

Raffinose

Intended Use

Raffinose Disc is used to differentiate bacteria on their ability to ferment carbohydrates.

Summary

In 1949, Soto developed miniaturized fermentation tests using carbohydrate impregnated paper discs. Sanders et al. subsequently developed a screening method for identification of *Enterobacteriaceae* using reagent impregnated discs.

The ability of an organism to ferment specific carbohydrate incorporated in a basal medium, resulting in production of acid and gas, has been used to characterize bacteria and help in differentiation.

Principle

Carbohydrate impregnated on the discs when added to culture medium diffuses through the medium. When the microorganism ferments the carbohydrate, acid or acid and gas is produced which lowers the pH of the medium. Indicator in the medium changes the colour; e.g., phenol red changes from red to orange to yellow.

Specimen sample

Discs are not intended for testing mixed flora. The organism to be tested should first be isolated as single colonies.

Directions

Solid Media

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 from (2 cm) from each other.

Incubation is carried out at 35°C-37°C and plates are examined after 18-48 hours.

Media recommended: Phenol Red Agar Base (201160080500 / 201160080500)

Purple Agar Base (201160350500 / 201160350500)

Liquid Media

Carbohydrate discs are transferred aseptically to tubes containing 5mL of appropriate broth. Inoculum is then introduced in the medium.

Incubation is carried out at 35°C-37°C and tubes are examined after 18-48 hours.

Media recommended: Phenol Red Broth Base (201160090100 / 201160090500)

Andrade Peptone Water (201010110100 / 201010110500)

Semi Solid Media

Transfer aseptically one carbohydrate disc to each tube of appropriate basal medium. Inoculate by stabbing the medium once with a straight needle to about one-half its depth. As the needle goes into the agar the disc is pushed aside into the agar near the surface.

Incubation is carried out at 35°C-37°C and tubes are examined after 15-18 hours.

Media recommended: OF Basal Medium (201150030100 / 201150030500)

Tryptone Agar Base (201200190100 / 201200190100)

Quality Control

Appearance: High quality absorbent paper discs of 10 mm diameter with printed 'Rf' on each side of the disc.

Cultural Response: The carbohydrate fermentation reactions after an incubation of 18-48 hours at 35°C-37°C, of various bacteria with Raffinose Differentiation disc were tested using Phenol Red Broth Base.

Organisms (ATCC)	Growth	Acid	Gas
<i>Citrobacter freundii</i> (8090)	Good	-	-
<i>Klebsiella aerogenes</i> (13048)	Good	+	+
<i>Escherichia coli</i> (25922)	Good	-	-
<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> (700603)	Good	+	+
<i>Proteus hauseri</i> (13315)	Good	-	-
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar <i>Typhimurium</i> (14028)	Good	-	-
<i>Shigella flexneri</i> serotype 2b (12022)	Good	+	-

Key :

- : Negative reaction, No colour change/ No Gas production

+ : Positive reaction, Yellow colour / Gas production

Storage and Stability

Discs are stored between 10°C-30°C.

Results

1. On solid media, a positive reaction is determined by formation of yellow zone around the disc.
2. In liquid media, reactions of *Enterobacteriaceae* and other nonfastidious bacteria are rapid, especially if the inoculum is large.
3. In semi solid media, the reaction begins near the disc and depend upon the of growth and size of the inoculum. Formation of gas is apparent from bubbles at the surface, around the disc or along the line of inoculation. The bottom of the tube serves as a control.

Precautions

1. For Laboratory Use.
2. Aseptic techniques and established precautions should be followed against microbiological hazards throughout all procedures.
3. Plates, tubes and other contaminated materials must be sterilized by autoclaving before discarding.
4. Discs which show signs of discoloration or deterioration should not be used.

Limitations

1. In solid and Liquid media reversal of pH may occur due to further breakdown of acid production, or due to accumulation of ammonia formed from breakdown of nitrogen compounds. Detection of gas formation is not reliable.
2. Rapid reversal of reactions in semisolid media is uncommon.
3. Frequent observation of results is necessary since the organisms do not necessarily ferment all carbohydrates at the same rate, one carbohydrate disc may indicate fermentation by a colour reaction and then that reaction may reverse, while the reaction with another carbohydrate is just the beginning.

Reference

1. Sanders, A.C., J.E. Faber and T.M. Cook. 1957. A rapid method for the characterization of enteric pathogens using paper discs. Appl. Microbiol. 5:36-40.
2. Soto, O.B. 1949. Fermentation reactions with dried paper discs containing carbohydrate and indicator. Puerto Rican J. Public Health Trop. Med. 25:96-100.
3. Bergey's Manual of Systematic Bacteriology, 1994, 9th ed., Williams and Wilkins, Baltimore.
4. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product	Symbol	Pack Size
206180330050	Raffinose	Rf	Single Vial (1x 50 Disc)

Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.