STANTON TERRITORIAL HEALTH AUTHORITY

Yellowknife, Northwest Territories

TITLE: API-20E	**Revision Date:** 06-March-2016	**Issue Date:** 06-March-2014
Document Number: MIC50200	**Status: Approved**	
Distribution: Microbiology Test Manual	**Page:** 1 of 5	
Approved by: C. Case, Manager of Diagnostic Services	**Signed by:** Cheryl Case	

PURPOSE:

The API-20E is a useful tool for the identification of *Enterobacteriaceae* and other non-fastidious Gram-negative bacilli. It uses 21 miniaturized biochemical tests and an online database for identification. Organisms tested should be Gram-negative, oxidase negative bacilli. A few oxidase positive non-fastidious organisms can be identified as well.

SAMPLE INFORMATION:

Storage Requirements	Store at 2-7°C, stable for 10 months after opening foil pouch.

REAGENTS and/or MEDIA:

- API 20E strip and incubation box (bioMerieux Inc, REF 20 100)
- Blood Agar Plate (BAP)
- 5 mL of 0.85% NaCl
- ~5 mL sterile water
- Ferric Chloride Reagent
- James or Kovacs Reagent
- VP1 and VP2 Reagent
- NIT1 and KOH Reagent
- Zn Powder
- Mineral Oil

SUPPLIES:

- Sterile Pipette

SPECIAL SAFETY PRECAUTIONS:

- Standard precautions should always be followed.

QUALITY CONTROL:

Performed on each shipment/lot # received:

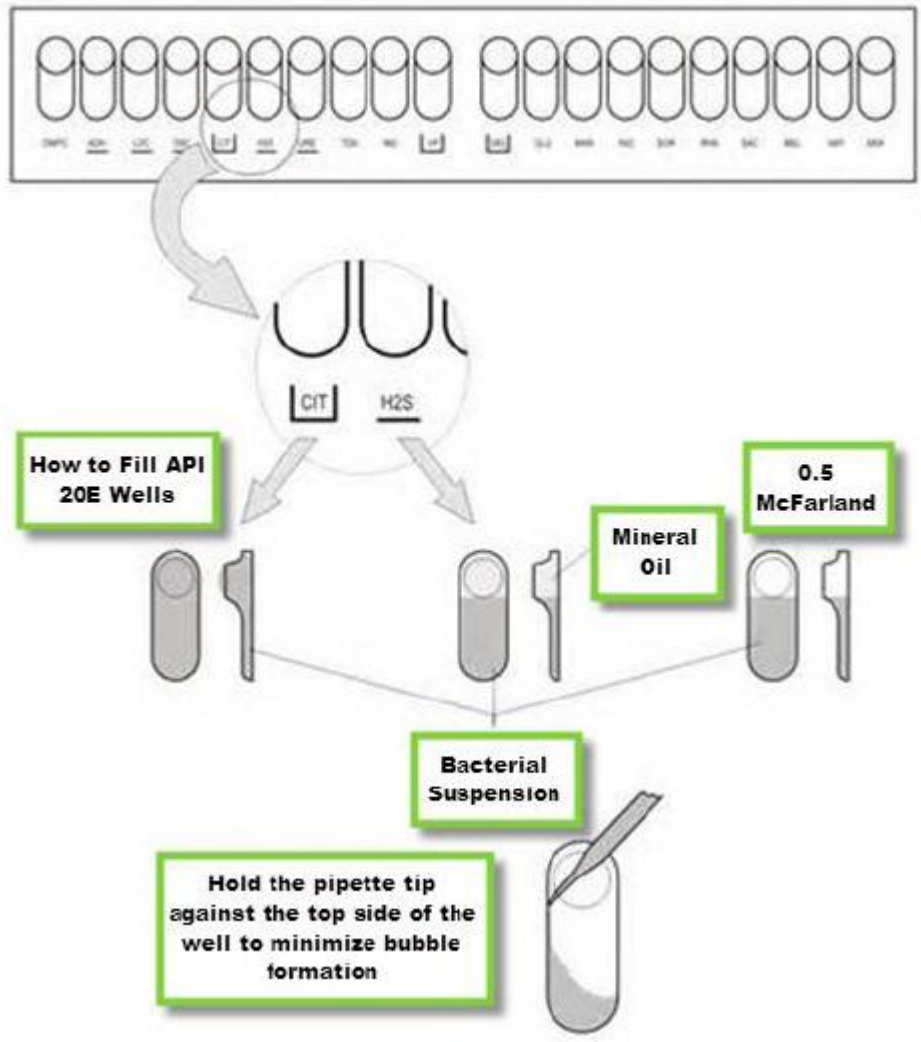
- E. coli ATCC25922

ONPG	ADH	LDC	ODC	CIT	H2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	NO2	N2
+	-	+	+	-	-	-	-	+	-	-	+	+	-	+	+	-	+	-	+	+	-

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 **^20E.**
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	Aliquot approximately 4 mLs of 0.85% saline into a plastic test tube.

6	Prepare a 0.5 McFarland suspension of the organism – the culture should be pure and 18-24 hours old.
7	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.
8	For the ICITI, IVPI, and IGELI – Fill both the tube and the cupule.
9	For all other tests, fill only the tube.
10	For all underlined tests: ADH, LDC, ODC, H2S, and URE – overlay with mineral oil to

	create an anaerobic environment.
11	Incubate at 36°C +/- 2°C for 18-24 hours.
12	Using 1 drop of the suspension, streak out a BAP for purity.
13	If **less than 3** tests are positive, re-incubate the strip for an additional 24 hours **BEFORE** adding any reagents.
14	Inspect the purity plate – if not pure, repeat API using a pure culture.
15	The following wells require the addition of reagent: **TDA:** Add 1 drop of ferric chloride **IND:** Add 1 drop of James (Kovacs) reagent ***Perform test last as the gas produced interferes with other tests. **VP:** Add 1 drop each of VP1 and VP2 reagent – wait 10 minutes.
16	Refer to the Reference Table for colour reactions in the supplied package insert. Package inserts are located in the MIC D3 drawer to the right of the wound bench.
17	Log in to the apiweb: https://apiweb.biomerieux.com
18	Login name: **NSTANTONTERRITORIALHOSPITAL** Password: **YKNIFE** Hit **Go**.
19	Select the appropriate API item (ie. API20E).
20	Input reactions and hit "**CONFIRM**".
21	Print out ID sheet and evaluate the outcome.

REFERENCES:

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

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	31Jul13	Initial Release	A. Darrach
1.1	06Mar14	Document control number changed from MTE10200 to MIC50200	C. Russell