275. TREPONEMA SUCCINIFACIENS MEDIUM

**Solution A:**
- CaCl$_2$ x 2 H$_2$O: 0.1 g
- MgSO$_4$ x 7 H$_2$O: 0.1 g
- KH$_2$PO$_4$: 0.5 g
- K$_2$HPO$_4$: 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
- (NH$_4$)$_2$SO$_4$: 0.5 g
- Na-resazurin solution (0.1% w/v): 0.5 ml
- L-Cysteine-HCl x H$_2$O: 0.5 g
- Distilled water: 600.0 ml

**Solution B:**
- Glucose: 10.0 g
- Distilled water: 50.0 ml

**Solution C:**
- 1 M K-phosphate buffer, pH 7.4: 20.0 ml
Combine 16.04 ml of 1 M K$_2$HPO$_4$ with 3.96 ml of 1 M KH$_2$PO$_4$ stock solution to reach a pH of around 7.4.

**Solution D:**
- Na$_2$CO$_3$: 1.5 g
- Distilled water: 30.0 ml

Dissolve ingredients of solution A, adjust pH to 6.2 – 6.3 and sparge with 100% N$_2$ 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 and C are prepared under 100% N$_2$ gas atmosphere and autoclaved. Solution D is prepared under 80% N$_2$ and 20% CO$_2$ gas atmosphere and autoclaved. To complete the medium appropriate amounts of solutions B to D are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be at 7.4.