



**DEPARTMENT OF THE ARMY**  
WALTER REED ARMY INSTITUTE OF RESEARCH  
503 ROBERT GRANT AVENUE, ROOM 2S04  
SILVER SPRING MARYLAND 20910-7500

## **Leishmania Scraping & Biopsy Procedures**

### **1) Criteria for scraping or biopsy:**

- Any patient who has had a non-healing lesion (does not have to be an open, weeping ulcer) for greater than 3 to 4 weeks needs to be suspected of having leishmania.
- These patients need to be placed on a course of antibiotic therapy for 7 to 10 days with an antibiotic, which has proven activity in Iraq (recommendation is Augmentin 875mg BID for 7 to 10 days).
- At the conclusion of therapy, the patient should be seen by the same practitioner and a decision needs to be made if there was any efficacy to the course of antibiotics. If the lesion has persisted or worsened, a scraping or biopsy should be performed.
- Photos of the lesion prior to scraping or biopsy being done should be accomplished if the practitioner has the capability. E-mail these photos to WRAIR since this may help in the diagnosis ([peter.weina@us.army.mil](mailto:peter.weina@us.army.mil)).

### **2) Scraping procedure:**

- Clean area with alcohol pads and allow to dry.
- Anesthetize with lidocaine 1% or 2% with epinephrine 1:100,000 (unless the epinephrine is contraindicated due to anatomic site).
- 2 tissue smears are performed by horizontally scraping (lightly enough to elicit an exudates, but not vigorously enough to cause bleeding) the base of the underlying ulceration with a #15 blade (this often requires removal of the overlying crusted debris). The dermal tissue is then thinly applied in a circular fashion to a dime to nickel sized area in the center of the slide. Minimize blood, epithelium (keratinocytes), and purulence on the specimen.
- Additionally, material from the scrapings (and even the overlying crusted debris) should be inserted into a small vial of 95-100% ethanol for PCR analysis.
- Ensure slides are labeled per the format of your affiliated pathology department and submit per their protocol. If pathology services unavailable locally, ship per address below. Work closely with pathologists to verify adequacy of tissue smear samples.

### **2) Biopsy/touch prep-impression smear procedure:**

- An area of the lesion needs to be cleaned thoroughly with alcohol pads and dried.
- The anticipated area of biopsy should be anesthetized as described above.
- A 4 mm sterile disposable punch or sterile scalpel (#15, #11, or #10) should to be used to remove a piece of tissue approximately 3 to 4 mm in circumference and approximately 1 mm deep from the edge of the lesion (see photo for preferred area of biopsy). Lesions on the face, anterior of the neck, and near larger vessels and/or nerves need to be biopsied with extreme caution and a simple surface scraping (described above) may be preferred to a true biopsy.
- The biopsy should be placed on a sterile, clean, dry gauze 2X2 briefly to absorb excess blood on the tissue that may interfere with the reading of the touch preparations.

- The tissue should be grasped with forceps and impression smears made on clean slides (4 for each biopsy) by rubbing the tissue gently across the surface of the slide in a circular motion.
- Dry thoroughly. Fix with methanol if available.
- The tissue biopsy (after the impression smears are made) should then be placed in a very small amount of ethyl alcohol (just enough to cover the specimen) in a leakproof vial (such as a “nunc” transport tube).
- The slides and the vial with the tissue should be shipped per your local pathology section protocol or via DHL or Federal Express to the address below. The container should be labeled as diagnostic specimens and no shipping permit is required (all MTFs have personnel and resources to ship diagnostic specimens correctly).
- **PLEASE LABEL WITH PATIENT NAME, SPECIMEN SOURCE, DATE, AND MATERIAL TRANSPORTED IN** (formalin, ETOH, etc.).
- Complete the patient information sheet (attachment #2 below) and include with the specimen for each patient biopsied.
- Procedural inquiries should be made to COL Peter Weina at (301) 319-9956.

### **SHIPPING ADDRESS**

Colonel Peter J. Weina, PhD, MD  
Director, Leishmania Diagnostic Laboratory  
Division of Experimental Therapeutics  
503 Robert Grant Avenue  
Walter Reed Army Institute of Research  
Silver Spring, Maryland 20910-7500

### **Preferred biopsy area:**

