SUMMARY AND EXPLANATION
The API 20 A system enables 21 tests to be carried out quickly and easily for the biochemical identification of anaerobes. Other tests such as colonial and microscopic morphology, Gram stain, etc. should be performed and the results used to confirm or complete the identification. 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 A strip consists of 20 microtubes containing dehydrated substrates. These tests are inoculated with a bacterial suspension which 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 by referring to the Analytical Profile Index or using the identification software.

CONTENT OF THE KIT (Kit for 25 tests) :
- 25 API 20 A strips
- 25 incubation boxes
- 25 ampules of API 20 A Medium
- 25 result sheets
- 1 package insert

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

Medium

<table>
<thead>
<tr>
<th>API 20 A Medium</th>
<th>Trypticase</th>
<th>Yeast extract</th>
<th>Sodium chloride</th>
<th>L-tryptophane</th>
<th>L-cystine</th>
<th>Hemin (porcine origin)</th>
<th>Vitamin K₁</th>
<th>Sodium sulfite</th>
<th>Demineralized water to make 1000 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 ml</td>
<td>5 g</td>
<td>5 g</td>
<td>2.5 g</td>
<td>0.2 g</td>
<td>0.4 g</td>
<td>0.005 g</td>
<td>0.01 g</td>
<td>0.1 g</td>
<td>pH 6.9-7.3</td>
</tr>
</tbody>
</table>

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED
Reagents :
- Mineral oil (Ref. 70 100)
- Reagents : BCP (Ref. 70 510)
- EHR (Ref. 70 520)
- XYL (Ref. 70 530)
- McFarland Standard (Ref. 70 900)
- API 20 A Analytical Profile Index (Ref. 20 390) or Identification software (consult bioMérieux)
- Hydrogen peroxide (3 %)

Material :
- Swabs
- Pipettes or PSIpettes
- Anaerobic atmosphere generator
- Ampule rack
- Ampule protector
- General microbiology laboratory equipment including Ultra violet lamp (365 nm)

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, ...
- 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 A 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**

**Preparation of the inoculum**

- Open an ampule of API 20 A Medium as indicated in the paragraph "Warnings and Precautions".
- Using a swab, harvest all the growth obtained on blood agar in anaerobic conditions. It is recommended to use young cultures (18-24 hours old). Check that the strain is pure. (If necessary, perform a subculture using a well-isolated colony).
- Hold the ampule upright and emulsify the organisms by rotating the swab and rubbing it against the side of the ampule without taking it out of the suspension medium. The final turbidity should be greater than or equal to 3 McFarland. This suspension must be used immediately after preparation. Slow growing organisms may require more than a single blood agar plate to achieve this inoculum density.

**NOTE**: To maintain anaerobic conditions, avoid introducing air into the medium when homogenizing.

**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 honeycombed wells of the tray to create a humid atmosphere.
- Record the strain references on the elongated flap of the tray. (Do not record the references on the lid as it may be misplaced during the procedure.)
- Remove an API 20 A strip from its packaging and place it in the incubation tray.
- Using a sterile pipette, inoculate the strip with the suspension in the ampule of API 20 A Medium, avoiding the formation of bubbles and tilting the strip slightly forwards.
  - For the [GEL] test, fill both the tube and cupule.
  - For the [IND] test, fill just the tube with API 20 A Medium and fill the cupule with mineral oil to prevent the indole from evaporating.
- Place the lid on the tray and incubate for 24 hours (± 2 hours) at 36°C ± 2°C in an anaerobic chamber, jar or bag.
  - The surplus API 20 A Medium can be used to check the purity and viability of the strain, by inoculating a set of 2 culture medium plates (one inoculated aerobically and the other anaerobically).

**READING AND INTERPRETATION**

**Reading the strip**

Many anaerobic bacteria produce reactions which are clear and easy to read within 24 hours, but some strains grow slowly and can only be identified after 48 hours of incubation.

- After incubation, read the strip by referring to the Reading Table.
- Record all spontaneous reactions (those not requiring the addition of reagents) on the result sheet.
- Reveal the tests which require the addition of reagents:
  - The BCP present in the reaction medium may be discolored by reduction. In this case, reveal the acidification reaction by adding 1 drop of BCP reagent to all microtubes containing carbohydrates. A yellow or yellow-green color indicates a positive reaction to be recorded on the result sheet.
  - IND test: add 1 drop of XYL reagent to the mineral oil overlay. Mix using an applicator stick and leave for 2-3 minutes. Add 1 drop of EHR reagent. The reagent should float on the xylene/mineral oil (so as not to dilute the color in the microtube). Read within 5 minutes. A red color indicates a positive reaction to be recorded on the result sheet.
  - CAT test: Catalase production is determined after strips have been exposed to air for 30 minutes. Add 2 drops of 3% H₂O₂ to a positive reaction microtube. The appearance of bubbles indicates a positive reaction to be recorded on the result sheet.

**Interpretation**

Identification is obtained with the **numerical profile**.

**Determination of the numerical profile**

The result sheet reproduces the outline of the API 20 A strip with its 20 tests, plus the catalase reaction and 3 morphological characteristics: SPOR for spore (+, –), GRAM (+, –) and COCC for coccus (+, –). On the result sheet, the tests are separated into groups of 3 and a value of 1, 2 or 4 is indicated for each. By adding together the values corresponding to positive reactions within each group, an 8-digit profile number is obtained.

**Identification**

This is performed using the database (V3.0)

* with the Analytical Profile Index:
  - Look up the numerical profile in the list of profiles.
* with the identification software:
  - Enter the 8-digit numerical profile manually via the keyboard.
QUALITY CONTROL

The strips, media and reagents are systematically controlled at various stages of their manufacture. For those users who wish to perform their own quality control tests with the strip, it is preferable to use the strain 1. *Clostridium perfringens ATCC 13124* or else one of the following strains:

2. *Bacteroides ovatus* ATCC 8483
3. *Clostridium sordellii* ATCC 9714

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

|   | IND | URE | GLU | MAN | LAC | SAC | MAL | SAL | XYL | ARA | GEL | ESC | GLY | CEL | MNE | MLZ | RAF | SOR | RHA | TRE | CAT |
|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1. | -   | -   | +   | -   | +   | +   | -   | -   | -   | -   | +   | -   | +   | -   | +   | -   | +   | -   | +   | -   |
| 2. | +   | -   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   |
| 3. | +   | +   | +   | -   | -   | +   | -   | -   | -   | -   | +   | -   | +   | -   | -   | -   | -   | -   | -   | -   |

* This result may vary depending on the culture medium used.

Profiles obtained after 24 hours of incubation after culture on Columbia sheep blood 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 A system is intended uniquely for the biochemical identification of those anaerobic bacteria included in the database (see Identification Table at the end of this package insert). It cannot be used to identify any other organisms 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

2967 collection strains and strains of various origins belonging to species included in the database were tested:
- 88.9% of the strains were correctly identified (with or without supplementary tests).
- 7.1% of the strains were not identified.
- 4.0% of the strains were misidentified.

WASTE DISPOSAL

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

<table>
<thead>
<tr>
<th>TESTS</th>
<th>ACTIVE INGREDIENTS</th>
<th>QTY (mg/cup.)</th>
<th>REACTIONS/ENZYMES</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>IND</td>
<td>L-tryptophane</td>
<td>0.98</td>
<td>INDole formation</td>
<td>XYL - mix / 2-3 min + EHR / 5 min yellow red</td>
</tr>
<tr>
<td>URE</td>
<td>urea</td>
<td>0.648</td>
<td>UREase</td>
<td>yellow-orange red</td>
</tr>
<tr>
<td>GLU</td>
<td>D-glucose</td>
<td>1.96</td>
<td>acidification (GLUCose)</td>
<td>purple yellow / yellow-green</td>
</tr>
<tr>
<td>MAN</td>
<td>D-mannitol</td>
<td>1.96</td>
<td>acidification (MANnitol)</td>
<td></td>
</tr>
<tr>
<td>LAC</td>
<td>D-lactose</td>
<td>1.96</td>
<td>acidification (LACTose)</td>
<td></td>
</tr>
<tr>
<td>SAC</td>
<td>D-saccharose (sucrose)</td>
<td>1.96</td>
<td>acidification (SACcharose)</td>
<td></td>
</tr>
<tr>
<td>MAL</td>
<td>D-maltose</td>
<td>1.64</td>
<td>acidification (MALtose)</td>
<td>BCP</td>
</tr>
<tr>
<td>SAL</td>
<td>salcin</td>
<td>1.64</td>
<td>acidification (SALcin)</td>
<td></td>
</tr>
<tr>
<td>XYL</td>
<td>D-xylose</td>
<td>1.64</td>
<td>acidification (XYLose)</td>
<td></td>
</tr>
<tr>
<td>ARA</td>
<td>L-arabinose</td>
<td>1.96</td>
<td>acidification (ARAbinose)</td>
<td></td>
</tr>
<tr>
<td>GEL</td>
<td>gelatin (bovine origin)</td>
<td>0.6</td>
<td>hydrolysis (protease) (GELatin)</td>
<td>no diffusion of pigment (1) diffusion of black pigment (1)</td>
</tr>
<tr>
<td>ESC</td>
<td>esculin</td>
<td>0.36</td>
<td>hydrolysis (β-glucosidase) (ESCUlin)</td>
<td>yellow (2) brown-black (2)</td>
</tr>
<tr>
<td>ESC</td>
<td>ferric citrate</td>
<td>0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLY</td>
<td>glycerol</td>
<td>1.82</td>
<td>acidification (GLYcerol)</td>
<td>purple yellow / yellow-green</td>
</tr>
<tr>
<td>CEL</td>
<td>D-cellobiose</td>
<td>1.86</td>
<td>acidification (CELlobiose)</td>
<td></td>
</tr>
<tr>
<td>MNE</td>
<td>D-mannose</td>
<td>1.96</td>
<td>acidification (ManNOSE)</td>
<td></td>
</tr>
<tr>
<td>MLZ</td>
<td>D-melezitose</td>
<td>1.96</td>
<td>acidification (MeLeZitose)</td>
<td></td>
</tr>
<tr>
<td>RAF</td>
<td>D-raffinose</td>
<td>2.18</td>
<td>acidification (RAFfinose)</td>
<td></td>
</tr>
<tr>
<td>SOR</td>
<td>D-sorbitol</td>
<td>2.18</td>
<td>acidification (SORbitol)</td>
<td></td>
</tr>
<tr>
<td>RHA</td>
<td>L-rhamnose</td>
<td>1.96</td>
<td>acidification (RHAmnose)</td>
<td></td>
</tr>
<tr>
<td>TRE</td>
<td>D-trehalose</td>
<td>1.96</td>
<td>acidification (TREhalose)</td>
<td></td>
</tr>
<tr>
<td>CAT</td>
<td>–</td>
<td></td>
<td>CATalase</td>
<td></td>
</tr>
<tr>
<td>SPOR</td>
<td>–</td>
<td></td>
<td>spores</td>
<td>absent present</td>
</tr>
<tr>
<td>GRAM</td>
<td>–</td>
<td></td>
<td>Gram reaction</td>
<td>pink violet</td>
</tr>
<tr>
<td>COCC</td>
<td>–</td>
<td></td>
<td>morphology</td>
<td>rod coccus</td>
</tr>
</tbody>
</table>

(1) With incubation in a round jar, the pigment only diffuses in the lower part of the tube.
(2) The brown-black color sometimes only develops after the strip has been exposed to air: this should be taken into consideration when reading.

A black color may be due to the formation of ferric sulphide (FeS) due to H₂S reacting with the ferric citrate. This does not indicate esculin hydrolysis. The two may be distinguished by the fact that the ferric sulphide forms a black precipitate at the base of the tube whereas esculin hydrolysis results in a brown-black area at the top of the tube. If the tube is completely black, and in case of doubt, the test should be read by examining for fluorescence in UV light.

- 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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