

# MEVAG/Agar

# 355-5544

## DEFINITION

Bacteria can use carbohydrates by means of two different metabolic paths:

- **A fermentative path** in which all reactions take place in the absence of oxygen in the air. In particular acid catabolites form and accumulate in the medium, causing a drop in its pH.
- **An oxidative path** in which air is necessarily required, with few catabolic acids forming. Acidity due to carbohydrate oxidation must therefore be detected out in a slightly buffered medium such as the MEVAG medium.

## PRESENTATION

### Ready-to-use

8 ml x 25 tubes

code 355-5544

## STORAGE

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

## THEORETICAL FORMULA

Macerated meat (500 g/l)	50 ml
KCl	5 g
Agar	3 g
Phenol red (0.2% aqueous solution )	10 ml
Distilled water q.s.p.	1,000 ml
Final pH (25°C) = 7.0 ± 0.2	

## MEVAG medium can be used for two purposes:

1. To determine the path of attack of glucose,
2. To test the carbohydrates attacked by oxidative bacteria.

## 1.DETERMINATION OF PATH OF ATTACK OF GLUCOSE (Hugh and Leifson test).

## PROTOCOL

- Melt the contents of 2 tubes of MEVAG in boiling water-bath.
- Cool to about 44°C- 47°C, and under aseptic conditions add to each tube a few drops of sterile aqueous glucose solution to obtain a final concentration of 1% (i.e. 7 drops of a 30% solution).

- Mix, then cool the medium by immersion in cold water.

- Inoculate each tube by centrally inserting a platinum wire loaded with inoculum from an 18-48 hours culture in a liquid medium, in such way that the inoculum is of moderate abundance and distributed evenly along the wire.  
In the case of bacteria requiring the presence of ascite or serum for their growth, a 10% concentration of this substance can be added to the liquefied medium cooled to about 44°C-47°C.

- Melt sterile vaseline in a water-bath heated to about 80°C.  
Under aseptic conditions, pour a 10 - 15 mm layer of vaseline onto one of the two tubes of MEVAG media, previously treated with glucose and inoculated, and immediately immerse the tube in cold water to cool the vaseline.

- Place the 2 tubes, referred to as "open" (without vaseline) and "closed" (with vaseline), in an autoclave at 37°C, taking care not to tighten the caps too firmly).

- Read results after 24 hours (or longer for oxidative bacteria and for certain slow growing fermentative bacteria).

## READING AND INTERPRETATION

The Hugh and Leifson test can distinguish between three categories of bacteria:

### • Fermentative bacteria:

Rapid, equal acidification in the 2 media, which within 24 hours become yellow along the top of the inoculation injection.

Any gas produced will be visible under the layer of vaseline.

### • Oxidative bacteria:

- In the "closed" tube: little or no culture, and no acidification (after several days, even in the absence of visible culture, the medium may take on an orange tint if the vaseline used is acidic).

- In the "open" tube: moderate and rather slow acidification, starting at the surface, within 24

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- 48 hours or sometimes longer. Gas is never produced.

***N.B.:*** *Certain strict aerobes (Neisseria, Flavobacterium) are capable of weak culture under the layer of vaseline; in this case gradual acidification is observed in the two tubes, beginning with the upper portion of the medium.*

### • Inactive bacteria:

- In the “closed” tube: little or no culture.
- In the “open” tube, culture with no change in pH (no acidification) or with a more or less marked alkalization on the surface (turning red-violet).

### Example of results:

A : acidification  
alc : alkalization  
NC : unchanged  
G+ : gas production  
G- : no gas production

	OPEN TUBE	CLOSED TUBE	
	pH	pH	gas
<b>Fermentative bacteria</b> • <i>Enterobacteriaceae</i> • <i>Vibrions</i> • <i>Aeromonas</i>	A A A	A A A	G+ or G G G+ or G-
<b>Oxidative or inactive</b> • <i>Pseudomonas</i> • <i>Acinetobacter, Moraxella</i> • <i>Moxarella lacunata</i> and <i>A. Iwoffii</i> • <i>Neisseria</i>	A (or NC) A (or NC) NC or alc A (or NC)	NC NC NC NC	

## 2. TEST OF CARBOHYDRATES ATTACKED BY OXIDATIVE BACTERIA

Bacteria oxidating glucose also have an oxidative metabolism for all the glucide they attack. To detect which carbohydrates are attacked by oxidative bacteria, there is no need to carry out the Hugh and Leifson test for each one: it is sufficient to study each carbohydrate in a single tube of “open” MEVAG medium.

### PROTOCOL

Preparation and inoculation of medium: follow the instructions above for glucose. The carbohydrates of interest for the diagnosis are: galactose, fructose, L-arabinose, D-arabinose, xylose, lactose, saccharose, maltose, etc.

### READING AND INTERPRETATION

Depending on the case, inoculated media should be placed at 30°C or at 20°C - 25°C.

Acidification always starts at the surface, but is often slow to appear; cultures should therefore be examined for at least 7 days.

### PRECAUTIONS

- Keep the tubes of MEVAG carefully-sealed in order to avoid their slow acidification on contact with the air. At the moment of use, unscrew the cap to allow the CO<sub>2</sub> to escape as the medium warm up.
- If, after incorporation of the carbohydrates (particularly with arabinose and xylose), the pH drops excessively (pale orange or even yellow medium), the medium must be re-alkalinized (pure red color) by means of a sterile 4% soda solution
- Comply with Good Laboratory Practice.

### PERFORMANCES / QUALITY CONTROL OF THE TEST

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

STRAINS	Result of 24–48h culture at 37°C	
	“Open” tube + Glc	“Closed” tube + Glc + paraffin
<i>Escherichia coli</i> ATCC 25922	Acidification + gas	Acidification + gas
<i>Ps.aeruginosa</i> ATCC 27853	Slow acidification on surface	Color unchanged

### 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

MEVAG / Carbohydrates / Fermentative path / Oxidative path / Phenol red / Medium.