



APPENDIX A

สำนักหอสมุด

Leptospire culture medium

1. EMJH basal medium

1.1 Liquid medium: This medium consisted of 2.3 g of EMJH (Difco, USA) and 1 ml of glycerol (Univar, USA) in a total volume of 900 ml of distilled water (DW).

The EMJH powder was dissolved in 800 ml of DW. One milliliter of glycerol was added to the solution, and the pH was adjusted to 7.4. Distilled water was adjusted to make 900 ml. The medium was then sterilized by autoclaving at 15 psi, 121°C for 15 min. The medium could be kept at 4°C for 6 months.

1.2 Semisolid medium: This medium consisted of 2.3 g of EMJH, 1 ml of glycerol and 1.8 g of Bacto agar (Oxoid, England) in a final volume of 900 ml.

EMJH powder was dissolved in 800 ml of DW. One milliliter of glycerol was then added to the solution. The pH and the volume of the medium were adjusted as described above. The Bacto agar was added to the medium and dissolved by heating and stirring. The medium was sterilized by autoclaving as described above.

2. Supplement solution (100 ml)

Supplement solution consisted of 10.0 g of bovine serum albumin (BSA; Fraction V; Sigma Chemical Co, St Louis, Minnesota, USA), 0.1 g of sodium acetate (Sigma Chemical Co) and 0.2 g of sodium pyruvate (Sigma Chemical Co) in a total volume of 50 ml of DW and mixed the minor ingredients as described below in a final volume of 26.6 ml.

Bovine serum albumin, sodium acetate and sodium pyruvate were dissolved in 50 ml of DW. The following stock solutions were added slowly to the solution while stirring:

0.4% ZnSO ₄ •7H ₂ O	1.0	ml
1.0% CaCl ₂ •2H ₂ O	0.5	ml
1.0% MgCl ₂ •6H ₂ O	0.5	ml
0.5% FeSO ₄ •7H ₂ O	10.0	ml
0.3% CuSO ₄ •5H ₂ O	0.1	ml
0.1% MnSO ₄ •4H ₂ O	1.0	ml
10% Tween 80	12.5	ml
0.02% Vitamin B12	1.0	ml

(All ingredients were from Sigma Chemical Co)

The solution was then adjusted to pH 7.4 and the final volume was made up to 100 ml. This solution was sterilized by filtered through a 0.22 μm Millipore membrane. Supplement solution was aliquoted and kept at 4°C with light protection.

3. Complete EMJH medium

The complete EMJH medium was consisted of one volume of the supplement solution to 9 volumes of either the liquid EMJH medium or melted semisolid EMJH medium.

