

**765. ISOSPHERA PALLIDA MEDIUM****Solution A:**

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1.000	g
KH <sub>2</sub> PO <sub>4</sub>	0.300	g
MgSO <sub>2</sub> x 7 H <sub>2</sub> O	2.000	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.200	g
KCl	4.000	g
NaCl	1.000	g
FeCl <sub>3</sub>	0.292	mg
Glucose	0.250	g
Casamino acids	0.250	g
Vitamin solution	1.000	ml
Trace element solution SL-7a (see below)	1.000	ml
Distilled water	1000.000	ml

*Trace element solution SL-7a:*

HCl (25%)	1.000	ml
MnCl <sub>2</sub> x 4 H <sub>2</sub> O	100.000	mg
H <sub>3</sub> BO <sub>3</sub>	60.000	mg
ZnCl <sub>2</sub>	70.000	mg
CoCl <sub>2</sub> x 6 H <sub>2</sub> O	200.000	mg
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	20.000	mg
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	40.000	mg
CuCl <sub>2</sub> x 2 H <sub>2</sub> O	20.000	mg
Distilled water	999.000	ml

**Solution B:**

NaHCO <sub>3</sub>	2.1	g
Distilled water	50.0	ml

Fill into 100 ml serum bottle, gas with CO<sub>2</sub>, seal as for anaerobic work and autoclave. Cool before adding to the final medium.

*Vitamin solution:*

Vitamin B <sub>12</sub>	0.1	mg
Nicotinic acid	20.0	mg
Thiamine-HCl x 2 H <sub>2</sub> O	10.0	mg
p-Aminobenzoic acid	0.2	mg
Distilled water	100.0	ml

Filter sterilise.

Prepare the medium by mixing 250 ml of solution A (without vitamins added) with 650 ml distilled water. Gas with air:CO<sub>2</sub> (95:5 v/v), and fill the medium into serum bottles so that the gas to liquid ratio is about 5:1 (v/v). Gas the serum bottles with air:CO<sub>2</sub> (95:5 v/v), seal the bottles as for anaerobic work and autoclave. To the cooled medium add (using a syringe) 10ml NaHCO<sub>3</sub>/ 90ml medium and 0.5ml sterile vitamin solution to 100ml of the resulting medium. Final pH 7.9.