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Microbial Diagnosis

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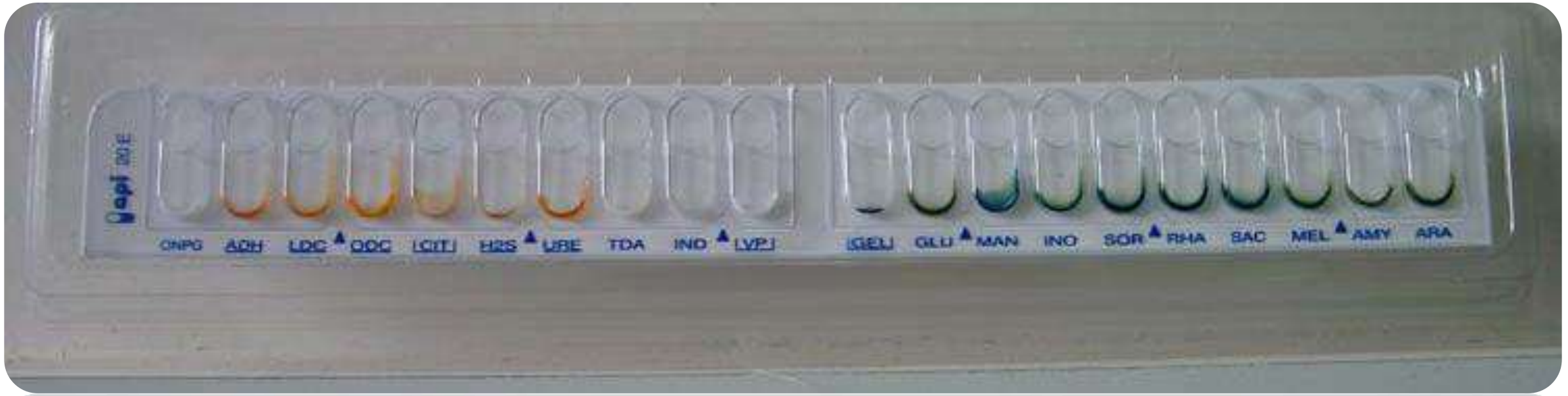
Identification of a microbial isolate usually follows from a sequence of morphological, biochemical, immunological, and genetic techniques. Delineation of the biochemical activities of a microbial isolate is the most convenient way to narrow the search path towards the identity of an unknown strain. Biochemical reactions, for the purpose of identification, often utilize a limited combination of metabolic and enzymatic activities prominent and pertinent to a specific group of microorganisms that share these common activities.

Variations in biochemical activities exhibited by members of the same group of organisms are then utilized to differentiate between these individuals and ultimately lead to identification. Among the most commonly utilized microbial biochemical activities are fermentation of sugars (carbohydrates), utilization of certain carbon sources, production of certain unique fermentation products, possession of specific enzymes, etc.



(API 20E)

Analytical Profile Index System for Identification of *Enterobacteriaceae*

- **The Analytical Profile Index (API)** is a miniaturized panel of biochemical tests compiled for identification of groups of closely related bacteria. Different test panels are prepared in dehydrated forms which are reconstituted upon use by addition of bacterial suspensions. After incubation, positive test results are scored as a seven-digit number (profile). Identity of the bacterium is then easily derived from the database with the relevant cumulative profile code book or software.

- **API 20E** presented herein is a biochemical panel for identification system and differentiation of members of the family *Enterobacteriaceae* and Gram negative rods .
- Other API panels for other groups of bacteria, such as staphylococci and streptococci, are also available in the same format.

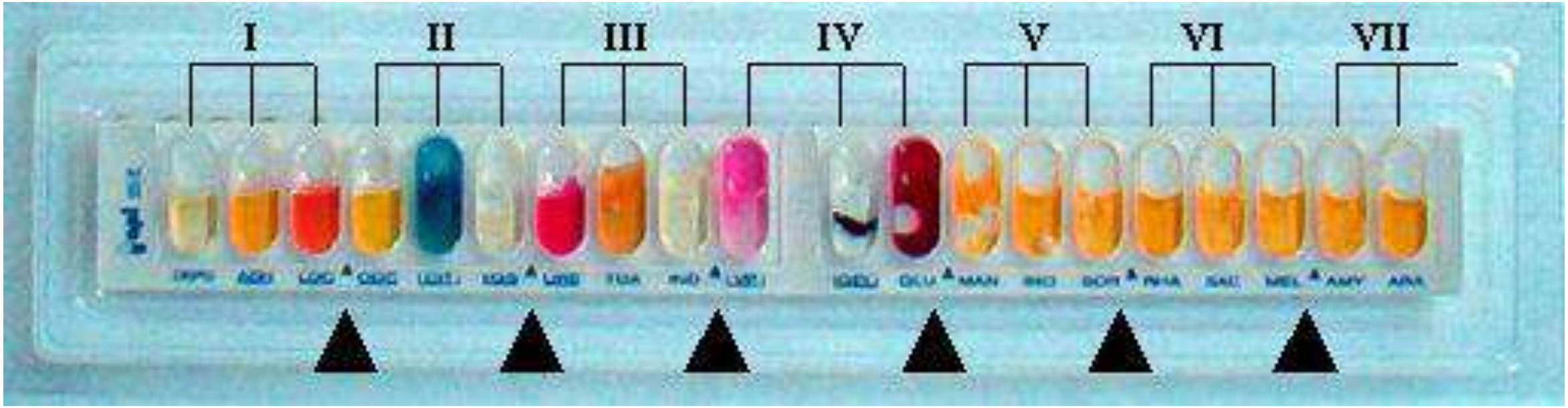
- **API 20E has a** plastic strip holds twenty mini-test chambers containing dehydrated media having chemically-defined compositions for each test, which uses 21 miniaturized biochemical tests and a database.
- The complete list of those organisms that it is possible to identify with this system is given in the Identification.
- Table at the end of this package insert.

These include:

- **1.ONPG:** test for b-galactosidase enzyme by hydrolysis of the substrate o-nitrophenyl-b-D-galactopyranoside
- **2.ADH:** decarboxylation of the amino acid arginine by arginine dihydrolase
- **3.LDC:** decarboxylations of the amino acid lysine by lysine decarboxylase
- **4.ODC:** decarboxylations of the amino acid ornithine by ornithine decarboxylase
- **5.CIT:** utilization of citrate as sole carbon source
- **6.H2S:** production of hydrogen sulfide
- **7.URE:** test for the enzyme urease
- **8.TDA:** detection of the enzyme tryptophan deaminase

- **9.IND:** production of indole from tryptophan by the enzyme tryptophanase. Indole is detected by addition of Kovac's reagent.
- **10.VP:** the Voges-Proskauer test for the detection of acetoin (acetyl methylcarbinol) produced by fermentation of glucose by bacteria utilizing the butylene glycol pathway
- **11.GEL:** test for the production of the enzyme gelatinase which liquefies gelatin
- **12.GLU:** fermentation of glucose (hexose sugar)
- **13.MAN:** fermentation of mannose (hexose sugar)

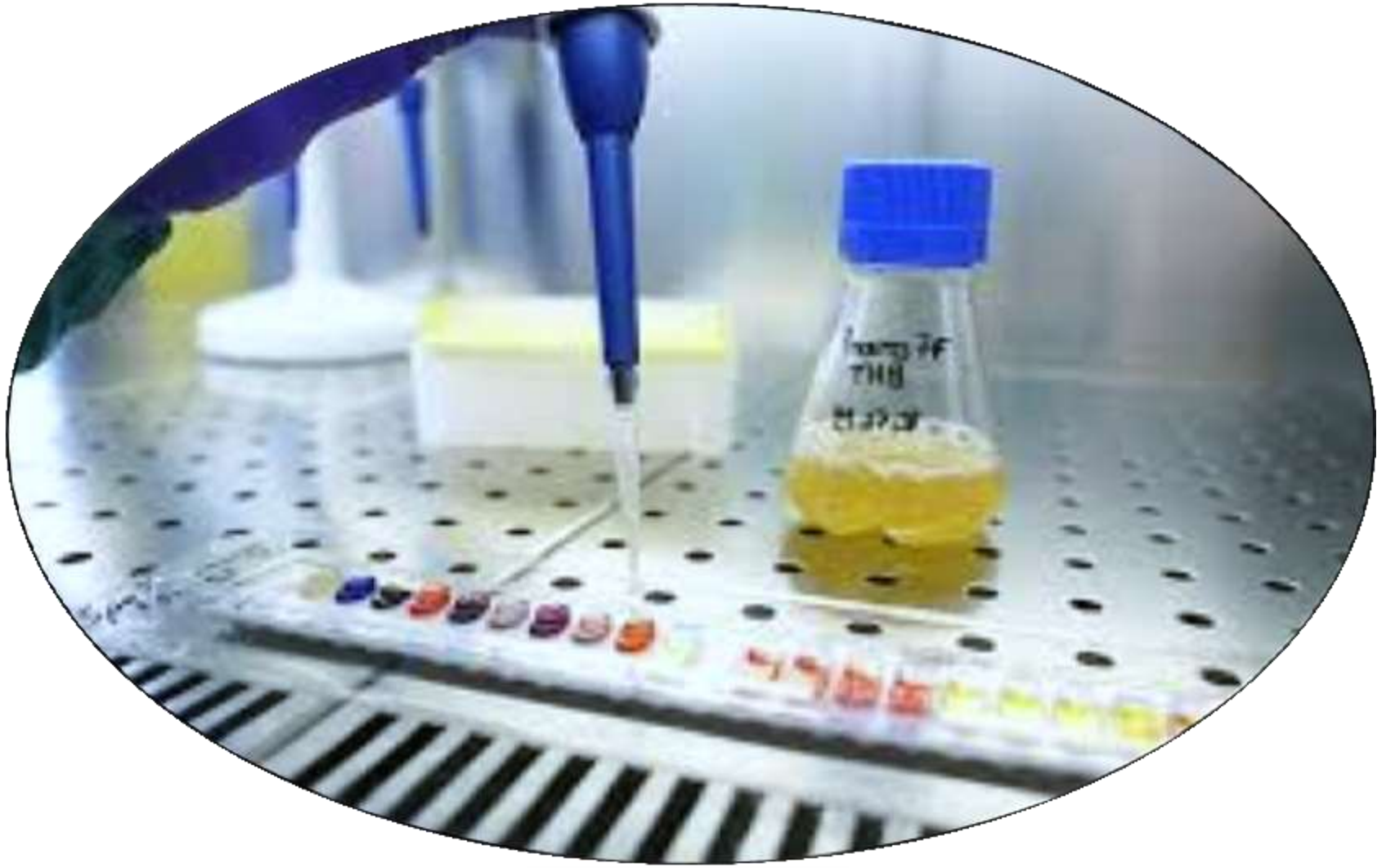
- **14.INO:** fermentation of inositol (cyclic polyalcohol)
- **15.SOR:** fermentation of sorbitol (alcohol sugar)
- **16.RHA:** fermentation of rhamnose (methyl pentose sugar)
- **17.SAC:** fermentation of sucrose (disaccharide)



- **18.MEL:** fermentation of melibiose (disaccharide)
- **19.AMY:** fermentation of amygdalin (glycoside)
- **20.ARA:** fermentation of arabinose (pentose sugar)
- **The OX test** is a test for cytochrome oxidase which is performed separately from the above tests. It is done using a portion of a bacterial colony on a paper strip impregnated by the oxidase reagent N,N,N',N'-tetramethylphenylenediamine which turns blue if cells possess oxidase enzyme.

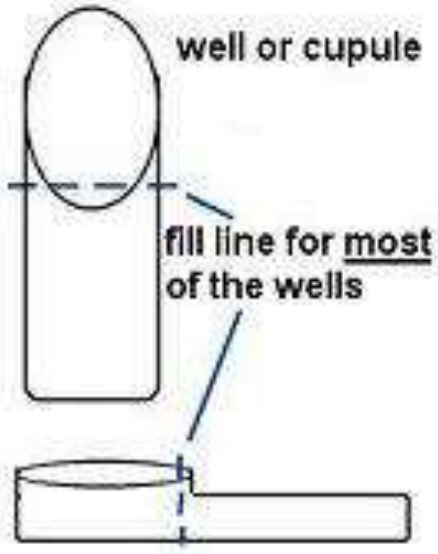
READING TABLE

TESTS	ACTIVE INGREDIENTS	QTY (mg/cup.)	REACTIONS/ENZYMES	RESULTS	
				NEGATIVE	POSITIVE
ONPG	2-nitrophenyl-ßD-galactopyranoside	0.223	ß-galactosidase (Ortho NitroPhenyl-ßD-Galactopyranosidase)	colorless	yellow (1)
ADH	L-arginine	1.9	Arginine DiHydrolase	yellow	red / orange (2)
LDC	L-lysine	1.9	Lysine DeCarboxylase	yellow	red / orange (2)
ODC	L-ornithine	1.9	Ornithine DeCarboxylase	yellow	red / orange (2)
CIT	trisodium citrate	0.756	CITrate utilization	pale green / yellow	blue-green / blue (3)
H_2S	sodium thiosulfate	0.075	H_2S production	colorless / greyish	black deposit / thin line
URE	urea	0.76	UREase	yellow	red / orange (2)
TDA	L-tryptophane	0.38	Tryptophane DeAminase	TDA / immediate	
				yellow	reddish brown
IND	L-tryptophane	0.19	INDole production	JAMES / immediate	
				colorless pale green / yellow	pink
VP	sodium pyruvate	1.9	acetoin production (Voges Proskauer)	VP 1 + VP 2 / 10 min	
				colorless	pink / red (5)
GEL	Gelatin (bovine origin)	0.6	GELatinase	no diffusion	diffusion of black pigment
GLU	D-glucose	1.9	fermentation / oxidation (GLUcose) (4)	blue / blue-green	yellow / greyish yellow
MAN	D-mannitol	1.9	fermentation / oxidation (MANnitol) (4)	blue / blue-green	yellow
INO	inositol	1.9	fermentation / oxidation (INOsitol) (4)	blue / blue-green	yellow
SOR	D-sorbitol	1.9	fermentation / oxidation (SORbitol) (4)	blue / blue-green	yellow
RHA	L-rhamnose	1.9	fermentation / oxidation (RHAmnose) (4)	blue / blue-green	yellow
SAC	D-sucrose	1.9	fermentation / oxidation (SACcharose) (4)	blue / blue-green	yellow
MEL	D-melibiose	1.9	fermentation / oxidation (MELibiose) (4)	blue / blue-green	yellow
AMY	amygdalin	0.57	fermentation / oxidation (AMYgdalin) (4)	blue / blue-green	yellow
ARA	L-arabinose	1.9	fermentation / oxidation (ARAbinose) (4)	blue / blue-green	yellow
OX	(see oxidase test package insert)		cytochrome-OXidase	(see oxidase test package insert)	



PRINCIPLE

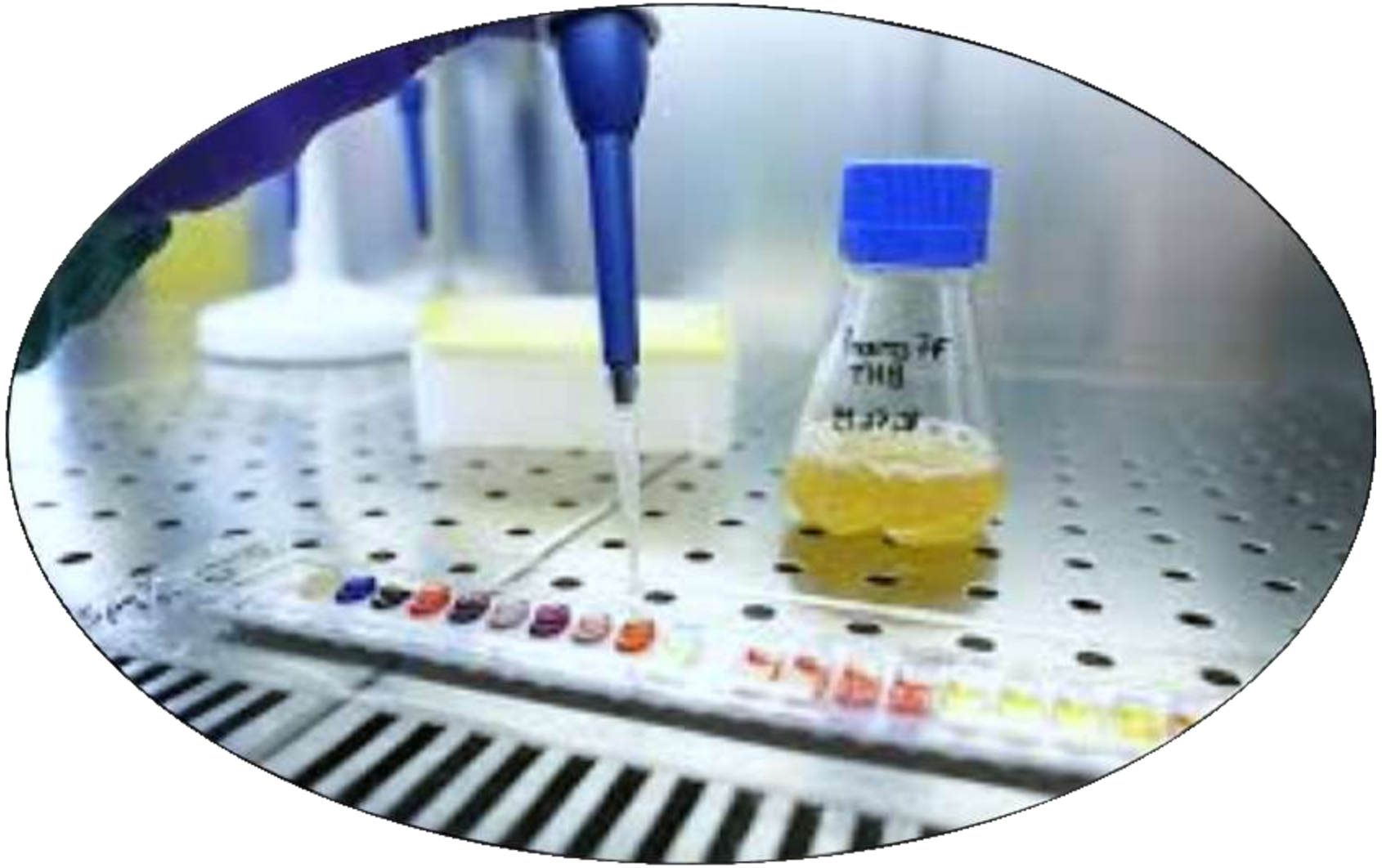
- API 20E Strip is miniaturized version of conventional tests.
- Identification of members of *Enterobactereaceae* and other Gram negative rods.
- This system utilizes a plastic strip with 20 separate compartments.
- Each compartment consists of a depression, or cupule, and a small tube that contains a specific dehydrated medium.



- Consists of 20 microtubules containing dehydrated substrates.
- These tests are inoculated with a bacterial suspension.
- During incubation, the metabolism of the organism produces color changes or revealed by the addition of reagents.
- The reactions are read according to the Reading Table and the identification is obtained by referring to the Analytical Profile Index or using the identification software.

MATERIALS:

- API 20 E Strips
- Incubation boxes
- Report sheets
- Disposable Plastic pipettes
- Disposable plastic inoculating
- 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 API 20E 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.

(Fill cupules)

5- Using same pipette fill only the tubes of the other test.

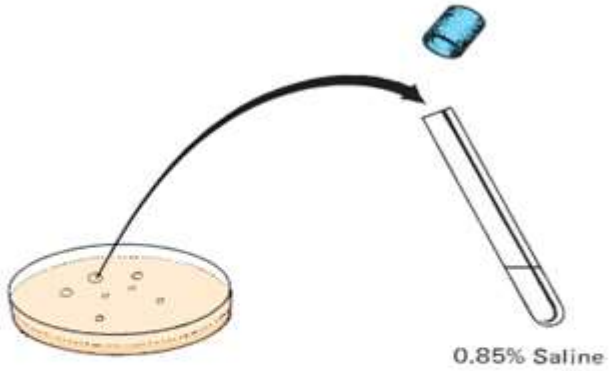
6- Creat an aerobaic in the tests ADH , LDC , ODC , URE and H2S by overlaying with mineral oil.

7- Inoculate and streak Mackoncey purity plate.

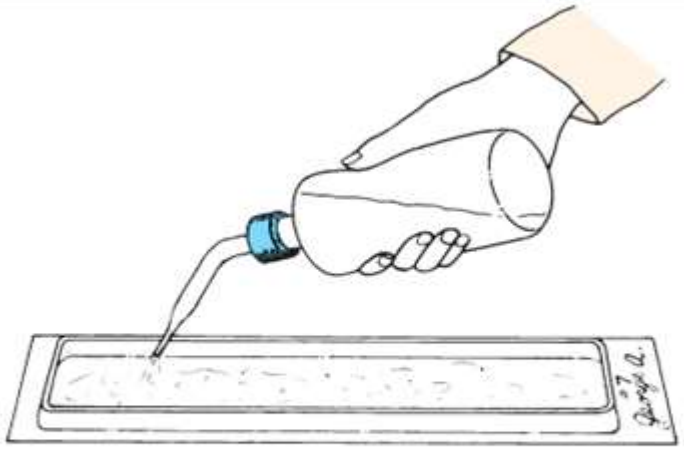
8- Mark the tray with identification number (Patient ID or Organism ID), date and your initials.

9- place a lid on the incubation tray and incubate both the strip and the MacKoncey plate at 37 C for 18 – 24 hours.

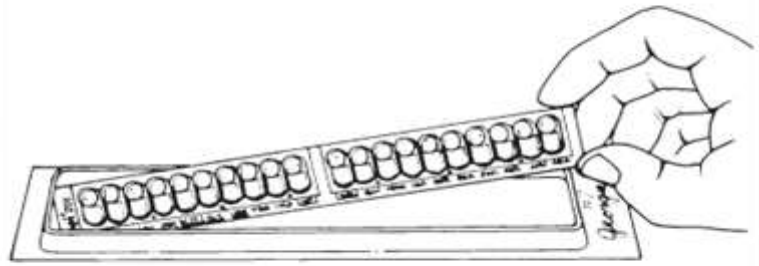
(Anaerobic condition)



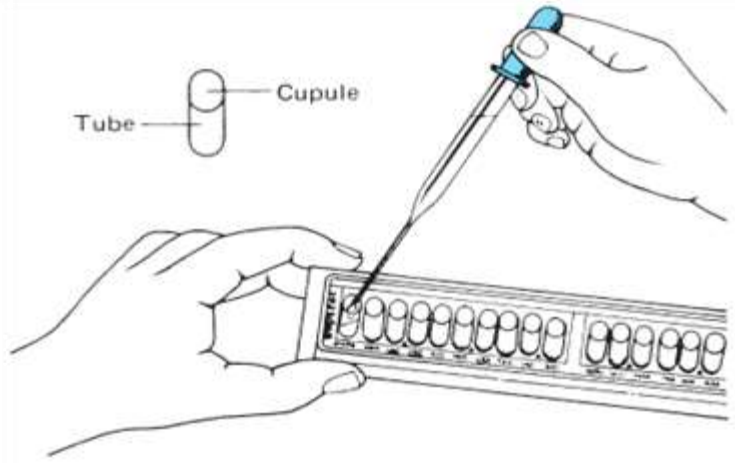
1 Select one well-isolated colony to make a saline suspension of the unknown organism. Suspension should be well dispersed with a Vortex mixer.



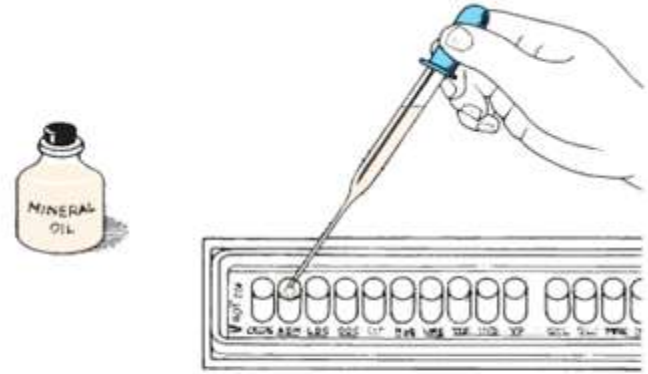
2 After labeling the end tab of a tray with your name and unknown number, dispense approximately 5 ml. of tap water into bottom of tray.



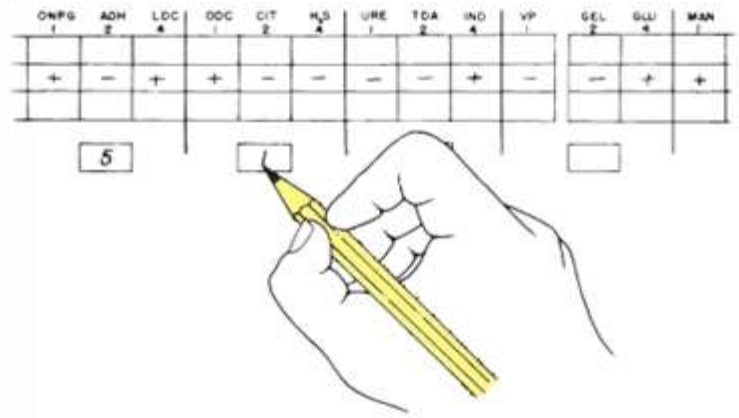
3 Place an API 20E test strip into the bottom of the moistened tray. Be sure to seal the pouch from which the test strip was removed to prevent contamination of remaining strips.



4 Dispense saline suspension of organisms into cupules of all twenty compartments. Slightly *underfill* ADH, LDC, ODC, H_2S, and URE. *Completely fill* cupules of CIT, VP, and GEL.



5 To provide anaerobic conditions for chambers ADH, LDC, ODC, H_2S, and URE, completely fill cupules of these chambers with sterile mineral oil. Use a fresh sterile Pasteur pipette.



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.

Results and Interpretation

- **For some of the compartments, you can just read the change in color straight way after 24 hours but for some you have to put reagents before reading.**

- **1-**Check the MacKoncey purity plate to ensure. If the culture is mixed repeat the test.

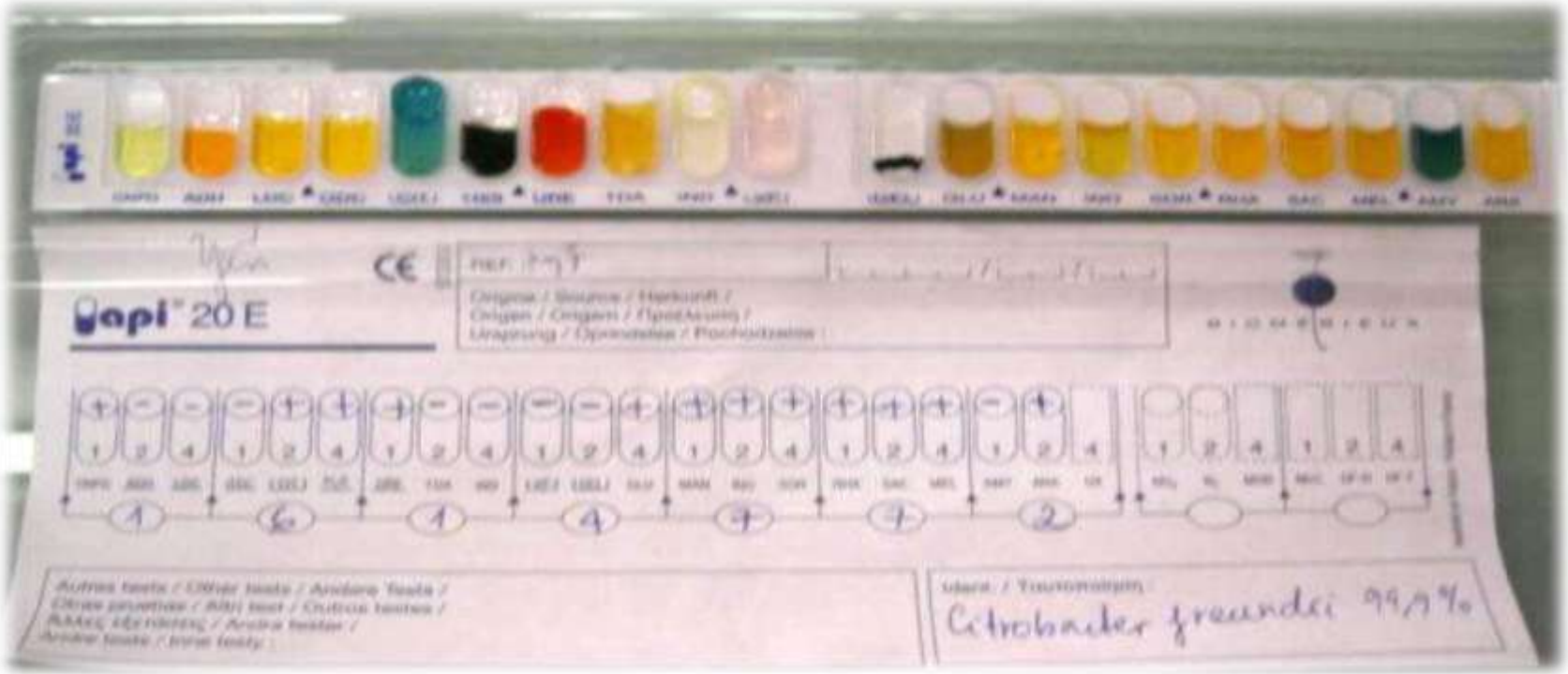
- **2-**Reveal the test which require the addition of reagent as follows:

- Vp test. Put one drop of 40 % KOH (VP reagent 1) & One drop of VP Reagent 2 (α-Naphthol) (you have to wait for 10 minutes before telling it negative).

- **For some of the compartments, you can just read the change in color straight way after 24 hours but for some you have to put reagents before reading.**
- **TDA test:** add one drop of TDA reagent (Put one drop of Ferric Chloride A reddish brown color indicates a positive reaction to be recorded on the result sheet.
- **IND test:** add one drop of James reagent (Put one drop of Kovacs reagent), add 1 drop of JAMES reagent.
- A pink color developed in the whole cupule indicates a positive reaction to be recorded on the result sheet.

- **3-** Get the API Reading Scale (color chart).
- Mark each test as positive or negative on the lid of the tray
- Read the API strip according to the interpretation table, and record the result on the report sheet.

- **4-** On the report sheet, the test are separated into groups of three and number 1 , 2 or 4 is allocated for each test. By adding the numbers corresponding to the positive reaction within each group, a7- digit profile number is obtained for 20 tests of the API 20E strip.
- **5-** The 7- digit profile is then compared with the numerical profile in the API 20 E analytical profile index book to obtain the organism identification.



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api® 20 E

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Origine / Source / Herkunft /
Origen / Origem / Προέλευση /
Ursprung / Oprindelse / Pochodzenie :

BIOMÉRIEUX

1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4
ONPG	ADH	LDC	ODC	CIT	H_2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	OX	NO_2	N_2	MOB	McC	OF-O	OF-F			
5	1	1	5	1	1	5	1	1	5	1	1	5	1	1	5	1	1	5	1	1									

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Autres tests / Other tests / Andere Tests /
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Άλλες εξετάσεις / Andra tester /
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Ident. / Ταυτοποίηση :

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Any Questions

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