Leishmania Diagnostics Laboratory
Diagnostics and Countermeasures Branch
Center of Infectious Disease Research
Walter Reed Army Institute of Research
503 Robert Grant Avenue, Silver Spring, MD 20910
Phone (240)595-7353 – Fax (301) 319-9997

Bone marrow Procedures

1. Criteria for Bone marrow aspiration

a. Visceral Leishmaniasis (VL) should be considered if the patient presents with a history of prolonged fever (2 weeks or more) associated with clinical hepatosplenomegaly, cytopenias or wasting (weight loss). There should be an associated history of prior travel (even remote in time) to an endemic region or blood transfusion.

b. For VL, bone marrow aspiration/biopsy (BM) is an excellent tissue specimen. Although the diagnostic sensitivity is typically higher for splenic aspirates than for specimens from other organs/tissues such as lymph node, liver, bone marrow, splenic aspiration can be associated with life-threatening hemorrhage, even if conducted under radiologic guidance; thus, bone marrow is preferred. WRAIR *Leishmania* Diagnostics Laboratory (LDL) accepts BM specimens for culture and for molecular testing.

c. Please consult with LDL Clinical Consultant (usarmy.detrick.medcom-wrair.mbx.leishmania-diagnostic@health.mil) before performing the procedure. Performing an initial VL rapid serologic test (Kalazar Detect™) should be considered.

2. Bone Marrow (BM) procedure

a. Obtain BM aspiration (and core biopsy if histopathology is planned) in accordance with your standard medical procedures.

b. For BM aspirate material, do the following:

i. For histopathology, make several smears of aspirate material on slides as you would for routine blood smear analysis.

ii. For culture, under aseptic conditions put approximately 0.5 to 1 ml of aspirate material into the LDL provided transport culture media (refer to Sections 4.c. and 6. below). Keep at culture at ambient (room) temperature; ship specimens by priority overnight, express courier for arrival on a weekday within 24 hours of collection of specimen (refer to # 4. a.-d. below)

Note: Please request media from the LDL prior to performing the BM procedure, see Section 4 for contact information; Section 6 to request a Specimen Collection Kit.

iii. For molecular tests (*Leishmania* Genus PCR), place 0.2mL of BM aspirate material into a leak-proof cryovial prefilled with 50ul of Alcohol (70-100% Ethanol, Methanol or Isopropanol).

c. If a core biopsy is obtained, divide aseptically, the collected core biopsy into three parts.

i. For *Leishmania* PCR, place the first portion (1/3) in a leak-proof vial in a small amount of alcohol (70-100% ethanol, methanol or isopropanol); use just enough alcohol to cover the specimen

ii. For culture, under aseptic conditions, place the second portion of the core biospy material into RPMI or LDL-provided media. Keep at culture medial at ambient (room) temperature; ship specimens by priority overnight, express courier for arrival on a weekday within 24 hours of collection of specimen (refer to # 4. a.-d. below)

iii. For Histology, use the remaining 1/3 portion of the core biopsy material to perform touch prep smears and for tissue examination (FFPE)

1) For smears, gently press the blotted surface of the core biopsy with a rolling or circular motion onto a glass microscope slide. Repeat in parallel rows down the slide.

2) Additionally, post touch prep, the core biopsy tissue can be studied locally per your institutional procedures by placing it in 10% formalin followed by paraffin block embedding.

3. Submission of Bone Marrow Specimens

a. Send the aspirates/core biopsy culture material, smears and vials with tissue in alcohol for *Leishmania* PCR and/or in media for culture directly to LDL at ambient temperature as directed in # 4.a-d.

b. Label the specimen legibly with the following information to prevent delay in testing:

- Patient name

- Unique identification number
- Date of birth; or barcode
- Date of collection/draw date
- Tissue type (bone marrow aspirate or bone marrow core biopsy tissue)

c. Wrap the primary specimen container in absorbent packing material.

d. Place the specimen tube in secondary leak-proof packaging.

e. Place the secondary package in an outer container approved for shipment of UN3373 Category Biological Substance Category B diagnostics specimens.

f. Include the WRAIR LDL test request form (CONUS or OCONUS) with patient's name, date of birth, brief clinical history, and travel history; specimen collection date; and test(s) requested.

g. Label the shipping container "Clinical Specimen" on the outside of the package.

h. Include the following information: submitter's name, address, phone number, fax number, and e-mail address.

i. Ship at ambient temperature by overnight carrier.

4. Shipment of Specimens

a. Send specimens and copies of the Leishmaniasis Test Request Form via Federal Express courier to the address below. Label as UN3373 Biological Category B diagnostic specimens.

b. POC: Laboratory Director, LDL at COM: 240-595-7353 (24 hours Emergency Number); Office: 301-319-2297; Cell: 240-406-6510; email: usarmy.detrick.medcom-wrair.mbx.leishmania-diagnostic@health.mil

c. Alternate POC: Associate Laboratory Director, LDL at cell: 301-661-2667, Office: 301-319-3512; email: usarmy.detrick.medcom-wrair.mbx.leishmania-diagnostic@health.mil

d. Shipping Address

Diagnostics and Countermeasures Branch
Walter Reed Army Institute of Research
ATTN: Leishmania Diagnostics Laboratory (LDL)
508 Research Drive, Silver Spring, MD 20910

5. Turn Around Time (TAT)

TAT for a histopath smear (Giemsa) is 24 hours; TAT for RT-PCR, and rK39 report is 24-48 hours, unless specimens are received on Friday. Culture results with speciation by Acetate Electrophoresis (CAE) assay may take up to 28 days for culture; 2 days for CAE. The Associate Laboratory Director will provide preliminary verbal reports to the Provider prior to issuance of a final report.

6. Request a Specimen Collection Kit.

Providers may request shipment of a LDL Specimen Collection Kit containing LDL culture media, slides, alcohol pre-filled vials for collection of dermal scrapings, aspirate, and/or biopsy material from LDL. POC is the LDL Associate Laboratory Director as listed in #4.c. above. Request kits with sufficient lead time prior to procedure(s) for LDL to priority express ship the kit to your facility.