REF 20 050

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api® 20 NE

IVD

Identification system for non-fastidious, non-enteric Gram-negative rods

SUMMARY AND EXPLANATION

API 20 NE is a standardized system for the identification of non-fastidious, non-enteric Gram-negative rods (e.g. *Pseudomonas*, *Acinetobacter*, *Flavobacterium*, *Moraxella, Vibrio, Aeromonas,* etc.), combining 8 conventional tests, 12 assimilation 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 NE strip consists of 20 microtubes containing dehydrated substrates.
The conventional tests are inoculated with a saline bacterial suspension which reconstitutes the media. During incubation, metabolism produces color changes that are either spontaneous or revealed by the addition of reagents.
The assimilation tests are inoculated with a minimal medium and the bacteria grow if they are capable of utilizing the corresponding substrate.
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)

- 25 API 20 NE strips
- 25 incubation boxes
- 25 ampules of API AUX Medium
- 25 result sheets
- 1 package insert

COMPOSITION

Strip

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

Medium

API AUX Medium 7 ml	Ammonium sulphate	2 g
	Agar	1.5 g
	Vitamin solution	10.5 ml
	Trace elements	10 ml
	Monosodium phosphate	6.24 g
	Potassium chloride	1.5 g
	Demineralized water to make	1000 ml
	Final pH : 7.0-7.2	

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED

Reagents :

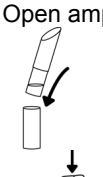
- API NaCl 0.85 % Medium, 2 ml (Ref. 20 070)
- Reagents : JAMES (Ref. 70 542)
  NIT 1 + NIT 2 (Ref. 70 442)
  Zn (Ref. 70 380)
- Oxidase (Ref. 55 635*)
  * reference not sold in certain countries : use an equivalent reagent.
- Mineral oil (Ref. 70 100)
- McFarland Standard (Ref. 70 900) No. 0.5
- API 20 NE Analytical Profile Index (Ref. 20 090) or identification software (consult bioMérieux)

Material :

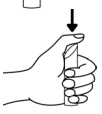
- Pipettes or PSIpettes
- Ampule protector
- Ampule rack
- General microbiology laboratory equipment

WARNINGS AND PRECAUTIONS

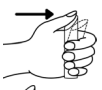
- **For *in vitro* diagnostic use and microbiological control.**
- **For professional use only.**
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore 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 and components are intact.
- Do not use strips which have been damaged : cupules deformed, etc.
- Open ampules carefully as follows :



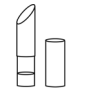
- Place the ampule in the ampule protector.
- Hold the protected ampule in one hand in a vertical position (white plastic cap uppermost).



- Press the cap down as far as possible.
- Cover the flattened part of the cap with the upper part of the thumb.



- Apply thumb pressure in an outward motion to the base of the flattened part of the cap to snap off the top of the ampule inside the cap.



- Take the ampule out of the ampule protector and put the protector aside for subsequent use.
- Carefully remove the cap.

- 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 and media should be stored at 2-8°C until the expiration date indicated on the packaging.

SPECIMENS (COLLECTION AND PREPARATION)

API 20 NE is not for use directly with clinical or other specimens.
The microorganisms to be identified must first be isolated on a suitable culture medium (e.g., Trypticase Soy agar) 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).

Selection of colonies

API 20 NE should only be used with non-fastidious Gram-negative rods which do not belong to the *Enterobacteriaceae*.

NOTE 1 : Some non-enteric Gram-negative rods are oxidase negative (*S. maltophilia*, *Acinetobacter*...). These microorganisms may also be identified with API 20 NE but their selection must be based on other bacteriological or clinical criteria.

NOTE 2 : Fastidious organisms having demanding nutritional requirements and requiring appropriate handling precautions (i.e. *Brucella* and *Francisella*) are not included in the API 20 NE database. Alternative procedures must be used to exclude or confirm their presence.

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 bottom of the tray to create a humid atmosphere.
- Record the specimen number on the elongated flap of the tray. (Do not record the number on the lid as it may be misplaced during the procedure.)
- Remove the strip from its individual packaging.
- Place the strip in the incubation box.

Preparation of the inoculum

- Open an ampule of API NaCl 0.85 % Medium (2 ml) as indicated in the paragraph "Warnings and Precautions" of the package insert for this product, or use any tube containing 2 ml of 0.85 % physiological saline without additives.
- Using a pipette or PSIpette, pick up 1-4 colonies of identical morphology from the agar plate, either by suction or by successive touches. It is recommended to use young cultures (18-24 hours old).
- Prepare a suspension with a turbidity equivalent to 0.5 McFarland. This suspension must be used immediately after preparation.

NOTE : It is very important that the density of the inoculum be adjusted to 0.5 McFarland ; the API 20 NE strip tests may otherwise not function correctly. In particular, a weaker inoculum may lead to false negative results. Do not touch the cupules while working with the strip and do not leave the strip exposed to air for a long period of time after inoculation.

Inoculation of the strip

- Inoculate tests NO_3 to PNPG by distributing the saline suspension into the tubes (and not the cupules) using the same pipette. To avoid the formation of bubbles at the base of the tubes, tilt the strip slightly forwards and place the tip of the pipette or PSIpette against the side of the cupule.
- Open an ampule of API AUX Medium as indicated in the paragraph "Warnings and Precautions" and add approximately 200 µl of the remaining saline suspension to the ampule. Homogenize well with the pipette, avoiding the formation of bubbles.
- Fill the tubes and cupules of tests |GLU| to |PAC| with the suspension. Take care to leave a flat or slightly convex, but not concave, meniscus. Cupules under or overfilled may give incorrect results.
- Add mineral oil to the cupules of the 3 underlined tests (GLU, ADH and URE) until a convex meniscus is formed.
- Close the incubation box and incubate at 29°C ± 2°C for 24 hours (± 2 hours).

READING AND INTERPRETATION

Reading the strip

- After the incubation period, read the strip by referring to the Reading Table.
- Record all spontaneous reactions (GLU, ADH, URE, ESC, GEL and PNPG) on the result sheet.
- The reading of the two tests NO_3 and TRP should be performed whilst protecting the assimilation tests from airborne contamination. To do this, cover the assimilation tests with the incubation box lid during the reading of the NO_3 and TRP tests.
- **NO_3 test :**
  - Add 1 drop of NIT 1 and 1 drop of NIT 2 reagents to the NO_3 cupule.
  - After 5 minutes, a **red** color indicates a **positive** reaction to be recorded on the result sheet.
  - A negative reaction may be due to the production of nitrogen (indicated by the presence of tiny bubbles) : add 2-3 mg of Zn reagent to the NO_3 cupule.
  - After 5 minutes, a cupule remaining **colorless** indicates a **positive** reaction to be recorded on the result sheet. If the cupule turns **pink-red**, the reaction is **negative** as nitrates were present in the tube and were reduced to nitrite by the zinc.

  The reaction used for the identification of the bacterium is the reduction of nitrates. It is positive when either of the above reactions (production of NO_2 or N_2) is positive.

  The production of N_2 may, however, be useful alone as a supplementary test (refer to the Analytical Profile Index).
- **TRP test :**
  Add 1 drop of JAMES reagent. The reaction takes place immediately : a **pink** color which develops in the whole cupule indicates a **positive** reaction to be recorded on the result sheet.

- **Assimilation tests :**
  Observe the bacterial growth. An **opaque** cupule indicates a **positive** reaction.
  Occasionally, a cupule may show weak growth. In this case, the results should be recorded as ∓ or ± by comparing the intensity to that of the other tests on the strip.
  Once these readings have been made, identification should be possible as indicated in the paragraph "Interpretation". However, in the following cases, the strip must be reincubated :
  - if the profile cannot be found in the API 20 NE Analytical Profile Index
  - if the following note is indicated for the profile obtained :

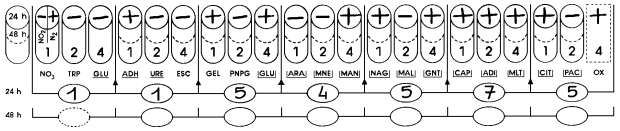
    IDENTIFICATION NOT VALID
    BEFORE 48-HR INCUBATION

  Using a pipette or PSIpette, remove the NIT 1, NIT 2 and JAMES reagents by suction and immediately cover tests NO_3 and TRP with mineral oil so that a convex meniscus is formed. Reincubate the strip at 29°C ± 2°C for a further 24 hours and read the all the tests again, except the first 3 (NO_3, TRP and GLU) which should only be read once at 24 hours.

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 number 1, 2 or 4 is indicated for each. By adding the values corresponding to positive reactions within each group, a 7-digit number is obtained ; the oxidase reaction constitutes the 21st test and has a value of 4 if it is positive.
- Identification :
  This is performed using the database (V6.0)
  * 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.



1 154 575 *Pseudomonas aeruginosa*

QUALITY CONTROL

The media, strips, and reagents are systematically quality controlled at various stages of their manufacture. For those who wish to perform their own quality control tests with the strip, it is preferable to use the strain **1. *Sphingobacterium multivorum* ATCC 35656** or else one of the following strains :

2. *Aeromonas hydrophila* ATCC 35654
3. *Pseudomonas aeruginosa* ATCC 27853
4. *Alcaligenes faecalis* ATCC 35655

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

	NO_3	TRP	GLU	ADH	URE	ESC	GEL	PNPG	GLU	ARA	MNE	MAN	NAG	MAL	GNT	CAP	ADI	MLT	CIT	PAC	OX
1.	**–**	**–**	**–**	**–**	**+**	**+**	**–**	**+**	**+**	**+**	**+**	**–**	**+**	**+**	**–**	**–**	**–**	**–**	**–**	**–**	**+**
2.	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	–	+	–*	–	+
3.	+	–	–	V	V	–	+	–	+	–	–	+	+	–	+	+	+	+	+	–	+
4.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+	+	+	+

* Weak reactions may occur.

Profiles for tests ADH to PAC obtained after 48 hours of incubation after culture of the colonies on Trypticase Soy agar.

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 NE system is intended uniquely for the identification of those non-fastidious, non-enteric 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.
- 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

5728 collection strains and strains of various origins belonging to species included in the database were tested :

- 92.53 % of the strains were correctly identified (with or without supplementary tests).
- 3.13 % of the strains were not identified.
- 4.34 % of the strains were misidentified.

WASTE DISPOSAL

Unused ampules of API AUX Medium may be considered as non hazardous waste and disposed of accordingly.
Dispose of all used or unused reagents (other than the ampules of API AUX Medium) as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.
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
NO_3	potassium nitrate	0.136	reduction of nitrates to nitrites	NIT 1 + NIT 2 / 5 min: colorless	pink-red
			reduction of nitrates to nitrogen	Zn / 5 min: pink	colorless
TRP	L-tryptophane	0.2	indole production (TRyptoPhane)	JAMES / immediate: colorless pale green / yellow	pink
GLU	D-glucose	1.92	fermentation (GLUcose)	blue to green	yellow
ADH	L-arginine	1.92	Arginine DiHydrolase	yellow	orange / pink / red
URE	urea	0.76	UREase	yellow	orange / pink / red
ESC	esculin ferric citrate	0.56 0.072	hydrolysis (β-glucosidase) (ESCulin)	yellow	grey / brown / black
GEL	gelatin (bovine origin)	0.6	hydrolysis (protease) (GELatin)	no pigment diffusion	diffusion of black pigment
PNPG	4-nitrophenyl-βD-galactopyranoside	0.22	β-galactosidase (Para-NitroPhenyl-ßD-Galactopyranosidase)	colorless	yellow
GLU	D-glucose	1.56	assimilation (GLUcose)	transparent	opaque
ARA	L-arabinose	1.4	assimilation (ARAbinose)	transparent	opaque
MNE	D-mannose	1.4	assimilation (ManNosE)	transparent	opaque
MAN	D-mannitol	1.36	assimilation (MANnitol)	transparent	opaque
NAG	N-acetyl-glucosamine	1.28	assimilation (N-Acetyl-Glucosamine)	transparent	opaque
MAL	D-maltose	1.4	assimilation (MALtose)	transparent	opaque
GNT	potassium gluconate	1.84	assimilation (potassium GlucoNate)	transparent	opaque
CAP	capric acid	0.78	assimilation (CAPric acid)	transparent	opaque
ADI	adipic acid	1.12	assimilation (ADIpic acid)	transparent	opaque
MLT	malic acid	1.56	assimilation (MaLaTe)	transparent	opaque
CIT	trisodium citrate	2.28	assimilation (trisodium CITrate)	transparent	opaque
PAC	phenylacetic acid	0.8	assimilation (PhenylACetic acid)	transparent	opaque
OX	(see oxidase test package insert)	-	cytochrome oxidase	(see oxidase test package insert)	

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

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