Technical Information

Tryptose Sulphite Neomycin Agar

Product Code: DM 1634

Application: Tryptose Sulphite Neomycin Agar is used for selective isolation of *Clostridium perfringens* in foods or other specimens.

Composition**

Ingredients	Gms / Litre
Tryptose	15.000
Yeast extract	10.000
Sodium sulphite	1.000
Ferric citrate	0.500
Neomycin sulphate	0.050
Polymyxin B sulphate	0.020
Agar	13.500
Final pH (at 25°C) **Formula adjusted, standardized to suit performance parameters	7.2±0.2

Principle & Interpretation

Clostridium perfringens food poisoning is one of the comments types of human foodborne illness [1]. The foods usually involved are cooked meat or poultry containing large numbers of viable cells. A heat labile enterotoxin produced only by sporulation cells [2] induces the major symptoms of diarrhea in perfringens poisoning [6]. Tryptose Sulphite Neomycin Agar is a modification of Mossel Medium [3] developed by Marshall et al [4] for the selective isolation and enumeration of *C.perfringens* from food. Thioglycollate addition is recommended if the cultured medium is to be incubated anaerobically [4, 5].

Tryptose and yeast extract provide nitrogenous compounds, vitamin B complex and other growth nutrients. The antibiotics neomycin and polymyxin B sulphate inhibit gram-negative enteric bacilli. Neomycin is also lethal for *C.bifermentans.* The colonies of *C.perfringens* are black due to the ferric sulphide formed after the sulphite reduction. The high incubation temperature of 46°C renders the medium specific for *C.perfringens.* The presumptive black colonies of *C.perfringens* should be confirmed biochemically. The selectivity of the medium results in the inhibition of some strains of *C.perfringens* [6].

Methodology

Suspend 40.07 grams of powder media in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. Dispense in screw capped containers. Autoclave with caps loose at 118°C for 12 minutes. Close the caps while the medium is still hot. 5 ml of sterile buffered thioglycollate solution may be added to every 200 ml of medium if desired. The buffered aqueous thioglycollate solution contains 35 ml buffer mixture (5.7% dipotassium phosphate and 28% sodium carbonate) and 15 ml sodium thioglycollate solution (13.3%).

Quality Control

Physical Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.35% Agar gel.

Colour and Clarity of prepared medium
Medium amber coloured clear to slightly opalescent gel forms in Petri plates.

Reaction
Reaction of 4.0% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH Range:-
7.00-7.40

Cultural Response/Characteristics
DM 1634: Cultural characteristics observed under anaerobic conditions, after an incubation at 46°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Clostridium perfringens ATCC 12924	50-100	good-luxuriant	>=50%	black
Escherichia coli ATCC 25922	>=10^3	inhibited	0%	
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	0%	

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

1. Doyle M. P., (Ed.), 1989, Foodborne Practical Pathogens, Marell Dekker, New York, N. Y.
2. Dunean C. L., 1973, A. J. Bacteriol., 113: 932
3. Mossel, 1959, J. Sci. Food Agric., 10:662.
4. Marshall, Steenbergen and McClung, 1965, Appl. Microbiol., 13:559.
5. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
6. Vanderzant C. and Splittstoesser D. F., (Eds.), 1992, Compendium of Methods for the Microbiological Examination of Foods, 3rd Ed., APHA, Washington, D.C.

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