AKI Medium

M1879

Intended Use:

For identification of *Vibrio* in accordance with FDA BAM, 1998.

Composition**

Ingredients	Gms / Litre
Peptone	15.000
Yeast extract	4.000
Sodium chloride	5.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 24.0 gms in 970ml purified / distilled water. Heat if necessary to dissolve the medium completely.Sterilise by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and add 30ml of freshly prepared, filter sterilised $NaHCO_3$ and mix. Adjust the final pH 7.4 ± 0.2. Dispense aseptically into screw capped tubes.

Principle And Interpretation

V. cholerae, the type species of the genus *Vibrio*, is the causative agent of cholera outbreaks and epidemics. Cholera enterotoxin (CT) is the primary virulence factor of these organisms(5,7). Most strains of *V. cholerae* isolated from foods or environment do not produce choleare toxin and are not considered to be virulent. Various biochemical properties and antigenic types are used to characterize the species.*V. mimicus* has been associated with diarrhoea following consumption of raw or undercooked seafood. Hence isolates of Vibrio should be tested for the production of CT or CTX gene. AKI medium is used for the serological identification of CT of these organisms in accordance with FDA BAM, 1998 (2). After enrichment plating, screening and confirmation of the toxins can be done by Y-1 mouse adrenal cell assay and immunoassay methods. Peptone and Yeast extract provide necessary nutrients and Sodium chloride maintains the osmotic equilibrium of the medium.

Blend the food sample to be analysed with Alkaline peptone water (APW) in appropriate ratio and incubate as per the recommendation by FDA BAM. Pure cultures can be isolated from APW by plating a loopful of the inoculums into TCBS agar. Crowded colonies are separated using Tryptone salt agar, w/ 1% NaCl (M1877). For immuno assays, Inoculate test cultures into AKI medium and incubate at 35 ±2°C 18 h with shaking at 100 rpm. Centrifuge 5 to 7 ml of culture at 8,000 x g for 10 min. Filter sterilize the supernatant through a 0.2 µm filter or used as is for immunological assays for the presence of cholera toxin (CT) .

Type of specimen

Food and dairy samples.

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,6,8). 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.Further isolation on selective media is required for identification.

2. Some isolates may show poor growth due to nutritional variations.

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

Colour and Clarity of prepared medium

Light yellow coloured clear solution without any precipitate

Reaction

Reaction of 2.4% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

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

Organism	Inoculum (CFU)	Growth
Vibrio cholerae ATCC 14035	50-100	luxuriant
Vibrio parahaemolyticus ATCC 17802 (00037)*	50-100	luxuriant

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°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 (3,4).

Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2. FDA, U.S. 1998. Bacteriological Analytical Manual. 8 ed. Gaithersburg, MD: AOAC International.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. 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.
5. Karaolis, D. K., Johnson, J.A., Bailey, C.C., Boedeker, E.C., Kaper, J.B. and Reeves, P.R 1998. Proc. Natl. Acad. Sci. U. S. A., 95(6): 3134-3139.
6. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
7. Spira, W. M . and Fedorka-Cray, P.J. 1984. Infect. Immun, 45: 679-684.
8. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

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