

# SPECIMEN SUBMISSION 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](mailto: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.

## OVERVIEW OF LEISHMANIASIS DIAGNOSTIC SERVICES PROVIDED BY CDC

- Provision of leishmanial **culture medium** (see below about obtaining medium in advance)
- **Leishmania species identification**, using PCR and DNA sequencing
- **In vitro culture** for species identification
- **Immuno-Histo-Chemistry (IHC)**
- **Examination of slides** (touch-preps and/or histopathologic slides)
- **Serologic testing for visceral leishmaniasis**

## “LEISHMANIAL MEDIUM” / CULTURE MEDIUM:

If possible, request “leishmanial medium” / culture medium from CDC before obtaining biopsy specimens or aspirates. CDC can provide tubes of leishmanial medium by overnight express mail at no charge. CDC’s leishmanial medium contains antimicrobial agents that will minimize the risk for overgrowth of skin flora. Request leishmanial medium by sending an email to [leishmania@cdc.gov](mailto:leishmania@cdc.gov); please remember to provide your complete shipping address, and your direct phone number.

If CDC-provided leishmanial medium for biopsies was not obtained prior to specimen collection, these are some options:

- Place the **biopsy specimens** 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).
  - o Use just enough fluid to cover the biopsy specimen.
  - o Do not wrap biopsy specimen in gauze.
  - o If no buffered medium is available, place the biopsy specimen in a sterile tube/vial.
  - o Keep the biopsy specimen refrigerated and ship it on cold pack, by overnight courier to CDC.



**U.S. Department of  
Health and Human Services**  
Centers for Disease  
Control and Prevention

- Ship **serum specimens** at ambient temperature or on cold packs (not on dry ice).
- Ship all **other specimens** (blood, bone marrow etc.) with a cold pack.
- If in doubt about the form or proper submission process, please email [leishmania@cdc.gov](mailto:leishmania@cdc.gov) with any questions before submitting the specimen and 50.34.

## INSTRUCTIONS FOR COLLECTION OF BIOPSY AND ASPIRATE SPECIMENS

See the "[Specimen Collection Guide](#)" for CDC laboratory diagnosis.

## LEISHMANIA SPECIES IDENTIFICATION (PCR/DNA SEQUENCE ANALYSIS)

### Acceptable specimen types:

- fresh tissue biopsies
- aspirates from skin lesions
- bone marrow
- spleen
- liver
- dermal scrapings
- EDTA whole blood

**Turn-around time:** 2 weeks after specimen receipt at CDC.

## IN VITRO CULTURE

Culture will routinely be attempted on all unpreserved (fresh) specimen types submitted for the *Leishmania* species identification test. However, culture results will be reported for clinical diagnostic purposes only if the culture results contradict the prior PCR/sequencing results (e.g., culture positive vs. PCR negative). Of note: additional specimens might be requested, if required to help resolve any discordant or inconclusive results.

### Acceptable specimen types:

- Fresh, unfixed sample in leishmanial medium.

**Turn-around time:** 6 weeks after specimen receipt at CDC, if applicable.

## IMMUNO-HISTO-CHEMISTRY (IHC)

Please note that tests on formalin-fixed specimens, including FFPE blocks, require pre-approval by the CDC Laboratory of Pathology. See specimen submission instructions below. If the pathologic examination/IHC supports a diagnosis of leishmaniasis, DNA may be extracted and forwarded to molecular lab for PCR testing, to obtain a species-level diagnosis. However, please note that PCR testing on formalin-fixed tissue has reduced sensitivity, compared to IHC.

### Acceptable specimen types:

- Formalin-fixed specimens

**Turnaround time:** 8 weeks after specimen receipt at CDC.

## EXAMINATION OF SLIDES

Touch-prep slides are prepared and diagnosed by microscopy at CDC using fresh biopsy specimens submitted for *Leishmania* species identification. Microscopy results are reported as "no parasites found" or "amastigotes present: species identification is not possible from microscopy alone."

### Acceptable specimen types:

- Histologic stained slides prepared outside of CDC are accepted for leishmaniasis clinical diagnostics. Unstained, fixed biopsy touch-prep smears may also be accepted.

**Turnaround time:** 7 days after specimen receipt at CDC.

## SEROLOGIC TESTING FOR VISCERAL LEISHMANIASIS

CDC offers the InBios Kalazar Detect Rapid Test for Visceral Leishmaniasis. It detects antibodies against organisms in the *Leishmania donovani* species complex.

### Acceptable specimen types:

- Serum

**Turnaround time:** 3 weeks after specimen receipt at CDC.

## SPECIMEN SUBMISSION INSTRUCTIONS

### A. For *Leishmania* species identification (PCR and culture on fresh/unpreserved specimens); Examination of slides; Serologic testing:

Please contact your State Public Health Department Lab (SPHDL) before submitting specimens to CDC for diagnostic testing. If your SPHDL would prefer that the specimen be routed through their facility, the specimen shall be submitted accordingly.

Please visit <https://www.cdc.gov/laboratory/specimen-submission/form.html> to obtain the specimen submission form (**form CDC 50.34**). One completed CDC 50.34 form is required per specimen submitted (e.g. two biopsies are collected on different dates, two specimen types from the same date, etc.). It is important to fill out this form correctly. The form shall be filled out electronically and then printed to generate barcodes. Please ensure that the following information is entered:

- Patient details (shall match the patient identifiers used on the specimen container)
- Specimen details
- SPHDL information: please use the drop-down list provided in the form
- Original Submitter information: please name a person as a Point of Contact for delivery of the diagnostic report, and include address, phone, and fax numbers. **Incomplete information in this section may delay or prevent delivery of the final CDC report.**
- On the second page, please include details about the patient's travel history and clinical manifestations. This information will assist the correct interpretation of the laboratory diagnostic test results.

If your SPHDL gives permission for **direct submission to CDC**, then send the completed form with the specimen(s)—ideally, by overnight courier, for weekday delivery—to:

#### Serum specimens:

**Centers for Disease Control and Prevention**  
RDSB/STATT **Unit 57**  
1600 Clifton Road  
Atlanta, GA 30329-4027

#### Biopsies, blood, and other specimens:

**Centers for Disease Control and Prevention**  
RDSB/STATT **Unit 52**  
1600 Clifton Road  
Atlanta, GA 30329-4027

**NOTE 1:** Submit biopsy specimens in the CDC-provided leishmanial medium at ambient temperature as soon as possible following collection. If shipping is delayed, refrigerate specimens in CDC-provided leishmanial medium, and then ship at ambient temperature.

## **B. For *Leishmania* IHC testing of formalin-fixed specimens;**

Analysis of formalin-fixed specimens, including FFPE tissue blocks, shall be approved in advance by the CDC Laboratory of Pathology. Please contact [pathology@cdc.gov](mailto:pathology@cdc.gov) for specific submission instructions (and [leishmania@cdc.gov](mailto:leishmania@cdc.gov)). Include the following information in your email:

- a)** A brief clinical history
- b)** A copy of the surgical pathology report or autopsy report (prelim reports are acceptable)
- c)** A listing of available formalin-fixed specimen types (wet-tissue, paraffin-blocks, and/or slides)
- d)** Relevant clinical, gross pathology, or microscopic pathology digital images, as available.

## **REPORTING OF RESULTS**

- Results will be issued electronically to the SPHDL indicated on the submission form. That SPHDL will be responsible for forwarding the CDC lab report to the address entered in the Original Submitter section.
- Allow sufficient time for results to be completed and delivered. If the CDC report is not received within the listed turnaround time of two weeks (*Leishmania* species identification on fresh/unpreserved specimens), three weeks (Leishmaniasis serology) or eight weeks (testing of formalin-fixed specimens, including FFPE tissue blocks) then please inquire from your SPHDL before reaching out to CDC.