Fermentation Medium Base for C. perfringens

M919

Intended Use:

For studying fermentation reaction of *Clostridium perfringens* with added carbohydrate.

Composition**

Ingredients	g / L
Tryptone	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 purified/distilled water. Heat to boiling to dissolve the medium completely. Dispense 9 ml amounts in 16 X 125 mm test tubes containing inverted Durham's tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Just before use, heat in a boiling water or free flowing steam for 10 minutes to remove dissolved oxygen and add 1 ml of 1% sterile carbohydrates - Salicin and Raffinose 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.

Tryptone 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.

Type of specimen

Clinical- stool, abscess; Food samples

Specimen Collection and Handling

Inoculate about 2 gram of food sample into 15 to 20 ml of CL 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. 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 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

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)

Organism	Growth	Salicin (24 hours)	Raffinose (72 hours)
Clostridium paraperfringens	luxuriant	acid and gas production	
Clostridium perfringens ATCC 12924	luxuriant		acid production, yellow colour

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 inorder 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 material sand 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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30°C
10°C **Storage temperature**

CE Marking

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