REF 20 100 / 20 160

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api® 20 E

IVD

Identification system for *Enterobacteriaceae* and other non-fastidious Gram-negative rods

SUMMARY AND EXPLANATION

API 20 E is a standardized identification system for *Enterobacteriaceae* and other non-fastidious, Gram-negative rods 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.

PRINCIPLE

The API 20 E strip consists 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 read according to the Reading Table and the identification is obtained by referring to the Analytical Profile Index or using the identification software.

CONTENT OF THE KIT

Kit for 25 tests (ref. 20 100)

- 25 API 20 E strips
- 25 incubation boxes
- 25 result sheets
- 1 clip seal
- 1 package insert

Kit for 100 tests (ref. 20 160)

- 100 API 20 E strips (4x25 strips)
- 100 incubation boxes
- 100 result sheets
- 1 clip seal
- 1 package insert

COMPOSITION OF THE STRIP

The composition of the API 20 E strip is given in the Reading Table of this package insert.

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED

Reagents :

- API NaCl 0.85 % Medium, 5 ml (Ref. 20 230) or API Suspension Medium, 5 ml (Ref. 20 150)
- API 20 E reagent kit (Ref. 20 120) or individual reagents : TDA (Ref. 70 402)
  JAMES (Ref. 70 542)
  VP 1 + VP 2 (Ref. 70 422)
  NIT 1 + NIT 2 (Ref. 70 442)
- Zn reagent (Ref. 70 380)
- Oxidase (Ref. 55 635*)
  * reference not sold in certain countries : use an equivalent reagent.
- Mineral oil (Ref. 70 100)
- API 20 E Analytical Profile Index (Ref. 20 190) or identification software (consult bioMérieux)

Material :

- Pipettes or PSIpettes
- Ampule protector
- Ampule rack
- General microbiology laboratory equipment

POSSIBLE ADDITIONAL REAGENTS :

- API OF Medium (Ref. 50 110) :
  Test for the determination of fermentative or oxidative metabolism.
- API M Medium (Ref. 50 120) :
  Test for motility of facultative anaerobic bacteria.

WARNINGS AND PRECAUTIONS

- **For *in vitro* diagnostic use and microbiological control.**
- **For professional use only.**
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest or inhale).
- All specimens, microbial cultures and inoculated products should be considered infectious and handled appropriately. Aseptic technique and usual precautions for handling the bacterial group studied should be observed throughout this procedure. Refer to "NCCLS M29-A, *Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue*; *Approved Guideline* - December 1997". For additional handling precautions, refer to "Biosafety in Microbiological and Biomedical Laboratories, HHS Publication No. (CDC) 93-8395, 3rd Edition (May 1993)", or to the regulations currently in use in each country.
- Do not use reagents past the expiration date.
- Before use, check that the packaging of the various components is intact.
- Do not use strips which have been damaged : cupules deformed, desiccant sachet open, ...
- The performance data presented were obtained using the procedure indicated in this package insert. Any change or modification in the procedure may affect the results.
- Interpretation of the test results should be made taking into consideration the patient history, the source of the specimen, colonial and microscopic morphology of the strain and, if necessary, the results of any other tests performed, particularly the antimicrobial susceptibility patterns.

STORAGE CONDITIONS

The strips are supplied in an aluminum pouch with desiccant sachets.
Once opened (*), the pouch should be re-sealed using the clip seal (included in the kit) to preserve the remaining strips with the desiccant sachets : place the open end of the pouch along the seal and carefully clamp between the two parts. The strips may then be kept for up to **10 months after the pouch has been opened**, at 2-8°C (or until the expiration date indicated on the packaging, if this comes before).
(*) *Recommended method for opening the pouches* : cut open the pouch just below the seal while holding the pouch upright, in order to avoid damaging the desiccant sachets.

SPECIMENS (COLLECTION AND PREPARATION)

API 20 E is not for use directly with clinical or other specimens.
The microorganisms to be identified must first be isolated on a culture medium adapted to the culture of *Enterobacteriaceae* and/or non-fastidious Gram-negative rods, according to standard microbiological techniques.

INSTRUCTIONS FOR USE

Oxidase test

The oxidase test must be performed according to the manufacturer's instructions for use. The result should be recorded on the result sheet as it is an integral part of the final profile (21st identification test).

Preparation of the strip

- Prepare an incubation box (tray and lid) and distribute about 5 ml of distilled water or demineralized water [or any water without additives or chemicals which may release gases (e.g., Cl_2, CO_2, etc.)] into the honeycombed wells of the tray to create a humid atmosphere.
- Record the strain reference on the elongated flap of the tray. (Do not record the reference on the lid as it may be misplaced during the procedure.)
- Remove the strip from its packaging.
- Place the strip in the incubation box.

NOTE : API 20 E should only be used with *Enterobacteriaceae* and/or non-fastidious Gram-negative rods. Fastidious organisms having demanding nutritional requirements and requiring appropriate handling precautions (i.e., *Brucella* and *Francisella*) are not included in the API 20 E database. Alternative procedures must be used to exclude or confirm their presence.

Preparation of the inoculum

- Open an ampule of API NaCl 0.85 % Medium (5 ml) or an ampule of API Suspension Medium (5 ml) as indicated in the paragraph "Warnings and Precautions" of the package insert for these products, or use any tube containing 5 ml of sterile saline or sterile distilled water, without additives.
- Using a pipette or PSIpette, remove a single well-isolated colony from an isolation plate. It is recommended to use young cultures (18-24 hours old).
- Carefully emulsify to achieve a homogeneous bacterial suspension.
  This suspension must be used immediately after preparation.

NOTE : most *Vibrio* species are halophilous. If a *Vibrio* is suspected, suspend the bacteria in API NaCl 0.85 % Medium.

Inoculation of the strip

- Using the same pipette, fill both tube and cupule of the tests CIT, VP and GEL with the bacterial suspension.
- Fill only the tube (and not the cupule) of the other tests.
- Create anaerobiosis in the tests ADH, LDC, ODC, H_2S and URE by overlaying with mineral oil.
- Close the incubation box.
- Incubate at 36°C ± 2°C for 18-24 hours.

READING AND INTERPRETATION

Reading the strip

- After the incubation period, read the strip by referring to the Reading Table.
- 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 :
  - TDA Test : add 1 drop of TDA reagent. A **reddish brown** color indicates a **positive** reaction to be recorded on the result sheet.
  - IND Test : 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.
  - VP Test : add 1 drop each of VP 1 and VP 2 reagents. Wait at least 10 minutes. A **pink** or **red** color indicates a **positive** reaction to be recorded on the result sheet. If a **slightly pink** color appears after 10 minutes, the reaction should be considered **negative**.

  NOTE : The indole production test must be performed last since this reaction releases gaseous products which interfere with the interpretation of other tests on the strip. The plastic incubation lid should not be replaced after the addition of the reagent.
- If the number of positive tests (including the GLU test) before adding the reagents is less than 3 :
  - Reincubate the strip for a further 24 hours (± 2 hours) without adding any reagents.
  - Reveal the tests requiring the addition of reagents (see previous paragraph).
  - To complete the identification, it may be necessary to perform supplementary tests (refer to Identification paragraph).

Interpretation

Identification is obtained with the **numerical profile**.

- Determination of the numerical profile :
  On the result sheet, the tests are separated into groups of 3 and a value 1, 2 or 4 is indicated for each. By adding together 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 it is positive.
- Identification :
  This is performed using the database (V4.0)
  * with the Analytical Profile Index :
    - Look up the numerical profile in the list of profiles.
  * with the identification software :
    - Enter the 7-digit numerical profile manually via the keyboard.

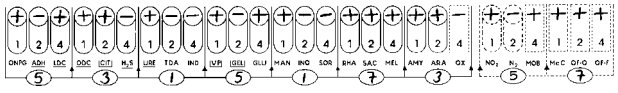
In some cases, the 7-digit profile is not discriminatory enough and the following supplementary tests need to be carried out :

- Reduction of nitrates to nitrites (NO_2) and N_2 gas (N_2) : add 1 drop each of NIT 1 and NIT 2 reagents to the GLU tube. Wait 2 to 5 minutes. A **red** color indicates a **positive** reaction (NO_2). A negative reaction (yellow) may be due to the reduction to nitrogen (as sometimes evidenced by gas bubbles) : add 2 to 3 mg of Zn reagent to the GLU tube. After 5 minutes, if the tube remains **yellow** this indicates a **positive** reaction (N_2) to be recorded on the result sheet. If the test turns **orange-red**, this is a **negative** reaction : the nitrates still present in the tube have been reduced by the Zinc. This reaction is useful when testing Gram-negative, oxidase positive rods.

NOTE : For the same reason as the indole test (see the note in the paragraph "Reading the strip"), the nitrate reduction test must be performed last.

- Motility (MOB) : Inoculate an ampule of API M Medium (see package insert).
- Growth on MacConkey agar medium (McC) : Streak a MacConkey agar plate (see package insert).
- Oxidation of glucose (OF-O) : Inoculate an ampule of API OF Medium (see package insert).
- Fermentation of glucose (OF-F) : Inoculate an ampule of API OF Medium (see package insert).

These supplementary tests, indicated in the introduction section (Profile coding) of the Analytical Profile Index, may be used to form a 9-digit profile. Identification is then obtained using the identification software.



5 315 173 (57) ***Enterobacter gergoviae***

Further tests may be proposed in case of low discrimination. Refer to the identification software or Analytical Profile Index.

QUALITY CONTROL

The media, strips, and reagents are systematically quality controlled at various stages of their manufacture. For those users who wish to perform their own quality control tests with the strip, it is preferable to use the strain **1. *Escherichia coli* ATCC 25922** or else one of the following strains :

2. *Stenotrophomonas maltophilia* ATCC 51331
3. *Enterobacter cloacae* ATCC 13047
4. *Proteus mirabilis* ATCC 35659
5. *Klebsiella pneumoniae* ssp *pneumoniae* ATCC 35657

ATCC : American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, USA.

	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*
1.	**+**	**–**	**+**	**+**	**–**	**–**	**–**	**–**	**+**	**–**	**–**	**+**	**+**	**–**	**+**	**+**	**–**	**+**	**–**	**+**	**+**	**–**
2.	+	–	V	–	V	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
3.	+	+	–	+	+	–	–	–	–	+	–	+	+	–	+	+	+	+	+	+	+	–
4.	–	–	–	+	V	+	+	+	–	–	V	+	–	–	–	–	V	–	–	–	+	–
5.	+	–	+	–	+	–	V	–	–	V	–	+	+	+	+	+	+	+	+	+	+	–

* The N_2 (+) state may be observed for the strain ATCC 13047 and the strain ATCC 25922.

- Profile obtained after 24-48 hours of incubation for the strain ATCC 51331, using colonies grown on Trypticase Soy agar + blood.
- Profiles obtained after 18-24 hours of incubation for the other strains, using colonies grown on Trypticase Soy agar + blood.
- Bacterial suspensions prepared in API NaCl 0.85 % Medium.

It is the responsibility of the user to perform Quality Control in accordance with any local applicable regulations.

LIMITATIONS OF THE METHOD

- The API 20 E system is intended uniquely for the identification of *Enterobacteriaceae* and those non-fastidious, Gram-negative rods included in the database (see Identification Table at the end of this package insert). It cannot be used to identify any other microorganisms or to exclude their presence.
- 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.
- On rare occasions, the glucose reactions for organisms such as *Klebsiella* or *Proteus* may revert from positive to negative, in which instance a bluish-green color is seen. This reaction will be recorded as a negative reaction. Such occurrences are reflected in the percentages indicated in the Identification Table.
- If *Salmonella* or *Shigella* are identified, serological identification must be performed to confirm the bacterial identification.
- Only pure cultures of a single organism should be used.

RANGE OF EXPECTED RESULTS

Consult the Identification Table at the end of this package insert for the range of expected results for the various biochemical reactions.

PERFORMANCE

- *Enterobacteriaceae* :
  5514 collection strains and strains of various origins belonging to species included in the database were tested:
  - 92.80 % of the strains were correctly identified (with or without supplementary tests).
  - 4.61 % of the strains were not identified.
  - 2.59 % of the strains were misidentified.

- Other non-fastidious Gram-negative rods :
  2386 collection strains and strains of various origins belonging to species included in the database were tested :
  - 90.32 % of the strains were correctly identified (with or without supplementary tests).
  - 6.16 % of the strains were not identified.
  - 3.52 % of the strains were misidentified.

WASTE DISPOSAL

It is the responsibility of each laboratory to handle waste and effluents produced according to their type and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

WARRANTY

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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	TDA / immediate: reddish brown
IND	L-tryptophane	0.19	INDole production	JAMES / immediate: colorless pale green / yellow	JAMES / immediate: pink
VP	sodium pyruvate	1.9	acetoin production (Voges Proskauer)	VP 1 + VP 2 / 10 min: colorless	VP 1 + VP 2 / 10 min: 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)	

(1) A very pale yellow should also 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, oxidation begins in the cupule.
(5) A slightly pink color after 10 minutes should be considered negative.

- The quantities indicated may be adjusted depending on the titer of the raw materials used.
- Certain cupules contain products of animal origin, notably peptones.

SUPPLEMENTARY TESTS

TESTS	ACTIVE INGREDIENTS	QTY (mg/cup.)	REACTIONS/ENZYMES	RESULTS	
				NEGATIVE	POSITIVE
Nitrate reduction GLU tube	potassium nitrate	0.076	NO_2 production	NIT 1 + NIT 2 / 2-5 min yellow	red
			reduction to N_2 gas	Zn / 5 min orange-red	yellow
MOB	API M Medium or microscope		motility	non-motile	motile
McC	MacConkey medium		growth	absence	presence
OF-F	glucose (API OF Medium)		fermentation : under mineral oil	green	yellow
OF-O			oxidation : exposed to the air	green	yellow

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