STANTON TERRITORIAL HEALTH AUTHORITY

Yellowknife, Northwest Territories

TITLE: API-NH	Revision Date: 20-April-2018	Issue Date: 20-April-2016
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Distribution: Microbiology Test Manual	Page: 1 of 7	
Approved by: S. Asmussen, Manager of Diagnostic Services	Signed by: 	

PURPOSE:

The system is used for identification of *Neisseria spp.*, *Haemophilus spp.* and *Moraxella catarrhalis.* It utilizes 10 microtubes containing dehydrated substrates to test for 12 separate enzymatic reactions or sugar fermentations, as well as the presence of a penicillinase. Only organisms belonging to the above genera should be tested.

SAMPLE INFORMATION:

Storage Requirements	Kit (strip + saline): 2-8°C James/ZymB reagent: 2-8°C in the dark, expire 1 month after opening

REAGENTS and/or MEDIA:

- apiNH strips (bioMerieux Inc, REF 10 400)
- 0.85% NaCl (provided with kit)
- Incubation box
- ~ 5 mLs sterile water
- James reagent
- Zym B reagent
- Mineral oil
- Densichek
- Chocolate Agar Plate
- 35-37C Incubator

SUPPLIES:

- Sterile pipette

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 where 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.

QUALITY CONTROL:

Performed on each shipment/lot# received using the following strain:

- *Neisseria gonorrhoeae* ATCC31426

PEN	GLU	FRU	MAL	SAC	ODC	URE	LIP	PAL	βGAL	ProA	GGT	IND
+	+	-	-	-	-	-	-	-	-	+	-	-

Generate TQC order via TQC Order Entry – result QC results in TQC

PROCEDURE INSTRUCTIONS:

Step	Action
Setting Up and Interpreting apiNH Strips	
1	In the plate log – Order **^NH**
2	Prepare the incubation tray by adding 5mL of sterile water – filling up the honeycomb wells
3	Remove the strip from its packaging and place it in the incubation tray
4	Write the Specimen# and date on the flap attached to the tray
5	Using the provided 0.85% NaCl prepare a suspension of the organism in question to the equivalent of 4.0 McFarland - the culture should be pure and 18-24hrs old
6	Tilt the API strip and using a sterile pipette slowly distribute the bacterial suspension into the tubes • Hold the pipette tip against the top side of the well to minimize bubble formation
7	Fill the tube of the first 7 microtubules PEN→URE
8	Fill the tube and cupules of the last 3 microtubules |LIP/ProA|, |PAL/GGT|, |BGAL/IND| Avoid the formation of a convex meniscus
9	Cover all underlined tests with mineral oil (PEN→URE)
10	Cover and Incubate the tray for 2-2.5hrs at 36C in O2
11	Using 1 drop of your suspension, inoculate a Chocolate Agar Plate for purity – Incubate in CO2
12	After the incubation period, read the reactions and record the results on the resulting sheet
13	To the following wells add the reagents: |ProA|, |GGT| - 1 drop of ZymB |IND| - 1 drop of James Wait 3 minutes then read the reactions See Appendix for reagent reconstitution instructions
14	**If after 2 hrs reactions are doubtful, re-incubate for an additional 2 hours
15	Refer to the attached "**Reference Table**" for color reactions or the supplied package

	insert
16	Log into the apiweb: https://apiweb.biomerieux.com
17	Login name: **NSTANTONTERRITORIALHOSPITAL** Password: **YKNIFE** Hit **Go**
18	Select the appropriate API item (ie. APINH)
19	Input reactions and hit "**CONFIRM**"
20	Print out ID sheet and evaluate the outcome
21	Limitations of test: 1. If the ID is *N.gonorrhoeae*, ensure that the |ProA| is positive – the ID must be confirmed at Dynalife 2. Certain species of *Moraxella* may be wrongly identified at *N. meningitis* or *N. gonorrhoeae* – confirm with serological testing at Dynalife

INTERPRETATION OF RESULTS:

TESTS	REACTIONS	RESULTS	
		NEGATIVE	POSITIVE
1) PEN	PENicillinase	Blue (penicillinase absent)	Yellow Yellow-green Yellow-blue (penicillinase present)
2) GLU 3) FRU 4) MAL 5) SAC	GLUcose (Acidification) FRUctose (Acidification) MALtose (Acidification)	red red-orange	yellow orange

	Saccharose/Sucrose (Acidification)		
6) ODC	Ornithine DeCarboxylase	yellow-green grey-green	blue
7) URE	UREase	yellow	pink-violet
8a) |LIP|	LIPase	colourless pale grey	blue (+ precipitate)
9a) |PAL|	ALKaline Phosphatase	colourless pale yellow	yellow
10a) |BGAL|	Beta GALactosidase	colourless	yellow
8b) |ProA|	Proline Arylamidase if LIP is + ProA is always -	ZYM B / 3 min	
		yellow pale orange (brown if LIP +)	orange
9b) |GGT|	Gamma Glutamyl Transferase	ZYM B / 3 min	
		yellow pale orange (yellow-orange if PAL +)	orange
10b) |IND|	INDole	JAMES / 3 min	
		Colourless	Pink

APPENDIX:

Type	ZYM B Reagent	James Reagent
Storage Requirements	2-8°C in the dark	
Stability	• Stable until the expiration date indicated on the packaging. • The reagents may be kept for up to 1 month after the ampoules have been opened and the reagents transferred into the dropper bottles.	
Reagent Preparation	1. Break the ampoule cap. 2. Turn the ampoule upside	1. Break open the ampoule of solvent associated with the

	down and maintain it in a vertical position. 3. Squeeze gently on the cap to release a drop to transfer the entire reagent into the dropper bottle.	James reagent (R1). 2. Take up the contents of the ampoule using a completely dry pipette and transfer this solvent into the dropper bottle (R2). 3. Fit the dropper to the bottle, carefully close it and shake the bottle containing the dehydrated active ingredient. 4. Wait 10 minutes until it is completely dissolved.
Notes	These reagents are VERY sensitive to light: check the appearance of the reagents before transferring them into the dropper bottles and before each use. After transferring the contents of the ampoules into the dropper-bottles, put the bottles into a used Vitek card foil package (or other aluminum foil).	
	The Zym B reagent is normally yellow to amber in colour. Dispose of the reagent if any tint of pink is observed (sign of deterioration). Exposure to laboratory lighting for a short period of time (approx. 1 hour) will damage the reagent.	The James reagent may only be used if it is pale yellow. If a pink colour appears when the reagent is reconstituted with the solvent, wait until this pink colour has completely disappeared before using the reagent.

REFERENCES:

- bioMerieux. (2010, 02). apiNH.

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	31Dec2013	Initial Release	A.Darrach
2.0	31Mar16	Update of “Special Safety Precautions” to reflect risk assessment recommendations.	C. Russell