

Catalase Test Protocol

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Information History

In order to survive, organisms must rely on defense mechanisms that allow them to repair or escape the oxidative damage of hydrogen peroxide (H_2O_2). Some bacteria produce the enzyme catalase which facilitates cellular detoxification. Catalase neutralizes the bactericidal effects of hydrogen peroxide (13) and its concentration in bacteria has been correlated with pathogenicity (8).

Enzyme-based tests play a crucial part in the identification of bacteria. In 1893, a publication by Gottstein brought attention to bacterial catalase, making it one of the first bacterial enzymes to be described (6, 9). Some 30 years later, McLeod and Gordon (9) developed and published what is thought to be the first bacterial classification scheme based on catalase production and reactions (6). Initial methods of catalase detection were cumbersome, labor-intensive, time-consuming, and required specialized equipment (6). Over the years, the techniques first described by Gagnon et al. (6) and particularly those of Thomas (12) have been modified and streamlined, thus greatly simplifying the performance of this test.

Purpose

The catalase test facilitates the detection of the enzyme catalase in bacteria. It is essential for differentiating catalase-positive *Micrococcaceae* from catalase-negative *Streptococcaceae*. While it is primarily useful in differentiating between genera, it is also valuable in speciation of certain gram positives such as *Aerococcus urinae* (positive) from *Aerococcus viridians* (negative) and gram-negative organisms such as *Campylobacter fetus*, *Campylobacter jejuni*, and *Campylobacter coli* (all positive) from other *Campylobacter* species (7, 8). Some have reported its value in the presumptive differentiation among certain *Enterobacteriaceae* (11). The catalase test is also valuable in differentiating aerobic and obligate anaerobic bacteria, as anaerobes are generally known to lack the enzyme (8, 9). In this context, the catalase test is valuable in differentiating aerotolerant strains of *Clostridium*, which are catalase negative, from *Bacillus*, which are catalase positive (8).

Theory

The catalase enzyme serves to neutralize the bactericidal effects of hydrogen peroxide (13). Catalase expedites the breakdown of hydrogen

peroxide (H_2O_2) into water and oxygen ($2\text{H}_2\text{O}_2 + \text{Catalase} \rightarrow 2\text{H}_2\text{O} + \text{O}_2$). This reaction is evident by the rapid formation of bubbles (2, 7).

RECIPE

For routine testing of aerobes, use commercially available 3% hydrogen peroxide (2, 7). Store the hydrogen peroxide refrigerated in a dark bottle.

For the identification of anaerobic bacteria, a 15% H_2O_2 solution is necessary (1). In this context, the catalase test is used to differentiate aerotolerant strains of *Clostridium*, which are catalase negative, from *Bacillus* species, which are positive (8).

The superoxol catalase test used for the presumptive speciation of certain *Neisseria* organisms requires a different concentration of H_2O_2 . Refer to the "Additional Recommendations" section for details.

PROTOCOL

There are many applications and method variations of the catalase test. These include the slide or drop catalase test, the tube method, the semiquantitative catalase for the identification of *Mycobacterium tuberculosis*, the heat-stable catalase used for the differentiation of *Mycobacterium* species, and the capillary tube and cover slip method (7). One of the most popular methods in clinical bacteriology is the slide or drop catalase method, because it requires a small amount of organism and relies on a relatively uncomplicated technique. This protocol delineates the procedure for the qualitative slide and tube catalase methods, which are primarily used for the differentiation of staphylococci and streptococci.

Slide (drop) method

Place a microscope slide inside a petri dish. Keep the petri dish cover available. The use of a petri dish is optional as the slide catalase can be properly performed without it. However, to limit catalase aerosols, which have been shown to carry viable bacterial cells (4), the use of a petri dish is strongly recommended. Using a sterile inoculating loop or wooden applicator stick, collect a small amount of organism from a well-isolated 18- to 24-hour colony and place it onto the microscope slide. Be careful not to pick up any agar. This is particularly important if the colony isolate was grown on agar containing red blood cells. Carryover of red blood cells into the test may result in a false-positive reaction (5, 7). Using a dropper or Pasteur pipette, place 1 drop of 3% H_2O_2 onto the organism on the microscope slide. Do not mix. Immediately cover the petri dish with a lid to limit aerosols and observe for immediate bubble formation ($\text{O}_2 + \text{water} = \text{bubbles}$). Observing for the formation of bubbles against a dark background enhances readability.

Positive reactions are evident by immediate effervescence (bubble formation) (Fig. 1). Place microscope slide over a dark background and use a magnifying glass or microscope to observe weak positive reactions.

If using a microscope, place a cover slip over the slide and view under 40x magnification. No bubble formation (no catalase enzyme to hydrolyze the hydrogen peroxide) represents a catalase-negative reaction (Fig. 1).

Quality control is performed by using organisms known to be positive and negative for catalase.

Note: If a platinum inoculating loop is used, do not add 3% H₂O₂ to the slide before the organism, as the platinum wire in the loop may produce a false-positive result. This is not the case with nichrome wire.

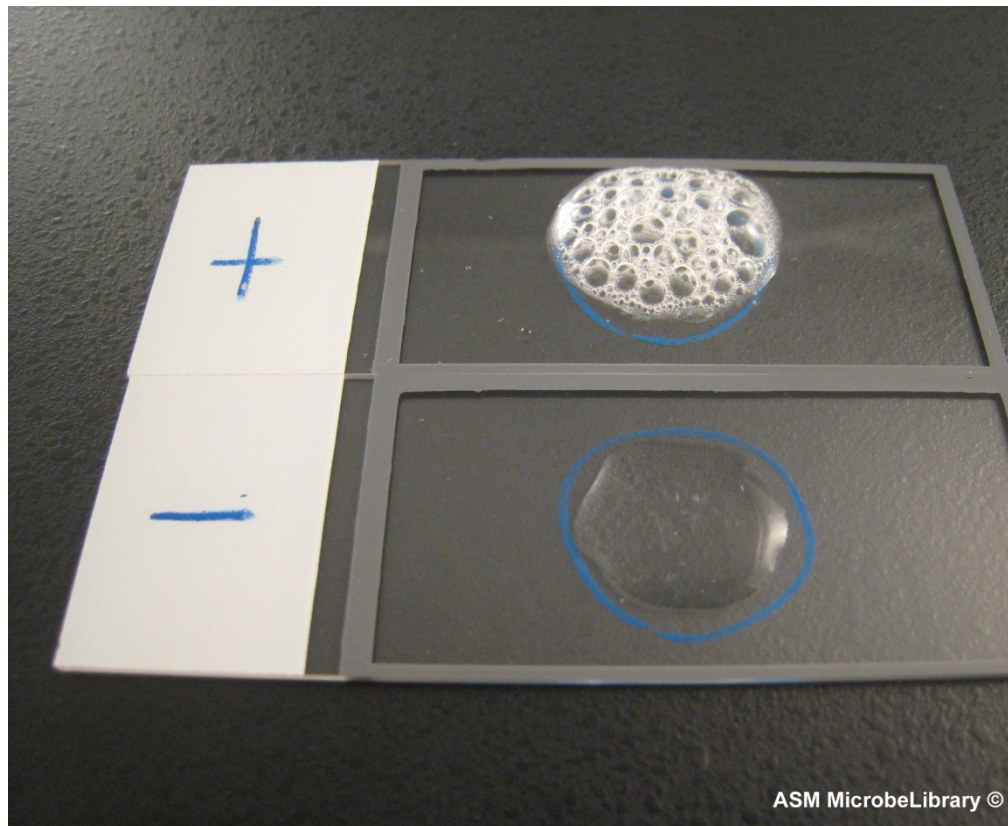


FIG. 1. Slide catalase test results. (Top) The positive reaction was produced by *Staphylococcus aureus*; (bottom) the negative reaction was produced by *Streptococcus pyogenes*.

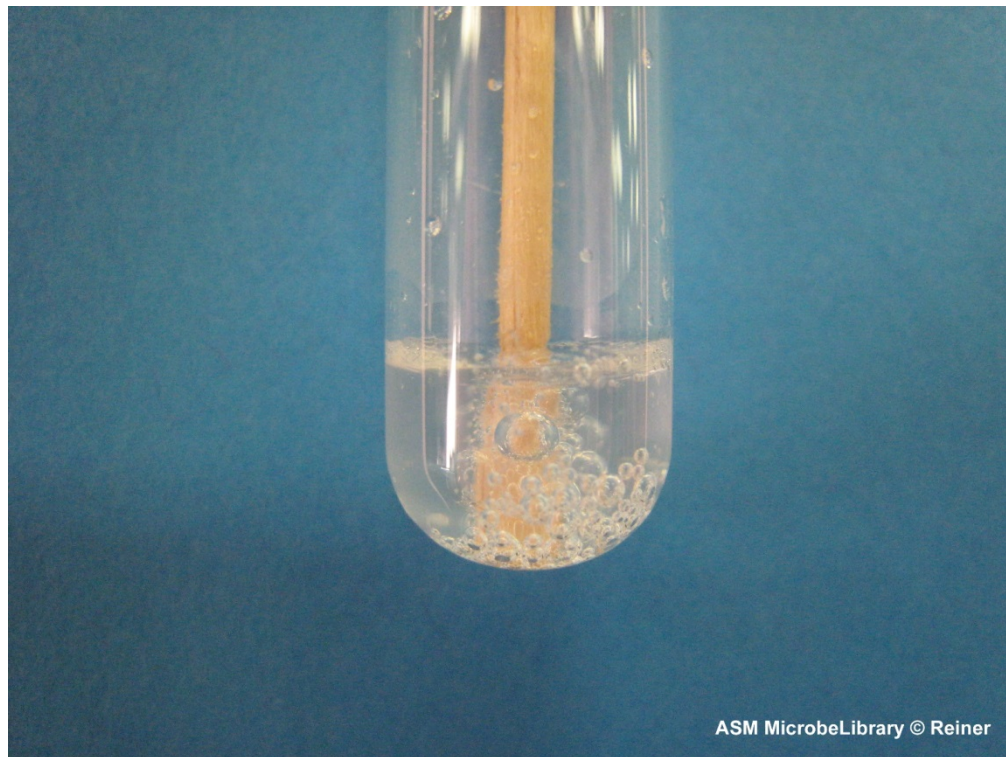
Tube method (10)

Add 4 to 5 drops of 3% H₂O₂ to a 12 x 75-mm test tube (10). Using a wooden applicator stick, collect a small amount of organism from a well-isolated 18- to 24-hour colony and place into the test tube. Be careful not to pick up any agar. This is particularly important if the colony isolate was grown on agar containing red blood cells. Carryover of red blood cells into the test may result in a false-positive reaction (5, 7). Place the tube against a dark background and observe for immediate bubble

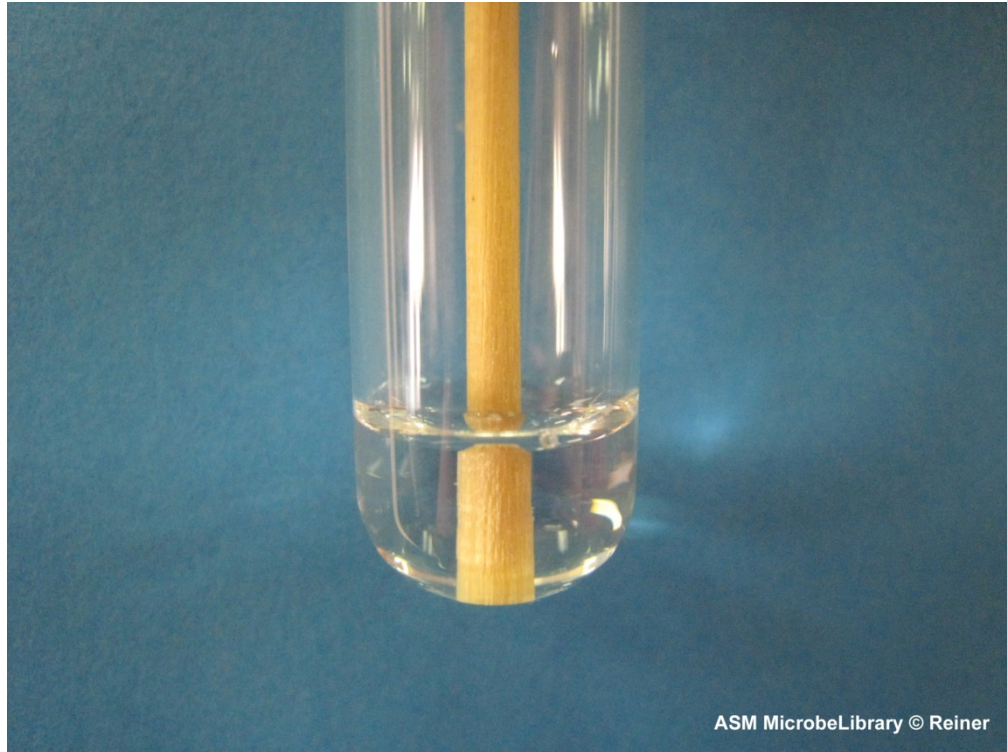
formation (O_2 + water = bubbles) at the end of the wooden applicator stick.

Positive reactions are evident by immediate effervescence (bubble formation) (Fig. 2A). Use a magnifying glass or microscope to observe weak positive reactions. No bubble formation (no catalase enzyme to hydrolyze the hydrogen peroxide) represents a catalase-negative reaction (Fig. 2B).

Quality control is performed by using organisms known to be positive and negative for catalase.



A



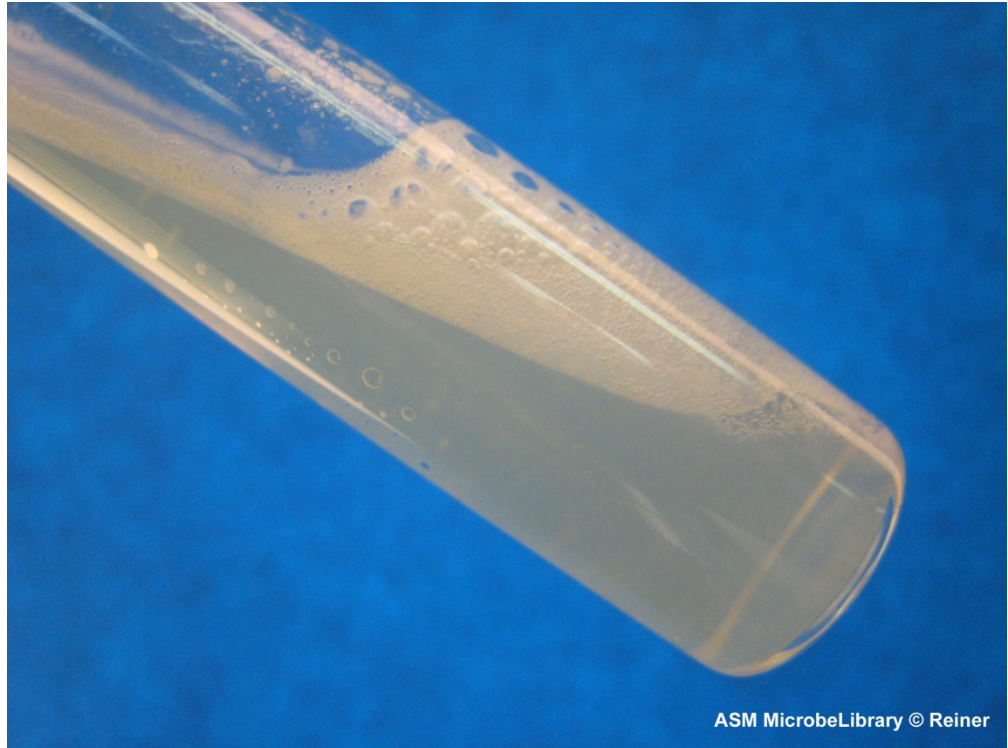
B

FIG. 2. Tube catalase test results. (A) The positive reaction was produced by *Staphylococcus aureus*; (B) the negative reaction was produced by *Streptococcus pyogenes*.

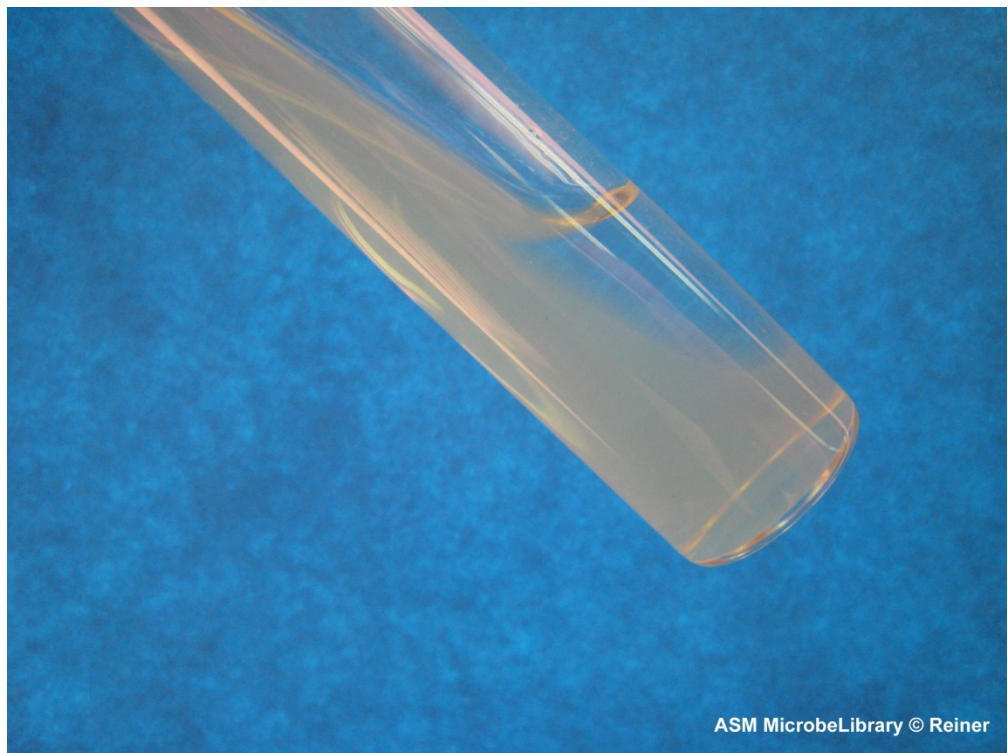
Tube (slant) method

Add 1.0 ml of 3% H₂O₂ directly onto an 18- to 24-hour heavily inoculated pure culture grown on a nutrient agar slant (7). Place the tube against a dark background and observe for immediate bubble formation.

Positive reactions are evident by immediate effervescence (bubble formation) (Fig. 3A). No bubble formation (no catalase enzyme to hydrolyze the hydrogen peroxide) represents a catalase-negative reaction (Fig. 3B).



A



B

FIG. 3. Tube (slant) catalase test results. (A) The positive reaction was produced by *Staphylococcus aureus*; (B) the negative reaction was produced by *Streptococcus pyogenes*.

SAFETY

The ASM advocates that students must successfully demonstrate the ability to explain and practice safe laboratory techniques. For more information, read the laboratory safety section of the [ASM Curriculum Recommendations: Introductory Course in Microbiology](#) and the [Guidelines for Biosafety in Teaching Laboratories](#).

COMMENTS AND TIPS

Always use strict aseptic techniques. If possible, perform the test under a biosafety hood. Do not use this procedure for *Mycobacteria* testing. In addition to potential self-contamination by aerosol exposure, *Mycobacteria* speciation by the catalase test is achieved by different techniques not discussed in this protocol (3, 5, 7).

Positive superoxol organisms such as *Neisseria gonorrhoeae* produce immediate, vigorous bubbling (8). Other *Neisseria* species produce delayed bubbling or none at all (8). The superoxol test is performed the same way as the 3% catalase test.

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