

Practical 10
API 20 E
(Analytical Profile Index System for Identification of Enterobacteriaceae)



Principles

- API 20E Strip is miniaturized version of conventional tests
- Identification of members of Enterobacteriaceae and other Gram negative rods,
- This system utilizes a plastic strip with 20 separate compartments.
- Each compartment consists of a depression, or cupule, and a small tube that contains a specific dehydrated medium
- Consists of 20 microtubes containing dehydrated substrates.
- These tests are inoculated with a bacterial suspension
- During incubation, the metabolism of the organism produces colour changes.

Materials

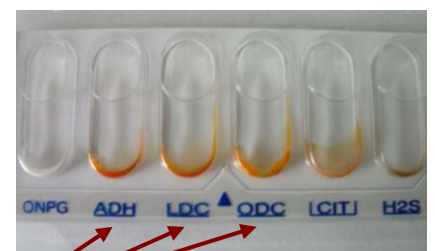
API 20 E Strips
Incubation boxes
Report sheets
Disposable Plastic pipettes
Disposable plastic inoculating loop
5 ml sterile distilled water
Mineral Oil
MacConkey agar plate

Procedure

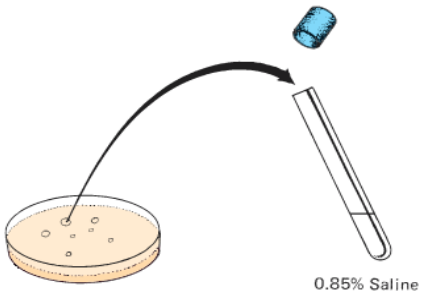
- 1-prepare an incubation tray and lid and distribute 5 ml of sterile distilled water into the tray to create a humid chamber.
- 2- Place the API20E strip in the tray.
- 3-Using a sterile disposable loop remove a single well-isolated colony from an isolation plate and carefully emulsify this in 5 ml of sterile distilled water.
- 4-With a sterile disposable pipette fill both the tube and cupule of the test CIT, VP and GGL with the bacterial suspension.
- 5-Using same pipette fill only the tubes of the other test.
- 6-Creat an aerobaic in the tests ADH , LDC , ODC , URE and H2S by overlaying with mineral oil.
- 7-Inoculate and streak Mackoncey purity plate.
- 8- place a lid on the incubation tray and incubate both the strip and the MacKoncey plate at 37 C for 18 – 24 hours.



(Fill cupules)

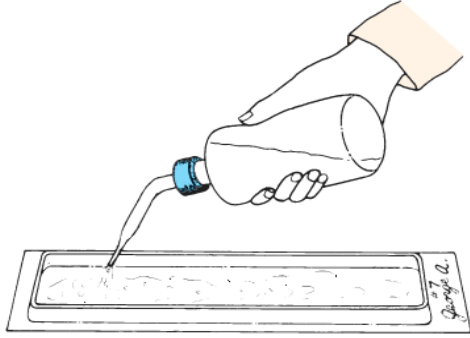


(Anaerobic condition)

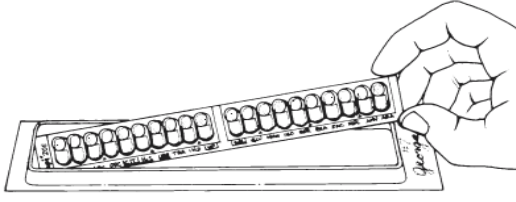


0.85% Saline

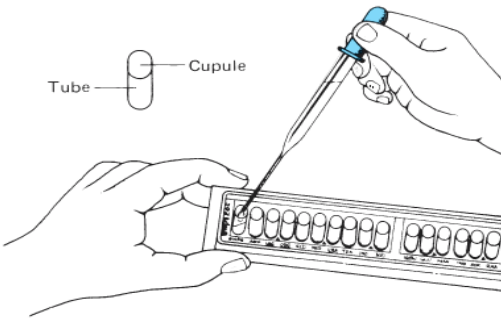
1 Select one well-isolated colony to make a saline suspension of the unknown organism. Suspension should be well dispersed with a Vortex mixer.



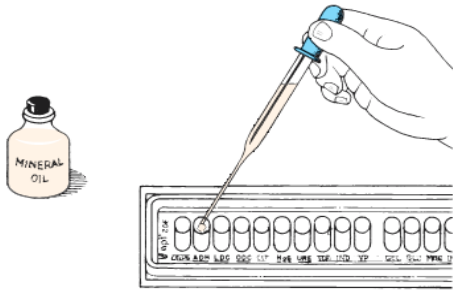
2 After labeling the end tab of a tray with your name and unknown number, dispense approximately 5 ml. of tap water into bottom of tray.



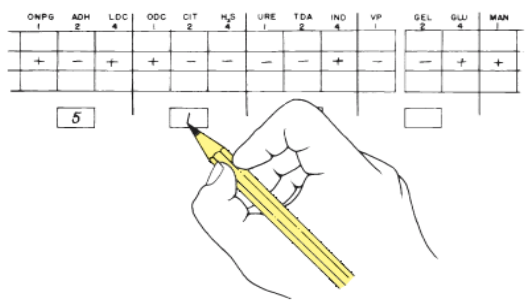
3 Place an API 20E test strip into the bottom of the moistened tray. Be sure to seal the pouch from which the test strip was removed to prevent contamination of remaining strips.



4 Dispense saline suspension of organisms into cupules of all twenty compartments. Slightly *underfill* ADH, LDC, ODC, H₂S, and URE. *Completely fill* cupules of CIT, VP, and GEL.



5 To provide anaerobic conditions for chambers ADH, LDC, ODC, H₂S, and URE, completely fill cupules of these chambers with sterile mineral oil. Use a fresh sterile Pasteur pipette.



ONPG	ADH	LDC	ODC	CIT	H ₂ S	URE	TDA	IND	VP	GEL	GLU	MAN
1	2	4	1	2	4	1	2	4	1	2	4	1
+	-	+	+	-	-	-	-	+	-	-	+	+

6 After incubation and after adding test reagents to four compartments, record all results and total numbers to arrive at 7-digit code. Consult the *Analytical Profile Index* to find the unknown.

READING THE API 20

TESTS	SUBSTRATE	REACTION TESTED	- RESULTS	+ RESULTS
ONPG	ONPG	beta-galactosidase	colorless	yellow
ADH	arginine	arginine dihydrolase	yellow	red/orange
LDC	lysine	lysine decarboxylase	yellow	red/orange
ODC	ornithine	ornithine decarboxylase	yellow	red/orange
CIT	citrate	citrate utilization	pale green/yellow	blue-green/blue
H2S	Na thiosulfate	H2S production	colorless/gray	black deposit
URE	urea	urea hydrolysis	yellow	red/orange
TDA	tryptophan	deaminase	yellow	brown-red
IND	tryptophan	indole production	yellow	red (2 min.)
VP	Na pyruvate	acetoin production	colorless	pink/red (10 min.)
GEL	charcoal gelatin	gelatinase	no diffusion of black	black diffuse
GLU	glucose	fermentation/oxidation	blue/blue-green	yellow
MAN	mannitol	fermentation/oxidation	blue/blue-green	yellow
INO	inositol	fermentation/oxidation	blue/blue-green	yellow
SOR	sorbitol	fermentation/oxidation	blue/blue-green	yellow
RHA	rhamnose	fermentation/oxidation	blue/blue-green	yellow
SAC	sucrose	fermentation/oxidation	blue/blue-green	yellow
MEL	melibiose	fermentation/oxidation	blue/blue-green	yellow
AMY	amygdalin	fermentation/oxidation	blue/blue-green	yellow
ARA	arabinose	fermentation/oxidation	blue/blue-green	yellow
OX	oxidase	oxidase	colorless/yellow	violet