169. TREPONEMA ZUELZERAE MEDIUM

**Solution A:**
- CaCl$_2$ x 2 H$_2$O: 0.04 g
- MgSO$_4$ x 7 H$_2$O: 0.50 g
- Yeast extract (OXOID): 4.00 g
- D-Glucose: 2.00 g
- Na-resazurin solution (0.1% w/v): 0.50 ml
- L-Cysteine-HCl x H$_2$O: 0.50 g
- Distilled water: 960.00 ml

**Solution B:**
- NaHCO$_3$: 1.00 g
- Distilled water: 20.00 ml

**Solution C:**
- 0.5 M K-phosphate buffer, pH 7.4: 20.00 ml

Combine 16.04 ml of 0.5 M K$_2$HPO$_4$ with 3.96 ml of 0.5 M KH$_2$PO$_4$ stock solution to reach a pH of around 7.4.

Dissolve ingredients of solution A (except cysteine) and sparge medium with 100% N$_2$ gas for 30 – 45 min to make it anoxic. Add cysteine and adjust pH 7.2 with KOH, then distribute under the same gas atmosphere in anoxic Hungate-type tubes or serum vials and autoclave. Solution B is autoclaved separately under 80% N$_2$ and 20% CO$_2$ gas atmosphere and solution C is autoclaved under 100% N$_2$ gas atmosphere. 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 complete medium should be 7.5. For solid medium add 10.00 g/l agar (BD Bacto) to solution A.