75315 OF Test Nutrient Agar

Medium for demonstrating oxidative and fermentative catabolism of carbohydrate acc. to Hugh and Leifson (1953). In particular for the differentiation and classification of gram-negative intestinal bacteria.

Composition:

Ingredients	Grams/Litre
Casein peptone (pancreatic)	2.0
Sodium chloride	5.0
Dipotassium hydrogen phosphate	0.3
Bromothymol blue	0.08
Agar	2.5

Final pH 7.1 +/- 0.2 at 25°C

Store prepared media below 8°C, protected from direct light. Store dehydrated powder, in a dry place, in tightly-sealed containers at 2-25°C. The prepared culture medium is dark-green to blue-green in color and clear.

Directions:
Dissolve 9.88 g in 1 litre distilled water. Sterilize by autoclaving at 121°C for 15 minutes. Let cool to approx. 50°C and add 100 ml of sterile filtered 10% D(+)-glucose (49140), lactose (61340), sucrose (84100) or other carbohydrate. Mix and dispense in 5 ml amounts in sterile tubes in duplicate for aerobic and anaerobic fermentation. For each carbohydrate, inoculate two tubes with an isolated pure culture of the microorganism to be examined down to the bottom of the tube by using a loop. Immediately after inoculation overlay one of the tubes with a 1 cm layer of sterile paraffin oil (paraffin viscous; e.g. 76235).
The organisms used for inoculation should be in the logarithmic phase of growth. Incubation: at least 48 hours at the optimal temperature.

Principle and Interpretation:
The added carbohydrate is degraded to carbon dioxide and acid, which is indicated by the pH indicator bromothymol blue. The color changes to yellow in the acidic environment. Some microorganisms are able to degrade the carbohydrate only in presence of oxygen (oxidative) and others can degrade also under exclusion of air (fermentative). A yellow coloration in both, the open and paraffin-sealed tubes, signifies fermentative degradation whereas yellow coloration of the open tube alone indicates that the carbohydrate in question is broken down by oxidation. Oxidative breakdown takes place at or close to the surface of the medium, while fermentative breakdown occurs both at the surface and within the solution. To be sure that microorganisms have grown, check the tubes for turbidity.
Casein peptone provides the nitrogen, vitamins and amino acids. Sodium chloride is for the osmotic balance and dipotassium hydrogen phosphate acts as a buffer substance.
Mossel and Martin (1961) reported that this test can be performed in one tube if yeast extract is added to improve the growth of fastidious microorganisms, if the agar content is also increased to 1.5 % and if the depth of the culture medium is at least 8 cm.

Carbohydrate metabolism of some important microorganisms (Hugh and Leifson, 1953)							
Microorganisms	Glucose		Lactose		Sucrose		Group
	aerob	anaerob	aerob	anaerob	aerob	anaerob	
Alcalescens faecalis	-	-	-	-	-	-	I non-oxyd. spec. non-ferm. spec.
Pseudomonas *aeruginosa* *Bact. anitratum* *Agrobacterium tumefaciens* *Malleomyces pseudomallei*	A A A A	- - - -	- A - A	- - - -	- - A A	- - - -	II oxid. spec. non-ferm. spec.
Shigella dysenteriae *Shigella sonnei* *Vibrio comma*	A A A	A A A	- A -	- A -	- - A	- - A	IIIa ferm. spec. (anaerogenic)
Salmoella enteritidis *E. coli* *Aeromonas liquefaciens* *Enterobacter aerogenes*	AG AG AG AG	AG AG AG AG	- AG - AG	- AG - AG	- - AG AG	- - AG AG	IIIb ferm. spec. (aerogenic)
Non-classified species Some Paracolonbacteria	A AG	A AG	A A	-? -?	variable variable	variable variable	IIIc oxid. spec. ferm. spec.

- neutral or alkaline reaction
A acid production
AG acid and gas production (sometimes observable)

OF test for the identification of some obligate and facultative aerobic, gram-negative rods of medical interest (modified according to Costin 1967)			
Glucose-degradation	**Oxidase**	**Type of reaction**	**Microorganisms**
Fermentative	Negative	I	*1. Enterobacteriaceae* *2. Yersinia pestis* *3. Yersinia malassezii (pseudotuberculosis)* *4. Yersinia enterocolitica*
	Positive	II	*1. Aeromonas spp.* *2. Vibrio cholerae* *3. Vibrio spp.* (NAG or NVC) *4. Vibrio parahaemolyticus* *5. Pasteurella haemolytica* *6. Pasteurella multocida* *7. Pasteurella pneumotropica* *8. Actinobacillus lignieresii* *9. Chromobacterium violaceum*
Oxidative	Negative	III	*1. Acinetobacter calcoaceticus* (produces acid) *2. Pseudomonas maltophilia*
	positive	IV	*1. Pseudomonas aeruginosa* *2. Pseudomonas stutzeri* *3. Pseudomonas fluorescens (putida)* *4. Pseudomonas mallei* *5. Pseudomonas pseudomallei* *6. Flavobacterium meningosepticum*

Negative	negative	V	*1. Acinetobacter calcoaceticus* (does not produce acid) *2. Bordetella parapertussis*
	positive	VI	*1. Alcaligenes faecalis (denitrificans)* *2. Pseudomonas alcaligenes* *3. Bordetella bronchiseptica* *4. Pseudomonas spp.* *5. Campylobacter (Vibrio fetus)* *6. Moraxella spp.*

Cultural characteristics after 18-48 hours at 35ºC.

Organisms (ATCC)	Growth	Color change to yellow with Dextrose	
		aerobic	anaerobic
Escherichia coli (25922)	+++	AG	AG
Alcalescens faecalis (19209)	+++	K	K
Acinetobacter calcoaceticus (19606)	+++	A	K
Pseudomonas aeruginosa (9027)	+++	A	K
Shigella flexneri (12022)	+++	A	A
Vibrio cholerae (15748)	+++	A	A

K neutral or alkaline reaction, green (no change)
A acid production (yellow)
G gas production (sometimes observable)

References:

1. R. Hugh, E. Leifson, The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram-negative bacteria, J. Bact., 66, 24 (1953)
2. J.A. Erasmus, Studies on coagulase-positive staphylococci from bovine milk, Onderstepoort J. Vet. Res., 52, 25 (1985)
3. D.A.A. Mossel, G. Martin, Milieu simplifié permettant l'étude des divers modes d'action des bactéries sur les hydrates des carbones, Ann. Inst. Pasteur de Lille, 12, 225 (1961)
4. I.D. Costin, An outline for the biochemical identification of aerobic and facultatively anaerobic gram-negative rods of medical interest., 5. Intern. Kongr. f. Chemotherapie Wien, B2/1, 73 (1967)
5. D.F. Welch, M.J. Muszynski, C.H. Pai, M.J. Marcon, M.M. Hribar, P.H. Gilligan, J.M. Matsen, P.A Ahlin, B.C. Holman, S.A. Chartrand, Selective and differential medium for recovery of Pseudomonas cepacia from the respiratory tracts of patients with cystic fibrosis, J. Clin. Microbiol., 25, 1730 (1987)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.