Wagatsuma Agar Base

M626

Intended Use:

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

Composition**

Ingredients	g / L
Peptone	10.000
Yeast extract	3.000
Sodium chloride	70.000
Dipotassium hydrogen 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 purified/distilled water. Heat to boiling to dissolve the medium completely. Steam for 30 minutes. Cool to 45-50°C. Add 2 ml of a suspension (approx. 0.5%) of freshly drawn citrated human red blood cells (previously washed 3 times in saline) to 100 ml agar. Mix well and pour 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.

Peptone 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.

Type of specimen

Clinical samples - Faeces sample; Food samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,8).
For food samples, follow appropriate techniques for sample collection and processing as per guidelines (6).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Further biochemical and serological tests must be carried out for complete identification.

2. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
3. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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

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 between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (7,8).

Reference

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IVD ***In vitro* diagnostic medical device**

10°C–30°C **Storage temperature**

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