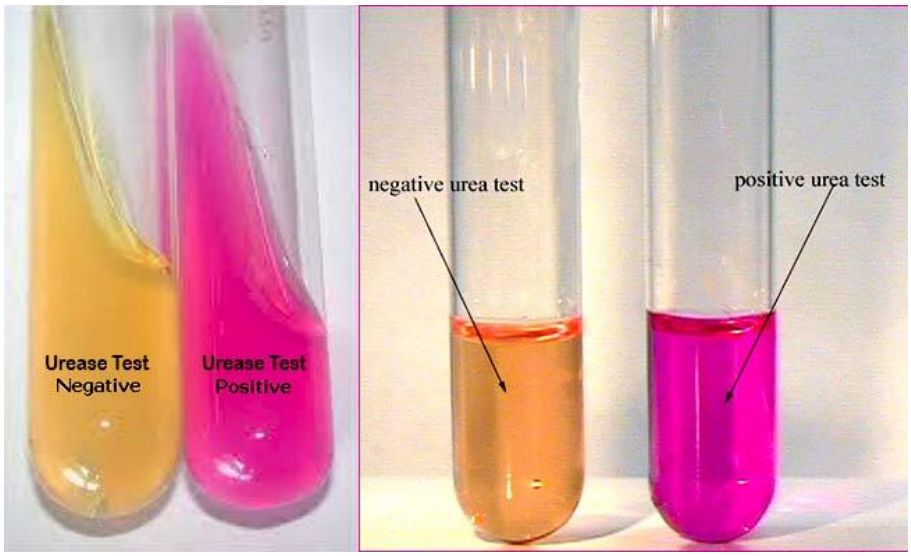
**► Results**

**Positive:** If organism produces urease enzyme, the color of the slant changes from light orange to pink.

**Examples:** *Proteus* spp., *Cryptococcus* spp., *Helicobacter* pylori, *Yersinia* spp., *Brucella* spp.

**Negative:** If organism do not produce urease the agar slant and butt remain light orange (medium retains original color).

**Examples:** *Escherichia coli*.




**Urease Test Results**

## 5. Triple Sugar Iron Agar (TSI) and $H_2S$ production test

Whenever you see the name of this test i.e. Triple Sugar Iron Agar, you have to remember that it's a test which has **three sugar (Lactose, Sucrose and Glucose)** and also iron; and it contains Agar Agar as solidifying agent (TSI is a semi solid media having slant and butt).

**► Composition of Triple Sugar Iron Agar (TSI)**

● **0.1% Glucose:** if only glucose is fermented, only enough acid is produced to turn the butt yellow. The slant will remain red.

● **1.0% lactose /1.0% sucrose:** a large amount of acid turns both butt and slant yellow, thus indicating the ability of the culture to ferment either lactose or sucrose.

● **Iron:** (Ferrous sulfate), indicator of **$H_2S$** formation

● **Phenol red:** indicator of acidification (it is **yellow in acidic condition and red under alkaline conditions**).

● It also contains **Peptone** which acts as source of nitrogen. (Remember that whenever peptone is utilized under aerobic condition ammonia is produced).

► **Procedure**

1. With a sterilized straight inoculation needle touch the top of a well-isolated colony.

2. Inoculate TSI Agar by **first stabbing** through the center of the medium to the bottom of the tube and then **streaking on the surface** of the agar slant.

3. Leave the cap on loosely and incubate the tube at 35 °C in ambient air for 18 to 24 hours.

► **Results**

1. If **lactose (or sucrose) is fermented**, a large amount of acid is produced, which turns the phenol red indicator yellow both the butt and the slant. Some organisms generate **gases**, which produces **bubbles/cracks** on the medium.

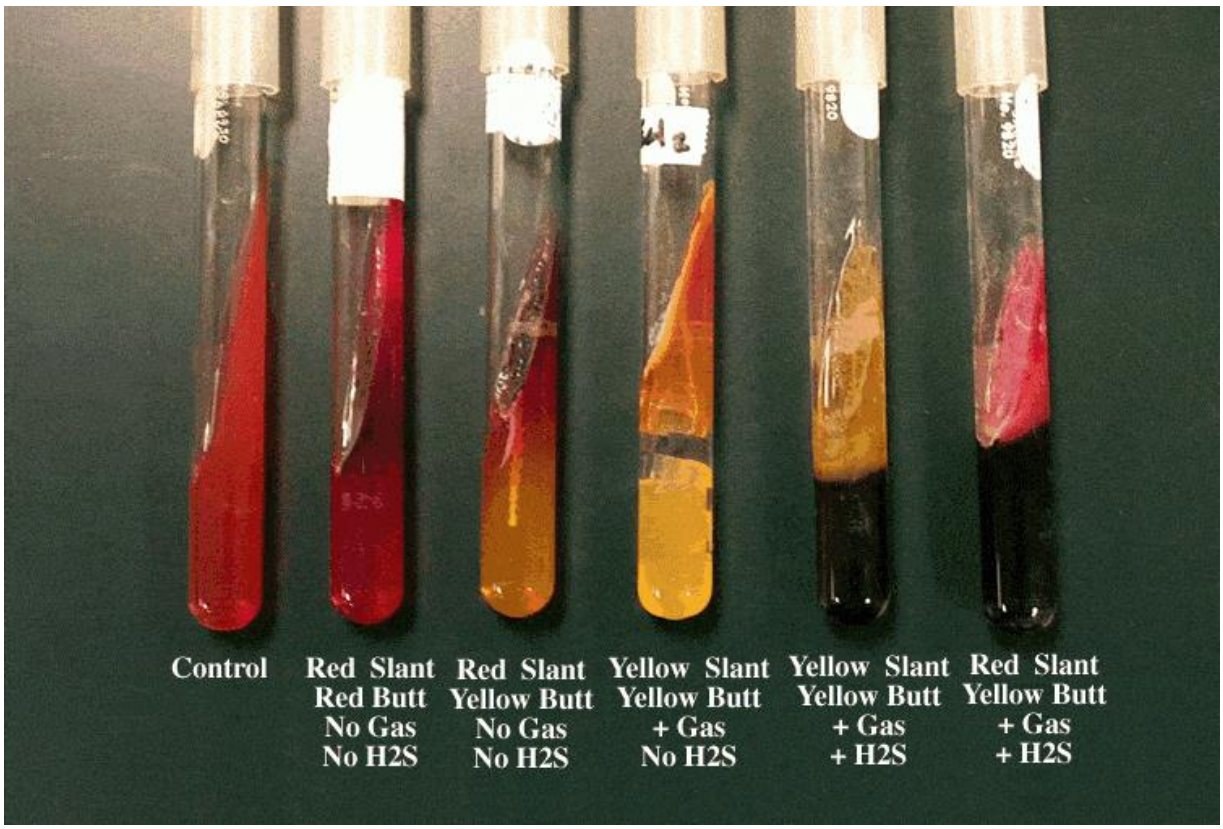
2. If **lactose is not fermented but the small amount of glucose is**, the oxygen deficient **butt will be yellow** (remember that butt comparatively have more glucose compared to slant i.e. more media more glucose), but on the slant the acid (less acid as media in slant is very less) will be oxidized to carbon dioxide and water by the organism and the **slant will be red** (alkaline or neutral pH.

3. If **neither lactose/sucrose nor glucose is fermented, both the butt and the slant will be red**. The slant can because a deeper red-purple (more alkaline) as a result of production of ammonia from the oxidative deamination of amino acids (remember peptone is a major constituents of TSI Agar).

4. If **$H_2S$ is produced**, the black color of ferrous sulfide is seen.

| **Results(slant/butt)** | **Symbol** | **Interpretation** |
|---|---|---|
| Red/yellow | K/A | Glucose fermentation only; Peptone catabolized |
| Yellow/yellow | A/A | Glucose and lactose and/or sucrose fermentation |
| Red/red | K/K | No fermentation; Peptone catabolized |
| Red/no color change | K/NC | No fermentation; Peptone used aerobically |

| | | |
|---|---|---|
| Yellow/yellow with bubbles | A/A,G | Glucose and lactose and/or sucrose fermentation; Gas produced |
| Red/yellow with bubbles | K/A,G | Glucose fermentation only; Gas produced |
| Red/yellow with bubbles and black precipitates | K/A,G,$H_2S$ | Glucose fermentation only; Gas produced; $H_2S$ produced |
| Red/yellow with black precipitate | K/A, $H_2S$ | Glucose fermentation only; $H_2S$ produced |
| Yellow/yellow with black precipitate | A/A,$H_2S$ | Glucose and lactose and/or sucrose fermentation; $H_2S$ produced |
| No change/no change | NC/NC | No fermentation |
| **A= acid production; K= alkaline reaction; G= gas production; $H_2S$= reduction** | | |




**Triple Sugar Iron Test Results**

**1. Alkaline slant/no change in butt (K/NC) i.e. Red/Red = glucose, lactose and sucrose non-fermenter.**

**2. Alkaline slant/Alkaline butt (K/K) i.e. Red/Red = glucose, lactose and sucrose non-fermenter.**

**3. Alkaline slant/acidic butt (K/A); Red/Yellow = glucose fermentation only, gas (+ or -), $H_2S$ (+ or -).**

**4. Acidic slant /acidic butt (A/A); Yellow/Yellow = glucose, lactose and/ or sucrose fermenter gas (+ or -) H2S (+ or -).**

**► Some examples of Triple Sugar Iron (TSI) Agar Reactions:**

| **Name of the organisms** | **Slant** | **butt** | **Gas** | **$H_2S$** |
|---|---|---|---|---|
| *Escherichia, Klebsiella, Enterobacter* | Acid (A) | Acid (A) | Pos (+) | Neg (-) |
| *Shigella, Serratia* | Alkaline (K) | Acid (A) | Neg (-) | Neg (-) |
| *Salmonella, Proteus* | Alkaline (K) | Acid (A) | Pos (+) | Pos (+) |
| *Pseudomonas* | Alkaline (K) | Alkaline (k) | Neg (-) | Neg (-) |