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Système d'identification des *Enterobacteriaceae* et autres bacilles à Gram négatif non fastidieux

INTRODUCTION ET OBJET DU TEST

API 20 E est un système standardisé pour l'identification des *Enterobacteriaceae* et autres bacilles à Gram négatif non fastidieux, comprenant 21 tests biochimiques miniaturisés, ainsi qu'une base de données. La liste complète des bactéries qu'il est possible d'identifier avec ce système est présente dans le Tableau d'identification en fin de notice.

PRINCIPE

La galerie API 20 E comporte 20 microtubes contenant des substrats déshydratés. Les microtubes sont inocués avec une suspension bactérienne qui reconstruit les tests. Les réactions produites pendant la période d'incubation se traduisent par des virages colorés spontanés ou révélés par l'addition de réactifs. La lecture de ces réactions se fait à l'aide du Tableau de Lecture et l'identification est obtenue à l'aide du Catalogue Analytique ou d'un logiciel d'identification.

PRESENTATION

Coffret de 25 tests (réf. 20 100)

- 25 galeries API 20 E
- 25 boîtes d'incubation
- 25 fiches de résultats
- 1 barrette de fermeture
- 1 notice

Coffret de 100 tests (réf. 20 160)

- 100 galeries API 20 E (4x25 galeries)
- 100 boîtes d'incubation
- 100 fiches de résultats
- 1 barrette de fermeture
- 1 notice

COMPOSITION DE LA GALERIE

La composition de la galerie API 20 E est reportée dans le Tableau de Lecture de cette notice.

REACTIFS ET MATERIEL NECESSAIRES MAIS NON FOURNIS

Réactifs

- API NaCl 0.85 % Medium, 5 ml (Réf. 20 230) ou API Suspension Medium, 5 ml (Réf. 20 150)
- API 20 E coffret de réactifs (Réf. 20 120) ou réactifs individuels : TDA (Réf. 70 402) JAMES (Réf. 70 542) VP 1 + VP 2 (Réf. 70 422) NIT 1 + NIT 2 (Réf. 70 442)
- Réactif Zn (Réf. 70 380)
- Oxydase (Réf. 55 635)
- référence non commercialisée dans certains pays : utiliser un réactif équivalent.
- Huile de paraffine (Réf. 70 100)
- Catalogue Analytique API 20 E (Réf. 20 100) ou logiciel d'identification *apiweb*™ (Réf. 40 011) (consulter bioMérieux)

Matériel

- Pipettes ou PSpipettes
- Protège-ampoule
- Potior pour ampoules
- Equipement général de laboratoire de bactériologie

REACTIFS COMPLEMENTAIRES

- API OF Medium (Réf. 50 110) : Test pour la détermination du métabolisme fermentatif ou oxydatif du glucose.
- API M Medium (Réf. 50 120) : Test pour la détermination de la mobilité des bactéries aéro-aérobie.

PRECAUTIONS D'UTILISATION

- Pour diagnostic *in vitro* et pour contrôle microbiologique.
- Pour usage professionnel uniquement.
- Ce coffret contient des composants d'origine animale. La maîtrise de l'origine et/ou de l'état sanitaire des animaux ne pouvant garantir de façon absolue que ces produits ne contiennent aucun agent pathogène transmissible, il est recommandé de les manipuler avec les précautions d'usage relatives aux produits potentiellement infectieux (ne pas ingérer, ne pas inhaler).
- Les prélèvements, cultures bactériennes et produits ensemencés doivent être considérés comme potentiellement infectieux et doivent être manipulés de façon appropriée. Les techniques aseptiques et les précautions usuelles de manipulation pour le groupe bactérien étudié doivent être respectées tout au long de la manipulation ; se référer à "CLSI M29-A, Protection of Laboratory Workers from Occupationally Acquired Infections, Approved Guideline - Révision en vigueur". Pour informations complémentaires sur les précautions de manipulation, se référer à "Biosafety in Microbiological and Biomedical Laboratories - CDC/NIH - Dernière édition", ou à la réglementation en vigueur dans le pays d'utilisation.
- Ne pas utiliser les réactifs après la date de péremption.
- Avant utilisation, s'assurer de l'intégrité de l'emballage des différents composants.
- Ne pas utiliser de galeries ayant subi une altération physique : cupule déformée, sachet déshydratant ouvert, ...
- Les performances présentées sont obtenues avec la méthodologie indiquée dans cette notice. Toute déviation de méthodologie peut altérer les résultats.
- L'interprétation des résultats du test doit être faite en tenant compte du contexte clinique ou autre, de l'origine du prélèvement, des aspects macro et microscopiques de la souche et éventuellement des résultats d'autres tests, en particulier de l'antibiogramme.

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 (2nd 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*, *Adinetobacter*...). 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. *Brunella* 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. Cl₂, CO₂, 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 PSpipette, 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 NO₂ to PNPNG 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 PSpipette 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 PNPNG) on the result sheet.
- The reading of the two tests NO₂ 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 NO₂ and TRP tests.

NO₂ test :

- Add 1 drop of NIT 1 and 1 drop of NIT 2 reagents to the NO₂ 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 NO₂ 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 NO₂ or N₂) is positive.

The production of N₂ 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.

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., Cl₂, CO₂, etc.)] into the honey-combed 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 P5ipette, 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, H₂S and URE by overlaying with mineral oil.
- Close the incubation box.
- Incubate at 36°C ± 2°C for 18-24 hours.

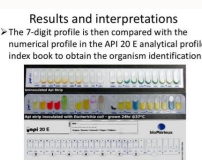
READING AND INTERPRETATION

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.



Committed to remaining at the forefront of innovation, bioMérieux brings a new dimension to the reference API® biochemical test strip range by making identification available to everyone, anytime, anywhere. A tool that leverages the technologies of today and tomorrow, APIWEB™ is the essential complement to manual identification. User-friendly APIWEB™ - it's as simple as navigating in a webpage! Internet platform Easy-to-use integrated intuitive interface that uses Internet navigation tools Available anytime 24/7 Reading assistance for each identification strip APIWEB™ user friendly-platform See how easy it is to use? Watch these videos done by our API® customers Reliable complement to manual identification For years, the API® ID strip range has been appreciated by microbiologists over the world for its quality and ease-of-use. In fact, the standardized, miniaturized strips of biochemical tests are the reference for bacterial and fungal identification. API® has a wide identification database on the market and a numerical identification calculation method that brings standardization and makes identification easy and accurate. Considering that 10 API® strips are used every minute, APIWEB™ is an invaluable tool for the microbiology community. Get high-performance results With APIWEB™, you get a reliable, state-of-the-art interpretation tool. It contains the regularly updated API®/ID32 databases. Plus, APIWEB™ interprets a great number of profiles enabling the identification of more than 700 species of bacteria and yeast. It's the straightforward and easy way to get high-performance results! Each identification includes a complete report: Species proposed Comment on the reliability of the identification, based on two indices: the identification percentage (probability of species identification) the typicity index (typical character of the profile studied) Complete biochemical profile Additional information and tests Secure connection Your privacy as a user and the confidentiality of your results are important to us. That's why we've made sure that APIWEB™ is protected by full security. Secure site, personalized access codes Data encoding to ensure the integrity and confidentiality of your results Highly reactive tool maintenance With Roles, you can now add co-organizers to your group and start collaborating on events. Co-organizers can edit group and event pages, access sales and attendee information, manage ticket sales and more. Co-organizers will be granted limited shared access to your group and events, hence please ensure this is a party you trust before assigning the role. What can co-organizers do? Invite your intended co-organizers to follow your group on Peatix. From the followers list, assign them as co-organizers. All products for: Microbiology Add to my selection OVERVIEW TECHNICAL DETAILS RESOURCES Right from the moment it launched, API® completely revolutionized the field of bacteriology. API® brings together high quality and ease of use with standardized, miniaturized strips of biochemical tests to use with comprehensive identification databases. With API®, bacterial and fungal identification is simple, rapid and reliable. That's why today, API® is recognized as THE REFERENCE for identification of bacterial and fungal species. API® strips are established as the reference technique worldwide: on top of routine use, it is also used to assess the performance of other identification products API® products have led to the identification of new bacterial species (e.g.: within the *Staphylococcus* and *Streptococcus* genera) Now supported by apiweb™ for easy interpretation Reliable and accurate With multiple biochemical reactions in limited wells along with a wide identification database on the market, API® makes bacterial identification reliable and accurate. 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Widely used and appreciated by our customers as you'll see in this video User-friendly internet platform: Easy-to-use interface which is integrated and intuitive High-performance: Contains all the regularly updated API®/ID32 databases Enables the identification of 700 species of bacteria and yeasts A complete report for each identification including proposed species and comments on reliability (through calculation of identification percentage and typicity index) Complete biochemical profile Fully secure site Check out apiweb™ Gram (-) rods API® 20E Identification of Gram (-) rods in 18-24 hrs Ref. 20 100 (25 strips) Ref. 20 160 (100 strips) RapID 20E Rapid identification of enterobacteriaceae in 4 hrs Ref. 20 701 - 25 strips API® 20 NE Identification of non-enteric Gram (-) rods in 24-48 hrs Ref. 20 050 - 25 strips + media API® 10 S Simplified identification of Gram (-) rods in 18-24 hrs Ref. 10 100 - 50 strips Yeasts API® Candida Identification of yeasts found in clinical infections in 18-24 hrs Ref. 10 500 - 10 strips + media API® 20 C AUX Identification of yeasts in 24-48 hrs Ref. 20 210 - 25 strips + media Staphylococci API® Staph Identification of staphylococci and micrococci in 18-24 hrs Ref. 20 500 - 25 strips + media Streptococci API® 20 Strep Identification of streptococci and related bacteria in 4 or 24 hrs Ref. 20 600 - 25 strips + media + swabs Anaerobic bacteria API® 20 A Identification of anaerobes in 24-48 hrs Ref. 20 300 - 25 strips + media Other bacterial groups API® Coryne Identification of corynebacteria and coryneform bacteria in 24 hrs Ref. 20 900 - 12 strips + media + McFarland Standard API® Listeria Identification of Listeria species in 24 hrs Ref. 10 300 - 10 strips + media + reagent API® NH Identification of *Neisseria*, *Haemophilus* and *B. catarrhalis* in 18h to 24 hrs Ref. 10 400 - 10 strips + media + reagents + swabs API® Campy Identification of *Campylobacter* in 24 hrs Ref. 20 800 - 12 strips + media + McFarland Standard API® 50 CH "Research" strip (metabolism of carbohydrates) Ref. 50 300 - 10 strips Please contact your local bioMérieux representative for product availability. API & ID32 Identification databases booklet OTHER PUBLICATIONS Evaluation of API 20 NE in routine diagnostics of nonfermenting gram-negative rod-shaped bacteria Geiss HK, Piotrowski HD, Hingst V. Rapid and economical method for species identification of clinically significant coagulase-negative staphylococci Ieven M, Verhoeven J, Pattyn SR, Goossens H. Evaluation of the RAPID ID 32A system for the identification of *Bacteroides fragilis* and related organisms Jenkens SA, Drucker DB, Keaney MG, Ganguli LA. University of Manchester

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