

Cutaneous Leishmaniasis Scrapings Procedures

1. Criteria for scraping

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

2. Collection Procedure for Acquisition of Scrapings

- a. Clean area with soap and sterile water or betadine then wash off thoroughly with alcohol pads, blot with gauze, and allow the area to dry. Note: residual betadine may inhibit parasite growth in culture.
- b. Anesthetize with lidocaine 1% or 2% with epinephrine 1:100,000 unless epinephrine is contra indicated due to the anatomic site. Avoid high concentrations of anesthetic that could inhibit parasite growth in culture.
- c. To ensure a clean ulcer based is sampled, debride any exudate and remove part of eschar/crust from the ulcerative lesion.
 - i. For culture, ensure culture media with a neutral pH (~7.0-7.4) such as RPMI is available locally; alternatively contact the *Leishmania* Diagnostics Laboratory (LDL) so that we can provide the requisite media by overnight priority courier *prior to the procedure* (see # 6 below).
 - ii. Sterilely obtain dermal scrapings that are about the size of a large grain of rice. Add scrapings to culture media. 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: While LDL successfully cultures *Leishmania* species from dermal scrapings, risk for contamination is high unless scrapings are sterilely acquired.
 - iii. For Histopathology, perform two (2) tissue smears by horizontally scraping the base of the ulcer with a sterile scalpel blade lightly enough to elicit an exudate, but not vigorously enough to cause much bleeding. Apply the dermal tissue *very thinly* in a circular

fashion to a dime-sized area in the center of the slide to make as thin a smear as possible. Minimize blood on the slide.

- iv. For Molecular Assays (*Leishmania* PCR), place material from another scraping and even the overlying crusted debris into a small leak-proof vial prefilled with 70-100% ethanol, isopropyl alcohol, or methanol (enough to cover the tissue).

3. Submission of specimens

- a. Send the scraping 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 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.
- h. Label the shipping container "Clinical Specimen" on the outside of the package.

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

- i. Include the following information: submitter's name, address, phone number, fax number, and e-mail address.
- j. Ship at room temperature by overnight priority 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 and/or biopsy material from LDL. POC is the LDL Associate Laboratory Director as listed in #4.c. above. Request kits with

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sufficient lead time prior to procedure(s) for LDL to priority express ship the kit to your facility.