FAQs for Health Care Providers
Leishmaniasis in Afghan Evacuees

All assays for molecular, serological, morphological identification, or characterization of parasitic diseases at CDC are temporarily offline and turn-around time is delayed. Diagnostic testing through CDC is expected to resume soon. CDC offers consultation regarding other options in the absence of diagnostic testing at CDC for some parasitic diseases. While CDC *Leishmania* testing is offline, Walter Reed Army Institute of Research (WRAIR) is able to accept *Leishmania* specimens from Afghan evacuees for testing.

Leishmaniasis is a parasitic infection transmitted by sand flies that is found in about 90 countries, including Afghanistan. There are more than 20 *Leishmania* species that affect humans. The most common include 5 Old World species, which occur primarily in Europe, the Mediterranean basin, Africa, the Middle East, and parts of Asia; and 7 New World species that occur in the Western hemisphere, primarily in Central and South America. These species are morphologically indistinguishable but can be differentiated by molecular methods. The two main forms of the disease are cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL). Different species are associated with diverse clinical manifestations and sequelae.

Epidemiology of leishmaniasis in Afghanistan

There is a high burden of leishmaniasis in Afghanistan. CL most commonly occurs in rural and semi-rural areas, especially the northern Afghan plains and outskirts of Kabul. Kandahar in the south and Herat in the west are also high transmission areas (Figure 1). However, CL is also found in urban dwellers, including residents of large cities, such as Kabul.

Information on leishmaniasis in Afghanistan is incomplete, but the most common species are:

- *L. major*:
    - Predominant species in northern Afghanistan; aggressive forms have been found
    - Main presentation: CL
    - Other presentations:
        - Mucosal leishmaniasis (ML): Low risk for ML but case reports exist of this unusual presentation.
- *L. tropica*:
    - Predominantly occurs in southern Afghanistan, including region around Kabul
    - Main presentation: CL
    - Other presentations:
        - Viscerotropic leishmaniasis: Uncommon (cases reports)
        - Leishmaniasis recidivans: Uncommon presentation of CL recurrence
        - ML: Low risk for ML, but case reports exist of this unusual presentation.

Other less common species of *Leishmania* in Afghanistan include:

- *L. donovani*
    - Main presentation: VL

- Other presentations:
    - Post kala-azar dermal leishmaniasis: an uncommon presentation
    - Localized cutaneous lesions: less common presentation than VL
- *L. infantum*
    - Main presentation: VL
    - Other presentations:
        - CL: limited published reports; some provider experience in the northern border with Pakistan
        - ML: Risk of ML is limited, but might be higher than *L. major* and *L. tropica* for immunocompromised individuals

What are the signs and symptoms of cutaneous leishmaniasis?

CL is characterized by one or more lesions involving areas of the skin where sand flies have fed (Figures 2,3). The sores are typically painless, although may be painful if superinfected, and may be associated with localized lymphadenopathy. Lesions usually develop within several weeks or months after exposure, but occasionally first appear years later (for example, in the context of trauma or immunosuppression.) The lesions change in size and appearance over time, often evolving from sores or papules to nodular plaques or ulcerative lesions with a raised border and central depression.

Old World CL is generally a self-limited skin disease. But depending on species, cutaneous dissemination might occur in immunocompromised individuals, or leishmaniasis recidivans may develop. Certain New World *Leishmania* species that cause CL can also cause mucosal leishmaniasis (ML) (Figure 4).

<u>Differential diagnosis of CL</u>:

- Superficial bacterial skin infection (such as impetigo)
- Cutaneous mycobacterial infection (tuberculous and nontuberculous)
- Fungal infection (such as sporotrichosis, blastomycosis, chromoblastomycosis)
- Leprosy
- Skin cancer
- Spider bite

Features of CL include its chronicity, typically painlessness, occurrence on exposed skin, the frequent clustering of lesions or presence of satellite papules, sporotrichoid spread, subcutaneous induration and subcutaneous nodules, and well-defined indurated borders.

How do I evaluate cutaneous leishmaniasis?

Diagnosis can be difficult, especially in austere settings. Various laboratory methods can aid in diagnosis, but some of the methods are available only in certain reference laboratories. Currently available serologic tests are not helpful in the diagnosis of CL. See **Diagnostic Testing** section for further information.

Potential approach to initial evaluation of a patient with possible CL:

1. Assess character of the lesion(s), as well as size, number, location (for example, involving face, close to joints), induration, presence or absence of pain, signs of scarring, nodules along lymphatics distal to skin lesion, and regional adenopathy. (See Fig. 5)
2. Ask about symptoms of ML (see section on ML) and examine mucosal surfaces of the naso-oropharynx for lesions.
3. If other diagnoses are possible, such as bacterial infection, consider a trial of appropriate treatment, and if no resolution of lesions, move to specimen collection (skin scraping/biopsy).
4. Specimens should be collected from active/ulcerative lesions without evidence of secondary infection. Ensure the lesion is clean prior to specimen collection. Ideally, collection should be done by an experienced clinician (for example, if biopsy, a dermatologist), if possible, and using special specimen collection requirements from the reference laboratory. See "What diagnostic tests are available for leishmaniasis?" section for more information on specimen collection.
5. Send specimens to a reference laboratory that can assess presence of intracellular amastigotes and perform molecular testing (PCR) and/or culture for diagnosis and (ideally) speciation. PCR should be obtained on all specimens. See "What diagnostic tests are available for leishmaniasis?" section for more information on specimen submission.
6. Commercially available serologic tests are not helpful in the diagnosis and management of CL.

What is the treatment for cutaneous leishmaniasis?

Treatment decisions should be individualized, with expert consultation if needed, as different species and presentations respond differently to treatment regimens. CDC can assist with diagnosis and treatment recommendations to healthcare providers. Not all cases of CL require treatment and **treatment should not be initiated without confirmation of the diagnosis**. Special groups who are particularly vulnerable (such as young children, elderly people, pregnant or lactating women, and immunocompromised people) might need different medications or dosage regimens. The following are options for treatment, in no particular order, based on the species of *Leishmania* and presentation. Pentavalent antimonials, potential treatment options in many countries, have been excluded from the list below as they are not currently available in the United States. Note that treatment choices might differ for *L. tropica* and *L. major* infections. When test results return indicating leishmaniasis, **use Figure 5 to determine if the presentation is simple or complex.**

If species is able to be determined

- *L. major*
    - Simple:
        - If lesion is healing or new lesion, consider observation (every 2-4 weeks) and wound care; lesions can spontaneously heal within 2-6 months
        - Heat treatment via ThermoMed radiofrequency wave device*
        - Cryotherapy with liquid nitrogen*
        - Topical paromomycin*
    - Complex:
        - Liposomal amphotericin B (IV)*

        - Miltefosine (oral)*
        - Fluconazole (oral)* has mixed results, a higher dose may be more effective
- *L. tropica*
    - Simple

*See Tables 1 and 3 for dosing, administration, monitoring, and comments

and wound care;

lesions can spontaneously heal within 6-15 months
        - Heat therapy via ThermoMed radiofrequency wave device*
        - Cryotherapy with liquid nitrogen*
        - Photodynamic therapy*
    - Complex
        - Miltefosine (oral)*
        - Liposomal amphotericin B (IV)*
        - Photodynamic therapy could be considered in consultation with an expert.

If leishmaniasis diagnosis is confirmed but species is unable to be determined

- Simple
    - If lesion is healing or a new lesion, then consider observation and wound care
    - Heat treatment via ThermoMed radiofrequency wave device*
    - Cryotherapy with liquid nitrogen*
- Complex
    - Miltefosine (oral)*
    - Liposomal amphotericin B (IV)*

How do you assess response to therapy in cutaneous leishmaniasis?

Response to therapy for CL depends on multiple factors including patient factors, location of the lesion, treatment, and species of *Leishmania*. Clinical criteria should be used to assess response to therapy including:

- Size of the area of ulceration
- Size of the area of induration
- Thickness of the induration
- Extent of scarring

The first sign of healing is usually flattening of the lesion. Generally, healing has started by 6-9 weeks after initiating treatment and healing may continue after completion of treatment. By 4-6 weeks after treatment, the lesion size should have decreased by more than 50%, ulcerative lesions should be re-epithelialized and no new lesions should be appearing. Clinical cure usually occurs by 3 months after treatment initiation. However, *L. tropica* infections can heal more slowly (6-15 months) than *L. major* (2-6 months). Paradoxical increase in inflammation around the lesion can occur in the first two weeks of treatment, which should not be concerning unless persistent or inflammation progresses. Lesions should be monitored for 6-12 months after treatment for signs of treatment failure, including

breakdown along the border of previously epithelialized ulcers (an initial sign of failure) and increasing size, induration and erythema of the ulcer.

Photographs of lesions are helpful in monitoring response to therapy. Observations should be made [illegible] The surface [illegible]ecorded.

*See Tables 1 and 3 for dosing, administration, monitoring, and comments

Document the size and location of the lesion(s) and associated subcutaneous nodules or adenopathy as well as any new findings around the original lesion(s) and along lymphatic drainage pathways. Note evidence of secondary infection.

Repeat parasitological testing is generally not recommended in healing lesions.

What are the signs and symptoms of visceral leishmaniasis?

Visceral leishmaniasis (VL) is a severe, systemic condition in which the parasite invades the spleen, liver, and bone marrow. It is associated with fever, weight loss, and chronic malaise; affected people often have enlarged livers and/or spleens. VL may be accompanied by various laboratory abnormalities, including hypergammaglobulinemia, anemia, leukopenia, thrombocytopenia, hypoalbuminemia, elevated acute inflammatory markers, and abnormal liver enzymes. VL can be fatal if not treated promptly.

Differential diagnosis of VL: (other causes of fever and hepato-splenomegaly, especially in children)

- Acute onset:
  - Malaria, typhoid fever, typhus, acute Chagas disease, acute schistosomiasis, miliary tuberculosis, amebic liver abscess, mononucleosis, viral hepatitis
- Subacute or chronic:
  - Miliary tuberculosis, brucellosis, prolonged or recurrent Salmonella infections, subacute bacterial endocarditis, histoplasmosis or other disseminated fungal diseases, malaria with tropical splenomegaly syndrome, hepatosplenic schistosomiasis with portal hypertension
- Non-infectious:
  - Lymphoma, leukemia, other myeloproliferative diseases, rheumatoid arthritis with Felty syndrome, other autoimmune processes, hemophagocytic lymphohistiocytosis (HLH)

How do I evaluate visceral leishmaniasis?

Diagnosis can be difficult. Various laboratory methods can aid in diagnosis; however, some of the methods are available only in certain reference laboratories.

- Suspected VL cases should be evaluated for other possible causes of disease concurrently.
- Serum should be collected for detection of antileishmanial antibodies.
- Tissue specimens (aspirates or biopsy) should be collected, with bone marrow the preferred specimen for diagnosis. Other diagnostic specimen sources include liver, enlarged lymph nodes, whole blood (buffy coat).
- In immunocompromised persons, blood should be collected for buffy coat examination, in vitro culture and molecular analyses, in addition to the specimens above.

Currently available serologic tests can be helpful in diagnosing VL (such as rK39 antibody test) and can be used as a screening assay to exclude *L. infantum* and *L. donovani* in children thought to have acquired the disease in Afghanistan, prior to the collection of tissue samples by aspirate or biopsy. Positive serologic test results should be followed by testing to confirm the diagnosis. However, if the serology test is negative but there is a strong clinical suspicion for VL, then providers may proceed with additional diagnostic testing. Definitive diagnosis requires demonstration of *Leishmania* in a clinical specimen (such as bone marrow) by histology, culture, and/or molecular (PCR) testing. Identification of

the *Leishmania* species using specialized testing can be important in determining optimum treatment and prognosis; however, this requires special isoenzyme assays or molecular testing.

What is the treatment for visceral leishmaniasis?

All patients with symptomatic VL should be treated. Treatment decisions should be individualized, with expert consultation if needed, as different species and presentations respond differently to treatment regimens. CDC can assist with diagnosis and treatment recommendations to healthcare providers. In Afghanistan, the most common causes of VL are *L. donovani* and *L. infantum*. Special groups who are particularly vulnerable (such as young children, elderly people, pregnant or lactating women, and immunocompromised people) might need different medications or dosage regimens. In children, secondary HLH can occur with VL and should be managed by treating the VL with antiparasitic therapy.

- **First line:** For VL, Liposomal amphotericin B* is considered first line.
- **Alternative:** Miltefosine* has been effective for *L. donovani* infection acquired in the Indian subcontinent. Evidence is sparse for *L. infantum.*

How do you assess response to therapy in visceral leishmaniasis?

Response to therapy in VL should be assessed by clinical monitoring, such as normalization of vital signs, laboratory abnormalities and physical exam findings. Fever should resolve in less than 1 week with treatment, but hepatomegaly and splenomegaly make take months to resolve. Laboratory abnormalities can normalize anywhere to within one month to up to a year. Repeat parasitological testing is generally not recommended for patients that are improving.

What diagnostic tests are available for leishmaniasis?

Diagnosis can be difficult, especially in austere settings. Various laboratory methods can aid in diagnosis; however, some of the methods are available only in specific reference laboratories.

Definitive diagnosis requires demonstration of *Leishmania* in a clinical specimen (such as skin or bone marrow) by histology, culture, and/or molecular (PCR) testing. Identification of the *Leishmania* species using specialized testing can be important in determining optimum treatment and prognosis; however, this requires special isoenzyme assays or molecular testing. Currently available serologic tests are not helpful in the diagnosis of CL but can be helpful in diagnosing VL (such as an rK39 antibody test) and can be used as a screening assay for children thought to have acquired the disease in Afghanistan (see "How do I evaluate visceral leishmaniasis?" section above).

In the United States, CDC provides reference diagnostic services for leishmaniasis.

All assays for molecular, serological, morphological identification, or characterization of parasitic diseases at CDC are temporarily offline, and turn-around time is delayed. Diagnostic testing through CDC is expected to resume soon.

*See Tables 2 and 3 for dosing, monitoring, and comments

While CDC *Leishmania* testing is offline, Walter Reed Army Institute of Research (WRAIR) can perform rK39 serology, histopathology, molecular testing and culture to assist in the diagnosis of leishmaniasis. Appendix 1 has the Leishmaniasis Test Request Form, which needs to be filled out completely and accompany the specimen. The procedures for CL scrapings, CL biopsies, needle aspirations, bone marrow aspiration/biopsies, and sera collection are detailed in appendices 2 through 6 respectively. **Prior to collecting a specimen please contact WRAIR Leishmania Diagnostic Lab (LDL) by phone at: Office: 301-319-2297; Cell: 240-406-6510; 24 hours Emergency Number 240-595-7353; or email: usarmy.detrick.medcom-wrair.mbx.leishmania-diagnostic@mail.mil as most test require special collection kits from LDL for specimen submission.** Please read these procedures and forms completely prior to obtaining and submitting a specimen. **Specimens need to be shipped by priority overnight, express courier for arrival on a weekday (except federal holidays) within 24 hours of specimen collection.** Questions on obtaining specimens and/or specimen submission should be directed to usarmy.detrick.medcom-wrair.mbx.leishmania-diagnostic@mail.mil.

CDC can be consulted for any patient if healthcare providers or health professionals have questions (CDC Parasitic Disease Inquiries: parasites@cdc.gov or at 404-718-4745 M-F 8a-4p EST, or after hours at 404-488-7100).

Who needs special considerations when treating leishmaniasis?
Patients who are immunocompromised or who have been treated without adequate response will need special consideration and should be managed with expert consultation.

Is leishmaniasis contagious? Are there special infectious disease precautions for someone with leishmaniasis? No, the infection is not spread person-to-person. There is no need to isolate affected people.

What are some additional clinical presentations of leishmaniasis?

Mucosal leishmaniasis (ML)

Certain New World *Leishmania* species that cause cutaneous leishmaniasis (especially *L. (Viannia) braziliensis*, *L. (Viannia) panamensis*, *L. (Viannia) guyanensis*, and *L. amazonensis*) can invade mucosal areas, particularly the nose, mouth, and palate. . Initially ML can manifest as persistent, unusual nasal symptoms (such as stuffiness or bleeding) or oral or pharyngeal symptoms. Persons who are at risk for ML and who have persistent mucosal symptom(s) or compatible naso-oropharyngeal mucosal lesions should be referred to a specialist for an otorhinolaryngologic exam. This exam typically should include fiber-optic endoscopy. If left untreated, ML can progress to ulcerative destruction of the cartilage and surrounding tissue. Generally, all clinically manifest cases of ML should be treated.

Leishmaniasis recidivans

May occur in *L. tropica* infection and occasionally other species, although uncommon. After healing of the initial lesion or following trauma, new papules form around the margin of a scar. Successful treatment can be challenging.

Diffuse cutaneous leishmaniasis

Caused by *L. mexicana*, *L. amazonensis*, *L. aethiopica*, and *L. major* (to a lesser extent). This is a chronic, non-ulcerative, plaque-like form of CL thought to be related to a host immunologic defect. Treatment is often unsatisfactory, and relapse is common with treatment is stopped. Some type of chronic or intermittent treatment may be required.

Disseminated cutaneous leishmaniasis

Caused mainly by *Viannia spp*. This form of CL has widely scattered small papules, nodules and ulcers. It has been reported primarily in northern and northeastern Brazil and is associated with an increased risk for ML, particularly in patients with head and neck lesions. It is a rare outcome but seems to most often affect young male adults. In the Old World, several cases of disseminated CL associated with *L. major* co-infected with HIV have been reported in the literature.

Resources and References

1. **Centers for Disease Control and Prevention:** https://www.cdc.gov/parasites/leishmaniasis/
2. **Centers for Disease Control and Prevention:** https://www.cdc.gov/parasites/leishmaniasis/health_professionals/index.html
3. Aronson N, Herwaldt BL, Libman M, Pearson R, Lopez-Velez R, Weina P, et al. Diagnosis and Treatment of Leishmaniasis: Clinical Practice Guidelines by the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH). Clin Infect Dis. 2016;63(12):1539-57.
4. Aronson NE, Joya CA. Cutaneous Leishmaniasis: Updates in Diagnosis and Management. Infect Dis Clin North Am. 2019;33(1):101-17.
5. WHO. Control of the leishmaniases. World Health Organ Tech Rep Ser. 2010(949):xii-xiii, 1-186,
6. Ghatee MA, Taylor WR, Karamian M. The Geographical Distribution of Cutaneous Leishmaniasis Causative Agents in Iran and Its Neighboring Countries, A Review. Front Public Health. 2020;8:11.
7. Leslie T, Saleheen S, Sami M, Mayan I, Mahboob N, Fiekert K, et al. Visceral leishmaniasis in Afghanistan. Cmaj. 2006;175(3):245.
8. Goldin H, Kohen S, Taxy J, Libman M, Cibull T, Billick K. Leishmania tropica infection of the ear treated with photodynamic therapy. JAAD Case Rep. 2020;6(6):514-7.

Figures:

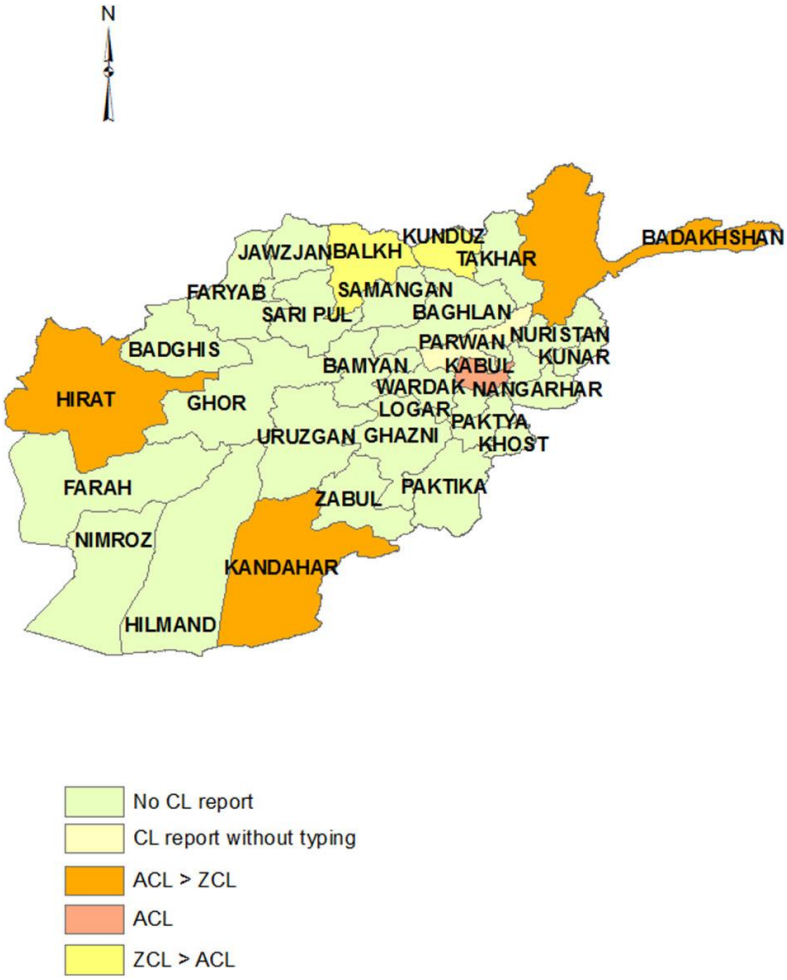

Figure 1. Map of Afghanistan with causes of cutaneous leishmaniasis identified by color. ACL (anthroponotic cutaneous leishmaniasis) is caused by *L. tropica*. ZCL (zoonotic cutaneous leishmaniasis) is caused by *L. major*. Note there are multiple areas where no cutaneous leishmaniasis is reported but this should not be interpreted to mean cutaneous leishmaniasis does not exist in that area but rather there is no information about cutaneous leishmaniasis in that area. Source: Ghatee MA, Taylor WR, Karamian M. The Geographical Distribution of Cutaneous Leishmaniasis Causative Agents in Iran and Its Neighboring Countries, A Review. Front Public Health. 2020;8:11.

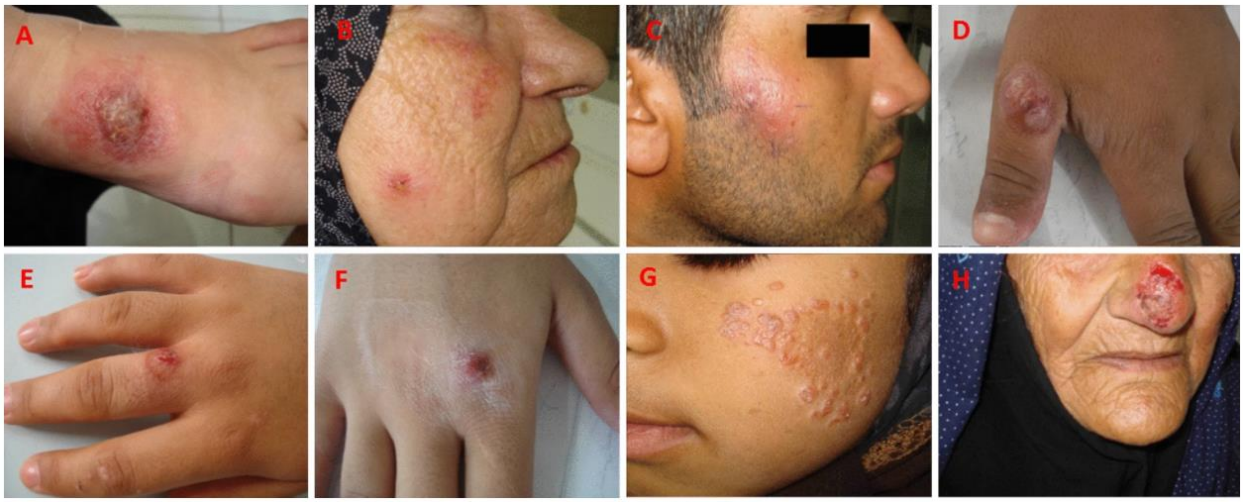

Figure 2: Representative pictures of anthroponotic cutaneous leishmaniasis cases obtained from kerman and Bam cities (A-F localized skin lesions; G lupoid leishmaniasis; H a chronic/unresponsive cutaneous lesion). Source: Karimi T, Sharifi I, Aflatoonian MR, Aflatoonian

B, Mohammadi MA, Salarkia E, et al. A long-lasting emerging epidemic of anthroponotic cutaneous leishmaniasis in southeastern Iran: population movement and peri-urban settlements as a major risk factor. Parasit Vectors. 2021;14(1):122.

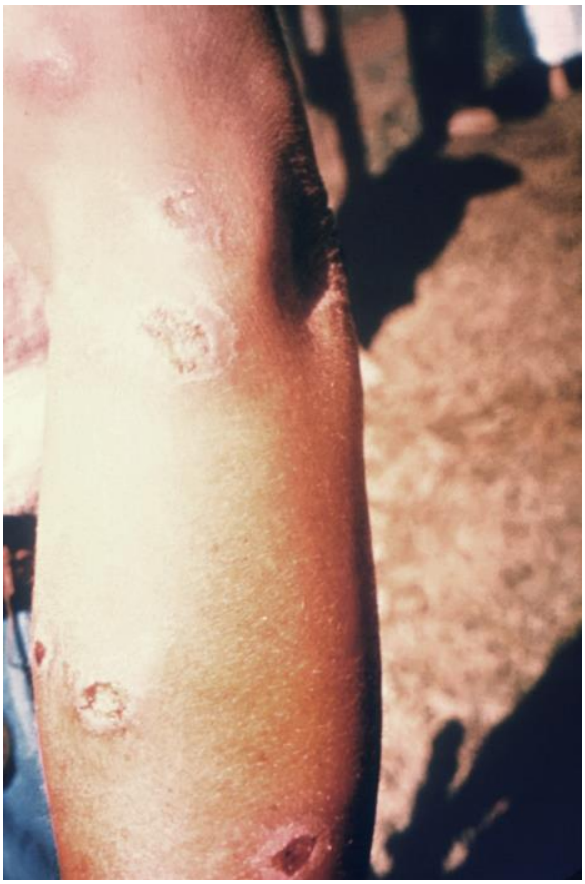

Figure 3. Multiple crater ulcers on this patient's left arm, due to leishmaniasis. Note the various stages of the ulcers, up and down the arm, with some undergoing resolution, leaving behind a depressed, crater-like scar, and others in an erythematous inflammatory phase, revealing their reddened, scabby interiors. Source: Public Health Image Library https://phil.cdc.gov/Details.aspx?pid=15066

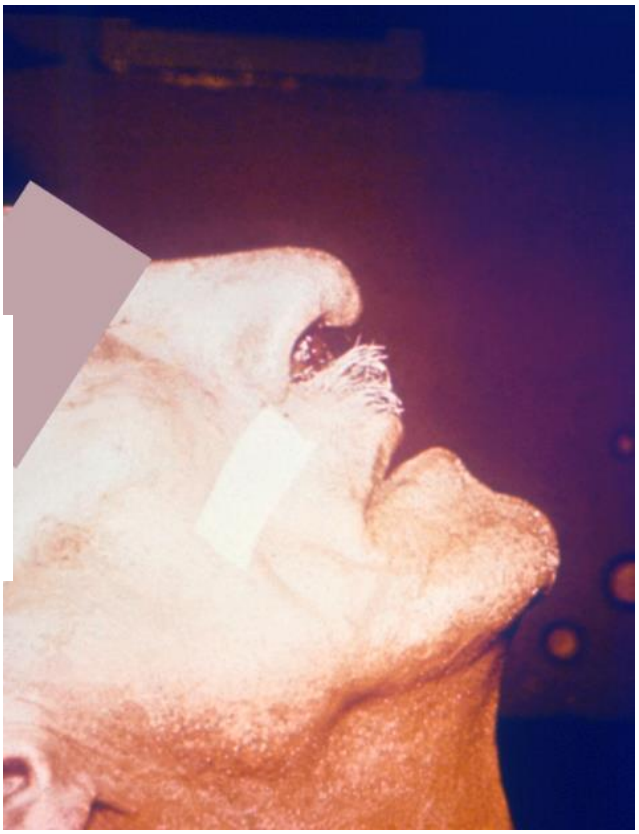

Figure 4. Right lateral view of a patient's head revealing a nasal deformation ("tapir nose") caused by erosion of the nasal septum due to mucocutaneous leishmaniasis. Source: Public Health Image Library https://phil.cdc.gov/Details.aspx?pid=15065

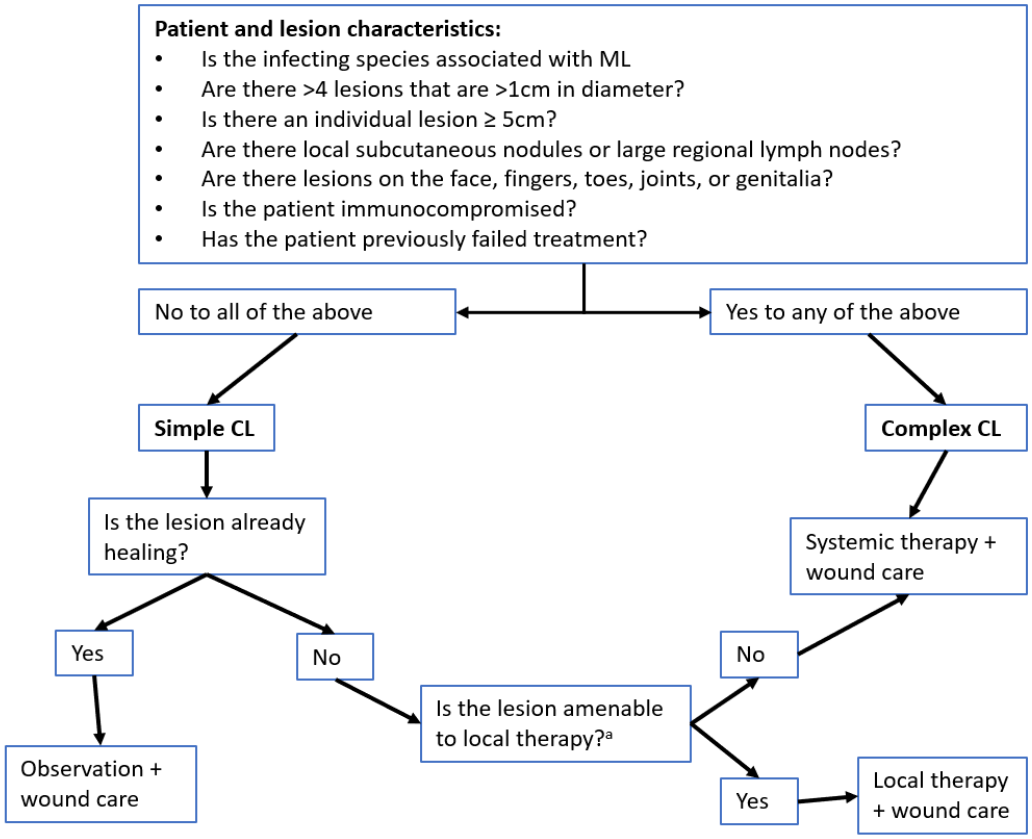

Figure 5. Treatment algorithm for CL: a basic approach. Although CL treatment should be individualized to the patient, this is a generalized flowchart to describe a basic approach to therapy. All patients should be educated about the natural history of leishmaniasis and risks and benefits of treatment and agree to treatment plan. This does not address the treatment of pregnant patients, those with evidence of ML, or those with unusual syndromes of leishmaniasis (leishmaniasis recidivans, diffuse CL, or disseminated leishmaniasis). [a]Few lesions (<5), small lesions (<4cm in diameter), lesions are not in a cosmetically important area (such as the face), and not in functionally important areas. Adapted from: Aronson NE, Joya CA. Cutaneous Leishmaniasis: Updates in Diagnosis and Management. Infect Dis Clin North Am. 2019;33(1):101-17.

Table 1. Approach to treatment of cutaneous leishmaniasis for patients currently located in North America					
Drug/Treatment	Proprietary Name	Route of Administration	Regimen	FDA Approval and Availability	Comments
Liposomal amphotericin B	AmBisome®	IV	3mg/kg/day on days 1-5 and 10 or on days 1-7 (total 18-21mg/kg)	Yes, but not for CL; off label use	No standard dosage regimens have been established; other regimens have been described in case reports/series from various settings.
Miltefosine	Impavido®	Oral	FDA-approved regimen: if 30-44kg, 50mg BID for 2 days; if ≥ 45kg, 50mg TID for 28 days	Yes, for CL caused by L. V*iannia* species; off-label use for other species	Target dose is ~ 2.5mg/kg/day, but doses > 150mg/day have not been studied. GI side effects may limit higher dose.
Fluconazole	Diflucan®	Oral	Adults 200 mg daily for 6 weeks	Yes, but not for CL; off label use	Has mixed results for *L. major* and a higher dose of 400mg daily vs 200mg daily may be more effective.
Topical paromomycin		Topical	For 15% paromomycin and 12% methylbenzethonium chloride ointment apply BID for 10 days, rest for 10 days and reapply BID for 10 days. For 15% paromomycin and 0.5% gentamicin cream* apply once per day for 20 days	The capsule formulation of paromomycin is FDA approved for other indications; use the capsules to compound antileishmanial ointment constitutes off-label use.	Local irritation, erythema and/or mild pain are common. Higher response rates noted for infection caused by *L. major* than *L. tropica.*
Heat therapy	ThermoMed®	Locally applied to skin	Apply under local anesthesia for 30 s doses in grid-like pattern extending 1-2mm into surrounding normal-appearing skin. Usually one session (sometimes up to 3)	Yes, cleared for CL indication	Avoid applying over eyelids, tip of nose, lips, mucous membranes, cartilaginous structures or superficial nerves. Use topical antibiotics for several days after the heat treatment. Keloids may be less common than with cryotherapy.
Cryotherapy with liquid nitrogen		Locally applied to skin	Multiple regimens, e.g., freeze 5-20 s until 1-2mm of normal circumferential skin frozen, thaw 20-60 s, repeat every 3 weeks for up to 3 total applications (few, if healed sooner)	Yes, “grandfathered in”	Increased efficacy has been noted if used in combination with intralesional pentavalent antimonials. Avoid applying over eyelids, tip of nose, lips, mucous membranes, cartilaginous structures or superficial nerves. Can cause permanent hypopigmentation.
Photodynamic therapy		Locally applied to skin	One report performed the following: Debrided the lesion, applied aminolevulinic acid hydrochloride 10% gel and occluded for 4 hours. Lesion was then exposed to 75J of red light (633 nm).	Yes, but not for CL.	See case report in reference 8. Adverse effects can include photosensitivity, burning sensation, erythema and swelling. Limited experience. Consultation with expert for complex lesions.

			Retreatment is usually performed but frequency and total number of treatments vary.		

* The paromomycin preparation will need to be specially prepared by a compounding pharmacy. Two recipes in no particular order and with no preference:

- Paromomycin sulfate 15% w/w, gentamicin sulfate ointment 0.5%, Aquaphilic® ointment base with 10% carbamide (Medco Lab, Inc, Sioux City, IA) 67.8%, and water 16.67%
- Unibase ointment (as a carrier)—with the following: paromomycin sulfate 15% (based on free base), gentamicin sulfate 0.5% (based on free base), urea 6.75%, and purified water 42.2%.

Table adapted from: Aronson N, Herwaldt BL, Libman M, Pearson R, Lopez-Velez R, Weina P, et al. Diagnosis and Treatment of Leishmaniasis: Clinical Practice Guidelines by the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH). Clin Infect Dis. 2016;63(12):1539-57.

Table 2. Approach to treatment of Visceral Leishmaniasis for patients currently located in North America						
Syndrome	Drug/Treatment	Proprietary Name	Route of Administration	Regimen	FDA Approval and Availability	Comments
VL	Liposomal amphotericin B	AmbBsome	IV	FDA approved regimen if immunocompetent: 3mg/kg/day on days 1-5, 14 and 21 (total dose 21mg/kg) FDA-approved regimen if immunosuppressed: 4mg/kg/day on days 1-5, 10,17, 24, 3 and 38 (total dose 40mgkg) An additional regimen of 10mg/kg/day for 2 days has been widely used for children in the Mediterranean, but this regimen is not FDA approved.	Yes, for this indication	Considered treatment of choice for VL. For treatment of VL in immunocompetent persons with VL acquired in East Africa, regimens with total doses ≥ 40mg/kg may be needed.
VL	Miltefosine	Impavido	Oral	FDA-approved regimen: if 30-44kg, 50mg BID for 28 days; if ≥ 45kg, 50mg TID for 28 days	Yes, for VL caused by *L. donovani*	On the basis of anecdotal experience in Europe and Brazil, not as effective for VL caused by *L. infantum.* In general, target dose is ~2.5mg/kg/day, but doses >150mg/day have not been studied. GI side effects may limit higher doses.
Adapted from: Aronson N, Herwaldt BL, Libman M, Pearson R, Lopez-Velez R, Weina P, et al. Diagnosis and Treatment of Leishmaniasis: Clinical Practice Guidelines by the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH). Clin Infect Dis. 2016;63(12):1539-57.						

Table 3. Antileishmanial therapy: Adverse events, monitoring for toxicity and mitigation approaches							
Drug	Route	Adverse Events[ǂ,§]	Laboratory Monitoring for Toxicity[ǂ,¶]	Mitigation and Management Approaches[ǂ,¶]	Pregnancy[¶,**]	Breastfeeding[¶,ǂǂ]	Comments
Liposomal amphotericin B	IV	Infusion-related reactions[§§] (eg, fever, rigors, headache, nausea, vomiting, hypotension, tachypnea), electrolyte abnormalities (eg, hypokalemia, hypomagnesemia), nephrotoxicity, anemia. Infusion-related reactions to liposomal amphotericin B also can be caused by liposome-induced complement activation-related pseudoallergy. Usually better tolerated than amphotericin B deoxycholate.	Baseline and frequent (eg, once or twice weekly) serum chemistry values and CBC. More frequent and/or additional testing (eg, ECG, urinalysis) may be indicated or prudent for some patients.	Examples: premedication; saline loading; test dose; slow infusions (~2hr); electrolyte supplementation, increased intervals between doses, and/or drug holidays, if indicated. Avoid/minimize use of other nephrotoxic agents.	Category B[¶¶]	Probably compatible; interruption of breastfeeding may be prudent.	
Miltefosine***	Oral	GI symptoms (nausea/vomiting>diarrhea) mainly early in treatment course; dizziness/motion sickness; scrotal pain (decreased/absent ejaculate); nephrotoxicity and/or hepatotoxicity.	Baseline and weekly assessment of renal function; also (particularly, if VL) monitor hepatic function and CBC.	To minimize GI symptoms take with food and use divided daily dosing. Encourage fluid intake if vomiting/diarrhea.	Female patients with reproductive potential should have a negative pretreatment pregnancy test and use effective contraception during and for 5 months after treatment, and should not rely on hormonal contraception if vomiting/diarrhea.	Breastfeeding not recommended during or for 5 mo after treatment.	Not FDA approved for patients < 12 years of age or <30kg. Has been used in other countries for younger children but capsule size may be an issue and pediatric pill is not available in the U.S. Contraindicated in patients with Sjögren-Larsson Syndrome (congenital ichthyosis).
Fluconazole	Oral	GI symptoms (eg, nausea, vomiting, abdominal pain); headache; hepatotoxicity; reversible hairloss and agranulocytosis	Baseline and weekly assessment of hepatic function; more frequent	Can be taken with or without food. Avoid/minimize use of other hepatotoxic agents.	Typically not warranted or recommended for antileishmanial	Generally considered compatible; on principle, interruption of	

			and/or additional types of monitoring (eg ECG, CBC) may be indicated or prudent for some patients	Hepatotoxicity may warrant interrupting therapy. Caution with drug-drug interactions.	treatment during pregnancy.	breastfeeding may be prudent.	

[ǂ] Not all-inclusive

[§] Selected examples are provided (eg, comparatively common or noteworthy adverse events); potential dermatologic effects and phlebitis (if IV) are not addressed. The types and rates of adverse events associated with a particular drug may vary, depending on interrelated factors such as the leishmanial syndrome, dosage regimen, and host characteristics (eg, immunologic status, comorbid conditions, concomitant/recent use of other medications).

[¶] Should be individualized.

** Use during pregnancy only if clearly indicated; expert consultation encouraged.

[ǂǂ] The potential for risk to breastfeeding infants cannot be excluded; expert consultation encouraged.

[§§] Some of the examples (eg, headache, GI symptoms) are not necessarily just infusion related.

[¶¶] Reproduction studies in animals have not demonstrated fetal risk; however, data from adequate, controlled studies in pregnant women are not available.

*** See black box warning in prescribing information.

Adapted from: Aronson N, Herwaldt BL, Libman M, Pearson R, Lopez-Velez R, Weina P, et al. Diagnosis and Treatment of Leishmaniasis: Clinical Practice Guidelines by the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH). Clin Infect Dis. 2016;63(12):1539-57.

Prior to collecting a specimen please contact WRAIR Leishmania Diagnostic Lab (LDL) by phone at: Office: 301-319-2297; Cell: 240-406-6510; 24 hours Emergency Number 240-595-7353; or email: usarmy.detrick.medcom-wrair.mbx.leishmania-diagnostic@mail.mil as most test require special collection kits from LDL for specimen submission. Specimens need to be shipped by priority overnight, express courier for arrival on a weekday (except federal holidays) within 24 hours of specimen collection.

Appendix 1

Leishmaniasis Test Request Form (CONUS-CIV)

Please complete the request form to ensure timely specimen processing.

Test Requested (Check one)	Specimen Requirement	Draw Tube/ medium	Lesion Location # Lesions	Shipping Conditions (Check one)
☐ **rK39 -** Kalazar *Detect*™ Rapid Test **(VL)**	☐ 1-2 ml serum	☐ SST Tubes ☐ Red-top tube		☐ 2-8° C post-centrifugation, shipped in cold box with ice packs
☐ **Histopathology (Smear ONLY)**	☐ Dermal Scrapings ☐ Touch prep-impression smears	☐ Microscope Slides		☐ Ambient 15-30°C ☐ Fixed with alcohol ☐ Stained Slides
☐ **Molecular test**	☐ Dermal Scrapings ☐ Punch Biopsy ☐ Shavings ☐ Needle Aspirates ☐ Bone Marrow (VL)	☐ 70-100% Ethanol ☐ Methanol ☐ Isopropanol		☐ Ambient 15-30°C
☐ **Culture**	☐ Dermal Scrapings ☐ Punch Biopsy ☐ Needle Aspirates ☐ Bone Marrow (VL)	☐ LDL Schneider's medium ☐ RPMI		☐ Ambient 15-30°C

Travel History:

…… …… …… ……

…… …… …… ……

Clinical History

Please include lesion location(s), duration of lesion and clinical appearance (nodule, ulcer, plaque, other, describe please)

…… …… …… ……

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…… …… …… ……

…… …… …… ……

PATIENT IDENTIFICATION	CONTACT INFORMATION
Patient identifiers <u>MUST INCLUDE</u>: **Full Name** _____ **Unique ID**_____ **DOB** _____ **Draw Date**_____ **Antibiotic Treatment (Type/dose/length):** _____	**Clinic/Center**_____ **Address** _____ **Physician Name** _____ **Phone** _____**Fax**_____ **Email** _____ **Alternate POC Name**_____ **Alternate POC Phone**_____ **Alternate POC Email** _____

PROCESSING LAB (For LDL use only)		
BARCODE	**DATE RECEIVED/LDL #/Initials**	**Quantity & Type Received**

Email (usarmy.detrick.medcom-wrair.mbx.leishmania-diagnostic@mail.mil**) a FedEx tracking number to ensure that all shipments sent to LDL can be tracked/received, IAW CAP GEN.40530**

Prior to collecting a specimen please contact WRAIR Leishmania Diagnostic Lab (LDL) by phone at: Office: 301-319-2297; Cell: 240-406-6510; 24 hours Emergency Number 240-595-7353; or email: usarmy.detrick.medcom-wrair.mbx.leishmania-diagnostic@mail.mil as most test require special collection kits from LDL for specimen submission. Specimens need to be shipped by priority overnight, express courier for arrival on a weekday (except federal holidays) within 24 hours of specimen collection.

Appendix 2

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.

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@mail.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@mail.mil

d. Shipping Address

Diagnostics and Countermeasures Branch
Walter Reed Army Institute of Research
ATTN: *Leishmania* Diagnostics Laboratory (LDL)
9100 Brookville Road, Building 508, Silver Spring, MD 20910

5. Turn Around Time (TAT)

TAT for a histopath smear (Giemsa) is 24 hours; TAT for RT-PCR 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 sufficient lead time prior to procedure(s) for LDL to priority express ship the kit to your facility.

Prior to collecting a specimen please contact WRAIR Leishmania Diagnostic Lab (LDL) by phone at: Office: 301-319-2297; Cell: 240-406-6510; 24 hours Emergency Number 240-595-7353; or email: usarmy.detrick.medcom-wrair.mbx.leishmania-diagnostic@mail.mil as most test require special collection kits from LDL for specimen submission. Specimens need to be shipped by priority overnight, express courier for arrival on a weekday (except federal holidays) within 24 hours of specimen collection.

Appendix 3

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@mail.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@mail.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@mail.mil

d. Shipping Address

Diagnostics and Countermeasures Branch
Walter Reed Army Institute of Research
ATTN: *Leishmania* Diagnostics Laboratory (LDL)
9100 Brookville Road, Building 508, Silver Spring, MD 20910

5. Turn Around Time

TAT for a histopath smear (Giemsa) is 24 hours; TAT for RT-PCR 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.

Appendix 4

Needle aspirates Procedures

1. Criteria for needle aspirate

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.

b. Please consult with the WRAIR *Leishmania* Diagnostics Laboratory (LDL) clinical consultant (usarmy.detrick.medcom-wrair.mbx.leishmania-diagnostic@mail.mil) before performing the procedure.

2. Needle Aspirate Procedure

a. Obtain needle aspirate in accordance with your standard medical procedures.

b. For culture, under aseptic conditions, put one-half (1/2) of aspirate volume into 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.

c. For *Leishmania* PCR, place the remainder of aspirate volume in a leak proof screw cap vial prefilled with 50ul of Alcohol (70-100% Ethanol, Methanol or Isopropanol).

3. Submission of specimens

a. Send the aspirate culture material, and vials with aspirate material in alcohol for *Leishmania* PCR 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

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@mail.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@mail.mil

d. Shipping Address

Diagnostics and Countermeasures Branch
Walter Reed Army Institute of Research
ATTN: *Leishmania* Diagnostics Laboratory (LDL)
9100 Brookville Road, Building 508, Silver Spring, MD 20910

5. Turn Around Time

TAT for a histopath smear (giemsa) is 24 hours; TAT for RT-PCR 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.

Appendix 5

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@mail.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 0.2 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@mail.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@mail.mil

d. Shipping Address

Diagnostics and Countermeasures Branch
Walter Reed Army Institute of Research
ATTN: *Leishmania* Diagnostics Laboratory (LDL)
9100 Brookville Road, Building 508, Silver Spring, MD 20910

5. Turn Around Time

TAT for a histopath smear (giemsa) is 24 hours; TAT for RT-PCR 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.

Appendix 6

Sera Procedures

1. Criteria for Sera Collection

Any individual presenting with these symptoms: fever, weight loss (cachexia; wasting) and/or hepatosplenomegaly (usually, the spleen is more prominent than the liver), who lived and/or travelled to *Leishmania* endemic region is suspected to have Visceral leishmaniasis (VL). Sera from such individuals can screened for antibody using the Kalazar Detect™ Rapid Test for Visceral Leishmaniasis (VL).

2. Sera Collection Procedure

Note: LDL recommends the use of serum separator blood collection tubes (SSTs) for acquisition of serum specimens for serological testing. If SSTs are not available, red top tubes can be used.

a. After blood collection, invert the blood collection tube gently 5 times. Further inversion may cause alterations in specimen integrity.

b. Position the tube in an upright position and allow the blood to clot for at least 30 minutes, but no longer than 2 hours after collection, before centrifugation.

c. Spin the blood collection tube as per manufacture's guidelines.

d. Transfer the sera to a secondary tube; indicate that the specimen is serum on the secondary tube as well as on the corresponding test request form.

3. Submission of specimens

a. Send sera directly to LDL on cold packs by overnight courier.

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
- Serum

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.

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@mail.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@mail.mil

d. Shipping Address

Diagnostics and Countermeasures Branch
Walter Reed Army Institute of Research
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9100 Brookville Road, Building 508, Silver Spring, MD 20910

5. Turn Around Time

TAT for a histopath smear (giemsa) is 24 hours; TAT for RT-PCR 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.