MR-VP Broth (Methyl-red VOGES-PROSKAUER Broth)

Test culture medium for the methyl red test (CLARK and LUBS 1915) and the VOGES-PROSKAUER Test (VOGES and PROSKAUER 1898), which are used for biochemical differentiation, particularly within the Coli-Aerogenes group.

This culture medium complies with the recommendations of the ISO (1975), the DIN Norm 10160 for the examination of meat and the DIN Norm 10181 for the examination of milk.

Mode of Action

a. Some bacteria utilize glucose to form large amounts of acid with the result that the pH value of the medium falls to below 4.4. Other species produce less acid so that the fall in pH is not as great. This difference can be visualized by using methyl red which is yellow above pH 5.1 and red at pH 4.4.

b. Many microorganisms metabolize glucose to produce acetoin (acetylmethyl carbinol), 2,3-butanediol or diacetyl. The presence of these metabolites is established by means of O'MEARA's reagent (1931) improved by LEVINE et al. (1934), copper sulfate solution according to LEIFSON (1932), BARRIT's reagent (BARRITT 1936) or other reagents (see references). According to HOLLÄNDER et al. (1982), addition of fumarate to the broth enhances this reaction.
Details and comparative studies on the various modifications of the MR-VP test are to found in EDDY (1961), SUASSUNA et al. (1961), IJUTOV (1963) and SKERMAN (1969).

Typical Composition (g/litre)

Peptone from meat 7.0; D(+)glucose 5.0; phosphate buffer 5.0.

Preparation

Suspend 17 g/litre, dispense 5 ml aliquots into tubes and autoclave (15 min at 121 °C).

pH: 6.9 ± 0.2 at 25 °C.

The broth is clear and yellowish-brown.

Preparation of the methyl red indicator solution: Suspend 0.04 g methyl red in 60 ml absolute ethanol, adjust the pH to a value of approx. 5.0. The solution then becomes orange.

Preparation of O'MEARA's reagent: Suspend 40 g potassium hydroxide in 100 ml distilled water. Allow to cool, add 0.3g creatine (monohydrate) and dissolve. The prepared reagent solution can be stored for about 4 weeks in the refrigerator (+4 °C).

Preparation of copper sulfate solution acc. to LEIFSON: Suspend 1 g copper sulfate in 40 ml concentrated ammonia and add 690ml of an approx. 10 % potassium hydroxide solution (prepared from potassium hydroxide).

Preparation of BARRITT's reagent: Suspend 5 g naphthol(1) in 100 ml absolute ethanol.

Experimental Procedure and Evaluation

Inoculate two tubes containing MR-VP Broth with a pure culture of the microorganisms under investigation.

Incubation: up to 4 days at 35 °C.

Carry out the following tests:

Methyl red test: Add about 5 drops of the methyl red indicator solution to the first tube.

VOGES-PROSKAUER test: Add 5 ml of copper sulfate solution acc. to LEIFSON or 3 ml BARRIT's solution and 1 ml 40 % potassium hydroxide solution (prepared from extra pure potassium hydroxide) or 5 ml O'MEARA's reagent to the second tube. With the first two reagents a positive reaction is indicated, if the colour of the medium changes to red within a few minutes.

In the case of O'MEARA's reagent, the reaction is positive if, after frequent shaking, a pink coloration appears after approx. 20minutes beginning at the surface and becoming more intense within 2hours.

Colour Reaction	Microorganisms
From orange to red	Escherichia coli, Citrobacter and others
From orange to yellow	Enterobacter aerogenes, Enterobacter cloacae and others
Red (positive)	Enterobacter aerogenes, Enterobacter cloacae and others
No colour change (negative)	Escherichia coli, Citrobacter and others

Literature

ISO International Organization for Standardization: Meat and meat products. Detection of Salmonellae. Reference method. – **International Standard ISO 3565**; (1975).

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Ordering Information

Product	Merck Cat. No.	Pack size
MR-VP Broth (Methyl-red VOGES-PROSKAUER Broth)	1.05712.0500	500 g
Ammonia solution 25 %	1.05432.1000	1 l
Copper sulfate	1.02790.0250	250 g
Creatine (monohydrate)	8.41470.0050	50 g
Ethanol absolute	1.00983.1000	1 l
Methyl red	1.06076.0025	25 g
Naphthol-(1)	1.06223.0050	50 g
Potassium hydroxide pellets	1.05033.0500	500 g

Quality control

Test strains	Growth	Methyl red	VOGES-PROSKAUER
Escherichia coli ATCC 25922	good / very good	+	-
Klebsiella pneumoniae ATCC 13883	good / very good	+	-
Klebsiella pneumoniae ATCC 10031	good / very good	+	-
Enterobacter cloacae ATCC 13047	good / very good	-	+
Serratia marcescens ATCC 14756	good / very good	±	+