275: TREPONEMA SUCCINIFACIENS MEDIUM

<table>
<thead>
<tr>
<th>Solution</th>
<th>Volume (ml)</th>
<th>Concentration (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution A</td>
<td>920.00</td>
<td></td>
</tr>
<tr>
<td>Solution B</td>
<td>50.00</td>
<td></td>
</tr>
<tr>
<td>Solution C</td>
<td>30.00</td>
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</tbody>
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Dissolve ingredients of solution A and sparge with 80% N\textsubscript{2} and 20% CO\textsubscript{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 is prepared under 100% N\textsubscript{2} gas atmosphere and autoclaved. Solution C is prepared under 80% N\textsubscript{2} and 20% CO\textsubscript{2} gas atmosphere and autoclaved. 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 medium should be at 7.4.

**Solution A**

- CaCl\textsubscript{2} x 2 H\textsubscript{2}O: 0.10 g
- MgSO\textsubscript{4} x 7 H\textsubscript{2}O: 0.10 g
- KH\textsubscript{2}PO\textsubscript{4}: 0.50 g
- K\textsubscript{2}HPO\textsubscript{4}: 0.50 g
- NaCl: 1.00 g
- **Clarified rumen fluid**: 300.00 ml
- Yeast extract (OXOID): 0.50 g
- Peptone (BD BACTO): 0.50 g
- (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}: 0.50 g
- Sodium resazurin (0.1% w/v): 0.50 ml
- L-Cysteine HCl x H\textsubscript{2}O: 0.50 g
- Distilled water: 620.00 ml

**Solution B**

- Glucose: 10.00 g
- Distilled water: 50.00 ml

**Solution C**

- Na\textsubscript{2}CO\textsubscript{3}: 1.50 g
- Distilled water: 30.00 ml

**Clarified rumen fluid** (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g.
for 20 min. The supernatant is made anoxic by sparging with 100% N₂ gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.