Catalase Test

I. PRINCIPLE

A. Reaction Principle

Catalase, an enzyme within the cytochrome enzyme system, is responsible for the decomposition of hydrogen peroxide (H_2O_2) formed during aerobic respiration. All organisms using the cytochrome system of respiration will give a positive catalase reaction when tested. Those organisms using a different system will not produce catalase and will yield a negative reaction.

The mechanism of action is as follows:

$3H_2O_2$ + catalase = $3H_2O$ + $2O_2$

B. Clinical Principle

The possession of the catalase enzyme helps to distinguish staphylococci from streptococci and is useful in the identification of other bacteria.

II. REAGENTS

A. Reagents and Supplies

1. 3% Hydrogen peroxide solution
   a. Store H_2O_2 at 4$\pm$ 2°C.
   b. Do not expose H_2O_2 to light, heat or dust.
   c. Mark the date opened on the bottle and record a 6 month outdate, from the date opened. Do not exceed manufacturer's outdate.
2. Glass slide
3. Wooden applicator stick or Bio-loop (do not use nickel loops)

III. INTERFERING SUBSTANCES

1. When picking up a bacterial colony, be careful not to pick up any blood agar. The red cells contain catalase and will give a false positive reaction.
2. When performing a catalase test on an isolate from a blood culture, do not select the colony from the first quadrant. The presence of blood will produce a false result.
3. Do not use nickel loops that will give a false positive reaction.
4. Do not use H_2O_2 that is past the expiration date.

IV. QUALITY CONTROL

A. Quality control is performed each new batch, lot number, and shipment.

B. Staph sp. is used as the positive control and a Strep sp. is used as the negative control.

C. Expected results:

1. Staph spp.: bubbling reaction

2. Strep sp.: absence of bubbling reaction

D. Controls must display expected results. If controls do not display expected results, quality control must be repeated.

E. Record results in the computer.

V. PROCEDURE

A. Place one drop of H_2O_2 on a glass slide.

B. Pick up the bacterial colony to be tested with a wooden applicator stick or a Bio-loop.

C. Suspend the colony in the drop of H_2O_2.

D. Observe the reaction over a dark background.

VI. INTERPRETATION

A. A positive test is a bubbling reaction caused by the release of O_2 from the H_2O_2 in the presence of catalase.

B. A negative test is the absence of any bubbling reaction.

VII. NOTES

A. Do not use expired reagent.

B. Care should be taken when working with a H_2O_2 solution. Do not splash. It may cause irritation to the skin or eyes. Rinse affected areas with water upon exposure.

VIII. REFERENCES

A. Burrows, L., “Textbook of Microbiology”. Philadelphia, W.B. Saunders Company, 127-128, 1963.

B. Blazevic and Ederer, Principles of Biochemical Tests in Diagnostic Microbiology, 13-14, 1975.

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