Kaper's Medium

M1169

Intended Use:

Recommended for the enumeration and identification of *Aeromonas hydrophila* from food samples in accordance with APHA.

Composition**

Ingredients	g / L
Proteose peptone	5.000
Yeast extract	3.000
Tryptone	10.000
L-Ornithine monohydrochloride	5.000
Mannitol	1.000
Inositol	10.000
Sodium thiosulphate	0.400
Ferric ammonium citrate	0.500
Bromocresol purple	0.020
Agar	3.000
Final pH (at 25°C)	6.7±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 37.92 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Dispense into tubes (5 ml). Sterilize by autoclaving at 15 lbs pressure (121°C) for 12 minutes.

Principle And Interpretation

Aeromonas hydrophila (often referred as motile aeromonads) is a facultative anaerobe, which is characterized by growth at 37°C and motility. The detection of *Aeromonas* species in foods and environmental samples is usually quite easy. However difficulties may arise when quantitative recovery is required or in cases where large number of other organisms are present (1). Kaper et al (2) described a single tube medium for the rapid presumptive identification of *A. hydrophila*, which is also recommended by APHA (3). This single tube medium shows the following reactions: mannitol and inositol fermentation, ornithine decarboxylation and deamination, motility, indole and H_2S production. The food samples should be processed as soon as possible upon arrival at the laboratory. Motile aeromonads are somewhat sensitive to pH values below 5.5; therefore, acidic foods should be processed soon after arrival in the laboratory. On the basis of biochemical characterization, *Aeromonas* can be differentiated as mannitol fermenters, inositol non-fermenters, absence of ornithine decarboxylase, and hydrogen sulfide not produced from thiosulphate.

Tryptone, proteose peptone and yeast extract provide essential nitrogenous compounds and B vitamin etc. Sodium thiosulphate and ferric ammonium citrate acts as indicators of H_2S production. Inositol and mannitol are the fermentable carbohydrates; L-ornithine hydrochloride is an amino acid. Bromocresol purple is the pH indicator, which is yellow at acidic pH and purple at neutral to alkaline pH values. Usually in tubes containing Kapers Medium inoculated with *A. hydrophila*, the butts turn yellow due to acid formation and an alkaline band is formed at the top of the medium. Small amount of agar facilitates motility determination.

A. hydrophilla is inoculated in Kapers Medium for the verification of the isolates. After 18-24 hours, *Aeromonas* shows motility, are H_2S negative and indole positive (add 2 drops of Kovacs Reagent (R008) to the tubes and look for a red colour).

Type of specimen

Food samples

Specimen Collection and Handling:

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (5). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Due to nutritional variation, some strains may show poor growth.
2. Further serological and biochemical testing is required for complete identification.

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 yellow homogeneous free flowing powder

Gelling

Semisolid, comparable with 0.3% Agar gel.

Colour and Clarity of prepared medium

Purple coloured, clear to slightly opalescent gel forms in tubes as butts

Reaction

Reaction of 3.79% w/v aqueous solution at 25°C. pH : 6.7±0.2

pH

6.50-6.90

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Medium
Aeromonas hydrophila ATCC 7966 (00063*)	50-100	luxuriant	acidic butt, with alkaline band at the top

Key :(*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-30°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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference

1. Corry J. E. L., Curtis G. D. W., and Baird R. M., Culture Media for Food Microbiology, Vol. 34, Progress in Industrial Microbiology, 1995, Elsevier, Amsterdam.
2. Kaper J., Seidler R. J., Lockman H. and Colwell R. R., 1979, Appl. Environ. Microbiol., 38:1023.
3. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed.,American Public Health Association, Washington, D.C.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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