IDENTIFICATION OF NON-ENTERIC GRAM-NEGATIVE RODS (API 20NE)

Principle

The API 20NE system facilitates the identification of non-fastidious Gram-negative rods not belonging to the *Enterobacteriaceae* within 48 hours.

The API 20NE strip consists of microtubes containing dehydrated media and substrates. The media microtubes containing conventional tests are inoculated with a bacterial suspension which reconstitutes the media. After incubation, the metabolic end products are detected by indicator systems or the addition of reagents. The substrate microtubes contain assimilation tests and are inoculated with a minimal medium. If the bacteria are capable of utilizing the corresponding substrate, then they will grow.

Materials

API 20NE strips - store at 2-8^{0}C
0.85% sterile saline
Mineral oil
Zinc dust
AUX Medium }
James Reagent }
Nitrate 1 - store at 2-8^{0}C } Store at 2-8^{0}C
Nitrate 2 - store at 2-8^{0}C }
Oxidase Reagent

Procedure

1. Preparation of Inoculum

   a) Add 2 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. The suspension turbidity should be equal to a 0.5 McFarland standard.

2. Preparation of the Strip

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

3. Inoculation of the Strip

a) Remove the cap from the tube containing the bacterial suspension and insert a sterile pipette.
b) Tilt the API 20NE incubation tray and fill the TUBE section of the NO_3 to PNPG microtubes by placing the pipette tip against the side of the cupule.
c) Open an ampule of AUX Medium and add 200 uL of the bacterial suspension to the ampule. Mix well with a pipette while avoiding the formation of air bubbles.
d) Using the AUX Medium bacterial suspension, fill both the TUBE and CUPULE section of [GLU] to [PAC]. Do not overfill the cupules. Fill to a flat or slightly convex meniscus.
e) After inoculation, completely fill the CUPULE section of the 3 underlined tests, GLU, ADH and URE tubes with mineral oil.
f) 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, and/or additional biochemical testing. Incubate the slant or plate with the API 20NE strip.

4. Incubation of the Strip

a) After inoculation, place the plastic lid on the tray and incubate the strip for 24 hours at 30^0C in a non-CO_2 incubator.

5. Reading the Strip

a) After 24 hours incubation, record all reactions not requiring the addition of reagents.
b) Perform the oxidase test.

A portion of the growth from the agar slate or plate, inoculated from the 20NE 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 positivereaction.

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

c) Assimilation tests are observed for bacterial growth. An opaque cupule indicates a positive reaction.
d) Protect the assimilation tests with the incubation tray lid during the reading of the Nitrate and TRP tests.
e) Perform the Nitrate test.

   i. Add one drop of Nitrate 1 and one drop of Nitrate 2 reagents to NO_3 cupule.
   ii. After 5 minutes a red color indicates a positive reaction.
   iii. A negative reaction may be due to the production of nitrogen. Add Zinc dust to the NO_3 cupule. After 5 minutes a colorless cupule indicates a positive reaction. A pink-red cupule indicates a negative reaction.

f) Perform the TRP test.

   i. Add one drop of JAMES Reagent.
   ii. The reaction takes place immediately, producing a pink color in the entire cupule if the reaction is positive.

Interpretation

1. Use the API 20NE analytical profile index.

2. The tests are separated into groups of three. The following numerical value is assigned to each positive 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 the third test of the group

   By adding the values corresponding to positive reactions in each group, a seven digit number is obtained.

3. The strip must be reincubated in the following cases:

   i. If the profile cannot be found in the Analytical Profile Index.
   ii. If the following note is indicated for the profile obtained:

   IDENTIFICATION NOT VALID
   BEFORE 48-HR INCUBATION

   iii. If the strip is to be reincubated, remove the reagents from the NO_3 and TRP cupules and then cover these tests with mineral oil.

iv. Reincubate the strip for another 24 hours at 30^0C in a non-CO_2 incubator.
v. Read all the tests again, except for NO_3, TRP and GLU.

READING TABLE

TESTS	SUBSTRATES	REACTONS/ENZYMES	NEGATIVE RESULTS	POSITIVE RESULTS
$N0_3$	Potassium nitrate	NITrate reduction to nitrites	**NIT 1 + NIT 2 / 5 min** colourless	pink-red
		NITrates to nitrogen	**Zn / 5 min** pink	colourless
TRP	tryptophane	indole production	**JAMES / immediate** colourless / pale green / yellow	pink
GLU	glucose	Acidification	blue to green	yellow
ADH	arginine	arginine dihydrolase	yellow	orange/pink/red
URE	urea	Urease	yellow	orange/pink/red
ESC	esculin	hydrolysis (β-glucosidase)	yellow	grey/brown/black
GEL	gelatine (with India ink)	hydrolysis (protease)	no pigment diffusion	diffusion of black pigment
PNPG	p-nitrophenyl-β-D-galactopyranoside	β-galactosidase	colourless	yellow
[GLU]	glucose	Assimilation	transparent	opaque
[ARA]	arabinose	Assimilation	transparent	opaque
[MNE]	mannose	Assimilation	transparent	opaque
[MAN]	mannitol	Assimilation	transparent	opaque
[NAG]	N-acetyl-glucosamine	Assimilation	transparent	opaque
[MAL]	maltose	Assimilation	transparent	opaque
[GNT]	gluconate	Assimilation	transparent	opaque
[CAP]	caprate	Assimilation	transparent	opaque
[ADI]	adipate	Assimilation	transparent	opaque
[MLT]	malate	Assimilation	transparent	opaque
[CIT]	citrate	Assimilation	transparent	opaque
[PAC]	phenyl-acetate	Assimilation	transparent	opaque
OX	see oxidase test	cytochrome oxidase	colorless/ light purple	dark purple