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Section: Parasitology Manual	Subject Title: Laboratory Procedures for Blood and Tissue Parasites	
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***Leishmania* spp.**

PRINCIPLE

Leishmania is found in macrophages around the point of infection.

SPECIMEN

Scrapings from infected areas.

SAFETY

Blood tubes should only be opened in a running biological safety cabinet. Any spills should be cleaned up immediately. Gloves must be worn when preparing samples and any contact with contaminated sharps should be reported. *Leishmania* can be transmitted from blood samples, therefore follow up of exposure is recommended.

REAGENTS

Absolute methanol
Giemsa stain.
NNN media (see appendix)

PROCEDURES

This is considered to be a non-routine procedure therefore it should only be performed by experienced personnel.

1) Smears:

- a) Prepare smears - from touch preps of biopsy material or from an aspirate from the edge of a lesion.
- b) Dry and fix in methanol.
- c) Stain with Giemsa (as for malaria smears) and examine under oil immersion (X1000) for the presence of amastigotes.

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***In Vitro* Cultivation**

- a) Inoculate biopsy material or aspirate from the lesion to prewarmed (room temperature) NNN media culture tubes and incubate at room temperature. Attending physician inoculates specimen at bedside.

- b) Examine culture using a wet prep at 10, 14, and 21 days for the presence of promastigotes.

QUALITY CONTROL

- Make sure that media is not outdated or contaminated.
- Ensure that the microscope has been calibrated in the last year and that the results of the calibration are displayed on the microscope base.
- Include a control slide if Giemsa Stain is used. Red cells should stain grayish, white cell nuclei stain red-purple and cytoplasm stains bluish.

REPORT

Report the presence of *Leishmania* sp. promastigotes from culture and amastigotes from biopsies or aspirates. Definitive species identification requires isoenzyme analysis or molecular methods.

AUTHOR

Ian Crandall

REFERENCES

Gillespie, S.H. and Hawkey, P.M. Medical Parasitology: A Practical Approach. IRL Press New York 1994 pp151-176