

Tests for enzymes

1. Catalase test
2. Oxidase test
3. Urease test
4. Coagulase test

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Catalase test

Enzymes that decompose hydrogen peroxide into water and oxygen.



Hydrogen peroxide forms as one of the oxidative end products of aerobic carbohydrate metabolism. If this is allowed to accumulate in the bacterial cells it becomes lethal to the bacteria. Catalase thus helps in converting H_2O_2 to H_2O and O_2 .

Reagent

3% hydrogen peroxide stored in dark brown bottle under refrigeration,
18 to 24 hrs. culture of the organism to be tested.

Examples: Positive: *Staphylococcus aureus*; Negative : *Streptococcus* spp.

Methods

Direct plate method

Slide method

Tube method



Oxidase test

Principle

Determine the presence of bacterial cytochrome enzyme oxidase. Cytochromes in aerobic respiration transfer electrons (H) to oxygen to form water.

The reagent used is a dye p-phenylenediamide dihydrochloride (PPDD) acts as an artificial electron acceptor substituting the oxygen.

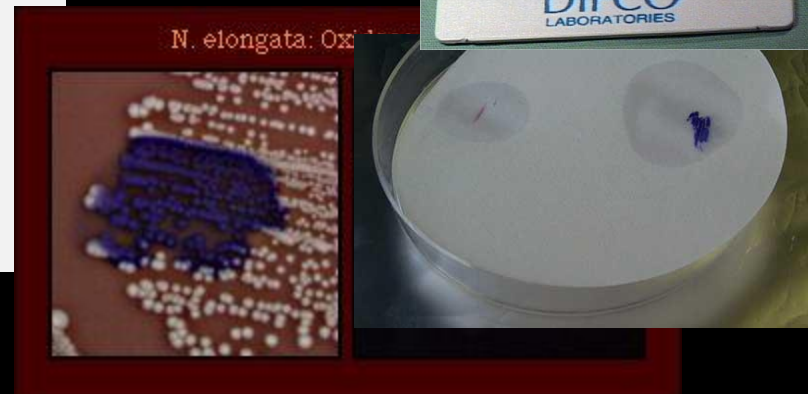
in the presence of enzyme cytochrome oxidase dye is oxidized to indophenol blue which is a dark purple colored end products.

Methods

1. Dry slide method
2. . Moist filter paper method
3. Direct plate method

Positive: *Pseudomonas spp*

Negative : Enterobacteriaceae ; *E.coli*



Urease test

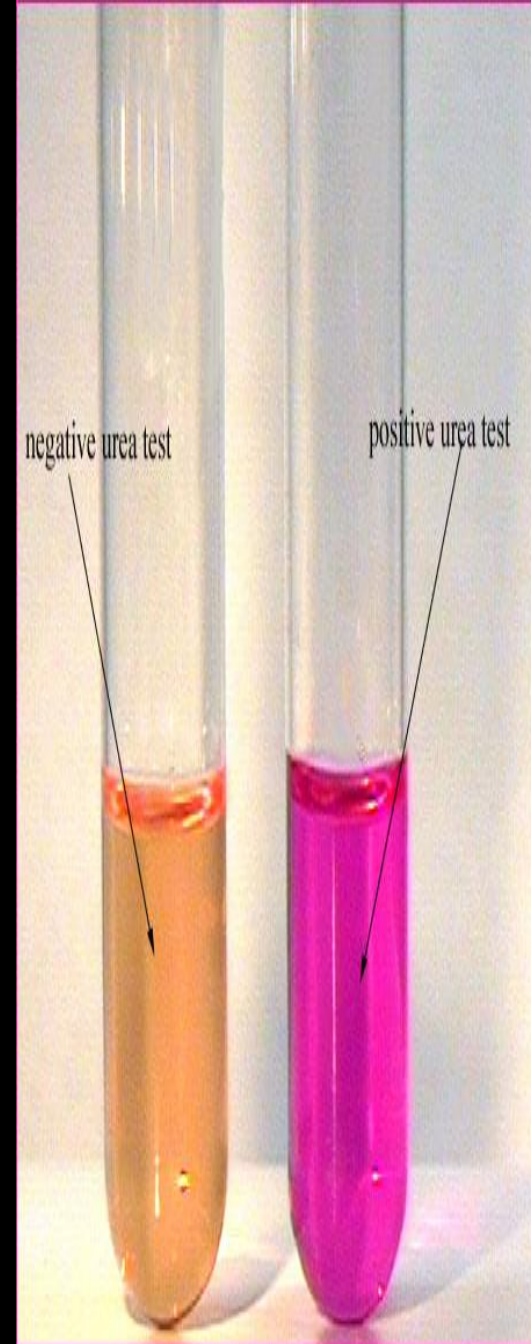
Principle

To determine the ability of the organism to split urea forming 2 molecules of ammonia by the action of the enzyme urease with resulting alkalinity. **pH indicator is phenol red which become red in alkalinity**

urease is an enzyme that hydrolyzes urea to carbon dioxide and ammonia.



Urease test media contain 2% urea and phenol red as a pH indicator. An increase in pH due to the production of ammonia results in a color change from yellow (pH 6.8) to bright pink (pH 8.2).



Procedure

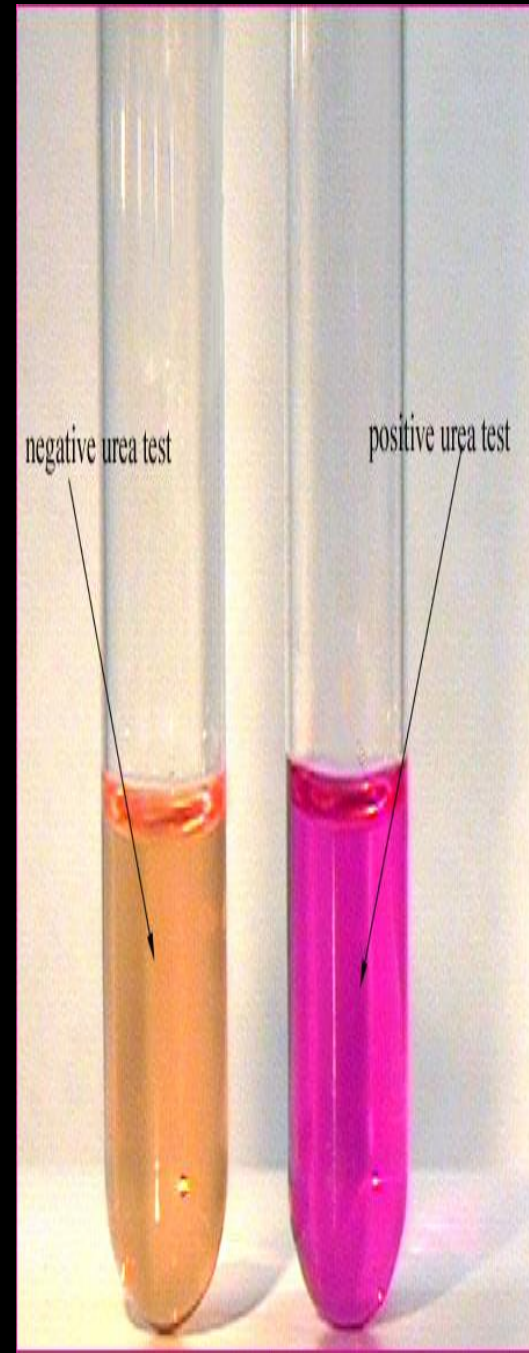
The surface of the agar slant is streaking with the test organism and incubated at 35°C for 18-24hrs.

Rapid hydrolysers gives positive results in 1-2 hrs. less active spp require 3 or more days.

Positive reaction: (red or pink) *Proteus* spp and *Klebsiella* spp

Negative reaction: (yellow or light pink)

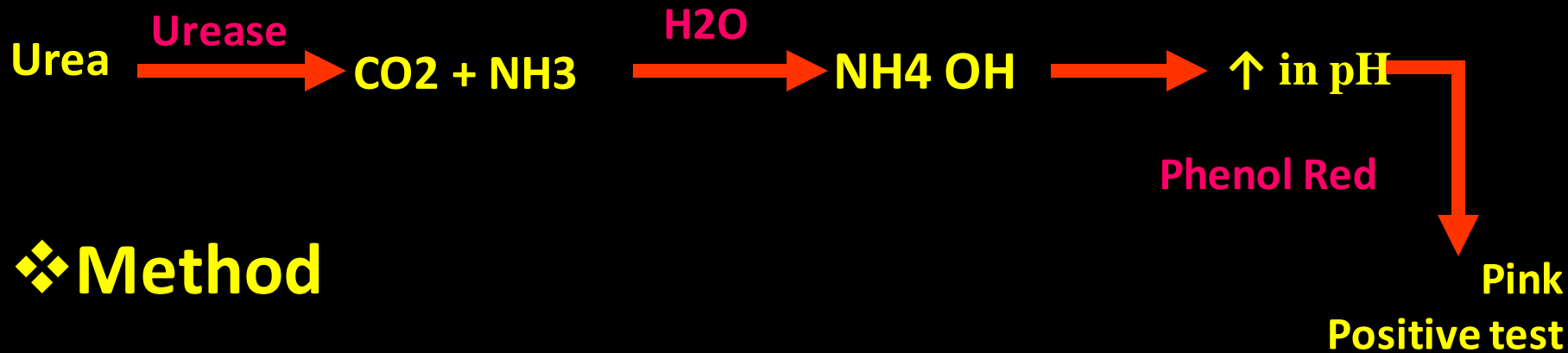
E.coli



Urease Test

❖ Principle

- Urea agar contains urea and phenol red
- Urease is an enzyme that catalyzes the conversion of urea to CO₂ and NH₃
- Ammonia combines with water to produce ammonium hydroxide, a strong base which ↑ pH of the medium.
- ↑ in the pH causes phenol red to turn a deep pink. This is indicative of a positive reaction for urease



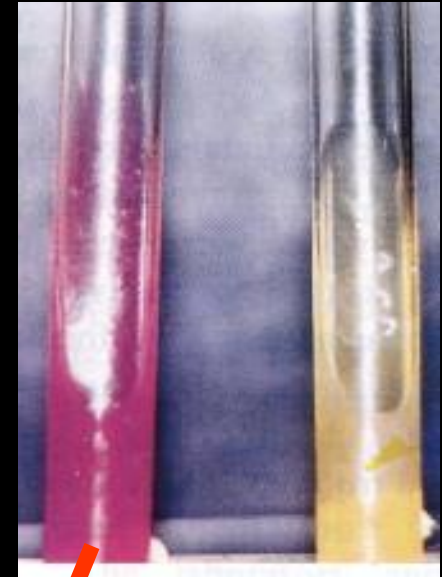
❖ Method

- Streak a urea agar tube with the organism
- incubate at 37°C for 24 h

Urease Test

❖ Result

- If color of medium turns from yellow to pink indicates positive test.
- *Proteus* give positive reaction after 4 h while *Kelebsiella* and *Enterobacter* gave positive results after 24 h



Positive test

Negative test

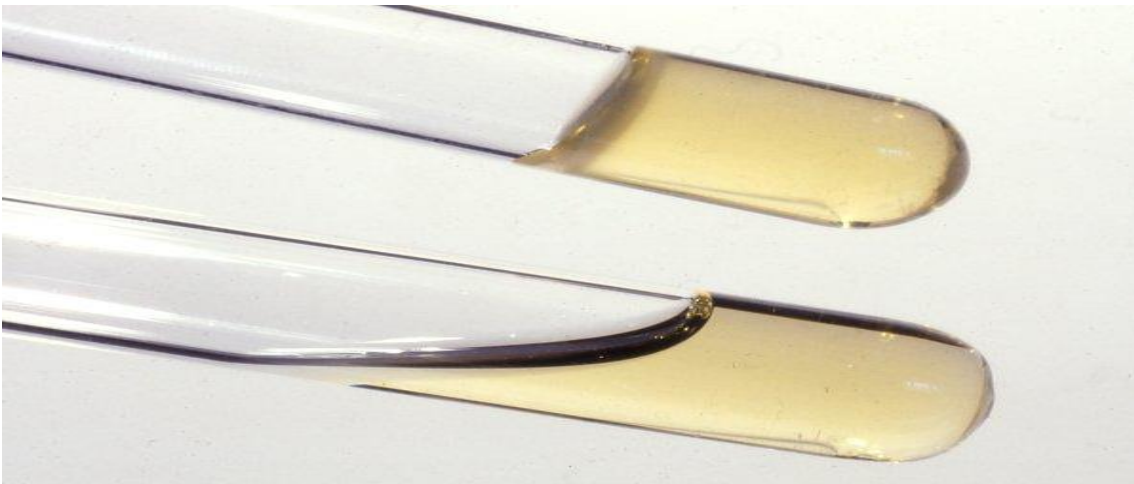
Coagulase test

Some bacteria produce coagulase, which is an enzyme that converts fibrinogen to fibrin, which means that it can coagulate plasma. The ability to produce coagulase is assumed to be associated to the virulence of staphylococci. The test is used to distinguish between coagulase positive and coagulase negative staphylococci.

method

- Suspend one colony from the suspected pure culture in 0.5 ml of plasma from horse, rabbit or man.
- Incubate at 37°C.
- Read the test after 4 h. If the result is negative (see below), continue with the incubation.
Perform the final read after 24 h

- **Positive reaction** eb ton tsum tl .elbats si etalugaoc eht dna setalugaoc amsalp eht fi .gnirrits nopu devlossid
- **Negative reaction** devlossid si etalugaoc eht fi ro etalugaoc ton seod amsalp eht fi .gnirrits nopu niaga



Coagulase test of *Staphylococcus.pps*

The upper tube shows positive result (the plasma has coagulated) and the lower tube shows a negative result .