140: RUMINICLOSTRIDIUM MEDIUM

KH$_2$PO$_4$ 0.50 g  
NaCl 1.00 g  
(NH$_4$)$_2$SO$_4$ 0.50 g  
MgSO$_4$ x 7 H$_2$O 0.10 g  
CaCl$_2$ x 2 H$_2$O 0.10 g  
K$_2$HPO$_4$ 0.50 g  
**Clarified rumen fluid** 300.00 ml  
**Sludge fluid**, alternative 300.00 ml  
Sodium resazurin (0.1% w/v) 0.50 ml  
Na$_2$CO$_3$ 2.50 g  
Cellobiose 5.00 g  
L-Cysteine HCl x H$_2$O 0.25 g  
Na$_2$S x 9 H$_2$O 0.25 g  
Distilled water 700.00 ml

Dissolve ingredients (except carbonate, cellobiose and reducing agents), bring medium to the boil, then cool to room temperature under 100% CO$_2$ gas atmosphere. Use either clarified rumen fluid or sludge fluid as supplement. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add cellobiose, sulfide and cysteine from sterile anoxic stock solutions prepared under 100% N$_2$ gas and carbonate from a sterile anoxic stock solution prepared under 80% N$_2$ and 20% CO$_2$ gas mixture. Cellobiose has to be sterilized by filtration. Adjust pH of complete medium to 6.8, if necessary.

**Sludge fluid** (from medium 119)

Yeast extract 4.00 g  
Sludge 1000.00 ml

Add 0.4% yeast extract to sludge from an anaerobic digester, and after gassing with nitrogen gas for a few minutes incubate it at 37°C for 24 hours. Then centrifuge the sludge at 13000 g and autoclave the resulting, clear supernatant in screw-capped vessels under nitrogen gas. The sludge fluid can be stored at 8-12°C in the dark.

**Clarified rumen fluid** (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N$_2$ gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.