

## Main sol. 509a

<b>Solution A</b>	962.00	ml
<b>Solution B</b>	20.00	ml
<b>Solution C</b>	1.00	ml
<b>Solution D</b>	10.00	ml
<b>Solution E</b>	10.00	ml

Solution A is sparged with 80% N<sub>2</sub> and 20% CO<sub>2</sub> 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% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. Solutions C, E and F are autoclaved under 100% N<sub>2</sub> gas atmosphere. Solution D is prepared under 100% N<sub>2</sub> 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.