

## Pre-Packaged Multi-Media for the Identification of Bacteria: The Enterotube II System

### Introduction

Now that you have completed the previous eight experiments using eight different, over-sized kinds of media, it is time to introduce you to one of the simplest methods used in the clinical laboratory for the rapid identification of bacteria. This method is via the use of the Enterotube II System™. The Enterotube II System™ is a pre-packaged multi-media diagnostic packet for the identification of bacteria in the *Enterobacteriaceae*. Representative bacteria that fall into this grouping include *Escherichia*, *Citrobacter*, *Klebsiella*, *Proteus*, *Morganella*, *Salmonella*, *Serratia*, *Shigella* and *Yersinia*.

The Enterotube II System™ is a plastic shell that is about 8" long and less than an inch wide and high that contains 12 mini-media "ports". **Table 1** itemizes these media and the positive and negative results -- REMEMBER: **do not deviate** from these results. This system also has a built-in inoculating wire that does not need to be sterilized (sterilizing this wire in flame will result in the Enterotube II System™ melting). There are two screw-on caps at either end of this system: one over the handle end of the wire and the other over the needle end of the wire.

This system was developed over years of research and data collection. All the data for the various bacteria and their various biochemical reactions were fed into a computer and analyzed statistically. From this data came a computer print-out that will identify bacteria specifically in *Enterobacteriaceae*. The manner in which one puts this to use is to "score" the different reactions in the media in a specific order, write down the score and then find the score in the print-out. When the score has been found in the print-out, one merely reads to the right of the score and reads the name of the microorganism. That is the microbe that has been grown in the system.

Where does one get this "score" mentioned above? When one uses the Enterotube II System™, one also receives a results pad. This results pad has a picture of the system on each sheet. Beneath each port of medium is a number. The number is the score for each positive result. Around each sets of numbers are parentheses. The results that are positive are circled. Those that are negative receive no score and are not considered for the identification number.

Medium	Positive Results (Score)	Negative Results (Score)
Glucose	Yellow color (2)	Red color
Gas Production (in with Glucose)	wax moved (1)	wax not moved
Lysine decarboxylase	Purple color (4)	Yellow color
Ornithine decarboxylase	Purple color (2)	Yellow color
H <sub>2</sub> S	Black color (1)	Beige color
Indole	Rose color (4)	Colorless
Adonitol	Yellow color (2)	Red color
Lactose	Yellow color (1)	Red color
Arabinose	Yellow color (4)	Red color
Sorbitol	Yellow color (2)	Red color
Voges-Proskauer	Red color	Colorless
Dulcitol	Yellow (1)	Green
Phenylalanine Deaminase	Black-smoky-gray color (4)	Green
Urease	Red/purple color (2)	Beige color
Citrate	Blue color (1)	Green color

**Table 1.** Results for media in the Enterotube II System™ .

Each positive score inside each set of parentheses is summed and written in the box below the arrow that is attached to the parentheses. Eventually this gives rise to a 5-digit number: this is your identification number. This number in the computer print-out will match with the bacteria for its identification. **Table 2** shows some of the scores/identification numbers for some bacteria in the lab at WNCC.

Bacteria	Identification Number	Bacteria	Identification Number
<i>Mycobacterium smegmatis</i>	2 0 0 0 2	<i>Serratia marcescens</i>	2 6 0 0 3
<i>Bacillus megaterium</i>	2 0 0 6 2 or 2 0 0 6 3	Atypical <i>Bacillus megaterium</i>	2 0 0 0 2
<i>Streptococcus faecalis</i>	2 0 0 4 3 or 2 0 0 0 2 or 2 0 0 4 2	<i>Escherichia coli</i>	2 4 1 4 2

**Table 2.** Typical Identification Numbers for Bacteria at WNCC.

Note that this system is by no means infallible IF YOU USE THE SYSTEM FOR THE WRONG PURPOSE, i.e., it is not to be used for gram positive bacteria. Although it is used here at WNCC in an incorrect manner, it serves a very valuable lesson as written above.

The purpose of this experiment is to acquaint you with the Enterotube II System™, how to use it and how to interpret the results from the media.

### Materials and Methods

#### *Materials*

Enterotube II	Bacteria	Paper towels	Disinfectant
		Incubator	

#### *Methods*

Unscrew the two caps from the ends of the Enterotube making certain that you do not touch the needle end of the wire. Obtain a bacterium per your professor. Using your system as an inoculating wire, inoculate the system by pulling and twisting the wire (by its handle) out of the system, then running it back through the system. After you have run the wire back through the system, pull it out to the notch in the wire and snap the wire off. Screw the caps back on the system.

OLD TUBES: Remove the blue tape from the adonitol, lactose, arabinose, sorbitol, Voges-Proskauer, dulcitol/phenylalanine deaminase, urease and citrate compartments (makes these aerobic) and slide the clear plastic slide over the glucose/gas compartment.

NEW TUBES: using the snapped off piece of inoculating wire (it is sterile), puncture the “keyholes” in the back of the Enterotubes as demonstrated by your professor.

ALL TUBES: Put your system in the incubator. Dispose of the wire as instructed. Interpret the results of your system next week.

In the table below, record the score for each compartment:

Glucose (2) Gas (1)	Lysine decarboxylase (4) Ornithine decarboxylase (2) H <sub>2</sub> S (1)	Indole (4) Adonitol (2) Lactose (1)	Arabinose (4) Sorbitol ((2) Dulcitol (1)	Phenylalanine deaminase (4) Urease (2) Citrate (1)
SCORE:	SCORE:	SCORE:	SCORE:	SCORE:

In the box below, write your identification number:

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With which bacteria in the table above do your results match?:

## REFERENCES

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5. Thomas, C.G.A.: **Medical Microbiology, Sixth Edition.** (Baillere Tindall: London) ©1988.