



Clostridium perfringens Agar Base

M2070

Intended Use

Recommended for isolation and identification of *Clostridium perfringens* from unheated food material.

Composition**

Ingredients	Gms / Litre
HMH extract #	5.000
Proteose peptone	10.000
Peptone	10.000
Sodium chloride	5.000
Lactose	10.000
Phenol red	0.050
Agar	20.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Heart extract

Directions

Suspend 60.05 grams in 900 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C and aseptically add 100 ml sterile Egg Yolk Tellurite Emulsion (FD046). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Clostridium perfringens, ranked behind *Salmonella* species and *Staphylococcus aureus*, has been the third most common etiological agent of food-borne disease (1). *Clostridium* species are spore forming, gram-positive rods occurring naturally in soil (2). *C.perfringens* food poisoning results from eating contaminated food. The major virulence factor of *C.perfringens* is the CPE enterotoxin, which is secreted upon invasion of the host gut, and contributes to food poisoning and other gastrointestinal illnesses (2). *C.perfringens* cells may lose viability if the suspected food samples are refrigerated, thereby making it difficult to incriminate the organisms in food poisoning outbreaks (3). *C.perfringens* Agar Base is recommended for detecting *C.perfringens* from raw foods. If desired, Kanamycin can be added to the medium which restricts the growth of other bacteria.

HMH extract, proteose peptone and peptone provides nitrogenous, carbonaceous nutrients, amino acids and other complex nutrients. Lecithinase of *C.perfringens* degrades lecithin of egg yolk, forming an insoluble opaque precipitate (4). Addition of tellurite and Kanamycin aids for selective isolation of *C.perfringens*.

Type of specimen

Food samples : meat and meat products

Specimen Collection and Handling:

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (4).

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 variable nutritional requirements, some strains show poor growth on this medium.
2. Further biochemical test must be carried out for confirmation.

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 light pink homogeneous free flowing powder

Gelling

Firm, comparable with 2.0% Agar gel.

Colour and Clarity of prepared medium

Basal medium - Red coloured, clear to very slightly opalescent gel. After addition of Egg Yolk Emulsion -Light red coloured, opaque gel forms in Petri plates

Reaction

Reaction of 6.0% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

M2070: Cultural characteristics observed with added Egg Yolk Tellurite Emulsion (FD046) when incubated anaerobically, at 35-37°C for 24 - 48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
<i>Clostridium perfringens</i> ATCC 13124 (00007*)	50-100	good-luxuriant	≥50%	black opaque zone around the colony
<i>Clostridium perfringens</i> ATCC 12916 (00080*)	50-100	good-luxuriant	≥50%	black opaque zone around the colony

Key:- (*) Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°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. Use before expiry date on the label. 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 (5,6).

Reference

1. Centre for Disease Control, 1982, CDC Surveillance Summaries, 35:7SS-16SS, 1986.
2. Czczulin J. R., Hanna P. C., Mcclane B., Infect. Immun., 61: 3429-3439 (1993).
3. Traci P. A., and Duncan C. L., 1974, Appl. Microbiol., 28:815
4. 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.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
6. 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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