BIOMÉRIEUX

REF 20100 / 20160

07584 - L - 2019-06

EN

API® 20 E

℞ only IVD

INTENDED USE

API® 20 E is a qualitative standardized system for the identification of *Enterobacteriaceae* and other non-fastidious Gram-negative rods. It uses miniaturized tests as well as a specially adapted database.

Inoculation and reading of the strip are performed manually and the identification is obtained using an identification software.

The complete list of those organisms that it is possible to identify with this system is given in the Technical Brochure - Information for Identification Software.

PRINCIPLE

The API® 20 E strip consists of 20 microtubes containing dehydrated substrates. These microtubes 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 using an identification software (ATB™ NEW or APIWEB™).

CONTENT OF THE KIT

KIT FOR 25 TESTS (Ref. 20100)

- 25 API® 20 E strips
- 25 incubation boxes
- 25 result sheets
- 1 clip seal
- 1 package insert provided in the kit or downloadable from www.biomerieux.com/techlib.

KIT FOR 100 TESTS (Ref. 20160)

- 100 API® 20 E strips
- 100 incubation boxes
- 100 result sheets
- 1 clip seal
- 1 package insert provided in the kit or downloadable from www.biomerieux.com/techlib.

COMPOSITION

Composition of the Strip

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

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED

Reagents

- API® NaCl 0.85 % Medium, 5 mL (Ref. 20230) or API® Suspension Medium, 5 mL (Ref. 20150)
- Mineral oil (Ref. 70100)
- API® 20 E reagent kit (Ref. 20120) or individual reagents:
  - NIT 1 + NIT 2 (Ref. 70442)
  - VP 1 + VP 2 (Ref. 70422)
  - TDA (Ref. 70402)
  - JAMES (Ref. 70542)
- Zn reagent (Ref. 70380)
- Oxidase (Ref. 55635*)
  * reference not sold in certain countries: use an equivalent reagent.

Materials

- Pipettes or PSIpettes (Ref. 70250)

- Ampule rack
- Ampule protector
- General microbiology laboratory equipment.
- ATB™ NEW and APIWEB™ software for identification (consult bioMérieux)

POSSIBLE ADDITIONAL REAGENTS
- API® OF Medium (Ref. 50110): Test for the determination of fermentative or oxidative metabolism.
- API® M Medium (Ref. 50120): Test for motility of facultative anaerobic bacteria.

WARNINGS AND PRECAUTIONS
- **For *in vitro* diagnostic use and microbiological control.**
- **For professional use only.** This test is intended for use by trained laboratory professionals.
- **For US Only: Caution: US Federal Law restricts this device to sale by or on the order of a licensed practitioner.**
- 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; do not 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 "CLSI M29-A, Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline - Current revision". For additional handling precautions, refer to "Biosafety in Microbiological and Biomedical Laboratories - CDC/NIH - Latest edition", or to the regulations currently in use in each country.
- Do not use reagents after the expiry date.
- Before use, check that the packaging and components are intact.
- Do not use strips which have been damaged: for example, cupules deformed, desiccant sachet open.
- The strip is for single use only and should not be reused.
- Allow media to come to room temperature before use.
- The performance data presented in the Technical Brochure 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’s history, the source of the specimen, the 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 and should be kept in the pouch until use. Once opened*, the pouch should be resealed 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 at +2°C/+8°C after the pouch has been opened (or until the expiry 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.

Ensure that the complete device identifier information on the package is marked on the storage container: item number (01), batch number (10) and expiry date (17).

SPECIMEN 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 suitable 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

1. 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 (for example, Cl_2, CO_2)] into the honeycombed wells of the tray to create a humid atmosphere.

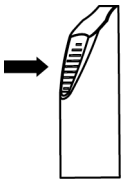
2. Record the strain reference on the elongated flap of the tray. (Do not record the reference on the lid as it may be misplaced during the procedure).
3. Remove the strip from its packaging just before use.
4. Place the strip in the incubation box.

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 (for example, *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

1. Open an ampule of API® NaCl 0.85 % Medium (5 mL) or an ampule of API® Suspension Medium (5 mL) as indicated below, or use any tube containing 5 mL of sterile saline or sterile distilled water, without additives.

   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.
   - Position the thumb tip on the striated part of the cap and press forward to snap off the top of the ampule.
   - Take the ampule out of the ampule protector and put the protector aside for subsequent use.
   - Carefully remove the cap.

2. 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).
3. 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

1. With the same pipette, distribute the bacterial suspension into the tubes of the strip (to avoid the formation of bubbles at the base of the tubes, tilt the strip slightly forward and place the tip of the pipette or PSIpette against the side of the cupule):
   - For the [CIT], [GEL] and [VP] tests, fill both the tube and the cupule,
   - For the other tests, fill only the tubes (and not the cupules),
   - For the ADH, LDC, ODC, H_2S and URE tests, create anaerobiosis by overlaying with mineral oil.
2. Close the incubation box.
3. Incubate at +36°C ± 2°C for 18-24 hours.

READING AND INTERPRETATION

Reading of 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:
   - 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.
3. 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 the previous paragraph).

- To complete the identification, it may be necessary to perform supplementary tests (refer to the "Identification" paragraph below).

Interpretation

Determination of the numerical profile
On the result sheet, the tests are separated into groups of three and a value 1, 2 or 4 is assigned to each. By adding together the values corresponding to positive reactions within each group, a 7-digit numerical profile 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 numerical profile with the APIWEB™ or ATB™ NEW identification software. For further instructions on the numerical profile, refer to the identification software.

- API® systems identify an organism by using a methodology based on the characteristics of the data and knowledge about the organism and reactions being analyzed. Sufficient data have been collected from known strains to estimate the typical reactions of the claimed species to a set of discriminating biochemicals. If a unique identification pattern is not recognized, a list of possible organisms is given or the strain is determined to be outside the scope of the database. The software comment and/or the printed lab report contains suggestions for any supplementary tests necessary to complete the identification. If the tests are not sufficient to complete the identification, then standard microbiology references and literature should be consulted.
- Certain species may belong to a slashline (mixed) taxa. This occurs when the biopattern is the same for the taxa listed. Supplementary tests may be used to separate slashline taxa.

The supplementary tests are listed in the Software Technical Brochure.

Concerning API® 20 E, in some cases, the profile is not discriminatory enough and the following tests need to be performed:

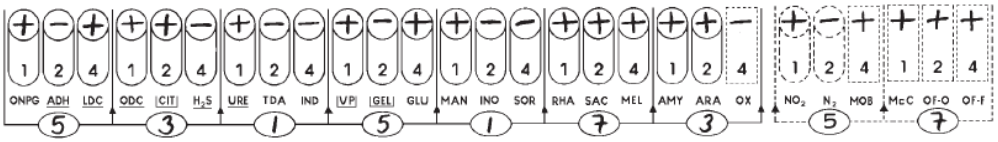
- **Reduction of nitrates to nitrites (NO_2) and N_2 gas (N_2)**:
  Add 1 drop each of NIT 1 and NIT 2 reagents to the GLU tube. Wait 2 to 5 minutes. A red color indicates a positive reaction (NO_2). A negative reaction (yellow) may be due to the reduction to nitrogen (as sometimes evidenced by gas bubbles): 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 of the Strip"), the nitrate reduction test must be performed last.

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

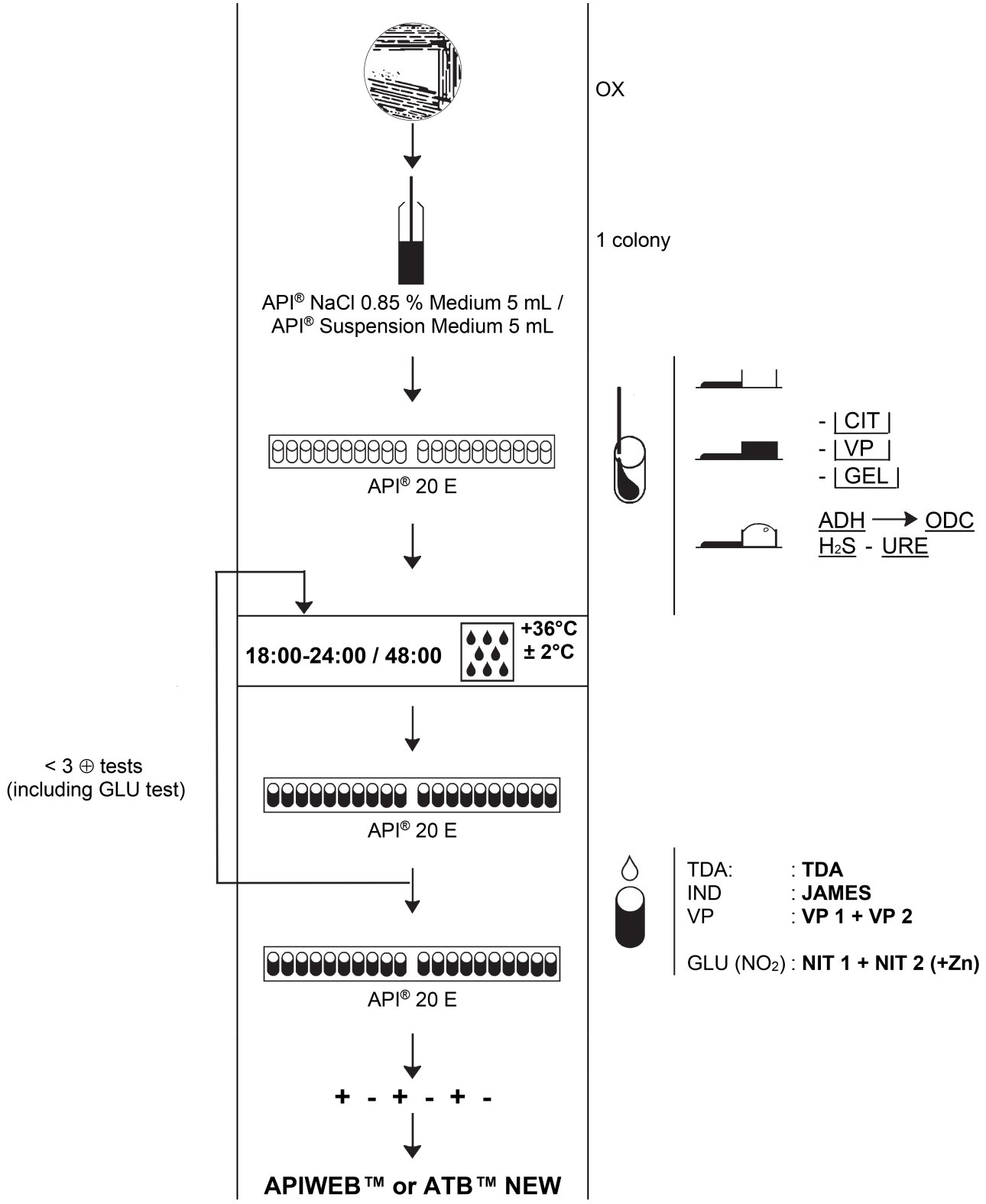
These tests may be used to form a 9-digit profile. Identification is then obtained using the identification software.

Below is an example of a numerical profile.



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

PROCEDURE


OX
1 colony
API® NaCl 0.85 % Medium 5 mL /
API® Suspension Medium 5 mL
API® 20 E
- CIT
- VP
- GEL
ADH → ODC
H2S - URE
18:00-24:00 / 48:00
+36°C
± 2°C
< 3 ⊕ tests
(including GLU test)
API® 20 E
TDA: : TDA
IND : JAMES
VP : VP 1 + VP 2
GLU (NO2) : NIT 1 + NIT 2 (+Zn)
API® 20 E
+ - + - + -
APIWEB™ or ATB™ NEW

READING TABLE

TESTS	ACTIVE INGREDIENTS	QTY (mg/ cupule)	REACTIONS/ENZYMES	RESULTS	
				NEGATIVE	POSITIVE
ONPG	2-Nitrophenyl-βD-galactopyranoside	0.223	β-Galactosidase (Ortho nitrophenyl-βD-galactopyranosidase)	Colorless	Yellow [1]
ADH	L-Arginine	1.9	Arginine dihydrolase	Yellow	Orangey-red [2]
LDC	L-Lysine	1.9	Lysine decarboxylase	Yellow	Orangey-red [2]
ODC	L-Ornithine	1.9	Ornithine decarboxylase	Yellow	Orangey-red [2]
[CIT]	Trisodium citrate	0.756	Citrate utilization	Pale green / Yellow	Blue-green / Blue [3]
H2S	Sodium thiosulfate	0.075	H_2S production	Colorless / Greyish	Black deposit / Thin line
URE	Urea	0.76	Urease	Yellow	Orangey-red [2]
				TDA immediate	
TDA	L-Tryptophan	0.38	Tryptophan deaminase	Yellow	Reddish brown
				JAMES immediate	
IND	L-Tryptophan	0.19	Indole production	Colorless / Pale green-yellow	Pink
				VP 1 + VP 2 / 10 min	
[VP]	Sodium pyruvate	1.9	Acetoin production (Voges Proskauer)	Colorless / Pale pink	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-Saccharose	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-Arabiose	1.9	Fermentation - oxidation (arabiose) [4]	Blue / Blue-green	Yellow
OX	See the oxidase test package insert		Cytochrome oxidase	See the oxidase test package insert	

[1] A very pale yellow should also be considered positive.

[2] An orange color after 36-48 hours of incubation must be considered negative.

[3] Reading performed 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.

QUALITY CONTROL

The media, strips and reagents are systematically quality controlled at various stages of their manufacture.

Streamlined quality control can be used to confirm acceptable performance of the system after shipping and storage. This control can be performed by following the instructions and the expected criteria below, in connection with the referential document CLSI® M50-A Quality Control for Commercial Microbial Identification Systems.

Testing may be conducted using *Proteus mirabilis* ATCC® 35659™ to evaluate the performance of the ODC and ARA tests. Tests performed by bioMérieux have shown that the the ODC and ARA tests are the most labile on the strip. When testing the strip, *Proteus mirabilis* ATCC®35659™ can be used to detect degradation.

For those users who are required to perform comprehensive quality control testing with the strip, the following strains should be used to demonstrate positive and negative reactivity for most of the tests.

1. *Proteus mirabilis* ATCC® 35659™.
2. *Stenotrophomonas maltophilia* ATCC® 51331™
3. *Enterobacter cloacae* ssp *cloacae* ATCC® 13047™
4. *Escherichia coli* ATCC® 25922™
5. *Klebsiella pneumoniae* ssp *pneumoniae* ATCC® 35657™

	ONPG	ADH	LDC	ODC	[CIT]	H2S	URE	TDA	IND	[VP]
1	-	-	-	+	V	+	+	+	-	-
2	+	-	V	-	V	-	-	-	-	-
3	+	+	-	V	+	-	-	-	-	+
4	+	-	+	+	-	-	-	-	+	-
5	+	-	+	-	+	-	V	-	-	V

	[GEL]	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	NO_2	N_2*
1	V	+	-	-	-	-	V	-	-	-	+	-
2	+	-	-	-	-	-	-	-	-	-	-	-
3	-	+	+	V	+	+	+	+	+	+	+	-
4	-	+	+	-	+	+	-	+	-	+	+	-
5	-	+	+	+	+	+	+	+	+	+	+	-

* The N_2 (+) state may be observed for the ATCC® 13047™, ATCC® 25922™ and ATCC® 35657™ strains.

- Profile obtained after 24-48 hours of incubation for the ATCC® 51331™ strain, 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 applicable local regulations.

Quality control strains are chosen for their reaction performance rather than for their identification performance.

In general, quality control strains are identified with a single taxon, low discrimination or mixed taxa.

It may happen that an ATCC® strain is misidentified when all expected quality control reactions are correct.

Note: As species names may change over time, please refer to the official taxonomy for the latest updates.

TECHNICAL BROCHURE: INFORMATION RELATED TO THE APIWEB™ AND ATB™ NEW IDENTIFICATION SOFTWARE

The following sections are fully documented in the Technical Brochure:

- Limitations of the method
- Identification Table (%)
- Performance

To consult the Technical Brochure, proceed as follows:

- APIWEB™
  - Click 

- Click “TECHNICAL BROCHURE”.

- ATB™ NEW:
  - Open the “TECHNICAL BROCHURE” available on your Documentation CD-ROM.

WASTE DISPOSAL

Dispose of used or unused reagents 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 nature and degree of hazardousness, and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

LITERATURE REFERENCES

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6. MURRAY P.R., BARON E.J., JORGENSEN J.H., PFALLER M.A., YOLKEN R.H.
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Evaluation of Five Test-Kits, API, AuxoTab, Enterotube, PathoTec and R/B, for Identification of *Enterobacteriaceae*. (1974) Med. Microbiol. Immunol. 159, 211-220.

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Use of the API 20E System to Identify Veterinary *Enterobacteriaceae*. (1980) J. Clin. Microbiol. 12, 10-14.

11. Clinical and Laboratory Standards Institute, M50-A, Quality Control for Commercial Microbial Identification Systems; Approved Guideline, Latest Edition.

INDEX OF SYMBOLS

Symbol	Meaning
REF	Catalogue number
IVD	*In Vitro* Diagnostic Medical Device
Rx only	For US Only: Caution: US Federal Law restricts this device to sale by or on the order of a licensed practitioner
	Manufacturer
	Temperature limit
	Use by date

Symbol	Meaning
	Batch code
	Do not reuse
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Date of manufacture
	Moistened atmosphere

LIMITED WARRANTY

bioMérieux warrants the performance of the product for its stated intended use provided that all procedures for usage, storage and handling, shelf life (when applicable), and precautions are strictly followed as detailed in the instructions for use (IFU).

Except as expressly set forth above, bioMérieux hereby disclaims all warranties, including any implied warranties of merchantability and fitness for a particular purpose or use, and disclaims all liability, whether direct, indirect or consequential, for any use of the reagent, software, instrument and disposables (the "System") other than as set forth in the IFU.

REVISION HISTORY

Change type categories

N/A	Not applicable (First publication)
Correction	Correction of documentation anomalies
Technical change	Addition, revision and/or removal of information related to the product
Administrative	Implementation of non-technical changes noticeable to the user

Note: *Minor typographical, grammar, and formatting changes are not included in the revision history.*

Release Date	Part Number	Change Type	Change Summary
2019/06	07584L	Administrative	Improvements to match the bioMérieux Templates and style guide and comply with RECAST regulations.

For users in the European Union (Regulation (EU) 2017/746) and in countries with similar requirements: Should a serious incident occur during the use of this device or as a result of its use, please report it to the manufacturer and/or their authorized representative as well as to your national authority.

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