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| **TITLE:** Catalase | **Revision Date:** 20-April-2018 | **Issue Date:** 20-April-2016 |
| **Document Number: MIC50400** | **Status: Approved** | |
| **Distribution: Microbiology Test Manual** | **Page:** 1 of 4 | |
| **Approved by:** S. Asmussen, Manager of Diagnostic Services | **Signed by:**  | |

## PURPOSE:

This test is used in the identification of Gram positive cocci to differentiate Staphylococci (catalase positive) from Streptococci (catalase negative).

## INTRODUCTION:

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.

## REAGENTS and/or MEDIA:

| | |
|---|---|
| **Type** | 3% Hydrogen peroxide |
| **Stability** | Do not use beyond expiry date |
| **Storage requirement** | 15-30$^{o}$C in a dark bottle<br>Do not freeze or overheat |

## SUPPLIES:

- Glass slide
- Sterile loop or wooden stick

## SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures.

- Lab gown must be worn when performing activities with potential pathogens.

- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used where there is a known or potential risk of exposure to splashes.
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
- The use of needles, syringes, and other sharp objects should be strictly limited.

Test reagent is an oxidizer; apply the use of proper personal protective equipment as outlined in Hydrogen Peroxide MSDS sheet.

## QUALITY CONTROL:

Quality Control is set up each day the test is performed using the following control organisms:

| | | |
|---|---|---|
| **Positive**: | *Staphylococcus aureus* | ATCC# 25923 |
| **Negative**: | *Streptococcus pyogenes* | ATCC# 19615 |

- A QC order will be generated in the TQC system

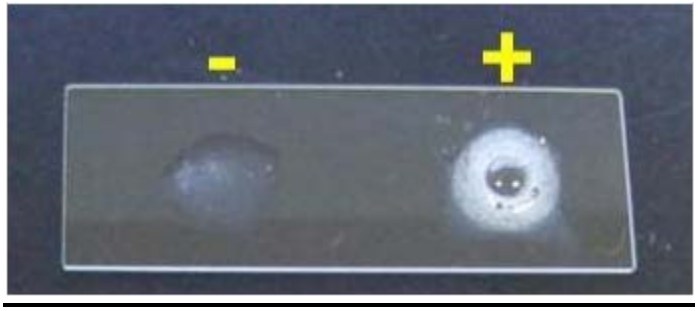
## PROCEDURE INSTRUCTIONS:

| Step | Action |
|---|---|
| **Testing Colonies for the Presence of the Catalase Enzyme** | |
| **1** | In the LIS system ORDER: **^CAT** |
| **2** | Place one drop of 3% hydrogen peroxide on a glass slide. |
| **3** | Using a loop or wooden stick, touch the top of a well isolated colony. |
| **4** | Apply colony to the hydrogen peroxide on the slide. |
| **5** | Observe for bubbles. |

## INTERPRETATION OF RESULTS:

**Positive:** Presence of bubbles

**Negative:** Absence of bubbles




## NOTES AND PRECAUTIONS:

1. Red blood cells contain catalase and contact with hydrogen peroxide will result in slow production of bubbles. Pick from centre of colony and avoid scraping surfaces of plate to prevent false positives.
2. Hydrogen peroxide is unstable and easily breaks down to water, especially when exposed to light and warm temperatures. False negative reactions will occur if reagent has completely broken down.
3. Catalase testing should be done on 18-24 hour cultures because the enzyme is present in viable cultures only. Older cultures may give false negative reactions.
4. 3% $H_2O_2$ is caustic – avoid exposure to skin. If $H_2O_2$ does get on the skin, immediately flood the area with 70% ethyl alcohol, not water.

## REFERENCES:


- Murray, P., Baron, E. J., Jorgensen, J., Landry, M. L., & Pfaller, M. (2007). *Manual of Clinical Microbiology* (Vol. 1). Washington, DC, USA: ASM Press.

**REVISION HISTORY:**

| REVISION | DATE | Description of Change | REQUESTED BY |
|---|---|---|---|
| 1.0 | 13 Nov 2012 | Initial Release | A.Darrach |
| 1.1 | 31-Jul-13 | Add illustrations | A. Darrach |
| 1.2 | 07-Mar-14 | Change from MTE10400 to MIC50400 | C. Russell |
| 2.0 | 31-Mar-16 | Update of “Special Safety Precautions” to reflect risk assessment recommendations. | C. Russell |
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