

**KCMC Biotechnology  
Laboratory, Microbiology**

**STANDARD  
OPERATING  
PROCEDURE**

**Effective Date**

**SOP-Number  
MIC.023**

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**Date  
27 August 2006**

**Title: API NH TEST**

**Author/Date: Anne Morrissey, 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:**

| <b>Name</b>                         | <b>Date</b> |
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**Title: API NH TEST**

**PURPOSE**

For the identification of *Neisseria* and *Haemophilus* spp. The strip may also be used for determining carbohydrate fermentation of fastidious Gram negative bacilli.

**PRINCIPLE**

The API-NH strip contains cupules of dehydrated substrates to demonstrate enzymatic activity, carbohydrate fermentation and penicillinase production of test organisms. The first 7 cupules contain substrates for 7 individual reactions. Cupules 8-10 each contain substrates for 2 reactions; one of the tests will react spontaneously, the other requires the addition of reagent. Test cupules are filled with a suspension of organism equal in turbidity to a #4 McFarland standard. Following a 2 hour incubation period, spontaneous reactions in all cupules are recorded and reagents are added to cupules 8-10. A profile number is calculated from the values of the positive reactions.

**SCOPE**

This Standard Operating Procedure applies to the identification of isolates suspected to be *Neisseria* or *Haemophilus* spp. or fastidious Gram negative bacilli by technical staff that are trained and competent in performing this test.

**PRECAUTIONS**

Inoculation of test strips with isolates resembling *Neisseria meningitidis* (Gram neg diplococci, smooth, gray nonhemolytic colonies on blood and/or chocolate agar) should be performed in a biosafety cabinet.

**MATERIALS**

API NH kit:

- 10 test strips
- 10 ampules of saline
- 1 ampule of James reagent
- 1 ampule ZYM B reagent
- 10 swabs

McFarland turbidity standard #4

Pasteur pipets

Mineral oil

Fresh 18-24 hr culture of test isolate.

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### **QUALITY CONTROL**

**Frequency** - Each shipment of each lot of test strips

**Control Organism** - *Haemophilus influenzae* ATCC 10211

**Acceptable results:** See API NH QC record sheet for acceptable reactions.

**Corrective actions for Unacceptable QC Results:**

1. Repeat results with fresh subculture of QC organism passed twice from freezer stock.
2. If still unacceptable, consult supervisor.

**Documentation:**

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

### **TEST PROCEDURE**

Consult MIC.014 IDENTIFICATION OF GRAM NEGATIVE COCCI and MIC.015 IDENTIFICATION OF FASTIDIOUS GRAM NEGATIVE BACILLI AND HAEMOPHILUS to establish eligibility for the API NH test.

1. Prepare a suspension in saline of the organism to be tested equal in turbidity to a #4 McFarland standard.
2. Place strip into inoculation tray which contains about 5 ml of tap water to provide humidity.
3. Using a pasteur pipet, fill the tube portions only of the first 7 tests (PEN to URE)
4. Fill tubes AND cupules of the last 3 tests (LIP/ProA, PAL/GGT, BGAL/IND).
5. Cover the first 7 test cupules with mineral oil.
6. Incubate at 35<sup>0</sup> C in aerobic incubator for 2 hours.

### **READING/INTERPRETATION**

1. After 2 hours incubation read the spontaneous reactions and record on result sheet. Refer to **TABLE 1.** below.
2. Add 1 drop of ZYM B reagent to cupules 8 (for ProA) and 9 (for GGT).
3. Add 1 drop of JAMES reagent to cupule 10 (for Indol).
4. Interpret results after 3 minutes.

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5. If, after the 2 hour incubation, several reactions are doubtful the strip may be reincubated for an additional 2 hours and reread (cupules 1-7 only)
6. Calculate a 4 digit profile number by adding the values of the positive reactions in each triplet of tests and consult numerical profile list in package insert or APIWeb. An identification table is included in the kit for additional assistance in interpreting results.

**Accessing APIWeb:**

- a. Software is loaded on supervisor's and ISAAC computer in technician's office.
- b. Load the CD into the CD drive.
- c. Click on the APIWeb icon to connect to website.
- d. Select API 20E.
- e. Enter the study (REF) number.
- f. Enter the octal code in spaces provided.
- g. Select CONFIRM.
- h. Print a copy of the APIWeb report and attach to the POSITIVE CULTURE form.

**REPORTING RESULTS**

1. If EXCELLENT IDENTIFICATION OR VERY GOOD IDENTIFICATION - Report results.
2. If ACCEPTABLE IDENTIFICATION OR GOOD LIKELIHOOD, LOW SELECTIVITY consult supervisor.
3. Organisms that cannot be identified will be reexamined at the Duke Medical Center Microbiology Laboratory. Consult supervisor for appropriate report verbiage.
4. Enter the code number and organism name on the POSITIVE CULTURE REPORT FORM.
5. If from blood culture enter organism name and code number on BLOOD CULTURE result form.
6. Freeze isolate at -80° C. Refer to MIC.003 FREEZING ISOLATES for procedure.

**Title: API NH TEST****Table 1. TEST REACTIONS**

| TEST  | REACTION                   | REAGENT       | NEG                                      | POS                                   |
|-------|----------------------------|---------------|--|---------------------------------------|
| PEN   | Penicillinase production   | None          | blue                                     | Yellow<br>Yellow-green<br>Yellow-blue |
| GLU   | Glucose acidification      | None          | Red<br>Red/orange                        | Yellow                                |
| FRU   | Fructose acidification     | None          | Red<br>Red/orange                        | Yellow                                |
| MAL   | Maltose acidification      | None          | Red<br>Red/orange                        | Yellow                                |
| SUC   | Sucrose acidification      | None          | Red<br>Red/orange                        | Yellow                                |
| ODC   | Ornithine decarboxylase    | None          | Yellow green<br>Gray green               | blue                                  |
| URE   | Urease production          | None          | yellow                                   | Pink-violet                           |
| LIP   | Lipase production          | None          | Colorless<br>Gray-green                  | Blue<br>(+ precipitate)               |
| PAL   | Alkaline phosphatase       | None          | Colorless<br>Pale yellow                 | Yellow                                |
| β GAL | Beta galactosidase         | None          | Colorless                                | Yellow                                |
| ProA  | Proline arylamidase        | ZYM B (3 min) | Yellow<br>Pale orange Brown if<br>LIP +* | Orange                                |
| GGT   | Gamma glutamyl transferase | ZYM B (3 min) | Yellow<br>Pale orange                    | Orange                                |
| IND   | Indol production           | JAMES (3 min) | Colorless                                | Pink                                  |

**\* If LIP is +, ProA is always negative.****REFERENCES**

Package Insert, API NH Test Strips, Sept. 2004, bioMerieux, Inc., Hazelwood, MI.

Barbe, G. et al. 1994. Evaluation of API NH, a new 2 hour system for identification of *Neisseria* and *Haemophilus* species and *Moraxella catarrhalis* in the routine laboratory. J. Clin. Micro. 32: 187.**APPENDIX A – API NH QC SHEET**