Rapid Perfringens Medium Base (Twin pack)

M1898

Intended Use:

Recommended for rapid detection of *Clostridium perfringens* in food.

Composition**

Ingredients	Gms / Litre
Part A	-
LM powder	70.000
Part B	-
Tryptone	15.13
Yeast extract	8.04
Dextrose (Glucose)	10.55
Sodium chloride	4.02
L-Cystine	0.51
Sodium thioglycollate	0.51
Resazurin sodium	0.001
Gelatin	60.00
Peptone	5.00
Dipotassium hydrogen phosphate	5.00
Iron (II) sulphate	0.50
Agar	0.755
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 70 grams of Part A in 500 ml purified / distilled water. Mix well and adjust the pH to 6.8. Sterilize by autoclaving at 15 lbs pressure (121°C) for 5 minutes. Cool to 45-50°C and aseptically add sterile rehydrated contents of one vial of Perfringens Selective Supplement (FD307).

Suspend 110 grams of Part B in 500 ml warm purified / distilled water. Heat to boiling to dissolve the medium completely. Adjust the pH to 7.1. Dispense 5 ml amount in screw-capped glass tubes. Sterilize by autoclaving at 15 lbs pressure (121° C) for 15 minutes. Cool to 45-50°C. Aseptically add 5 ml of previously cooled Part A solution to Part B. Mix well and store at 2-8°C. Before use, liquefy the medium by placinggthe tubes in a water bath 45-50°C for 30 minutes.

Principle And Interpretation

Rapid Perfringens Medium Base is formulated by Erickson & Deibel (1) for the detection *Clostridium perfringens* in food (8). The Mesophilic spore forming anaerobes belonging to the genus Clostridia of food concern are Gram-positive, catalase negative, rods of varying sizes.

The medium can be used to initiate growth from small inocula and to obtain the highest viable count of Clostridia. Rapid Perfringens Medium Base is a liquid medium with a litmus milk base and is prepared in tubes. Selectivity is provided by the antibiotics Polymyxin B sulfate and neomycin sulfate, coupled with an incubation temperature of 46°C (3). Tryptone, yeast extract peovides amino acids and other complex nitrogenous, carbonaceous substances and vitamin B complex Glucose is an energy source. Sodium chloride maintains the osmotic equilibrium. Dibasic potassium phosphate acts as a buffer to control pH. Whereas L-cystine, an amino acid, also serves as source of essential growth factors. Sodium thioglycollate and L-cystine lower the oxidation reduction potential of the medium by removing oxygen to maintain a low pH. Sodium thioglycollate also helps to neutralize the toxic effects of mercurial preservatives (6,7).

Litmus Milk cultures, enzymes of *Clostridium perfringens* attack the proteins and carbohydrates of the milk producing a stormy fermentation with clotting and gas formation (2).

Type of specimen

Food samples.

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (9).
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.Some strains 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

Part A : Pinkish purple to grey homogeneous free flowing powder Part B : Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light Brown coloured opaque solution in tubes.

Reaction

Reaction of 7.0% w/v of Part A + 11.0% w/v of Part B at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

Cultural characteristics observed in an anaerobic atmosphere after an incubation at 46°C for 48 hours.

Organism	Inoculum (CFU)	Growth
Clostridium perfringens ATCC 13124 (00007*)	50-100	good - luxuriant with stormy fermentation
Proteus mirabilis ATCC 25933	50-100	good - luxuriant

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. 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 (4,5).

Reference

1. Erickson,J.E. and Deibel,R.H.(1978)New medium for rapid screening and enumeration of *Clostridium perfringens* in foods. Appl.Environ.Microbiol.36,567-571.

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3. Handbook of Culture Media for Food and Water Microbiology,3rd edition. Edited by Janet E.L. Corry, Gordon D.W. Curtis and Rosamund M. Baird.

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5. 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.

6. Nungester, Hood and Warren, 1943, Proc. Soc. Exp. Biol. Med., 52: 287

7. Portwood, 1944, J. Bacteriol., 48: 255

8. Smith, M.and Mood,T.J.(1983)Direct testing of gelatin hydrolysis in rapid perfringens medium.J. Assoc.Off.Anal Chem.66,1045-1046.

9. 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.

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