

Wagatsuma HiVeg™ Agar Base**MV626**

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

Composition ** :

Ingredients	Grams/Litre
HiVeg peptone	10.0
Yeast extract	3.0
Sodium chloride	70.0
Dipotassium phosphate	5.0
Mannitol	10.0
Crystal violet	0.001
Agar	15.0

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 (approx 0.5%) suspension of freshly drawn citrated human red blood cells (previously washed 3 times in saline) to 100 ml medium. Mix well before pouring into sterile petriplates.

Principle and Interpretation :

This medium is prepared by using HiVeg peptone in place of peptic digest of animal tissue which makes the medium free of BSE/TSE risks. Wagatsuma HiVeg Agar is the modification of Wagatsuma Agar which is formulated as per formulation of Wagatsuma (1) and recommended by APHA (2) for differentiation of pathogenic *Vibrio parahaemolyticus* from nonpathogenic strains. Differentiation is based on the ability of this organism to produce hemolytic reaction on a medium with high salt concentration, termed as Kanagawa phenomenon(3).

Extensive investigation in animal model suggests that Kanagawa haemolysin is the primary virulence factor in *Vibrio parahaemolyticus* (4). It has been well established that enteropathogenic *Vibrio parahaemolyticus* strains are always Kanagawa positive and seafood isolates are almost always Kanagawa negative. A positive Kanagawa test consists of observation of beta haemolysis - a zone of transparent clearing of blood cells around colonies within 24 hours incubation at 37°C.

HiVeg peptone and yeast extract provide nitrogenous compounds, vitamin B complex and other growth nutrients. High salt concentration and alkaline pH make the medium selective.

Product Profile :

Vegetable based (Code MV)©	Animal based (Code M)
MV626 HiVeg peptone	M626 Peptic digest of animal tissue

Recommended for : The performance of Kanagawa test to identify virulent *Vibrio parahaemolyticus*.

Reconstitution : 113.0 g/l

Quantity on preparation (500g) : 4.42 L

pH (25°C) : 8.0 ± 0.2

Supplement : Human red blood cells

Sterilization : Steam for 30 minutes.

Storage : Dry Medium - Below 30°C, Prepared Medium 2 - 8°C.

Quality Control :**Appearance of powder**

Light yellow coloured, may have slightly greenish tinge, homogeneous, free flowing powder.

Gelling

Firm, comparable with 1.5% Agar gel.

Colour and Clarity

Very light bluish coloured, clear to slightly opalescent gel forms in petri plates.

Reaction

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

Cultural Response

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

Organisms (ATCC)	Inoculum	Growth	Recovery	Haemolysin*
<i>V. parahaemolyticus</i> (11344) (avirulent)	10 ² -10 ³	luxuriant	>50%	-
<i>Vibrio parahaemolyticus</i>	10 ² -10 ³	luxuriant	>50%	+ (virulent)

Key : + = transparent zone of clearing of blood cells around colony

* = production

References :

- Wagatsuma S., 1968, Media Circle, 13 : 159.
- Downes FP and Ito K (Eds.), 2001, Compendium of Methods For The Microbiological Examination of Foods, 4th ed., APHA, Washington, D.C.
- Sakazaki R., et al, 1968, Jpn. J. Med. Sci. Biol., 21:325.
- Twedt R.M., Peeler J.T. and Spaulding P.L., 1980, Appl. Environ. Microbiol., 40:1012.