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| Author/Date: Modified by\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_Daniel Eibach Charity Wiafe Akenten |
| **Reviewed/Approved by/Date:**  \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ |
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**This SOP has been read and understood by:**

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| **Name** | **Signature** | **Date** |
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# 

# INTRODUCTION

The large-volume methods once used for microbial characterization tests have been miniaturized and presented in different easy-to-use formats. The API NH is one of these formats used in the identification of *Neisseria* spp*., Haemophilus* spp*.* and *Moraxella catarrhalis.*

# PRINCIPLE

The API-NH strip contains cupules of dehydrated substrates and relies on detection of preformed enzymes, 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 use of the API NH test strip by technical staff, who is trained and competent in performing this test

# MATERIALS

API NH Test kit (10 test/kit)

10 ampoules of saline

1 ampoule of James reagent

1 ampoule ZYM B reagent

10 swabs

McFarland turbidity standard 4

Pasteur pipettes

Mineral oil

Fresh 18-24h culture of test isolate.

# SAFETY

• **Aerosols from heavy suspensions of *Neisseria meningitides* are dangerous.**

Suspensions of suspected *N*.*meningitides* **must** be prepared in the safety cabinet

PROCEDURE

1. Label the API carrier tray with the patient’s barcode
2. Using a sterile swab or loop, make a heavy suspension (McFarland 4) of the test organism in 2ml sterile saline (provided with the kit).
3. Place strip into the inoculation tray, after addition of 2 ml of tap water to provide humidity.
4. Using a Pasteur pipette, fill the tube portions only of the first 7 tests (PEN to URE).
5. Fill tubes and cupules of the last 3 tests (LIP/ProA, PAL/GGT, BGAL/IND).
6. Cover the first 7 test cupules with mineral oil.
7. Incubate at 35°C-37 o C aerobically for 2 hours.

# RESULTS

1. After 2 hours incubation before adding any reagents 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.
5. If, after the 2 hour incubation, several reactions are doubtful the strip may be re-incubated for an additional 2 hours and reread (cupules 1-7 only)
6. Calculate a 4-digit profile by adding the values of the positive reactions in each triplet of tests and record the result on the API form, ignoring the Pen result and starting with GLU, FRU, MAL triplet. The third digit is derived from the upper three tests in the bifunctional wells and the fourth from the lower three.
7. 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:**

1. Load the CD into the CD drive.
2. Click on the APIWeb icon to connect to website.
3. Select API NH.
4. Enter the patient’s barcode number.
5. Enter the octal code in spaces provided.
6. Select CONFIRM.
7. Print a copy of the APIWeb report.

1. If EXCELLENT IDENTIFICATION OR VERY GOOD IDENTIFICATION - Report

Results.

1. If ACCEPTABLE IDENTIFICATION OR GOOD LIKELIHOOD, LOW SELECTIVITY consult supervisor.

# 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.

# 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 Interpretation Table

**APPENDIX B** – API NH QC sheet

# APPENDIX A API NH interpretation Table

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

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

**API NH QC Sheet APPENDIX B**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **API NH QC**  ***H. influenzae* ATCC 10211** | | | **PEN** | **GLU** | **FRU** | **MAL** | **SAC** | **ODC** |
| *Acceptable Results* | | | **-** | **+** | **V** | **-** | **-** | **+** |
| **QC DATE** | **QC BY** | **LOT#/EXP DATE** |  |  |  |  |  |  |
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**V: variable \*A: acceptable, NA: not acceptable**