**509a. SPIROCHAETA RI 19.B1 MEDIUM**

**Solution A:**
- NaCl 4.00 g
- MgCl₂ x 6 H₂O 0.80 g
- KCl 0.50 g
- NH₄Cl 0.30 g
- KH₂PO₄ 0.20 g
- CaCl₂ x 2 H₂O 0.03 g
- Selenite-tungstate solution (see medium 385) 1.00 ml
- Na-resazurin solution (0.1% w/v) 0.50 ml
- Distilled water 950.00 ml

**Solution B:**
- Na₂CO₃ 1.00 g
- Distilled water 20.00 ml

**Solution C:**
- Trace element solution SL-10 (see medium 320) 1.00 ml

**Solution D:**
- Vitamins solution (see medium 141) 10.00 ml

**Solution E:**
- Starch 1.00 g
- Distilled water 10.00 ml

**Solution F:**
- Na₂S x 9 H₂O 0.30 g
- Distilled water 10.00 ml

*Solution A* is sparged with 80% N₂ and 20% CO₂ gas mixture to reach a pH below 6 (at least 30-45 min), then distributed under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclaved. *Solution B* is autoclaved separately under 80% N₂ and 20% CO₂ gas atmosphere. *Solutions C, E and F* are autoclaved under 100% N₂ gas atmosphere. *Solution D* is prepared under 100% N₂ gas atmosphere and sterilized by filtration. To complete the medium appropriate amounts of *solutions B to F* are added to the sterile *solution A* in the sequence as indicated. Final pH of the medium should be 6.8 – 7.0.