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^{o}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^{o}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

TESTS	REACTIONS	SUBSTRATES	QTY (mg)	RESULTS	
				NEGATIVE	POSITIVE
1) PEN	PENicillinase	Penicillin G	1.36	Blue (penicillinase absent)	Yellow Yellow-green Yellow-blue (penicillinase present
2) GLU 3) FRU 4) MAL 5) SAC	GLUcose (Acidification) FRUctose (Acidification) MALtose (Acidification) SACcharose/Sucrose (Acidification)	Glucose Fructose Maltose Sucrose	0.5 0.1 0.1 0.5	Red Red-orange	Yellow Orange
6) ODC	Ornithine DeCarboxylase	Ornithine	0.55	Yellow-green Grey-green	Blue
7) URE	UREase	Urea	0.41	Yellow	Pink-violet
8a) LIP	LIPase	5-bromo-3-indoxyl-caprate	0.033	Colorless Pale grey	Blue (+precipitate)
9a) PAL	Alkaline Phosphatase	Para-Nitrophenyl-phosphate 2CHA	0.038	Colorless Pale yellow	Yellow
10a) βGAL	Beta GALactosidaase	Para-Nitrophenyl-BD galactopyranoside	0.04	Colorless	Yellow

Reactions Table (Cont'd)

TESTS	REACTIONS	SUBSTRATES	QTY (mg)	RESULTS	
				NEGATIVE	POSITIVE
8b) ProA	Proline Arylamidase If LIP is +. ProA is always -	Proline-4-methoxy- β naphthylamide	0.056	ZYM B / 3 min Yellow Pale orange (brown if LIP +)	ZYM B / 3 min Orange
9b) GGT	Gamma Glutamyl Transferase	Gamma glutamyl 4-methoxy- β naphthylamide	0.049	ZYM B / 3 min Yellow Pale orange (yellow-orange if PAL +)	ZYM B / 3 min Orange
10b) IND	INDole	Tryptophane	0.036	JAMES / 3 min Colorless	JAMES / 3 min Pink

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.