

Practical Guide for Specimen Collection and Reference Diagnosis of Leishmaniasis

— CDC's Division of Parasitic Diseases and Malaria —

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

CONTACT information & SHIPPING ADDRESS:

Please contact CDC laboratory staff at 404-718-4175 or by email (DPDx@cdc.gov) about all issues related to diagnosing leishmaniasis—such as questions about obtaining and shipping specimens to CDC for various types of testing. Each situation/case should be individualized, with expert consultation. Please visit our website (<http://www.cdc.gov/laboratory/specimen-submission/form.html>) to obtain the specimen submission form (**form CDC 50.34**). Please include the submitter's fax number, as well as details about the patient's travel history and clinical manifestations. Send the completed form and the slides/specimens—ideally, by **overnight mail, for weekday delivery**—to:

SMB/STAT, ATTN: Unit 52
Dr. Marcos de Almeida
1600 Clifton Road
Atlanta, GA 30333
Telephone: 404-718-4175; email: DPDx@cdc.gov

OVERVIEW of diagnostic services provided gratis by CDC:

- **Examination of slides** (e.g., of biopsy specimens, impression smears, and dermal scrapings)
- Provision of leishmanial **culture medium** (*see below about obtaining medium in advance*)
- ***In vitro* culture and PCR** for diagnosis of leishmaniasis and species identification. *Of note:*
 - PCR does not require additional specimens besides the tissue obtained for culture (*see below*)
 - Cultures typically are monitored for ~4 weeks before they are considered negative
- **Serologic testing** using the rK39 Rapid Test, for detection of antibodies against organisms in the *Leishmania donovani* species complex; useful primarily for visceral leishmaniasis

CULTURE MEDIUM—2 scenarios (*with vs without obtaining medium in advance from CDC*):

If possible, request culture medium before obtaining biopsy specimens or aspirates. CDC can provide tubes of culture medium by overnight express mail. Keep the tubes refrigerated until they are used; bring to room temperature shortly before inoculation. Once inoculated, keep at room temperature and mail to CDC **as soon as possible**, by overnight mail (*preferably, for arrival within 24 hours of when the specimens were obtained*). Please do not ship on a Friday; we do not have weekend delivery service. Do not ship cultures on cold packs or dry ice, but keep the cultures away from heat.

If culture medium was not obtained in advance, consult CDC to discuss the options below:

- If possible, place the specimen in a tube/vial that contains a sterile buffered medium (e.g., buffered saline, RPMI, Eagle's growth, Schneider's, Tobie's), with a neutral pH (~7–7.4). Use enough fluid to cover the specimen. Ship at room temperature, if the specimen will arrive at CDC within 24–48 hours. Otherwise, consult CDC about whether to refrigerate and ship on a cold pack, to minimize the potential for overgrowth of skin flora. (*Of note, CDC's culture medium contains antimicrobial agents.*)
- If no buffered medium is available, place the specimen in a sterile tube/vial; refrigerate the specimen; and ship it on a cold pack, by overnight mail, to CDC.

Summary of TYPES OF SPECIMENS (see figure on p. 4; also see text on pages 2–3):

If possible, to increase sensitivity, use several techniques and obtain several specimens per technique (e.g., from different lesions or different portions of the same lesion). Preferentially sample areas that appear the **youngest, most active, and least apt to be superinfected**.

- Obtain sterile biopsy specimens for **culture and PCR**. Also use biopsy specimens for **impression smears** (i.e., touch preparations) and **histologic examination**.
- Obtain sterile lesion aspirates for leishmanial **culture** (PCR).

Of note, dermal scrapings can be obtained for Giemsa-stained thin smears. However, obtain the dermal scrapings last, to minimize the risk of contaminating the site.

PREPARATION of the skin:

Inject anesthetic (e.g., 1% lidocaine with epinephrine 1:100,000) through intact skin—**cleansed with 70% alcohol**—into the dermis underlying the area that will be sampled. Avoid high concentrations of anesthetic, which could inhibit parasite growth in culture.

- 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.
- 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.

BIOPSY specimens:

Obtain sterile, full-thickness, punch-biopsy specimens 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 **3 portions** (or obtain multiple biopsy specimens):

- Use 1 sterile portion for **leishmanial and other cultures** (*bacterial, mycobacterial, and fungal*); the portion placed in leishmanial culture medium also can be used by CDC for **PCR**.
- Use 1 portion for **impression smears** (see below).
- Use 1 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.

Tissue 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 not sterile but still is usable (e.g., for PCR). Consult CDC about handling/shipping (e.g., place tissue in a tube/vial of buffered medium); see above.

Needle ASPIRATES:

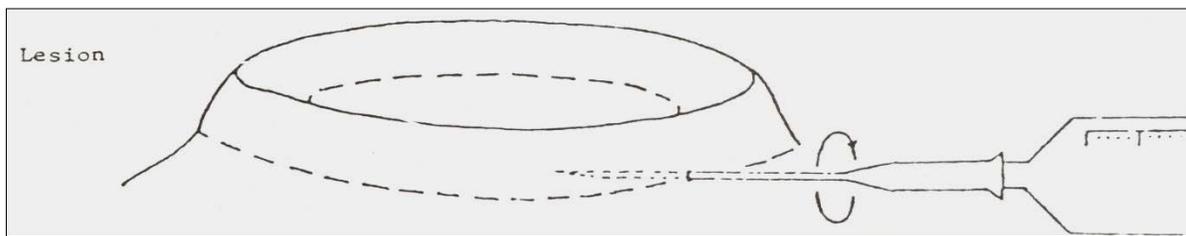
“Draw up” ~0.1 mL of preservative-free sterile 0.9% saline into a 1.0–3.0 mL syringe (*the better suction obtained with syringes at the larger end of the range may be advantageous*).

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 cytospin preparation is used.

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



Dermal SCRAPINGS (*for thin smears*):

As discussed above: 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 several millimeter long and deep slit 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 (e.g., 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.

Figure: **Types of tissue specimens and testing**
(see text for more details and consult CDC lab staff)

