api® 20 C AUX

IVD

Yeast identification system

SUMMARY AND EXPLANATION

API 20 C AUX is a system for the precise identification of the most frequently encountered yeasts. The complete list of those species 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 C AUX strip consists of 20 cupules containing dehydrated substrates which enable the performance of 19 assimilation tests. The cupules are inoculated with a semi-solid minimal medium. The yeasts will only grow if they are capable of utilizing each substrate as the sole carbon source.
The reactions are read by comparing them to growth controls. 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 C AUX strips
- 25 incubation boxes
- 25 ampules of API C Medium
- 25 result sheets
- 1 package insert

COMPOSITION

Strip

The composition of the API 20 C AUX strip is given below in the list of tests:

TESTS	ACTIVE INGREDIENTS	QTY (mg/cup.)
0	None	-
GLU	D-GLUcose	1.2
GLY	GLYcerol	1.2
2KG	calcium 2-Keto-Gluconate	1.2
ARA	L-ARAbinose	1.2
XYL	D-XYLose	1.2
ADO	ADOnitol	1.2
XLT	XyLiTol	1.2
GAL	D-GALactose	1.9
INO	INOsitol	2.36
SOR	D-SORbitol	1.2
MDG	Methyl-αD-Glucopyranoside	1.2
NAG	N-Acetyl-Glucosamine	1.2
CEL	D-CELlobiose	1.2
LAC	D-LACtose (bovine origin)	1.2
MAL	D-MALtose	1.2
SAC	D-SACcharose (sucrose)	1.2
TRE	D-TREhalose	1.2
MLZ	D-MeLeZitose	1.2
RAF	D-RAFfinose	1.9

Medium

API C Medium 7 ml	Ammonium sulfate	5 g
	Monopotassium phosphate	0.31 g
	Dipotassium phosphate	0.45 g
	Disodium phosphate	0.92 g
	Sodium chloride	0.1 g
	Calcium chloride	0.05 g
	Magnesium sulfate	0.2 g
	L-Histidine	0.005 g
	L-Tryptophan	0.02 g
	L-Methionine	0.02 g
	Gelling agent	0.5 g
	Vitamin solution	1 ml
	Trace elements	10 ml
	Demineralized water	to make 1000 ml
	final pH : 6.4-6.8 (at 20-25°C)	

Although API C Medium contains gelling agent, **it requires no prior heating** and may be as easily pipetted as a liquid medium. It is preferable to warm it at room temperature a few hours before use. **Do not shake**.

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED

Reagents / Instrumentation

- API Suspension Medium, 2 ml (Ref. 70 700) or API NaCl 0.85 % Medium, 2 ml (Ref. 20 070)
- Sabouraud Medium (Ref. 42 026 or 43 171 or equivalent)
- McFarland Standard (Ref. 70 900) No. 2
- API 20 C AUX Analytical Profile Index (Ref. 20 290), **api*web*™** identification software (Ref. 40 011), ATB ™ instrument or ***mini API***® (consult bioMérieux)
- RAT Medium [Rice Agar Tween]

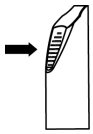
Material

- Pipettes or PSIpettes
- Ampule protector
- Ampule rack
- 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, yeast cultures and inoculated products should be considered infectious and handled appropriately. Aseptic technique and usual precautions for handling yeasts 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, 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.

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 20 C AUX 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 the 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. Cl_2, CO_2, etc.)] into the honeycombed 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 and place it in the incubation tray.

Preparation of the inoculum

- Open an ampule of API Suspension Medium (2 ml) or an ampule of API NaCl 0.85 % Medium (2 ml) as indicated in the paragraph "Warnings and Precautions" of the package insert for these products, or use any tube containing 2 ml of the same solution without additives.
- Using a pipette, pick up a portion of a yeast colony either by suction or by successive touches. It is recommended to use young cultures (18-24 hours old).
- Prepare a suspension with a turbidity equal to 2 McFarland. This suspension must be used immediately after preparation.
- Open an ampule of API C Medium as indicated in the paragraph "Warnings and Precautions" and transfer approximately 100 µl of the previous suspension into it. Gently homogenize with the pipette, avoiding the formation of bubbles.

Inoculation of the strip

- Fill the cupules with the suspension obtained in the ampule of API C Medium. Avoid the formation of bubbles by placing the tip of the pipette against the side of the cupule. Care should be taken not to overfill or underfill the cupules (the surface should be flat or slightly convex, but never concave), otherwise incorrect results may be obtained.
- Place the lid on the tray and incubate at 29°C ± 2°C for 48-72 hours (± 6°hours).

READING AND INTERPRETATION

Reading the strip

After 48 hours of incubation, or 72 hours (if the tests, in particular glucose, are not clearcut after 48 hours), compare growth in each cupule to the 0 cupule, which is used as a negative control. A cupule **more turbid than the control** indicates a **positive** reaction to be recorded on the result sheet.
In order to minimize the risks of contamination when reincubation is necessary, remove the lid only when reading the strip and replace immediately.

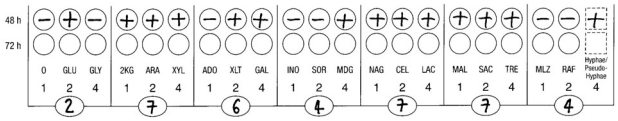
Morphology test

Determine the presence of hyphae (mycelium) or pseudohyphae (pseudomycelium) using RAT Medium [Rice Agar Tween].
Dispense 1 drop of the suspension obtained in the ampule of API Suspension Medium or API NaCl 0.85 % Medium onto RAT Medium or follow the manufacturer's recommendations. This test constitutes the 21st test of the strip. It is considered positive if hyphae or pseudohyphae are detected.

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 number 1, 2 or 4 is indicated for each. By adding the numbers corresponding to positive reactions within each group, a 7-digit number is obtained which constitutes the numerical profile.
- 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 ATB ™ instrument, ***mini API*** ®, or **api*web*** ™ identification software :
    - Enter the 7-digit numerical profile manually via the keyboard.



2 764 774 ***Trichosporon asahii***

QUALITY CONTROL

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

Streamlined quality control may be used to confirm acceptable performance of the API 20 C AUX 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.

As there are no substrates that are consistently sensitive to degradation during shipping conditions, streamlined quality control may be conducted by testing two strains: ***Cryptococcus laurentii*** **ATCC® 18803** that is mostly positive and ***Candida glabrata*** **ATCC 15126**, which is mostly negative for reactions on the API 20 C AUX system.

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 of the API 20 C AUX tests.

1. *Cryptococcus laurentii* ATCC 18803
2. *Candida glabrata* ATCC 15126
3. *Candida guilliermondii* ATCC 6260

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

	0	GLU	GLY	2KG	ARA	XYL	ADO	XLT	GAL	INO		SOR	MDG	NAG	CEL	LAC	MAL	SAC	TRE	MLZ	RAF
1.	–	+	–	+	+	+	+	+	+	+		+	+	+	+	+	+	+	–	+	+
2.	–	+	V	–	–	–	–	–	–	–		–	–	–	–	–	–	–	+	–	–
3.	–	+	+	+	+	+	+	+	+	–		+	+	+	+	–	+	+	–	+	+

Profiles obtained after 48 hours of incubation after culture on Sabouraud 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 C AUX system is intended uniquely for the identification of yeasts 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.

PERFORMANCES

5156 collection strains and strains of various origins belonging to species included in the database were tested :

- 89.7 % of the strains were correctly identified (with or without supplementary tests).
- 6.1 % of the strains were not identified.
- 4.2 % of the strains were misidentified.

WASTE DISPOSAL

Unused ampules of API C Medium may be considered as non hazardous waste and disposed of accordingly.
Dispose of all used or unused reagents (other than ampules of API C Medium) 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

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BIOMÉRIEUX

bioMérieux SA
RCS LYON 673 620 399
69280 Marcy-l'Etoile / France
Tél. 33 (0)4 78 87 20 00
Fax 33 (0)4 78 87 20 90
www.biomerieux.com

bioMérieux, Inc
Box 15969,
Durham, NC 27704-0969 / USA
Tél. (1) 919 620 20 00
Fax (1) 919 620 22 11
Printed in France

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