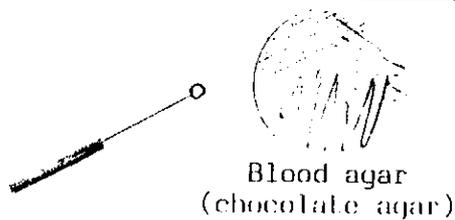


PRESERVATION OF CULTURES
 ~~~~~

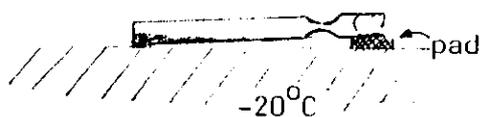
Pre-drying cultural conditions / Harvesting:



Incubation:  
 optimal atmosphere  
 suboptimal or  
 optimal temperature  
 Time: 16h ..... 4 days  
 as short as possible!

Suspending fluid\*  
 (glucose-serum-peptone)  
 (the old NCTC procedure)

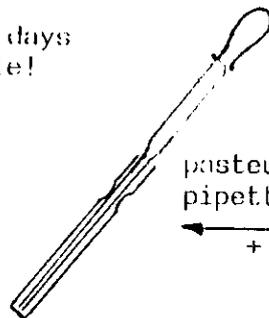
Filling and lyophilizing:



Lyophilized over night (18h)

Condenser temperature: -56°C  
 Final vacuum: 0,06 torr  
 Final temperature: +20°C  
 (Edwards freeze dryer  
 chamber type)

Transported to  
 freezer as soon as  
 possible



+ pen  
 mark

Ampoules for freeze drying  
 neutral glass, flat bottom  
 with constriction and with  
 breaking ring.  
 Outer diameter 8,65mm Height 110mm

Sealing: Ampoules are transferred to the manifold of a second lyophilizer and sealed (under vacuum) as soon as possible.

Labeling: Type-written labels are applicated. Collection number, identity if known, and batch-date are indicated.

Storage: At +4°C in the dark.

Quality control:

|              |                                   |                                         |
|--------------|-----------------------------------|-----------------------------------------|
| First batch  | : Minimum 4 amp<br>Maximum 20 amp | → "biochemistry"<br>→ viability, purity |
| Second batch | : Minimum 6 amp<br>Maximum 30 amp | → viability, purity, "biochemistry"     |
| Third batch  | : ?                               | → viability, purity, "biochemistry"     |
| Fourth batch | : ?                               | (prepared from the second batch)        |
| Fifth batch  | : ?                               | (prepared from the second batch)        |

We do our best to keep a few ampoules of old or original batches

\*Suspending fluid = Mist. desiccans (Fry and Greaves, 1951):

|                             |        |                  |
|-----------------------------|--------|------------------|
| Horse serum .....           | 300 ml | sterile-filtered |
| Glucose, pro analysis ..... | 50 g   |                  |
| 'Lab Lemco' Oxoid L29 ..... | 0,5 g  |                  |
| Bacto-peptone Difco 0018.   | 0,8 g  |                  |
| Water, high purity(SuperQ)  | 100 ml |                  |