275. TREPONEMA SUCCINIFACIENS MEDIUM

Solution A:
CaCl₂ x 2 H₂O 0.1 g
MgSO₄ x 7 H₂O 0.1 g
KH₂PO₄ 0.5 g
K₂HPO₄ 0.5 g
NaCl 1.0 g
Rumen fluid, clarified (see medium 1310) 300.0 ml
Yeast extract (OXOID) 0.5 g
Peptone (BD BACTO) 0.5 g
(NH₄)₂SO₄ 0.5 g
Na-resazurin solution (0.1% w/v) 0.5 ml
L-Cysteine-HCl x H₂O 0.5 g
Distilled water 600.0 ml

Solution B:
Glucose 10.0 g
Distilled water 50.0 ml

Solution C:
Na₂CO₃ 1.5 g
Distilled water 30.0 ml

Dissolve ingredients of solution A and sparge with 80% N₂ and 20% CO₂ gas atmosphere to make it anoxic. Then distribute medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solutions B is prepared under 100% N₂ gas atmosphere and autoclaved. Solution C is prepared under 80% N₂ and 20% CO₂ gas atmosphere and autoclaved. To complete the medium appropriate amounts of solutions B and C are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be at 7.4.