SYSTEM FOR IDENTIFICATION OF NEISSERIA & HAEMOPHILUS (API NH)

**Principle**

The API NH strip consists of 10 microtubes containing dehydrated substrates, which enable the performance of 12 identification tests (enzymatic reactions or sugar fermentations), as well as the detection of a penicillinase (particular interest in *Haemophilus influenzae*, *Haemophilus parainfluenzae*, *Branhamella catarrhalis* (*Moraxella catarrhalis*) and *Neisseria gonorrhoeae*).

The reactions produced during incubation result in spontaneous color changes or are revealed by the addition of reagents.

After a 2-hour incubation period at a temperature of 35-37°C, the reading of the reactions is performed visually and identification is obtained by consulting the profile list.

**Reagents**

- API NH strips
- NaCl 0.85% Medium (2 ml)
- JAMES reagent
- ZYM B reagent
- Swab
- Incubation box
- Result sheet
- 1 package insert
- McFarland Standard, point 4 on the scale
- Mineral oil
- Pipettes
- Ampule rack
- Ampule protector

**Procedure**

1. **Specimen Processing**

The microorganisms to be identified must first be isolated as separate colonies by streaking the specimen onto Blood agar, Chocolate agar or Martin-Lewis agar according to standard
microbial techniques.

2. **Preparation of Strip**

Each strip is composed of 10 cupules. Each cupule has an open and closed area (cupule and tube). An incubation tray is supplied for each strip. It serves as a support and individual chamber while both protecting the strip from contaminants in the air and assuring the humid atmosphere necessary to avoid dehydration during incubation.

- Remove the strip from its individual packaging
- Place the strip in the incubation box
- Discard the desiccant sachet

Record the specimen number on the flat portion of the tray (do not record the number on the lid as it may be misplaced during handling).

3. **Preparation of the Inoculum**

- Open an ampule of NaCl 0.85% Medium (2 ml) with the ampule protector.
- Using a swab, pick up a few well-isolated colonies and prepare a suspension with a turbidity equivalent to 4 McFarland, ensuring it is well mixed.
- The suspension should be used immediately after preparation.

4. **Inoculation of the Strip**

- Distribute the prepared bacterial suspension into the cupules, avoiding the formation of bubbles (tilt the strip slightly forwards and place the tip of the pipette or PSIpette against the side of the cupule):
  - Only fill the tube part of the first 7 microtubes (PEN to URE): about 50 µl.
  - Fill tube and cupule of the last 3 microtubes LIP/ProA, PAL/GGT, βGAL/IND: about 150 µl, avoiding the formation of a convex meniscus.
- Cover the first 7 tests (PEN to URE) with mineral oil (underlined tests).

**NOTE:** The quality of the filling is very important: tubes which are insufficiently or excessively full may cause false positive or false negative results.

- Close the incubation box.
- Incubate for 2 hours at 35-37°C **in aerobic conditions.**
5. Incubation

Incubate for 2 hours at 35-37°C in aerobic conditions.

6. Reading the Strip

Refer to the Reactions Table for a description of how to read the reactions.

Note all spontaneous reactions (PEN to βGAL) and record them as + or -.
- Add 1 drop of ZYM B reagent to microtubes 8 and 9: LIP/ProA and PAL/GGT.
- Add 1 drop of JAMES reagent to microtube 10: βGAL/IND.
- **Wait 2 minutes** then read the reactions by referring to the Reading Table in this package insert and record them on the result sheet.
  - If the LIP reaction is positive (blue pigment), interpret the ProA reaction as **negative**, whether the ZYM B reagent has been added or not.
  - If, after a 2-hour incubation period, several reactions (fermentation, penicillinase) are doubtful, re-incubate the strip for another 2 hours and read the reactions again (the enzymatic tests should not be re-read in this case).

### Reactions Table

<table>
<thead>
<tr>
<th>TESTS</th>
<th>REACTIONS</th>
<th>SUBSTRATES</th>
<th>QTY (mg)</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) PEN</td>
<td>PENicillinase</td>
<td>Penicillin G</td>
<td>1.36</td>
<td>Blue (penicillinase absent)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Yellow</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Yellow-green</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Yellow-blue (penicillinase present)</td>
</tr>
<tr>
<td>2) GLU</td>
<td>GLUcose (acidification)</td>
<td>Glucose</td>
<td>0.5</td>
<td>Red</td>
</tr>
<tr>
<td>3) FRU</td>
<td>FRUctose (acidification)</td>
<td>Fructose</td>
<td>0.1</td>
<td>Red-orange</td>
</tr>
<tr>
<td>4) MAL</td>
<td>MALtose (acidification)</td>
<td>Maltose</td>
<td>0.1</td>
<td>Yellow</td>
</tr>
<tr>
<td>5) SAC</td>
<td>SACcharose/Sucrose (acidification)</td>
<td>Sucrose</td>
<td>0.5</td>
<td>Orange</td>
</tr>
<tr>
<td>6) ODC</td>
<td>Ornithine DeCarboxylase</td>
<td>Ornithine</td>
<td>0.55</td>
<td>Yellow-green</td>
</tr>
<tr>
<td>7) URE</td>
<td>UREase</td>
<td>Urea</td>
<td>0.41</td>
<td>Pink-violet</td>
</tr>
<tr>
<td>8a) LIP</td>
<td>LIPase</td>
<td>5-bromo-3-indoxyl- caprate</td>
<td>0.033</td>
<td>Colorless Pale grey</td>
</tr>
<tr>
<td>9a) PAL</td>
<td>Alkaline Phosphatase</td>
<td>Para-Nitrophenyl-phosphate 2CHA</td>
<td>0.038</td>
<td>Colorless Pale yellow</td>
</tr>
<tr>
<td>10a) βGAL</td>
<td>Beta GALactosidase</td>
<td>Para-Nitrophenyl-BD galactopyranoside</td>
<td>0.04</td>
<td>Colorless</td>
</tr>
</tbody>
</table>
Reactions Table (Cont'd)

<table>
<thead>
<tr>
<th>TESTS</th>
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<th>SUBSTRATES</th>
<th>QTY (mg)</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ZYM B / 3 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Yellow</td>
</tr>
<tr>
<td>8b)</td>
<td>ProA</td>
<td>Proline Arylamidase</td>
<td>0.056</td>
<td>Pale orange (brown if LIP +)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Proline-4-methoxy-β naphthylamide</td>
<td></td>
<td>Orange</td>
</tr>
<tr>
<td>9b)</td>
<td>GGT</td>
<td>Gamma Glutamyl Transferase</td>
<td>0.049</td>
<td>Yellow</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gamma glutamyl 4-methoxy-β naphthylamide</td>
<td></td>
<td>Pale orange (yellow-orange if PAL +)</td>
</tr>
<tr>
<td>10b)</td>
<td>IND</td>
<td>INDole</td>
<td>0.036</td>
<td>Colorless</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tryptopane</td>
<td></td>
<td>Pink</td>
</tr>
</tbody>
</table>

Quality Control

To be performed on receipt of every new lot of strip by the Q.C bench technologist.

Reference

QC organisms to be used:

- Neisseria gonorrhoea ATCC 31426
- Haemophilus influenzae ATCC 10211
- Branhamella catarrhalis ATCC 23246
- Haemophilus paraphrophilus ATCC 49917

Reference Package Insert - api NH system for the identification of Neisseria and Haemophilus bioMerieux Inc., Missouri USA.