TITLE: API-NE	Revision Date: 20-April-2018	Issue Date: 20-April-2016
Document Number: MIC50215	Status: Approved	
Distribution: Microbiology Test Manual	Page: 1 of 6	
Approved by: S. Asmussen, Manager of Diagnostic Services	Signed by: 	

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:

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 or lot number received:

1. *Aeromonas hydrophilia* ATCC35654
2. *Alcaligenes faecalis* ATCC35655

QC Org	NO3	TRP	GLU	ADH	URE	ESC	GEL	PNPG	GLUI	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 o After the addition of Zn – **Pink** colour develops – test is **negative** as the

	Zn reacts with the nitrates left in the tube o 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
2.0	31Mar16	Update of “Special Safety Precautions” to reflect risk assessment recommendations.	C. Russell