TITLE: API-NE	Revision Date: 06-March-2016	Issue Date: 06-March-2014
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Distribution: Microbiology Test Manual	Page: 1 of 6	
Approved by: C. Case, Manager of Diagnostic Services	Signed by: Cheryl Case	

PURPOSE:

This system is used for the identification of non-fastidious, non-enteric Gram negative rods. The strip consists of 20 microtubes containing dehydrated substrates. The reactions are read and inputted into the database for identification.

SAMPLE INFORMATION:

Storage Requirements	Store at 2-8°C

REAGENTS and/or MEDIA:

- API 20NE strip and incubation box (bioMerieux Inc, REF 20 050)
- Blood Agar Plate (BAP)
- 5 mL of 0.85% NaCl
- 5 mL API AUX medium (supplied)
- ~5 mL sterile water
- Ferric Chloride Reagent
- James or Kovacs Reagent
- NIT1 and KOH Reagent
- Zn Powder
- Mineral Oil
- 29°C Incubator

SUPPLIES:

- Sterile Pipette
- Densichek

SPECIAL SAFETY PRECAUTIONS:

- Standard precautions should always be followed.

QUALITY CONTROL:

Performed on each shipment/lot # received:

1. Aeromonas hydrophilia ATCC35654
2. Alcaligenes faecalis ATCC35655

QC Org	NO3	TRP	GLU	ADH	URE	ESC	GEL	PNPG	GLU	ARA	MNE	MAN	NAG	MAL	GNT	CAP	ADI	MLT	CIT	PAC	OX
1	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	-*	-	+
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+

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

PROCEDURE INSTRUCTIONS:

Step	Action
Setting Up an API-20E	
1	In your plate log – Order **^NE.**

2	Prepare the incubation tray by adding 5 mL 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 number on the flap attached to the tray and date.
5	Order and perform an oxidase **(^OX)** and record the result on the result sheet.
6	Aliquot approximately 3 mLs of 0.85% saline into a plastic test tube.
7	Prepare a 0.5 McFarland suspension of the organism – the culture should be pure and 18-24 hours old.
8	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.
9	Inoculate tests NO3 → PNPG filling only the tubes.
10	Open the API AUX Medium ampule and add approximately 200 µL of the 0.5 McFarland – mix well avoiding the formation of bubbles.
11	Fill the tubes AND cupules from IGLUI → IPACI with the suspension ensuring a slightly convex meniscus.
12	Overlay GLU, ADH, and URE with mineral oil until a convex meniscus is formed.
13	Using one drop of the suspension – streak out a BA Purity Plate.
14	Incubate at 29°C +/- 2°C for 24 hours.
15	Inspect the Purity Plate – if not pure, repeat API using pure culture.

16	The following wells require the addition of reagent:
	1. **NO3:** Add 1 drop NIT1 and 1 drop 40% KOH – wait 5 minutes
	• **Red** colour develops: **Positive**
	• No colour develops: add 2-3 mg of Zn to the cupule – wait 5 minutes
	  ○ After the addition of Zn – **Pink** colour develops – test is **negative** as the Zn reacts with the nitrates left in the tube
	  ○ After the addition of Zn – No **Pink** develops – test is **positive** as all of the NO3 has been converted to nitrogen gas and has dissipated into the air

	2. TRP: Add 1 drop of James reagent
17	Refer to the **Reference Table** for colour reactions or the supplied package insert. **Assimilation Tests:** Observe for bacterial growth. An opaque cupule indicates a positive reaction.
18	Log in to the apiweb: https://apiweb.biomerieux.com
19	Login name: **NSTANTONTERRITORIALHOSPITAL** Password: **YKNIFE** Hit **Go**.
20	Select the appropriate API item (ie. APINE).
21	Input reactions and hit "**CONFIRM**".
22	Print out ID sheet and evaluate the outcome.
23	Re incubation for an additional 24 hours is necessary if: 1. Low discrimination 2. Unacceptable/doubtful profile 3. "Identification not valid before 48 hour incubation"
24	If re-incubation is required – remove the NIT1, KOH and James Reagents by suction with a pipette.
25	Re-incubate at 29°C for an additional 24 hours.
26	All tests from ADH → IPACI can be re-read at 48 hours.

EXPECTED RESULTS:

Consult the Identification Table at the end of the package insert for a range of expected results.

REFERENCES:

- bioMerieux. (2006, 02). api 20E.

REFERENCE TABLE:

TESTS	REACTIONS	RESULTS	
		NEGATIVE	POSITIVE
$N0_3$	Reduction of nitrates to nitrites	NIT 1 + KOH ~5 minutes	
		Colorless*	Pink-red
	Reduction of nitrates to nitrogen	*Addition of Zn ~5 mins	
		Pink	colorless
TRP	Indole Production	JAMES - IMMEDIATELY	
		Colorless Pale green/Yellow	Pink
GLU	Fermentation	Blue to Green	Yellow
ADH	Arginine DiHydrolase	Yellow	Orange/pink/red
URE	UREase	Yellow	Orange/pink/red
ESC	Hydrolysis(B-glucosidase)	Yellow	Grey/brown/black
GEL	Hydrolysis (prolease)	No pigment diffusion	Diffusion of black pigment
PNPG	B-galactosidease	Colorless	Yellow
|GLU|	Assimilation (GLUcose)	Transparent	Opaque
|ARA|	Assimilation (ARAbinose)	Transparent	Opaque
|MNE|	Assimilation (MANNosE)	Transparent	Opaque
|MAN|	Assimilation (MANnitol)	Transparent	Opaque
|NAG|	Assimilation (N-Acetyl-)	Transparent	Opaque
|MAL|	Assimilation (MALtose)	Transparent	Opaque
|GNT|	Assimilation(potassium GlucoNate)	Transparent	Opaque
|CAP|	Assimilation (CAPric Acid)	Transparent	Opaque
|ADI|	Assimilation (Adipic Acid)	Transparent	Opaque
|MLT|	Assimilation (MaLaTe)	Transparent	Opaque
|CIT|	Assimilation (trisodium CITrate)	Transparent	Opaque
|PAC|	Assimilation (PhenylAcelic acid)	Transparent	Opaque
OX	Cytochrome oxidase	Transparent	Opaque

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	31Jul13	Initial Release	A. Darrach
1.1	06Mar14	Document control number changed from MTE10215 to MIC50215	C. Russell