Lab Activity #15 – API Tests

Analytical Profile Index test strips are often used in Clinical Microbiology labs to identify organisms. These are essentially miniaturized versions of many of the tests we have already conducted.

A. API ZYM – tests for hydrolytic enzymes present in already grown cells – uses high density inoculum – does not depend on growth.

1. Harvest several mg cells from an actively growing TSA culture with a disposable loop and suspend in 3 mL sterile 0.85% NaCl solution by vortexing.

2. Zero a spectrophotometer at 600 nm with a 13 x 100mm tube with 3 mL sterile 0.85% NaCl solution
   - top right dial to set wavelength to 600 nm
   - no tube, left front dial to 0% transmittance
   - blank tube (13 x 100 with sterile 0.8% NaCl, right front dial to 100% transmittance
   - press mode button to change to absorbance

3. Measure absorbance of resuspended cells and add cells or dilute to achieve a turbidity of "5-6 McFarland" which is equivalent to an Absorbance of ~0.75. (Add, vortex, measure, repeat).

4. Label the tab on a plastic API strip incubation tray with the name of the organism and your initials. Fill the dimples in the tray with water from your squirt bottle. Remove the test strip from its package and place in the tray.

5. Add 65 uL of the 5-6 McFarland cell suspension to each cupule on the strip. Cover and incubate at 30C for 4-8 hrs.

6. After incubation, add 1 drop ZYM reagent A, one drop ZYM reagent B, incubate for 5 min, observe and record intensity of color change. Score in a range from 1-5.

B. API 20 NE (Non-Enteric) – Tests characteristics that distinguish many non-*Enterobacteriaceae*

1. Zero a spectrophotometer at 600 nm with a 13 x 100mm tube with 3 mL sterile 0.85% NaCl solution.

2. Add resuspended cells from part A to a 13 x 100 mm tube to achieve a turbidity of "0.5 McFarland" which is equivalent to an Absorbance of ~0.132. (Add, vortex, measure, repeat).

3. Label the tab on a plastic API strip incubation tray with the name of the organism and your initials. Fill the dimples in the tray with water from your squirt bottle. Remove the test strip from its package and place in the tray.

4. Add the 0.5 McFarland suspension to the NO3 - PNPG **tubes** (not the cupules) on the test strip.

5. Open an AUX medium ampule and add 200 uL of the 0.5 McFarland cell suspension. Mix by pipetting up and down.

6. Fill the tubes and cupules of the GLU – PAC tests with the AUX cell suspension

7. Fill the cupules of the 3 underlined tests <u>GLU, ADH, URE</u> with sterile mineral oil. Cover and incubate at 30C for 24-48 hrs.

REF 25 200

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api® ZYM

System for the research of enzymatic activity

SUMMARY AND EXPLANATION

API ZYM is a semi-quantitative micromethod designed for the research of enzymatic activities. The technique is applicable to all specimens (microorganisms, cell suspensions, tissues, biological fluids, etc.). It allows the systematic and rapid study of 19 enzymatic reactions using very small sample quantities. The system consists of a strip with 20 microwells (cupules), the base of which contains the enzymatic substrate and its buffer. This base allows contact between the enzyme and the generally insoluble substrate.

API ZYM has not been developed in view of achieving the precision of spectrophotometric or electrophoretic procedures but has mainly been developed to permit the detection of enzymatic activities in a complex sample which has not been purified. It can be used to screen specimens, thus providing a spectrum of enzymatic determinations which can be further tested by spectrophotometric and/or electrophoretic procedures.

PRINCIPLE

The API ZYM strip is composed of 20 cupules, specially designed for the study of enzymatic reactions. The base of the strip, containing synthetic substrates, is made of non-woven fibers. This base allows enzymatic reactions to take place, even if the substrates are insoluble.

The enzymatic tests are inoculated with a dense suspension of organisms, which is used to rehydrate the enzymatic substrates. The metabolic end products produced during the incubation period are detected through colored reactions revealed by the addition of reagents.

The reactions are read according to the Reading Table.

CONTENT OF THE KIT (Kit for 25 tests) :

- 25 API ZYM strips
- 25 incubation boxes
- 25 result sheets
- 1 package insert

COMPOSITION OF THE STRIP

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

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED

Reagents :

- API Suspension Medium, 2 ml (Ref. 70 700) or API NaCl 0.85 % Medium, 2 ml (Ref. 20 070)
- ZYM A + ZYM B Reagents (Ref. 70 472)
- McFarland Standard (ref. 70 900) or DENSIMAT (ref. 99 234)

Material :

- Pipettes or PSIpettes
- Ampule rack
- Ampule protector
- General microbiology laboratory equipment

WARNINGS AND PRECAUTIONS

- **This system is designed for the research of enzymatic activity only. Not for use in diagnostic procedures.**
- **For professional use only.**
- 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* – 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 expiration date.
- Before use, check that the packaging of the various components is intact.
- Do not use strips which have been damaged : cupules deformed, etc.

STORAGE CONDITIONS

The strips should be stored at 2-8°C until the expiration date indicated on the packaging.

SPECIMEN PREPARATION

Dilute the specimen in a minimum volume of 2 ml of distilled water or in another diluter such as normal saline **without any buffer**.

- **For microorganisms :**
  Prepare a suspension with a turbidity of 5-6 McFarland in API Suspension Medium (2 ml) (open the ampule as indicated in the paragraph "Warnings and Precautions" of the package insert for this medium), distilled water or an isotonic medium. Pure growth from an agar slant or sediment from a centrifuged broth culture can be used to prepare the suspension. In order to obtain reproducible results, it is important that the micro-organisms to be compared be initially grown on the same isolation medium, the diluter be the same and the suspension be of the same optical density. This technique assays for constitutive enzymes. Inductive enzymes can be detected by adding the corresponding inducer(s) to the culture medium.
- **For other specimens (cell suspensions, tissues, biological fluids, ...) :**
  Refer to literature or develop a specific procedure.

Based on the applications, the user must determine what test conditions are appropriate and how to interpret the results of API ZYM.

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. Cl_2, CO_2, etc.)] into the honey-combed wells of the tray to create a humid atmosphere.
- Record the sample 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.

Inoculation of the strip

- Using a pipette or PSIpette, dispense 65 µl of specimen into each cupule.
- After inoculation, place the plastic lid on the tray and incubate generally for 4 - 4 ½ hours at 37°C (optimum temperature). The time of incubation and temperature may vary depending on the sample to be tested. However, when samples are being compared, all test conditions (time, temperature, growth media, density of suspension) must be the same. The inoculated strip should not be placed in bright light.

READING AND INTERPRETATION

Reading the strip

After incubation :

- Add 1 drop of ZYM A reagent and 1 drop of ZYM B reagent to each cupule.
  By placing a surface-active agent (ZYM A reagent) in the cupule, solubilization of the ZYM B reagent in the medium is facilitated.
- Let the color develop for at least 5 minutes.
- If possible, put the strip under a powerful light source (1000 W bulb) for about 10 seconds with the bulb placed about 10 cm (4") above the cupules. The procedure will eliminate any yellow color which may appear in the cupules due to any excess of Fast Blue BB which has not reacted. After light exposure, negative reactions become colorless. Placing the strip in daylight for a few minutes will produce comparable results.

Recording the reactions

Read the reactions and record the results on the result sheet. A value ranging from 0-5 can be assigned, corresponding to the colors developed : 0 corresponds to a negative reaction, 5 to a reaction of maximum intensity and values 1, 2, 3 or 4 are intermediate reactions depending on the level of intensity (3, 4 or 5 being considered as positive reactions).
The colors remain stable for several hours after the strip has been inoculated with the reagents. After 24 hours, colors may deteriorate, interferring with test interpretation.

QUALITY CONTROL

For all applications, we strongly recommend that quality control be performed, prior to using API ZYM, which is adapted to the specimen analyzed and the procedure adopted.
The suspension media, strips and reagents are systematically quality controlled at various stages of their manufacture. The API ZYM quality control is performed using bacterial strains or purified enzymes, such as those indicated in the table below :

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.	–	+	+	+	+	+	–	–	–	–	V	–	–	–	–	–	–	–	–	–
2.	–	–	–	–	–	–	–	–	–	–	+	+	+	+	–	–	+	+	+	–
3.	–	–	+	V	–	–	–	–	V	+	–	–	–	–	–	–	–	–	–	–

1. *Pseudomonas aeruginosa* ATCC* 27853 (Profile obtained after 18-24 hr. culture on trypcase soy agar. Inoculum adjusted to between 5 and 6 McF using DENSIMAT.)
2. ß-glucosidase Sigma G0395 (Profile obtained using a concentration of 0.2 g/l.)
3. α-chymotrypsin Sigma C4129 (Profile obtained using a concentration of 1 g/l.)

- Reading and interpretation 7-10 minutes after addition of the reagents.

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

LIMITATIONS OF THE METHOD

- API ZYM should not be considered as an identification product.
- API ZYM is a research product and is not designed to produce biological analysis results for patients.
- All applications, other than those which have been quality controlled by bioMérieux, are under the responsibilty of the user. We recommend that you follow your institution's internal policies and procedures to verify and validate the methodology and accuracy of API ZYM.
- bioMérieux refuses to accept any responsibility concerning the use of results obtained with API ZYM.

WASTE DISPOSAL

Unused reagents may be considered as non hazardous waste and disposed of accordingly.
Dispose of used 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

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READING TABLE

No.	ENZYME ASSAYED FOR	SUBSTRATE	pH	RESULT	
				POSITIVE	NEGATIVE
1	Control			Colorless or color of the sample if it has an intense coloration	
2	Alkaline phosphatase	2-naphthyl phosphate	8.5	Violet	Colorless or Very pale yellow *
3	Esterase (C 4)	2-naphthyl butyrate	6.5	Violet	
4	Esterase Lipase (C 8)	2-naphthyl caprylate	7.5	Violet	
5	Lipase (C 14)	2-naphthyl myristate	"	Violet	
6	Leucine arylamidase	L-leucyl-2-naphthylamide	"	Orange	
7	Valine arylamidase	L-valyl-2-naphthylamide	"	Orange	
8	Cystine arylamidase	L-cystyl-2-naphthylamide	"	Orange	
9	Trypsin	N-benzoyl-DL-arginine-2-naphthylamide	8.5	Orange	
10	α-chymotrypsin	N-glutaryl-phenylalanine-2-naphthylamide	7.5	Orange	
11	Acid phosphatase	2-naphthyl phosphate	5.4	Violet	
12	Naphthol-AS-BI-phosphohydrolase	Naphthol-AS-BI-phosphate	"	Blue	
13	α-galactosidase	6-Br-2-naphthyl-αD-galactopyranoside	"	Violet	
14	ß-galactosidase	2-naphthyl-ßD-galactopyranoside	"	Violet	
15	ß-glucuronidase	Naphthol-AS-BI-ßD-glucuronide	"	Blue	
16	α-glucosidase	2-naphthyl-αD-glucopyranoside	"	Violet	
17	ß-glucosidase	6-Br-2-naphthyl-ßD-glucopyranoside	"	Violet	
18	N-acetyl-ß-glucosaminidase	1-naphthyl-N-acetyl-ßD-glucosaminide	"	Brown	
19	α-mannosidase	6-Br-2-naphthyl-αD-mannopyranoside	"	Violet	
20	α-fucosidase	2-naphthyl-αL-fucopyranoside	"	Violet	

* Colorless or color of the control if the strip has been exposed to an intense light source after addition of the reagents ; if the strip has not been exposed to intense light, a very pale yellow color is obtained.

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