ALBERTA PRECISION LABORATORIES
Leaders in Laboratory Medicine

LEISHMANIA SPECIMEN COLLECTION

Version 1.0 Effective May 28, 2022

AVAILABLE TESTS

- Microscopic examination of slides (tissue and fluid aspirates, biopsy impression smears, skin scrapings etc)
- In vitro culture and PCR for detection of leishmaniasis and species identification
    - *NOTE: PCR does not require additional specimens besides the tissue/fluid obtained for culture*
- Serologic testing (including the K39 test for detection of antibodies against *Leishmania donovani* species complex; used for visceral leishmaniasis)

INSTRUCTIONS FOR SPECIMEN COLLECTION: CUTANEOUS LESIONS

PREPARATION OF THE SKIN:

- Clean lesion thoroughly with 70% alcohol. Avoid using iodine as it can inhibit parasite growth in culture. Local anesthesia (e.g., 1% lidocaine) may be used to reduce discomfort. *Avoid high concentration of anesthetic – it can inhibit parasite growth*
- Use a scalpel blade to debride scabs and devitalized tissue from the relevant areas. Wipe off any blood with sterile gauze (and apply pressure if needed).

BIOPSY SPECIMENS

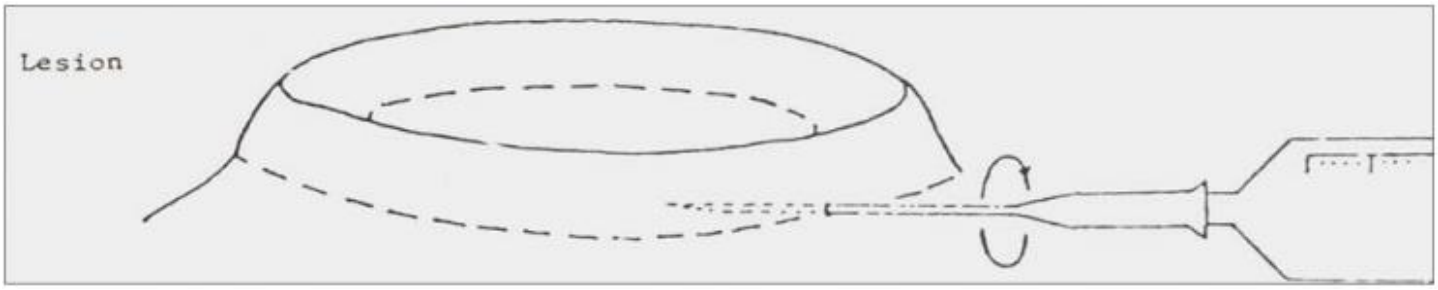
Obtain sterile, full-thickness, punch-biopsy specimens at the active border of the lesion (multiple biopsy specimens if possible to increase diagnostic yield)

- Use 1 sterile portion for leishmania cultures (this portion can also can be used for PCR).
- Use **1 <u>separate</u> portion for impression smears** (see below).
- Place the specimen for culture in a sterile tube or vial that contains a small amount of sterile saline (enough to cover the sample with some room to spare) or, if available, in leishmania culture/transport medium
- Forward the specimen to the laboratory as soon as possible

NEEDLE ASPIRATES

- Draw ~0.1 mL of preservative-free sterile 0.9% saline into a 1.0–3.0 mL syringe. Use the 23- to 27-gauge needle (or smaller for facial lesions)
- Move the needle back and forth 3-5 times, tangentially to the ulcer, simultaneously rotating the syringe and applying suction, until pink-tinged fluid is noted in the hub of the needle. If necessary, inject 0.05–0.1 mL saline under the skin and resume suction (see below).
- Withdraw the needle and discharge the aspirate into a sterile tube or vial that contains a small amount (appx 0.5 mL) of sterile saline (if available, place aspirate in leishmanial culture/transport medium)
- Forward the specimen(s) to the laboratory as soon as possible

NOTE: For ulcerative skin lesions, insert the needle, into the dermis of the active border of the lesion



(from: CDC — Diagnosis of Leishmaniasis — September 2015)

NOTE - smears of aspirates are suboptimal. Consult microbiologist on call for advice.

SKIN SCRAPINGS

- For **ulcerative lesions**, obtain specimens from area closest to or beneath the active border (the necrotic lip of the lesion). Gently scrape the upper dermis with a sharp instrument (e.g., a scalpel blade) to collect fluid and flecks of tissue
- Modified (slit-skin) technique
    - Use a scalpel blade to make slit through intact skin into the upper dermis - if possible, starting the incision in the active border and proceeding radially out from the ulcer
    - This technique can also be used for **nodular lesions**.
- Place the specimen in a sterile tube or vial that contains a small amount of sterile saline (enough to cover the sample with some room to spare) or, if available, in leishmania culture/transport medium.
- Using a separate blade collect and smear material on glass slides for direct staining (see below)
- Forward the specimen(s) to the laboratory as soon as possible

NOTE: if leishmania culture/transport medium is used for collection
- ➔ Store media in the refrigerator at 4 ^{0}C until used
- ➔ Prior to inoculation, bring media to room temperature
- ➔ After inoculation, media must be kept at room temperature at all times

INSTRUCTIONS FOR SPECIMEN COLLECTION (OTHER THAN SKIN)

BIOPSY SPECIMENS

Obtain biopsy specimens (bone marrow, spleen, liver, lymph node, other tissues) as per routine; multiple specimens if possible to increase diagnostic yield)

- Use 1 sterile portion for leishmania cultures (this portion can also can be used for PCR).
- Use **1 <u>separate</u> portion for impression smears** (see below).
- Place the specimen for culture in a sterile tube or vial that contains a small amount of sterile saline (enough to cover the sample with some room to spare) or, if available, in leishmania culture/transport medium
- Forward the specimen to the laboratory as soon as possible

DIRECT SMEARS FOR MICROSCOPICAL EXAMINATION

TISSUE IMPRESSION SMEARS (TOUCH PREPARATIONS)

- Grasp the biopsy specimen with forceps.
- 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 and forward to the laboratory

Note: after making the smears, the tissue is not sterile but still is usable (e.g., for PCR).

PRESS-IMPRINT-SMEAR METHOD

Recommended (quick, low cost, and relatively sensitive).

- Place biopsy sample on a glass slide, and use another glass slide to cover the tissue fragment in the form of a sandwich.
- On a firm surface, squeeze the tissue fragment between both slides. Apply pressure to the middle of the slides causing tissue cells and fluids to spread out across both slides' surfaces that are in contact with the sample.
- Air dry the slide and forward to the laboratory

SPECIMEN SHIPMENT

Forward all specimens (culture, PCR, smears/touch preparations) to the Provincial Laboratory as soon as possible after collection. Biopsies, aspirates and scrapings for leishmania cultures MUST be received by the lab within 24 hrs of collection (preferably 12 hrs or less)
If any delays are anticipated, consult Prov Lab Microbiologist on Call re whether to ship on a cold pack, to minimize the potential for overgrowth of skin flora (for cutaneous lesions).

NOTE – ***AFTER INOCULATION WITH PATIENT SAMPLE LEISHMANIA TRANSPORT MEDIA MUST BE KEPT AT ROOM TEMPERATURE AT ALL TIMES!!***