SUMMARY AND EXPLANATION

API® Staph is a standardized system for the identification of the genera *Staphylococcus*, *Micrococcus* and *Kocuria*, which uses miniaturized biochemical tests and a specially adapted database. The complete list of those bacteria that it is possible to identify with this system can be found in the Identification Table at the end of this package insert.

PRINCIPLE

The API Staph strip consists of 20 microtubes containing dehydrated substrates. These microtubes are inoculated with a bacterial suspension, prepared in API Staph Medium, that reconstitutes the tests. 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 Staph strips
- 25 incubation boxes
- 25 ampules of API Staph Medium
- 25 result sheets
- 1 package insert provided in the kit or downloadable from www.biomerieux.com/techlib

COMPOSITION

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

Medium

<table>
<thead>
<tr>
<th>API Staph Medium</th>
<th>Yeast extract</th>
<th>0.5 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 mL</td>
<td>Bactopeptone</td>
<td>10 g</td>
</tr>
<tr>
<td></td>
<td>(bovine/porcine origin)</td>
<td>5 g</td>
</tr>
<tr>
<td></td>
<td>NaCl</td>
<td>10 mL</td>
</tr>
<tr>
<td></td>
<td>Trace elements</td>
<td>10 mL</td>
</tr>
<tr>
<td></td>
<td>Demineralized water</td>
<td>qsp 1000 mL</td>
</tr>
<tr>
<td></td>
<td>pH : 7.0 - 7.4</td>
<td></td>
</tr>
</tbody>
</table>

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED

Reagents
- Mineral oil (Ref. 70 100)
- Reagents : VP 1 + VP 2 (Ref. 70 422)
- NIT 1 + NIT 2 (Ref. 70 442)
- ZYM A (Ref. 70 494)
- ZYM B (Ref. 70 493)
- McFarland Standard (Ref. 70 900)
- API Staph Analytical Profile Index (Ref. 20 590) or apiweb™ identification software (Ref. 40 011) (consult bioMérieux)

Material
- Pipettes or PSIpettes
- Ampule rack
- Ampule protector
- 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 reagents past the expiry date.
- Before use, check that the packaging and components are intact.
- Do not use strips which have been damaged : cupules deformed, desiccant sachet open, etc.
- 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.
- It is recommended to perform a quality control test when a new ampule of ZYM B reagent is opened.
STORAGE CONDITIONS
The strips and media should be stored at 2-8°C until the expiry date indicated on the packaging.

SPECIMENS (COLLECTION AND PREPARATION)
API® Staph 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 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 individual packaging.
• Place the strip in the incubation box.

Preparation of the inoculum
• Subculture the organism onto Columbia blood agar (or P agar) 18-24 hrs. at 36°C ± 2°C.
• Check that the strain belongs to the Micrococcaceae family (morphology, Gram stain, catalase etc.) and also check that the culture is pure.
• Open an ampule of API Staph Medium as indicated in the paragraph "Warnings and Precautions".
• Prepare a homogeneous bacterial suspension with a turbidity equivalent to 0.5 McFarland. It is recommended to use young cultures (18-24 hours old). This suspension must be used immediately after preparation.

Inoculation of the strip
• Using a pipette or PSIpette, fill the microtubes with the inoculated API Staph Medium. Only fill the tube portion of the microtubes, not the cupules (slightly underfill the microtubes). 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.
• Ensure anaerobiosis in the ADH and URE tests by filling the cupules with mineral oil to form a convex meniscus.
• Close the incubation box.
• Incubate at 36°C ± 2°C for 18-24 hours.

READING AND INTERPRETATION
Reading the strip
• After the incubation period, develop the reactions by adding 1 drop of each of the following reagents and then read all the reactions by referring to the Reading Table:
  - VP test : VP 1 and VP 2 reagents. Wait 10 minutes. A violet-pink color indicates a positive reaction. A pale pink or light pink color obtained after 10 minutes should be considered negative.
  - NIT test : NIT 1 and NIT 2 reagents. Wait 10 minutes. A red color indicates a positive reaction.
  - PAL test : ZYM A and ZYM B reagents (*). Wait 10 minutes. A violet color indicates a positive reaction.
(*) It is recommended to control each ampule of ZYM B before using for the first time.
To do this, it is recommended to use the strain ATCC® 700404™ indicated in the Quality Control paragraph in order to eliminate any defective reagents.
• Record the results on the result sheet.

Lysostaphin resistance test
Determine resistance to lysostaphin on P agar, as indicated in the following procedure or according to the manufacturer's recommendations. To perform the test, inoculate the surface of a P agar plate, by flooding it with a bacterial suspension (approximately 10⁷ organisms/mL). Leave to dry for 10-20 minutes at 36°C ± 2°C. Place a drop of lysostaphin solution (200 µg/mL) on the surface of the agar. Incubate for 18-24 hrs. at 35-37°C. Total or partial lysis of the bacterial culture indicates susceptibility to the enzyme. This test constitutes the 21st test of the strip. It is considered positive if resistance to lysostaphin is determined.

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 of 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.
• Identification:
  This is performed using the database (V 4.1)
  * with the Analytical Profile Index:
    - Look up the numerical profile in the list of profiles.
  * with the apiweb™ identification software:
    - Enter the 7-digit numerical profile manually via the keyboard.

6 706 113 Staphylococcus epidermidis
QUALITY CONTROL

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

Streamlined quality control may be used to confirm acceptable performance of the API® Staph system after shipping/storage. This methodology may be performed by following the instructions above for testing and meeting the criteria stated in CLSI® M50-A Quality Control for Commercial Microbial Identification Systems.

Testing may be conducted using Staphylococcus capitis ATCC® 35661™ to evaluate the performance of the XYL test. Tests performed by bioMérieux has shown that the XYL test is the most labile on the API Staph strip. When testing the strip, Staphylococcus capitis ATCC® 35661™ can be used to detect degradation.

For those users who are required to perform comprehensive quality control testing with the strip, the following three strains should be tested to demonstrate positive and negative reactivity for the most the API Staph test.

1. Staphylococcus capitis ATCC® 35661™
2. Staphylococcus xylosus ATCC® 700404™
3. Staphylococcus lentus ATCC® 700403™

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

<table>
<thead>
<tr>
<th></th>
<th>GLU</th>
<th>FRU</th>
<th>MNE</th>
<th>MAL</th>
<th>LAC</th>
<th>TRE</th>
<th>MAN</th>
<th>XLT</th>
<th>MEL</th>
<th>NIT</th>
<th>PAL</th>
<th>VP</th>
<th>RAF</th>
<th>XYL</th>
<th>SAC</th>
<th>MDG</th>
<th>NAG</th>
<th>ADH</th>
<th>URE</th>
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</tr>
</tbody>
</table>

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

Profiles obtained after culture of the strains on 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 Staph system is designed uniquely for the identification of the species included in the database (see Identification Table 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.

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

- Staphylococci
  2104 collection strains and strains of various origins belonging to species included in the database were tested:
  - 92.49% of the strains were correctly identified (with or without supplementary tests).
  - 4.42% of the strains were not identified.
  - 3.09% of the strains were misidentified.

- Micrococc/Kocuria
  171 collection strains and strains of various origins belonging to species included in the database were tested:
  - 87.72% of the strains were correctly identified (with or without supplementary tests).
  - 7.60% of the strains were not identified.
  - 4.68% of the strains were misidentified.

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.

WARRANTY

bioMérieux disclaims all warranties, express or implied, including any implied warranties of MERCHANTABILITY AND FITNESS FOR A PARTICULAR USE. bioMérieux shall not be liable for any incidental or consequential damages. IN NO EVENT SHALL BIOMERIEUX’S LIABILITY TO CUSTOMER UNDER ANY CLAIM EXCEED A REFUND OF THE AMOUNT PAID TO BIOMERIEUX FOR THE PRODUCT OR SERVICE WHICH IS THE SUBJECT OF THE CLAIM.
## READING TABLE

<table>
<thead>
<tr>
<th>TESTS</th>
<th>ACTIVE INGREDIENTS</th>
<th>QTY (mg/cup.)</th>
<th>REACTIONS / ENZYMES</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No substrate</td>
<td></td>
<td>Negative control</td>
<td>red</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>GLU</strong> D-glucose 1.56</td>
<td>(Positive control) (D-GLUcose)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>FRU</strong> D-fructose 1.4</td>
<td>acidification (D-FRUCtose)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>MNE</strong> D-mannose 1.4</td>
<td>acidification (D-ManNosE)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>MAL</strong> D-maltose 1.4</td>
<td>acidification (MALtose)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>LAC</strong> D-lactose (bovine origin) 1.4</td>
<td>acidification (LACtose)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>TRE</strong> D-trehalose 1.32</td>
<td>acidification (D-TREhalose)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>MAN</strong> D-mannitol 1.36</td>
<td>acidification (D-MANnitol)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>XLT</strong> xyitol 1.4</td>
<td>acidification (XyLitol)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>MEL</strong> D-melibiose 1.32</td>
<td>acidification (D-MELibiose)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>NIT</strong> potassium nitrate 0.08</td>
<td>Reduction of NITrates to nitrates</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>PAL</strong> ß-naphthyl phosphate 0.0244</td>
<td>ALkaline Phosphatase</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>VP</strong> sodium pyruvate 1.904</td>
<td>Acetyl-methyl-carbinol production (Voges Proskauer)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>RAF</strong> D-raffinose 1.56</td>
<td>acidification (RAFfinose)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>XYL</strong> D-xylitol 1.4</td>
<td>acidification (XYLose)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>SAC</strong> D-saccharose (sucrose) 1.32</td>
<td>acidification (SACcharose)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>MDG</strong> methyl-α-D-glucopyranoside 1.28</td>
<td>acidification (Methyl-αD-Glucopyranoside)</td>
</tr>
<tr>
<td></td>
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<td><strong>NAG</strong> N-acetyl-glucosamine 1.28</td>
<td>acidification (N-Acetyl-Glucosamine)</td>
</tr>
<tr>
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<td></td>
<td><strong>ADH</strong> L-arginine 1.904</td>
<td>Arginine DiHydrolase</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>URE</strong> urea 0.76</td>
<td>UREase</td>
</tr>
</tbody>
</table>

The acidification tests should be compared to the negative (0) and positive (GLU) controls.

* When MNE and XLT are preceded or followed by positive tests, then an orange test 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.
METHODOLOGIE / PROCEDURE / METHODIK / TECNICA / PROCEDIMENTO / ΔΙΑΔΙΚΑΣΙΑ / MÉTOD / METODYKA

- Cocci / Kokken / Cocos / Cocchi / Κόκκοι / Kocker / Coccer / Ziaimiaki
- Gram +
- Catalase + / Katalase + / Catalasa + / Katalasi + / Καταλάση + / Katalas + / Katalaza +

Gélose Colombia au sang / Columbia blood agar / Columbia Blutagar / Agar Columbia con sangre / Agar Columbia al sangue / Gelose Colombia de sangue / Αιματούχο όγαρ Columbia / Columbia blodagar / Agar Columbia z krwią

API Staph Medium

Résistance à la lysostaphine
Resistance to lysostaphin
Lysostaphinresistenz
Test de la resistencia a la lisostafina
Test di resistenza alla lisostafina
Resistência à lisostafina
Ανθεκτικότητα στη λυσοσταφίνη
Lysostasinresistens
Resistens mod lysostafin
Test oporności na lisostafinę

18:00 - 24:00 36°C ± 2°C

0.5 McF

URE

VP : VP 1 + VP 2
NIT : NIT 1 + NIT 2
PAL : ZYM A + ZYM B
### TABLEAU D'IDENTIFICATION / IDENTIFICATION TABLE / TABLA DE IDENTIFICACION / TABella Di IDENTIFICAzione / QUADRO DE IDENTIFICAÇÃO / ΠΙΝΑΚΑΣ ΤΑΥΤΟΠΟΙΗΣΗΣ / IDENTIFIERINGSTABELL / IDENTIFIKATIONSTABEL / TABELA IDENTYFIKACJI

% de réactions positives après 18-24 h à 36°C ± 2°C / % of reactions positive after 18-24 hrs. at 36°C ± 2°C / % der positiven Reaktionen nach 18-24 h bei 36°C ± 2°C / % de las reacciones positivas después de 18-24 H a 36°C ± 2°C / % di reazioni positive dopo 18-24 ore a 36°C ± 2°C / % das reacções positivas após 18-24 H a 36°C ± 2°C / % θετικών αντιδράσεων μετά από 18-24 ώρες στους 36°C ± 2°C / % positiva reaktioner efter 18-24 time. vid 36°C ± 2°C / % af positive reaktioner efter 18-24 timer ved 36°C ± 2°C / % pozytywnych reakcji po 18-24 godzinach w 36°C ± 2°C

<table>
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<tr>
<th>API STAPH V4.1</th>
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<th>GLU</th>
<th>FRU</th>
<th>MNE</th>
<th>MAL</th>
<th>LAC</th>
<th>TRE</th>
<th>MAN</th>
<th>XLT</th>
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<th>XYL</th>
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<th>NAG</th>
<th>ADH</th>
<th>URE</th>
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*Note: The table represents the identification of various bacterial species using the API Staph system.*

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*Source: bioMérieux SA*


### TABLE DES SYMBOLES / INDEX OF SYMBOLS / SYMBOLE / CUADRO DE SIMBOLOS / TABELLA DEI SIMBOLI / QUADRO DOS SIMBOLOS / ΠΙΝΑΚΑΣ ΣΥΜΒΟΛΩΝ / SYMBOLER / SYMBOLFORTEGNELSE / TABELA SYMBOLI

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### Limites de température / Temperature limitation
- Températurbegrenzung / Limit de temperatura
- Limiti di temperatura / Limites de temperatura
- Περιορισμοί θερμοκρασίας / Temperaturbegrensning
- Temperaturbegränsning / Przestrzegać zakresu temperatury

### Utiliser jusque / Use by / Verwendbar bis
- Fecha de caducidad / Utilizzare entro / Prazo de validade
- Ημερομηνία λήξης / Använd före / Holdbar til / Użyć przed

### 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 επαρκής για «ν» εξετάσεις
- Räcker till "n" antal tester
- Wystarczy na wykonanie <n> testów