

## Main sol. 861

<b>Solution A</b>	952.00	ml
<b>Solution B</b>	30.00	ml
<b>Solution C</b>	10.00	ml
<b>Solution D</b>	1.00	ml
<b>Solution E</b>	10.00	ml
<b>Solution F</b>	1.00	ml

1. 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 dispensed under the same gas atmosphere into serum vials (use at least bottles of 50 ml volume filled with 30 ml medium) and autoclaved. Solutions B and F are prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere and sterilized. Sterile solutions C, D and E are prepared under 100% N<sub>2</sub> gas. Solutions D and F are 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 7.0 - 7.2.

2. Note: For transfer of cultures use at least 10% (v/v) inoculum.