Fermentation Medium Base for C. perfringens

M919

Fermentation Medium Base for C. perfringens is a basal medium recommended for determination of fermentation reaction of *Clostridium perfringens* with added carbohydrate.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	10.000
Peptone, special	10.000
Sodium thioglycollate	0.250
Agar	2.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 22.25 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense 9 ml amounts in test tubes containing inverted Durhams tube. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Before use heat in boiling water or free flowing steam for 10 minutes to remove dissolved oxygen and add 1 ml of 1% sterile Salicin and Raffinose solution in separate tubes.

Principle And Interpretation

Contamination of foods with clostridia is largely derived from soil (1) and is usually responsible for *Clostridium perfringens* food poisoning. A heat labile enterotoxin produced by sporulating cells induces the major symptoms of diarrhea in perfringens poisoning. Although the enterotoxin is not preformed in the foods, the foods in which conditions are favorable for sporulation may contain enterotoxin (2, 3). Therefore *Clostridium* are common food contaminants responsible for spoilage of canned foods, chill stored products etc (4).

Fermentation Medium base for C. perfringens was formulated by Spray (5) and is recommended by APHA (6) for determination of fermentation reaction of *C.perfringens* . This medium helps in identification of *C.perfringens* from other *Clostridium* species.

Casein enzymic hydrolysate and peptone special provide the necessary growth nutrients. Sodium thioglycollate creates low oxygen tension required in the medium to facilitate the growth of anaerobic organisms.

Inoculate about 2 gram of food sample into 15 to 20 ml of Chopped Liver Broth (M606). Incubate at 35-37°C for 20-24 hours. Streak Tryptose Sulphite Cycloserine (T.S.C.) Agar Base (M837) containing Egg Yolk Emulsion (FD045) to obtain presumptive Clostridium perfringens @. Select representative black colonies and inoculate Fluid Thioglycollate Medium (M009). Incubate at 35-37°C for 18-24 hours. Perform gram staining and isolate on Tryptose Sulphite Cycloserine (T.S.C.) Agar Base (M837). Incubate anaerobically at 35-37°C for 18-24 hours to obtain isolated colonies. The Fluid Thioglycollate Medium (M009) tubes can be further used to confirm *C.perfringens* by performing biochemical identification including carbohydrate fermentation. *C. perfringens* can be differentiated from other clostridia on the basis of acid production from carbohydrates. To test acid, transfer 1 ml of culture from Fermentation Medium Base for *C. perfringens* (containing Salicin / Raffinose) to a test tube and add 2 drops of 0.04 % bromothymol blue. A yellow colour indicates acid production. Salicin is rapidly fermented by Clostridia other than *C. perfringens* , while *C. perfringens* produces acid from raffinose within 3 days, which is not shown by other species.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Semisolid, comparable with 0.2% Agar gel

Colour and Clarity of prepared medium
Light amber coloured, clear solution without any precipitate

Reaction
Reaction of 2.22% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

Cultural Response
Cultural characteristics observed under anaerobic condition with added 1% Salicin and Raffinose solutions in 2 separate tubes containing media after an incubation at 35-37°C for 24-72 hours.(Acid production is tested by addition of 0.04% Bromothymol blue)

Cultural Response

Organism	Inoculum (CFU)	Growth	Salicin (24 hours)	Raffinose (72 hours)
Cultural Response				
Clostridium paraperfringens	50-100	luxuriant	acid and gas production	
Clostridium perfringensATCC 12924	50-100	luxuriant		acid production,yellow colour

Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

Reference
1. Gibbs B. M. and Freame B., 1965, J. Appl. Bacteriol., 28, 95-111
2. Craven S. E., Blankenship L. C. and McDonel J. L., 1981, Appl. Microbiol. 41:1184
3. Naik H. S. and Duncan C. L., 1977, A. J. Food Safety., 1: 74. 4. 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.
5. Spray R. S., 1936, J. Bacteriol., 32:135.
6. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., APHA, Washington, D.C.

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