

**KCMC Biotechnology
Laboratory, Microbiology**

**STANDARD
OPERATING
PROCEDURE**

**Effective Date
10 May 2006**

**SOP-Number
MIC.026**

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**Date
10 May 2006**

Title: CATALASE TEST

Author/Date: Anne Morrissey, 10 May 2006

Approvals/Date:

John A. Crump, MB, ChB, Microbiology Laboratory Director

Anne Morrissey, MS, MT(ASCP)SM, Microbiology Lab Supervisor

This SOP has been read and understood by:

Name	Date
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Annual Review	
By	Date

Title: CATALASE TEST

Document History:

Version Number	Reason for Changes	Date

Copies distributed to:

Name	Date

Title: CATALASE TEST

PURPOSE

Aids in the differentiation of several bacterial species, commonly staphylococci (Pos) from streptococci (Neg), *Bacillus* spp. (Pos) from *Clostridium* sp. (Neg) and is useful in the identification of *Listeria*, *Campylobacter*, anaerobes and several of the fastidious gram negative bacilli.

PRINCIPLE

The enzyme catalase is present in most cytochrome containing aerobic and facultatively anaerobic bacteria, with the exception of streptococci. Catalase breaks down hydrogen peroxide (H₂O₂), an oxidative end product of aerobic degradation of carbohydrates, into water and oxygen. To test for catalase production several colonies of the test organism are transferred to a glass slide and a drop of hydrogen peroxide is added. Immediate and profuse bubble formation indicates presence of catalase.

SCOPE

This Standard Operating Procedure applies to the testing of bacterial cultures for catalase enzyme by technical staff in the microbiology laboratory that have been trained and are competent in performing this procedure.

SAFETY PRECAUTIONS

Dispose of contaminated glass slide in sharps bucket.

MATERIALS /STORAGE

3% Hydrogen peroxide (H₂O₂)/Refrigerate

Glass slide

18-24 hr culture of test organism

QUALITY CONTROL

Frequency – Each new lot/shipment of reagent.

Control Organisms/Acceptable Results:

Staphylococcus aureus/Positive

Streptococcus pyogenes/Negative

Corrective actions for Unacceptable QC results:

1. Repeat testing with fresh subcultures of control organisms.
2. If still unsatisfactory, retest with fresh reagent.

Documentation:

1. Record QC results on CATALASE QC sheet.
2. Document all corrective actions on QC DEVIATION FORM and submit to supervisor for review.
3. Supervisor will review QC results monthly

Title: CATALASE TEST

TEST PROCEDURE

1. Using a loop or wooden stick transfer colonies from 3rd or 4th streak area or subculture on to a glass slide. Allow anaerobes 30 min. exposure to air.

NOTE: Take care not to pick up blood agar as it will cause a false positive reaction.

2. Add 1 drop of 3% H₂O₂.

3. Watch for immediate and profuse production of bubbles due to release of O₂ gas.

Note: False positive, usually weak, reactions can occur when testing isolates from blood agar and some organisms may demonstrate weak catalase activity (e.g. enterococci, non-streptococcal Gram positive cocci and some fastidious Gram negative bacilli). Depending on the isolate, this can be misleading if interpreted as positive. Consult supervisor.

INTERPRETATION

Immediate/profuse bubbling – Positive

No bubble production – Negative

Weak bubble production – Weak (consult supervisor)

REPORTING

Enter test results on internal work card.

DISPOSAL

Dispose of slide in sharps bucket.

NOTES

1. An 18- 24 hour culture should be used. Older cultures may give a false negative result.
2. Red blood cells contain catalase; carryover of medium from a BAP may result in a false positive reaction.
3. The test should be done from a single colony from the second or third streak. Inoculum from the specimen may also result in a false positive.
4. Enterococci, other Gram positive cocci and some fastidious gram negative rods may be weak catalase producers. Other methods for identification should be used to characterize these organisms.

REFERENCES

Bannerman, TL. 2003. Staphylococcus, Micrococcus and other catalase positive cocci that grow aerobically. In: Manual of Clinical Microbiology. 8th Ed. ASM press, Washington, DC.

Ruoff, KL, etal. 2003. Streptococcus. In: Manual of Clinical Microbiology. 8th Ed. ASM Press, Washington, DC.

APPENDIX A – Catalase Test QC sheet

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APPENDIX A

**KCMC Biotechnology Laboratory
Microbiology**

**Quality Control
CATALASE TEST**

CONTROL ORGANISMS/Acceptable Results:

Staphylococcus aureus - Positive

Streptococcus pyogenes - Negative

QC DATE	QC BY	LOT#	<i>S. aureus</i> (+)	<i>S. pyogenes</i> (-)	A/NA*

*A/NA = Acceptable/Not acceptable
DEVIATION FORM

DOCUMENT ALL CORRECTIVE ACTION ON QC

Supervisor Review :						

CATALASE QC/QC SHEETS