



## Raffinose

DD029

Carbohydrate Differentiation Discs are used to differentiate bacteria on the basis of carbohydrate fermentation abilities.

### Directions

A Sugar free medium base is prepared as desired, dispensed and sterilized. Following media are recommended for this test.

#### Liquid Media

- M885 Andrade Peptone Water
- MV885 Andrade HiVeg Peptone Water
- M909 Andrade Peptone Water with Meat Extract
- MV909 Andrade Peptone Water w/ HiVeg Extract No. 1
- M054 Phenol Red Broth Base
- MV054 Phenol Red HiVeg Broth Base
- M279 Phenol Red Broth Base w/ Meat Extract
- MV279 Phenol Red Broth Base w/ HiVeg Extract No. 1
- M284 Purple Broth Base
- MV284 Purple HiVeg Broth Base
- M676 Yeast Fermentation Broth
- MV676 Yeast Fermentation HiVeg Broth Base

#### Semisolid Media

- M159 Cystine Tryptone Agar
- MV159 Cystine Tryptone Agar, HiVeg
- M395 OF Basal Medium
- MV395 OF Basal HiVeg Medium
- M319 Tryptone Agar Base
- MV319 Tryptone Agar Base, HiVeg

#### Solid Media

- M053 Phenol Red Agar Base
- MV053 Phenol Red HiVeg Agar Base
- M098 Purple Agar Base
- MV098 Purple HiVeg Agar Base

Any medium- liquid, semisolid or solid can be used as per choice. Liquid and semisolid media are dispensed in 5 ml amounts in test tubes and sterilized. On cooling to 45 - 50°C a single Carbohydrate disc is added to each tube aseptically and inoculated with the test organisms. In semisolid medium the disc is pushed in the medium along with the inoculum just below the surface of the medium, so that the medium at the bottom can serve as control while fermentation can be detected at the surface level. Using solid media it is possible to detect fermentation of number of sugars on the same plate. Sterile plates containing the agar medium of choice are surface seeded with test organism(s) and required Carbohydrate discs are placed and pressed gently on the surface of the plate at sufficient distance (2cm) from each other. Incubation is carried out at  $36 \pm 1.0^{\circ}\text{C}$  for 18-48 hours

and results are recorded at 18 - 24 hours and again at 48 hours. The results should be frequently observed since reversal of fermentation reaction can take place. In case of liquid medium gas produced during fermentation is collected in the inverted Durham's tube while acid produced changes colour of the medium. In semisolid media gas produced is trapped and seen as bubbles. On agar plates fermentation is visualized by change in colour around the disc.

## Principle And Interpretation

Ability of an organism to ferment a specific carbohydrate added in the basal medium, results in the production of acid or acid and gas. This ability has been used to characterize a specific species of bacteria which helps in differentiation of species as well (2,3). When carbohydrate impregnated disc is added to a culture medium the carbohydrate diffuses through the medium. When a carbohydrate is fermented by a microorganism, the acid (or acid and gas) produced lowers the pH of the medium and the indicator in the basal medium thus changes colour (e.g. phenol red changes from red to orange to yellow).

Bacteria capable of fermentation grow in Andrade Peptone and produce acid due to fermentation of the added carbohydrate and change the colour of the indicator from light straw colored to pink(1).

## Quality Control

### Appearance

Filter paper discs of 10 mm diameter bearing letters "Rf" in continuous printing style.

### Cultural response

The carbohydrate fermentation reactions after an incubation of 18-48 hours at 35-37°C, of various bacteria with Raffinose. Differentiation discs were tested using Phenol Red Broth Base (M054).

Organism	Growth	Acid	Gas
<i>Citrobacter freundii</i> ATCC 8090	Luxuriant	Negative reaction: no colour change	Positive reaction
<i>Enterobacter aerogenes</i> ATCC 13048	Luxuriant	Positive reaction: yellow colour	Positive reaction
<i>Escherichia coli</i> ATCC 25922	Luxuriant	Negative reaction: no colour change	Negative reaction
<i>Klebsiella pneumoniae</i> ATCC 13883	Luxuriant	Positive reaction: yellow colour	Positive reaction
<i>Serratia marcescens</i> ATCC 8100	Luxuriant	Negative reaction: no colour change	Negative reaction
<i>Proteus vulgaris</i> ATCC 13315	Luxuriant	Negative reaction: no colour change	Negative reaction
<i>Salmonella Typhi</i> ATCC 6539	Luxuriant	Negative reaction: no colour change	Negative reaction
<i>Salmonella Typhimurium</i> ATCC 14028	Luxuriant	Negative reaction: no colour change	Negative reaction
<i>Shigella flexneri</i> ATCC 12022	Luxuriant	Negative reaction: no colour change	Negative reaction

## Storage and Shelf Life

Store between 10-30°C. Use before expiry date on the label.

## Reference

1. Maxted W. R., 1953, J. Clin. Path., 6:234.

2. Eaton A.D, Clesceri L.S. Greenberg. A.W, 2005, Standard Methods for the Examination of Water and wastewater, 21st edn, APHA. Washington. DC.
3. Mackie and McCartney, 1996, Practical Medical Microbiology, 14th ed., Vol. 2, Collee, Duguid, Fraser and Marmion (Eds.), Churchill Livingstone, Edinburgh.

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