Technical Data Sheet

CE OF Basal Medium acc. to Hugh and Leifson

Ordering number: 1.10282.0500

Test culture medium proposed by Hugh and Leifson (1953) for detecting **o**xidative and **f**ermentative 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.

IVD in vitro diagnosticum - For professional use only

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 color 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

Peptone from Casein	2 g/l
Yeast Extract	1 g/l
NaCl	5 g/l
K_2HPO4	0.2 g/l
Bromothymol Blue	0.08 g/l
Agar-Agar	2.5 g/l

Preparation

Suspend 11 g/l. Autoclave 15 min at 121 °C. Cool to about 50 °C. Add 100 ml/l 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 appearance of the prepared medium is clear and dark-green to blue-green.

The pH value at 25 °C is in the range of 6.9-7.3.

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 h 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 OF Basal Medium acc. to Hugh and Leifson the culture medium is at least 8 cm.
  A yellow coloration in both, the open and paraffin-sealed tubes, signifies fermentative degradation whereas yellow coloration 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**	
Alcaligenes faecalis	-	-	-	-	-	-	**I** non-oxyd. spec. non-ferm. spec.
Pseudomonas aeruginosa *Bacterium 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. (an-aerogenic)
Salmonella enteritidis *Escherichia 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 *Paracolon*-bacteria	A AG	A AG	A A	-? -?	var. var.	var. var.	**IIIc** oxid. spec. ferm. spec.

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

Use of the OF test for the diagnostic identification of some obligate and facultative aerobic, gramnegative rods of medical interest (modified according to Costin 1967):

Glucose degradation	Oxidase	Type of Reaction	Microorganisms
	Negative	I	1. *Enterobacteriaceae* *2. Yersinia pestis* 3. *Yersinia malassezii (pseudotuberculosis)* 4. *Yersinia enterocolitica*
Fermentatitve	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*
	Negative	III	1. *Acinetobacter calcoaceticus* (produces acid) 2. *Pseudomonas maltophilia*
Oxidative	Positive	IV	1. *Pseudomonas aeruginosa* 2. *Pseudomonas stutzeri* 3. *Pseudomonas fluorescens (putida)* 4. *Pseudomonas mallei* 5. *Pseudomonas pseudomallei* 6. *Flavobacterium meningosepticum*
	Negative	V	1. *Acinetobacter calcoaceticus* (does not produce acid) 2. *Bordetella parapertussis*
Negative	Positive	VI	1. *Alcaligenes faecalis (denitrificans)* 2. *Pseudomonas alcaligenes* 3. *Bordetella bronchiseptica* 4. *Pseudomonas spp.* 5. *Campylobacter (Vibrio fetus)* 6. *Moraxella spp.*

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

Specimen

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

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

Quality Control

Control Strains	ATCC #	Incubation*	Expected Results
Escherichia coli	25922	24 h at 37 °C	Growth good to very good, color change to yellow, color change to yellow anaerobic
Staphylococcus aureus	25923	48 h at 35 °C	Growth good to very good, color change to yellow, color change to yellow anaerobic
Kocuria rhizophila	9341	48 h at 35 °C	Growth good to very good, color change to yellow
Pseudomonas aeruginosa	27853	24 h at 37 °C	Growth good to very good, color change to yellow, no color change to yellow anaerobic
Alcaligenes faecalis	19209	48 h at 35 °C	Growth good to very good, no color change to yellow, no color change to yellow anaerobic
Pseudomonas alcaligenes	14909	48 h at 35 °C	Growth good to very good, no color change to yellow, no color change to yellow anaerobic

* Tested with D(+)-Glucose

Please refer to the actual batch related Certificate of Analysis.

Literature

Costin, I.D. (1967): 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.

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

Mossel, D. A. and Martin, G. (1961): Simplified medium permitting study of the various modes of action of bacteria on carbohydrates. Annales de l'Institut Pasteur de Lille. **12**: 225.

Welch, D. F., Muszynski, M. J., Pai, C. H., Marcon, M. J., Hribar, M. M., Gilligan, P. H., Matsen, J.M.., Ahlin, P.A., Hilman B.C. and Chartrand, S. A. (1987): Selective and differential medium for recovery of *Pseudomonas cepacia* from the respiratory tracts of patients with cystic fibrosis. Journal of Clinical Microbiology. **25**: 1730-1734.

Ordering Information

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

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