1793: Chlamydia serpentis & poikilotherma Medium

MEM-Medium (+ Glutamine) 500.00 ml
Fetal bovine serum 100.00 ml
D-glucose solution 6.00 ml

1. Filter-sterilize (0.2 µm) and keep no longer than 4 weeks. Store at room temperature to facilitate detection of contamination.

2. Prepare a 25 cm² flask and seed cells according to standard protocols. Incubate at 37°C plus 5% CO₂. When a confluent layer has formed, infection can be carried out.

   Cycloheximid solution 0.70 ml

3. Exchange medium with Infection Medium (as above with the addition of cycloheximide) and add 500 - 1000 µl of EB stock solution (thawed quickly to 37°C).

4. Centrifuge for 1 h onto the cell layer at 1000 x g at >20°C.

5. Incubate at 37°C + 5% CO₂. Control cells daily and look for inclusions. Not all Chlamydiae form well-visible inclusions, ultimately, immunofluorescence or in situ-hybridization techniques are necessary to visualize inclusions.

D-glucose solution

   D-Glucose 3.00 g
   Demineralized water 50.00 ml

Filtrate.

Cycloheximid solution

   Cycloheximid 10.00 mg
   Demineralized water 10.00 ml

Filtrate.