Hayflick Broth Base

ME1885

Hayflick Broth Base with added horse serum and penicillin is recommended for detection of mycoplasmas in pharmaceutical products, in vaccines, cell banks and virus cultures in accordance with European Pharmacopoeia.

Composition**

Ingredients	Gms / Litre
Beef Heart Infusion Broth	17.790
Yeast Extract	19.800
Deoxyribonucleic acid (DNA)	0.019
Phenol Red	0.0237
Final pH (at 25°C)	7.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 18.82 gms in 416 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes or as per validated cycle. Cool to 45-50°C. Add 5 ml of the rehydrated contents of 1 vial of Hayflick Supplement (FD300). Aseptically add unheated 79 ml Horse serum (RM1239) to the prepared medium. Mix well and dispense in sterile test tubes or as desired .

Principle And Interpretation

Mycoplasma, members of class Mollicutes represents a group of minute bacteria devoid of cellwalls (1). These are common and are responsible for causing serious contamination in cell and or tissue cultures used to generate compendial articles. They may also cause contamination of filtered sterilized Soyabean Casein Digest Broth. Infection of cells in a culture can affect nearly every pathway of cell metabolism including alteration of the cells phenotypical characterization and normal growth. The presence of mycoplasma species does not always result in turbid growth in cultures or visible alteration of the cells.

Hayflick et al have reported complex medias for growth of Mycoplasmas (2,3) . Testing of mycoplasmas is necessary to assure reliably pure biotech products and allied materials used to generate these products. Hayflick broth media (liquid) is recommended for general detection of Mycoplasmas in Pharmacopoeias (4,5) for testing of products for Mycoplasma. When testing for Mycoplasmas, at least two known Mycoplasma species or strains as positive controls, one of which should be dextrose fermenter (i.e. *M.pneumoniae* or equivalent species and strain) and one of which should be an arginine hydrolyzer (i.e. *M. orale* or equivalent species and strain) should be included in each test. Only when testing insect cell lines should one include a Spiroplasma control strain (e.g., *S.citri* ATCC 29747, *S. melliferum* ATCC 29416, or equivalent species and strains.) Additionally these strains may be a little more fastidious in their nutritional requirements. They require lower incubation temperatures (as do insect cell lines).

This medium contains Beef heart infusion broth containing beef heart infusion and peptone which provides nitrogen, vitamins, aminoacids and carbon sources. Sodium chloride maintains the osmotic balance. Many Mycoplasmas require serum for their good growth. Addition of Penicillin supress growth of unwanted flora. Phenol red in the medium indicates the growth of Mycoplasma on change of colour of medium from red to yellow or purple. Added Horse serum provides growth factors including lipid components to Mycoplasma . DNA provides additional nutrients to Mycoplasma .Yeast extract serves rich source of Nicotinamide- Adenine Dinucleotide (NAD) required by *M.synoviae* .

Mycoplasma species are either aerobic or facultative anaerobic but some are microaerophilic. Few are anaerobic saprophytic Mycoplasma which grow best at 22-35°C while pathogenic strains grow at 35°C. Tubes should be incubated in an atmosphere containing 5-10% carbon dioxide and examined after incubation of 48 hours but they should not be discarded as negative until after incubation for three weeks. *M. synoviae* is not able to grow on Hayflick broth medium because growth depends on

NAD. *M. hyorhinis* ATCC 29052 which is recommended as a fastidious strain for use in indicator cell method, is not able to grow on this medium.

A 10 ml of the product to be tested is inoculated in 100 ml of Hayflick Broth. The bottles are tightly closed and incubated for 20-21 days at 35-37°C. They are monitored every 2-3 days and are subcultured, if a colour change occurs. Subcultures are incubated for 7 days and microaerophilic conditions at 35-37°C. On days 2-4, 6-8, 13-15 and 19-21 after inoculation the liquid media are subcultured on atleast one plate of each type of Hayflick Agar (ME1886) and incubated for 7 days under microaerophilic conditions at 35-37°C.

In addition 0.2 ml of the product to be tested are inoculated directly onto each of the Hayflick Agar (ME1886) and incubated for not less than 14 days under microaerophilic conditions (5-10% CO2) and sufficient humidity at 35-37°C.

Positive and negative controls have to be performed. According to the recommendations of EP the solid media are viewed for typical mycoplasma colonies.

Quality Control

Appearance
Light yellow to pink coloured homogeneous free flowing powder

Colour and Clarity of prepared medium
Orange-pink coloured clear solution without any precipitate with added supplement (FD300) and Horse serum (RM1239) in tubes.

Reaction
Reaction of 3.76% w/v aqueous solution at 25°C. pH : 7.8±0.2

pH
7.60-8.00

Cultural Response
Cultural characteristics observed with added sterile supplement (FD300) and Horse serum (RM1239)in presence of 10% carbon dioxide (CO2) after an incubation at 35-37°C for upto 7 days.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery (on ME1886)
Cultural Response			
Mycoplasma gallisepticum ATCC 19610	50-100	good-luxuriant	>=70%
Mycoplasma orale ATCC 23714	50-100	good-luxuriant	>=70%
Mycoplasma pneumoniae ATCC 15531	50-100	good-luxuriant	>=70%

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2-8°C . Use before expiry date on the label.

Reference

1. Murray P.R., Baron J.H., Pfaller M.A.., Jorgensen J.H. and Yolken R.H., (Eds.) 2003, Manual of Clinical Microbiology , 8th ed., American Society for Microbiology ,Washington, D.C.
2. Hayflick and Chanock, 1965, Bacteriol.Rev., 29: 185.
3. Hayflick and Stanbridge, 1967,Ann. N.Y.Acd.Sci.,143;608.
4. Microbiological tests/<63>Mycoplasma Tests , United States Pharmacopoeia, 2011, The United States Pharmacopoeial Convention, RockVille,MD.
5. Mycoplasmas 2.6.7, European Pharmacopoeia 2011 , European Department for the Quality of Medicines.

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