

LDC-ODC-ADH/Broth

355-3725

DEFINITION

Media used for differential diagnosis of species belonging to *Enterobacteriaceae*, *Vibrionaceae*, *Pseudomonadaceae*, etc. in the analysis of food products and water.

STANDARDS

FOOD MICROBIOLOGY

- **NF EN ISO 6579 (July 2002):** Food microbiology - Horizontal method for the detection of *Salmonella* spp.

PRESENTATION

Ready-to-use

5 ml x 3 tubes

355-3725

STORAGE

- Ready-to-use: + 2°C to +8°C.
- Expiration date and batch number are shown on the package.

THEORETICAL FORMULA

	ODC	ADH	LDC
L-ornithine (monohydrochloride)	5 g	-	-
L-arginine (monohydrochloride)	-	5 g	-
L-lysine (monohydrochloride)	-	-	5 g
Yeast extract	3 g	3 g	3 g
NaCl *	5 g	5 g	5 g
Glucose	1 g	1 g	1 g
L-lysine (1,6 g /100 ml of 95° alcohol)	1 ml	1 ml	1 ml
Distilled water	1,000 ml	1,000 ml	1,000 ml
Final pH (25°C)	6.8 ± 0.2	6.9 ± 0.2	6.9 ± 0.2

* NaCl has been added to these media to enhance the growth of halophile bacteria such as *Vibrio parahaemolyticus*, *V. alginolyticus*, etc.

4.5 ml volume of these media is dispensed in screw-capped 95 X 10 mm tubes and then sterilized in autoclave at 121°C for 15 minutes.

PERFORMANCES / QUALITY CONTROL OF THE TEST

The growth performances of the media are verified with the following strains:

STRAINS	Results after 24 h culture at 37°C		
	LDC	ODC	ADH
<i>Shigella flexneri</i> ATCC 12022	-	-	-
<i>Shigella sonnei</i> ATCC 25931	-	+	-
<i>Klebsiella pneumoniae</i> ATCC 13883	+	-	-
<i>Pseudomonas aeruginosa</i> ATCC 27853	-	-	+

QUALITY CONTROL OF MANUFACTURER

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product.

Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

KEY WORDS

LDC / ODC / ADH / *Enterobacteriaceae* / Food products / Water / Glucose / Medium.

BIBLIOGRAPHY

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• **RICHARD, C. (1968):** Techniques rapides de recherche des lysine décarboxylase, ornithinedécarboxylase et arginine-dihydrolase dans les genres *Pseudomonas*, *Alcaligenes* et *Moraxella*. Ann. Ins. Pasteur 114: 425-430.

APPLICATIONS

FACULTATIVE GRAM-NEGATIVE AERO - ANAEROBIC BACILLI WITH FERMENTING METABOLISM (*Enterobacteriaceae* and *Vibrionaceae*)

PRINCIPLE

These bacilli ferment glucose and at first the media acidify (causing the B.C.P. indicator to turn from violet to yellow). Decarboxylases and dihydrolases present maximum activity at acid pH. Afterwards, when the bacteria under study possess these enzymes, the amine metabolites formed from amino acids render the media alkaline and change the pH indicator to violet (formation of putrescine from ornithine, of cadaverine from lysine, and of agmatine, then putrescine, from arginine).

PROTOCOL

• Inoculation and incubation

Using a culture of the test strain on nutrient agar (code 356-4485) or on Trypto-casein-soy agar (code 356-4554), a suspension is prepared in physiological water containing about 10⁹ bacteria/ml.

Each of the 3 tubes is inoculated with 2 drops of this suspension. The tubes are then almost full: culture of bacteria is carried out in anaerobiosis suitable for the detection of decarboxylases and it is not necessary to cover the surface of the media with sterile vaseline, as is the case with media such as Moeller or Taylor.

READING AND INTERPRETATION

Reading is limited to 4 days.

Acid indicator (yellow): negative result Alkaline indicator (violet): positive result Refer to Table 1a and b.

N.B.:

• If a result appears positive (violet medium), the tubes must be checked to be certain that growth has really taken place (turbid medium).

• Inoculation of a 4th, control tube without amino acid can be avoided as, except in the case of *Plesiomonas shigelloïdes* (LDC+, ODC+, ADH+), at least 1 of the 3 tubes presents a negative result on the first day of reading, and this tube constitutes a control of primary acidification by fermentation of glucose.

ENTERO-BACTERIACEAE	LDC	ODC	ADH
<i>E.coli</i>	[+]	d	-
<i>Alkalescens dispar</i>	d	[-]	-
<i>Sh.dysenteriae</i>	-	-	-
<i>Sh.boydii</i>	-	-	-
<i>Sh.flexneri</i>	-	-	-
<i>Sh.sonnei</i>	-	+	-
S. Typhi	+	-	-
S. Paratyphi A	-	+	- or (+)
Other <i>Salmonella</i> serotypes (including <i>S.arizona</i>)	+	+	- or (+)
<i>Citrobacter</i>	-	[-]	-
<i>Edwardsiella</i>	+	+	- or (+)
<i>L.malonatica</i>	-	+	(+) or -
<i>L.amalonatica</i>	-	+	(+) or -
<i>Y.enterocolitica</i>	-	[+]	-
<i>Y.pseudotuberculosis</i>	-	-	-
<i>Y.pestis</i>	-	-	-
<i>E.aerogenes</i>	+	+	-
<i>K.pneumoniae</i>	+	-	-
<i>K.oxytoca</i>	+	-	-
<i>K.ozaenae</i>	d	-	-
<i>K.rhinoscleromatis</i>	-	-	-
<i>E.cloacae</i>	-	+	+
<i>E.agglomerans</i>	-	-	-
<i>H.alvei</i>	+	+	-
<i>S.marcescens</i>	+	+	-
<i>S.liquefaciens</i>	+ or (+)	+	-
<i>S.rubidaea</i>	+ or (+)	-	-
<i>P.mirabilis</i>	-	+	-
<i>P.morganii</i>	-	+	-
<i>P.vulgaris</i> , <i>P.rettgeri</i> <i>Providencia</i>	-	-	-

Table 1a

Legend:

- + positive in 1 or 2 days, [+] generally positive in 1 or 2 days, (+) positive in 3 or 4 days
- [-] generally negative, - negative.
- d: variable results depending on the strains.

VIBRIONACEAE	LDC	ODC	ADH
<i>V.cholerae</i>	+	[+]	-
<i>Vibrio NAG</i>	+	[+]	-
<i>V.paraeolyticus</i>	+	d	-
<i>V.alginolyticus</i>	-	-	+
<i>V.anguillarum</i>	-	-	+
<i>A.hydrophila</i>	-	-	[+]
<i>A.salmonicida</i>	-	-	-
<i>Pl.shigelloïdes</i>	+	+	+

Table 1b

Legend:

- + positive in 1 or 2 days, [+] generally positive in 1 or 2 days, (+) positive in 3 or 4 days
- [-] generally negative, - negative.
- d: variable results depending on the strains.

STRICT GRAM-NEGATIVE AEROBIC BACILLI WITH OXIDATIVE METABOLISM
(*Pseudomonas*, *Alteromonas*, *Flavobacterium*, *Xanthomonas*, *Alcaligenes*, *Acinetobacter*)

PROTOCOL

Unlike *Enterobacteriaceae* and *Vibrionaceae*, these strictly aerobic bacilli degrade glucides by oxidation and produce only few acid catabolites. As a result, the color of the three media remains practically unaltered: pale mauve to violet after 24 - 48 hours of incubation, except in the case of *Acinetobacter glucidolytica* (which turns a dirty yellow).

It is possible to detect decarboxylase or dihydrolase in these bacilli by adopting the following procedure:

A pH 4 buffer solution (Titrisol Merck) is poured drop by drop in each of the three tubes of medium. Shake for a few seconds after the addition of each drop. The same number of drops of pH 4 buffer is added to each tube until at least 1 of the 3 tubes turns acidic, which serves as the control (indicator turns yellow).

3 additional drops of pH 4 buffer are added to the tubes of medium that have remained alkaline (mauve coloration). Shake. The absence of an acid indicator corresponds to the presence of decarboxylase or dihydrolase.

For the results, refer to the following table:

PSEUDOMONADACEAE	LDC	ODC	ADH
<i>P.aeruginosa</i>	-	-	+
<i>P.fluorescens</i>	-	-	+
<i>P.putida</i>	-	-	+
<i>P.pseudomallei</i>	-	-	+
<i>P.mallei</i>	-	-	+
<i>P.stutzeri</i>	-	-	-
<i>P.pseudoamcaligenes</i>	-	-	+ weak
<i>P.cepacia</i>	+	d	-
<i>P.maltophilia</i>	+	-	-
<i>P.acidovorans</i>	-	-	-
<i>P.diminutavesicularis</i>	-	-	-

OTHER STRICT GRAM-NEGATIVE AEROBES	LDC	ODC	ADH
<i>Alteromonas putrefaciens</i>	-	+	-
<i>Alcaligenes</i>	-	-	-
<i>Flavobacterium</i>	-	-	-
<i>Xanthomonas</i>	-	-	-
<i>Acinetobacter L. woffi</i>	-	-	-
<i>Acinetobacter glucidolytica</i>	-	-	- OR +