# O Antisera

## Preparation:

An overnight slope culture Hartley's Digest Agar was washed into 5 ml. of saline filtered through cotton wool, washed, and resuspended in saline. This suspension was then autoclaved at 10 lbs for 20 minutes. It contained about 8400 x  $10^6$  organisms /ml.

## K Antiserum

### Preparation

Slope culture grown and washed of as before and resuspended in 5 cc saline. It was then treated with 25 cc methyl alcohol and shaken vigorously at intervals for 2 hours. The alcohol was then centrifuged off and the deposit washed twice in saline. The final suspension had a strength of  $3700 \times 10^6$  organisms /ml.

# H Antiserum

#### Preparation

A wellgrown, overnight broth culture was treated with formalin to make a 0.2% solution of this substance. It is important to use only highly motile strains for this work. Strength 4000 x 10<sup>6</sup> organisms /ml.

#### Injection Routine

The same routine was followed with all the above suspensions. At two day intervals the following doses were given:

0,25 ml. 0.5 ml. 0.5 ml. 1.0 ml. 1.5 ml.

The rabbits were then bled five days after the last injection.