OF Basal Medium acc. to HUGH and LEIFSON

Test culture medium proposed by HUGH and LEIFSON (1953) for deltecting oxidative and fermentative carbohydrate degradation. It is used primarily for the differentiation and classification of gram-negative intestinal bacteria.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

A selective and differential agar for Pseudomonas cepacia was conceived by WELCH et al. (1987) on the basis of this medium, with the addition of agar-agar, lactose, polymyxin B and bacitracin.

Principle

Microbiological method

Mode of Action

A carbohydrate is added to the culture medium, degradation of the carbohydrate to acid is indicated by the pH indicator bromothymol blue which changes its colour to yellow. The degradation is allowed to take place while the medium is exposed to air (degradation may be oxidative or fermentative) or under exclusion of air (degradation by fermentation only).

Typical Composition (g/litre)

Peptone from casein 2.0; yeast extract 1.0; sodium chloride 5.0; di-potassium hydrogen phosphate 0.2; bromothymol blue 0.08; agar-agar 2.5.

also to be added:

carbohydrate 10.0 g/l.

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25° C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25° C.

Suspend 11 g/litre, autoclave (15 min at 121 °C). Cool to about 50 °C, add 100 ml/litre of a 10 % filter-sterilized solution of D(+)glucose, lactose, sucrose or other carbohydrates, mix. Dispense into tubes to give a depth of approx. 5 cm. Immediately after cooling overlay half of the tubes with an 1 cm layer of sterile paraffin oil (paraffin viscous). The prepared culture medium is dark-green to blue-green in colour and clear.

pH: 7.1 ± 0.2 at 25 °C.

Specimen

e.g. Isolated bacteria from, stool, urine, etc.

Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure and Evaluation

For each carbohydrate, inoculate one tube with and one tube without a paraffin seal with a pure culture of the microorganism to be examined down to the bottom of the tube by the stabbing technique. The organisms used for inoculation should be in the logarithmic phase of growth.

Incubation: at least 48 hours at 35 °C.

- **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.**
  A yellow colouration in both, the open and paraffin-sealed tubes, signifies fermentative degradation whereas yellow colouration of the open tubes alone indicate that the carbonhydrate in question is broken down by oxidation. Oxidative breakdown takes place at or close to the surface of the medium, whilst fermentative breakdown occurs both at the surface and throughout the butt. The tubes should finally be checked to see whether microbial growth produces turbidity solely along the puncture line (immotile strain) or throughout the whole medium (motile strain).

OF Basal Medium acc. to HUGH and LEIFSON

Carbohydrate metabolism of some important species (HUGH and LEIFSON, 1953):

Microorganisms	Glucose		Lactose		Sucrose		Group
	aerob	anaerob	aerob	anaerob	aerob	anaerob	
Alcalig. faecalis	-	-	-	-	-	-	I non-oxyd. spec. non-ferm. spec.
Ps. aeruginosa	A	-	-	-	-	-	II
Bact. anitratum	A	-	A	-	-	-	oxid. spec
Agrobacterium tumefaciens	A	-	-	-	A	-	non-ferm.
Malleomyces pseudomallei	A	-	A	-	A	-	spec.
Shig. dysenteriae	A	A	-	-	-	-	IIIa
Shig. sonnei	A	A	A	A	-	-	ferm. spec.
Vibrio comma	A	A	-	-	A	A	(anaerogenic)
S. enteritidis	AG	AG	-	-	-	-	IIIb
E. coli	AG	AG	AG	AG	-	-	ferm. spec.
Aerom. liquefaciens	AG	AG	-	-	AG	AG	(aerogenic)
Ent. aerogenes	AG	AG	AG	AG	AG	AG	
Non-classified species							IIIc
Some Paracolon-bacteria	A	A	A	-?	variable	variable	oxid. spec.
	AG	AG	A	-?	variable	variable	ferm. spec.

Signs and symbols: - = neutral or alkaline reaction, A = acid production, AG = acid and gas production

OF Basal Medium acc. to HUGH and LEIFSON

Use of the OF test for the diagnostic 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
Fermntative	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.

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Literature

COSTIN, I.D.: 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**; 73-76 (1967).

HUGH, R., a. LEIFSON, E.: The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram-negative bacteria. **- J. Bact., 66**; 24-26 (1953).

MOSSEL, D.A.A., et MARTIN, G.: Milieu simplifié permettant l'étude des divers modes d'action des bactéries sur les hydrates des carbone.**- Ann. Inst. Pasteur de Lille, 12**; 225-226 (1961).

WELCH, D.F., MUSZYNSKI, M.J., PAI, C.H., MARCON, M.J., HRIBAR, M.M., GILLIGAN, P.H., MATSEN, J.M., AHLIN, P.A:, HOLMAN, B.C., a. CHARTRAND, S.A.: Selective and differential medium for recovery of Pseudomonas cepacia from the respiratory tracts of patients with cystic fibrosis. **- J. Clin. Microbiol., 25**; 1730-1734 (1987).

Ordering Information

Product	Merck Cat. No.	Pack size
OF Basal Medium acc. to HUGH and LEIFSON	1.10282.0500	500 g
D(+)Glucose monohydrate	1.08342.1000	1 kg
Lactose monohydrate	1.07657.1000	1 kg
Paraffin viscous	1.07160.1000	1 l
Sucrose	1.07651.1000	1 kg

Quality control

Test strains	Growth	Colour change to yellow	
		with layer (aerobic)	with layer (anaerobic)
Escherichia coli ATCC 25922	good / very good	+	+
Staphylococcus aureus ATCC 25923	good / very good	+	+
Micrococcus luteus ATCC 9341	good / very good	+	(-)
Pseudomonas aeruginosa ATCC 27853	good / very good	+	-
Alcaligenes faecalis ATCC 19209	good / very good	-	-
Pseudomonas alcaligenes ATCC 14909	good / very good	-	-