API 20 E

I. Principle

API 20 E is a standardized identification system for *Enterobacteriaceae* and other non-fastidious, Gram-negative bacilli. The API 20 E system consists of a series of 20 microtubes containing dehydrated substrates. These tests are inoculated with a bacterial suspension that reconstitutes the media. During incubation, metabolism produces color changes that are either spontaneous or revealed by the addition of reagents. The reactions are interpreted according to the Reading Table, and the identification is obtained by referring to the bioMèrieux online database.

II. Specimen

Use only pure, well-isolated colonies of the test isolate.

III. Materials and Reagents

1. API 20 E test strip, store at 2-8 °C until expiration date stated on box
2. Sterile NaCl 0.85% suspension medium
3. Sterile applicators
4. API incubation trays and covers
5. Distilled water
6. Sterile Pasteur pipettes
7. Sheep blood agar plates
8. Individual reagents: TDA, Kovacs, VP 1 & VP 2, NIT 1 & NIT 2
9. Zn reagent
10. Oxidase reagent
11. Mineral oil
12. API 20 E report sheets
13. API code book or identification software

IV. Quality Control

1. Quality control should be performed on each lot number of API 20 E received into the laboratory.
2. Remove the following organisms from the –70 °C freezer for subculture:
    i. *Escherichia coli* ATCC 25922
    ii. *Stenotrophomonas maltophilia* ATCC 51331
    iii. *Enterobacter cloacae* ATCC 13047
    iv. *Proteus mirabilis* ATCC 35659
    v. *Klebsiella pneumoniae* ssp. *pneumoniae* ATCC 35657
3. Subculture each organism to a TSA 5% sheep blood agar plate.
4. Allow organisms to incubate overnight at 36 °C. Repeat subculture to a new TSA plate, and allow culture to grow overnight before performing QC.
5. Using a plate with overnight growth, prepare inoculum and set-up strips following the API 20 E procedure.
6. After 18-24 h of incubation in a non-CO_2 incubator, interpret results as indicated.

7. Results should be logged into the computer. Acceptable results are as follows:

	ONPG	ADH	LDC	ODC	CIT	H_2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	NO_2	N_2
i.	+	-	+	+	-	-	-	-	+	-	-	+	+	-	+	+	-	+	-	+	+	-
ii.	+	-	V	-	V	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
iii.	+	+	-	+	+	-	-	-	-	+	-	+	+	-	+	+	+	+	+	+	+	-
iv.	-	-	-	+	V	+	+	+	-	-	V	+	-	-	-	-	V	-	-	-	+	-
v.	+	-	+	-	+	-	V	-	-	V	-	+	+	+	+	+	+	+	+	+	+	-

V. Safety

1. Wear gloves to protect skin.
2. Observe routine biosafety procedures.

VI. Procedure

1. Preparation of the strip
    i. Prepare an incubation tray, and distribute about 5 mL of distilled water into the honeycombed wells of the tray to create a humid atmosphere.
    ii. Record the patient's specimen number on the elongated flap of the tray.
    iii. Remove a strip from its packaging, and place it into the incubation tray.
2. Preparation of the inoculum
    i. Open a tube of sterile saline (NaCl 0.85%).
    ii. Aseptically remove a single well-isolated colony from the isolation plate. It's recommended that an 18-24 h old culture be used.
    iii. Carefully emulsify the colony in the sterile saline to achieve a homogenous suspension. This suspension should be used immediately after preparation.
3. Inoculation of the strip
    i. Using the same pipette, fill both the tube and cupule of the tests CIT, VP and GEL with the bacterial suspension.
    ii. Fill only the tube (and not the cupule) of the rest of the tests.
    iii. Create anaerobiosis in the tests ADH, LDC, ODC, H_2S, and URE by overlaying with mineral oil.
    iv. Place the lid on the incubation tray.
    v. Incubate at 36 °C ± 2 °C for 18-24 h.

VII. Identification of Organisms

Reading the strip

1. After the incubation period, read the strip by referring to the Reading Table.
2. If 3 or more tests (GLU test + or -) are positive, record all the spontaneous reactions on the result sheet, and then reveal the tests which require the addition of reagents:
    i. VP test: add 1 drop each of VP 1 and VP 2 reagents. Wait at least 10 min. A pink or red color indicates a positive reaction. If a slightly pink color develops after 10 min, the reaction should be considered negative.

ii. TDA test: add 1 drop of TDA reagent. A reddish brown color indicates a positive reaction.
iii. IND test: add 1 drop of JAMES / Kovacs reagent. A pink color developed in the whole cupule indicates a positive. This test should be performed last since the reaction releases gasses that interfere with the interpretation of other tests on the strip. The plastic lid should not be replaced after the addition of the reagent.

3. If the number of positive tests (including the GLU test) before adding the reagents is less than 3:
   i. Reincubate the strip for another 24 h without adding any reagents.
   ii. Then reveal the tests that require the addition of reagents.

Reading Table

Tests	Substrates	Enzymatic Activity or Reaction Tested	Negative	Positive
ONPG	2-nitrophenyl-ßD-galactopyranoside	ß-galactosidase	Colorless	Yellow (1)
ADH	L-arginine	Arginine DiHydrolase	Yellow	Red / orange (2)
LDC	L-lysine	Lysine DeCarboxylase	Yellow	Red / orange (2)
ODC	L-ornithine	ornithine DeCarboxylase	Yellow	Red / orange (2)
CIT	trisodium citrate	CITrate utilization	Pale green / yellow	Blue-green / blue (3)
H2S	sodium thiosulfate	H_2S production	Colorless / greyish	Black deposit / thin line
URE	Urea	UREase	Yellow	Red / orange (2)
TDA	L-tryptophane	Tryptophane DeAminase	TDA (1 drop) / immediate	
			Yellow	Reddish brown
IND	L-tryptophane	INDole production	JAMES (1 drop) / immediate	
			Colorless/pale green/yellow	Pink
VP	Sodium pyruvate	acetoin production (Voges Proskauer)	VP1 (1 drop) + VP2 (1 drop) / 10 min	
			Colorless	Pink / red (5)
GEL	Gelatin (bovine origin)	GELitinase	No diffusion	Diffusion of black pigment
GLU	D-glucose	Fermentation / oxidation (4)	Blue / blue-green	Yellow
MAN	D-mannitol	Fermentation / oxidation (4)	Blue / blue-green	Yellow
INO	Inositol	Fermentation / oxidation (4)	Blue / blue-green	Yellow
SOR	D-sorbitol	Fermentation / oxidation (4)	Blue / blue-green	Yellow
RHA	L-rhamnose	Fermentation / oxidation (4)	Blue / blue-green	Yellow
SAC	D-sucrose	Fermentation / oxidation (4)	Blue / blue-green	Yellow
MEL	D-melibiose	Fermentation / oxidation (4)	Blue / blue-green	Yellow
AMY	Amygdalin	Fermentation / oxidation (4)	Blue / blue-green	Yellow
ARA	L-arabinose	Fermentation / oxidation (4)	Blue / blue-green	Yellow
OX	on filter paper	Cytochrome-OXidase	Colorless	Violet

(1) A very pale yellow should be considered positive.
(2) An orange color after 36-48 hours incubation must be considered negative.
(3) Reading made in the cupule (aerobic).
(4) Fermentation begins in the lower portion of the tubes and oxidation begins in the cupule.
(5) A slightly pink color after 10 minutes should be considered negative.

Interpretation

1. Determination of the numerical profile
   i. On the result sheet. The tests are separated into groups of 3 and a value of 1, 2, or 4 is indicated for each. By adding the values

corresponding to positive reactions within each group, a 7-digit profile number is obtained for the 20 tests of the API 20 E strip. The oxidase reaction constitutes the 21st test and has a value of 4 if positive.

2. Identification
    i. Access online database at: https://apiweb.biomerieux.com/jsp/ident/index.jsp
    ii. Enter Login: **JSHMICRO** and Password: **SHMICRO**
    iii. Select API 20E from list of tests.
    iv. Enter reactions and click Confirm to see results.
3. In some cases the 7-digit profile is not discriminatory enough, and the following supplementary tests need to be carried out to form a 9-digit profile:
    i. Reduction of nitrates to nitrites (NO_2) and N_2 gas: Add 1 drop each of NIT 1 and NIT 2 reagents to the GLU tube. Wait 2-5 min. A red color indicates a positive reaction (NO_2). A negative reaction (yellow) may be due to the reduction to nitrogen gas. Add 2-3 mg of Zn reagent to the GLU tube. After 5 min, if the tube remains yellow, this indicates a positive reaction (N_2). If the test turns orange-red, this indicates a negative reaction.
    ii. Motility: perform a microscopic motility test.
    iii. Growth on MacConkey agar: Streak a MacConkey agar plate, and incubate overnight at 36 °C
    iv. Oxidation of glucose: Inoculate an OF glucose tube by stabbing the inoculum into the bottom of the hourglass tube, and incubate it with the lid loose in a non-CO_2 incubator overnight.

Supplementary Tests

Tests	Substrates	Enzymatic Activity or Reaction Tested	Negative	Positive
Nitrate reduction GLU tube	Potassium nitrate	NO_2 production	NIT 1 (1 drop) + NIT 2 / 2-5 minutes: Yellow	Red
		Reduction to N_2 gas	Zn / 5 minutes: Orange-red	Yellow
MOB	Microscopic test		Non-motile	Motile
McC	MacConkey medium	Growth	Absence	Presence
OF	Glucose (OF medium)	Fermentation: bottom tube	Green	Yellow
		Oxidation: top of tube	Green	Yellow

VIII. Result Reporting

1. If an acceptable identification is obtained, report the genus and species of the organism isolated.
2. If no identification is obtained, the isolate should be brought to the attention of the supervisor and the microbiology director for further workup.

IX. Limitations of the Testing

1. The API 20 E is intended uniquely for the identification of *Enterobacteriaceae* and the non-fastidious, Gram-negative rods included in the database (see

package insert). It cannot be used to identify any other microorganisms or to exclude their presence.
2. Discrepancies with respect to conventional methods may be observed. They are due to the different principles of the reactions used in the API technique. In addition, substrate variations exist that also account for percentage differences.
3. On rare occasions the glucose reactions for organisms such as *Klebsiella* or *Proteus* may revert from positive to negative, in which instance a bluish color is seen. This reaction will be recorded as a negative reaction. Such occurrences are reflected in the percentages indicated in the Identification Table of the package insert.
4. If *Salmonella* or *Shigella* are identified, serological identification must be performed to confirm the bacterial identification.
5. Only a pure culture of a single organism should be used.

X. References

1. API 20 E package insert, May 2004

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Updates and Revisions: