PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC53200 – API NH Test	Policy Number:
Program Name: Laboratory Services	
Applicable Domain: Lab, DI and Pharmacy Services	
Additional Domain(s):	
Effective Date:	Next Review Date:
Issuing Authority: Director of Health Services	Date Approved:
Accreditation Canada Applicable Standard: N/A	

GUIDING PRINCIPLE:
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.

PURPOSE/RATIONALE:
This standard operating procedure describes how to perform the API NH test.

SCOPE/APPLICABILITY:
This procedure applies to Medical Laboratory Technologists (MLTs) performing the API NH test.

SAMPLE INFORMATION:

Type	Few, well-isolated colonies 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 strip
Stability and Storage Requirements	• Store strips at 2°C to 8°C

SUPPLIES:

- Sterile water
- Ampule 0.85% saline
- Ampule protector
- Plastic Vitek tubes and caps
- Disposable inoculation needles
- Sterile pipettes
- Mineral oil
- Blood agar
- James reagent
- ZYM B reagent

EQUIPMENT

- 35° ambient air incubator

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 Alberta Precision Laboratories 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-Referral of Category B Specimens to *Dyna*LIFE and Alberta Precision Labs**

SPECIAL SAFETY PRECAUTIONS:

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

- Ensure that appropriate hand hygiene practices be used.
- 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 of 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. Routine Practices 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
- 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 test	
1	**Preparation of the strip:** a. Dispense 2 mL of sterile water into the honey-combed wells of the tray b. Record the specimen accession number and date and time on the elongated flap of the tray 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 **NOTE:** This suspension must 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 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 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 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 g. Read the reactions by referring to the Reading Table on APIweb
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)

INTERPRETATION OF RESULTS:

Step	Action
1	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" c. Record the profile number and result in the LIS

LIMITATIONS:

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.

CROSS-REFERENCES:

- MIC10510-Referral of Category B Specimens to *Dyna*LIFE and Alberta Precision Labs

REFERENCES:

1. bioMérieux. (2016-12*).* *API NH* package insert

APPROVAL:

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Date

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REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	23 Mar 19	Initial Release	L. Steven
2.0	30 Jun 21	Procedure reviewed and added to NTHSSA policy template	L. Steven