Wagatsuma Agar Base

M626

Wagatsuma Agar is recommended for the performance of Kanagawa test to identify virulent *Vibrio parahaemolyticus* strains.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	10.000
Yeast extract	3.000
Sodium chloride	70.000
Dipotassium phosphate	5.000
Mannitol	10.000
Crystal violet	0.001
Agar	15.000
Final pH (at 25°C)	8.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 11.3 grams in 100 ml distilled water. Heat to boiling to dissolve the medium completely. Steam for 30 minutes. Cool to 50°C. Add 2 ml of a suspension of freshly drawn citrated human red blood cells (previously washed 3 times in saline) to 100 ml agar. Mix well before pouring into sterile Petri plates.

Principle And Interpretation

Vibrio species cause intestinal or extra-intestinal human infections. *Vibrio parahaemolyticus* is a well-demonstrated cause of acute gastroenteritis (1, 2). Pathogenic strains of *V.parahaemolyticus* are differentiated from non-pathogenic strains by the ability of the former to produce a thermostable direct haemolysin whose production is termed as Kanagawa phenomenon (3). The extensive investigation in animal model suggests that Kanagawa haemolysin is the primary virulence factor in *V.parahaemolyticus* (4). It has been well established that enteropathogenic *V.parahaemolyticus* strains are always Kanagawa positive and seafood isolates are almost always Kanagawa negative. Wagatsuma Agar is formulated as described by Wagatsuma (5) and recommended by APHA (6) for the performance of Kanagawa test to identify virulent *V.parahaemolyticus* strains.

Peptic digest of animal tissue and yeast extract in the medium are the source of nitrogen and other growth factors. Mannitol is the energy and carbon source. The selective action is attributed to crystal violet, which is inhibitory to most of the gram-positive bacteria. High salt concentration and alkaline pH makes the medium selective for *V. parahaemolyticus* . Enteropathogenic *V. parahaemolyticus* strains are Kanagawa positive and produce haemolysin, which forms a transparent, clearing zone of blood cells around the colony.

Quality Control

Appearance
Cream to beige homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium
Very light bluish coloured clear to slightly opalescent gel forms in Petri plates.

Reaction
Reaction of 11.3% w/v aqueous solution at 25°C. pH : 8.0±0.2

pH
7.80-8.20

Cultural Response

M626: Cultural characteristics observed with added freshly drawn citrated human red blood cell suspension, after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Haemolysin production
Vibrio parahaemolyticus ATCC 11344 (avir	50-100	luxuriant	negative, no clear zone
Vibrio parahaemolyticus (virulent)	50-100	luxuriant	positive, transparent clear zone of blood cells around the colony

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1. Fujino T., Sakaguchi G., Sakazaki R. and Takeda Y., (Eds.), 1974, International Symposium on Vibrio parahaemolyticus , Saikon Publishing Company Ltd., Tokyo.
2. Balows A., Truper H. G., Dworkin M., Harder W., Schleifer K. H. (Ed.), The Prokaryotes, 1992, 2nd Edition, Vol. III, Springer-Verlag.
3. Sakazaki R., Tamura K., Kato T., Obora Y., Yamai S., Hobo K., 1968, Japan, J. Med. Sci. Biol., 21:325.
4. Twedt R. M., Peeler J. T. and Spaulding P. L., 1980, Appl. Environ. Microbiol., 40:1012.
5. Wagatsuma S., 1968, Media Circle, 13:159.
6. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., APHA, Washington, D.C

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