

Practical Guide for Laboratory Diagnosis of Leishmaniasis

— CDC's Parasitic Diseases Branch —

(See summary figure on the last page)

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

CONTACT information & SHIPPING ADDRESS:

Please contact **Frank Steurer** about all laboratory issues related to diagnosing leishmaniasis—such as questions about obtaining culture medium from CDC, collecting specimens for various types of testing, shipping specimens to CDC (*including the address and forms to use*), and getting test results. Each situation/case should be individualized, with expert consultation.

Contact information:

Frank Steurer (tele: 404-718-4175; email: fsteurer@cdc.gov; fax: 404-718-4195)

CDC, Parasitic Diseases Branch; 1600 Clifton Road, Mailstop D-64 (Bldg 23, Room 9-439)
Atlanta, GA 30329

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 getting it in advance*)
- **In vitro culture** (followed by isoenzyme analysis) and **PCR** analysis—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** (*not discussed below*)—which generally is more useful for visceral leishmaniasis (and some cases of mucosal leishmaniasis) than for cutaneous leishmaniasis

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

If possible, request culture medium before obtaining tissue specimens. 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 on a weekday, within 24 hours of when the specimens were obtained)**. 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, leishmanial culture medium from CDC 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 & obtain several specimens per technique (e.g., from different lesions or different portions of the same lesion). Preferentially sample lesions (or portions thereof) that appear the **youngest, most active, and least apt to be superinfected**.

- Obtain sterile **biopsy specimens** for **culture / 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, if biopsy specimens and/or aspirates will be obtained from the same lesions, obtain dermal scrapings last, to minimize the risk for contamination of the site.

PREPARATION of skin (*before obtaining specimens*):

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 (e.g., with 70% alcohol) the pertinent area of skin. 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.

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 also might remove amastigotes that are present on the surface.

- Filet the specimen to increase the 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; then fix it in methanol and stain with Giemsa. Alternatively, CDC can fix/stain the slide (as well as make impression smears after receipt of the tissue).
- After making the smears, the tissue no longer is 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:

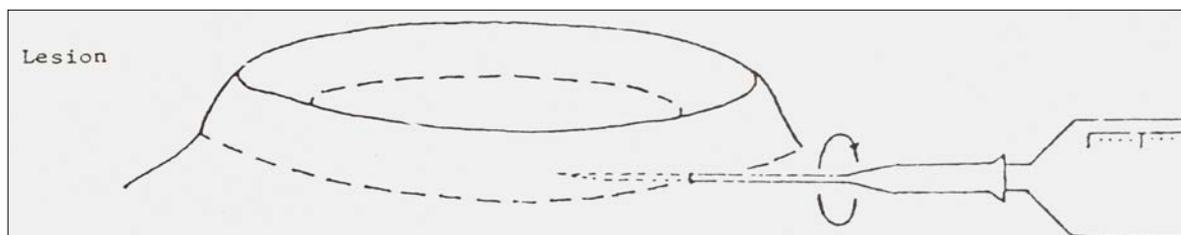
“Draw up” ~0.1 mL of preservative-free sterile 0.9% saline into a 1.0–3.0 mL syringe (*the better suction obtained with syringes at the larger end of the range may be advantageous*).

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. 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, stainless steel spatula, or dental/dendritic broach). After obtaining as much tissue pulp as possible, make as thin a smear as possible. Air dry the slide; then 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; also consult CDC lab staff)

