

VACUUM ASPIRATORY PUNCTURE SYSTEM FOR *LEISHMANIA* CULTURING, ISOLATION AND TRANSPORT. PRELIMINARY REPORT

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Different *Leishmania* species transmitted by several phlebotomine sandfly species are responsible for various clinical forms of tegumentary and visceral diseases that occur both in man and wild and domestic animals³, affecting 12 million people in many parts of the world⁸. Exposure of fighting troops to desert zoonotic leishmaniasis in the recent Middle East conflict will place this infection in evidence worldwide. In Brazil, 26,000 new cases of tegumentary and visceral leishmaniasis are annually recorded by the Brazilian Ministry of Health.

The isolation for taxonomic characterization of these parasites in human beings, reservoirs and vectors is crucial for the clinical assessment (prognosis and therapeutics), as well as for the epidemiologic evaluation aimed at control measures.

The aspiratory puncture for "in vitro" culturing and isolation of *Leishmania* has been referred to as the most effective method for cutaneous^{1,2,4,7}, mucosal^{1,7}, and visceral^{5,6} diseases.

We are proposing, for aspiratory puncture being carried out in field conditions on patients and animals a safer collection and culture system with acceptable contamination risk based on the adaptation of the blood collection vacuum system available in the market (VACUTAINER[®] - Becton - Dickinson) as show in Fig. 1.

The system consists of a plastic-made support that holds a needle with points at both ends and a sterile glass tube with rubber cover containing vacuum and classic culture medium NNN (blood agar with 3% Bacto agar 3ml and Liver Infusion Trypticase - Brain Heart Infusion liquid phase, or Schneider's *Drosophila*

medium, 1-2 ml with 10% inactivated fetal calf serum, streptomycin 250µg/ml and 5 fluorocytosine 500µg/ml).

Blood agar fused at 50°C is added into sterile glass tubes which are kept in inclined position until cooling. LIT-BHI or Schneider overlay is then added. The monophasic overlay can also be used alone.

After the rubber cover is placed, the vacuum is resumed through the aspiration of 5 to 10ml of air from the tube using a 20ml syringe with a 13 gauge needle.

The external side of the border of ulcerated or nodular, skin or mucosa lesion, lymph nodes, liver or spleen should be punctured and aspirated after previous asepsis and anesthesia.

On use, the system should be reverted and inclined so that the medium's liquid phase can cover the hollow center of the tube cover. The needle should be introduced the selected area with rotatory movements, and pulled out a few millimeters. After 15-30 seconds, the glass tube should be pushed while firmly holding the support with the thumb so that the rubber cover is pierced by the rear end of the needle and the negative pressure inside the tube aspirates a small amount of inter-tissue fluid into the medium's liquid phase. The tube should be removed from the support and the needle kept submerged in the puncture zone so as to avoid air coming into from outside. The tube is then returned to the vertical position, the sample identified and then sent to the laboratory.

In bone marrow puncture, the aspirate can be made by introducing the system's needle into the special bone puncture needle after this has been introduced into the bone marrow.

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TABLE I

Comparison of sensitivity and contamination of samples obtained by saline aspiratory puncture and vacuum puncture system for isolation of *Leishmania* from humans suspected of cutaneous leishmaniasis (C.L.) and dogs suspected of visceral leishmaniasis (V.L.)

Diseases and procedures	n° of paired culture tubes	Positivity (%)		Contamination (%)		TOTAL samples/individuals
		saline puncture	vacuum puncture	saline puncture	vacuum puncture	
Humans (C.L.) (skin puncture)	14	4(28,6)	4(28,6)	2(14,3)	4(28,6)	14/14
Dogs (V.L.) (Hepatic puncture)	22	5(22,7)	6(27,3)	3(16,6)	4(18,2)	22/6

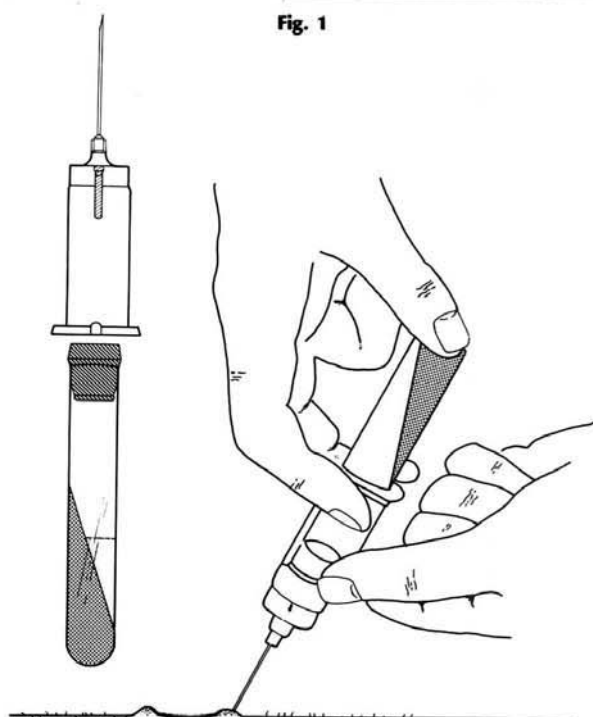


Fig. 1

Fig. 1. The vacuum aspiratory puncture system for *Leishmania* culturing.

The system can also be adapted (-1 to -2ml vacuum) to aspirate the gut contents of sandflies during dissection under sterile conditions on Microscope plates. Only a small fraction of the insect's gut emulsion should be carefully aspirated whenever positive.

In the laboratory, the tube should be kept at 24°C and its content examined every 5 days using an inverted microscope and transferred to a new medium.

In a preliminary assay we showed that this technique performed on 14 individuals suspected of cutaneous disease (unique ulcerated skin

lesion) in 14 paired samples gave comparable results to the former ones obtained by saline needle aspiration¹ (28,6% and 28,6%, respectively). In 22 paired samples from 6 dogs suspected of visceral disease (hepatic puncture) the comparative results were 27,3% by vacuum and 22,7% by saline (Table 1).

In our hands this system gives similar results to the classical technique of needle aspiration, while being safer, quicker, cheaper (tubes-medium and disposable needles *versus* tubes-medium, tubes-saline and disposable syringes and needles) and much more convenient in field conditions.

This system, with the proper adaptations, can also be used for isolation and culturing of other pathogens such as virus, fungi, bacteria and protozoa from animals and plants, as well as for the identification of neoplasm cells in fixing fluid.

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