

SPECIMEN COLLECTION GUIDE

for Laboratory Diagnosis of Leishmaniasis

This guide focuses on laboratory diagnosis of cutaneous leishmaniasis. However, many of the principles also apply to mucosal and visceral leishmaniasis.

The tests offered are performed at CDC by the Laboratory of Reference Diagnostics, Division of Parasitic Diseases and Malaria (DPDM) and the Laboratory of Pathology, Infectious Diseases Pathology Branch (IDPB).

CONTACT INFORMATION

Pre-approval is required before submitting specimens for leishmaniasis testing, as each situation/case should be individualized with expert consultation. Please contact CDC, Parasitic Inquiries office by emailing leishmania@cdc.gov, or calling 404-718-4175, for pre-approval or for any questions related to diagnosing leishmaniasis—such as questions about obtaining and shipping specimens to CDC for various types of testing.

PREPARATION OF THE SKIN

- Thoroughly **cleanse** the pertinent area of skin (e.g., with 70% alcohol). It is preferable not to use iodine because it could inhibit parasite growth in culture. If iodine is used, it should be thoroughly washed off.
- Inject **anesthetic** (e.g., 1% lidocaine with epinephrine 1:100,000) through intact, cleansed skin, into the dermis underlying the area that will be sampled. Avoid high concentrations of anesthetic, which could inhibit parasite growth in culture.
- If **biopsy** specimens and/or dermal scrapings will be obtained, use a scalpel blade to debride scabs and devitalized tissue from the relevant areas.
- Then apply **pressure**, with sterile gauze, to achieve hemostasis.

SUMMARY OF TYPES OF SPECIMENS

To increase sensitivity, obtain several specimens using several techniques laid out in this document. For example, collect from different lesions or different portions of the same lesion. Be sure to sample the areas that appear the youngest, most active, and least apt to be superinfected with bacteria.

Place sterile samples collected into leishmanial culture medium for submission to CDC for *Leishmania* culture, PCR, and light-microscopic examination of touch preparations.

1. Obtain sterile **biopsy specimens** for:
 - a. culture and PCR
 - b. **impression smears** / touch preparations and histologic examination
2. Obtain sterile lesion, **needle aspirates** for PCR.
3. Of note, dermal **scrapings** can be obtained for Giemsa-stained thin smears.
Obtain the dermal scrapings last, to minimize the risk of contaminating the site.



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BIOPSY SPECIMENS

- Obtain sterile, full-thickness punch-biopsy specimens (~2–4 mm) at the active border of the lesion. Some practitioners recommend having the specimen include both “affected” and “unaffected” (e.g., nonulcerated) tissue. Divide the specimen into several portions (or obtain multiple biopsy specimens); the first portion described below can suffice for the testing at CDC:
- Place sterile tissue into leishmanial culture medium (*obtained by sending a request to leishmania@cdc.gov*) for submission to CDC—for *Leishmania* culture, PCR, and light-microscopic examination of touch preparations.

For local testing – other than by CDC:

- If indicated, use sterile portion(s) for other cultures (e.g., bacterial, mycobacterial, fungal).
- Use a portion for impression smears (see below; note that CDC can make the smears).
- Use a portion for histologic examination of tissue sections (fixed in 10% formalin; embedded in paraffin)—stained with H&E, Giemsa, and other special stains—to help exclude mycobacterial, fungal, and other infectious etiologies.

IMPRESSION SMEARS / TOUCH PREPARATIONS

Grasp the biopsy specimen with forceps. To avoid making a bloody smear, some practitioners recommend briefly placing the cut surface on gauze or a paper towel to remove excess blood. However, blotting the specimen might remove amastigotes that are present on the surface.

- Filet the specimen to increase surface area. Gently press the tissue—with a rolling or circular motion—onto a glass microscope slide. Repeat in a parallel row down the slide.
- Air dry the slide, fix it in methanol, and stain with Giemsa. Alternatively, CDC can fix/stain the slide (as well as make the impression smears after receipt of the tissue).
- After making the smears, the tissue is no longer sterile but still is usable for PCR. Consult CDC about handling/shipping by emailing leishmania@cdc.gov.

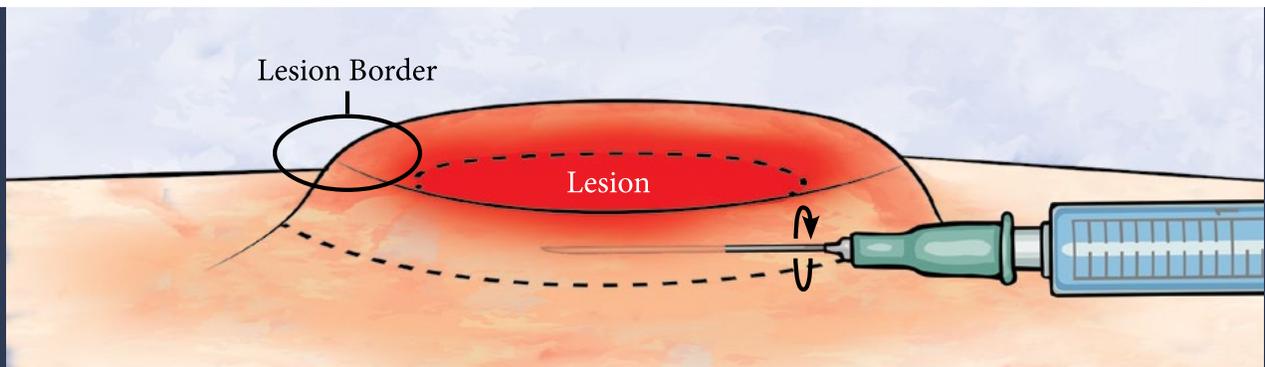
NEEDLE ASPIRATES

“Draw up” ~0.1 mL of preservative-free sterile 0.9% saline into a 1.0–3.0 mL syringe (the better suction obtained with syringes at the larger end of the range may be advantageous).

For ulcerative skin lesions, insert the needle, through intact sterile skin, into the dermis of the active border (see figure below). Use a 23- to 27-gauge needle; small-gauge needles are particularly useful for facial lesions.

Repeatedly move the needle back and forth under the skin, tangentially to the ulcer, simultaneously rotating the syringe and applying gentle suction, until pink-tinged tissue juice is noted in the hub of the needle. If necessary (if no aspirate is obtained), inject 0.05–0.1 mL saline under the skin and resume suction. After the aspirate is obtained, withdraw the needle from the skin and discharge the aspirate into the leishmanial culture medium (each aspirate into a different tube).

Although thin smears of aspirates can be made, they typically are suboptimal, unless a cytopsin preparation is used.



NOTE: If pertinent, consult CDC about obtaining/handling other types of fluids/aspirates (e.g., **bone marrow**).

SCRAPINGS (for thin smears)

- **As described above in 'Preparation of the Skin':** First thoroughly debride the relevant portions of ulcerative lesions; then apply pressure to ensure good hemostasis (to avoid making a bloody smear). A convenient location for obtaining specimens from ulcerative lesions is the area immediately adjacent to or beneath the active border (e.g., beneath the necrotic lip of the lesion).
- **Slit-skin smear technique:** Some practitioners first make an incision before obtaining a scraping. For this technique, pinch the skin to exclude blood and use a scalpel blade to incise a slit, several millimeters long and deep, through intact skin into the upper dermis. For ulcerative lesions, consider starting the incision in the active border and proceeding radially out from the ulcer, across several millimeters of intact skin. (This technique also can be used for nodular lesions.)
- Obtain tissue juice and flecks of tissue by scraping the upper dermis (e.g., beneath the necrotic lip of the lesion or along the walls of the incision, if one was made in the skin) with a sharp instrument such as a scalpel blade or stainless steel spatula. After obtaining as much tissue pulp as possible, make as thin a smear as possible. Air dry the slide, fix it in methanol, and stain with Giemsa. Alternatively, CDC can fix/stain the slide. Although dermal scrapings also can be cultured, the risk for contamination is high.