IDENTIFICATION OF *ENTEROBACTERIACEAE* (API 20E)

Principle

The API 20E system facilitates the 24-hour identification of *Enterobacteriaceae* as well as 24 or 48-hour identification of other Gram negative bacteria.

The API 20E strip consists of microtubes containing dehydrated substrates for the demonstration of enzymatic activity and carbohydrate (CHO) fermentation. The substrates are reconstituted by adding a bacterial suspension. After incubation, the metabolic end products are detected by indicator systems or the addition of reagents. CHO fermentation is detected by colour change in the pH indicator.

Materials

API 20E strips - store at 2-8^{0}C
0.85% sterile saline
Nitrate A - store at 2-8^{0}C
Nitrate B - store at 2-8^{0}C
Mineral oil
Zinc dust
Kovacs Reagent
Voges - Proskauer Reagents
Ferric Chloride
H_2O_2
Oxidase Reagent

} Store at 2-8^{0}C

OF Dextrose
Motility Medium

} ID of non-*Enterobacteriaceae*

Procedure

1. Preparation of Inoculum

   a) Add 5 ml. of 0.85% saline to a sterile test tube.

   b) Using a sterile inoculating loop, carefully touch the centre of a well isolated colony (2-3 mm. Diameter) and thoroughly emulsify in the saline.

2. Preparation of the Strip

   a) An incubation tray and lid is supplied for each strip.
   b) Dispense 5 ml of water in to the tray.

3. Inoculation of the Strip

   a) Remove the cap from the tube containing the bacterial suspension and insert a 5 ml. Pasteur pipette.
   b) Tilt the API 20E incubation tray and fill the tube section of the microtubes by placing the pipette tip against the side of the cupule.

      Note: The ADH, LDC, ODC, H2S, AND URE reactions can be interpreted best if these microtubes are slightly underfilled.
   c) Fill both the TUBE and CUPULE section of [CIT], [VP] and [GEL] tubes.
   d) After inoculation, completely fill the cupule section of the ADH, LDC, ODC, H2S and URE tubes with mineral oil.
   e) Using the excess bacterial suspension, inoculate an agar slant or plate (non-selective media such as nutrient agar, blood agar or tryptic (trypticase) soy agar is suggested) as a purity check and for oxidase testing, serology, and/or additional biochemical testing. Incubate the slant or plate for 18-24 hours at 35^0C.

4. Incubation of the Strip

   a) After inoculation, place the plastic lid on the tray and incubate the strip for 18-24 hours at 35^0C in a non-CO_2 incubator.

   b) Weekend incubation: The biochemical reactions of the API 20E should be read after 18-24 hours incubation. If the strips cannot be read after 24 hours incubation at 35^0C, the strips should be removed from the incubator and stored at 2-8^0C (refrigerator) until the reactions can be read.

5. Reading the Strip

   a) After 18 hours of incubation and before 24 hours incubation, record all reactions not requiring the addition of reagents.

   b) If the GLU tube is negative (blue or green), do not add reagents. Reincubate a further 18-24 hours.

c) If the GLU is positive (yellow):

i. Perform the oxidase test.

A portion of the growth from the agar slate or plate, inoculated from the 20E bacterial suspension, should be rubbed onto filter paper to which a drop of oxidase reagent (1% tetramethyl-p-phenylenediamine dihydrochloride) has been added. The area where the growth has been added will turn dark purple within 10 seconds if the reaction is positive and will be colourless or light purple if negative.

Note: (a) Nichrome wire loops should NOT be used in performing the oxidase test. Nichrome wire can cause a false positive reaction.

(b) The oxidase test should NOT be performed using bacterial growth from selective media such as MacConkey, EMB, etc.

Note: (a) Before addition of reagents, observe GLU tube (positive or negative) for bubbles.

(b) The nitrate reduction and indole tests must be performed last since these reactions release gaseous products which interfere with the interpretation of other tests on the strip. The plastic incubation lid should not be replaced after the addition of these reagents.

ii. Add the reagents to TDA and VP tubes. If positive, the TDA reactions will be immediate, whereas the VP reaction may be delayed up to 10 minutes.

iii. The Kovacs' reagent should then be added to the IND tube.

iv. The Nitrate Reduction test should be performed on all oxidase positive organisms. The reagents should be added to the GLU tube after the Kovacs Reagent has been added to the IND tube.

Interpretation

a) Use the API 20E analytical profile index. (For 18-24 hour tests, use white pages. For 36-48 hour tests, use blue pages.)

b) The tests are separated into groups of three. The following numerical value is assigned to each reaction recorded:

1- positive reaction in the first test of the group
2- positive reaction in the second test of the group
4- positive reaction in any test
0- negative reaction in any test

Reference

1. Murray P.A., et al. Manual of Clinical Microbiology, 7th ed., 1999.

SUMMARY OF RESULTS - 18-24 HOUR PROCEDURE

TUBE	INCUBATION	POSITIVE	NEGATIVE	COMMENTS
ONPG		Yellow	Colourless	(1) Any shade of yellow is a positive reaction. (2) VP tube, before the addition of reagents, can be used a negative control.
ADH	18-24 hr 36-48 hr	Red or Orange Red	Yellow Yellow or Orange	Orange reactions occurring at 36-48 hours should be interpreted as negative.
LDC	18-24 hr 36-48 hr	Red or Orange Red	Yellow Yellow or Orange	Any shade of orange within 18-24 hours is a positive reaction. At 36-48 hours, orange decarboxylase reactions should be interpreted as negative.
ODC	18-34 hr 36-48 hr	Red or Orange Red	Yellow Yellow or Orange	Orange reactions occurring at 36-48 hours should be interpreted as negative.
CIT		Turquoise or Dark Blue	Light Green Or Yellow	(1) Both the tube and cupule should be filled. (2) Reaction is read in the aerobic (cupule) area.
H_2S		Black Deposit	No Black Deposit	(1) H_2S production may range from a heavy black deposit to a very thin black line around the tube bottom. Carefully examine the bottom of the tube before considering the reaction negative. (2) A "browning" of the medium is a negative reaction unless a black deposit is present. "Browning" occurs with TDA positive organisms.
URE	18-24 hr 36-48 hr	Red or Orange Red	Yellow Yellow or Orange	A method of lower sensitivity has been chosen. *Klebsiella*, *Proteus* and *Yersinia* routinely give positive reactions.
TDA	Add 1 drop 10% Ferric chloride.	Brown-Red	Yellow	(1) Immediate reaction. (2) Indole positive organisms may produce a golden orange colour due to indole production. This is a negative reaction.
IND	Add 1 drop Kovacs Reagent	Red Ring	Yellow	(1) The reaction should read within 2 minutes after the addition of the Kovacs reagents and the results recorded. (2) After several minutes, the HCl present in Kovacs reagent may react with the plastic of the cupule resulting in a change from a negative (yellow) colour to a brownish-red. This is a negative reaction.

SUMMARY OF RESULTS - 18-24 HOUR PROCEDURE (cont'd)

TUBE	INCUBATION	POSITIVE	NEGATIVE	COMMENTS
VP	Add 1 drop of 40% Potassium Hydroxide, then 1 drop of alpha-napthol.	Red	Colourless	(1) Wait 10 minutes before considering the reaction negative. (2) A pale pink colour which appears immediately after the addition of reagents but which turns dark `pink or red after 10 minutes should be interpreted as positive. Motility may be observed by hanging drop or wet mount preparation.
GEL		Diffusion of the pigment	No diffusion	(1) The solid gelatin particles may spread throughout the tube after inoculation. Unless diffusion occurs, the reaction is negative. (2) Any degree of diffusion is a positive reaction.
GLU		Yellow Or Gray	Blue or Blue-Green	COMMENTS FOR ALL CARBOHYDRATES Fermentation (*Enterobacteriaceae, Aeromonas, Vibrio*) (1) Fermentation of the carbohydrates begins in the most anaerobic portion (bottom) of the tube. Therefore, these reactions should be read from the bottom of the tube to the top. (2) A yellow colour at the bottom of the tube only indicates a weak or delayed positive reaction.
MAN INO SOR RHA SAC MEL AMY ARA		Yellow	Blue or Blue-Green	Oxidation (Other Gram-negatives) (1) Oxidative utilization of the carbohydrates begins in the most aerobic portion (top) of the tube. Therefore, these reactions should be read from the top to the bottom of the tube. (2) A yellow colour in the upper portion of the tube and blue in the bottom of the tube indicate oxidative utilization of the sugar. This reaction should be considered positive only for non-*Enterobacteriaceae* gram negative rods. This is a negative reaction for fermentative organisms such as *Enterobacteriaceae.*

SUMMARY OF RESULTS - 18- 24 HOUR PROCEDURE (cont'd)

TUBE	INCUBATION	POSITIVE	NEGATIVE	COMMENTS
GLU	After reading GLU reaction, add 2 drops 0.8% sulfanilic acid and 2 drops 0.5% N. N-dimethyl-alpha-naphthylamine			(1) Before addition of reagents, observe GLU tube (positive or negative) for bubbles. Bubbles are indicative of reduction of nitrate to the nitrogenous (N_2) state. (2) A positive reaction may take 2-3 minutes for the red colour to appear. (3) Confirm a negative test by adding zinc dust or 20 mesh granular zinc. A pink-orange colour after 10 minutes confirms a negative reaction. A yellow colour indicates reduction of nitrates to the nitrogenous (N_2) state.
	NO_2	Red	Yellow	
	N_2 gas	Bubbles: Yellow after reagents and zinc	Orange after reagents and zinc	
MAN INO SOR Catalase	After reading carbohydrate reaction, add 1 drop 1.5% H_2O_2	Bubbles	No bubbles	(1) Bubbles may take 1-2 minutes to appear. (2) Best results will be obtained if the test is run in tubes which have no gas from fermentation.