



## **Lecture-7,8: Complementary diagnostic methods**

### **API 20 (analytical Profile Index)**

API 20 is a classification of bacteria based on biochemical tests, allowing fast identification. This system is developed for quick identification of clinically relevant bacteria. The API range introduced a standardized, miniaturized version of existing techniques, which up until then were complicated to perform and difficult to read. The API systems is specific for differentiating between members of:

**1-Gram negative** bacterial Family Enterobacteriaceae and is called API-20E

**2-Gram positive** bacteria, including *Staphylococcus* species, *Micrococcus*, species, and related organisms, and is called API-Staph.

API test strips consists of wells containing dehydrated substrates to detect enzymatic activity, usually related to fermentation of carbohydrate or catabolism of proteins or amino acids by the inoculated organisms.

#### **API 20 E after incubation...Positive results for all tests :**



#### **API 20 E after incubation...Negative results for all tests :**



**API System The different tests in API 20E**

A bacterial suspension is used to rehydrate each of the wells and the strips are incubated, after 5 hours' incubation, the metabolic profile of the organism is constructed from color changes in the micro tubes, these color changes indicate the presence or absence of the bacteria's ability to metabolize a particular substrat. All positive and negative test results are compiled to obtain a profile number, which is then compared with profile numbers in a commercial codebook to determine the identification of the bacterial species.



### Identify the organism by using API catalog

**The test kit enables the following tests:**

- 1- ON PG test:** The test is used to detect the enzyme  $\beta$ -galactosidase
- 2- ADH:** Decarboxylation of the amino acid arginine
- 3- LDC:** Decarboxylation of the amino acid lysine
- 4- ODC:** Decarboxylation of the amino acid ornithine
- 5- CIT:** Use of citrate as sole carbon source
- 6- H<sub>2</sub>S:** Hydrogen sulfide production
- 7- URE:** Urease enzyme test
- 8- TDA:** (Tryptophan deaminase): Detection of the tryptophan deaminase enzyme.
- 9- IND:** Indole test: production of indole from tryptophan by the enzyme tryptophanase

**10- VP:** The Voges-Proskauer test for the detection of acetoin (acetyl methyl carbinol) produced by fermentation of glucose by bacteria

**11- GEL:** Production of the enzyme gelatinase which liquefies gelatin

**12- GLU:** Glucose fermentation

**13- MAN:** Fermentation of mannose

**14- INO:** Fermentation of inositol

**15- SOR:** Fermentation of sorbitol

**16- RHA:** Rhamnose fermentation

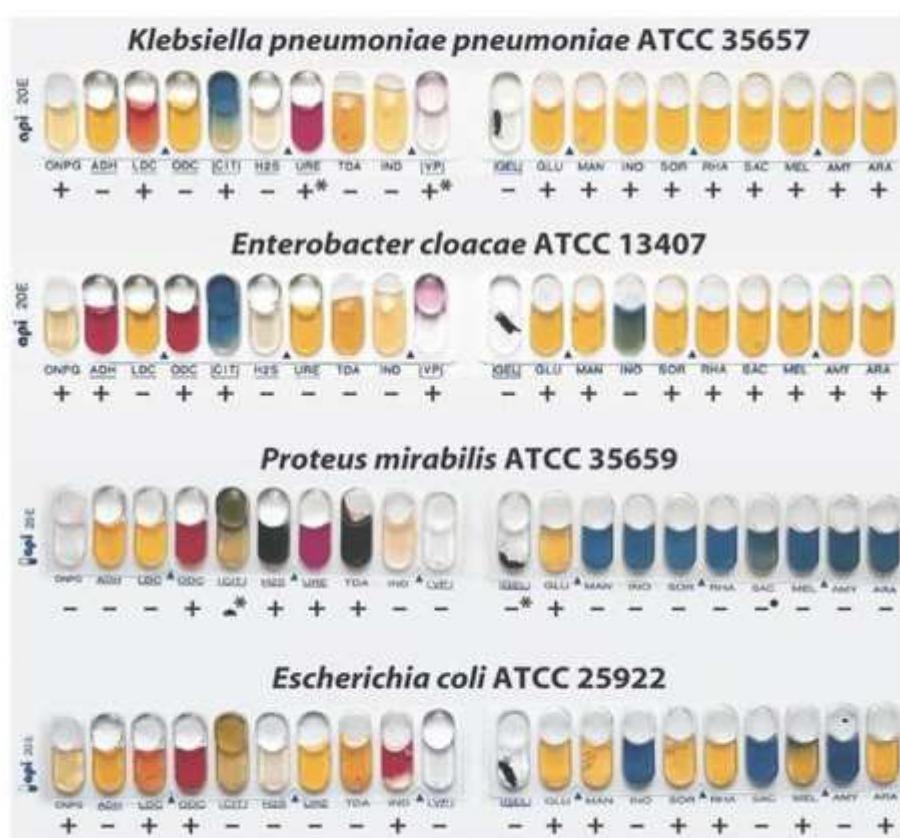
**17- SAC:** Fermentation of sucrose

**18- MEL:** Fermentation of melibiose

**19- AMY:** Fermentation of amygdalin (glycoside)

**20- ARA:** Fermentation of arabinose.

**The API System: Various species of Enterobacteriaceae are shown here with differences in reactions that enable them to be identified**



## **Advantages of API Test**

1. It is a fast and efficient method of identification and differentiation of organisms (18 - 24-hour identification of Enterobacteriaceae and other non-fastidious gram-negative bacteria).
2. It is also useful for fungal identifications (yeasts).
3. It is an easy-to-run, user-friendly, and standardized method of identification and differentiation of microorganisms.
4. API strips have a long shelf life, enabling every laboratory to keep the test kits on hand, and are very useful in medium-level set-up laboratories where identification of organisms is difficult using conventional methods
5. This allows accurate identifications based on extensive databases.

## **Automated systems for bacterial identification**

### **VITEK 2 Compact system**

Microbiology laboratories are increasingly using automated methods to identify bacterial pathogens as in the Vitek System which is a small plastic reagent cards containing micro liter quantities of various biochemical test media that allows for organism identification.

### **Principles**

The VITEK 2 is an automated microbiology system utilizing growth-based technology. The system is available in three formats (VITEK 2 compact, VITEK 2, and VITEK 2 XL) that differ in increasing levels of capacity and automation. All three systems accommodate the same colorimetric reagent cards that are incubated and interpreted automatically.

### **VITEK 2 Compact**

This format focuses on the industrial microbiology-testing environment while also having application for low to middle volume clinical laboratories, used to identify the spore-forming Gram-positive bacilli (i.e., *Bacillus* and related genera). The other colorimetric reagent cards (GN, GP, YST) apply to all system formats for both industrial and clinical laboratories.

## **VITEK 2 and VITEK 2 XL**

These formats are more focused on the clinical microbiology laboratory and provide increased levels of automation and capacity for higher volume laboratories. They also provide an option of automatic pipetting and dilution for antimicrobial susceptibility testing.

### **Reagent Cards**

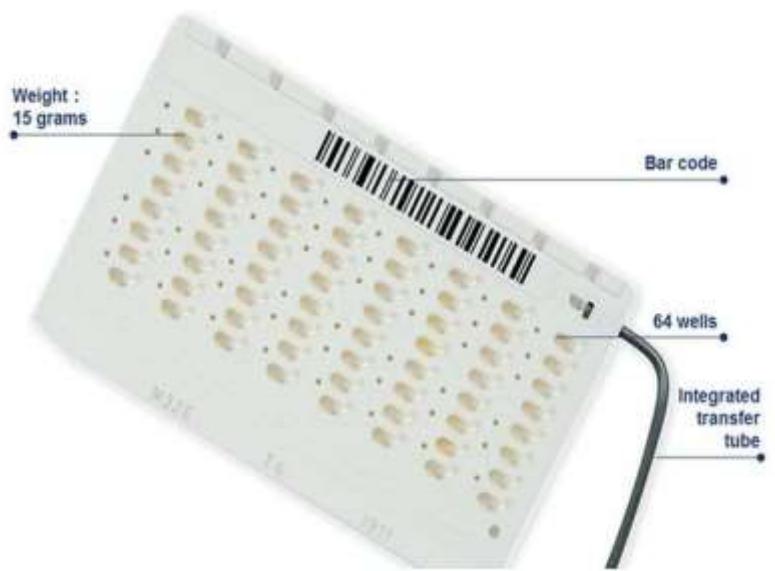
The reagent cards have 64 wells that can each contain an individual test substrate. Substrates measure various metabolic activities such as acidification, alkalinization, enzyme hydrolysis, and growth in the presence of inhibitory substances. An optically clear film present on both sides of the card allows for the appropriate level of oxygen transmission while maintaining a sealed vessel that prevents contact with the organism-substrate admixtures. Each card has a pre-inserted transfer tube used for inoculation. Cards have bar codes that contain information on product type, lot number, expiration date, and a unique identifier that can be linked to the sample either before or after loading the card onto the system.

There are currently four reagent cards available for the identification of different organism classes as follows:

- 1- GN - Gram-negative fermenting and non-fermenting bacilli**
- 2- GP - Gram-positive cocci and non-spore-forming bacilli**
- 3- YST - yeasts and yeast-like organisms**
- 4-BCL - Gram-positive spore-forming bacilli**

**VITEK 2 Compact system**





**The reagent cards**

**Advantages:**

- 1-Provide** rapid identification.
- 2-provides** large selection of cards that are ready to use for identifying a wide range of organisms and testing their sensitivity to a large number of different antimicrobials.
- 3-The testing** process is automated which reduces manual work error.