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

Cutaneous Leishmaniasis Biopsy Procedures

1. Criteria for biopsy

a. Any patient who has had a non-healing lesion (does not have to be an open ulcer) for greater than 3 to 4 weeks should be suspected of having leishmaniasis in the setting of past travel to an endemic region.

b. For OCONUS sites, please consult with your local dermatologists or with the WRAIR *Leishmania* Diagnostics Laboratory (LDL) clinical consultant (usarmy.detrick.medcom-wrair.mbx.leishmania-diagnostic@health.mil) before performing the biopsy.

2. Biopsy procedure

a. Thoroughly clean the area of the lesion with betadine, then carefully wash it off and allow the area to dry. Remove all betadine prior to acquisition of the specimens as betadine may inhibit parasite growth in culture.

b. Anesthetize the anticipated area of biopsy by infiltration with 1% lidocaine. Avoid high concentrations of anesthetic that could inhibit parasite growth in culture.

c. Take a 3-4 mm punch biopsy with a sterile disposable punch, or if acquiring a shave biopsy, use a sterile scalpel (#15, #11, or #10) to acquire the specimen from the indurated edge of lesion. Lesions on the face, anterior of the neck, and near larger vessels and/or nerves need to be biopsied with extreme caution.

> **Note:** a simple surface scraping may be preferred over a biopsy for ulcerative lesions.

d. Divide, aseptically, the collected biopsy into three parts.

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

ii. For culture, place the second portion of the biopsy under aseptic conditions in RPMI or LDL-provided media (see # 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: Culture media may be requested from LDL prior to performing the biopsy (see Section 6.0 below).

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

1) For touch press smears, gently press the blotted surface of the tissue with a rolling or circular motion onto a glass microscope slide; repeat in a parallel row down the slide.

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

3. Submission of Slides and/or Biopsy Specimens

a. Send the biopsy specimens, smears and vials with tissue in alcohol for *Leishmania* PCR and/or in media for culture directly to LDL as directed in # 4.a-d. below.

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

c. If slides, or specimens are acquired from multiple lesion sites provide, designation as to which anatomic site such as A –right arm, B right hand, etc.

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

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

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

g. Include the LDL Test Request Form (CONUS or OCONUS) with patient's name, date of birth, brief clinical history, travel history, specimen collection date, and test(s) requested.

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

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

j. 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, and alcohol pre-filled vials for collection of dermal scrapings 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.