



How to use the API system (analytical profile index) to identify bacteria.

Part 1 Video available on YouTube at: <http://www.youtube.com/watch?v=umD3QFwNAkU>

This video provides an entire overview of how to use and inoculate the strip in the laboratory.

Part 2 Video and transcript. Video available on YouTube at:

<http://www.youtube.com/watch?v=qfs2bByiYXU>

This video by Malgorzata and narrated by Amrat provides more detail of how to use the strip. The following document is a transcript of the narration.

The following video will demonstrate how to perform an identification test using the API 20E strip test.

API (Analytical Profile Index)

The API 20E strip is an example of a commercially available strip that enables identification of Enterobacteriaceae and other non-fastidious Gram-negative rods.

API strip represents a set of different biochemical tests such as, enzyme and fermentation tests.

There are 20 separate microtubes on the API strip. Each tube contains dehydrated substrates for the biochemical tests. The results are based on colour changes observed within each tube after the incubation time. Each microtube consists of a tube and a cupule.

The *Enterobacteriaceae* are all Grams (-) negative as well as oxidase negative. Therefore the above two tests should always be performed prior to the API 20E[®] identification.

Other characteristic common to *Enterobacteriaceae* is fact that they grow on the bile containing media such as, MacConkey Agar.

Depending on the ability to ferment lactose, *Enterobacteriaceae* may be divided into two groups;

- Lactose fermenters such as, *Escherichia coli*, that appears pink on MacConkey medium
- Lactose non-fermenters such as, *Pseudomonas aeruginosa* that appears colourless on MacConkey medium

Preparation of bacterial suspension

1. The straight wire is flamed.
2. Approximately 3-4 colonies of the unknown bacteria are taken from the agar culture.
3. The colonies are transferred into 5ml of saline solution.
4. A cloudy suspension should be obtained.

Setting up an API strip

1. The bottom tray of the API strip holder is filled with sterile water in order to prevent dehydration of the strip during the incubation time.
2. Pasteur pipette is used to inoculate the strip with bacterial suspension.
3. The API strip should be elevated to an angle of about 30 degrees whilst filling the tubes.
4. In order to avoid bubble formation the suspension is added on one side of the tube by touching the inside wall of the cupule with the end of the pipette.
5. Each of the tubes on the API strip is filled with the bacterial suspension from left to right.

ONPG test detects the presence of the enzyme **β -galactosidase** (enzyme that hydrolyses the sugar Lactose).

ADH test for presence of enzyme- **arginine dihydrolase**.

LDC test for the presence of **lysine decarboxylase** enzyme.

ODC test for the presence of **ornithine decarboxylase** enzyme.

CIT Citrate utilization test.

H₂S test for **hydrogen sulphide** production.

URE Urease test allow detect bacteria that produce **urease** enzyme.

TDA test for **tryptophane deaminase**.

IND test for **indole** production.

VP Voges Proskauer test for **acetoin** production.

GEL test for **gelatinase**.

Bacteria may break down different sugars. The following tests are for different carbohydrates.

GLU fermentaion / oxidation test for **Glucose**.

MAN fermentaion / oxidation test for **Mannitol**.

INO fermentaion / oxidation test for **Inositol**.

SOR fermentaion / oxidation test for **Sorbitol**.

RHA fermentaion / oxidation test for **Rhamnose**.

SAC fermentaion / oxidation test for **Saccharose**.

MEL fermentaion / oxidation test for **Melibiose**.

AMY fermentaion / oxidation test for **Amygdalin**.

ARA fermentaion / oxidation test for **Arabinose**.

6. Some of the tubes need to be filled with the bacterial suspension right to the very top end including cupule area.

Ehen filling the cupule area the API strip should be laying flat/horizontal on the laboratory bench

These tubes are marked with the box underneath the name:

CIT VP GEL

7. Some of the tubes are required to have oil added at the top of the microtube in the cupule area. Usually sterile liquid paraffin or mineral oil is used.

These tubes are marked with the line underneath the name:

ADH LDC ODC H₂S URE

The oil not only excludes the air and introduces anaerobic conditions but also stops the end gaseous products of certain reactions to be released. These gaseous substances

would react with the other substances from adjacent tubes and consequently they would affect the final results.

8. The strip is then incubated at 37°C for 18-24h aerobically.

After the incubation time most of the tests are ready to be read. However there are a few tests which require the additional reagents to be added to the cupules in order for the test to be developed and interpreted.

Adding the reagents

1. The TDA reagent is added to the TDA cupule.
2. The Voges Proskauer test (VP) requires two reagents VP1 and VP2.
3. Firstly the VP1 reagent is added to the VP cupule. Secondly the VP2 reagent is added to the VP cupule.

It allows detecting bacteria that utilise glucose through the neutral pathway. Acetoin is detected via colour change reaction with VP1 and VP2.

4. The James (or Kovacs reagent) is added to the indole test (IND) cupule.

Indole as an end up product may be detected by the reaction with JAMES reagent. Pink colour change indicates a (+) positive reaction.

IND test should be performed last due to possible interference of released gaseous products with other tests.

Approximately 10 minutes is allowed for the reaction to develop and the API strip is ready to be read.

MOVE ON TO PART 3