## EVALUATION OF TWO BIPHASIC CULTURE MEDIA FOR ISOLATION OF LEISHMANIA PARASITES FROM CUTANEOUS ULCERS IN IRANIAN PATIENTS

Keywords: Leishmania, Isolation, Culture media.

For isoenzyme characterization of leishmania organisms from patients with cutaneous lesions, isolation of organisms was required on a suitable medium. Not much success was achieved by using the classical NNN medium.<sup>2,4,5</sup> A modification of Evans'

modified Tobie's medium<sup>1,2</sup> was produced by addition of 1.5% filter sterilized glucose solution. 12.5% defibrinated and inactivated horse blood was added as required.<sup>1,2</sup> Proline balanced salt solution was used as the liquid phase of this medium.<sup>2</sup> The role of glucose

Table 1. Percent of culture positive results according to the medium used.

Culture medium	NNN	Modified	
Result	ididid		
Smear positive	58	22	
Culture positive	18 (31%)	20 (91%)	
Smear negative	76	30	
Culture positive	3 (3.9%)	6 (20%)	

and proline in the metabolic pathway of leishmania organisms has been well documented.<sup>1,3</sup>

From 134 specimens of patients suspected of CL and cultured on NNN medium, amastigotes of leishmania were observed in only 58 cases. From smear positive specimens, 18 cases (31%) were also culture positive, while among smear negative cases only 3 cases (3.9%) were culture positive.

From 52 specimens of patients suspected of CL that were cultured on modified medium, amastigotes of leishmania were observed in the direct smear in 22 cases with 20 cases (91%) being culture positive. Six smear negative cases (20%) were shown to be culture positive (Table I).

Eight patients with unusual manifestations of CL and lack of response to Glucantime were also cultured on these media (Table II). Positive cultures were obtained from all 8 cases on the modified medium; however, no growth was observed on the NNN medium.

The modified medium appeared also to be suitable for maintenance of the organisms, as some of the isolates were viable even after 7 months. Use of this modified medium is recommended both for isolation and for maintenance of leishmania organisms isolated from cutaneous lesions in this region.

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Table II. Geographical distribution and site of lesion in 8 patients with cutaneous leishmaniasis. Culture results of these cases on modified Tobie's medium were all positive, while all results were negative on NNN medium.

Isolate code	Locality	Direct smear	Duration of lesion (years)	Site of lesion
MHOM/IR/95/Hat. 140	Esfahan	+	2.5	around eye
MHOM/IR/95/Hat. 144	Semnan		2	nose
• MHOM/IR/95/Hat. 145	-	-	12	left arm
MHOM/IR/95/Hat. 141	Iranshahr	+	2	nose
MHOM/IR/95/Hat. 164	Mehran	-	1.5	face-nose
MHOM/IR/95/Hat. 165	Saveh	+	9	face
MHOM/IR/95/Hat. 168 MHOM/IR/95/Hat. 169	Kabol	-	2.5	wrist
	Kabol	-	2	arm

<sup>\*</sup> This patient underwent leishmamsation in 1983.

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