BD Difco™ Leptospira Medium Base EMJH
Difco™ Leptospira Enrichment EMJH

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INTENDED USE

Leptospira Medium Base EMJH is used with Leptospira Enrichment EMJH in cultivating *Leptospira*.

SUMMARY AND EXPLANATION

In 1816, Adolf Weil described the first recognized leptospiral infections in humans.[1] These cases were caused by *Leptospira icterohaemorrhagiae* and the disease was subsequently named Weil's Disease.[1] Leptospirosis is a zoonotic disease, having its reservoir in wild, domestic, and peridomestic animals. Infection usually results from direct or indirect exposure to the urine of leptospiruric animals.[2]

Indirect exposure through contaminated water and soil accounts for most sporadic cases. Direct exposure occurs in pet owners, veterinarians and persons working with livestock.[3]

The basal medium and enrichment are prepared according to the formulations described by Ellinghausen and McCullough[4] as modified by Johnson and Harris.[5] They modified the formula by replacing rabbit serum medium with polysorbate 80-albumin. Leptospira Medium EMJH was used in cultivation studies of *Leptospira*.[6]

Leptospira Medium EMJH is recommended for the clinical isolation of *Leptospira*.[7,8]

PRINCIPLES OF THE PROCEDURE

Leptospira Medium Base EMJH contains ammonium chloride, a nitrogen source, and thiamine, a growth factor. Sodium phosphate dibasic and potassium phosphate monobasic are buffering agents. Sodium chloride maintains the osmotic balance of this formula.

Leptospira Enrichment EMJH contains albumin, polysorbate 80 and additional growth factors for *Leptospira*.

REAGENTS

Leptospira Medium Base EMJH

Approximate Formula* Per Liter

Disodium Phosphate	1.0	g
Monopotassium Phosphate	0.3	g
Sodium Chloride	1.0	g
Ammonium Chloride	0.25	g
Thiamine	0.005	g

Final pH 7.5 ± 0.2

*Adjusted and /or supplemented as required to meet performance criteria.

Leptospira Enrichment EMJH

A solution of albumin, polysorbate 80 and additional growth factors for *Leptospira*.

Warnings and Precautions

For Laboratory Use

Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage Instructions: On receipt, store Leptospira Enrichment EMJH at 2 – 8°C. Avoid freezing and overheating. Do not open until ready to use.

Product Deterioration: Do not use bottles if they show evidence of microbial contamination, discoloration, or other signs of deterioration. The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

SPECIMEN COLLECTION AND HANDLING

Obtain and process specimens according to the techniques and procedures established by laboratory policy. Blood, cerebrospinal fluid (CSF) and urine are the specimens of choice for the recovery of leptospires from patients with leptospirosis.[3,5]

Observe established precautions against microbiological hazards throughout all procedures. All specimens should be handled according to CDC-NIH recommendations for any potentially infectious human serum, blood or other body fluids. Prior to discarding, sterilize specimen containers and other contaminated materials by autoclaving.

PROCEDURE

Materials Provided: Leptospira Enrichment EMJH

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

Culture Procedures[7]

Blood and Spinal Fluid

Freshly drawn blood is preferable; otherwise, use blood taken with SPS, sodium oxalate or heparin.

1. Inoculate four 5 mL tubes of Leptospira Medium EMJH with 1 – 2 drops of fluid per tube.
2. Incubate in the dark at 28 – 30°C or at room temperature.

Urine

A total of 12 tubes will be inoculated for each urine specimen.

1. Prepare 1:10 and 1:100 dilutions of urine using Leptospira Medium EMJH to dilute potential inhibitory substances.
2. Inoculate two 5 mL tubes each of Leptospira Medium EMJH with:
   Urine undiluted, 1 drop per tube;
   Urine diluted 1:10, 1 drop per tube;
   Urine diluted 1:100, 1 drop per tube.
3. Duplicate the above inoculations using medium containing 200 µg/mL 5- fluorouracil to inhibit contaminants.
4. Incubate the tubes in the dark at 28 – 30°C or at room temperature.

User Quality Control:

1. Examine bottles for signs of deterioration.
2. Prepare the complete Leptospira Medium EMJH per label directions. Check performance by inoculating a representative sample of bottles with pure cultures of stable control organisms that give known, desired reactions. The test strains listed below are recommended.
3. Inoculate tubes with 1 drop of an undiluted *Leptospira* culture. Incubate at 30 ± 2°C for up to 7 days.

Test Strain	**Expected Results**
Leptospira interrogans serotype australis ATCC™ 23605	Growth
Leptospira interrogans serotype canicola ATCC 23470	Growth
Leptospira kirschneri ATCC 23604	Growth

RESULTS[3,7]

1. Examine tubes weekly for signs of growth (turbidity, haze, or a ring of growth).
2. Examine tubes microscopically each week. Take a small drop from a few millimeters below the surface, and examine it with dark-field illumination. Use 400X magnification.
3. Leptospires will be seen as tightly coiled spirochetes about 1 µm wide and 6 – 20 µm long. Leptospires rotate rapidly on their long axes and usually have hooked ends.
4. If the specimen is positive, subculture about 0.5 mL taken from the area of growth to two tubes of fresh medium.

LIMITATIONS OF THE PROCEDURE

Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

AVAILABILITY

Cat. No.	**Description**
279510	**Difco™** Leptospira Enrichment EMJH

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