

Tryptose Serum Agar Base

Intended use

Recommended for routine cultivation and isolation of Mycoplasma mycoides clusterfrom clinical and non-clinical specimen.

Composition**

Ingredients	g / L
Tryptose	20.000
Sodium chloride	5.000
Disodium hydrogen phosphate	2.500
Agar	15.000

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 42.50 grams in 1000 ml purified / distilled water containing 5 ml glycerol. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add the rehydrated contents of one vial of PDTY Selective Supplement (FD334) and 108 ml of pig serum (RM10415) (Inactivate at 56°C for not more than 30 minutes). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Mycoplasma mycoides cluster comprises: M. mycoides subsp. capri; M. mycoides subsp. Mycoides small colony (SC); M. mycoides subsp. mycoides large colony (LC); bovine serogroup 7; M.capricolum subsp.capricolum (Mcc); and M. capricolum subsp. capripneumoniae (Mccp). All groups are pathogens and the principal hosts are cattle, goats or sheep (1). Tryptose serum agar was initially developed by Gourlay (2) for cultivatiYon of Mycoplasma mycoides var. mycoides. There are several reports that Mycoplasmas can be grown in Gourlay's broth or agar (1%) medium (modified Newings tryptose broth; Gourlay, 1964), containing thallous acetate 0.04% and ampicillin 0.4 mg/ml unless otherwise stated, at 37°C, in an atmosphere containing CO2 5%.(3). Other modifications of broth and agar media also support the growth of Mycoplasma such as VFG or WJB, Modified Friis, Hayflick media, PPLO media (4,5). The medium ingredients and all the supplements should be free of any toxic substances even in small amounts. Many Mycoplasma require serum for their good growth and also presence of antibiotic is necessary to prevent the growth of contaminating organisms. Mostly the Mycoplasma species are aerobic or facultatively anaerobic but some are microaerophilic. Few are anaerobic saprophytic Mycoplasma which grow best 22-35°C while 35°C. at pathogenic strains grow at Tryptose serum agar has tryptose which serves as a source of nitrogeneous and carbonaceous compounds, long chain amino acids, vitamins and other growth nutrients. Sodium chloride maintains osmotic balance. Dextrose serves as an energy source. Yeast extract provides vitamins especially Group B Vitamins. Glycerol serves as a carbon source. Disodium hydrogen phosphate are act as buffer salts. Penicillin G and thallium acetate inhibits contaminating flora. Pig serum provides good growth.

Type of specimen

Clinical samples - Sputum samples, Food and dairy samples; Water samples

Specimen Collection and Handling:

For clinical samples, follow appropriate techniques for sample collection and processing as per guidelines (6,7). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (8,9,10). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (11). 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.

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Limitations :

- 1. The medium ingredients and all the supplements should be free of any toxic substances even in small amounts.
- 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

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light amber coloured opalescent gel forms in Petri plates

Cultural Response

Cultural characteristics observed in presence of 10% Carbon dioxide with added Pig serum (RM10415), inactivated at 56°C for not more than 30 minutes and PDTY Selective Supplement (FD334), after an incubation at 22-35°C for 48 hours.

Organism	Growth
Mycoplasma mycoides var.mycoides	good-luxuriant
Mycoplasma capricolum subsp. capricolum	good-luxuriant
Mycoplasma capricolum subsp. capripneumoniae	good-luxuriant

Storage and Shelf Life

Store between 10-30°C in a tightly closed container. Use freshly prepared medium. 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

Reference

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