

**KCMC Biotechnology
Laboratory, Microbiology**

**STANDARD
OPERATING
PROCEDURE**

Effective Date
7 Nov 2006

SOP-Number
MIC.035.01

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Date
7 Nov 2006

Title: O.B.I.S. PYR TEST

Author/Date: Anne Morrissey

Approvals/Date:

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

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

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Document History:

| Version Number | Reason for Changes | Date |
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Title: O.B.I.S. PYR TEST

PURPOSE

For the identification of *Streptococcus pyogenes* (Group A streptococci) and enterococci. May also aid in the differentiation of *Citrobacter* spp. from *Salmonella*.

PRINCIPLE

Both Group A streptococci and enterococci possess the enzyme "PYRase" (L-pyrroglutamyl-peptide hydrolase). The O.B.I.S. PYR test utilizes the chromogenic substrate L-pyroglumatic acid 7-amino-4-methyl-coumarin and a dimethylamino-cinnamaldehyde color developer to detect PYRase activity. The enzymatic hydrolysis of the substrate results in the development of a purple color on addition of Developing solution.

SCOPE

This Standard Operating Procedure applies to the testing of isolates suspected to be enterococci or Group A streptococci based on colonial morphology by technical staff in the microbiology laboratory who have been trained and are competent in the use and performance of this test.

SPECIMEN COLLECTION – NA

SAFETY PRECAUTIONS

Dispose of test cards in infectious waste.

MATERIALS/STORAGE

- O.B.I.S. PYR Test kit (Store at 5-30° C)
- Test reagent cards (1 per test or QC organism)
- Developing solution – 1 dropper bottle containing 7 ml
- Phosphate Buffered Saline (PBS) - 1 dropper bottle containing 7 ml
- Inoculating loop or wooden applicator stick.
- Fresh culture (24-48 hrs) of test organism

EQUIPMENT -NA

QUALITY CONTROL

Frequency: Each shipment of each lot.

Title: O.B.I.S. PYR TEST

Controls/Acceptable Results:

Streptococcus pyogenes/ Positive

Streptococcus agalactiae/Negative

Corrective Actions for Unacceptable QC results:

1. Repeat test using fresh control organisms.
2. If still unsatisfactory, do not use for identification of isolates.
3. Arrange for replacement.

Documentation:

1. Record QC results on PYR TEST QC sheet.
2. Record unacceptable results and corrective actions on a QC DEVIATION form and submit to supervisor for review.
3. Supervisor will review QC monthly.

OPENING TEST KIT

1. Remove pouch containing test cards and allow to reach room temperature before opening.
2. Cut the pouch open just below the seal.
3. Remove the number of cards needed within 10 minutes of opening the pouch. Cards may be cut along the indicated lines if fewer tests are being performed.

TEST PROCEDURE

1. Apply 1-2 colonies of test organism to the test reaction area using an inoculating loop. Obtain enough growth to make a visible smear.
2. Moisten area with a drop of Buffer.
3. Incubate the inoculated test card at room temperature for 5 minutes.
4. Dispense 1 drop of Developing Solution onto the test area.
5. Look for color development within 20 seconds (no longer).

TEST INTERPRETATION

Vivid purple color – POSITIVE

No color development - NEGATIVE

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RECORDING RESULTS

Enter PYR results on POSITIVE CULTURE Form.

TEST LIMITATIONS

1. Other organisms may be PYRase positive e.g. lactococci, lactobacilli, *Abiotrophia*, *Aerococcus*, *Helcococcus*, *Gemella* and *Globiicatella*.
2. A slight color change at the site of inoculation may develop with negative reactions. Refer to positive and negative control results for comparison.

REFERENCES

Package Insert, May 2004. O.B.I.S. PYR Test, Oxoid Ltd. Basingstoke, Hampshire, UK.

Ellner, PD, et al. 1985. Preliminary evaluation of a rapid colorimetric methods for the presumptive identification of Group A streptococci and enterococci. J. Clin. Micro. 22:880.

APPENDIX A – PYR Test QC sheet

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APPENDIX A

**A KCMC Biotechnology Laboratory
Microbiology**

**Quality Control
PYR Test**

CONTROLS/Acceptable Results:

Streptococcus pyogenes(POS)/Positive

Streptococcus. agalactiae (NEG)/Negative

YEAR: _____

| QC DATE | QC BY | LOT# | POS(+) | NEG(-) | A/NA* |
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*A/NA = Acceptable/Not acceptable
DEVIATION FORM

DOCUMENT ALL CORRECTIVE ACTION ON QC

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| Supervisor Review : | | | | | | |
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PYR TEST QC/QC SHEETS