347: SYNTROPHOMONAS SAPOVORANS MEDIUM

<table>
<thead>
<tr>
<th>Solution</th>
<th>Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>921.00</td>
</tr>
<tr>
<td>B</td>
<td>30.00</td>
</tr>
<tr>
<td>C</td>
<td>1.00</td>
</tr>
<tr>
<td>D</td>
<td>25.00</td>
</tr>
<tr>
<td>E</td>
<td>10.00</td>
</tr>
<tr>
<td>F</td>
<td>10.00</td>
</tr>
<tr>
<td>G</td>
<td>10.00</td>
</tr>
</tbody>
</table>

Dissolve ingredients of solution A, adjust pH to 7.0, then sparge medium with 80% N\textsubscript{2} and 20% CO\textsubscript{2} gas mixture for 30 - 45 min to make it anoxic. Dispense under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solution B is filter-sterilized and then equilibrated with 80% N\textsubscript{2} and 20% CO\textsubscript{2} gas mixture for at least 15 min. Solution C is prepared under 100% N\textsubscript{2} gas and sterilized by filtration. Solutions D, E, F and G are autoclaved under 100% N\textsubscript{2} gas. To complete the medium, appropriate amounts of the solutions B to G are added to solution A in the sequence indicated. Adjust pH of complete medium to 7.2, if necessary.

**Solution A**

- KH\textsubscript{2}PO\textsubscript{4} 0.50 g
- MgCl\textsubscript{2} x 6 H\textsubscript{2}O 0.33 g
- NaCl 0.40 g
- NH\textsubscript{4}Cl 0.40 g
- CaCl\textsubscript{2} x 2 H\textsubscript{2}O 0.05 g

**Trace element solution SL-10**

- Clarified rumen fluid 50.00 ml
- Trypticase peptone (BD BBL) 1.00 g
- PIPES (SIGMA) 15.00 g
- Sodium resazurin (0.1% w/v) 0.50 ml
- Distilled water 870.00 ml

**Solution B**

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

**Solution C**

- Seven vitamins solution 1.00 ml

**Solution D**

- Na-laurate 2.78 g
Distilled water 25.00 ml

**Solution E**

CaCl$_2$ x 2 H$_2$O 1.84 g
Distilled water 10.00 ml

**Solution F**

L-Cysteine HCl x H$_2$O 0.30 g
Distilled water 10.00 ml

**Solution G**

Na$_2$S x 9 H$_2$O 0.30 g
Distilled water 10.00 ml

**Trace element solution SL-10** *(from medium 320)*

HCl (25%) 10.00 ml
FeCl$_2$ x 4 H$_2$O 1.50 g
ZnCl$_2$ 70.00 mg
MnCl$_2$ x 4 H$_2$O 100.00 mg
H$_3$BO$_3$ 6.00 mg
CoCl$_2$ x 6 H$_2$O 190.00 mg
CuCl$_2$ x 2 H$_2$O 2.00 mg
NiCl$_2$ x 6 H$_2$O 24.00 mg
Na$_2$MoO$_4$ x 2 H$_2$O 36.00 mg
Distilled water 990.00 ml

First dissolve FeCl$_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 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% N$_2$ gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.

**Seven vitamins solution** *(from medium 503)*

Vitamin B$_{12}$ 100.00 mg
p-Aminobenzoic acid 80.00 mg
D-(-)-biotin 20.00 mg
Nicotinic acid 200.00 mg
<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium pantothenate</td>
<td>100.00</td>
<td>mg</td>
</tr>
<tr>
<td>Pyridoxine hydrochloride</td>
<td>300.00</td>
<td>mg</td>
</tr>
<tr>
<td>Thiamine-HCl x 2 H₂O</td>
<td>200.00</td>
<td>mg</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000.00</td>
<td>ml</td>
</tr>
</tbody>
</table>