

IVD in vitro diagnosticum - For professional use only



OF Basal Medium acc. to HUGH and LEIFSON

OF basal medium

Cat. No. 1.10282.0500
(500 g)

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

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.

See also General Instruction of Use

Warnings and precautions see www.merck-chemicals.com®

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

Cat. No. 1.10282. OF Basal Medium acc. to HUGH and LEIFSON (500 g)

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 carbohydrate 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).

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

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

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

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
	negative	I	1. Enterobacteriaceae 2. <i>Yersinia pestis</i> 3. <i>Yersinia malassezii</i> (pseudotuberculosis) 4. <i>Yersinia enterocolitica</i>
Fermentative	positive	II	1. <i>Aeromonas</i> spp. 2. <i>Vibrio cholerae</i> 3. <i>Vibrio</i> spp. (NAG or NVC) 4. <i>Vibrio parahaemolyticus</i> 5. <i>Pasteurella haemolytica</i> 6. <i>Pasteurella multocida</i> 7. <i>Pasteurella pneumotropica</i> 8. <i>Actinobacillus lignieresii</i> 9. <i>Chromobacterium violaceum</i>
	negative	III	1. <i>Acinetobacter calcoaceticus</i> (produces acid) 2. <i>Pseudomonas maltophilia</i>
Oxidative	positive	IV	1. <i>Pseudomonas aeruginosa</i> 2. <i>Pseudomonas stutzeri</i> 3. <i>Pseudomonas fluorescens</i> (putida) 4. <i>Pseudomonas mallei</i> 5. <i>Pseudomonas pseudomallei</i> 6. <i>Flavobacterium meningosepticum</i>
	negative	V	1. <i>Acinetobacter calcoaceticus</i> (does not produce acid) 2. <i>Bordetella parapertussis</i>
Negative	positive	VI	1. <i>Alcaligenes faecalis</i> (denitrificans) 2. <i>Pseudomonas alcaligenes</i> 3. <i>Bordetella bronchiseptica</i> 4. <i>Pseudomonas</i> spp. 5. <i>Campylobacter</i> (<i>Vibrio fetus</i>) 6. <i>Moraxella</i> spp.

Quality control

Test strains	Growth	Colour change to yellow	
		without layer (aerobic)	with layer (anaerobic)
Escherichia coli ATCC 25922	good / very good	+	+
Staphylococcus aureus ATCC 25923	good / very good	+	+
Mirococcus 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	-	-

Additives

Merck Cat.No.	Product	Pack Size
1.07160.1000	Paraffin viscous	1 l
1.07651.1000	Sucrose	1 kg
1.07657.1000	Lactose monohydrate	1 kg
1.08342.1000	D(+)-Glucose monohydrate	1 kg

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).

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