Api NH

System for the Identification of *Neisseria* and *Haemophilus*

*** See package insert for more information***

Storage of the Reagents:

The reagents should be stored in the dark at 2-8°C until the expiration date indicated on the packaging. The reagents may be kept for up to 1 month after the ampules have been opened and the reagents transferred into the dropper-bottles: record the date opened on the bottle. The ZYM B and JAMES reagents are very sensitive to light: wrap the bottles in aluminum and only leave them out of the refrigerator while being used. Do not leave them on the bench for prolonged periods of time. The ZYM B reagent is normally yellow in color. Dispose of the reagent if any tint of pink is observed.

Use of the Reagents:

ZYM B reagent: see package insert

JAMES reagent: see package insert for reconstitution directions

Selection of Colonies:

As the API NH procedure requires an inoculum adjusted to 4 McFarland, it is generally necessary to perform a subculture onto Thayer Martin Medium. The incubation of the subculture should be carried out in a CO2-enriched atmosphere for 18-24 hours at 35-37°C.

Preparation of the strip:

- Prepare the incubation box
- Record the strain reference on the elongated flap of the tray
- Remove the strip from packaging and place in the box

Preparation of the inoculum:

- Open an ampule of NaCl 0.85 % Medium
- 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.

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: about 150 µL avoiding the formation of a convex meniscus.
- Cover the first 7 tests with mineral oil.
- Close the incubation box and incubate for 2 hours at 35-37°C in aerobic conditions

Reading the Strip:

After a 2-hour incubation period, read the reactions by referring to the Reading Table in the package insert:

- Read the spontaneous reactions and record them as + or – on the result sheet.

Warning: The last 3 microtubules are bi-functional and enable the performance of 2 reactions in the same tube:

- 8. **LIP** (spontaneous reaction) / **ProA** (reaction after adding reagent)
- 9. **PAL** (spontaneous reaction) / **GGT** (reaction after adding reagent)
- 10. **βGAL** (spontaneous reaction) / **IND** (reaction after adding reagent)

The results of reactions **LIP**, **PAL**, and **βGAL** should be recorded before the addition of the reagent.

- Add 1 drop of ZYM B reagent to microtubes 8 and 9.
- Add 1 drop of JAMES reagent to microtube 10.
- **Wait 3 minutes** then read the reactions by referring to the Reading Table in the 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 a2-hour incubation period, several reactions 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).

Interpretation:

1. Identification is obtained with the **numerical profile**.
   - On the result sheet, the tests are separated into groups of three and a value 1, 2 or 4 is assigned to each. By adding together the values corresponding to positive reactions within each group, a 4-digit numerical profile is obtained.

   Warning: do not code the first test (penicillinase).
   - Identification is obtained by searching for the numerical profile in the list of profiles in the package insert.
2. Penicillinase test:
   - A positive reaction (yellow, yellow-green or yellow-blue coloration) indicates the presence of a penicillinase. The presence of this enzyme prohibits the use of penicillins. A susceptibility test is required for the other β-lactams.
   - A negative reaction (blue coloration) indicates the absence of penicillinase.