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Instruction for GNB-API Test

INTENDED USE

GNB-Enterotube API Test is a ready-to-use identification system for the identification of *Enterobacteriaceae* and other pathogenic Gram-negative bacilli in Microbiology Laboratory.

PRINCIPLES OF THE PROCEDURE

GNB-API Test is a plastic tube with chambers that contains ten different media and allows the determination of 11 biochemical reactions including Citrate, Urea, Malonate, Lactose, Mannose, Arabinose, Indole, Hydrogen sulfide (H₂S), Lysine, Ornithine, and Glucose.

The inoculation wire inside the system makes it possible to inoculate all chambers in one step from one or two individual colonies. The combination of changes in reactions with diagnostic codes allows the identification of *Enterobacteriaceae* and other clinically important Gram-negative bacilli.

REAGENTS

The compounds used in the GNB-API test, the way of interpretation and the color of the specific culture media as base, negative and positive are fully explained in Table 1.

Table 1: Characteristics and interpretation of positive and negative reactions in the GNB-API test

Reaction				Interpretation
Test	Base	Negative	Positive	
Citrate	Green	Green	Blue	This test identifies microorganisms that are able to use citrate, in the form of sodium salt, as the only source of carbon in the environment. Organisms that are able to use citrate produce alkaline metabolites that change the color of the indicator from green (acidic) to dark blue (alkaline). Any degree of blue should be considered positive. Note: Some microorganisms do not always produce the ideal "strong" positive color change. Lighter shades than the same main color should also be

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				considered positive.
Urea	beige to light amber	beige to light amber	light pink to red	Urease, an enzyme produced by various microorganisms that hydrolyzes urea into ammonia, causing the color of the indicator to change from yellow (acidic) to red-pink (alkaline) in the environment. The urease test is strongly positive for <i>Proteus</i> species and may become apparent 4 to 6 hours after incubation. A weak reaction is possible after 18-24 hours of incubation for <i>Klebsiella</i> and <i>Enterobacter</i> species (light pink color).
Malonate Lactose Mannose Arabinose	Green	Green to blue	Yellow	Bacterial fermentation of malonate, lactose, mannose and arabinose leads to the formation of acidic final products with a color change. The indicator in the environment ranges from green (alkaline) to yellow (acidic). Note: Any degree of yellowness should be interpreted as a positive reaction. Blue should also be considered negative.
Indole	colorless	colorless	pink-red	Indole production from tryptophan metabolism is recognized by the bacterial enzyme tryptophanase from tryptophan. The development of a pink-red color is detected after the addition of indole reagent (Kovacs), which is added to the chamber after 18 to 24 hours of incubation.
Hydrogen Sulfide (H₂S)	Beige to light amber	Beige to light amber	Black	Hydrogen sulfide is created by bacteria that are able to reduce sulfur-containing compounds such as peptones and sodium thiosulfate in the environment and a black precipitate of iron sulfide is usually created along the inoculation line. Note: If the test is not read after 24 hours, the black precipitate may fade or return to negative.
Lysine	Yellow	Yellow	Purple	Bacterial decarboxylation of lysine leading to the

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				<p>formation of the alkaline end product cadaverine, indicated by a change in the color of the indicator in the medium from yellow (acidic) to purple (alkaline). If decarboxylation of lysine does not occur, the medium remains yellow.</p> <p>Note: The medium is covered with wax to provide anaerobic conditions</p>
Ornithine	Yellow	Yellow	Purple	<p>Decarboxylation of ornithine leads to the formation of the alkaline end product putrescine, indicated by a change in the color of the indicator in the medium from yellow (acidic) to purple (alkaline). If ornithine decarboxylation does not occur, the medium remains yellow.</p> <p>Note: The medium is covered with wax to provide anaerobic conditions</p>
Glucose	Green	Green to blue	Yellow	<p>The final products of bacterial fermentation are glucose and acid. A change in pH due to acid production is indicated by a change in the color of the indicator in the medium from green (alkaline) to yellow (acidic).</p> <p>Note1: Any degree of yellowness should be interpreted as a positive reaction. Blue should also be considered negative.</p> <p>Note2: The medium is covered with wax to provide anaerobic conditions</p>

PROCEDURE

For GNB-API Test, bacteria grown on McConkey Agar (MAC), Eosin Methylene Blue (EMB), Salmonella Shigella (SS) Agar, Hecton Enteric Agar (HE) or non-selective agar media can be used. The culture used for inoculation should be at least 18 hours, but generally not longer than 48 hours. Ensure that the colony to be detected by GNB-API is a pure culture of Gram-negative bacilli. The method of doing the work according to Figure 1 is as follows:

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1. Prepare a coding sheet for isolation by entering patient name, sample number and date.
2. Take a tube of GNB-API and write the patient's name, sample number and date on the label.
3. Remove both caps. Do not hold the tip of the wire on the flame.
4. Pick one or two single colonies directly with the tip of the wire (Figure 1-A). A visible amount of inoculation should be seen at the tip and side of the wire. Avoid touching the agar with the wire.
5. Pull out the GNB-API first by twisting the wire so that the wire is inoculated all 10 compartments by applying a turning motion (Figure 1-B).
6. Again insert and withdraw the wire (without sterilization) 1-2 times using a turning motion through all 10 wells to completely inoculate the media with bacteria.

Note: DO NOT remove the wire completely from the tube.

7. Then align the cut place of the wire (near the end of the wire) with the opening of the pipe and bend the wire at the cut place (Figure 1-C).
8. With the broken off part of the wire, make holes in the air inlet of the last six compartments (Arabinose, Mannose, Lactose, Malonate, Urea, and Citrate) (Figure 1-D). Aerobic growth takes place in these chambers. Then close both lids.
9. Incubate at 35-37°C for 18-24 hours with the GNB-API on a flat surface or in an upright position. Allow air to circulate between the incubated tubes (Figure 1-E).
10. After 24 hours, interpret and record all reactions except the indole test. (For complete instructions on how to read GNB-API results, see the Results section) All other tests should be read before the indole test is performed.
11. To perform the indole test: Prepare 2-3 drops of Kovacs reagent and add it to the H₂S/indole chamber. Allow the reagent to contact the surface of the medium. A positive test is indicated by the development of a pink-red color within 10 seconds.

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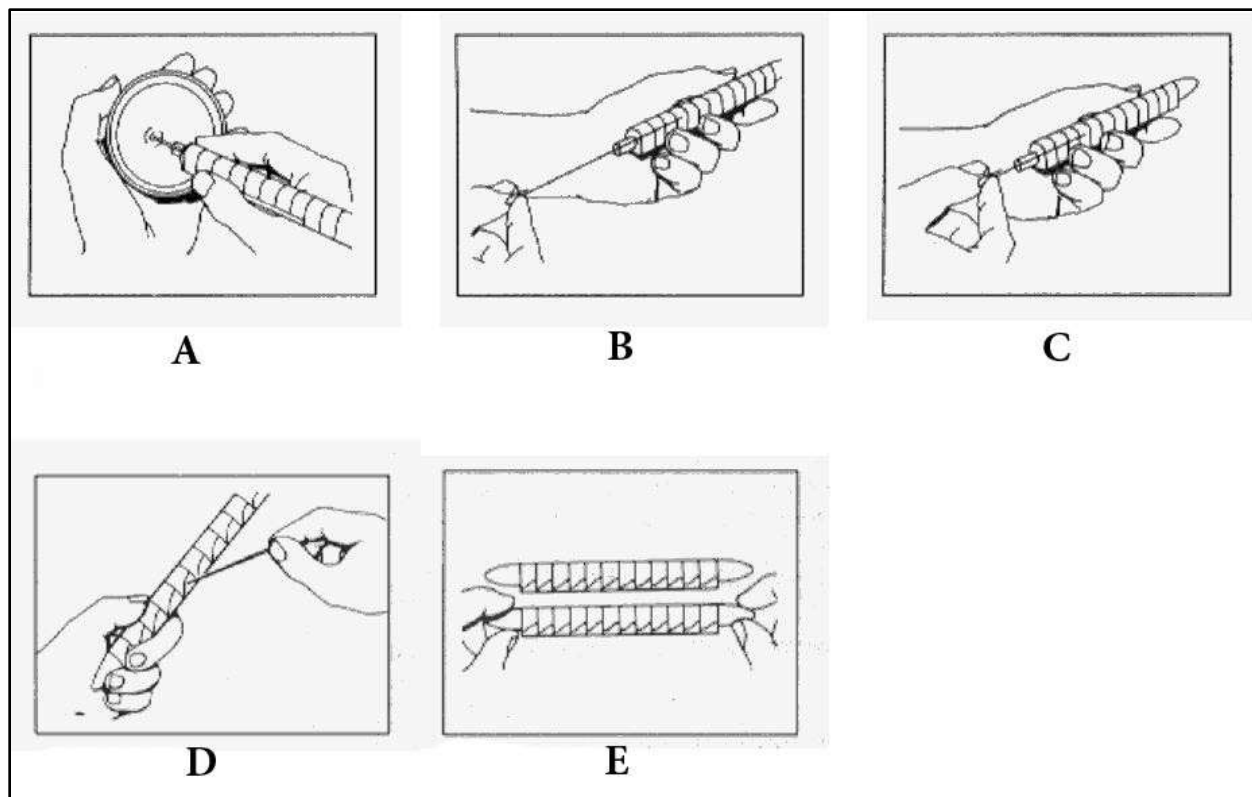


Figure 1: How to perform the test in 5 steps is shown schematically.

Results

Follow the instructions below to identify the isolate. Information about the positive and negative reactions of compounds is presented in Table 1.

1. After 18 to 24 hours of incubation, all reactions except the indole test should be interpreted and recorded. By comparing the colors of the medium in the tube with the colors in Table 1 and (finally) with an uninoculated GNB-API tube, the reactions are sequentially read as positive or negative. All reactions that should be positive may occur with equal, greater or lesser intensity.
2. Mark each positive test result by circling the number indicated under the corresponding compartment on the coding sheet (Figure 2).
3. Finally, do the indole test. If positive, circle the number.
4. Add the circled numbers in each part and enter the total number in the space below the arrow (Figure 3).

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5. Find the four-digit number in the Interpretation Guide page. In the example presented in Figure 2, the isolate performed was identified as *Escherichia coli*.

Coding system

Diagnostic codes for GNB-API were constructed using percentage data available in references for biochemical tests performed by GNB-API. To interpret the GNB-API test, each four-digit number is divided into 3 tests (except the last part which consists of two tests). Each test has a code of 4, 2 and 1 respectively. Then, by adding the positive numbers, a 4-digit number is obtained, which can be identified as a single code (Figure 2). In cases where two or more microorganisms are mentioned, confirmatory tests are required.

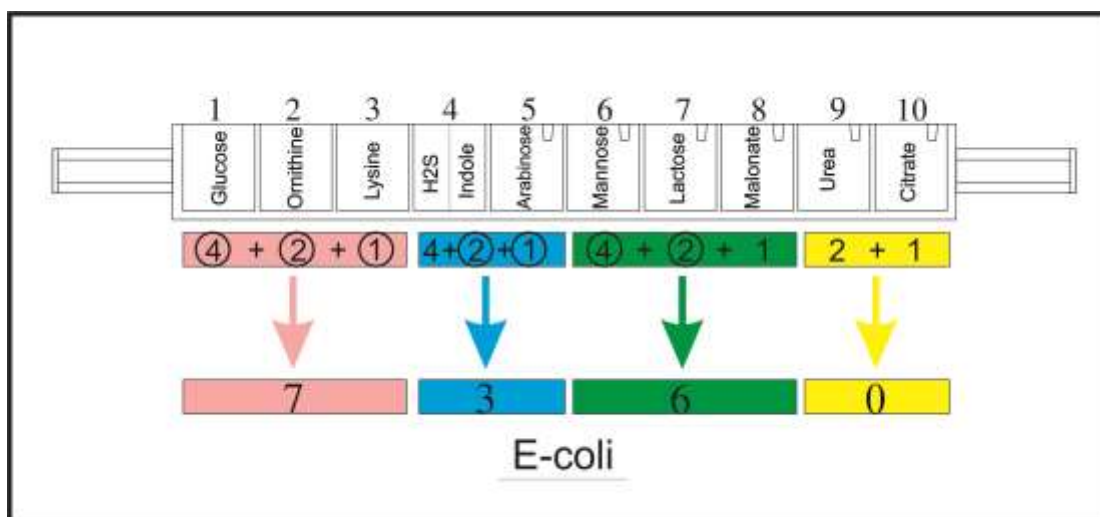


Figure 2: How to code 4 digits to detect Gram-negative bacilli

PRECAUTIONS

1. Designed for laboratory use and single use only.
2. Unused tests should be stored inside the box inverted (similar to the original arrangement) at the temperature of the refrigerator (4°C).
3. If there is evidence of microbial contamination, dehydration (drying) or liquefaction or any discoloration of the media, **DO NOT USE**. It could interfere with the accuracy of GNB-API.

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4. Examples of bacteria that are not able to ferment glucose in anaerobic conditions (chamber 1) belong to non-*Enterobacteriaceae* members, which include *Acinetobacter*, *Pseudomonas*, *Burkholderia* and *Stenotrophomonas maltophilia* species.

Materials Not Provided

Kovacs reagent for indole testing is not available in the kit.

QUALITY CONTROL

In order to check the quality control, the GNB-API test can be tested with the strains listed in Table 2.

Table 2: Standard strains to check the quality control of GNB-API test

Organisms	Glucose	Ornithin	Lysine	H ₂ S	Indole	Arabino	Mannos	Lactose	Malonat	Urea	Citrate	Code
<i>Proteus mirabilis</i> ATCC 12453	+	+	-	+	-	+	+	-	-	+	-	6542
<i>Escherichia coli</i> ATCC 25922	+	+	+	-	+	+	+	+	-	-	-	7360
<i>Serratia marcescens</i> ATCC 13880	+	+	+	-	-	-	+	-	+	-	-	7050
<i>Klebsiella pneumoniae</i> ATCC 700603	+	-	+	-	-	+	+	+	+	+	-	5172
<i>Pseudomonas aeruginosa</i> ATCC 27853	-	-	-	-	-	+	+	-	-	-	+	0141

PACKAGING/AVAILABILITY

GNB Enterotube API Test Cat. No. 202306 10 tubes

FURTHER INFORMATION

For further information please visit the address: www.mashhadgeneazma.com