PURPOSE: The API NH system is used for the identification of *Neisseria* spp., *Haemophilus* spp. and *Moraxella catarrhalis*. The strip consists of 10 microtubes containing dehydrated substrates, which enable the performance of 12 identification tests as well as the detection of a penicillinase. The reactions are read and recorded into the APIweb electronic database. The software interprets the reactions and provides identification for the organism.

SAMPLE INFORMATION:

Type	Few, well-isolated colonies from an isolation plate belonging to the genera: • *Neisseria* (Gram-negative cocci in pairs), • *Haemophilus* (small, Gram-negative coccobacilli) • *Moraxella catarrhalis* (Gram-negative cocci in pairs)

REAGENTS and/or MEDIA:

Type	BioMerieux API NH reagent strip, tray and lid
Stability and Storage Requirements	• Store strips at 2°C to 8°C until the expiry date indicated on the packaging.

PLEASE NOTE:

- **The API NH is only to be used on Gram-negative diplococci that are oxidase positive and small, Gram-negative bacilli resembling *Haemophilus* spp.**
- **Any positive result from a sterile site for *Haemophilus influenzae* or *Neisseria meningitidis* must be sent immediately to Provincial Lab Edmonton for typing as soon as identification is confirmed. Assure there is a purity plate made that can be used for this purpose and can be sent out the day the identification is confirmed. Refer to MIC10510.**

SUPPLIES:

- Sterile water
- Ampule 0.85% saline
- Ampule protector
- Sterile swabs
- Sterile pipettes
- Mineral oil
- 35° ambient air incubator
- James reagent (see Appendix)
- ZYM B reagent (see Appendix)

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures.

- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used when there is a known or potential risk of exposure to splashes.
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
- The use of needles, syringes and other sharp objects should be strictly limited.

All patient specimens are assumed to be potentially infectious. Universal precautions must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

QUALITY CONTROL:

- Quality control is performed on new shipments/new lot numbers:
  - *Neisseria gonorrhoeae* ATCC 31426, profile number 1001. ProA should be positive.
- A TQC order is automatically generated when a new kit is received to record the QC results.

PROCEDURE INSTRUCTIONS:

Step	Action
Performing the API NH	
1	**Preparation of the strip:** a. Dispense 2 mL of sterile water into the honey-combed wells of the tray to create a humid atmosphere. b. Record the specimen accession number and date and time on the elongated flap of the tray. Do not record on the lid as it may be misplaced during the procedure. c. Remove the strip from its individual packaging. d. Place the strip in the incubation box.
2	**Preparation of the inoculum:** a. Open an ampule of API NaCl 0.85% with the ampule protector. b. Using a sterile swab, pick up a few, well-isolated colonies and prepare a suspension with a turbidity equivalent to a 4 McFarland ensuring it is well mixed. c. The suspension should be used immediately after preparation.
3	**Inoculation of the strip:** a. Tilt the incubation tray and fill the tube section of the microtubes by placing the pipette tip against the side of the cupule to minimize bubble formation. b. Only fill the tube part of the first 7 microtubes (PEN to URE) c. Fill the tube and cupule of the last 3 microtubes [LIP/ProA], [PAL/GGT] and [βGAL/IND] avoiding the formation of a convex meniscus. d. Cover the first 7 tests (PEN to URE) with mineral oil. **NOTE:** The quality of filling is very important. Tubes which are insufficiently or excessively filled may cause false positive or false negative results. e. Close the incubation box and incubate at 36°C ± 2°C for 2 to 2 ¼ hours in the O_2 incubator.

4	**Reading the strip:** a. After 2 hours, read the strip by referring to the Reading Table in the appendix of this procedure. b. Read the spontaneous reactions and record them as + or – on the results sheet. c. Add the following reagents: **[LIP/ProA]** and **[PAL/GGT]** add: 1 drop of ZYM B reagent. **[βGAL/IND]** add: 1 drop of James reagent. d. Wait 3 minutes then read the reactions by referring to the Reading Table. e. If the [LIP] reaction is positive, interpret the [ProA] reaction as negative whether the ZYM B reagent has been added or not. f. If, after 2 hour incubation, several reactions are doubtful, re-incubate the strip for a further 2 hours and read the reactions again.
5	**Interpretation of the strip:** a. Identification is obtained with the numerical profile of the organism. b. The tests are separated into groups of three. c. The following numerical value is assigned to each reaction recorded: 1 – Positive reaction in the first test of the group 2 – Positive reaction in the second test of the group 4 – Positive reaction in the third test of the group 0 – Negative reaction in any test d. By adding together the values corresponding to positive reactions within each group, a 4-digit profile number is obtained for the 12 tests of the API NH strip. e. Do not code the first test (penicillinase).
6	**Identification:** a. Log into APIweb: https://apiweb.biomerieux.com. ➢ At login, type: **laura_steven@gov.nt.ca** ➢ At password, type: **YKNIFE** ➢ Select **"Go"** ➢ Select **"API NH"** from the list. b. Enter the profile number results and select "Confirm". Record the profile number and result in the LIS.

LIMITATIONS/PRECAUTIONS:

1. The API NH system is intended uniquely for the identification of those species included in the database (see Identification Table at the end of the package insert) i.e., those belonging to the genera *Neisseria* and *Haemophilus* (and related genera) and to the species *Moraxella catarrhalis*. It cannot be used to identify any other microorganisms or to exclude their presence.
2. Certain species of the genera *Moraxella*, *Oligella*, etc. may be wrongly identified as *Neisseria meningitidis* and *Neisseria gonorrhoeae* since their biochemical profile on the API NH strip is very similar. *Neisseria meningitidis* profiles need to be confirmed by serological testing.
3. If the result of the [ProA] test is negative when *Neisseria gonorrhoeae* is identified, this identification must be confirmed using an alternative method.
4. Only pure cultures of a single organism should be used.

REFERENCES:

- BioMerieux, API NH package insert and API NH reagents package insert, 2016/12

APPENDIX (APPENDICIES):

I. James reagent:

- Store at 2°C to 8°C in the dark. Reagent may be kept up to 1 month after the ampules have been opened and the reagents reconstituted in the dropper-vial. Record the opening date on the bottle label.
- JAMES reagents are very sensitive to light.
- After transferring the contents of the ampules into the dropper-vials, wrap the bottles of JAMES reagents in aluminum foil.
- Make sure that the reagents are put back in the refrigerator immediately after use.
- **Reconstitution:** Open the ampule of solvent associated with the JAMES reagent (R1). Take up the content of the ampule using a completely dry pipette and transfer this solvent into the dropper-bottle (R2). Fit the dropper to the bottle and carefully close. Shake the bottle containing the dehydrated active ingredient. Wait approximately 10 minutes until the active ingredient is completely dissolved. Carefully close the bottle after use and store as indicated.
  NOTE: The JAMES reagent must only be used if it is pale yellow. If a pink color appears when the reagent is reconstituted with the solvent, wait until this pink color has completely disappeared before using the reagent.

II. ZYM B reagent:

- Store at 2°C to 8°C in the dark. Reagent may be kept up to 2 weeks after the ampules have been opened and the reagents reconstituted in the dropper-vials. Record the opening date on the bottle label.
- Check the appearance of the ZYM B reagent after reconstitution in the dropper-vial. After reconstitution, the ZYM B reagent is normally yellow to amber in color.
- Make sure that the reagents are put back in the refrigerator immediately after use.
- **Reconstitution:** Open the ampule of ZYM B (R1) solvent and transfer the content in the bottle of ZYM B (R2) reagent. Carefully close the bottle after use and store as indicated.

III. Reading Table:

READING TABLE

TESTS	ACTIVE INGREDIENTS	QTY (mg/cup.)	REACTIONS/ENZYMES	RESULTS	
				NEGATIVE	POSITIVE
1) PEN	potassium benzylpenicillin	1.36	PENicillinase	blue (penicillinase absent)	yellow yellow-green yellow-blue (penicillinase present)
2) GLU	D-glucose	0.5	acidification (GLUcose)	red red-orange	yellow orange
3) FRU	D-fructose	0.1	acidification (FRUctose)		
4) MAL	D-maltose	0.1	acidification (MALtose)		
5) SAC	D-saccharose (sucrose)	0.5	acidification (SACcharose)		
6) ODC	L-ornithine	0.552	Ornithine DeCarboxylase	yellow-green grey-green	blue
7) URE	urea	0.41	UREase	yellow	pink-violet
8a) LIP	5-bromo-3-indoxyl-caprate	0.033	LIPase	colorless pale grey	blue (+ precipitate)
9a) PAL	4-nitrophenyl-phosphate 2CHA	0.038	ALkaline Phosphatase	colorless pale yellow	yellow
10a) ßGAL	4-nitrophenyl-ßD-galactopyranoside	0.04	ß GALactosidase	colorless	yellow
8b) ProA	proline-4-methoxy-ß-naphthylamide	0.056	Proline Arylamidase if LIP is +, ProA is always –	ZYM B / 3 min yellow pale orange (brown if LIP +)	orange
9b) GGT	γ-glutamyl-4-methoxy-ß-naphthylamide	0.049	Gamma Glutamyl Transferase	ZYM B / 3 min yellow pale orange (yellow-orange if PAL +)	orange
10b) IND	L-tryptophane	0.036	INDole	JAMES / 3 min colorless	pink

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	23 MAR 19	Initial Release	L. Steven