CATALASE REAGENT

INTENDED USE

Catalase Reagent is used to detect the presence of the enzymes, catalase and peroxidase, produced by some bacteria. Catalase Reagents are useful in the presumptive identification and differentiation of many bacteria. Beta-hemolytic organisms, such as *Streptococcus* species (catalase-negative), *Staphylococcus* species (catalase-positive), and *Listeria* species (catalase-positive) can be differentiated by their catalase reaction using 3% hydrogen peroxide. For catalase testing of anaerobic bacteria, 15% hydrogen peroxide appears to be more sensitive than 3% hydrogen peroxide.

SUMMARY AND EXPLANATION

Most cytochrome containing organisms produce a catalase enzyme which breaks down hydrogen peroxide into oxygen and water. When a small amount of a catalase producing organism is introduced into hydrogen peroxide, bubbles of oxygen form as a result of the enzyme's activity.

PRINCIPLE

In the respiratory chain of all bacteria, reduced flavoproteins and iron-sulfur proteins unite with oxygen and oxidases to form two compounds, hydrogen peroxide and the superoxide radical. These compounds, if allowed to accumulate, are toxic to bacteria and results in their death. Bacterial survival is accomplished by the production of specific enzymes that allow bacteria to neutralize the toxic compound.

Hydrogen peroxide is decomposed by the action of two enzymes: catalase and either a peroxidase, NADH, NADPH, cytochrome c, or glutathione. To observe the action of these enzymes, catalase reagent, a dilute solution of hydrogen peroxide, is added to a pure bacterial culture. Any immediate bubbling is indicative of a positive result since oxygen is a byproduct of hydrogen peroxide decomposition.

REAGENTS (FORMULA)

Hydrogen Peroxide ..... 3.0 %

PROCEDURE

A. Slide Method

1. With a sterile inoculating loop or wooden applicator stick, pick the center a colony derived from an overnight culture plate and place it on a clean, glass slide.

2. Place a drop of catalase reagent onto the smear.

3. Observe for immediate bubbling. It may be necessary to use a hand lens to detect weakly positive reactions.

B. Tube Method

1. Add 1.0-mL of Catalase Reagent directly to an overnight, heavily inoculated pure agar slant culture. (Do not use a blood agar)

2. Observe for immediate bubbling.

EXPECTED RESULTS

Observe for the immediate evolution of gas bubbles indicating a positive test. Formation of rare bubbles after 20 to 30 seconds is considered a negative catalase test.

Positive: Immediate bubbling (Oxygen is released)

Negative: No bubbling

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification.

Aerobic organisms must be taken from an 18 to 24 hour old culture. Organisms lose their catalase activity with age. For slower growing anaerobic organisms, an older (24-72 hours) culture may be acceptable. Anaerobic cultures should be exposed to ambient air for a minimum of 30 minutes before testing.

It is recommended that colonies to be tested with the Catalase Test be taken from non-blood containing media due to the endogenous catalase activity present in animal red blood cells. For genera that require blood-containing media for growth, use isolates from a nonselective blood agar and avoid touching the agar with the loop.

Do not introduce a metallic loop into the drop of Catalase Reagent, because this often causes a false-positive reaction.

Bacteria grown on media with low levels or no glucose may yield conflicting results from pseudocatalase, a non-iron enzyme. The pseudocatalase reaction can be prevented by using media with 1% glucose.

QUALITY CONTROL

All lot numbers have been tested and have been found to be acceptable. Customers can test products using the following quality control organisms. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, sample results should not be reported.

Organisms	Incubation	Results
Staphylococcus aureus ATCC 25923	35 ± 2°C for 18-24 hours	Catalase (+)
Streptococcus pyogenes ATCC 19615	35 ± 2°C for 18-24 hours	Catalase (-)

ATCC® is a registered trademark of American Type Culture Collection.

WARNING AND PRECAUTIONS

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

STORAGE AND SHELF LIFE

Store between 2-30°C in tightly closed container and away from direct light. Use before expiry date on label. On opening, product should be properly stored in dry ventilated area protected from extremes of temperature and sources of ignition. Product is light and temperature sensitive; protect from light, excessive heat and freezing. Seal the container tightly after use.

DISPOSAL

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques.

BIBLIOGRAPHY

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3. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

CBSA life

155-196 Innovation Drive, Winnipeg, MB, R3T 2N2, Canada

Phone: +1 (204) 269-2255 Email: info@cbsalife.com Website: https://cbsalife.com