**EMJH medium recipe for *Leptospira* spp. *in vitro* culture**

**A. Stock solutions for EMJH:**

**Zinc sulfate (ZnSO4.7H2O) – Sigma cat#Z0251**

0.4 g in 100 ml of autoclaved ultrapure water. Filter 0.22 µm and store at 4 °C.

**Calcium chloride (CaCl2.2H2O) – Fisher cat#C-79**

1. g in 100 ml of autoclaved ultrapure water. Filter 0.22 µm and store at 4 °C.

**Magnesium chloride (MgCl2.6H2O) – Fisher cat#BP214**

1.0 g in 100 ml of autoclaved ultrapure water. Filter 0.22 µm and store at 4 °C.

**Thiamine chloride – Sigma cat#T1270**

0.5 g in 100 ml of autoclaved ultrapure water. Filter 0.22 µm and store at 4 °C.

**Vitamin B12 – Sigma cat#V-6629**

0.02 g in 100 ml of autoclaved ultrapure water. Filter 0.22 µm and store at 4 °C.

**Manganese sulfate (MnSO4.H2O) – Sigma cat#8179**

0.36 g in 100 ml of autoclaved ultrapure water. Filter 0.22 µm and store at 4 °C.

**Ferrous sulfate (FeSO4.7H2O) – Sigma cat#F-7002**

0.5 g in 100 ml of autoclaved ultrapure water. Prepare this solution the day supplement is prepared. Filter 0.22 µm.

**Glycerol 10% solution – Fisher cat#BP229**

10 ml in 100 ml total of autoclaved ultrapure water. Filter 0.22 µm and store at 4 °C.

**Ammonium chloride (NH3Cl) 25% solution – Sigma cat#A-5666**

25 g in 100 ml of autoclaved ultrapure water. Filter 0.22 µm and store at 4 °C.

**Tween 80 – Sigma cat#P-4780**

20 ml in 180 ml ultrapure water. Filter 0.22 and store at 4 °C.

**5-Fluorouracil Stock Solution (5-FU) – Sigma cat#F6627**

Dissolve 1 g of 5-FU in 80 ml of filtered basal media (see below) at 37 °C for at least 1 hour. Adjust pH to 7.4 using 1N HCl (filtered) and add sterile basal medium to a final volume of 100 ml. Filter 0.22 µm and store at 4 °C. Final concentration will be 10 µg/ml.

**Superoxide dismutase – Sigma cat#S5395-75KU**

Dissolve 10 mg/ml in PBS. Filter 0.22 µm. Add 100 µL (0.001 g) to 100 ml of supplement.

B. Preparation of EMJH Supplement

1. Add 100 g Bovine Albumin Fraction V (Millipore Probumin® Universal Grade cat# 810037) slowly to 500 ml of distilled water. Stir slowly to avoid the formation of foam. Alternatively, add the BSA to water and hold at 4 °C overnight. Stir slowly while solution is warming to room temperature.

*Note: The quality of the albumin used for the cultivation of leptospires is critical. Please verify that the albumin used for DMC implantation supports the growth of virulent leptospires under standard in vitro growth conditions. Other sources of BSA were tested (e.g., Millipore Probumin*® *Vaccine Grade cat# 840644 and Sigma-Aldrich cat#A-9647) with similar results.*

1. When the BSA is fully dissolved, add the following stock solutions slowly, in order, while stirring. Wait at least 10 min before adding each reagent. Continue stir for one hour.

Thiamine chloride 10 ml

Calcium chloride 10 ml

Magnesium chloride 10 ml

Zinc sulfate 10 ml

Manganese sulfate 1 ml

Ferrous sulfate 100 ml

Vitamin B12 10 ml

Tween 80 (for EMJH) 125 ml

1. Adjust pH to 7.4 with 10% NaOH (filtered).
2. Adjust volume to one liter.
3. Filter through a 0.22 µm Millipore Stericup® filter unit and dispense aseptically in 50 ml conical tubes aliquots. Store at -20°C.

C. Preparation of EMJH Basal Medium

1. Before start, autoclave 1 L ultrapure water in a 2 L beaker, with a stir bar. Wait to cool down at room temperature (can be overnight in the biosafety cabinet).
2. Weight and add to the 1 L autoclaved water:

Disodium phosphate (Na2HPO4) – Sigma cat#RES20908-A702X 1.0 g

Monopotassium phosphate (KH2PO4) – Sigma cat#P5655 0.3 g

Sodium Chloride (NaCl) - Sigma cat#746398 1.0 g

1. Add the following filtered stock solutions:

Glycerol 10% 1.0 ml

Ammonium chloride 25% 1.0 ml

1. Stir ingredients until fully dissolved.
2. Adjust pH to 7.4 using 10% NaOH (filtered)
3. If not used immediately, filter through a 0.22 µm Millipore Stericup® filter unit and store at 4 °C. Can be used immediately for complete medium preparation as follows.

D. Preparation of Complete EMJH Medium

1. Autoclave 1 empty 200ml beaker with a stir bar inside.
2. Thaw 100 ml of supplement and 10 ml of normal rabbit sera (NRS) for each final 1 L of complete EMJH. Transfer supplement to the autoclaved beaker.
3. Add the following:

Lactalbumin hydrolase (BD cat#259962) 1 g

Sodium pyruvate (Sigma cat# P2256) 0.04 g

Superoxide dismutase 0.001 g (or add 100 uL of stock solution)

1. Add 10 ml NRS and 10 ml of 5-FU stock solution to the 100 ml supplement.
2. Combine 880 ml Basal EMJH medium and all 120 ml of supplement + NRS + 5-FU.
3. Filter through a 0.22 µm Millipore Stericup® filter unit.
4. Store at 4 °C.