BIOMÉRIEUX

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EN

API® 20 NE

℞ only IVD

INTENDED USE

API® 20 NE is a qualitative standardized system for the identification of non-fastidious, non-enteric Gram-negative rods (for example, *Pseudomonas, Acinetobacter, Moraxella, Vibrio, Aeromonas*). 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 NE 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 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 using an identification software (ATB™ NEW or APIWEB™).

CONTENT OF THE KIT

KIT FOR 25 TESTS

- 25 API® 20 NE strips
- 25 ampules of API® AUX Medium
- 25 incubation boxes
- 25 result sheets
- 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.

Composition of the Media

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 MATERIALS REQUIRED BUT NOT PROVIDED

Reagents

- API® NaCl 0.85% Medium, 2 mL (Ref. 20070)
- Oxidase (Ref. 55635*)
  * reference not sold in certain countries: use an equivalent reagent.
- Mineral oil (Ref. 70100)
- McFarland Standard (Ref. 70900), No. 0.5 on the scale
- Reagents:

- JAMES (Ref. 70542)
- NIT 1 + NIT 2 (Ref. 70442)
- Zn (Ref. 70380)

Materials

- Pipettes or PSIpettes
- Ampule rack
- Ampule protector
- DENSIMAT (Ref. 99234) (optional)
- General microbiology laboratory equipment
- ATB™ NEW or APIWEB™ software for identification (consult bioMérieux)

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 reagents 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 and media should be stored at +2°C/+8°C until the expiry date indicated on the packaging.

SPECIMEN 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 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 the Colonies

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

Note: Some non-enteric Gram-negative rods are oxidase negative (for example,*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: Fastidious organisms having demanding nutritional requirements and requiring appropriate handling precautions (*Brucella* and *Francisella*) are not included in the API® 20 NE database. Alternative procedures must be used to exclude or confirm their presence.

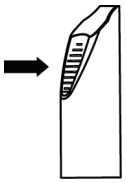
Preparation of the Strip

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 sample identification on the elongated flap of the tray. (Do not record the sample identification 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.

Preparation of the Inoculum

1. Open an ampule of API® NaCl 0.85% Medium (2 mL), or use any tube containing 2 mL of 0.85% physiological saline 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, 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).
3. 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

1. Using a pipette or PSIpette, fill the microtubes for tests NO_3 to PNPG with the inoculated suspension medium. Only fill the tube portion of the microtubes, not the cupules. 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.
2. Open an ampule of API® AUX Medium and add approximately 200 µL of the remaining saline suspension to the ampule. Homogenize well with the pipette, avoiding the formation of bubbles.
3. 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.
4. Ensure anaerobiosis to the 3 underlined tests (GLU, ADH, and URE) by filling the cupules with mineral oil to form a convex meniscus.
5. Close the incubation box and incubate at +29°C ± 2°C for 24 hours (± 2 hours) in aerobic conditions.

READING AND INTERPRETATION

Reading of the Strip

1. After the incubation period, read the strip by referring to the Reading Table.
2. Record all spontaneous reactions (GLU, ADH, URE, ESC, GEL, and PNPG) on the result sheet.
3. The reading of the two tests NO_3 and TRP should be performed while 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.
   - **$N0_3$ test:**
     1. Add 1 drop of NIT 1 and 1 drop of NIT 2 reagents to the NO_3 cupule.
     2. After 5 minutes, a **red** color indicates a **positive** reaction to be recorded on the result sheet.
     3. 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.
     4. 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.

- **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".

  Reincubation is necessary in the following cases:
  - low discrimination;
  - unacceptable or doubtful profile;
  - if the following note is indicated for the profile obtained:
    ID 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 all the tests again, except the first 3 (NO_3, TRP, and GLU) which should only be read once at 24 hours.

Interpretation

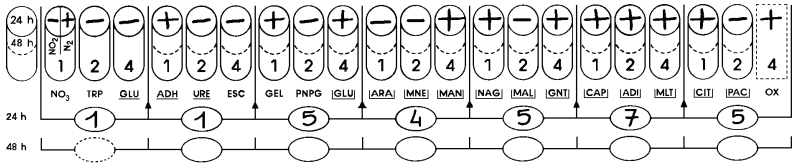
Determination of the numerical profile
On the result sheet, the tests are separated into groups of three and a value 1, 2 or 4 is assigned to each. By adding together the values corresponding to positive reactions within each group, a 7-digit numerical profile is obtained; the oxidase reaction constitutes 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.

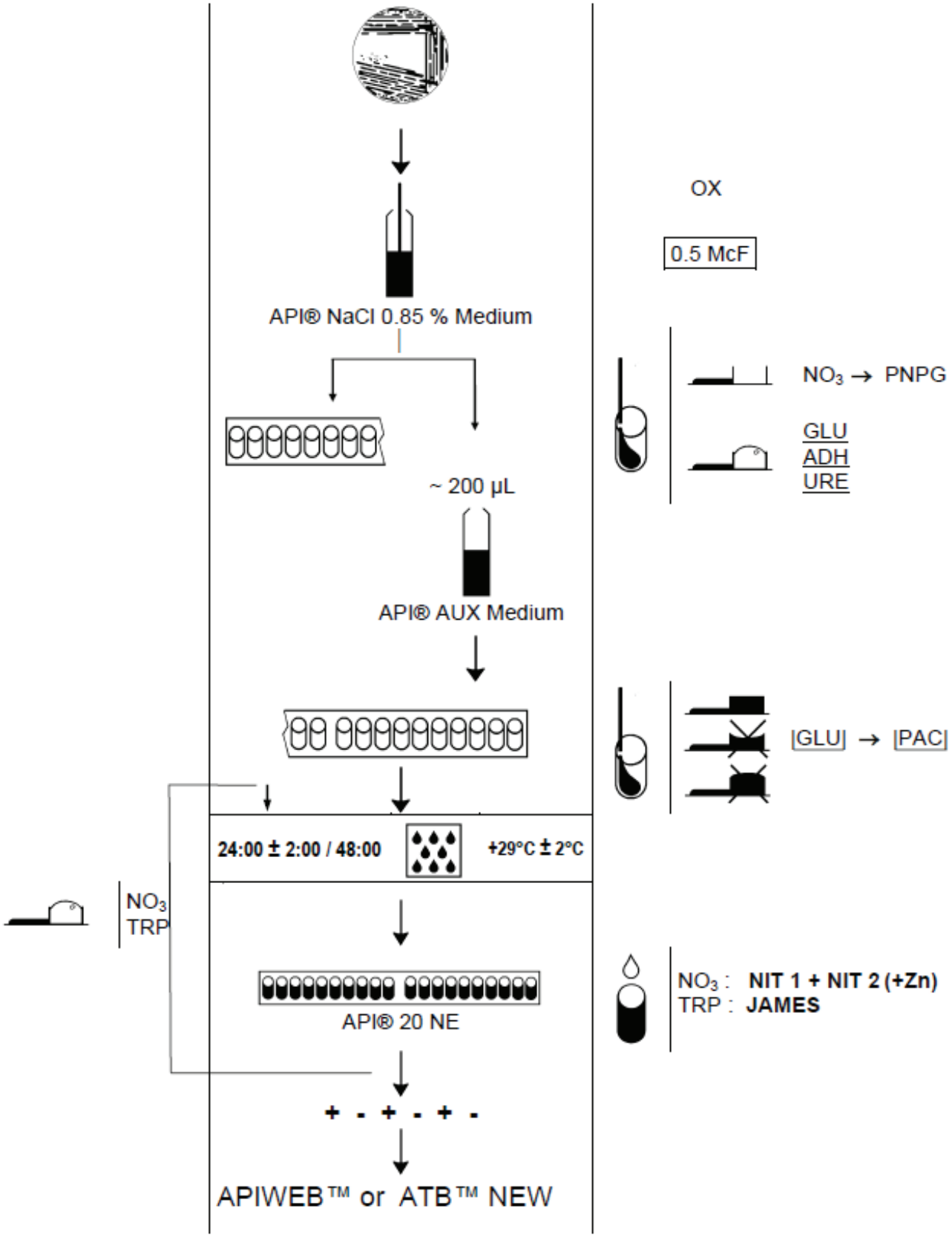
The supplementary tests are listed in the Technical Brochure.

Below is an example of a numerical profile.



1 154 575 *Pseudomonas aeruginosa*

PROCEDURE


OX
0.5 McF
API® NaCl 0.85 % Medium
NO3 → PNPG
GLU
ADH
URE
~ 200 µL
API® AUX Medium
|GLU| → |PAC|
24:00 ± 2:00 / 48:00
+29°C ± 2°C
NO3
TRP
NO3 : NIT 1 + NIT 2 (+Zn)
TRP : JAMES
API® 20 NE
+ - + - + -
APIWEB™ or ATB™ NEW

READING TABLE

TESTS	ACTIVE INGREDIENTS	QTY (mg/ cupule)	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-Tryptophan	0.2	Indole production (tryptophan)	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	0.56	Hydrolysis (β-glucosidase) (esculin)	Yellow	Grey/Brown/Black
	Ferric citrate	0.072			
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.

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.

As there are no substrates that are consistently sensitive to degradation during shipping conditions, streamlined quality control may be conducted by testing two strains: *Aeromonas hydrophila* ATCC® 35654™ that is mostly positive and *Alcaligenes faecalis* ssp *faecalis* ATCC® 35655™, which is mostly negative for reactions on the API® 20 NE system.

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. *Aeromonas hydrophilia* ATCC® 35654™
2. *Alcaligenes faecalis* ssp *faecalis* ATCC® 35655™
3. *Sphingobacterium multivorum* ATCC® 35656™
4. *Pseudomonas aeruginosa* ATCC® 27853™

	NO_3	TRP	GLU	ADH	URE	ESC	GEL	PNPG	|GLU|	|ARA|
1	+	+	+	+	-	+	+	+	+	+
2	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	+	+	-	+	+	+
4	+	-	-	V	V	-	+	-	+	-

	|MNE|	|MAN|	|NAG|	|MAL|	|GNT|	|CAP|	|ADI|	|MLT|	|CIT|	|PAC|	OX
1	+	+	+	+	+	+	-	+	-*	-	+
2	-	-	-	-	-	+	-	+	+	+	+
3	+	-	+	+	-	-	-	-	-	-	+
4	-	+	+	-	+	+	+	+	+	-	+

* Weak reactions may occur.

Profiles obtained from colonies grown on Trypticase Soy agar and after 48 hours of incubation for tests ADH to |PAC|.

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

Unused ampules of API® AUX Medium may be considered as non hazardous waste and disposed of accordingly.

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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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
LOT	Batch code
	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
2019/09	07615 L	Administrative	Improvements to match the bioMérieux Templates and style guide and comply with RECAST regulations.

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