## Skin Specimen

small piece of skin are examined for Onchocerciasis (river blindness). The worm live in the subcutaneus tissues. cornescleral punches are most commonly used to take bloodless skin snips.

### Collection of Specimens

- The nodules are round and hard, 1-5cm in diameter, when pushed with the finger they slide about under the skin. Take the specimen from the skin in the center of nodules.
- if a corneoscleral punch is not available, use a sterile disposable scalpel, and needles or punches
- Disinfect the skin area with a gauze pad dipped in alcohol.
- Place the cutting edge of the scalpel or razor blade on the stretched skin above the point of the needle. cut with a quick stroke the piece of skin pulled up by the point of the needle. The specimen should be about 2-3 mm wide it should remain attached to the tip of needles
- The specimen should not be blood-stained.
- the biopsy must be bloodless to avoid possible contamination with blood parasites.

### Examination of Specimen

1. put a drop of distilled water or saline on microscope slide.
2. put the small piece of skin in the drop and place a cover slide, not press on the skin or coverslip.
3. Let the mount stand for 30 min, then examine under the microscope with 10x, if the microfilaria are present they can usually be seen wringgling around in the saline, if they not present, skin snip should be allowed to stand in saline solution for 4 hours.

   Quantitative examination
   1. Wieght a skin with a balance (1-10mg).
   2. Transfer the specimen into one hole of microtitration plate.
   3. Add 0.1 ml of saline solution
   4. cover the plate to avoid evaporation of water and leave to incubate at room temprature for 24 hours
   5. count the number of microfilaria in the saline by 10x and express the result per wet wieght of specimen.

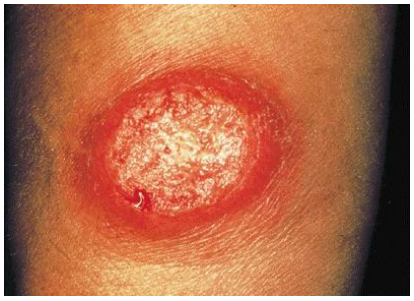
6. collaginase digestion of the skin over more than 24h. may increase the sensitivity of examination in specimen from patients with low parasite load. the digestion can also be performed on ethanol fixed material stored at ambient temprature .

## Lieshmaniasis Sample

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

**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:



biopsy edge of ulcer
smaer- gimsa
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 cannot 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.
- **Histology**: The second punch biopsy should be fixed in 10% buffered formol saline. Giemsa and H&E stained sections should be prepared for microscopy.

**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 reattached and the contents are blown out rapidly onto a clean, polished and alcoholfree 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.

## Sputum Sample

Microscopic examination of sputum is used in identifying *Paragonimus westermani* eggs, *Strongyloides stercoralis* larvae, *Ascaris lumbricoides* larvae, hookworm larvae, and rarely *Entamoeba histolytica*. Sputum should be obtained from the lower respiratory passages rather than a sample consisting mainly of saliva. Sputum specimens should be collected first thing in the morning. A sputum sample can be examined in several ways:

- The unfixed specimen may be centrifuged and then the sediment examined as a direct wet mount.
- If the sputum is too viscous, an equal volume of 3% sodium hydroxide may be added, then centrifuge, and examine the sediment.
- The specimen may be preserved in 10% formalin and a formalin-ethyl acetate concentration procedure may be completed and the sediment examined using either a wet mount or a stained preparation.
- The specimen may also be preserved in PVA if protozoa are suspected and stained with trichrome stain.