PLET Agar Base, Modified

M1451

PLET Agar Base medium is recommended for the selective isolation and cultivation of *Bacillus anthracis* .

Composition**

Ingredients	Gms / Litre
Beef heart, infusion from	500.000
Tryptose	10.000
Sodium chloride	5.000
EDTA	0.350
Thallous acetate	0.040
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40.40 grams in 990 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C. Aseptically add rehydrated contents of 1 vial of Anthracis Selective Supplement (FD185). Mix well and dispense as desired.

Principle And Interpretation

Anthrax is an infectious disease caused by spores of the bacterium *Bacillus anthracis* .

In human anthrax, the bacillus is usually demonstrable in material from a malignant pustule, sometimes in sputum from pulmonary anthrax and also in the blood in the septicemic stage of all forms of the infections. Man is relatively resistant to anthrax and laboratory workers are rarely infected. However great care should be taken to avoid escape of the long surviving spores into laboratory environment and all the procedures should be carried out in safety cabinet. Anthrax cannot spread directly from human to human but anthrax spores can be transported by human clothings, shoes etc. In humans, anthrax is caused by exposure to dead infected animals, consumptions of infected animal tissue or exposure to light density anthrax spores from animal wool, fur, hide, etc.

PLET Agar Base originally formulated by Knisley (1) is the best selective medium for cultivation of *B. anthracis* (2, 3, 4) from suspected environmental specimens, animal products or clinical specimens, inhibiting *Bacillus cereus* .PLET Agar Base, Modified Medium is similar to base except rhat it contains increased concentration of EDTA, which helps in inhibiting *Staphylococcus aureus* . Beef heart infusion from solids and tryptose provide the carbonaceous and nitrogenous compounds necessary for growth whereas sodium chloride provides the osmotic equilibrium. Thallous acetate and Polymyxin (FD185) are inhibitory agents allowing growth of *B.anthracis* while inhibiting contaminants. Lysozyme (FD185) specifically suppresses the growth of gram-negative contaminants. The suspected specimen may be used directly for streaking or heat-treated or alcohol-treated specimens can be used for streaking. On incubation at 37°C for 24 hours colonies develop from 30-100% of the *B.anthracis* spores that would grow on non-selective Heart Infusion Agar (M169), being smaller and smoother than on the later medium. PLET Agar Base, Modified inhibits growth of most strains of *B.cereus, B. subtilis* , other *Bacillus* species, *Enterobacteriaceae* and *Pseudomonas* species. Some strains of *B.cereus* from soil form colonies but they are smaller than those of *B.anthracis* , minute after 24 hours and moderately sized after 48 hours. Colonies of *B.anthracis* appear in 36-40 hours after incubation at 37°C. Roughly circular, creamy- white colonies with a ground-glass texture are further subcultured on blood agar plates for identification. Capsule production can be seen directly or on blood agar plates (4).

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 clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 4.04% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH
7.10-7.50

Cultural Response
M1451: Cultural characteristics observed after an incubation at 35-37°C for 36-40 hours

Organism	Growth
Bacillus anthracis ATCC 14578	luxuriant
Bacillus cereus ATCC 10876	inhibited
Staphylococcus aureus ATCC 25923	inhibited

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.Knisely R.F. 1966, J. Bacteriol, 92:784-786.
2.Norris J. R., Berkley C.W., Logan N.A., and ODonnell A.G., 1981. In M. P. Starr et al (ed) The Prokaryotes : a Handbook on Habitats, Isolation and Identification of Bacteria, Vol. 2, Springer Verlag, Berlin.
3.Parry J.M., Turnbull P.C.B. and Gibson J.R., 1983, A Colour Atlas of Bacillus species. Wolfe Medical Publications, London, United Kingdom.
4.Murray et al, 1999, Manual of Clinical Microbiology, 7th Edition, ASM Press, Washington, D.C.

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