



Craig's Medium

M974

Intended Use:

Recommended for cultivation of *Vibrio cholerae* to determine its enterotoxigenicity.

Composition**

Ingredients	Gms / Litre
Acicase™	30.000
Yeast extract	4.000
Dipotassium hydrogen phosphate	0.500
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 34.5 grams in 1000 ml purified / distilled water. Heat if necessary to ensure complete solution. Dispense and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add 2 ml of filter sterilized 20% glucose solution per 100 ml. Mix well.

Principle And Interpretation

Vibrio cholerae is a gram-negative bacterium that causes cholera in humans. Cholera is the prototype of diarrheal syndromes in which the disease is caused through the production of toxins that interrupt normal intra-intestinal exchange of water and electrolytes. A variety of laboratory diagnosis of cholera and toxin detection have been developed.

Craig's Medium has similar composition to that of CAYE Broth, which was formulated as per APHA (4) for cultivating *V. cholerae* while testing their enteropathogenicity.

For testing the enterotoxin production of *V. cholerae*, the culture is to be grown in Craig's Medium as this medium enhances the production of *Vibrio* enterotoxin. Medium contents like Acicase and yeast extract provide the essential nitrogenous nutrients and B-complex vitamins to the growing *Vibrio*'s. Dipotassium hydrogen phosphate helps in maintaining buffering conditions in the medium. Glucose is the energy source.

Inoculate test cultures from TN Agar (M950) slants to tubes of Craig's Medium and incubate overnight at $30 \pm 2^\circ\text{C}$ which is then further used for immunological testing of enterotoxigenicity.

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 (1,4,5).

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. Further biochemical and serological tests must be carried out for further 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

Light yellow to beige homogeneous free flowing powder

Colour and Clarity of prepared medium

Amber coloured, clear solution without any precipitate

Reaction

Reaction of 3.45% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

Cultural characteristics observed after an incubation at 25-30°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth
<i>Vibrio cholerae</i> ATCC 15748	50-100	good-luxuriant

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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. 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.
4. 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.
5. 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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Disclaimer :

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