

api® 50 CHB/E Medium

IVD

Bacillus and related genera / *Enterobacteriaceae* and *Vibrionaceae*

SUMMARY AND EXPLANATION

API 50 CHB/E Medium is intended for the identification of *Bacillus* and related genera, as well as Gram-negative rods belonging to the *Enterobacteriaceae* and *Vibrionaceae* families. It is a ready-to-use medium which allows the fermentation of the 49 carbohydrates on the API 50 CH strip to be studied.

PRINCIPLE

A suspension is made in the medium with the microorganism to be tested and each tube of the strip is then inoculated with the suspension. During incubation, the carbohydrates are fermented to acids which produce a decrease in the pH, detected by the change in color of the indicator. The results make up the biochemical profile which is used by the identification software to identify the strain.

The API 20 E strip may be used in association with the API 50 CH strip to provide supplementary tests (optional for *Bacillus* and related genera but essential for *Enterobacteriaceae* and *Vibrionaceae*).

CONTENT OF THE KIT (Kit for 10 tests)

- 10 ampules of API 50 CHB/E Medium
- 1 package insert provided in the kit or downloadable from www.biomerieux.com/techlib

COMPOSITION OF THE MEDIUM

API 50 CHB/E Medium 10 ml	Ammonium sulfate	2 g
	Yeast extract	0.5 g
	Tryptone (bovine/porcine origin)	1 g
	Disodium phosphate	3.22 g
	Monopotassium phosphate	0.12 g
	Trace elements	10 ml
	Phenol red	0.17 g
	Demineralized water pH 7.4-7.8 at 20-25°C	1000 ml

The quantities indicated may be adjusted depending on the titer of the raw materials used.

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED

Reagents / Instrumentation for *Bacillus* and related genera :

- API 50 CH strip (Ref. 50 300)
- API 20 E strip (Ref. 20 100)
- API 20 E reagent kit (Ref. 20 120)
- **apiweb™** identification software (Ref. 40 011), **ATB™** instrument or **mini API** (consult bioMérieux)
- Mineral oil (Ref. 70 100)
- API NaCl 0.85 % Medium, 5 ml (Ref. 20 230)
- McFarland Standard (Ref. 70 900), point 2 on the scale or DENSIMAT (Ref. 99 234) or ATB Densitometer

Reagents / Instrumentation for *Enterobacteriaceae* and *Vibrionaceae* :

- API 50 CH strip (Ref. 50 300)
- API 20 E strip (Ref. 20 100)
- API 20 E reagent kit (Ref. 20 120)
- **apiweb** identification software (Ref. 40 011), **ATB** instrument or **mini API** (consult bioMérieux)

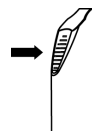
- Mineral oil (Ref. 70 100)
- API Suspension Medium, 5 ml (Ref. 20 150)
- McFarland Standard (Ref. 70 900), point 0.5 on the scale or DENSIMAT (Ref. 99 234) or ATB Densitometer

Material :

- Pipettes or PSIPettes
- Ampule rack
- Small and large ampule protectors
- Sterile distilled water or sterile saline, 1 ml
- 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 "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 media past the expiry date.
- Before use, check that the ampules are intact.
- Allow media to come to room temperature before use.
- 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.



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

SPECIMENS (COLLECTION AND PREPARATION)

API 50 CHB/E Medium 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

For *Bacillus*

Selection of the colonies

- Check the purity of the strain.
- Check that it belongs to the *Bacillus* genus : aerobic, spore-forming rod, usually Gram-positive.
- Culture it on a nutrient agar plate.
 - If the optimum growth temperature of the micro-organism is unknown, incubate several plates at different temperatures.
 - For slow growing strains, use two plates so as to have enough bacteria :
 - mesophiles grow at temperatures between 25°C and 45°C during 16-18 hours ;
 - psychrophiles grow at 20°C during 18-48 hours ;
 - thermophiles grow at 55°C during 12-16 hours.
- Growth of *Bacillus lentus* is encouraged by the addition of 1 g of urea/litre into the nutrient agar before sterilization.

Preparation of the strips

See the package inserts for API 50 CH and API 20 E (use optional).

Preparation of the inoculum

The solutions must be used immediately after preparation.

- If the DENSIMAT or ATB™ Densitometer is used :
 - 1) Suspension for inoculation of the API 50 CH strip :
 - Open an ampule of API 50 CHB/E Medium as indicated in the paragraph "Warnings and Precautions".
 - Pick up several identical colonies.
 - Prepare a suspension with a turbidity equivalent to 2 McFarland in the ampule of API 50 CHB/E Medium.
 - 2) Suspension for inoculation of the API 20 E strip :
 - Open an ampule of API NaCl 0.85 % Medium (5 ml) as indicated in the paragraph "Warnings and Precautions".
 - Pick up several identical colonies.
 - Prepare a suspension with a turbidity equivalent to 2 McFarland.

- If the DENSIMAT or ATB Densitometer is not used :
 - 1) Suspension for inoculation of the API 20 E strip :
 - Open a tube containing 1 ml of sterile saline.
 - Pick up all the bacteria from the culture using a swab.
 - Prepare a heavy suspension (S) in the tube.
 - Open an ampule of API NaCl 0.85 % Medium (5 ml) as indicated in the paragraph "Warnings and Precautions".
 - Prepare a suspension with a turbidity equivalent to 2 McFarland by transferring a certain number of drops of suspension S into the ampule : record this number of drops (n).
 - 2) Suspension for inoculation of the API 50 CH strip :
 - Open an ampule of API 50 CHB/E Medium as indicated in the paragraph "Warnings and Precautions".
 - Inoculate the ampule of API 50 CHB/E Medium by transferring twice the number of drops of suspension (i.e. 2n) into the ampule.
- Homogenize.

NOTE : If the API 20 E strip is be used in association with the API 50 CH strip, follow the instructions in the API 20 E package insert.

Inoculation of the strips

(see the API 50 CH and API 20 E package inserts)

- Fill the tubes (not the cupules) with the inoculated API 50 CHB/E Medium.

NOTE : The addition of mineral oil is optional ; it is not however recommended for strict aerobic bacteria.
- Inoculate the first 12 tests only of the API 20 E strip, as the last 8 tests are duplicated on the API 50 CH strip, and inoculate the GLU test to reveal the NIT reaction.

Incubation of the strips

- Incubate :
 - thermophilic species at 55°C ± 2°C for 3 - 3 ½ hours, 6 - 6 ½ hours and 24 hours (± 2 hours), slightly tilting the API 50 CH strip, base of the tubes uppermost, in order to trap any gas produced,
 - other species at 29°C ± 2°C for 24 hours (± 2 hours) and 48 hours (± 6 hours).

NOTE : For the API 20 E strip, the same incubation conditions must be observed.

Reading the strips

(see the API 50 CH and API 20 E package inserts)

- Read the results :
 - for thermophilic species after 3 - 3 ½ hours, 6 - 6 ½ hours and 24 hours (± 2 hours) of incubation,
 - for other species after 24 hours (± 2 hours) and 48 hours (± 6 hours) of incubation.
- For the API 50 CH strip :
 - A positive test corresponds to acidification revealed by the phenol red indicator contained in the medium changing to YELLOW.
 - For the esculin test (tube no. 25), a change in color from red to BLACK is observed.

NOTE : If a positive test becomes negative at the second reading, only the positive result should be taken into account (this is caused by an alkalization due to the production of ammonia from peptone).

 - Record the results on the result sheet.

- For the API 20 E strip :
 - The reagents are added just before the last reading.
 - To read the tests, refer to the API 20 E package insert. The results of the first 11 tests and the NIT reaction in the GLU test should be recorded for final interpretation.

Interpretation

The biochemical profile obtained for the strain after the final reading can be identified using the ATB™ instrument, **mini API**, or **apiweb™** identification software with the database (V4.0).

NOTE :

The biochemical profile may also be :

- used with other results for a taxonomic study.
- recorded as it is, to characterize the strain and to make comparisons.

For *Enterobacteriaceae*

Selection of the colonies

- Check the purity of the strain.
- Culture it on a nutrient medium (Trypcase soy agar for example), and incubate for 18-24 hours at 37°C.
- Check that it belongs to the *Enterobacteriaceae* or *Vibrionaceae* family.

Preparation of the strips

See the API 50 CH and API 20 E package inserts.

Preparation of the inoculum

The solutions must be used immediately after preparation.

- If the DENSIMAT or ATB Densitometer is used :
 - Open an ampule of API 50 CHB/E Medium as indicated in the paragraph "Warnings and Precautions".
 - Pick up several identical colonies.
 - Prepare a suspension with a turbidity equivalent to 0.5 McFarland in the ampule of API 50 CHB/E Medium.
- If the DENSIMAT or ATB Densitometer is not used:
 - Open an ampule of API 50 CHB/E Medium as indicated in the paragraph "Warnings and Precautions".
 - Pick up a few colonies and suspend them in 1 ml of sterile distilled water to obtain a turbidity equivalent to 4 McFarland.
 - Transfer this suspension into the ampule of API 50 CHB/E Medium.
- Homogenize.
- Prepare the inoculum to be used for the inoculation of the API 20 E strip as indicated in the API 20 E package insert.

Inoculation of the strips

(see the API 50 CH and API 20 E package inserts)

- Fill the tubes (not the cupules) with the inoculated API 50 CHB/E Medium, and cover all the tests with mineral oil.
- Inoculate the first 11 tests of the API 20 E strip.

Incubation of the strips

- Incubate aerobically at 36°C ± 2°C for 24 hours (± 2 hours) and 48 hours (± 6 hours).

Reading the strips

(see the API 50 CH and API 20 E package inserts)

- Read after 24 hours (± 2 hours) and 48 hours (± 6 hours) of incubation.
- For the API 50 CH strip :
 - A positive test corresponds to acidification revealed by the phenol red indicator contained in the medium changing to YELLOW.
 - For the esculin test (tube no. 25), a change in color from red to BLACK is observed.
- **NOTE :** If a positive test becomes negative at the second reading, only the positive result should be taken into account (this is caused by an alkalization due to the production of ammonia from peptone).
 - Record the results on the result sheets.
- For the API 20 E strip :
 - The reagents are added just before the last reading.
 - To read the tests, refer to the API 20 E package insert. The results of the first 11 tests should be recorded for final interpretation.

Interpretation

The biochemical profile obtained for the strain after the final reading can be identified using the ATB instrument, **mini API**, or **apiweb** identification software with the database (V3.1).

NOTE :

The biochemical profile may also be :

- used with other results for a taxonomic study.
- recorded as it is, to characterize the strain and to make comparisons.

QUALITY CONTROL

The media and strips are systematically controlled at various stages of their manufacture. For those users who wish to perform their own quality control tests with the strip, the following strains may be used :

For *Bacillus* : ***Paenibacillus polymyxa* ATCC® 43865**

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	
24	-	+	-	-	+	+	+	-	-	V	+	+	+	+	-	V	-	-	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-
48	-	+	-	-	+	+	+	-	-	+	+	+	+	+	-	V	-	-	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	V	+	+	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-

Results obtained after incubation at 30°C.

For *Enterobacteriaceae* : preferably **1. *Klebsiella pneumoniae* ssp *pneumoniae* ATCC 35657** or else :

2. *Providencia alcalifaciens*

ATCC 9886

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49										
1. 24	-	+	-	-	+	+	+	-	+	-	+	+	+	+	-	+	-	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	+	-	-	-	-	+	+	-	+	+	-										
48	-	+	-	V	+	+	+	-	+	-	+	+	+	+	-	+	-	+	+	+	+	-	+	V	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	+	V	-	-	-	+	+	-	+	+	-										
2. 24	-	-	-	-	+	-	-	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	V	+	-	-	-	-	-	-	-	-	-
48	-	V	-	-	-	+	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-

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

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

LIMITATIONS OF THE METHOD

- The API 50 CHB/E system is intended uniquely for the identification of those species included in the database (see Identification Tables 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.

- For *Enterobacteriaceae* and *Vibrionaceae*

2930 collection strains and strains of various origins belonging to species included in the database were tested :

- 93.93% of the strains were correctly identified (with or without supplementary tests).
- 4.47% of the strains were not identified.
- 1.60% of the strains were misidentified.

RANGE OF EXPECTED RESULTS

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

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 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.

PERFORMANCE

- For *Bacillus* and related genera
1378 collection strains and strains of various origins belonging to species included in the database were tested :
- 91.1% of the strains were correctly identified (with or without supplementary tests).
- 3.9% of the strains were not identified.
- 5.0% of the strains were misidentified.

WARRANTY

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ATCC is a trademark belonging to American Type Culture Collection.

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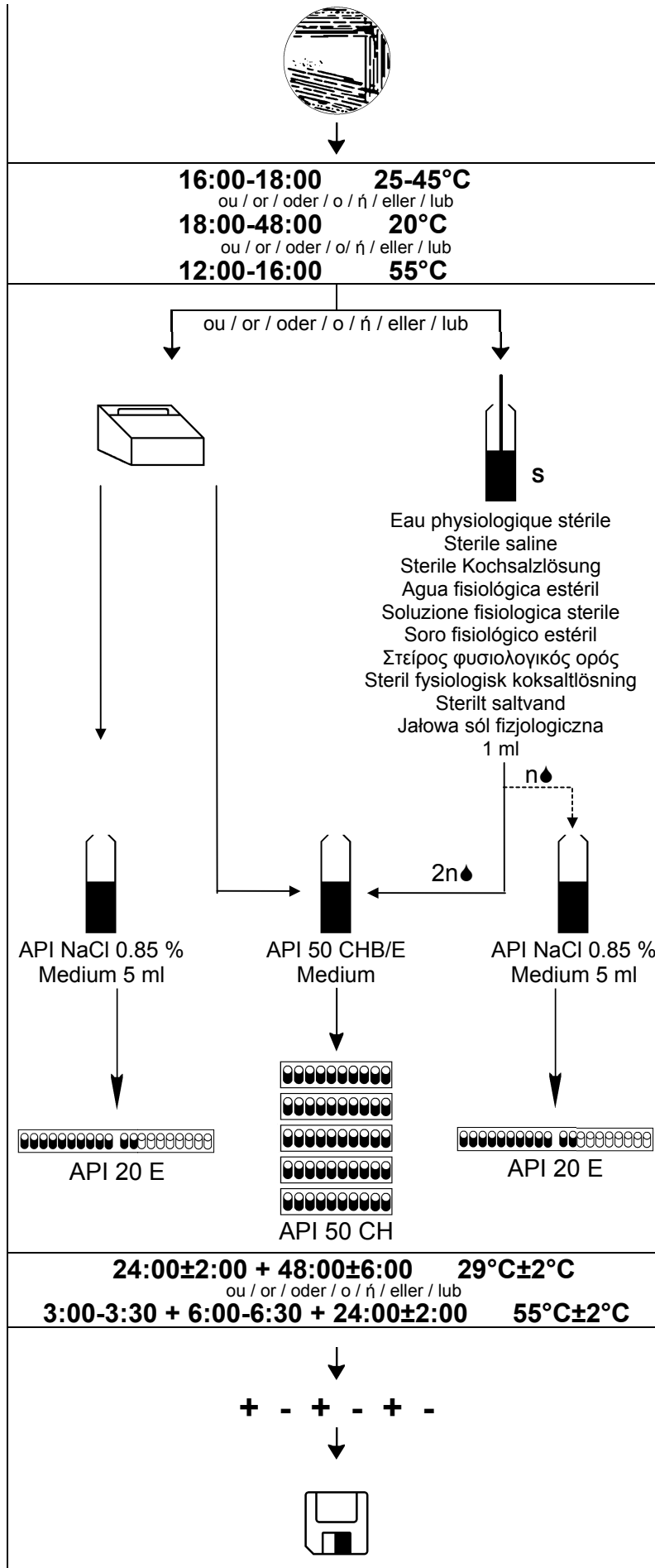
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METHODOLOGIE / PROCEDURE / METHODIK / TECNICA / PROCEDIMENTO / ΔΙΑΔΙΚΑΣΙΑ / METOD / METODYKA

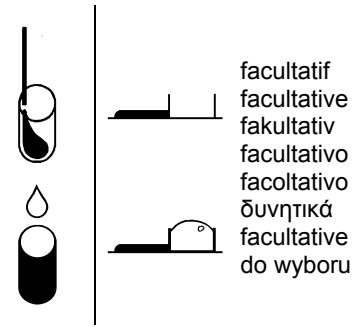
Bacillus



Bacillus et genres apparentés
Bacillus and related genera
Bacillus und verwandte Gattungen
Bacillus y géneros próximos
Bacillus e generi affini
Bacillus e géneros semelhantes
Bacillus και σχετικά γένη
Bacillus och närstående släkten
Bacillus og relaterede genera
Bacillus i rodzaje spokrewnione

Toute la culture
 All the culture
 Alle Keime
 Todo el cultivo
 Tutta la coltura
 Toda a cultura
 Όλη η καλλιέργεια
 Hela kulturen
 Alle bakterier
 Całość hodowli

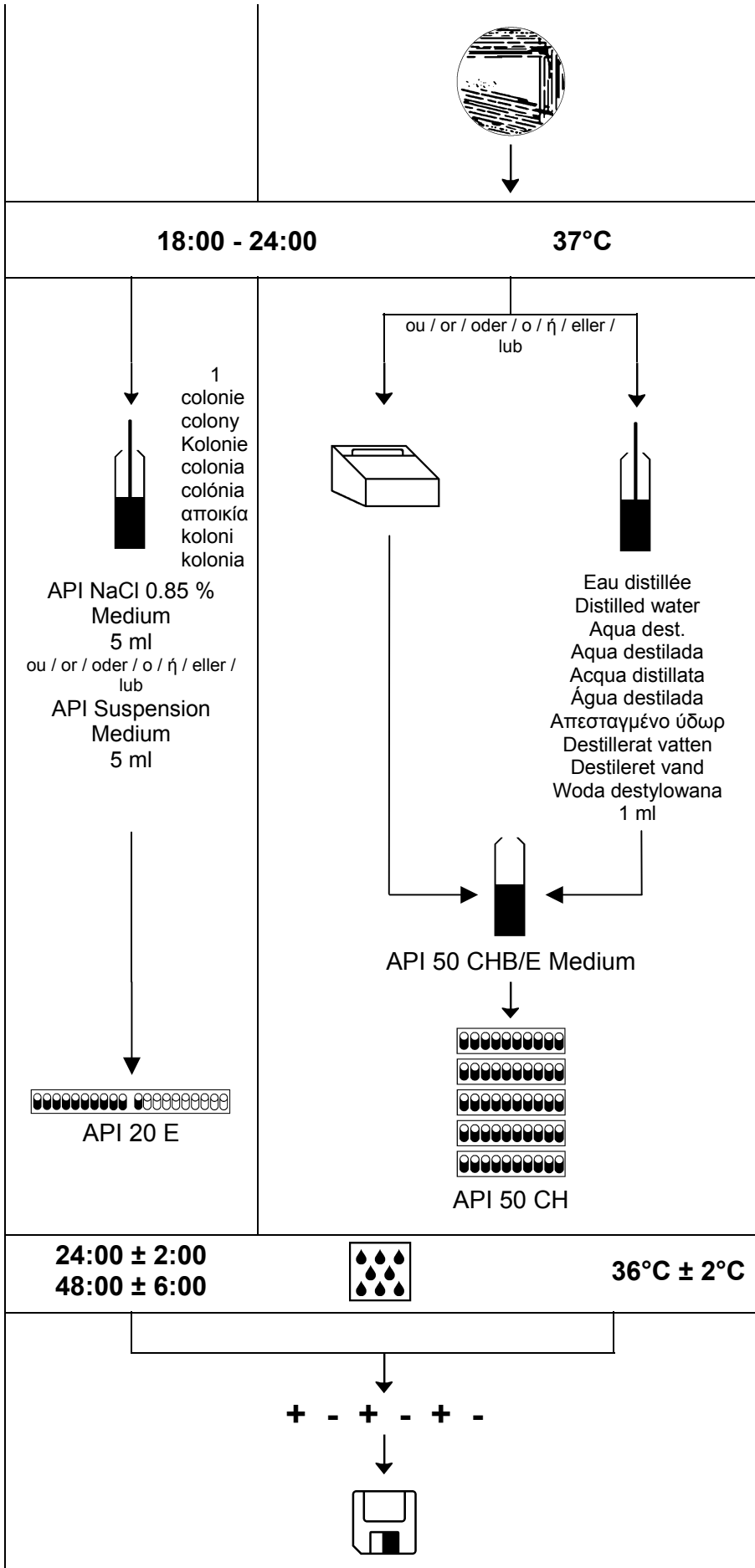
2 McF



TDA : TDA
 IND : JAMES (IND)
 VP : VP 1 + VP 2
 GLU (NO₂) : NIT 1 + NIT 2 (+Zn)

API 20 E

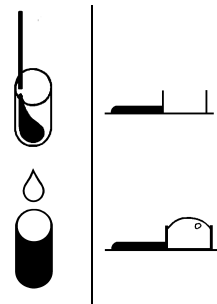
METHODOLOGIE / PROCEDURE / METHODIK / TECNICA / PROCEDIMENTO / ΔΙΑΔΙΚΑΣΙΑ / METOD / METODYKA
Enterobacteriaceae



Enterobacteriaceae, Vibrionaceae

4 McF

0.5 McF



24:00 ± 2:00
 48:00 ± 6:00

36°C ± 2°C

+ - + - + -

API 20 E | TDA : TDA
 IND : JAMES (IND)
 VP : VP 1 + VP 2
 GLU (NO₂) : NIT 1 + NIT 2 (+Zn)

TABLEAU D'IDENTIFICATION / IDENTIFICATION TABLE / PROZENTABELLE / TABLA DE IDENTIFICACION / TABELLA DI IDENTIFICAZIONE / QUADRO DE IDENTIFICAÇÃO / ΠΙΝΑΚΑΣ ΤΑΥΤΟΠΟΙΗΣΗΣ / IDENTIFIZIERINGSTABELL / IDENTIFIKATIONSTABEL / TABELA IDENTYFIKACYJNA

Enterobacteriaceae

% de réactions positives après 48 H (± 6 H) à 36°C ± 2°C / % of positive reactions after 48 hrs. (± 6 hrs.) at 36°C ± 2°C / % der positiven Reaktionen nach 48 Std. (± 6 Std.) bei 36°C ± 2°C / % de las reacciones positivas después de 48 H (± 6 H) a 36°C ± 2°C / % di reazioni positive dopo 48 ore (± 6 ore) a 36°C ± 2°C / % das reacções positivas após 48 H (± 6 H) a 36°C ± 2°C / % θετικών αντιδράσεων μετά από 48 ώρες (± 6 ώρες) στους 36°C ± 2°C / % positiva reaktioner efter 48 tim. (± 6 tim.) vid 36°C ± 2°C / % positive reaktioner efter 48 timer (± 6 timer) ved 36°C ± 2°C / % pozytywnych reakcji po 48 godzinach (± 6 godz.) w 36°C ± 2°C

Table with columns for API 50 CHE (0-49) and API 20 E (GLY-URE). Rows list various bacterial species such as Aeromonas caviae, Citrobacter amalonaticus, Escherichia coli 1, etc., with numerical values indicating reaction results.

**BIBLIOGRAPHIE / LITERATURE REFERENCES / LITERATUR / BIBLIOGRAFIA /
ΑΝΑΦΟΡΕΣ ΑΡΘΡΟΓΡΑΦΙΩΝ / REFERENSLITTERATUR / LITTERATUR / ΠΙΣΜΙΕΝΝΙCTWO**

Bacillus









**et apparentés / and related genera / und verwandte Gattungen / y microorganismos próximos /
e generi affini / e semelhantes / και σχετικά γένη / och närstående släkten / og relaterede genera /
i rodzaje pokrewne**

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Enterobacteriaceae + Vibrionaceae

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Symbole / Symbol / Símbolo / Simbolo / Σύμβολο	Signification / Meaning / Bedeutung / Significado / Significato / Επεξήγηση / Betydelse / Betydning / Znaczenie
REF / 	Référéncie du catalogue / Catalogue number (GB) / Catalog number (US) Bestellnummer / Número de catálogo / Numero di catalogo Referência de catálogo / Αριθμός καταλόγου Artikelnummer / Katalognummer / Numer katalogowy
	Dispositif médical de diagnostic in vitro / In Vitro Diagnostic Medical Device In Vitro Diagnostikum / Producto sanitario para diagnóstico in vitro Dispositivo medico-diagnostico in vitro / Dispositivo médico para diagnóstico in vitro In Vitro Διαγνωστικό Ιατροτεχνολογικό προϊόν / Medicinsk utrustning för in vitro-diagnostik / Medicinsk udstyr til in vitro-diagnostik Urządzenie medyczne do diagnostyki in vitro
	Fabricant / Manufacturer / Hersteller / Fabricante / Fabbicante Κατασκευαστής / Tillverkad av / Producent
	Limites de température / Temperature limitation Temperaturbegrenzung / Limite de temperatura Limiti di temperatura / Limites de temperatura Περιορισμοί θερμοκρασίας / Temperaturgräns Temperaturbegrænsning / Przechowywać w temperaturze
	Utiliser jusque / Use by / Verwendbar bis / Fecha de caducidad Utilizzare entro / Prazo de validade / Ημερομηνία λήξης / Används före Holdbar til / Zużyć do
LOT / 	Code du lot / Batch code / Chargenbezeichnung / Código de lote Codice del lotto / Código do lote / Αριθμός Παρτίδας / Batchnummer / Lotnummer / Numer serii
	Consulter les instructions d'utilisation / Consult Instructions for Use Gebrauchsanweisung beachten / Consulte las instrucciones de uso Consultare le istruzioni per l'uso / Consulte as instruções de utilização Συμβουλευτείτε τις οδηγίες χρήσης / Se användarinstruktionerna Se brugsanvisning / Odnies się do instrukcji użycia
	Contenu suffisant pour "n" tests / Contains sufficient for <n> tests Inhalt ausreichend für <n> Prüfungen / Contenido suficiente para <n> ensayos Contenuto sufficiente per "n" saggi / Conteúdo suficiente para "n" ensaios Περιεχόμενο επαρκές για «n» εξετάσεις / Innehållet räcker till <n> tester Indeholder tilstrækkeligt til "n" undersøgelser Zawartość wystarczy do wykonania <n> oznaczeń