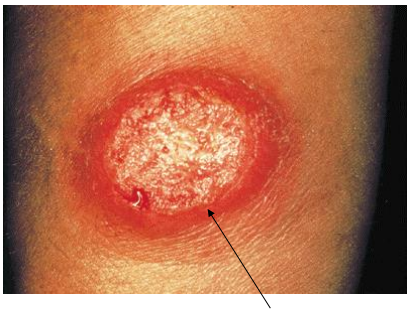
CLINICAL DIAGNOSTIC PARASITOLOGY LABORATORY

NOTES FOR TAKING MATERIAL FOR SUSPECTED LEISHMANIASIS

Material may be sent to examine for amastigotes from patients with suspected cutaneous or visceral leishmaniasis. Parasites may be difficult to identify and smears, cultures and histology may be necessary to make a diagnosis. It is important to know patients' travel details and suspected country where they were infected to enable correct primers to be used in the test.

Cutaneous leishmaniasis

If the lesion is old and 'crusted' it is probably too late to find parasites as these lesions are usually self-limiting. If the lesion has a raised, red margin the following specimens should be taken:



Diagnosis

- •Biopsy edge of ulcer
- •Impression smear -> geimsa
- •Histology
- •PCR
- •Culture

Sampling of Lesions

A rigorous approach to making a parasitological diagnosis is important because of the toxicity associated with systemic sodium stibogluconate treatment, which remains the mainstay of treatment in these guidelines. Suspicious lesions should be sampled by aspiration (without dilution), scraping (either directly from an ulcer or from skin slits) and biopsy (usually with a 4 mm punch biopsy tool). Skin biopsy is the most useful of these methods, but lesions that can not be biopsied (*eg.* on a finger, ear or face) should still be aspirated and scraped. Sampling should only be performed by staff that are properly trained in these techniques, otherwise poor results and complications may occur.

Biopsy

Two standard 4 mm punch biopsies from the nodular part or raised edge of the lesion should usually be performed. Blood contamination can be prevented by using local anesthetic with adrenaline (where anatomically-permitted), applying pressure haemostasis before removing the core and gently rolling the core on gauze after its removal. The use of iodine should be avoided as this causes problem with subsequent PCR.

- **Impression smears:** One of the punch biopsies should be cut in half, the freshly cut surface is firmly pressed (not smeared) to create a series of touch preparations onto **clean** microscope slides, once dry smears should be methanol fixed for 1 minute.
- **PCR:** The tissue used for the smear (size of a rice grain) should then be placed in 300µl PCR (Qiagen ATL) buffer OR in 10 % ethanol OR placed in a dry, sterile container.
- If wishing to perform **Histology**: The second punch biopsy should be fixed in 10% buffered formol saline. Giemsa and H&E stained sections should be prepared for microscopy. The CDPL cannot prepare slides for histology but will examine stained sections if required.
- If only fixed, histological block is available, several **10µm** thick sections should be cut, placed onto microscope slides BUT NOT STAINED and sent for PCR.

Aspirate smears

If unable to perform a punch biopsy aspirate smears should be taken, sensitivity of these smears is variable.

Aspiration is performed from the nodular part or raised edge of a lesion.

- A 0.5 mm diameter (orange) needle is tightly connected to an empty syringe.
- The needle is slowly advanced with negative pressure applied in a straight line (to avoid blood contamination) along the edge of an ulcer or into the centre of a solid lesion. It is withdrawn in a similar manner, taking care not to draw any air into the syringe.
- The needle is then disconnected, air is drawn into the syringe, the needle is re-attached and the contents are blown out rapidly onto a clean, polished and alcohol-free microscope slide. The aspirate should be gently spread on the slide using the tip of the needle to achieve best results on microscopy. This slide should be labelled appropriately (to identify the patient and the sampling technique used) and, once dry, methanol fixed for 1 minute.

Scraping

Scrapings may be performed either from the surface of an ulcer or from skin slits. Ulcer scraping should be taken from both the edge and the centre of the lesion. Slit skin scrapings should be taken from the nodular part or raised edge of a lesion.

- scrapings should be smeared onto clean, polished and alcohol-free microscope slides. This slide should be labelled appropriately (to identify the patient and the sampling technique used) and, once dry, methanol fixed for 1 minute.

Smears
Should be fixed in methanol for 1 minute and stained with Giemsa or Leishman's stain using buffered water, pH 7.2.

PCR
Tubes containing ATL (lesions) or AL (marrow/blood) buffer (Qiagen) can be supplied on request. The buffer contains a guanidine thiocyanate based solution and should be stored at room temperature in the dark both before and after adding sample material.
Please note that the PCR screening test gives a Positive/Negative result. Patients positive with screening PCR will have a second speciation PCR test performed.

Histology
Tissue should be processed at source hospital, we are happy to examine tissue sections stained with Giemsa and H&E.

Visceral leishmaniasis
For suspected visceral leishmanisis the following specimens are recommended

- **Smears: thin marrow** smears should be made, fixed in methanol and stained with Giemsa, pH7.2. Alternatively fix the dry smears in methanol for 1 minute and send for staining.
- **PCR**: **Marrow** collected in EDTA may also be used for PCR.
- It is sometimes possible to isolate parasites from **blood**. Anticoagulated (**EDTA**) blood should be sent.

Serological tests are useful in cases of suspected VL who do not have HIV infection, in suspected VL patients who are HIV positive sera is often non-reactive. In CL serology is of little value.