An Improved Medium for the Cultivation of the Eaton Agent

D. Herderschee

The Laboratory of Medical Microbiology, University of Amsterdam, The Netherlands


The composition of an improved medium for the cultivation of the Eaton Agent is described; growth obtained on this medium permits serum inhibition tests. Sera from patients with primary atypical pneumonia caused visible growth inhibition.

P.P.L.O. strains, in this laboratory, are usually typed by means of the growth inhibition method. The technique used is to allow a drop of P.P.L.O. antiserum to penetrate into a dried agar medium; then the P.P.L.O. strain is streaked across the area in which the antiserum was applied. If the P.P.L.O. strain is of the same type as the strain against which the antiserum was prepared, a zone of growth inhibition will in due time mark the area with the antiserum. Most P.P.L.O.'s give a layer of colonies sufficiently even and dense to permit observation of growth inhibition with the naked eye, or, in more difficult cases, with the microscope. With some strains, however, growth on the solid medium is so poor that growth inhibition can hardly be observed.

The Eaton agent which is the cause of primary atypical pneumonia (P.A.P.) in man is a P.P.L.O. (Chanock et al. 1962). However, it grows slowly and generally in smaller colonies than other P.P.L.O. strains.

We studied the growth inhibiting effect of sera of patients with P.A.P. on the Mac strain of the Eaton agent, isolated by M. D. Eaton et al. (1945). Dr. J. F. Ph. Hers, Leiden, supplied us with a subculture in chick embryo. As growth was rather poor, several alterations of the medium were tried.

According to Chanock et al. (1962) fresh, non-inactivated horse serum is beneficial to growth. We tested several methods for preparing the serum. The best results were obtained with a P.P.L.O. medium enriched with serum prepared as follows: horse blood was drained into a bottle with trypsin solution. Defibrination was faster than in the control bottle without trypsin. The serum was inactivated and added to the commonly used P.P.L.O. broth or to the agar medium. The plates inoculated with the Eaton agent were incubated anaerobically at 37 C for 10 days.

The largest colonies were obtained when an agar medium was inoculated
with a 10 days-old culture in a liquid medium and incubated for 10 days. Most colonies then showed the fried egg appearance. When we made subcultures on a fresh agar medium by streaking it with an agar block with large colonies, we saw, after about 10 days' incubation, a very dense growth of small colonies like a haze on the plate. On these plates growth inhibition became visible with the naked eye.

The serum of a patient with primary atypical pneumonia taken on the 12th day of illness caused growth inhibition with the Mac strain. In this case, the diagnosis was corroborated with the fluorescent antibody technique by Dr. Hers. The sera of four other patients with the clinical picture of this disease also showed growth inhibition on the 11th, 12th, 12th and 17th day of illness, respectively. Sera of two patients received on the 3rd and 4th day, respectively, gave negative results.

Yeast-trypsin-serum medium (Y.T.S.)

Basic broth: 500 g minced beef heart is boiled for 10 min in 1 liter of aqua dest. The broth is roughly filtered, 1% of proteose peptone added and the pH adjusted to 8. The broth is boiled once more for 10 min and filtered through paper. To 240 ml of the broth 24 g of sucrose is added, and 3 g of agar if a solid medium is desired. The broth, or the nutrient agar, is sterilized at 120 C for 20 min. After transferring the broth to a boiling water bath, 15 ml of sheep or horse blood is added and the mixture left at 100 C for 10 min. The broth, or the agar medium, is centrifuged and the supernatant cooled to 45 C; then 60 ml of the horse serum with yeast extract is added.

Serum: 400 ml horse blood is drained into a sterile 500 ml bottle which contains 7.5 ml of a 10% sterile trypsin solution, and glass beads. The bottle is shaken until fibrin clots become visible. In the laboratory 10 ml of yeast extract is added. The serum is separated by centrifugation, inactivated by keeping it at 56 C for 30 min, and used the same day.

Yeast-extract: 1000 g of active bakers yeast is mixed in 1000 ml of aqua dest. The mixture is heated to 80 C and the pH adjusted to 4.5 with 38% HCl. The mixture is kept at 80 C for 20 min. It is filtered, first through paper and then through a sterilizing glass filter.

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